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Research Article/Araştırma Makalesi

New and Additional Notes on the Aleocharinae Subfamily (Coleoptera: Staphylinidae) of Türkiye

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Abstract: In this study, new and additional records of 15 species of the Aleocharinae subfamily (Coleoptera: Staphylinidae) are presented from Türkiye. Among them, *Dinusa cretica* Assing, 2013 is the first record from Türkiye. Besides, *Autalia longicornis* Scheerpeltz, 1947 and *Maurachelia roubali* (Lohse, 1970) from the Marmara Region and *Enalodroma hepatica* (Erichson, 1839) from the Aegean Region are reported for the first time. In addition, new records are given for many provinces of Türkiye.

Keywords: New records, fauna, endemism, Anatolia.

Türkiye Aleocharinae (Coleoptera: Staphylinidae) Altfamilyası Üzerine Yeni ve Ek Notlar

Öz: Bu çalışmada, Aleocharinae (Coleoptera: Staphylinidae) alt familyasına ait 15 türün Türkiye'den yeni ve ek kayıtları sunulmaktadır. Bunlar arasından, *Dinusa cretica* Assing, 2013, Türkiye'den ilk kez kaydedilmiştir. Ayrıca, *Autalia longicornis* Scheerpeltz, 1947 ve *Maurachelia roubali* (Lohse, 1970) Marmara Bölgesi'nden ve *Enalodroma hepatica* (Erichson, 1839) Ege Bölgesi'nden ilk kez rapor edilmiştir. Ayrıca, Türkiye'nin birçok ili için yeni kayıtlar verilmiştir.

Anahtar kelimeler: Yeni kayıtlar, fauna, endemizm, Anadolu.

1. Introduction

The Staphylinidae family is the most diverse group of the Coleoptera order, comprising more than 64.000 valid species belonging to 33 subfamilies in the world, over 16.500 of them belonging to the Aleocharinae subfamily (Newton, 2019).

According to Anlaş (2007), Turkey, especially Anatolia, is one of the most interesting countries in terms of staphylinid taxonomy and biogeography. The Turkish fauna comprises most of the staphylinid subfamilies occurring the Palaearctic region and includes many endemic species.

The Staphylinidae family currently includes more than 2000 species from Turkey (Anlaş, 2009: updated, Schülke & Smetana, 2015). About 700 of them are restricted to Anatolia and represent more than 30% of the Turkish staphylinid fauna (Anlaş, 2009; updated). Even some genera have an endemism rate of almost one hundred percent. The staphylinid genera and subgenara with the highest diversity of endemic species in Turkey are *Geostiba* (83 endemics of 88 species), *Sunius* (43 endemics of 39 species), and *Eurysunius* (24 endemics of 25 species) (Anlaş, 2009, 2020, 2021, 2022; Örgel & Anlaş, 2020; Örgel, 2021).

In Turkey, the Aleocharinae subfamily contains 620 species (Anlaş, 2009; updated). The Aleocharinae species can be found in many habitats, mostly in moist places, under stones, on streams, in leaf litter of forest and meadows, and in decaying matter. In addition, many species are known as predator and parasitoid of pests. Especially some *Aleochara* species are parasitoids of Diptera pest species and, at the same time, they can be very effective as biological control agents (Özgen & Mamay, 2022).

The aim of this study is to give new faunistic records on Turkish Aleocharinae fauna.

2. Material and Methods

The present paper is based on the material collected during many field studies from different provinces of Turkey (Fig. 1 and Table 1) between the years of 2013 and 2017. The specimens have been collected by pitfall trap, shifting, and aspirator.

Figure 1. Locations in Anatolia in which the material used in this study were collected.

The morphological studies were carried out by a Stemi 508 (Zeiss Oberkochen, Germany) stereomicroscope. Habitus and forebody photographs of the new record for Turkey (*Dinusa cretica*) were taken with a Zeiss Axiocam ERC5s digital camera. Adobe Photoshop 2020 was used for focus stacking of these photos.

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CorelDRAW Graphics Suite X7 was used for editing photographs and drawings of median lobe of aedeagus in lateral view and spermatheca. The map was generated using the Google Earth Pro. Classification and nomenclature were made according to Newton (2019) and Schülke and Smetana (2015). The material referred to in this study is stored in the Alaşehir Zoological Museum, Manisa (AZMM).

Table 1. Information about coordinates of locations in distribution map.

Species	Province	Latitude	Longitude
Aleochara (*Coprochara*) *bipustulata*	Denizli	38°02'50"N	28°46'11"E
	Kütahya	39°15'37"N	28°48'37"E
		38°57'04"N	29°39'26"E
Aleochara (*Ceranota*) *erythroptera*	Denizli	37°37'01"N	29°26'55"E
	İzmir (Bozdağlar)	unknown	unknown
Aleochara (*Xenochara*) *grandeguttata*	Afyonkarahisar	38°39'42"N	30°06'05"E
	Kütahya	38°56'15"N	29°35'45"E
Aleochara (*Aleochara*) *lata*	Antalya	37°07'57"N	31°33'48"E
Aleochara (*Rheochara*) *leptocera*	Denizli	37°40'37"N	29°17'10"E
Aleochara (*Ceranota*) *subtumida*	İzmir (Bozdağlar)	unknown	unknown
Amarochara (*Lasiochara*) *siculifera*	Antalya	36°30'49"N	32°10'02"E
Autalia longicornis	Bursa	39°48'40"N	28°56'04"E
Dinusa cretica	Aydın	37°39'54"N	27°13'04"E
Ocalea (Ocalea) ruficollis	Aydın	37°39'45"N	28°15'51"E
	Denizli	38°18'03"N	29°54'39"E
	Manisa	38°51'40"N	28°49'11"E
Maurachelia roubali	Balıkesir	39°13'17"N	28°01'50"E
Tetralaucopora longitarsis	Aydın	37°41'10"N	27°14'33"E
	Balıkesir	39°18'57"N	28°23'53"E
	İzmir	38°23'41"N	28°02'54"E
Liogluta longiuscula	Adıyaman	37°53'07"N	37°43'05"E
	Afyonkarahisar	38°39'42"N	30°06'05"E
	Antalya	36°30'49"N	32°10'02"E
	Denizli	37°19'57"N	29°10'47"E
	Elazığ (Hazarbaba)	unknown	unknown
Enalodroma hepatica	Kütahya	39°02'52"N	29°17'46"E
Pseudosemiris kaufmanni	İzmir	38°17'26"N	28°01'06"E

3. Results

Family: Staphylinidae Latreille, 1802

Subfamily: Aleocharinae Fleming, 1821

Tribe: Aleocharini Fleming, 1821

Aleochara (*Coprochara*) *bipustulata* (Linnaeus, 1760)

Material examined. Denizli: 4 exs., 30.V.2014, Buldan, Yaylagöl, 1155 m, 38°02'50"N, 28°46'11"E, leg. Örgel. Kütahya: 5 exs., 01.VI.2015, Simav, Akdağ, 1963 m, 39°15'37"N, 28°48'37"E, leg. Örgel & Yağmur; 2 exs., 19.VI.2013, Gediz, Murat Mts., 2073 m, 38°57'04"N, 29°39'26"E, leg. Örgel & Yağmur.

Distribution in the world. This species is widely distributed in Europe, Asia, and North Africa (Schülke & Smetana, 2015).

Distribution in Turkey. Widespread in Turkey (Anlaş, 2009; Özgen, 2011; Sert et al., 2014, 2015, 2021; Tezcan et al., 2019).

Aleochara (*Ceranota*) *erythroptera* Gravenhorst, 1806

Material examined. Denizli: 3 exs., 17.IV.2015, Acıpayam, Elmadağ, 1659 m, 37°37'01"N, 29°26'55"E, leg. Örgel & Altın. İzmir: 1 ex., 15.IV.2014, Bozdağ Mts., Sazlı, leg. Örgel.

Distribution in the world. A. erythroptera is widely distributed in Europe and known from Israel and Turkey (Schülke & Smetana, 2015).

Distribution in Turkey. This species is known from Aydın, Denizli, Erzurum, İzmir, Kahramanmaraş, Kayseri, Kırşehir, Konya, Muğla, and Osmaniye provinces (Anlaş, 2009; Assing, 2018; Sert et al., 2015).

Aleochara (*Xenochara*) *grandeguttata* Assing, 2009

Material examined. Afyonkarahisar: 2 exs., 02.V.2015, Ahır Mts., 1925 m, 38°39'42"N, 30°06'05"E, leg. Örgel & Altın. Kütahya: 2 exs., 03.V.2015, Murat Mts., 1754 m, 38°56'15"N, 29°35'45"E, leg. Örgel & Yağmur.

Distribution in the world. A. grandeguttata is known from

Armenia, Hungary, Romania, and Turkey (Schülke & Smetana, 2015).

Distribution in Turkey. This species is known from Adana, Afyonkarahisar, Aksaray, Artvin, Bitlis, Burdur, Erzurum, Eskişehir, Gümüşhane, Hakkari, Isparta, Kayseri, Konya, Ordu, Samsun, Sinop, and Yozgat provinces (Anlaş, 2009; Assing, 2009a, 2013a; Sert et al., 2015; Örgel & Yağmur, 2021).

Aleochara (*Aleochara*) *lata* Gravenhorst, 1802

Material examined. Antalya: 1 ex., 30.IV.2016, İbradı, İbradı-Derebucak Hwy., 1337 m, 37°07'57"N, 31°33'48"E, leg. Kunt.

Distribution in the world. According to Schülke and Smetana (2015), this species widely distributed in Europe and Asia and known from Afrotropical Nearctic and Neotropical regions.

Distribution in Turkey. Ankara, Antalya, Karaman, and Konya (Anlaş, 2009; Anlaş & Rose, 2011; Sert et al., 2014, 2015).

Aleochara (*Rheochara*) *leptocera* Eppelsheim, 1889

Material examined. Denizli: 3 exs., 13.VI.2013, Honaz Mts., 2500 m, 37°40'37"N, 29°17'10"E, leg. Örgel & Anlaş.

Distribution in the world. The known distribution of this species is confined to Iran, Israel, Lebanon, Syria, and Turkey (Schülke & Smetana, 2015).

Distribution in Turkey. This species was known from Denizli, Kahramanmaraş, and Karaman provinces (Anlaş, 2009; Assing, 2007, 2009a, 2011, 2018).

Aleochara (*Ceranota*) *subtumida* (Hochhuth, 1849)

Material examined. İzmir: 2 exs., 14.IV.2014, Bozdağ Mts., Sorguncuk, leg. Örgel.

Distribution in the world. This species is distributed in Azerbaijan, Armenia, Georgia, Russia, and Turkey (Schülke & Smetana, 2015).

Distribution in Turkey. Bitlis, Bolu, Düzce, Gümüşhane, İzmir, Kahramanmaraş, Kastamonu, Kocaeli, Samsun, Sinop, and Zonguldak (Anlaş, 2009; Assing, 2013a, 2018).

Amarochara (*Lasiochara*) *siculifera* Assing, 2002

Material examined. Antalya: 2 exs., 30.III.2016, Alanya, Mahmutlar, Laertes Ancient City, 872 m, 36°30'49"N, 32°10'02"E, leg. Kunt.

Distribution in the world. This species is endemic to Anatolia (Schülke & Smetana, 2015).

Distribution in Turkey. A. siculifera was known from Adana, Hatay, and Mersin provinces (Anlaş, 2009; Assing, 2013a, 2015).

Tribe: Autaliini Thomson, 1859

Autalia longicornis Scheerpeltz, 1947

Material examined. Bursa: 3 exs., 02.VI.2015, Büyükorhan 5 km NE, 779 m, 39°48'40"N, 28°56'04"E, leg. Yağmur.

Distribution in the world. According to Schülke and Smetana (2015), this species widely distributed in Europe and known from Iran, Syria, and Turkey.

Distribution in Turkey. Antalya, Bartın, and Sinop (Anlaş, 2009; Assing, 2014).

Tribe Oxypodini Thomson, 1859

Dinusa cretica Assing, 2013 (Fig. 2)

Material examined. Aydın: 3 exs., 05.IV.2015, Kuşadası National Park, 866 m, 37°39'54"N, 27°13'04"E, leg. Örgel & Yağmur.

Distribution in the world. This species was known only from Crete and Karpathos Islands (Greece) until this study (Assing, 2016; Schülke & Smetana, 2015).

Distribution in Turkey. D. cretica is recorded for the first time from Turkey.

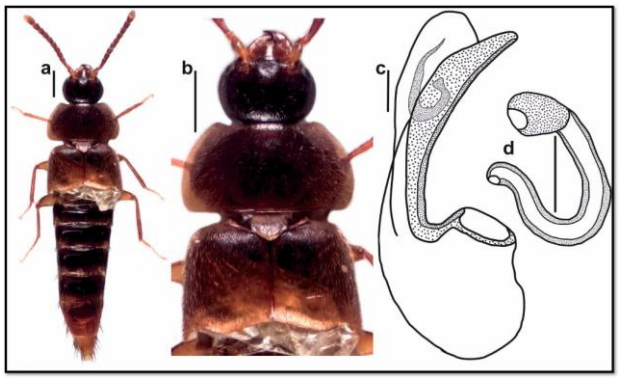

Figure 2. *Dinusa cretica* Assing, 2013. a) habitus; b) forebody; c) median lobe of aedeagus in lateral view; d) spermatheca. Scale bars: 0.5 mm (a, b) and 0.1 mm (c, d).

Ocalea (*Ocalea*) *ruficollis* Eppelsheim, 1888

Material examined. Aydın: 4 exs., 02.V.2013, Bozdoğan, Madran Yaylası, 1103 m, 37°39'45"N, 28°15'51"E, leg. Örgel & Yağmur. Denizli: 5 exs., 14.X.2013, Çivril, Işıklı, 843 m, 38°18'03"N, 29°54'39"E, leg. Örgel & Yağmur. Manisa: 2 exs., 14.III.2014, Selendi, Dedeler, 972 m, 38°51'40"N, 28°49'11"E, leg. Örgel & Yağmur.

Distribution in the world. This species is distributed in Algeria, Bosnia Herzegovina, Bulgaria, Greece, and Turkey (Schülke & Smetana, 2015).

Distribution in Turkey. Aksaray, Antalya, Artvin, Bolu, Çankırı, Eskişehir, Giresun, İstanbul, Kayseri, Kırşehir, Manisa, Nevşehir, Niğde, Sivas, Yozgat, and Zonguldak (Anlaş, 2009; Anlaş & Rose, 2011; Assing 2014; Sert et al., 2015, 2021).

Maurachelia roubali (Lohse, 1970)

Material examined. Balıkesir: 2 exs., 31.III.2016, Sındırgı, Koçu Mts., 1023 m, 39°13'17"N, 28°01'50"E, leg. Örgel & Yaman.

Distribution in the world. This species is distributed in Austria, Greece, Hungary, Slovakia, and Turkey (Schülke & Smetana, 2015).

Distribution in Turkey. M. roubali was only recorded from Isparta province in southern Anatolia by Assing (2013a).

Tetralaucopora longitarsis (Erichson, 1839)

Material examined. Aydın: 3 exs., 03.V.2013, Güzelçamlı, 3 km N, 503 m, 37°41'10"N, 27°14'33"E, leg. Örgel & Yağmur. Balıkesir: 2 exs., 13.IV.2014, Sındırgı, Ulus Mts., 1675 m,

39°18'57"N, 28°23'53"E, leg. Örgel & Yağmur. İzmir: 1 ex., 15.IV.2014, Bozdağlar, Çamurhamamı, 1513 m, 38°23'41"N, 28°02'54"E, leg. Örgel.

Distribution in the world. This species is widely distributed in Europe, Asia, and North Africa (Schülke & Smetana, 2015).

Distribution in Turkey. Adana, Amasya, Antalya, Artvin, Gümüşhane, İzmir, Konya, Manisa, Mersin, Niğde, and Yozgat (Anlaş, 2009; Anlaş & Rose, 2011; Assing, 2014, 2021; Sert et al., 2015, 2021; Örgel & Yağmur, 2021).

Tribe: Athetini Casey, 1910

Liogluta longiuscula (Gravenhorst, 1802)

Material examined. Adıyaman: 3 exs., 06.IV.2017, Gölbaşı-Kapıdere road, 1173 m, 37°53'07"N, 37°43'05"E, leg. Örgel & Yağmur. Afyonkarahisar: 3 exs., 02.V.2015, Ahır Mts., 1925 m, 38°39'42"N, 30°06'05"E, leg. Örgel & Altın. Antalya: 1 ex., 30.III.2016, Alanya, Mahmutlar, Laertes Ancient City, 872 m, 36°30'49"N, 32°10'02"E, leg. Kunt. Denizli: 2 exs., 03.V.2014, Bozdağ Mts., ski center, 2036 m, 37°19'57"N, 29°10'47"E, leg. Örgel. Elazığ: 1 ex., 23.IV.2017, Sivrice, Hazarbaba, leg Özgen.

Distribution in the world. This species is widely distributed in Europe, Asia, and North Africa (Schülke & Smetana, 2015).

Distribution in Turkey. Widespread in Turkey (Anlaş, 2009; Assing, 2010, 2013a, 2014).

Tribe: Geostibini Seevers, 1978

Enalodroma hepatica (Erichson, 1839)

Material examined. Kütahya: 3 exs., 10.V.2015, Şaphane Mts., 1736 m, 39°02'52"N, 29°17'46"E, leg. Örgel & Yağmur.

Distribution in the world. This species is widely distributed in Europe and known from South Korea and Turkey (Schülke & Smetana, 2015).

Distribution in Turkey. *E. hepatica* was only recorded from Bitlis and Konya provinces (Assing 2009b, 2013a).

Pseudosemiris kaufmanni (Eppelsheim, 1887)

Material examined. İzmir: 5 exs., 25.III-12.X.2017, Ödemiş, 794 m, 38°17'26"N, 28°01'06"E, Pitfall trap, leg Yağmur.

Distribution in the world. P. kaufmanni is widely distributed in Europe and known from Turkey (Schülke & Smetana, 2015).

Distribution in Turkey. This species was only recorded from Antalya and Muğla provinces (Anlaş, 2009).

4. Discussion

In the study, a total of 70 individuals belonging to the Aleocharinae subfamily were examined and 15 species from Turkey were identified. Among them, *Aleochara* (*Coprochara*) *bipustulata* is widespread in Turkey but it is recorded for the first time from Denizli and Kütahya provinces with this study. *Amarochara* (*Lasiochara*) *siculifera* was only known from Adana, Hatay, and Mersin provinces and it is most probably endemic to the Taurus Mountains (southern of Anatolia) and it is recorded for the first time from Antalya Province. *Autalia longicornis* was recorded from the Mediterranean, Western and Central Black Sea regions of Turkey and it is recorded for the first time from the Marmara Region. Therefore, this species is most probably widespread in Turkey but its distribution could not be revealed due to insufficient studies. *Dinusa cretica* was described from two specimens collected in the Dikti Oros (Crete Island (Greece) (Assing, 2013b). Then, it was recorded from Karpathos Island (Greece) (Assing, 2016). This species is associated with ants of the genus *Messor* Forel, 1890. *D. cretica* is recorded for the first time from Turkey in this study. *Ocalea* (*Ocalea*) *ruficollis* is recorded for the first time from Aydın and Denizli provinces. *Maurachelia roubali* was known from the Western Taurus Mountains (Isparta Province) (Assing, 2013a) and it is recorded for the first time from Marmara Region. This species is most probably widespread in Turkey. *Tetralaucopora longitarsis* is widespread in Turkey and recorded from Aydın and Balıkesir for the first time. *Enalodroma hepatica*, previously only known from the Eastern and Central Anatolia Regions, is recorded for the first time from the Aegean Region. *Pseudosemiris kaufmanni* is recorded for the first time from İzmir Province.

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References

Anlaş, S. (2007). The present situation of the Staphylinidae fauna of Turkey (Coleoptera). *Linzer biologische Beiträge*, 41(1), 5-9.

Anlaş, S. (2009). Distributional checklist of the Staphylinidae (Coleoptera) of Turkey, with new and additional records. *Linzer biologische Beiträge*, 41, 215-342.

Anlaş, S. (2020). A new micropterous species of the genus *Sunius* Stephens, 1829 from Central Anatolia (Coleoptera: Staphylinidae). *Zoology in the Middle East*, 66(4), 342-346. https://doi.org/10.1080/09397140.2020.1826676

Anlaş, S. (2021). On the genus *Sunius* Stephens, 1829 of Turkey IX. A new species from Northwestern Anatolia (Coleoptera: Staphylinidae: Paederinae). *Journal of Insect Biodiversity*, 26(3), 35-38. https://doi.org/10.12976/jib/2021.26.2.2

Anlaş, S. (2022). The subgenus *Eurysunius* Reitter in Turkey, with the description of three new species (Coleoptera: Staphylinidae: *Astenus*). *Zoology in the Middle East*, 68(1), 49-58. https://doi.org/10.1080/09397140.2022.2034305

Anlaş, S. & Rose, A. (2011). Some additional notes about Aleocharinae (Coleopetera: Staphylinidae) fauna of Turkey. *Munis Entomology & Zoology*, 6(1), 181-185.

Assing, V. (2007). On the Aleocharini of Turkey, with notes on some species from adjacent regions (Coleoptera: Staphylinidae, Aleocharinae). *Beiträge zur Entomologie*, 57, 177-209. https://doi.org/10.21248/contrib.entomol.57.1.177-209

Assing, V. (2009a). On the taxonomy and zoogeography of some Palaearctic *Aleochara* species of the subgenera *Xenochara* Mulsant & Rey and *Rheochara* Mulsant & Rey (Coleoptera: Staphylinidae: Aleocharinae). *Beiträge zur Entomologie*, 59(1), 33-101. https://doi.org/10.21248/contrib.entomol.59.1.33-101

Assing, V. (2009b). On the Staphylinidae of Turkey. VI. Thirteen new species and additional records (Coleoptera). *Koleopterologische Rundschau*, 79, 117-172.

Assing, V. (2010). On the Staphylinidae of Turkey. VII. Five new species and additional records (Coleoptera: Staphylinidae). *Koleopterologische Rundschau*, 80, 71-102.

Assing, V. (2011). On the Staphylinidae of Turkey VIII. Eleven new species, two new synonymies, a new combination, and additional records (Coleoptera: Staphylinidae). *Koleopterologische Rundschau*, 81, 179-227.

Assing, V. (2013a). On the Staphylinidae (Coleoptera) of Turkey IX. Five new species, a new synonymy, and additional records. *Stuttgarter Beiträge zur Naturkunde* A, Neue Serie 6, 103-125.

Assing, V. (2013b). On the Staphylinidae (Coleoptera) of Crete, Greece. *Stuttgarter Beiträge zur Naturkunde* A, Neue Serie 6, 83-102.

Assing, V. (2014). On the Staphylinidae of Turkey X. Two new species and additional records (Insecta: Coleoptera). *Linzer biologische Beitr*äge, 46(2), 1133-1146.

Assing, V. (2015). A revision of *Amarochara* Thomson of the Holarctic Region V. A new species from China, a new combination, the male of *A. caeca* Assing, and additional records (Coleoptera: Staphylinidae: Aleocharinae: Aleocharini. *Linzer biologische Beiträge*, 47(1), 63-71.

Assing, V. (2016). On the Staphylinidae of the Greek island Karpathos (Insecta: Coleoptera). *Linzer Biologische Beiträge*, 48(1), 235-263.

Assing, V. (2018). On the *Aleochara* subgenera *Ceranota* and *Xenochara*. IV. A revision of types, a new species, and additional records (Coleoptera: Staphylinidae: Aleocharinae). *Linzer Biologische Beiträge*, 50(1), 129-148.

Assing, V. (2021). On the taxonomy of *Parocyusa*, *Tectusa*, and miscellaneous genera of Oxypodina (Insecta: Coleoptera: Staphylinidae: Aleocharinae: Oxypodini). *Annalen des Naturhistorischen Museums in Wien*, 123, 99-218.

Newton, A.F. (2019). StaphBase: Staphyliniformia World catalog database (version Nov 2018). In: Species 2000 & ITIS Catalogue of Life, 2019 Annual Checklist (Roskov Y., Ower G., Orrell T., Nicolson D., Bailly N., Kirk P.M., Bourgoin T., DeWalt R.E., Decock W., Nieukerken E. van, Zarucchi J., Penev L., eds.). Retrieved from www.catalogueoflife.org/annual-checklist/2019.

Örgel, S. (2021). Descriptions of *Geostiba dindymosensis* sp. n. and *Geostiba yagmuri* sp. n. (Coleoptera: Staphylinidae: Aleocharinae), and additional records for *Geostiba* Thomson, 1858 from Turkey. *Turkish Journal of Entomology*, 45(3), 361-370. https://doi.org/10.16970/entoted.952885

Örgel, S., & Anlaş, S. (2020). Description of three new species of *Geostiba* Thomson 1858 (Coleoptera: Staphylinidae, Aleocharinae) from central western Anatolia. *Turkish Journal of Zoology*, 44(2), 156-164. https://doi.org/10.3906/zoo-1907-35

Örgel, S., & Yağmur, E.A. (2021). New locality records on Aleocharinae (Coleoptera: Staphylinidae) fauna of Turkey. *International Journal of Fauna and Biological Studies*, 8(5), 30-32. https://doi.org/10.22271/23940522.2021.v8.i5a.854

Özgen, İ. (2011). New records of Staphylinidae (Coleoptera) from Bulgaria, Iran and Turkey. *Acta Zoologica Bulgarica*, 63(2), 203-204.

Özgen, İ., & Mamay, M. (2022). Aleocharinae (Coleoptera: Staphylinidae) subfamily and its role in biological control of agricultural pests. *Journal of Research (Science)*, 33, 11-20.

Schülke, M., & Smetana, A. (2015). Staphylinidae. In: Löbl, I., & Löbl, D. (Ed). Catalogue of Palaearctic Coleoptera. Volume 2. Hydrophiloidea – Staphylinoidea. Revised and updated edition. Leiden, Brill, 304-1134.

Sert, O., Turan, Y., Fırat, S., Şabanoğlu, B., & Kabalak, M. (2014). Faunistical, ecological and zoogeographical assessments on some subfamilies of the family Staphylinidae (Coleoptera) in Ankara province. *Hacettepe Journal of Biology and Chemistry*, 42(4), 517-529.

Sert, O., Turan, Y., Fırat, S., & Şabanoğlu, B. (2015). Faunistic composition, ecological properties and zoogeographical composition of the subfamily Aleocharinae (Coleoptera: Staphylinidae) of the Central Anatolian Region of Turkey. *Transactions of the American Entomological Society*, 141(1), 197-221. https://doi.org/10.3157/061.141.0112

Sert, O., Turan, Y., & Kabalak, M. (2021). Contribution to the knowledge of Aleocharinae (Coleoptera: Staphylinidae) fauna of Turkey with new records. *Transactions American Entomological Society*, 147, 21-48. https://doi.org/10.3157/061.147.0103

Tezcan, S., Örgel, S., & Gülperçin, N. (2019). Staphylinidae (Insecta: Coleoptera) fauna associated with cow dung in Aspat (Strobilos), Bodrum, Muğla, Western Turkey. *Munis Entomology & Zoology*, 14(1), 192-196.

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Research Article/Araştırma Makalesi

Cleome quinquenervia (Cleomaceae): A New Plant Record for the Flora of Türkiye

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Abstract: In this article, *Cleome quinquenervia* (Cleomaceae) species is reported as a new record for the flora of Turkey based on the samples collected from Şanlıurfa, Mardin, and Adıyaman provinces. A detailed description and particular photographs of this species are given based on the large number of specimens collected. The meticulous comparison of the descriptions of the *C. quinquenervia* species and the information obtained in this study were made by examining the floras of the nearby countries. In addition, the systematical, ethnobotanical, and pharmacological features of the genus *Cleome* are summarized.

Keywords: Adıyaman, plant diversity, Mardin, spider flower, Şanlıurfa, taxonomy.

Cleome quinquenervia (Cleomaceae), Türkiye Florası İçin Yeni Bir Bitki Kaydı

Öz: Bu makalede, Şanlıurfa, Mardin ve Adıyaman illerinden toplanan örneklere dayanarak *Cleome quinquenervia* (Cleomaceae) türü, Türkiye Florası için yeni kayıt olarak rapor edilmektedir. Toplanan çok sayıda örneklere dayalı olarak bu türün, ayrıntılı betimi ve detaylı fotoğrafları verilmiştir. Yakın ülke floraları incelenerek bunlarda yer alan *C. quinquenervia* türünün betimlemeleri ile bu çalışmada elde edilen bilgilerin detaylı karşılaştırması yapılmıştır. Ayrıca *Cleome* cinsinin sistematik, etnobotanik ve farmakolojik özellikleri özetlenmiştir.

Anahtar kelimeler: Adıyaman, bitki çeşitliliği, Mardin, saçakgülü, Şanlıurfa, taksonomi.

1. Giriş

Cleomaceae familyası 14 cins (*Atamisquea* Miers ex Hook. & Arn., *Boscia* Lam., *Cadaba* Forssk., *Capparis* L., *Cleome* L., *Cleomella* DC., *Crateva* L., *Forchhammeria* Standl., *Koeberlinia* Zucc., *Maerua* Forssk., *Morisonia* L., *Oxystylis* Torr. & Frém., *Polanisia* Raf., *Wislizenia* Engelm.) ve bunlara bağlı yaklaşık 270 tür içermektedir. *Cleome* cinsi familyadaki en büyük cins olup dünyanın farklı bölgelerinde yetişen 200'den fazla tür içermektedir (Singh et al., 2018). Hem moleküler hem de morfolojik olarak Brassicaceae familyası ile yakın ilişkilidir (Thulin & Roalson, 2017).

Bu familya türleri Antarktika dışındaki tüm kıtalarda çöllerden tropik ormanlara ve aradaki her biyoklimatik bölgelere kadar bulunabilir (Bayat et al., 2018).

Cleome quinquenervia DC. türü, *Cleome* cinsinin *Thylacophora* Franch. seksiyonunun *Droserifolia* kladında yer alır. Bu klad içinde yer alan türler, eski dünyanın çöl ve yarı çöl bölgelerinde yaşamaktadır. Morfolojik olarak basit yapraklı, dört stamenli ve üçgenimsi, kısa kabzalı, dimorfik taç yapraklara sahip olması, ayrıca epipetal bezler taşıması ile bu klad diğer kladlardan ayrılmaktadır (Thulin & Roalson, 2017).

Droserifolia kladında 12 tür yer alır. Bunlardan *Cleome quinquenervia*, *C. fimbriata* ve *C. droserifolia* birbiri ile yakın ilişkilidir (Feodorova et al., 2010; Patchell et al., 2014). Bu türler yapılan bir çalışmada *Rorida quinquenervia* (DC.) Thulin & Roalson adı altında birleştirilmiştir (Thulin & Roalson, 2017). *R. quinquenervia* türü günümüzde, *C. quinquenervia*'nın homotipik sinonimi olarak kabul edilmektedir (POWO, 2022).

Cleome türleri, genellikle baharat olarak taze veya toz halde kullanılışı oldukça yaygındır. Bunun yanı sıra iltihap önleyici, ağrı kesici, ateş düşürücü, depresyon engelleyici, ishal kesici, solucan düşürücü olarak çeşitli tıbbi özelliklerinin de olduğu farklı yayınlarda rapor edilmiştir (Ansari et al., 2016; Fouche et al., 2016; Singh et al., 2018).

Cleome cinsi Türkiye'de *C. iberica* DC. (saçakgülü), *C. ornithopodioides* L. (taş saçakgülü) ve *C. stevensiana* Schult. & Schult.f. (bayır saçakgülü) olmak üzere 3 türle temsil edilmektedir. Hepsi sect. *Ornithodioides* içinde yer almaktadır (Coode & Cullen, 1965; Carlström, 1984).

Bu makalede, Türkiye florası için yeni kayıt olarak rapor edilen *Cleome quinquenervia* türü ise diğerlerinden farklı olarak *Thylacophora* seksiyonundadır. Türkiye florasında bu seksiyona ait bir tür ilk kez bu makalede rapor edilmiştir. Türkiye'de *Cleome* cinsine ait tür sayısı bu çalışma ile 4'e yükselmiştir.

2. Materyal ve Metot

Araştırma materyalini oluşturan bitki örnekleri 2021–2022 yılları arasında Şanlıurfa, Mardin ve Adıyaman illerinden toplanmıştır. Taze ve herbaryum örnekleri üzerinde steriomikroskop yardımıyla incelemeler yapılmıştır. Mikroskobik ölçümler AlaMet S. 0.06 programı ile yapılmıştır. IUCN tehlike kategorisi "Geospatial Conservation Assessment Tool" (GeoCAT) programı ile analiz edilmiştir. Bitki teşhisinde Thulin & Roalson (2017), Coode & Cullen (1965), Carlström (1984) ve komşu ülke floralarından; U.S.S.R (Bobrov, 1939), Irak (Blakelock & Townsend, 1980), İran (Hedge & Lamond, 1970) ve Pakistan (Jafri, 1973) floralarından yararlanılmıştır.

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Toplanan örnekler herbaryum materyali haline getirilerek Harran Üniversitesi Herbaryumu (HARRAN)'nda muhafaza altına alınmıştır.

3. Bulgular

Cleome quinquenervia DC., Prodr. 1: 239 (1824) (Şekil 1-2)

Tip: IRAN. "Perse," Olivier s. n. (holo: P00741872!; iso: BM001235008!) (Şekil 3).

Sin. (Thulin & Roalson, 2017):

Cleome fimbriata Vicary in J. Asiat. Soc. Bengal 16: 1158 (1847).

Cleome noeana Boiss., Diagn. Pl. Orient., Ser. 2, 1: 48 (1854).

Cleome noeana Boiss. var. *hispida* Regel & Schmalh. in Regel, Descr. Pl. Nov. Rar.: 12 (1882).

Cleome noeana Boiss. var. *persepolitana* Bornm. in Beih. Bot. Centralbl. 28: 127 (1911).

Cleome griffithiana Rech.f. in Anz. Österr. Akad. Wiss., Math.-Naturwiss. Kl. 87: 296 (1950).

Cleome dolichostyla Jafri in Kew Bull. 12: 174 (1957).

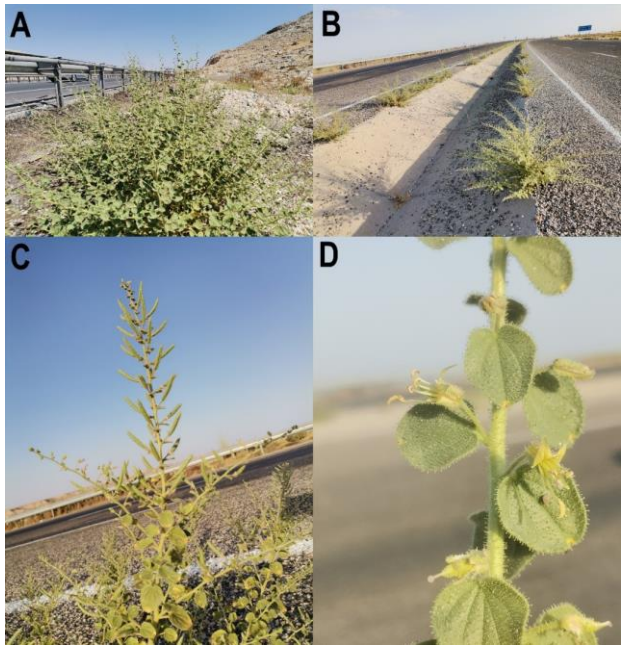
Rorida quinquenervia (DC.) Thulin & Roalson, Syst. Bot. 42: 576 (2017).

Türkçe Bilimsel Ad. Urfa saçakgülü, yeni bilimsel isim.

Yörede yerel ismine rastlanmamış olması ve ilk toplamanın Şanlıurfa ilinden olması nedeniyle bu türe Türkçe bilimsel ad olarak "Urfa saçakgülü" adı önerilmiştir (Menemen et al., 2016).

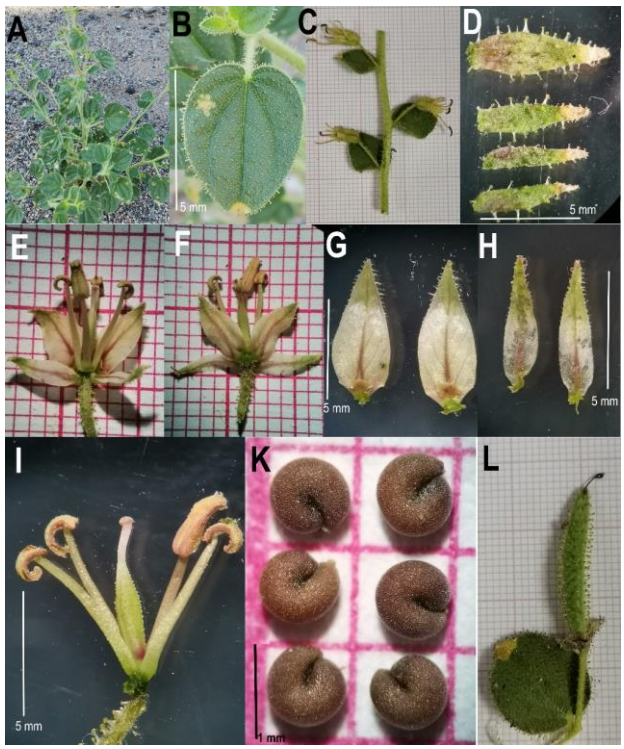
İncelenen materyaller. Türkiye, Şanlıurfa: Şanlıurfa-Mardin karayolu, Tek Tek Dağları, Çobanboğazı geçidi, yol kenarı boyunca, taşlık dere kenarı, 550-700 m, 27.08.2021, M. Balos 5246!; Şanlıurfa, Harran Üniversitesi, Osmanbey kampüsü, Tıp Fakültesi binası yakını, kaldırım üstü ve yol kenarı, 515 m, 03.08.2022, M. Balos 5402!; Şanlıurfa-Bozova yolu, Tülmen-Kesmetaş köyleri arası, yol kenarı, 745 m, 30.08.2022, M. Balos 5403!; Şanlıurfa-Adıyaman yolu, Adıyaman, Kuyulu köyü erozyon sahası yol üstü, 583 m, 30.08.2022, M. Balos 5404!; Adıyaman: Şanlıurfa-Adıyaman yolu, Adıyaman, Akpınar köyü arası, yol kenarı, 630 m, 30.08.2022, M. Balos 5405!; Şanlıurfa-Hilvan yolu, cezaevi kavşağı, kaldırım üstünde, 797 m, 31.08.2022, M. Balos 5406!; Mardin: Midyat'ın batısı, Acırlı mevkii, ormanlık alan yakını yol kenarı, 940 m, 04.09.2022, M. Balos 5407!.

Betim. Bitki tek ya da çok yıllık, 20–90 cm boyunda. Gövde dik, tabandan yukarıya doğru çok dallı, yoğun bir şekilde saplı yapışkan salgı tüyleriyle kaplı. Yapraklar basit, lamina geniş yumurtamsı ila dairesel, taban yapraklar 3.5–5 × 3–4 cm çapında, taban kordat, kenarlar saplı salgı tüylü, tepe apikulat veya mukronat uçlu yuvarlak, 5–7 damarlı, damarlar alt kısımda belirgin, laminanın üstü ve altı salgı tüylü, yaprak sapı 1–5 cm uzunluğunda. Çiçek durumu az çiçekli, gevşek, meyvede uzamış, terminal. Brakteler yapraksı, oval ila yumurtamsı, kısa saplı, 3 damarlı, çiçek durumu üst kısmına doğru küçülür. Çiçekler 9–13 mm, pedisellerin koltuklarından tekli çıkar, çift eşeyli, bir simetrili. Pediseller ince, 5–10 mm uzunluğunda, meyvede 12–15 mm uzunluğuna kadar, meyvede belirgin olarak dik. Sepaller 4, eşit değil, doğrusal, dikdörtgensi, mızraksı, valvat (valf şeklinde),



Şekil 1. *Cleome quinquenervia*'nın A-B: Doğal habitatındaki fotoğrafları, C-Meyve durumu, D-Çiçek durumu.

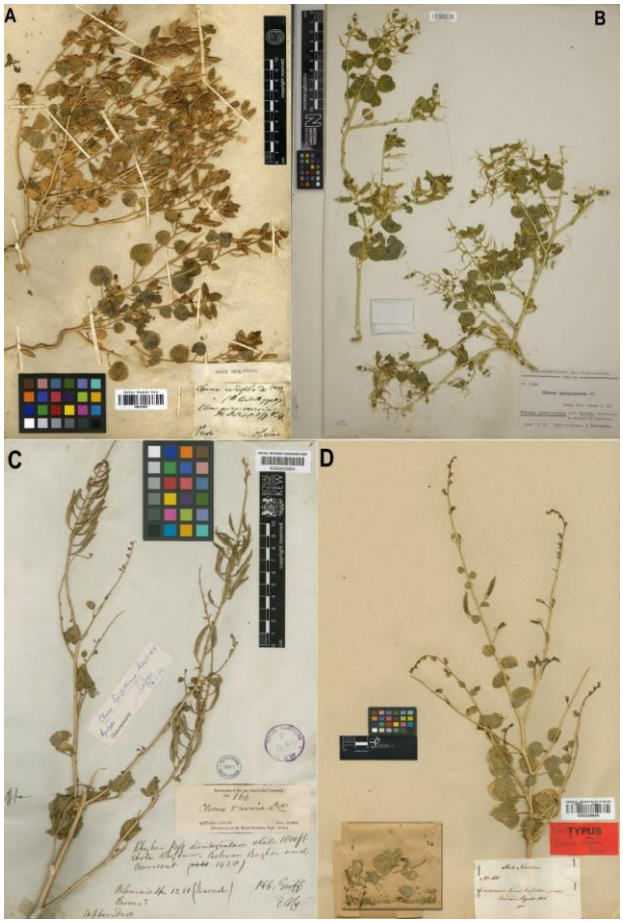
Figure 1. A-B: Photographs of *Cleome quinquenervia* in its natural habitat, C-Fruit status, D-Flower status.



Şekil 2. *Cleome quinquenervia;* A- Genel görünüm, B-Yaprak, C-Çiçeklerin gövde üzerindeki dizilişi, brakte ve stipul durumu, D-Sepaller, E-Çiçeğin sepal olmadan önden görünüşü, F-Çiçeğin sepal olmadan sırttan görünümü, G-H-Petal çiftleri, I-Ovaryum, stilus, anter ve flament durumu, K-Tohumlar, L-Stipullu meyve ve yaprak benzeri brakte.

Figure 2. *Cleome quinquenervia*; A- General view, B-Leaf, C-Arrangement of flowers on the stem, bract and stipule status, D-Sepals, E-Front view of flower without sepal, F-Rear view of flower without sepal, G-H-Petal pairs, I-Ovary, stylus, anther and filament state, K-Seeds, L-Stipuled fruit and leaf-like bract.

tabanda hafifçe birleşir, tepe sivri, tüylü, 3.5–6.5 × 1–2 mm, yoğun salgı tüylü. Petaller 4 adet, sarı veya beyazımsı sarı, kahverengimor damarlı, yuvarlak ve dalgalı kenarlı bazal pullu, uç kısma doğru yeşilimsi, dimorfik, bir çifti mızrak şeklinde, 6–8 × 1.5–3.75 mm, kısa bir kabzanın üstünde geniş oval şeklinde aniden genişler, yukarı doğru daralarak küt bir uçla sonlanır, uca doğru ince fakat yoğun salgı tüylü, diğer çift ise daha dar olup 5.5–7 × 1.4–1.8 mm boyutlarındadır. Stamen 4 adet, kaliksten ileriye doğru uzamış. Ovaryum üst durumlu, dikdörtgensi ya da uzun elips şeklinde, alt kısım kırmızımsı, 1.5–5.5 × 0.5–1.25 mm çapında, iki karpelli, tek bölmeli, yan 2 plesenta üzerinde birçok ovül bulunur; üst kısım sapsız ve salgı tüylü. Meyve kapsül şeklinde, 2–3.5 × 0.3–0.5 cm, hafif kavisli, her iki ucunda sivrilen, siliquiform yapıda, üzeri az buruşuk, ince ağ damarlı, yoğun salgı tüylü. Stilus kısa 0.5–3.2 mm, stigma kesik, şişkin. Anterler sarımsı-açık kahverengi, 1.75–4.2 mm uzunluğunda, apikulat, flament 5–9 mm boyunda. Tohumlar 0.75–0.9 mm çapında, kahverengi, çok sayıda, basık, yuvarlak, iki ucu kaynaşmış, merkeze kadar yarık, düzenli sivilcemsi çıkıntılı, yüzeyi tüysüz.



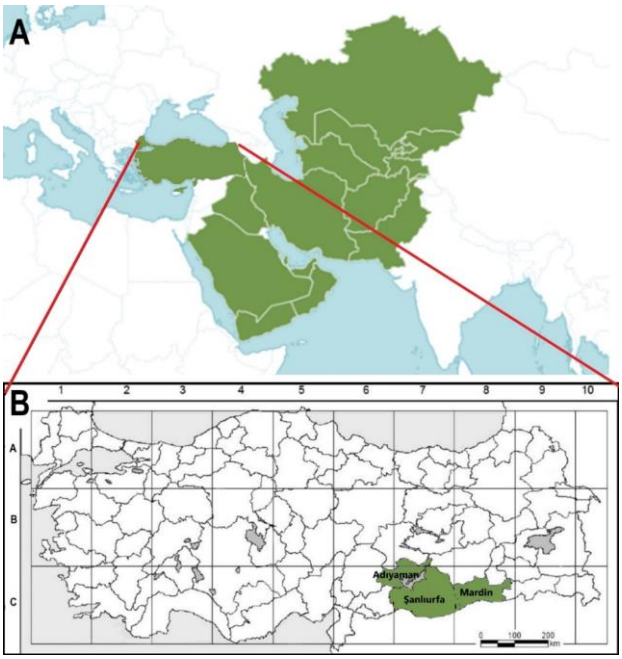
Şekil 3. *Cleome quinquenervia*'nın A- Holotip örneği (P00741872). B-İzotip örneği (BM001235008), C-İzolektotip örneği (K000643924), D-Lektotip (G00330648) (Thulin & Roalson, 2017).

Figure 3. A-Holotype specimen of *Cleome quinquenervia* (P00741872). B-Isotype sample (BM0011235008), C-Isolectotype (K000643924), D-Lectotype (G00330648) (Thulin & Roalson, 2017).

Genel yayılış. Suudi Arabistan, Bahreyn, Katar, Birleşik Arap Emirlikleri, Umman, Irak, İran, Afganistan, Pakistan, Türkmenistan, Özbekistan ve Tacikistan'dan biliniyor (Thulin & Roalson, 2017). Türkiye'de ise Şanlıurfa, Adıyaman ve Mardin'de yayılış gösterir (Şekil 4).

Fenoloji. Bu türün tespit edilen çiçeklenme dönemi Temmuz ve Ağustos aylarıdır. Daha sonra kısa sürede meyve dönemine girmektedir. Bitki meyveye dönerken genellikle üzerinde hem meyve hem de çiçek bulunabilir. Yetiştiği alanların ortalama yükseltisi 515–940 metre arasında değişmektedir.

Tehlike ve koruma statüsü. Bu türün Şanlıurfa, Mardin ve Adıyaman popülasyonları arasındaki mesafe 6358 km²'ye ulaşmaktadır. IUCN tehlike kategorisi Geospatial Conservation Assessment Tool (GeoCAT, 2022) programı ile analiz edilmiş ve IUCN (2022) kategorileri için en uygun VU (Zarar görebilir) kategorisi önerilmiştir (Şekil 5).



Şekil 4. *Cleome quinquenervia*'nın Dünya'daki (A) (POWO, 2022) ve Türkiye'deki (B) dağılım haritası.

Figure 4. Distribution map of *Cleome quinquenervia* in the World (A) (powo.science.kew.org) and Turkey (B).

Şekil 5. *Cleome quinquenervia*'nın IUCN tehlike kategorisinin GeoCAT programı ile analizi (GeoCAT, 2022).

Figure 5. Analysis of the IUCN risk category of *Cleome* quinquenervia with GeoCAT program (GeoCAT, 2022).

Habitat. *Cleome quinquenervia* türünün yetiştiği alanlar taşlık, kuru dere yatakları, dökülmüş harfiyat üstü alanlar, otoyol kenarları ve yol aralarındaki kanalların çatlaklarıdır. *Echinops spinosissimus* Turra, *Picnomon acarna* (L.) Cass, *Chrozophora tinctoria* (L.) A.Juss. ve *Lactuca serriola* L. gibi bitkiler ile aynı habitatı paylaşmaktadır.

4. Tartışma ve Sonuç

Cleome quinquenervia bu çalışmadan önce Türkiye'de kayıt altına alınmamıştı. Bu türün genel görünüşü Türkiye'de bulunan diğer *Cleome* türlerinden (*C. iberica*, *C. ornithopodioides* ve *C. stevensiana*) oldukça farklıdır. Bahsi

geçen türler *Ornithodioides* seksiyonunda yer alır. *C. quinquenervia* türü ise *Thyllacophora* seksiyonunda yer alır. Bu seksiyonun temel ayrım karakterleri şu şekildedir: 4 serbest stamen, kenarı dalgalı yuvarlak bazal pullu (claw), mızraksı tipte petaller, nispeten geniş siliquiform kapsüller ve kendine özgü basit yapraklar (Bobrov, 1939).

Araştırma bulguları Flora of U.S.S.R (Bobrov, 1939), Flora of Irak (Blakelock & Townsend, 1980), Flora Iranica (Hedge & Lamond, 1970) ve Flora of Pakistan (Jafri, 1973) ile karşılaştırılmıştır (Tablo 1). Tablo 1'de komşu floralardaki türler ve son çalışmalarda sinonim olarak geçen türler floradaki ismi ile aynen alınmıştır. Bu karşılaştırmada en göze çarpan farklılık, Türkiye'deki örneklerin bitki boyu uzunluğunun 90 cm'e kadar uzamış olmasıdır. Ayrıca petal renginde, yapraklardaki damar sayısında, yaprak sapı, çiçek sapı, sepal ve petal uzunluklarında bazı farklılıklar tespit edilmiştir. Farklılıklarda habitat ve ekolojik koşullardaki varyasyonun bir yansımasının olması muhtemeldir. Tablo 1'de görülebileceği gibi çiçek uzunluğu, brakte şekli, geniş ve dar petal ölçüleri, ovaryum ölçüleri, anter rengi, anter ve flament ölçüleri İran florasında verilmemiştir. Sepal şekli İran florasında *Cleome quinquenervia* ve *C. noeana* türlerinde verilmemiştir. Pedisel uzunluğu, sepal şekli, sepal ölçüleri, petal uzunluğu, geniş ve dar petal ölçüleri, ovaryum ölçüleri, stilus, anter rengi, anter uzunluğu, flament uzunluğu ve tohum ile ilgili veriler Rusya florasında verilmemiştir. Sepal şekli, dar ve geniş petal ölçüleri, ovaryum ölçüleri, anter şekli, anter ve flament ölçüleri de Pakistan florasında belirtilmemiştir.

Tablo 1. *Cleome quinquenervia*'nın araştırma sonuçlarının komşu ülke floraları ile karşılaştırılması

Table 1. Comparison of the research results of *Cleome quinquenervia* with the flora of neighboring countries

Karakterler	*C. quinquenervia* (Araştırma sonuçları)	*C. quinquenervia* (İran)	*C. quinquenervia* (U.S.S.R)	*C. noeana* (İran)	*C. noeana* (U.S.S.R)	*C. noeana* (Irak)	*C. dolichostyla* (İran)	*C. fimbriata* (Pakistan)
Bitki boyu (cm)	20–90	8–35	10–20	40–60	20–60	46–60	15–35	20–60
Taban yapraklar (boy x en cm)	3.5–5 × 3–4	3.5 x 2	3 × 3	4 × 4	≤5 × 5	4 × 4	3 × 4	4 × 3.5
Yaprak laminası	yuvarlak-geniş yumurtamsı, kordat	yuvarlak-geniş yumurtamsı, kordat	yuvarlak, kordat	geniş yumurtamsı, kordat	yuvarlak, kordat	yumurtamsı-yuvarlak, kordat veya kesik	geniş yumurtamsı, kordat	yumurtamsı-dairesel, yuvarlak-subkordat
Yaprak damar sayısı	5–7	5	3–7	5–7	7	5–7	5	-
Yaprak sapı uzunluğu (cm)	1–5	≤4	≤6	-	±5	1.5–3	≤4	1–2
Pedisel (çiçek sapı) (mm)	5–10	≤12	-	3–10	-	4–12	≤6	5–12
Çiçek uzunluğu mm	9–12	-	±7	-	±8	-	-	30-40
Brakte	yapraksı, kısa saplı	-	mızrak şeklinde, sapsız	-	yuvarlak kordat, sivri uçlu, sapsız	-	-	yapraksı, sapsız
Sepaller	dikdörtgensi, mızraksı, valvat	-	-	-	-	doğrusal-dikdörtgen, akut	ovat	-
Sepaller (boy x en mm)	3.5–6.5 × 1–2	3–4 mm boy	-	3–4 mm boy	±7 mm boy	3–4 mm boy	±4 mm boy	3–4 × 1.5–2
Petal boyu (mm)	5.5–8 × 1.4–3.75	5 × 1.5–2.5	-	6 × 1.5–2.5	-	5–8	6–8 × 1.5–3.5	6–8 × 1.5–2.5
Geniş petal (boy x en mm)	6–8 × 1.5–3.75	-	-	-	-	-	-	-
Dar petal (boy x en mm)	5.5–7 × 1.4–1.8	-	-	-	-	-	-	-
Petal rengi	sarı veya beyazımsı sarı	-	sarı	sarı	sarımsı	sarı (veya beyazımsı)	koyu sarı, leylak damarlı	mor veya menekşe damarlı sarı
Ovaryum (mm)	1.5–5.5 × 0.5–1.25	-	-	-		-	-	-
Meyve kapsülü (boy x en cm)	2–3.5 × 0.3–0.5	1–1.8 × 0.4–0.5	1.5–2 × 0.6	1.5–3 × 0.3–0.4	2–4 × 0.2–0.4	1.5–3.2 × 0.3–0.4	1.8–2.8 × 0.4	25–35 × 3–4
Stilus (mm)	0.5–3.2	1–2	-	±3	-	3	8–12	1
Anter rengi	sarı-açık kahverengi	-	-	-	-	-	-	-
Anter uzunluğu (mm)	1.75–4.2	-	-	-	-	-	-	-
Flament uzunluğu (mm)	5–9	-	-	-	-	-	-	-
Tohum çapı (mm)	0.75-0.9	≤1	-	≤1	-	±1	≤1	±1
Tohum yüzeyi	verrüküloz, tüysüz	granüllü, tüysüz	-	granüllü, tüysüz	-	verrüküloz, tüysüz	pürüzsüz, tüysüz	tanecikli
Tohum rengi	kahverengi	kahverengi	-	koyu kahverengi	-	kahverengi	parlak samani	koyu kahverengi

Petallerin dimorfik özellik göstermesinden dolayı geniş olan ve dar olan petal ölçüleri ayrı ayrı ve ilk kez bu makale içinde verilmiştir. Anter ve flament özellikleri de ilk kez bu çalışmada ortaya konmuştur.

Thulin & Roalson (2017) tarafından yapılan araştırmada *Cleome* cinsi yerine *Rorida* cinsi yeniden diriltilerek kullanılmıştır. *C. quinquenervia, C. fimbriata, C. noeana ve C. dolichostyla* arasında net bir ayırım yapamadıklarını belirtmekte ve bu türleri *Rorida quinquenervia* türünün sinonimi olarak ele almaktadırlar. Bu türlerin genel görünüşleri, yaprak şekli ve indumentum özelliği bakımından benzerdir. Tablo 1'de verilen morfolojik karşılaştırmada verilen karakterlerin birbirine yakın oldukları görülmektedir. Parsa (1951) daha önce *C. noeana*'yı *C. quinquenervia* türünün varyetesi olarak ele almış, Jafri (1973) hem *C. noeana* hem de *C. griffithiana*'yı *C. fimbriata* türünün sinonimi olarak ele almış ve bunun da aslında *C. quinquenervia*'ya "çok benzer" olduğunu söylemiştir. Blakelock & Townsend (1980), *C. noeana* türünün "*C. quinquenervia* türüne rahatsız edici derecede yakın" olduğunu söyleyerek benzer bir açıklama yapmışlardır. *Cleome* cinsinin *Thylacophora* Franch. seksiyonunun *Droserifolia* kladında yer alan türlerin statülerinin durumu için geniş çapta bir monografa ihtiyaç vardır.

Antik çağlardan beri *Cleome* türleri, halk hekimliğinde antimikrobiyal, antidiyabetik, antelmintik, antiinflamatuar, antikonvülsan, ateş düşürücü, ishal ve gaz giderici, yara iyileştirici, epilepsi, konvülsiyon, spazm, ağrı ve cilt rahatsızlıkların tedavisinde kullanıldığı rapor edilmektedir (Devi et al., 2002; Parimaladevi et al., 2003; Bose et al., 2011; Motaal et al., 2011; Thomas et al., 2014; Archi et al., 2016; Khuntia et al., 2022).

Fitokimyasal araştırma sonuçlarına göre, *Cleome* türlerinde alkaloidler, uçucu yağlar, yağ asitleri, flavonoidler, terpenler, steroller ve antosiyaninler dahil olmak üzere geniş yelpazede yararlı biyoaktif bileşikler içerdiği rapor edilmiştir. *Cleome* cinsi üzerinde yapılan biyolojik aktivite çalışmalarının incelenmesi sonucunda bazı türlerin Anti-hiperglisemik, Analjezik, antipiretik, antiinflamatuar, antioksidan, antiartritik, anthelmintik, antimikrobiyal, müshil, diüretik, antiromatizmal, antitahriş, antiirritan, antikanser, tümör büyümesini önleyici, tip 2 diyabet ve cilt rahatsızlıklarını giderici aktivite gösterdiği rapor edilmiştir (Khuntia et al., 2022).

Cleome quinquenervia çok yoğun bir kokuya sahiptir. İran'daki *C. quinquinervia*'nın kök, yaprak ve tohum uçucu yağının kimyasal içeriği araştırılmıştır. Çalışma sonucunda yaprakta β-pinen (%31), α-pinen (%26.1), trans-pinokarvil asetat (%6.6) olduğu, kökte α-eudesmol (%29), β-eudesmol (%27.5) ve γ-eudesmol (%13), tohumda trans-pinokarvil asetat (%12.5), β-eudesmol (%10.8) ve β-pinen (%10.8), α-eudesmol (%9.6) olduğu rapor edilmiştir (Mirza et al., 2015). *C. quinquenervia*'nın sinonimi olan ve Suudi Arabistan'dan toplanan *C. dolichostyla* türünün tohum yağındaki asit içerikleri araştırılmış, yağın insan ve hayvan tüketimi için gıda potansiyelinin olabileceği rapor edilmiştir (Sawaya et al., 1985). Bu türün biyolojik aktivite özelliklerinin araştırılması bu çalışmanın bir sonraki adımı olarak düşünülebilir.

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Kaynaklar

Ansari, P., Debnath, M., Ahmad, M.F., Azam, S., Akther, S., Mustakim, G.M., ..., & Sarwar, S. (2016). Evaluation of antinociceptive activity of methanol extract from Cleome rutidosperma in Mice. *Chinese Herbal Medicines*, 8(3), 273–279. https://doi.org/10.1016/S1674-6384(16)60050-3

Archi, F.F., Islam, S., Babu, M.A.H.K., Ullah, A., Azam, S., Chowdhury, M. R., ..., & Goswami, S. (2016). Potential evaluation of central nervous system anti-depressant activity of *Cleome rutidosperma* in mice. *Biomedical Research and Therapy,* 3(10), 889–901.

Bayat, S., Schranz, M.E., Roalson, E.H., & Hall, J.C. (2018). Lessons from Cleomaceae, the sister of crucifers. *Trends in Plant Science*, 23(9), 808–821. https://doi.org/10.1016/j.tplants.2018.06.010

Blakelock, R.A., & Townsend, C.C. (1980). Cleomaceae. In: Flora of Iraq. Townsend, C. C. and Guest, E. (Eds.). Baghdad: Ministry of Agriculture and Agrarian Reform, 4(1), 144–155.

Bobrov, E.G. (1939). Capparidaceae. In: Flora of U.S.S.R. Komarov (Ed.). Israel program for scientific translations, Israel, 8, 5–13.

Bose, U., Bala, V., Ghosh, T.N., Gunasekaran, K., & Rahman, A.A. (2011). Antinociceptive, cytotoxic and antibacterial activities of *Cleome viscosa* leaves. *Revista Brasileira de Farmacognosia,* 21(1), 165–169. https://doi.org/10.1590/S0102-695X2011005000023

Carlström, A. (1984). A revision of *Cleome* series Ornithopodioides Tzvelev (Capparaceae). *Willdenowia*, 14, 119–130.

Coode, M.J.E., & Cullen, J. (1965). *Cleome* L. In: Davis PH (ed). Flora of Turkey and the East Aegean Islands. Edinburgh: Edinburgh University Press. 1: 498.

Devi, B.P., Boominathan, R., & Mandal, S.C. (2002). Evaluation of anti-diarrheal activity of *Cleome viscosa* L. extract in rats. *Phytomedicine*, 9 (8), 739–742. https://doi.org/10.1078/094471102321621368

Feodorova, T.A., Voznesenskaya, E.V., Edwards, G.E., & Roalson, E.H. (2010). Biogeographic patterns of diversification and the origins of C4 in *Cleome* (Cleomaceae). *Systematic Botany*, 35(4), 811–826. https://doi.org/10.1600/036364410X539880

Fouche, G., Leboho, T., Wellington, K.W., Sakong, B.M., Adenubi, O.T., Pauw, E., & Eloff, J.N. (2016). Anthelmintic activity of acetone extracts from South African plants used on egg hatching of Haemonchus contortus. *Onderstepoort Journal of Veterinary Research*, 83(1), 1–7.

GeoCAT, (2022). Geospatial Conservation Assessment Tool Program. Retrieved from http://geocat.kew.org/

Hedge, I., & Lamond, J. (1970). Capparidaceae. In: K. H. Rechinger (Ed.), Flora Iranica. Austria: Academische Druck-U-verlagsanstalt Graz, 68, 13–30.

IUCN, (2022). Guidelines for Using the IUCN Red List Categories and Criteria. Version 15. Prepared by the Standards and Petitions Committee. Retrieved from https://www.iucnredlist.org/resources/redlistguidelines

Jafri, S.M.H. (1973). Capparidaceae. In: Nasir, E., Ali S. I. (Eds.), Flora of Pakistan, Karachi: The Herbarium, Dept. of Botany, University of Karachi, Pakistan, 34, 20–32.

Khuntia, A., Martorell, M., Ilango, K., Bungau, S.G., Radu, A.F., Behl, T., & Sharifi-Rad, J. (2022). Theoretical evaluation of *Cleome* species' bioactive compounds and therapeutic potential: A literature review. *Biomedicine & Pharmacotherapy*, 151, 113–161. https://doi.org/10.1016/j.biopha.2022.113161

Menemen, Y., Aytaç, Z., & Kandemir, A. (2016). Türkçe Bilimsel Bitki Adlandırma Yönergesi. *Bağbahçe Bilim Dergisi,* 3(3), 1–3.

Mirza, M., Navaei, M.N., & Mahnesaee, A. (2015). Study on Root, Leaf and Seed Chemical Composition of *Cleome quinquenervia* DC. from Iran. *Journal of Essential Oil Bearing Plants*, 18(2), 510–514. https://doi.org/10.1080/0972060X.2014.1001136

Motaal, A.A., Ezzat, S.M., & Haddad, P.S. (2011). Determination of bioactive markers in *Cleome droserifolia* using cell-based bioassays for antidiabetic activity and isolation of two novel active compounds. *Phytomedicine*, 19(1), 38–41. https://doi.org/10.1016/j.phymed.2011.07.003

Parimaladevi, B., Boominathan, R., & Mandal, S.C. (2003). Studies on analgesic activity of *Cleome viscosa* in mice. *Fitoterapia*, 74(3), 262–266. https://doi.org/10.1016/S0367-326X(03)00020-0

Parsa, A. (1951). *Cleome*. In: Flore de l'Iran (La Perse). Teheran: Imprimerie Danesh, 1(1), 908–914.

Patchell, M.J., Roalson, E.H., & Hall, J.C. (2014). Resolved phylogeny of Cleomaceae based on all three genomes. *Taxon*, 63(2), 315–328. https://doi.org/10.12705/632.17

POWO, (2022). *Cleome* L. In: Plants of the World Online. Retrieved from http://www.plantsoftheworldonline.org

Sawaya, W.N., Khalil, J.K., & Ahmad, S. (1985). Chemical composition and nutritional quality ofCleome dolichostyla seeds. *Plant Foods for Human Nutrition*, 35(1), 27–33.

Singh, H., Mishra, A., & Mishra, A.K. (2018). The chemistry and pharmacology of *Cleome* genus: a review. *Biomedicine & Pharmacotherapy*, 101, 37–48. https://doi.org/10.1016/j.biopha.2018.02.053

Thomas, B., Arumugam, R., Veerasamy, A., & Ramamoorthy, S. (2014). Ethnomedicinal plants used for the treatment of cuts and wounds by Kuruma tribes, Wayanadu districts of Kerala, India. *Asian Pacific Journal of Tropical Biomedicine*, 4, 488–491. https://doi.org/10.12980/APJTB.4.2014B571

Thulin, M., & Roalson, E.H. (2017). Resurrection of the genus Rorida (Cleomaceae), a distinctive Old World segregate of *Cleome*. *Systematic Botany*, 42(3), 569–577. https://doi.org/10.1600/036364417X695989

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Research Article/Araştırma Makalesi

Protective Effect of 3-Benzoyl-7-Hydroxy Coumarin on Lipid Peroxidation and Minerals on Rat Liver Tissues Induced Oxidative Stress with Lead Acetate

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Abstract: Lead is a toxic substance and damages human metabolism. Coumarin-derived substances have many effects such as antioxidant, anticancer, and antibacterial ones. In this study, the effects of 3-benzoyl-7-hydroxy coumarin on rat liver tissues under oxidative stress with lead acetate were investigated. In the study, rats were divided into 4 groups. Control group (K), Coumarin group (KUM), Lead acetate group (P) and Coumarin+Lead acetate group (KUMP) groups were formed. Malondialdehyde (MDA), lead (Pb), iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu) levels were determined in the liver tissues of the rats. MDA level of P group increased compared to the other groups. A decrease was observed in the KUMP group compared to the P group MDA level. While the Pb and Fe levels of the P group increased compared to the K group, the Pb and Fe levels of the KUMP group decreased compared to the P group. As a result, it is concluded that the 3-benzoyl-7-hydroxy coumarin molecule protects the high lipid peroxidation, lead, and iron metabolism caused by lead acetate in the liver.

Keywords: Sprague-Dawley, toxic, antioxidant, malondialdehyde.

Kurşun Asetat İle Oksidatif Stres Oluşturulan Rat Karaciğer Dokuları Üzerine 3-Benzoil-7-Hidroksi Kumarin'in Lipit Peroksidasyon ve Mineraller Üzerine Koruyucu Etkisi

Öz: Kurşun, toksik bir maddedir ve insan metabolizmasına hasar vermektedir. Kumarin türevli maddelerin antioksidant, antikanser ve antibakteriyel gibi birçok etkileri vardır. Bu çalışmada, kurşun asetat ile oksidatif stres oluşturulan rat karaciğer dokuları üzerine 3-benzoil-7-hidroksi kumarin'in etkileri araştırıldı. Çalışmada ratlar 4 gruba ayrıldı. Kontrol grubu (K), Kumarin grubu (KUM), Kurşun asetat grubu (P) ve Kumarin+Kurşun asetat grubu (KUMP) grupları oluşturuldu. Ratların karaciğer dokularında malondialdehit (MDA), kurşun (Pb), demir (Fe), çinko (Zn), mangan (Mn) ve bakır (Cu) düzeyleri tespit edildi. P grubu MDA düzeyi diğer gruplara göre arttı. P grubu MDA düzeyine göre KUMP grubunda azalma gözlendi. P grubu Pb ve Fe düzeyi K grubuna göre artarken, KUMP grubu Pb ve Fe düzeyi P grubuna göre azaldı. K grubu Mn düzeyi diğer gruplara göre azaldığı gözlendi. Sonuç olarak kurşun asetatın karaciğerde oluşturduğu yüksek lipit peroksidasyonu, kurşun ve demir metabolizmasını 3-benzoil-7-hidroksi kumarin molekülünün koruduğunu düşünmekteyiz.

Anahtar kelimeler: Sprague-Dawley, toksik, antioksidant, malondialdehit.

1. Giriş

Kurşun, toksik bir maddedir ve endüstride yoğun olarak kullanılmaktadır. İnsan ve hayvan metabolizmasını olumsuz olarak etkilemektedir. İnsanlar bu toksik maddeyi solunum, içme suyu ve yiyecekler yoluyla metabolizmasına alır (Fischbein, 1992; Xiang et al., 2010). Kurşun toksisitesi özellikle kan, böbrek ve karaciğer dokularında etkilidir. Uzun dönem kurşun toksisitesine maruz kalınmada karaciğer dokusu yüksek düzeyde hasar görür (Lockitch, 1993; Patra et al., 2001; Özkaya et al., 2016). Kurşunun neden olduğu karaciğer hasarı birkaç mekanizma ile açıklanmıştır. Kurşun, hepatik biyotransformasyon ve aktiviteyi, kolesterol metabolizmasını ve nükleik asit sentezindeki değişikliklerden kaynaklanabilecek hepatosit fonksiyon bozukluğunu indüklemektedir (Mudipalli, 2007; Abdel Moneim et al., 2011). Kurşunun karaciğer hücrelerinde biriktiği; hücresel alanda nekroz ve hidropik dejenerasyon gibi yapısal düzensizliklere neden olduğu bildirilmiştir (Özkaya et al., 2016). Ayrıca kurşun ile indüklenen hepatotoksisitenin reaktif oksijen türlerini arttırdığı öne sürülmüştür (Aykin-Burns et al., 2003; Abdel Moneim et al., 2011). Kurşun, lipit peroksidasyona neden olmaktadır. Ayrıca, kurşunun antioksidan enzim aktivite sistemine negatif etkileri vardır (Oyagbemi et al., 2014; Özkaya et al., 2018).

Kumarinler polifenolik yapıya sahiptir ve "2H-1-benzopyran-2-one" yapısındadır. Bu yapılar bitkilerde yoğun olarak bulunduğu gibi laboratuvar şartlarında da sentezleri yapılmaktadır (Venugopala et al., 2013). Kumarinlerin kimyasal yapıları yoğun π-konjuge bağ sistemlerine sahiptir. Özellikle molekülün ana iskeletine farklı konumlarda ve farklı türden fonksiyonel grupların bağlanması moleküle önemli işlevsellikler kazandırır. Ayrıca, kumarinlerin fotofiziksel, kimyasal ve spektroskopik gibi özelliklerinin değişmesine ve farklı uygulama alanlarının gelişmesine katkı sağlamaktadır (Kurt & Koca, 2016; Kurt et al., 2016; Kurt & Topsoy, 2017; Kurt et al., 2019). 3-benzoil-7-hidroksi kumarin molekülü hidroksikumarinler yapısındadır. Hidroksikumarinler fenolik yapıda olup serbest radikalleri temizlemede etkilidir. Antioksidan özelliği olan bu yapıların metal şelatör yapma özellikleri de bulunmaktadır (Lien et al., 1999).

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Kumarin molekülleri tarçın, sinameki yaprağı, yeşil çay ve hindiba gibi birçok bitki yapısında bulunmaktadır (Skehan et al., 1990). Bu moleküllerin antikoagülan (Poole & Poole, 1994), anti_HIV (Patil et al., 1993; Spino et al., 1998), antibakteriyel (Rosselli et al., 2009), antioksidan (Whang et al., 2005), antihipertansif (Crichton & Waterman, 1978), antitüberküler (Shin et al., 2010), antikonvülsan (Baek et al., 2000), antifungal (Teng et al., 1994), antihiperglisemik (Fort et al., 2000), antiinflamatuar (Piller, 1975) ve antikanser (Luo et al., 2011) özellikleri vardır.

İnsan metabolizmasında minerallerin düzeyleri çok önemlidir. Özellikle enzimlerin yapısında kofaktör rolünde yol alırlar. Ayrıca antioksidan etkileri de mevcuttur. Minerallerin metabolizmada eksikliği ve fazlalığı hastalıklara yol açar (Yang et al., 2016).

Oksidatif strese karşı fraxin, esculetin ve agosyllin (Venugopala et al., 2013) gibi kumarinlerin etkinliği çalışılmış olup, 3-benzoil-7-hidroksi kumarin molekülünün ise oksidatif strese karşı etkinliğinin çalışmadığı tespit edilmiştir. Çalışmamızda kurşun asetat ile oksidatif stres oluşturulan sıçan karaciğerlerinde lipid peroksidasyon düzeyi ve mineral düzeyleri üzerine 3-benzoil-7-hidroksi kumarin molekülünün etkinliği araştırıldı.

2. Materyal ve Metot

2.1. Deneysel Hayvanlar ve Deneysel Protokol

Çalışmanın deney protokolü Adıyaman Üniversitesi Yerel Etik Kurulu tarafından onaylandı (Etik kurul karar no:2020/066). Deney hayvanlarının bakım ve uygulamaları ulusal ve uluslararası yasalara uygun olarak yapıldı. Deneyde 28 adet yetişkin (250±10 gr) *Sprague-dawley* erkek sıçan kullanıldı. Sıçanlar Adıyaman Üniversitesi Deney Hayvanları Üretim Uygulama ve Araştırma Merkezinde temin edildi. Sıçanlar rastgele dört gruba ayrıldı. Bu gruplar Kontrol (K), 3-benzoil-7-hidroksi kumarin (KUM), Kurşun asetat (P) ve Kurşun asetat+3-benzoil-7-hidroksi kumarin (KUMP) gruplarıdır. Kurşun asetat 500 ppm olacak şekilde içme sularına karıştırılarak sıçanlara uygulaması yapıldı (Bennet et al., 2007). Kumarinler ile ilgili çalışmalarda uygulanan doz aralığı 10-35 mg/kg düzeylerindedir (Khan et al., 2004a). Bu nedenle çalışmada sıçanlara 20 mg/kg olacak şekilde gün aşırı oragastrik uygulaması yapıldı. Deneysel uygulamalar 30 gün boyunca sürdü. Deney uygulamaları tamamlanınca sıçanlara servikal dislokasyon uygulanarak elde edilen karaciğer dokuları çıkartıldı. Dokular deneysel uygulamalara kadar -80°C'de muhafaza edildi.

2.2. Karaciğer Dokusu Homojenizasyonu

Karaciğer dokuları 0.1 M potasyum fosfat tamponu, 0.15 M KCI, 1mM EDTA ve 1 mM DTT çözeltisinde 1:4 total doku ağırlığı oranında olacak şekilde homojenizasyonu gerçekleştirildi. Homojenizasyon işlemi Heidolph RZ 2021 markalı cihazla yapıldı. Homojenatlar 4°C'de 30 dakika süresince 15.000 g devrinde Hettich ROTINA 420 R cihazında gerçekleştirildi. Santrifüj işleminden sonra süpernatantlar elde edildi. Elde edilen süpernatantlarda lipit peroksidasyon düzeyini teşkil eden malondialdehit (MDA) düzeyleri tespit edildi.

2.3. Malondialdehit Düzeyleri Tespiti

MDA düzeyi Placer et al. (1966)'un belirttiği yönteme göre yapıldı. 500 µL homojenat üzerine % 15 trikloroasetik asit, %0.375 thiobarbütirik asit ve 0.25 N HCI (1:1:1,w/v) olacak şekilde ilave edildi. Karışım su banyosunda 100°C'de 30 dakika ısıtıldı. Karışım oda sıcaklığında soğutulduktan sonra 15 dakika 15.000 g devirde santrifüj edildi. Elde edilen süpernatant örnekleri mikroplaka kuyucuklarına aktarılarak 532 nm'de MDA düzeyleri tespiti yapıldı. MDA düzeyleri (nmol/g ıslak doku ağırlığı) olarak ifade edildi (Placer et al., 1966).

2.4. Karaciğer Dokusu Mineral Analizleri

Sıçanların karaciğer dokularındaki kurşun (Pb), demir (Fe), bakır (Cu), çinko (Zn) ve mangan (Mn) düzeylerinin ölçümü, İndüklenmiş Eşleşmiş Plazma-Kütle Spektrofotometrisi (ICP-MS) cihazında yapıldı. Karaciğer dokuları (250 mg) %65'lik 5 mL HNO_3 ile mikrodalgada parçalandı. Elde edilen çözeltideki mineral düzeyleri ICP-MS cihazında yapıldı (Özkaya & Türkan, 2021). Mineral düzeyleri ppb olarak verilmiştir.

2.5. 3-Benzoil-7-Hidroksi Kumarin Molekülü Sentezi

3-benzoil-7-hidroksi kumarin molekülü sentezi için, etil benzoil asetat (3.844 g), 2,4-dihidroksibenzaldehit (2.762 g), 50 mL aseton ve üç damla piperidin bir magnetik karıştırıcı üzerinde 2 saat sureyle riflaks edildi. Karışım aşırı methanol içine aktarılarak 3-benzoil-7-hidroksi kumarin molekülünün çökmesi sağlandı. Bu molekül etanolde kristallendirilerek saflaştırıldı (Kurt et al., 2018).

3. Bulgular

Karaciğer dokusu mineral ve MDA düzeyleri Tablo'1 de gösterilmiştir. P grubu Pb ve Fe mineral düzeyleri K grubuna göre arttığı tespit edildi ($p<0.001$). KUMP grubu Pb ve Fe düzeyleri P grubuna göre azaldığı gözlendi ($p<0.001$). Tüm grupların Zn düzeyleri arasında istatistiksel fark olmadığı tespit edildi ($p>0.05$). K grubu Mn düzeyleri diğer gruplara göre düşük çıktığı saptandı ($p<0.05$, $p>0.001$). Tüm grupların Cu düzeyleri arasında istatistiksel fark olmamasına rağmen, P grubu Cu düzeyinin diğer gruplara göre nispi oranda arttığı gözlendi ($p>0.05$). P grubu MDA düzeyi diğer gruplara göre yüksek çıktığı tespit edildi ($p<0.001$). KUMP grubu MDA düzeyi P grubuna göre azaldığı gözlendi ($p<0.001$).

4. Tartışma

Bu çalışmada, kurşun asetat ile oksidatif stres oluşturulan sıçan karaciğer dokuları üzerine 3-benzoil-7-hidroksi kumarin molekülünün lipit peroksidasyon ve bazı mineraller üzerine etkinliği araştırıldı. Çalışmada, kurşun asetat verilen grubun MDA düzeyi kontrol grubuna göre yüksek çıktığı tespit edildi. Kumarin grubu MDA düzeyi kontrol grubuyla istatistiksel fark gözlenmezken, kombinasyonlu grubun MDA düzeyi kurşun asetat grubundan düşük çıktığı gözlendi.

Kurşun, metabolizmadaki dokularda oksidatif stresi arttırarak antioksidan savunma sistemini olumsuz etkilemektedir. Ayrıca, dokulardaki mineral metabolizmasının işlevselliğini bozmaktadır. Hücresel faaliyetler için eser elementlere ihtiyaç duyar (Alonso et al., 2004; Mansouri & Cauli, 2009).

Sıçanlara 500 ppm düzeyinde 8 hafta süresince kurşun asetat uygulaması sonucunda karaciğer dokusunun MDA düzeyinin arttığı rapor edilmiştir (Wang et al., 2012; Yoloğlu, 2017).

Tablo 1. Karaciğer Dokusu Mineral ve MDA Düzeyleri

Table 1. Liver Tissue Mineral and MDA Levels

Mineraller	K	KUM	P	KUMP
Pb	61.62±3.22	0.10±0.02^{z}	1682.82±62.17^{c}	1345.41±63.57^{cz}
Fe	102735.02±1799.22	106244.92±1838.25^{z}	120946.81±948.65^{c}	95708.16±2706.18^{z}
Zn	30304.25±551.98	28535.76±377.48	28641.77±300.82	29834.86±795.51
Mn	2138.92±114.05	2536.85±61.31^{a}	2801.55±75.78^{c}	2759.11±76.09^{c}
Cu	5391.27±357.76	5340.40±150.75	5803.15±71.50	5332.67±205.08
MDA	45.78± 4.12	48.51±6.75^{z}	96.64±8.56^{c}	61.23±5.78^{az}

K grubuna göre karşılaştırma. a: $p < 0.05$, b: $p < 0.01$, c: $p < 0.001$

P grubuna göre karşılaştırma. x: $p < 0.05$, y: $p < 0.01$, z: $p < 0.001$

Anlamsal farkın olmaması: $p > 0.05$

Başka bir çalışmada, 4 hafta süresince 500 ppm kurşun asetat uygulaması yapılan erkek sıçanların karaciğer dokusu MDA düzeylerinin kontrol grubuna göre önemli düzeyde arttığı bildirilmiştir (Özkaya et al., 2016; Yoloğlu, 2017).

Kurşun toksisitesi ile ilgili önemli bir çalışmada, kurşunun sıçan karaciğer dokularında süperoksit radikalleri, hidrojen peroksit ve singlet oksijen düzeylerini önemli düzeyde arttığı belirtilmiştir. Ayrıca, protein, lipit ve amino asitlerin önemli düzeyde hasar gördüğü bildirilmiştir (Liu et al., 2011; Liu et al., 2012; Yoloğlu, 2017).

Son yıllarda yapılan önemli bir çalışmada, kurşun asetat 500 ppm olarak, 20 gün süresince sıçanlara uygulanmıştır. Deneysel sonuçlarında sıçanların karaciğer dokularında MDA düzeyi artarken antioksidan enzimlerden glutatyon redüktaz (GR), Glutatyon S-transferaz ve karboksil esteraz (Ces) enzim aktivitelerinin önemli düzeyde azaldığı rapor edilmiştir (Özkaya et al., 2018).

Çalışmamızda kurşun-asetatın arttırdığı MDA düzeyini 3-benzoil-7-hidroksi kumarin molekülünün azalttığı tespit edildi. Ayrıca, kurşun asetat grubunun Pb, Fe ve Mn düzeylerinin kontrol grubuna göre arttığı gözlendi. Kombinasyonlu grubun Pb ve Fe düzeylerini kurşun asetat grubuna göre azalttığı tespit edildi. Bireysel kumarin grubundan Pb düzeyinin ise kontrol grubuna göre azaldığı gözlendi. Ayrıca, kurşun grubu Cu düzeyi diğer gruplara göre nispi oranda arttığı tespit edilirken, kombinasyonlu grubun Cu düzeyi ise kontrol grubu seviyesine düştüğü gözlendi. Yapılan birçok çalışmada, kurşun maruziyetine kalmış hayvan dokularında (böbrek, karaciğer ve beyin) kurşun seviyesinin arttığı tespit edilmiştir (Xia et al., 2010; Mehana et al., 2012; Özkaya et al., 2018).

Kurşun uygulanan sıçan karaciğer, kalp ve testis dokularında Fe düzeyinin arttığı bildirilirken, karaciğer ve kalp dokularında Cu ve Zn düzeylerinde kontrol grubuna göre istatistiksel fark olmadığı rapor edilmiştir (Aksu et al., 2017).

Çalışmada 3-benzoil-7-hidroksi kumarin molekülünün kurşun asetatın peroksidan etkilerine karşı lipit peroksidasyon ve bazı mineral düzeylerine karşı antioksidan özellikler sergilediğini göstermektedir. 3-benzoil-7-hidroksi kumarin'in karaciğer dokularında hem Pb ve Fe konsantrasyonlarını düzelttiğini hem de MDA düzeylerine pozitif etki gösterdiğini tespit ettik.

Kumarinlerin antioksidan aktiviteleri serbest radikalleri temizleme ve metal iyonlarını şelatlama kabiliyetlerinden kaynaklanmaktadır. Kumarinlerin bu özellikleri moleküler yapılarından kaynaklanmaktadır (Khan et al., 2004b).

Kumarinlerin yapılarında bulunan hidroksi, asetoksi ve metoksi grupları antioksidan kapasitelerini arttırmaktadır. Bu fonksiyonel gruplar kumarinlerin detoksifikasyon fonksiyonları için önemlidir (Khan et al., 2004b).

Yapılan bir çalışmada kumarin özelliği yapısındaki esculetin ve scaparone'nin hepatoprotektif etkileri olduğu rapor edilmiştir (Atmaca et al., 2011).

Yapılan başka bir çalışmada ferrik nitrilotriasetat (Fe-NTA) ile oluşturulan oksidatif strese karşı 1,2- benzopiron kumarin molekülün etkisi araştırılmıştır. Fe-NTA'nın dokularda MDA düzeyini arttırdığı ve antioksidan enzim sistemini olumsuz etkilediği bildirilirken, 1,2- benzopiron molekülünün Fe-NTA'nın oluşturduğu bu olumsuz etkileri düzelttiği rapor edilmiştir (Khan et al., 2004b).

Ayrıca birçok çalışmada, kumarinlerin çeşitli oksidatif stres ajanlarına karşı antioksidan etkiye sahip olduğu bildirilmiştir (Tseng, 1991; Wu et al., 2007; Lin et al., 2008).

Karbontetraklorür'ün sıçan karaciğerlerinde oluşturduğu hasarlara karşı bazı kumarinlerin antioksidan etki göstererek karaciğer hasarını engellediği rapor edilmiştir (Atmaca et al., 2011).

Fraxin, esculetin, agasillin, osthol ve grandivittin gibi kumarinlerin serbest radikalleri süpürücü etkileri bildirilmiştir (Venugopala et al., 2013).

Sonuç olarak, kurşun asetatın neden olduğu yüksek lipit peroksidasyonu, kurşun ve demir metabolizmasını olumsuz olarak etkilemiş olup, bu negatif etkiyi 3-benzoil-7-hidroksi kumarin molekülünün düzelttiğini düşünmekteyiz.

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Kaynaklar

Abdel Moneim, A.E., Dkhil, M.A., & Al-Quraishy, S. (2011). The redox status in rats treated with flaxseed oil and lead-induced hepatotoxicity. *Biological trace element research,* 143(1), 457–467. https://doi.org/10.1007/s12011-010-8882-z

Aksu, D.S., Saglam, Y.S., Yildirim, S., & Aksu, T. (2017). Effect of pomegranate (Punica granatum L.) juice on kidney, liver, heart and testis histopathological changes, and the tissues lipid peroxidation and antioxidant status in lead acetate-treated rats. *Cellular and molecular biology,* 63(10), 33-42. https://doi.org/10.14715/cmb/2017.63.10.5

Alonso, M.L., Montaña, F.P., Miranda, M., Castillo, C., Hern´andez, J., & Benedito, J.L. (2004). Interactions between toxic (As, Cd, Hg and Pb) and nutritional essential (Ca, Co, Cr, Cu, Fe, Mn, Mo, Ni, Se, Zn) elements in the tissues of cattle from NW Spain. *Biometals,* 17(4), 389-97. https://doi.org/10.1023/B:BIOM.0000029434.89679.a2

Atmaca, M., Bilgin, H.M., Obay, B.D., Diken, H., Kelle, M., & Kale, E. (2011). The hepatoprotective effect of coumarin and coumarin derivates on carbon tetrachloride-induced hepatic injury by antioxidative activities in rats. *Journal of physiology and biochemistry,* 67(4), 569–576. https://doi.org/10.1007/s13105-011-0103-5

Aykin-Burns, N., Laegeler, A., Kellogg, G., & Ercal, N. (2003). Oxidative effects of lead in young and adult Fisher 344 rats. *Archives of environmental contamination and toxicology,* 44(3), 417–420. https://doi.org/10.1007/s00244-002-2023-4

Baek, N.I., Ahn, E.M., Kim, H.Y., & Park, Y.D. (2000). Furanocoumarins from the root of *Angelica dahurica. Archives of pharmacal research,* 23(5), 467-470. https://doi.org/10.1007/BF02976574

Bennet, C., Bettaiya, R., Rajanna, S., Baker, L., Yallapragada, P.R., Brice J.J., …, & Bokara, K.K. (2007). Region specific increase in the antioxidant enzymes and lipidperoxidation products in the brain of rats exposed to lead. *Free radical research,* 41(3), 267–273. https://doi.org/10.1080/10715760600889855

Crichton, E.G., & Waterman, P.G. (1978). Dihydromammea c/ob: a new coumarin from the seed of Mammea africana. *Phytochemistry,* 17(10), 1783-1786. https://doi.org/10.1016/S0031-9422(00)88695-1

Fischbein, A. (1992). Occupational and environmental lead exposure. In: Ed. Rom WN. *Environmental and occupational medicine*, 2nd Ed. Boston: Little Brown & Co, 735– 758.

Fort, D., Rao, K., Jolad, S., Luo, J., Carlson, T., & King, S. (2000). Antihyperglycemic activity of Teramnus labialis (Fabaceae). *Phytomedicine : international journal of phytotherapy and phytopharmacology,* 6(6), 465-467. https://doi.org/10.1016/S0944-7113(00)80075-6

Khan, N., Sharma, S., & Sultana. S. (2004a). Attenuation of potassium bromate-induced nephrotoxicity by coumarin (1,2-benzopyrone) in Wistar rats: chemoprevention against free radical-mediated renal oxidative stress and tumor promotion response. *Redox report: communications in free radical research,* 9(1), 19-28. https://doi.org/10.1179/135100004225003860

Khan, N., Sharma, S., & Sultana, S. (2004b). Amelioration of ferric nitrilotriacetate (Fe-NTA) induced renal oxidative stress and tumor promotion response by coumarin (1,2-benzopyrone) in Wistar rats. *Cancer letters,* 210(1), 17–26. https://doi.org/10.1016/j.canlet.2004.01.011

Kurt, A., & Koca, M. (2016). Synthesis, characterization and thermal degradation kinetics of poly(3-acetylcoumarin-7-yl-methacrylate) and its organoclay nanocomposites. *Journal of Engineering Research,* 4(4), 46-65.

Kurt, A., Kaya, M., & Koca, M. (2016). Synthesis and characterization of coumarin derived surface active monomer. *Adıyaman University Journal of Science,* 6(1), 110-121.

Kurt, A., & Topsoy, O.K. (2017). Preparation of novel coumarin cyclic polymer/montmorillonite based nanocomposites. *Russian Journal of Applied Chemistry,* 90(12), 2019−2027. https://doi.org/10.1134/S1070427217120199

Kurt, A., Ayhan, A.F., & Koca, M. (2018). Kumarin Türevli Kopolimerlerin Sentezi ve Karakterizasyonu. *Sakarya Üniversitesi Fen Bilimleri Enstitüsü Dergisi,* 22(3), 880-887. https://doi.org/10.16984/saufenbilder.322354

Kurt, A., B. Gunduz, B., & Koca, M. (2019). A detailed study on the optical properties of 3-benzoyl-7-hydroxy coumarin compound in different solvents and concentrations. *Macedonian Journal of Chemistry and Chemical Engineering,* 38(2), 227-236. https://doi.org/10.20450/mjcce.2019.1403

Lien, E.J., Ren, S., Bui, H.H., & Wang, R. (1999). Quantitative structure-activity relationship analysis of phenolic antioxidants. *Free Radical Biology and Medicine,* 26(3–4), 285–294. https://doi.org/10.1016/S0891-5849(98)00190-7

Lin, H.C., Tsai, S.H., Chen, C.S., Chang, Y.C., Lee, C.M., Lai, Z.Y., & Lin, C.M. (2008). Structure-activity relationship of coumarin derivatives on xanthine oxidase-inhibiting and free radical-scavenging activities. *Biochemical pharmacology,* 75(6), 1416–1425. https://doi.org/10.1016/j.bcp.2007.11.023

Liu, C.M., Ma, J.Q., & Sun, Y.Z. (2011). Protective role of puerarin on lead-induced alterations of the hepatic glutathione antioxidant system and hyperlipidemia in rats. *Food and chemical toxicology,* 49(12), 3119-3127. https://doi.org/10.1016/j.fct.2011.09.007

Liu, C.M., Ma, J.Q., & Sun, Y.Z. (2012). Puerarin protects the rat liver against oxidative stress-mediated DNA damage and apoptosis induced by lead. *Experimental and Toxicologic Pathology,* 64(6), 575-582. https://doi.org/10.1016/j.etp.2010.11.016

Lockitch, G. (1993). Perspectives on lead toxicity. *Clinical Biochemistry,* 26(5), 371–381. https://doi.org/10.1016/0009-9120(93)90113-K

Luo, K., Sun, J., Chan, J.Y.W., Yang, L., Wu, S., Fung, K.P., & Liu, F. (2011). Anticancer effects of imperatorin isolated from Angelica dahurica: induction of apoptosis in HepG2 cells through both death-receptor-and mitochondriamediated pathways. *Chemotherapy,* 57(6), 449-459. https://doi.org/10.1159/000331641

Mansouri, M.T., & Cauli, O. (2009). Motor alterations induced by chronic lead exposure. *Environmental toxicology and pharmacology,* 27(3), 307–313. https://doi.org/10.1016/j.etap.2009.01.003

Mehana, E.E., Meki, A.R., & Fazili, K.M. (2012). Ameliorated effects of green tea extract on lead induced liver toxicity in rats. *Experimental and toxicologic pathology,* 64(4), 291–295. https://doi.org/10.1016/j.etp.2010.09.001

Mudipalli, A. (2007). Lead hepatotoxicity & potential health effects. *The Indian journal of medical research,* 126(6), 518–527.

Oyagbemi, A., Saba, A., Omobowale, T., Akinrinde, A., Ogunpolu, B., & Daramola, O. (2014). Lack of reversal from lead acetate-induced hepatotoxicity, free radical generation and oxidative stress in Wistar rats (1139.17). *The FASEB journal,* 28(1 supplement). https://doi.org/10.1096/fasebj.28.1_supplement.1139.17

Özkaya, A., Sahin, Z., Dag, U., & Ozkaraca, M. (2016). Effects of Naringenin on Oxidative Stress and Histopathological Changes in the Liver of Lead Acetate Administered Rats. *Journal of biochemical and molecular toxicology,* 30(5), 243-248. https://doi.org/10.1002/jbt.21785

Özkaya, A., Sahin, Z., Kuzu, M., Saglam, Y.S., Ozkaraca, M., Uckun, M., … & Yologlu, S. (2018). Role of geraniol against lead acetate-mediated hepatic damage and their interaction with liver carboxylesterase activity in rats. *Archives of physiology and biochemistry,* 124(1), 80-87. https://doi.org/10.1080/13813455.2017.1364772

Özkaya, A., & Türkan, K. (2021). Protective Effects of 3-benzoyl-7-hydroxy Coumarin on Liver of Adult Rat Exposed to Aluminium Chloride. *Acta chimica Slovenica,* 68(1), 222–228. http://doi.org/10.17344/acsi.2020.6390

Patil, A.D., Freyer, A.J., Eggleston, D.S., Haltiwanger, R.C., Bean, M.F., Taylor, P.B., …, & Bartus, H.R. (1993). The inophyllums, novel inhibitors of HIV-1 reverse transcriptase isolated from the Malaysian tree, Calophyllum inophyllum Linn. *Journal of medicinal chemistry,* 36(26), 4131-4138. https://doi.org/10.1021/jm00078a001

Patra, R.C., Swarup, D., & Dwivedi, S.K. (2001). Antioxidant effects of alpha tocopherol, ascorbic acid and L-methionine on lead induced oxidative stress to the liver, kidney and brain in rats. *Toxicology,* 162(2), 81–88. https://doi.org/10.1016/S0300-483X(01)00345-6

Piller, N.B. (1975). A comparison of the effectiveness of some anti-inflammatory drugs on thermal oedema. *British journal of experimental pathology,* 56(6), 554–559.

Placer, Z.A., Cushman, L.L., & Johnson, B.C. (1966). Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems.

Analytical biochemistry, 16(2), 359–364. https://doi.org/10.1016/0003-2697(66)90167-9

Poole, S.K., & Poole, C.F. (1994). Thin-layer chromatographic method for the determination of the principal polar aromatic flavour compounds of the cinnamons of commerce. *Analyst,* 119, 113-120. https://doi.org/10.1039/AN9941900113

Rosselli, S., Maggio, A.M., Faraone, N., Spadaro, V., Morris-Natschke S.L., Bastow, K.F., & Bruno, M. (2009). The cytotoxic properties of natural coumarins isolated from roots of Ferulago campestris (Apiaceae) and of synthetic ester derivatives of aegelinol. *Natural product communications,* 4(12), 1701-1706. https://doi.org/10.1177/1934578X0900401219

Shin, E., Choi, K.-M., Yoo, H.S., Lee, C.K., Hwang, B.Y., & Lee, M.K. (2010). Inhibitory effects of coumarins from the stem barks of *Fraxinus rhynchophylla* on adipocyte differentiation in 3T3-L1 cells. *Biological & pharmaceutical bulletin,* 33(9), 1610-1614. https://doi.org/10.1248/bpb.33.1610

Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., …, & Boyd, M.R. (1990). New colorimetric cytotoxicity assay for anticancer-drug screening, *Journal of the National Cancer Institute,* 82(13), 1107-1112. https://doi.org/10.1093/jnci/82.13.1107

Spino, C., M. Dodier, M., & Sotheeswaran, S. (1998). Anti-HIV coumarins from Calophyllum seed oil. *Bioorganic & medicinal chemistry letters*, 8(24), 3475-3478. https://doi.org/10.1016/S0960-894X(98)00628-3

Teng, C.M., Lin, C.H., Ko, F.N., Wu, T.S., & Huang, T.F. (1994). The relaxant action of osthole isolated from Angelica pubescens in guinea-pig trachea. *Naunyn-Schmiedeberg's archives of pharmacology*, 349(2), 202-208. https://doi.org/10.1007/BF00169838

Tseng, A. (1991). Proc. *American Association for Cancer Research,* 32, Abstract No. 2257

Venugopala, K.N., Rashmi, V., & Odhav, B. (2013). Review on natural coumarin lead compounds for their pharmacological activity. BioMed Research International, 963248, 1-14. https://doi.org/10.1155/2013/963248

Wang, J., Yang, Z., Lin, L., Zhao, Z., Liu, Z., & Liu, X. (2012). Protective effect of naringenin against lead-induced oxidative stress in rats. *Biological trace element research,* 146(3), 354-359. https://doi.org/10.1007/s12011-011-9268-6

Whang, W.K., Park, H.S., Ham, I., Oh, M., Namkoong, H., Kim, H.K., …, & Park, Y.G. (2005). Natural compounds, fraxin and chemicals structurally related to fraxin protect cells from oxidative stress. *Experimental & molecular medicine,* 37(5), 436-446. https://doi.org/10.1038/emm.2005.54

Wu, C.R., Huang, M.Y., Lin, Y.T., Ju, H.Y., & Ching, H. (2007). Antioxidant properties of Cortex Fraxini and its simple coumarins. *Food Chemistry,* 104(4), 1464–1471. https://doi.org/10.1016/j.foodchem.2007.02.023

Xia, D., Yu, X., Liao, S., Shao, Q., Mou, H., & Ma, W. (2010). Protective effect of Smilax glabra extract against lead-induced oxidative stress in rats. *Journal of ethnopharmacology,* 130(2), 414–420. https://doi.org/10.1016/j.jep.2010.05.025

Xiang, J.J., Zhai, Y.F., Tang, Y., Wang, H., Liu, B., & Guo, C.W. (2010). A competitive indirect enzyme-linked immunoassay for lead ion measurement using mAbs against the leadDTPA complex. *Environmental Pollution,* 158(5), 1376–1380. https://doi.org/10.1016/j.envpol.2010.01.002

Yang, Y., Wang, H., Guo, Y., Lei, W., Wang, J., Hu, X., …, & He, Q. (2016). Metal Ion Imbalance-Related Oxidative Stress Is Involved in the Mechanisms of Liver Injury in a Rat Model of Chronic Aluminum Exposure. *Biological trace element research,* 173(1), 126–131. https://doi.org/10.1007/s12011-016-0627-1

Yoloğlu, S. (2017). Kurşuna maruz kalan rat dokularında geraniol'ün etkileri (465222). Retrieved from https://tez.yok.gov.tr/UlusalTezMerkezi/giris.jsp.

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Research Article/Araştırma Makalesi

Identifying the Past, Present, and Future Distribution Patterns of the Balkan Wall Lizard (Sauria: Lacertidae: *Podarcis tauricus*) by Ecological Niche Modelling

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Abstract: Pleistocene glacial and interglacial periods have been greatly affected the distribution pattern of the species. The impact of the global climate change upon species distributions such as range shifts in latitude or elevation has been widely studied. In this study, it was aimed to have a better understanding on the effects of the Late-Pleistocene climatic oscillation and the global climate changes on a widely distributed reptile species, the Balkan wall lizard (*Podarcis tauricus*). To find out the dynamics of the species' range shifts, ecological niche modelling approach was applied. Bioclimatic variables and regenerated species occurrence records were used to construct models. The chosen model was projected to the present, reconstructed past and predicted future bio-climatic conditions. Moreover, distribution change and landscape connectivity analyzes were executed. Under present conditions, model prediction for the Balkan wall lizard was largely caught its known distribution area. The LGM distribution prediction was limited to a few spots (57,596.19 km^2) in the southern Balkans, mainly due to the negative effect of the mean winter temperature. From the LGM to the present, distribution area of the species remarkably extended, particularly noticeable during Mid-Holocene (1,254.59%). The model predicted the distribution area of the species would extend due to high mean summer and high mean winter temperatures in the future and move basically towards northern latitudes and at higher elevations. A connectivity pattern in between the southwestern and northeastern populations of the Balkan wall lizard was found with high connectivity predicted predominantly over the southern Balkans.

Keywords: Late-Quaternary climatic oscillations, glacial refugia, global climate change, maxent, wallace.

Ekolojik Niş Modellemesi ile Balkan Duvar Kertenkelesinin (Sauria: Lacertidae: *Podarcis tauricus*) Geçmiş, Günümüz ve Gelecek Yayılış Örüntüsünün Tanımlanması

Öz Pleyistosen buzul ve buzullar arası dönemler, türlerin dağılım desenini büyük ölçüde etkilemiştir. Bu çalışmada, geç Pleyistosen iklim dalgalanmalarının ve küresel iklim değişikliklerinin, yaygın olarak yayılış gösteren bir sürüngen türü olan Balkan duvar kertenkelesi (*Podarcis tauricus*) üzerindeki etkilerinin daha iyi anlaşılması amaçlanmıştır. Türün dağılım değişimine yönelik dinamikleri bulmak için ekolojik niş modellemesi yaklaşımı uygulanmıştır. Biyoiklimsel değişkenler ve yeniden oluşturulmuş tür gözlem kayıtları kullanılarak, ekolojik niş modelleri hazırlanmıştır. Seçilen model, yeniden oluşturulmuş geçmiş ve tahminlenen gelecek bio-iklimsel koşullara projekte edilmiştir. Ayrıca, dağılım değişikliği ve peyzaj bağlantısallığı analizleri gerçekleştirilmiştir. Mevcut koşullar altında, Balkan duvar kertenkelesi için model tahmini, büyük ölçüde bilinen dağılım alanını yakalamıştır. SBM dağılım tahmini, esas olarak ortalama kış sıcaklığının olumsuz etkisinden dolayı güney Balkanlar'da birkaç noktayla (57,596.19 km^2) sınırlanmıştır. SBM'den günümüze, türün dağılış alanı, özellikle Orta Holosen'de belirgin şekilde (1,254.59%) genişlemiştir. Model, türün gelecekte yüksek ortalama yaz ve yüksek ortalama kış sıcaklıkları nedeniyle yayılış alanını genişleteceğini ve temel olarak kuzey enlemlere ve daha yüksek rakımlara doğru hareket edeceğini öngörmüştür. Balkan duvar kertenkelesinin güneybatı ve kuzeydoğu populasyonları arasında bir bağlantısallık bulunmuş olup, ağırlıklı olarak güney Balkanlar'da yüksek bağlantısallık tahminlenmiştir.

Anahtar kelimeler: Geç Kuvaterner iklim dalgalanmaları, buzul sığınağı, küresel iklim değişikliği, maxent, wallace.

1. Introduction

The wall lizards of the genus *Podarcis* (Wagler, 1830) is a member of the family Lacertidae (Reptilia: Squamata: Sauria). They are abundant and diverse taxon with 26 currently recognized species mainly due to several vicariance events (Yang et al., 2021). The origin of the taxon is from Western Europe (Oliverio et al., 2000; Psonis et al., 2018) but also distributed in North Africa and introduced into North America due to animal trade (Kolbe et al., 2013).

Among the wall lizards, the Balkan wall lizard (*Podarcis tauricus*, Pallas, 1814) is one of a few species of the wall lizard genus having large distribution range from the Crimean Peninsula, and southwestern Ukraine to the southeastern part of Czech Republic (Fischer et al., 2019; Rehák et al., 2022) in the north and Greece (Gasc et al., 1997; Sindaco & Jeremčenko, 2008; Uetz & Hallermann, 2022) and northwestern part of Türkiye (Başoğlu & Baran, 1977; Baran et al., 1992; Tok & Çiçek, 2014; Bülbül et al., 2015; Gül & Tosunoğlu, 2017) in the south. The Balkan wall lizard is a diurnal, heliothermic, medium-size, actively foraging ground-dwelling lizard having a total body length up to 22 cm (Başoğlu & Baran, 1977; Ljubisavljević et al., 2010). The coloration varies geographically and seasonally and matches its surrounding environment. The Balkan wall lizards as a dominant group of Mediterranean lacertids (Böhme & Corti, 1993) are known to be ecologically generalists, occupying wide variety of

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habitats (Arnold, 1987) and a common species in suitable habitats such as stony scrublands, meadows, field and forest edges, open parts of steppes and grasslands, sandy dunes with sparsely halophyte vegetation, cultivated lands, sides of highways, and rural gardens (Covaciu-Marcov et al., 2006; Kati et al., 2007). It was found that the presence of the Balkan wall lizards is being affected positively by tussock height which distributes generally in open, sandy patches with low vegetation cover (Mizsei et al., 2020). The altitudinal distribution of the species ranges from 0 to 2350 m (Böhme et al., 2009). According to the IUCN Red List categories, conservation status of the Balkan Wall Lizard is evaluated as Least Concern (LC, IUCN 3.2, accessed in June, 2022) and listed in Appendix II of the Bern Convention (1979) and in Annex IV of the European Union Habitats Directive (1992) as a strictly protected species due to local threats in parts of its range such as habitat loss and pollution.

The spatiotemporal responses of the species (e.g. range contractions and expansion) during Quaternary climatic oscillations have been studied for some decades (Hewitt, 1996; Taberlet et al., 1998; Stewart et al., 2010; Gür et al., 2013). Pleistocene glacial periods were characterized by cold and dry climate. Most of the temperate species could not survive in their current range but usually at lower latitudes glacial refugia. During the interglacial periods, species expanded their ranges mostly towards higher latitudes from their refugia (Hewitt, 1996, 1999, 2000, 2004). The Balkan Peninsula, as one of the lower latitude glacier refugia, has played a key role for postglacial re-colonization of central and northern Europe (Taberlet et al., 1998; Hewitt, 1999, 2000; Feliner, 2011). Several studies have highlighted the impact of global climate change upon species distributions and climate-driven range shifts in latitude or elevation (Parmesan et al., 1999; Thomas, 2010; Chen et al., 2011; Vicenzi et al., 2017; Bezeng et al., 2018). As it is mentioned in Le Galliard et al. (2012), substantial range shifts with northward or upward movements have been predicted for most of the reptiles in Europe in the future according to niche modelling studies.

Ecological niche models (ENMs) have been widely used to understand further response of species to global climate changes through the past and future time periods (Guisan & Zimmermann, 2000; Peterson et al., 2002; Hijmans & Graham, 2006; Waltari et al., 2007; Nogués-Bravo, 2009). ENM makes a relation between georeferenced occurrence data where species has been observed and environmental data to construct models of species' potential geographical distribution (Guisan & Thuiller, 2005). Under the assumptions of species-climate equilibrium and stability of ecological niches through time, ENMs are able to be extrapolated to other scenarios, either in time (past or future projections; Peterson et al., 2002; Nogués-Bravo, 2009) or in space (projections to other study areas: Peterson et al., 2007).

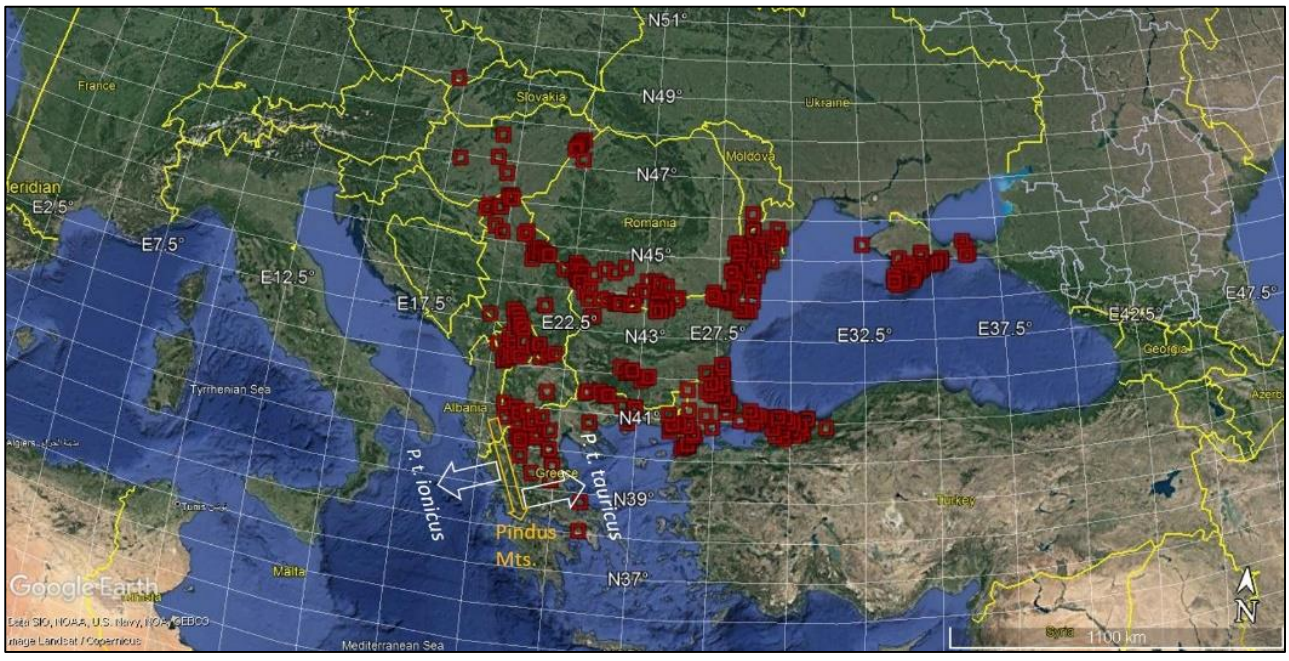

Figure 1. Species occurrence data and its distribution in the range of the Balkan Wall Lizard.

Dispersal capacity of reptile species is basically limited as they are highly dependent on their environment (Huey, 1982; Joger et al., 2007) which makes it possible to expect relationship between distribution areas and climate of their habitats. Distribution pattern of the evolution history of reptile species has been studied either only with ecological niche modelling approach (Kaliontzopoulou et al., 2008; Sillero & Carretero, 2013; Gül et al., 2015; Yousefkhani et al., 2016; Ćorović et al., 2018; Mothes et al., 2019; Kurnaz & Yousefkhani, 2019; Kurnaz & Yousefkhani, 2021) or together with molecular phylogeography (Melville et al., 2016; Psonis et al., 2018; Promnun et al., 2021). In a detailed phylogeographical study on *Podarcis* taxon (Psonis et al., 2018), existence of extensive genetic structure within *P. tauricus* and the distribution of two main clades corresponding with the eastern and western sides of the Pindus Mountains (See Fig. 1) was confirmed (see also Çördük et al., 2018). The potential distribution of *P. tauricus* for the recent and past time periods (the LIG and the LGM) has been shown as a supportive analysis to the molecular studies. On the basis of this study, the Taxonomic Committee of the European Herpetological Society accepted the split of the species into two species: eastern species retained the name *P. tauricus*, including

nominate subspecies *P. tauricus tauricus* and *P. t. thasopulae (Kattinger, 1942)*, and western species took the name *P. ionicus* (*Lehrs, 1902*). Therefore, *P. tauricus tauricus* and *P. tauricus thasopulae* have been included in this study.

In the face of the global climate change, suitable habitats reduce and as an outcome, the distance between convenient habitat patches and the cost of dispersal increase (Le Galliard et al., 2012). Therefore, landscape connectivity is an enabling tool to measure connections among populations and to draw connecting corridors among populations of the species (Hodgson et al., 2009; Brown, 2014). Landscape connectivity tool together with species distribution models (SDMs) and/or population genetics data has been applied formerly in a spatially explicit framework (e.g. Chen et al. 2011; Yu et al., 2015; Zhang et al., 2019). To estimate the least costly routes in which a population can move through (cost accepted as the inverse of habitat suitability), the least-cost corridors (LCCs) and least-cost paths (LCPs) methods are used (Rudnick et al., 2012).

The main goal of this study is to assess the distribution pattern of the Balkan wall lizard as a temperate and widely distributed reptile species in present, under the past and the future global climate models and scenarios. To predict the potential geographical distribution of the species, ecological niche modelling, with the presence records (recorded by the author and reconstructed from published articles) and bioclimatic data (from the WorldClim v. 1.4 database for the present, the past, and the future, Hijmans et al., 2005) was used. Distribution change of the Balkan wall lizard among different time periods and potential glacial refugium were assessed to understand the response of the species to the global climate changes throughout the Quaternary glacial–interglacial cycles and to the predicted future climate changes. Furthermore, the bioclimatic variables were evaluated in terms of contribution to the model prediction and effects of climate on shaping the distribution pattern of the species. The LCCs and the LCPs analyses were executed and bioclimatic connectivity in the distribution area of the species populations was analyzed.

2. Material and Methods

2.1. Occurrence data

The species occurrence records were obtained from the former published articles and the previous field research of the authors. In most of the articles, the geographical coordinates of the occurrence data are not given, instead the locations of the species specimens are explained. Therefore, the information in the papers was transformed into decimal coordinate data on Google Earth pro v.7.3.2 (http://www.google.com/earth) where possible. Totally, 370 occurrence records were gathered from Crimea, Czech Republic, Hungary, Ukraine, Moldova, Romania, Serbia, Kosovo, North Macedonia, Albania, Bulgaria, Greece, and Türkiye (Fig. 1) between the years 1977 and 2019 (mostly after 1990) and 10% of the occurrence records have coordinates. The species occurrence records that were used to construct the articles were as follows: Altunışık et al., 2016; Başoğlu & Baran, 1977; Bülbül et al., 2015; Cogălniceanu et al., 2013; Çördük et al., 2018; Eroğlu et al., 2017; Fischer et al., 2019; Iftime & Iftime, 2016; Koç et al., 2018; Kukushkin & Doronin, 2013; Mollov & Valkanova, 2009; Petrov et al., 2006; Poulakakis et al., 2005a, 2005b; Psonis et al., 2017; Sokolov, 2019; Stănescu et al., 2013; Tomovic et al., 2018; Tok & Çiçek, 2014; Urošević et al., 2015. The location information of the collection of COMU Zoology Research Laboratory ZDEU-COMU and new specimens collected for the current study under COMU-Ethical Committee permission (No: 2018/04-01) were used for modelling. They are ZDEU-49/2009-1/1♂ Demirköy/Kırklareli, Leg. C. V. Tok, B. Y. Yakın; ZDEU-58/2010/1♂ Dupnisa/Kırklareli, Leg. C. V. Tok, B. Y. Yakın; ZDEU-17/2011-1/1♂ Saray/Tekirdağ, Leg. C. V. Tok, B. Y. Yakın; ZDEU-127/2009/1♀ Dereköy/Kırklareli, Leg. C. V. Tok, B. Y. Yakın; ZDEU-28/2018/1♂ Karasu/Sakarya, Leg. C. V. Tok, Ç. Göcek; ZDEU-27/2019/1♀ Çardak/Çanakkale, Leg. C. V. Tok, Ç. Göcek.

The Maxent algorithm assumes that all occurrence records on the study area are equally likely to be sampled (Merow et al., 2013). To reduce the clustering of occurrence points, caused by the survey bias, they were filtered (Boria et al., 2014; Kadmon et al., 2004) by 10 km, 15 km, and 20 km distance thresholds via using 'spatial thinning' application on the Wallace platform v. 1.0.6 (Kass et al., 2018) and; as a result, 20 Km spatial thinning with 153 occurrence points was used to construct the model due to 10 Km (243 occurrence points) and 15 Km (193 occurrence points) spatial thinning were not able to remove clustering (Fig. 2).

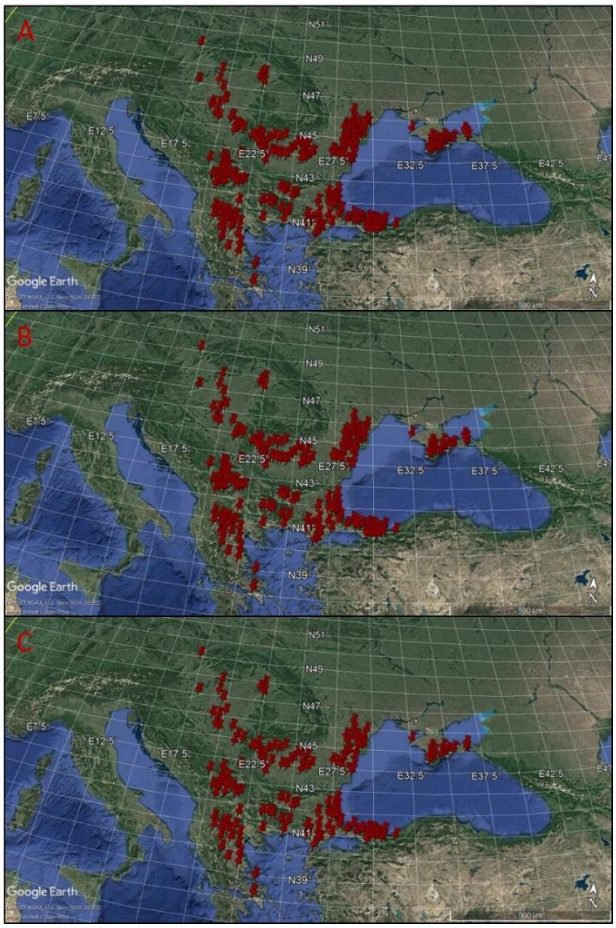

Figure 2. A, B, and C maps are10 Km, 15 Km, and 20 Km spatially rarified species occurrence points, respectively.

The background extent of the study region has been defined as a rectangular bounding box around the boundary of the occurrence points with 2^0 (~200 km) buffer

zone (i.e. the study area, 15.25° to 38.33° E and 36.08° to 50.92° N; Supplemental Fig. 1) considering species dispersal limits (Soberón & Peterson, 2005).

2.2. Bioclimatic data

The bioclimatic data were downloaded from the WorldClim database version 1.4 database (Hijmans et al., 2005) at a spatial resolution of 5 arc-minutes to be in accordance with the certainty of the occurrence data (~ 8.3 km at the equator; Araújo et al., 2019; Feng et al., 2019; Sofaer et al., 2019). The bioclimatic data includes the present (between years 1960–1990); two past time periods: the Last Glacial Maximum (LGM, ~22 kya) and Mid-Holocene (~7 kya); and two future time periods (2050, the average of 2041-2060 and 2070, the average of 2061-2080).

Under Coupled Model Intercomparison Project Phase 5 (CMIP5), three global climate models (CCSM4 - The Community Climate System Model Version 4, Gent et al., 2011; MPI-ESM - Max Planck Institute Earth System Model, Giorgetta et al., 2013; and MIROC-ESM - Model for Interdisciplinary Research on Climate, Watanabe et al., 2011) for the past and the future time periods were used for modelling. These global climate models were taken from four Representative Concentration Pathways (RCPs) scenarios (from the low to the medium to the high forcing levels: RCP2.6 (low), RCP4.5 (medium), RCP6.0 (medium), and RCP8.5 (high). Via using different models and scenarios, the uncertainty in the ecological niche modelling due to a broad range of the global climate change in the past and the future time periods were taken into account (Varela et al., 2015). The data include 19 bioclimatic variables derived from monthly temperature and precipitation values (for detailed descriptions, see http://www.worldclim.org and Supplemental Table 1). The bioclimatic variables, having known spatial artefacts (i.e. artificial discontinuities in climate gradients; Mean Temperature of Wettest Quarter (BIO8), Mean Temperature of Driest Quarter (BIO9), Precipitation of Warmest Quarter (BIO18), Precipitation of Coldest Quarter (BIO19), variables with extreme values and variables that are not in accordance with the study aims, Max Temperature of Warmest Month (BIO5), Min Temperature of Coldest Month (BIO6), Temperature Annual Range (BIO7), Precipitation of Wettest Month (BIO13), and Precipitation of Driest Month (BIO14) were excluded. The exclusion of these bioclimatic variables reduced uncertainty of the predictions (Varela et al., 2015) and; as a result, 10 bioclimatic variables, Annual Mean Temperature (BIO1), Mean Diurnal Range (BIO2), Isothermality (BIO3), Temperature Seasonality (BIO4), Mean Temperature of Warmest Quarter (BIO10), Mean Temperature of Coldest Quarter (BIO11), Annual Precipitation (BIO12), Precipitation Seasonality (BIO15), Precipitation of Wettest Quarter (BIO16), and Precipitation of Driest Quarter (BIO17) were used for constructing the model. The correlation between the bioclimatic variables were checked using 0.8 threshold value in SDM ToolBox v. 2.4 (Brown et al., 2017). Four different bioclimatic data sets emerged, with the largest number of variables in each and without including the climatic variables having a correlation of 0.8 ≥ among each other in the same data set (Supplemental Table 2, Supplemental Table 3).

Table 1. Model evaluation statistics of candidate models for each group ordered based on decreasing Average AUCTEST value.

Candidate models: Used variables	FC	RM	Average AUC_{TEST}	Variance AUC_{TEST}	Average AUC_{DIFF}	Variance AUC_{DIFF}	Average Test OR_{MIN}	Variance Test OR_{MIN}	Average Test OR_{10}	Variance Test OR_{10}	AICc	Delta AICc	PM
M:1 BIO 1,2,4,12,15	L	5.0	0.788	0.016	0.061	0.006	0.033	0.001	0.221	0.04	3,025.769	57.172	5
M:2 BIO 1,2,4,15,16	L	5.0	0.788	0.017	0.058	0.007	0.033	0.002	0.221	0.04	3,027.198	67.859	5
M:3 BIO 2,10,11,12,15,17	LQ	5.0	0.8	0.013	0.059	0.005	0.038	0.006	0.247	0.031	2,984.674	26.649	9
M:4 BIO 2,10,11,15,16,17	LQ	5.0	0.794	0.015	0.06	0.05	0.038	0.06	0.215	0.022	2,991.321	30.373	9

For detailed descriptions of variables, see Supplemental Table 1. FC, feature classes (L = Linear, Q = Quadratic, H = Hinge, P = Product). RM, regularization multiplier. AUCTEST, value of the area under the curve of the receiver operating characteristic (ROC) plot calculated based on testing bins. AUCDIFF, the difference between training and testing AUC. ORMIN, minimum training presence omission rate. OR10, 10% training omission rate. AICc, the Akaike information criterion corrected for small sample sizes. Delta AICc, the difference between the lowest AICc and each AICc. PM, number of parameters in candidate model. Bold values refer to the chosen model details.

2.3. Ecological niche modelling

Maximum entropy modeling algorithm Maxent v. 3.4.1 (Phillips et al., 2017) was used for modelling the ecological niche and concluding bioclimatic suitability throughout the study area under the present, past, and future climatic conditions for the Balkan wall lizard since it is one of the most effective ecological niche modelling algorithms (Phillips et al., 2004; 2006; Elith et al., 2006) requiring presence-only data for creating a model (Peterson et al., 2011). WALLACE v. 1.0.6 software which is an open-source platform, having R-scripted user-friendly workflow (Kass et al., 2018; Available from Url: https://wallaceecomod.github.io/; for methodological descriptions, see Muscarella et al., 2014, and for a Turkish tutorial, see Gür, 2019) was used for selecting optimal set of variables and model settings (Elith et al., 2011; Merow et al., 2013). The model settings were as follows on WALLACE interface: 20 km thinned occurrence dataset was used to eliminate clustering. Final model were projected onto the present, past, and future climatic conditions for 3^0 (~ 300 km) buffer zone encompassing occurrence records (i.e. 14.25° to 39.33°E and 35.08° to 51.92°N).

All pixels within the buffer zone were sampled as the background data (n=36,619 pixels) and; thus, full representation of environments available for the Balkan wall lizard was provided (Guevara et al., 2018). To adjust the model complexity, following combinations were used: 1) Four sets of non-collinear variables (Supplemental Table 3), 2) 5 combinations of feature classes **(L**inear -L, **L**inear-

Quadratic -LQ, **H**inge -H, **L**inear-**Q**uadratic-**H**inge -LQH, **L**inear-**Q**uadratic-**H**inge-**P**roduct -LQHP), and 3), and 5 different regularization multiplier values (1 to 5 in increments of 1). As a result, 100 candidate models were tested to select the most favorable set of variables. To evaluate model significance and the performance, the partial ROC analysis was executed (Peterson et al., 2008) in NicheToolBox v.0.6.0.1 software (Osorio-Olvera et al., 2020) with the following settings: Proportion of omission = 0.001, Random points % = 50, Number of iterations for the bootstrap = 1000.

To calculate model evaluation statistics, the spatial k-fold cross-validation method was used (Supplementary Fig. 1). This method is suitable for transferring to other geographical extents and time periods (for methodology see Muscarella et al., 2014; Kass et al., 2018). In this method, the full dataset (presence and background data) was partitioned into 4 (k=4) spatially distinct, non-overlapping bins with equal numbers of observation points by the latitude and longitude lines. Then, for each candidate model, five models were tested. Four out of five models were built iteratively via using 3 bins for training the model and one bin is left out for model testing and; then, threshold-independent evaluation statistics (the area under ROC -A receiver operating characteristic- curve AUC_{TEST}, AUC_{DIFF}) and threshold-dependent evaluation statistics (10% training omission rate, OR_{10} and 'Minimum Training Presence' omission rate, OR_{MIN}) as averaged over the iteration were calculated. One out of five models was built using non-partitioned, full dataset to calculate the Akaike information criterion corrected for small sample sizes (AICc). The highest average AUC_{TEST} value was chosen as the best performing model for discrimination ability (Table 1; Phillips et al., 2004) and; thus, the most favorable set of variables and model settings were used (Elith et al., 2011; Merow et al., 2013).

After choosing the final ecological niche model, the final model with partitioned dataset (153 presence records and 36,619 background points) was projected onto the present, past, and future climatic conditions with both extrapolation and fade by clamping options to regulate the probable model reaction to the environmental values that were more extreme than those of the training dataset (Elith et al., 2010). The multivariate environmental similarity surface analysis (MESS) (Elith et al., 2010) were executed on Maxent both for analyzing prediction capability of the model in novel bioclimatic conditions and finding out possible similar and/or non-similar conditions between training and projection datasets. The limiting bioclimatic variables driving the MESS value in each grid cell for the future and the past time projections were also provided in the analysis (Elith et al., 2010). The response curves of the model (bioclimatic suitability vs. response variable) were analyzed to discover how the bioclimatic conditions affect the model predictions (Anderson, 2013). The relative contributions of the variables to the final model were checked via Jackknife test and percent contribution of each variable were assessed. For bioclimatic suitability maps, the cloglog output format, having values indicating suitability from 0 to 1 was used (Phillips et al., 2017). To identify the main drivers for the bioclimatic suitability, Maxent Explain tool (Elith et al., 2010) was checked at any pixel chosen from the study area.

Each bioclimatic suitability map was divided into five classes to interpret easily: very low suitability (< 0.2), low suitability (0.2-0.4), moderate suitability (0.4-0.6), high suitability (0.6-0.8), and very high suitability (> 0.8). Areas of moderate, high, and very high suitability were also defined as suitable bioclimatic areas based on the '10 percentile training presence' threshold (=0.415). To simplify the explanation of the past and future bioclimatic suitability maps, for each time period (for the past, LGM and Mid-Holocene; for the future, 2050, average of 2041-2060 and 2070, average of 2061-2080), and for each scenario, only one consensus bioclimatic suitability map was presented as an average of three global climate models (1 average of: LGM, Mid-Holocene, 2050/RCP2.6, 2050/RCP4.5, 2050/RCP7.0, and 2050/RCP8.5, 2070/RCP2.6, 2070/RCP4.5, 2070/RCP6.0, and 2070/RCP8.5) via using 'raster calculator' in SDM Toolbox 2.4. Since there is no MPI-ESM-LR model for RCP6.0 2050 and RCP6.0 2070, averages of only two global climate models were taken for this scenario. To execute distributional change analyses initially, consensus bioclimatic suitability maps were transformed into presence/absence binary maps using the threshold value (0.415). The outcomes were represented as follows: range expansion, no occupancy (absence in both), no change (presence in both), and range contraction under reconstructed past and projected future. For the workflow of ecological niche modelling, Gür (2022) was followed as a methodological curriculum.

2.4. Dispersal corridors and paths

Landscape connectivity analysis for the present potential distribution area was executed to find out population connectivity. The least Cost Corridors and Least Cost Paths (LCCs and LCPs) maps were generated with 70 Km spatially thinned occurrence dataset and; as a result, 50 occurrence points were used to deduce connectivity in a spatially explicit framework among the Balkan wall lizard populations (Chen et al., 2011; Brown, 2014; Yu et al., 2015). For calculations, prediction under the present bioclimatic conditions were inverted to use as friction layers (i.e. areas of high suitability were converted to areas of low dispersal cost) and used together with spatially rarified occurrence records. All the analyses, unless stated otherwise, were executed in SDM ToolBox v. 2.4. All GIS operations were conducted using ArcGIS v.10.5 (https://www.esri.com/en-us/arcgis/products/arcgis-desktop/resources).

3. Results

Out of 100 tested candidate models based on four different non-correlated bioclimatic variable datasets and different model settings, the final model was provided by the 3rd bioclimatic data set with six input bioclimatic variables (BIO2, BIO10, BIO11, BIO12, BIO15, BIO17); the feature classes of linear and quadratic (LQ); and a regularization multiplier of 5. The highest Avg.AUC_{TEST} (0.80) among all models was in M3 (Model 3, Table 1) and this model performed better than a random prediction (statistics for AUC ratio, mean ± SD = 1.69 ± 0.03, range=1.60–1.77, P<0.001).

The univariate response curves gave more insights into the precise effect of each variable on the distribution of the species in the study area. The response curves of

bioclimatic variables BIO11 and BIO17 were bell-shaped which means for these bioclimatic variables, background extent contains the full range of conditions that are inhabitable for the species. The rest four bioclimatic variables were truncated when habitat suitability was decreasing (Supplemental Fig. 2). Marginal response curves indicated that the Balkan wall lizard primarily prefers areas having not mild winter, not too hot and dry summer, and annually stable precipitation regime with moderate amount of summer rain (Supplemental Fig. 3). Nevertheless, the mean temperature of the coldest quarter (BIO11, 56.2%) and the precipitation seasonality (BIO15, 40.9%) gave the most contribution to the model (together, 97.1%) almost equally and mostly shaped the geographic distribution of the species (having the most useful information that is not present in the other variables and having the most useful information by itself) (Supplemental Fig. 4, Table 2). The bioclimatic suitability decreased with increasing winter (especially>5°C) and summer temperatures (especially28>°C) and precipitation seasonality (highest around 10%) characterized by hot and dry summers and wet winters at lower latitudes and cooler summer temperatures and spring and summer precipitation at higher latitudes of the study area (see Fig. 3, Supplemental Fig. 3).

Table 2. Contributions of the bioclimatic variables to the model.

Variables	Percent contribution	Permutation importance
BIO11	56.2	62.3
BIO15	40.9	28.1
BIO12	1.6	4.8
BIO10	0.8	2.2
BIO17	0.6	2.4
BIO2	0	0.2

The predicted suitable bioclimatic areas for the species for the present time conditions (sum of moderate, high, and very high suitability, 778,605.39 km², 28.7% of the study area) were mainly including the Crimean Peninsula, high latitudes of the east, and the central Europe until southeastern edge of the Czech Republic (forming the northernmost distribution border) and covering mostly the Balkan Peninsula at low latitudes (excluding high mountain ranges). The potential distribution area of the Balkan wall lizard was found mostly to be similar to its known distribution area (Fig. 3). In addition, high mountain ranges (Rhodope, Balkan, Carpathian, and Crimean Mountains) were predicted correctly low bioclimatic suitability due to low winter temperatures (BIO11; Supplemental Fig. 6). Besides, areas where the species is not known to occur also have been over predicted such as southwestern part of Russia that is adjacent to the Crimean Peninsula, southeastern coastline of the Black Sea, and inner parallel lines (where the elevation is low), adjacent scattered areas in central Anatolia, the Italian Peninsula, and the northern coastline of the Adriatic Sea. (Fig. 3, for the IUCN Red List present distribution, see Böhme et al., 2009).

3.1. Past Projection

For the reconstructed past climatic conditions, final summary predictions were generated for the LGM and Mid Holocene (averaged over global climate models). According to the average LGM prediction, distribution area was withdrawn to some specific locations (northern coastline of the Aegean Sea, the Italian Peninsula and Sicily, totally 57,596.19 km²) mainly due to the negative effect of mean winter temperature (BIO11; see Fig. 4; Supplemental Fig. 7). From LGM to the present, distribution area of the species remarkably extended (1,251.83%, Table 3; Fig. 4; Fig. 6).

Mid-Holocene predictions were considerably different than the LGM and had much closer distribution pattern and distribution extent to the present geographical distribution of the species (Distributional change from Mid-Holocene to present is -0.2% and from the LGM to Mid-Holocene is 1,254.59%). Mid-Holocene distribution projection expanded to more northern latitudes and inner side of the Balkan and the Crimean Peninsulas, the Europe, and also vicinity of the Black Sea due to total contribution of bioclimatic variables with changing amounts from pixel to pixel within the study area (totally 780,190.61 km²; Table 3; Fig. 5; Fig. 6; Supplemental Fig. 8).

Table 3. Bioclimatic suitability (km²) under present and past conditions (i.e. for each time period, LGM and Mid-Holocene) for the Balkan wall lizard (*Podarcis tauricus*).

	Present	LGM	Mid-Holocene
Expansion	--	28,181.62	79,260.81
Suitable areas in both	--	29,414.57	700,929.79
Total suitable areas	778,605.39	57,596.19	780,190.61
Contraction	--	749,190.82	7,7675.60
Unsuitable areas in both	--	1,903,580.54	1,852,501.34
Total unsuitable areas	1,931,762.16	2,652,771.36	1,930,176.94

3.2. Future Projections

The effect of the global climate change on the bioclimatic suitability was quite clear for each scenario for each time period in the future. Consistent with each other in all scenarios, potential suitable bioclimatic areas have expanded mostly to the north-northwestern direction and at higher elevations in the south including interior of the Europe, north of Ukraine, but also inner Anatolia (Fig. 7). These results are broadly agreed upon by all global climate models. The differences of potential suitable bioclimatic areas among scenarios were more marked for 2070 than for 2050. In the most drastic scenario (RCP 8.5 scenarios for 2070), the distribution pattern of the species expanded heavily towards northernmost of the distribution range. Under the future bioclimatic conditions, suitable bioclimatic areas were projected to increase by 77.42 – 124.41% (RCP2.6 on the low and RCP8.5 on the high end) and 75.96 – 174.95% (RCP2.6 on the low and RCP8.5 on the high end) in 2050 and 2070, respectively (Table 4; Fig. 7; Fig. 8) mainly due to the increase in summer and winter temperature in northern latitudes of the study area (BIO10 and BIO11; Supplemental Fig. 9).

The analog and the non-analog conditions and extrapolation risks in model transfers were identified by MESS analysis (Elith et al., 2010). The extrapolation in high-predicted areas of distribution was not a critical issue except for LGM projections showing extrapolation in limited areas at the northern coastline of the Aegean Sea for CCSM4 and MIROC-ESM models (Supplemental Fig. 10) due to mean diurnal range (BIO2). For the future

climatic models, novel conditions were located mainly in the southeastern part of the study area where bioclimatic suitability is very low and in a few pixels (around Thessaloniki) due to the mean summer temperature (BIO10) where high bioclimatic suitability was projected (Supplemental Fig. 11). To sum, the model was required to extrapolate into some limited novel bioclimatic conditions.

Table 4. Bioclimatic suitability (km²) under present and future conditions (i.e. for each scenario, RCP2.6, RCP4.5, RCP7.0, and RCP8.5, for each time period, 2050 and 2070) for the Balkan wall lizard (*Podarcis tauricus*).

	Present	2050				2070			
		RCP2.6	RCP4.5	RCP6.0	RCP8.5	RCP2.6	RCP4.5	RCP6.0	RCP8.5
Expansion	--	629,418.93	686,927.05	823,872.12	1,009,254.36	614,975.84	1,144,614.21	1,050,205.78	1,437,967.29
Suitable areas in both	--	752,008.98	753,770.34	755,883.96	738,006.24	755,091.35	733,602.86	724,355.77	702,779.21
Total suitable areas	778,605.39	1,381,427.91	1,440,697.39	1,579,756.08	1,747,260.60	1,370,067.19	1,878,217.08	1,774,561.55	2,140,746.50

Table 4. (Continued)

	Present	2050				2070			
		RCP2.6	RCP4.5	RCP6.0	RCP8.5	RCP2.6	RCP4.5	RCP6.0	RCP8.5
Contraction	--	26,596.41	24,835.05	22,721.43	40,599.15	23,514.04	45,002.53	54,249.62	75,826.18
Unsuitable areas in both	--	1,302,343.23	1,244,835.11	1,107,890.04	922,507.80	1,316,786.31	787,147.94	881,556.38	493,794.87
Total unsuitable areas	1,931,762.16	1,328,939.64	1,269,670.16	1,130,611.47	963,106.95	1,340,300.36	832,150.47	935,806.00	569,621.05

3.3. Dispersal Corridors and Paths

Putative dispersal corridor and paths as a sign of population connectivity under present conditions were visualized (Fig. 9). High population connectivity generally followed the way beneath Carpathian Mountains and in between Balkan and Rhodope Mountains in the north and Pindus Mountains in the south. This route partially overlaid with the valleys in between these mountains.

4. Discussion

This study presents a first attempt to assess the detailed past (the LGM and Mid-Holocene) and the future (2050 and 2070) potential distribution patterns of the Balkan wall lizard as a common lizard species having large range of distribution area. Accordingly, ENM (Franklin, 2010; Peterson et al., 2011) approach was used to predict the probable suitable bioclimatic areas.

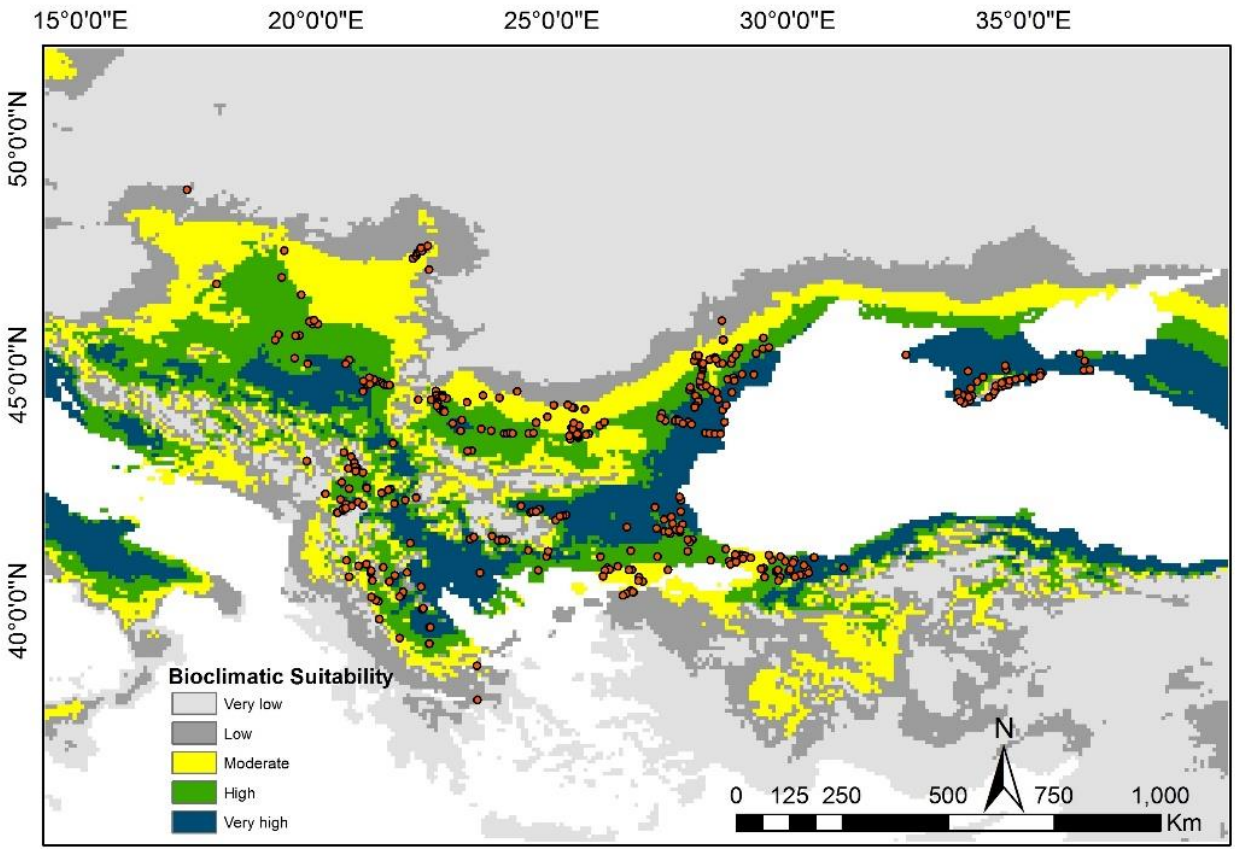

Figure 3. Bioclimatic suitability under present (1960-1990) conditions for the Balkan wall lizard (*Podarcis tauricus*). Red circles indicate 153 presence records. The visible area in maps is 14.25° to 39.33°E and 35.08° to 51.92°N.

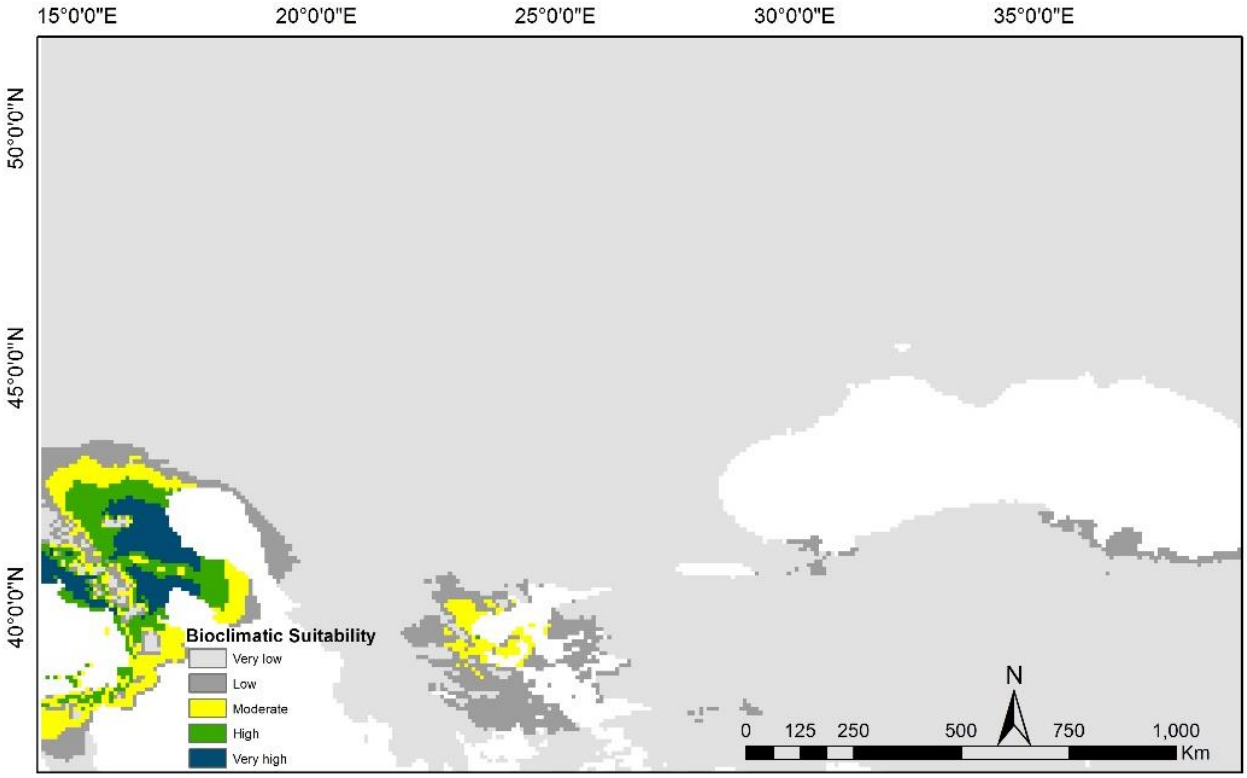

Figure 4. Habitat suitability and glacial refugia under reconstructed LGM conditions as averaged over global climate models (CCSM4, MIROC-ESM, MPI-ESM-LR) for each scenario for each time period for the Balkan wall lizard (*Podarcis tauricus*). The visible area in maps is 14.25° to 39.33°E and 35.08° to 51.92°N.

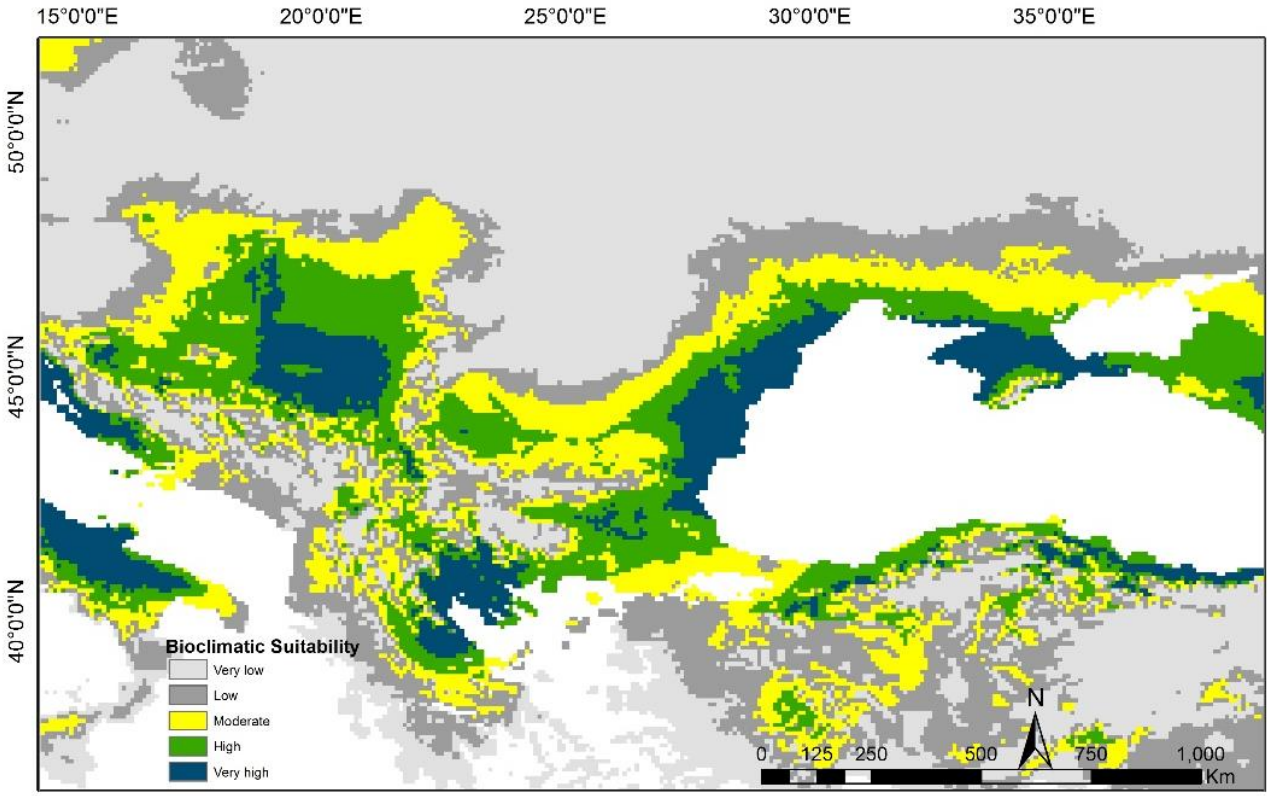

Figure 5. Habitat suitability under reconstructed Mid-Holocene conditions averaged over global climate models (CCSM4, MIROC-ESM, MPI-ESM-LR) for each scenario for each time period for the Balkan wall lizard (*Podarcis tauricus*). The visible area in maps is 14.25° to 39.33°E and 35.08° to 51.9°N.

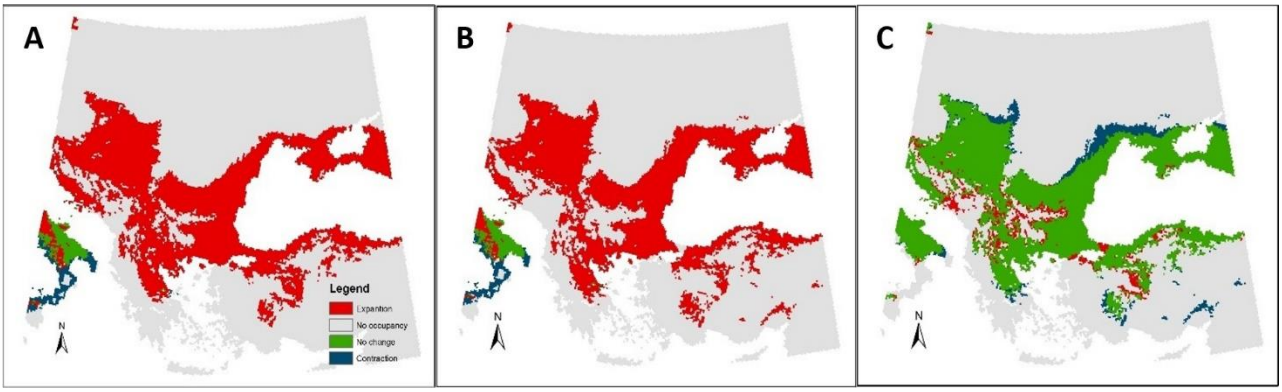

Figure 6. Areas of expansion, no change, contraction, and no occupancy in suitable bioclimatic areas under past conditions (for each time period, LGM and Mid-Holocene as averaged over global climate models) for the Balkan wall lizard (*Podarcis tauricus*). A: From LGM to present, B: From LGM to Mid-Holocene, C: From Mid-Holocene to present, respectively.

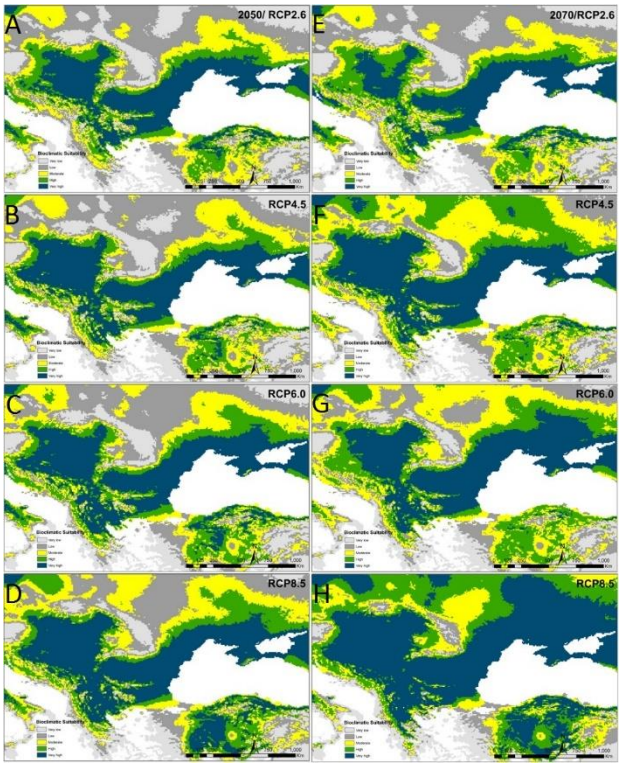

Figure 7. Habitat suitability under future bioclimatic conditions averaged over global climate models (CCSM4, MIROC-ESM, MPI-ESM-LR) for each scenario for each time period (A, B, C, D for 2050 and E, F, G, H for 2070) for the Balkan wall lizard (*Podarcis tauricus*). The visible area in maps is 14.25° to 39.33°E and 35.08° to 51.92°N.

4.1. The Present, the Past and the Future Distribution Pattern of the Balkan Wall Lizard

The results of the study indicate that the Balkan wall lizards have responded to global climate changes through the Late Quaternary in a fashion that in the LGM (22,000 years ago), the potential distribution range considerably contracted and during the interglacial period (Mid-Holocene and the present) it was expanded. To sum, the classical paradigm of glacial range contraction and interglacial range expansion for temperate species (Hewitt, 1996, 1999, 2000, 2004) has been met for the Balkan wall lizard.

Under the present bioclimatic conditions, areas having high bioclimatic suitability were predicted across most of the known distribution area of the species. The model correctly predicted as very low suitability for the Balkan wall lizard at the western part of the Pindus Mountains, which is the distribution area of *P. ionicus*. This shows that the predictive ability of the model was very high for this area. In Psonis et al. (2018), it was found in niche similarity analysis that there was low niche overlap between *P. tauricus* and *P. ionicus*. These findings are also in accordance with the study result. However, the model over-predicted the distributions in some areas at southwestern and northeastern parts of the study area including south of Dinaric Alp Mountains, the Italian Peninsula, Sicily, east of the Black Sea Basin, and inner Anatolia (Fig. 3). This could be due to several reasons: 1) Known distribution area of the species is expanded with new studies (Tok & Çiçek, 2014; Bülbül et al., 2015; Gül & Tosunoğlu, 2017; Fischer et al., 2019) and the results of the study might support it, 2) Italy and the south part of Dinaric Alp Mountains are not inside the distribution area of the species historically, even though it had been, the barrier status of the Alps could have blocked northwards directed range expansions from the Italian Peninsula (Sudhaus et al., 1997). Likewise Italy, Sicily was also over-predicted and these findings could be the result of modelling weakness. The temporal accordance between the occurrence data and the bioclimatic data is a limitation of this study (Roubicek et al., 2010). Bioclimatic data were from the period 1960-1990 and the occurrence data were from the period 1977-2019 (mostly after 1990).

Podarcis taxon came from the western parts of Europe and they colonized the Balkan Peninsula (Oliverio et al., 2000). The phylogeographic scenario suggests in Psonis et al. 2017 and 2018 that the diversification within *Podarcis* started in the Upper Miocene (~9.60 Mya) due to orogenic activity and went on with differentiation of the Balkan species group (started at 8.63 Mya in Mid-Aegean Trench). This was followed by the differentiation of *P. tauricus* species subgroup during the Messinian Salinity Crisis in Late Miocene (MSC=5.96-5.33 Mya) due to the geomorphological alterations and climatic oscillations. Finally, genetically different lineages have been revealed which could be due to geographic fragmentation in the glacial and inter-glacial periods as a result of the Pleistocene climatic oscillation.

The Balkan Peninsula was a source for postglacial colonization of central and northern Europe by species

populations (Hewitt, 1999, 2000). The southern Balkans is considered by other phylogeographic studies as a local climatic refugium (Taberlet et al., 1998; Joger et al., 2007; Sagonas et al., 2014; Marzahn et al., 2016). In this study, it was found that during the LGM, the distribution range restricted to a few spots in the southern Balkans. The LGM projections could catch southern Balkan refuge since the coastline of the Aegean Sea was predicted as suitable bioclimatic area (Fig. 4). During Mid-Holocene, the Balkan wall lizard substantially expanded its range from the restricted area of the LGM glacial refugium to its present range. Therefore, recent findings support former suggestion in that the species is a post-glacial colonizer (Psonis et al., 2018; Fig. 5). Mid-Holocene potential distribution area and the present distribution area of the Balkan wall lizard were almost the same in size which may indicate that the climatic conditions during Mid-Holocene was the main driver for expansion of the distribution area of the species after significant LGM contraction due to the positive effects of bioclimatic variables with changing contributions from one pixel to the other (Supplemental Fig. 8). These findings are mostly in accordance with Psonis et al. (2018) in that being Southern Balkan as a LGM refuge and present range expansion of the species. Yet, the difference between the studies could be due to several factors such as using different bioclimatic variables, occurrence records, and Maxent calibration values to contract a model. These findings are compatible with the results of demographic and ENM analyzes in Psonis et al. (2018) in that the LGM possibly caused a bottleneck for the species and a recent spatial expansion of the species population occurred from the south to the north after the end of the LGM.

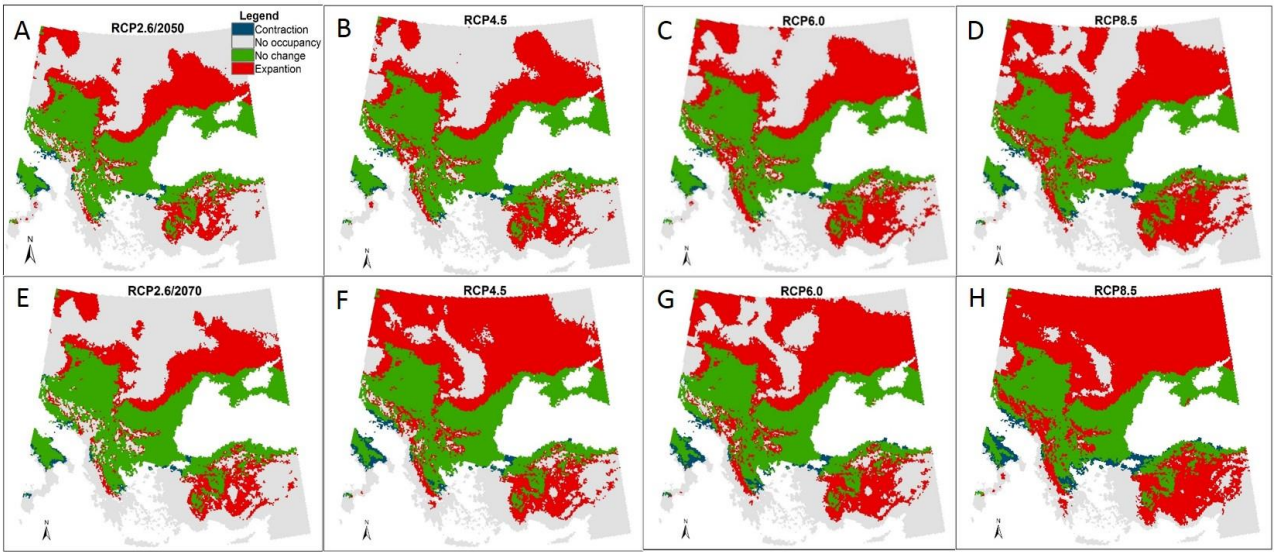

Figure 8. Areas of expansion, no change, contraction and no occupancy in suitable bioclimatic areas under future conditions (for each time period (A, B, C, D for 2050 and E, F, G, H for 2070) and for each scenario (RCP2.6, RCP4.5, RCP6.0 and RCP8.5) as averaged over global climate models for the Balkan wall lizard (*Podarcis tauricus*).

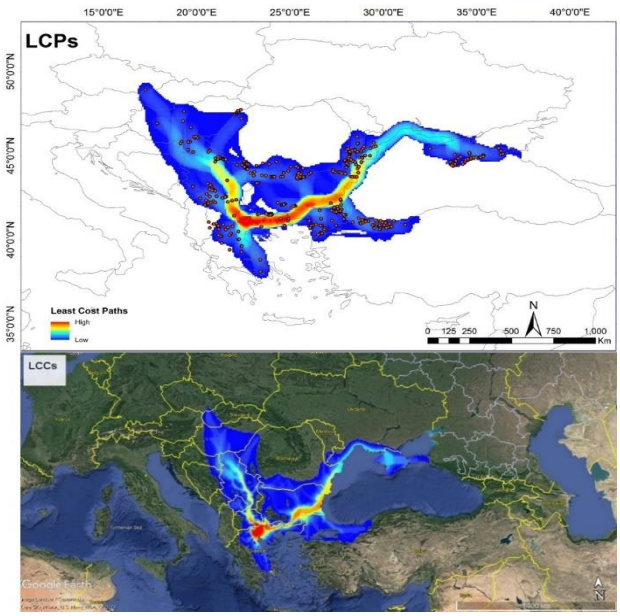

Figure 9. Construction of dispersal corridors (LCCs) and paths (LCPs) for the Balkan wall lizard (*Podarcis tauricus*). Warmer colors depict higher population connectivity.

Furthermore, together with the global climate change, the predicted distribution area of the species will expand towards northern latitudes, higher elevations of the mountain ranges, and inlands of the European and the Asian continents (Fig. 7). Many studies suggest that global warming is driving species ranges northwards and toward higher elevations at temperate latitudes (Parmesan, 2006; Wilson et al., 2007; Chen et al., 2011). The bioclimatic suitability for the species was predicted to increase in northern latitudes due to mainly high mean summer and winter temperatures (BIO10 and BIO11; Supplemental Fig. 9). According to IPCC 5th Assessment Report (IPCC, 2014), global average temperature would increase between 0.3 and 4.8 °C by the end of 21st century to be in agreement within all RCPs. Mediterranean region was projected to be much drier and hotter in the warm seasons (Giorgi & Lionello, 2008) and the central/north Europe to be much warmer and wetter in the cold seasons (Kjellstrom & Ruosteenoja, 2007) compared to recent climatic conditions. These projections are in accordance with the predictions of the northernmost distribution expansion of the Balkan wall lizards under the future bioclimatic conditions. All these results were evaluated under the assumption that the ecological requirements of the species have remained the

same through time periods (Nogués-Bravo, 2009). However, given low dispersal capacity, it is unlikely that the species could reach all of the potential distribution area (Huey, 1982).

4.2. Dispersal Corridors and Paths

Connectivity among populations was assessed via LCCs and LCPs analyses. By virtue of the approach integrated with ENMs, possible dispersal corridors among populations of the Balkan wall lizard in present bioclimatic conditions have been identified. Even though distribution area of the species included several high mountain ranges, no noticeable barriers have been observed between south-western and north-eastern populations of the Balkan wall lizard. Moreover, there is a high connectivity via valleys among Dinaric Alp, Balkan, Rhodope, Pindus Mountains and until the coastline of the Black Sea. Predicted high connectivity followed the way beneath Carpathian Mountains and above Dinaric Alp Mountains and went on between Balkan and Rhodope Mountains on the north and Pindus Mountains on the south (Fig. 9).

Although validity of the lineages was not clear due to low representation, three lineages were found within *P. tauricus* (Psonis et al., 2018). The current LCCs analyses could catch high connectivity in one of that lineage (including populations from Albania, Bulgaria, FYROM, Greece, Hungary, Romania, Serbia, and Türkiye) and also low connectivity among those three lineages.

4.3. Effects of the Bioclimatic Variables

Our results offer insights into the ecological factors related to the distribution of the Balkan wall lizard in the study area. The most significant bioclimatic variables in predicting the present potential distribution (which characterized environmental space) of *P. tauricus* is BIO11 and BIO15 (Supplemental Fig. 3). The bioclimatic suitability decreased with increasing BIO15 and increasing BIO10 and BIO11. Moreover, for BIO11 and BIO17, the background extent contained the full range of conditions that are inhabitable for the species. These findings emphasize the preference of the species for annually stable precipitation regime with summer rains and avoiding too warm and too cold winter conditions. These preferences also express itself in the distribution area of the Balkan wall lizards that mainly includes Balkans and more northern latitudes. Moreover, western part of Pindus Mountains was predicted as bioclimatically very low suitable for the species due to BIO12 and BIO15 (Supplemental Fig. 12). These findings are mostly in accordance with former studies. Kaliontzopoulou et al. (2008) mentioned the preference of *Podarcis* in the northern Africa for humid conditions but not extremely high temperatures without giving details about the species specific preferences. Psonis et al. (2018) discussed the most important climatic parameter was the annual range of the temperature.

The species spatial distributions depend on three general, interacting types of factors: the abiotic environment (e.g. temperature, humidity), the biotic environment (e.g. competition), and accessibility of areas across landscapes (migration) (Pulliam, 2000; Soberón & Peterson, 2005; Soberón, 2007). It is known that ecological competition among Balkan Podarcis species has been one of the main driver of their evolutionary history (Oliverio et al., 2000; Poulakakis et al., 2005a, 2005b). Moreover, changing climatic conditions may reform the community and new competitors may arise (Le Galliard et al., 2012). Besides, herptiles are known to be poor dispersers but dispersal ability may vary substantially within taxa (Smith & Green, 2005). In this study, biotic factors and dispersal ability of the species in shaping the distribution pattern remained unknown and bioclimatic variables as abiotic factors were taken into account exclusively. Moreover, in the face of the global climate change, land-use practices are also changing and habitat loss appears as an important driver shaping the species distributions (Huey, 1982; Zakkak et al., 2015) and the Balkan wall lizards have been facing this threat in some of their distribution range (Böhme et al., 2009). Therefore, using bioclimatic data and land-use data together in the future ecological niche modelling studies would give deeper insight for distributional pattern of the Balkan wall lizard.

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References

Altunışık, A., Kalaycı, T., Uysal, İ., Tosunoğlu, M., & Özdemir, N. (2016). Age, adult survival rate, and adult life expectancy of a *Podarcis tauricus* population (Reptilia: Lacertidae) from Saros Bay, Türkiye. *Russian Journal of Herpetology*, 23(4), 278-282.

Anderson, R.P. (2013). A framework for using niche models to estimate impacts of climate change on species distributions. *Annals of the New York Academy of Sciences*, 1297(1), 8-28. https://doi.org/10.1111/nyas.12264

Araújo, M.B., Anderson, R.P., Barbosa, A.M., Beale, C.M., Dormann, C.F., Early, R., …, & Rahbek, C. (2019). Standards for distribution models in biodiversity assessments. *Science Advances*, 5(1), eaat 4858. https://doi.org/10.1126/sciadv.aat4858

Arnold, E.N. (1987). Resource partition among lacertid lizards in southern Europe. *Journal of Zoology*, 1(4), 739-782. https://doi.org/10.1111/j.1096-3642.1987.tb00753.x

Baran, İ., Yılmaz, İ., Kete, R., Kumlutaş, Y., & Durmuş, H. (1992). Batı ve Orta Karadeniz Bölgesinin herpetofaunası. *Turkish Journal of Zoology*, 16(1), 275-288.

Başoğlu, M., & Baran, İ. (1977). The Reptiles of Turkey, Part 1: The Turtles and Lizards, *Ege University, Science Faculty Book Series*, No: 76, 133-135

Bezeng, B.S., Tesfamichael, S.G., & Dayananda, B. (2018). Predicting the effect of climate change on a range-restricted lizard in southeastern Australia. *Current Zoology*, 64(2), 165-171. https://doi.org/10.1093/cz/zox021

Böhme, W., & Corti, C. (1993). Zoogeography of the lacertid lizards of the western Mediterranean basin. In: Böhme, Perez Mellado, Maragou (ed).

Lacertids of the Mediterranean region. *Hellenic Zoological Society*, Athens, Bonn, Alicante, Chapter 2, 17-33.

Böhme, W., Lymberakis, P., Ajtic, R., Tok, V., Ugurtaş, I.H., Sevinç, M., …, & Avci, A. (2009). *Podarcis tauricus*. *The IUCN Red List of Threatened Species*. Retrieved from https://www.iucnredlist.org/species/61554/12515695 & https://doi.org/10.2305/IUCN.UK.2009.RLTS.T61554A12515695.en. Accessed on 15 June 2022.

Boria, R.A., Olson, L.E., Goodman, S.M., & Anderson, R.P. (2014). Spatial filtering to reduce sampling bias can improve the performance of ecological niche models. *Ecological Modelling*, 275(2014), 73-77. http://doi.org/10.1016/j.ecolmodel.2013.12.012

Brown, J.L. (2014). SDMtoolbox: a python-based GIS toolkit for landscape genetic, biogeographic and species distribution model analyses. *Methods in Ecology and Evolution*, 5(7), 694-700. https://doi.org/10.1111/2041-210X.12200

Brown, J.L., Bennett, J.R., & French, C.M. (2017). SDMtoolbox 2.0: the next generation Python-based GIS toolkit for landscape genetic, biogeographic and species distribution model analyses. *PeerJ*, 5, e4095. https://doi.org/10.7717/peerj.4095

Bülbül, U., Kurnaz, M., Eroğlu, A.İ., Koç, H., & Kutrup, B. (2015). New locality record of *Podarcis tauricus tauricus* (Pallas, 1814) (Squamata: Lacertidae) from the western Black Sea region of Turkey. *Turkish Journal of Zoology*, 39(5), 981-986. https://doi.org/10.3906/zoo-1411-25

Chen, I., Hill, J.K., Ohlemüller, R., Roy, D.B., & Thomas, C.D. (2011). Rapid Range Shifts of Species Associated with High Levels of Climate Warming. *Science*, 333(6045), 1024-1026. https://doi.org/10.1126/science.1206432

Cogălniceanu, D., Rozylowicz, L., Székely, P., Samoilă, C., Stănescu, F., Tudor, M., Székely, D., & Losif, R. (2013). Diversity and distribution of reptiles in Romania. *ZooKeys*, 341, 49-76. https://doi.org/10.3897/zookeys.341.5502

Çördük, N., Gül, Ç., Tosunoğlu, M., & Özdemir, N. (2018). Evaluating the taxonomic status of the Balkan wall lizard (*Podarcis tauricus*, Pallas, 1814) from the Anatolian part of Çanakkale (Turkey) using mitochondrial DNA. *American Journal of Innovative Research and Applied Sciences*, 6(5), 227-233.

Ćorović J., Popović, M., Cogălniceanu, D., Carretero, M.A., & Crnobrnja-Isailović, J. (2018). Distribution of the meadow lizard in Europe and its realized ecological niche model. *Journal of Natural History*, 52(29-30), 1909-1925. https://doi.org/10.1080/00222933.2018.1502829

Covaciu-Marcov, S.D., Ghira, I., Cicort-Lucaciu, A.Ş., & Sas-Kovacs, I. (2006). Contributions to knowledge regarding the geographical distribution of the herpetofauna of Dobrudja, Romania. *North-Western Journal of Zoology*, 2(2), 88-125.

Elith, J., Graham, C.H., Anderson, R.P., Dudík, M., Ferrier, S., Guisan, A., …, & Zimmermann, N.E. (2006). Novel methods improve prediction of species' distributions from occurrence data. *Ecography*, 29(2), 129-151. https://doi.org/10.1111/j.2006.0906-7590.04596.x

Elith, J., Kearney, M., & Phillips, S. (2010). The art of modeling range-shifting species. *Methods in Ecology and Evolution*, 1(4), 330-342. https://doi.org/10.1111/j.2041-210X.2010.00036.x

Elith, J., Phillips, S.J., Hastie, T., Dudík, M., Chee, Y.E., & Yates, C.J. (2011). A statistical explanation of MaxEnt for ecologists. *Diversity and Distributions*, 17(1), 43-57. https://doi.org/10.1111/j.1472-4642.2010.00725.x

Eroğlu, A.İ., Bülbül, U., & Kurnaz, E. (2017). Age structure and growth in a Turkish population of the Crimean Wall Lizard, *Podarcis tauricus* (Pallas, 1814) (Squamata: Sauria: Lacertidae). *Herpetozoa*, 29(3-4), 125-133.

Feliner, G.N. (2011). Southern European glacial refugia: A tale of tales. *Taxon*, 60(2), 365-372. https://doi.org/10.1002/tax.602007

Feng, X., Park, D.S., Walker, C.M., & Peterson, A.T. (2019). A checklist for maximizing reproducibility of ecological niche models. *Nature Ecology & Evolution*, 3(2019), 1382-1395. https://doi.org/10.1038/s41559-019-0972-5

Fischer, D., Babická, K., Fischerová, J., Lerch, Z., Mikátová, B., Reiter, A., & Rehák, I. (2019). Discovery of the *Podarcis tauricus* population in the Czech Republic (Squamata: Lacertidae). *Acta Societatis Zoologicae Bohemicae*, 83, 239-254.

Franklin, J. (2010). Mapping species distributions: spatial inference and prediction. Cambridge University Press, 320 pp.

Gasc, J.P., Cabela, A., Crnobrnja-Isailovic, J., Dolmen, D., Grossenbacher, K., Haffner, P., …, & Zuiderwijk, A. (1997). Atlas of Amphibians and Reptiles in Europe. *Societas Europaea Herpetologica and Muséum national d'Histoire naturelle*, Paris, 133-134 pp.

Gent, P.R., Danabasoglu, G., Donner, L.J., Holland, M.M., Hunke, E.C., Jayne, S.R., …, & Zhang, M. (2011). The Community Climate System Model Version 4. *Journal of Climate*, 24(19), 4973-4991. https://doi.org/10.1175/2011JCLI4083.1

Giorgetta, M.A., Jungclaus, J., Reick, C. H., Legutke, S., Bader, J., Böttinger, M., …, & Stevens, B. (2013). Climate and carbon cycle changes demo 1850 to 2100 in MPI-ESM simulations for the Coupled Model Intercomparison Project phase 5. *Journal of Advances in Modeling Earth Systems*, 5(3), 572-597. https://doi.org/10.1002/jame.20038

Giorgi, F., & Lionello, P. (2008). Climate change projections for the Mediterranean region. *Global and Planetary Change*, 63(2-3), 90-104. https://doi.org/10.1016/j.gloplacha.2007.09.005

Guevara, L., Gerstner, B.E., Kass, J.M., & Anderson, R.P. (2018). Toward ecologically realistic predictions of species distributions: A cross-time example from tropical montane cloud forests. *Global Change Biology*, 24(4), 1511-1522. https://doi.org/10.1111/gcb.13992

Guisan, A., & Thuiller, W. (2005). Predicting species distribution: offering more than simple habitat models. *Ecology Letters*, 8(9), 993-1009. https://doi.org/10.1111/j.1461-0248.2005.00792.x

Guisan, A., & Zimmermann, N.E. (2000). Predictive habitat distribution models in ecology. *Ecological Modelling*, 135(2-3), 147-186. https://doi.org/10.1016/S0304-3800(00)00354-9

Gül, Ç., & Tosunoğlu, M. (2017). *Podarcis tauricus* (Pallas, 1814), new to the Anatolian part of the Province of Çanakkale, Turkey. *Herpetozoa*, 30(1-2), 78-80.

Gül, S., Kumlutaş, Y., & Ilgaz, Ç. (2015). Climatic preferences and distribution of 6 evolutionary lineages of *Typhlops vermicularis* Merrem, 1820 in Turkey using ecological niche modeling. *Turkish Journal of Zoology*, 39, 235-243. https://doi.org/10.3906/zoo-1311-9

Gür, H. (2013). The effects of the Late Quaternary glacial–interglacial cycles on Anatolian ground squirrels: range expansion during the glacial periods? *Biological Journal of the Linnaean Society*, 109(1), 19-32. https://doi.org/10.1111/bij.12026

Gür, H. (2019). Tür Dağılım Modellemesi ile İklim Değişikliği Uygulamaları. *Ekoloji ve Evrimsel Biyoloji Derneği Yayınları*, 1. Retrieved from http://www.ekoevo.org/wp-content/uploads/2020/04/GurH_EkoEvo_2019.pdf

Gür, H. (2022). The future impact of climate and land-use changes on Anatolian ground squirrels under different scenarios. *Ecological Informatics*, 70(2022), 101693. https://doi.org/10.1016/j.ecoinf.2022.101693

Hewitt, G.M. (1996). Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society*, 58(3), 247-276. https://doi.org/10.1006/bijl.1996.0035

Hewitt, G.M. (1999). Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society*, 68(1-2), 87-112. https://doi.org/10.1111/j.1095-8312.1999.tb01160.x

Hewitt, G.M. (2000). The genetic legacy of the Quaternary ice ages. *Nature*, 405(6789), 907-913. https://doi.org/10.1038/35016000

Hewitt, G.M. (2004). Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences*, 359(1442), 183-195. https://doi.org/10.1098/rstb.2003.1388

Hijmans, R.J., & Graham, C.H. (2006). The Ability of Climate Envelope Models to Predict the Effect of Climate Change on Species Distributions. *Global Change Biologie*, 12(12), 2272-2281. http://doi.org/10.1111/j.1365-2486.2006.01256.x

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., & Jarvis. A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, 25(15), 1965-1978. https://doi.org/10.1002/joc.1276

Hodgson, J.A., Thomas, C.D., Wintle, B.A., & Moilanen, A. (2009). Climate change, connectivity and conservation decision making: back to basics. *Journal of Applied Ecology*, 46(5), 964-969. https://doi.org/10.1111/j.1365-2664.2009.01695.x

Huey, R.B. (1982). Temperature, physiology, and the ecology of reptiles. In: Gans, C., Pough, F.H., (ed). *Biology of the Reptilia, Physiology C, Physiological Ecology*. Academic Press, London, Vol.12, 25-91.

Iftime, A., & Iftime, O. (2016). Contributions to the Knowledge on the Amphibians and Reptiles of Teleorman County (Southern Romania). *Travaux du Muséum National d'Histoire Naturelle "Grigore Antipa"Antipa"*, 58(1-2), 63-71. https://doi.org/10.1515/travmu-2016-0009

IPCC, (2014). The Fifth Asseessment reports from the Intergovernmental Panel on Climate Change. Impacts, Adaptation and Vulnerability:

Summary for Policymakers. Cambridge, United Kingdom and New York, Cambridge University Press, 32 pp.

Joger, U., Fritz, U., Guicking, D., Kalyabina-Hauf, S., Nagy, Z. T., & Wink, M. (2007). Phylogeography of western Palearctic reptiles, spatial and temporal speciation patterns. *Zoologischer Anzeiger*, 246(4), 293-313. https://doi.org/10.1016/j.jcz.2007.09.002

Kadmon, R, Farber, O., & Danin, A. (2004). Effect of roadside bias on the accuracy of predictive maps produced by bioclimatic models. *Ecological Applications*, 14(2), 401-413. https://doi.org/10.1890/02-5364

Kaliontzopoulou, A., Brito, J.C., Carretero, M.A., Larbes, S., & Harris, D.J. (2008). Modelling the partially unknown distribution of wall lizards (*Podarcis*) in North Africa: Ecological affinities, potential areas of occurrence, and methodological constraints. *Canadian Journal of Zoology*, 86(9), 992-1001. https://doi.org/10.1139/Z08-078

Kass, J.M., Vilela B., Aiello-Lammens, M.E., Muscarella, R., Merow, C., & Anderson, R.P. (2018). Wallace: A flexible platform for reproducible modeling of species niches and distributions built for community expansion. *Methods in Ecology and Evolution*, 9(4), 1151-1156. https://doi.org/10.1111/2041-210X.12945

Kati, V., Foufopoulos, J., Ioannidis, Y., Papaioannou, H., Poirazidis, K., & Lebrun, P. (2007). Diversity, ecological structure and conservation of herpetofauna in a Mediterranean area (Dadia National Park, Greece). *Amphibia-Reptilia*, 28, 517-529. https://doi.org/10.1163/156853807782152471

Kjellstrom, E., & Ruosteenoja, K. (2007). Present-day and future precipitation in the Baltic Sea region as simulated in a suite of regional climate models. *Climatic Change*, 81, 281-291. https://doi.org/10.1007/s10584-006-9219-y

Koç, H., Bülbül, U., Kurnaz, M., Eroğlu, İ., & Kutrup, B. (2018) Phylogenetic relationships of *Podarcis siculus* (Rafinesque-Schmaltz, 1810) and *Podarcis tauricus* (Pallas, 1814) in Turkey, based on mitochondrial DNA. *Mitochondrial DNA Part A*, 29(5), 664-673. https://doi.org/10.1080/24701394.2017.1342245

Kolbe, J.J., Lavin, B.R., Burke, R.L., Rugiero, L., Capula, M., & Luiselli, L. (2013). The desire for variety: Italian wall lizard (*Podarcis siculus*) populations introduced to the United States via the pet trade are derived from multiple native-range sources. *Biological Invasions*, 15, 775-783. https://doi.org/10.1007/s10530-012-0325-7

Kukushkin, O.V., & Doronin, I.V. (2013). Distribution peculiarities of the rare colour aberrations of the Balkan wall lizard, *Podarcis tauricus* (Sauria: Lacertidae) from the Crimea. *Труды Зоологического института РАН Том*, 317(4), 474-493.

Kurnaz, M., & Yousefkhani, S.S.H. (2019). Ecological niche divergence between *Darevskia rudis* and *D. bithynica* (Lacertidae) in Turkey. *Biologia*, 75, 1307–1312. https://doi.org/10.2478/s11756-019-00374-0

Kurnaz, M., & Yousefkhani, S.S.H. (2021). A Contribution to the Biogeography of *Ablepharus anatolicus and A. budaki* (Squamata: Scincidae) Using Ecological Niche Modeling in Turkey. *Folia Biologica (Kraków)*, 69(4). https://doi.org/10.3409/fb_69-4.17

Le Galliard, J.F., Massot, M., Baron, J. P., & Clobert, J. (2012). Ecological effects of climate change on European reptiles. *Wildlife conservation in a changing climate*, 179-203. https://doi.org/10.13140/RG.2.1.3523.0248

Ljubisavljević, K., Džukić, G., & Kalezić, M.L. (2010). Female reproductive characteristics of the Balkan wall lizard (*Podarcis taurica*) in the northwestern periphery of its range. *Central European Journal of Biology*, 5(3), 391-395. https://doi.org/10.2478/s11535-010-0016-2

Marzahn, E., Mayer, W., Joger, U., Ilgaz, Ç., Jablonski, D., Kindler, C., …, & Fritz, U. (2016). Phylogeography of the *Lacerta viridis* complex: mitochondrial and nuclear markers provide taxonomic insights. *Journal of Zoological Systematics and Evolutionary Research*, 54(2), 85-105. https://doi.org/10.1111/jzs.12115

Melville, J., Haines, M.L., Hale, J., Chapple, S., & Ritchie, E.G. (2016). Concordance in phylogeography and ecological niche modelling identify dispersal corridors for reptiles in arid Australia. *Journal of Biogeography*, online published. https://doi.org/10.1111/jbi.12739

Merow, C., Smith, M.J., & Silander, J.A. (2013). A practical guide to MaxEnt for modeling species' distributions: what it does, and why inputs and settings matter. *Ecography*, 36(10), 1058-1069. https://doi.org/10.1111/j.1600-0587.2013.07872.x

Mizsei, E., Fejes, Z., Malatinszky, Á., Lengyel, S., & Vadász, C. (2020). Reptile responses to vegetation structure in a grassland restored for an endangered snake. *Community Ecology*, 21(2020), 203-212. https://doi.org/10.1007/s42974-020-00019-2

Mollov, I.A., & Valkanova, M.V. (2009). Risks and Opportunities of Urbanization – Structure of Two Populations of the Balkan Wall Lizard *Podarcis tauricus* (Pallas, 1814) in the City of Plovdiv. *Ecologia Balkanica*, 1, 27-39.

Mothes, C.C., Stroud, J.T., & Clements, S.L. (2019). Evaluating ecological niche model accuracy in predicting biotic invasions using South Florida's exotic lizard community. *Journal of Biogeography*, 46(2), 432-441. https://doi.org/10.1111/jbi.13511

Muscarella, R., Galante, P.J., Soley-Guardia, M., Boria, R.A., Kass, J.M., Uriarte, M., & Anderson, R.P. (2014). ENMeval: An R package for conducting spatially independent evaluations and estimating optimal model complexity for MAXENT ecological niche models. *Methods in Ecology and Evolution*, 5(11), 1198-1205. https://doi.org/10.1111/2041-210X.12261

Nogués-Bravo, D. (2009). Predicting the past distribution of species climatic niches. *Global Ecology and Biogeography*, 18(5), 521-531. https://doi.org/10.1111/j.1466-8238.2009.00476.x

Oliverio, M., Bologna, M.A., & Mariottini, P. (2000). Molecular biogeography of the Mediterranean lizards *Podarcis* Wagler, 1830 and Teira Gray, 1838 (Reptilia, Lacertidae). *Journal of Biogeography*, 27(6), 1403-1420. https://doi.org/10.1046/j.1365-2699.2000.00517.x

Osorio-Olvera L., Lira-Noriega, A., Soberón, J., Peterson, T.A., Falconi, M., Contreras-Díaz, R.G., …, & Barve, N. (2020). ntbox: an R package with graphical user interface for modeling and evaluating multidimensional ecological niches. *Methods in Ecology and Evolution*, 11(10), 1199-1206. https://doi.org/10.1111/2041-210X.13452

Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics*, 37, 637-669. https://doi.org/10.1146/annurev.ecolsys.37.091305.110100

Parmesan, C., Ryrholm, N., Stefanescu, C., Hill, J.K., Thomas, C.D., Descimon, H., Huntley, B., & Warren, M. (1999). Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature*, 399(6736), 579-583. https://doi.org/10.1038/21181

Peterson, A.T., Ortega-Huerta, M.A., Bartley, J., Sánchez-Cordero, V., Soberón, J., Buddemeier, R.H., & Stockwell, D.R.B. (2002). Future projections for Mexican faunas under global climate change scenarios. *Nature*, 416, 626-629.

Peterson, A.T., Papeş, M., & Eaton, M. (2007). Transferability and model evaluation in ecological niche modeling: a comparison of GARP and Maxent. *Ecography*, 30(4), 550-560. https://doi.org/10.1111/j.0906-7590.2007.05102.x

Peterson, A.T., Papeş, M., & Soberón, J. (2008). Rethinking receiver operating characteristic analysis applications in ecological niche modeling. *Ecological modelling*, 213(1), 63-72. https://doi.org/10.1016/j.ecolmodel.2007.11.008

Peterson, A.T., Soberón, J., Pearson, R.G., Anderson, R.P., Martínez-Meyer, E., Nakamura, M., & Araújo, M.B. (2011). Ecological niches and geographic distributions (MPB-49). *Princeton and Oxford, Princeton University Press*, 328 pp.

Petrov, B.P., Tzankov, N., Strijbosch, H., Popgeorgiev, G., & Beshkov, V. (2006). The herpetofauna (Amphibia and Reptilia) of the Western Rhodopes Mountain (Bulgaria and Greece). *Herpetofauna*. In: Beron P. (ed.) Biodiversity of Bulgaria, 3. Biodiversity of Western Rhodopes (Bulgaria and Greece) *Valakos I. Pensoft & Nat. Mus. Natura History*, Sofia, 863-912 pp.

Phillips, S.J., Anderson, R.P., & Schapire, R.E. (2006). Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, 190(3-4), 231-259. https://doi.org/10.1016/j.ecolmodel.2005.03.026

Phillips, S.J., Anderson, R.P., Dudík, M., Schapire, R.E., & Blair, M.E. (2017). Opening the black box: an open-source release of Maxent. *Ecography*, 40(7), 887-893. https://doi.org/10.1111/ecog.03049

Phillips, S.J., Dudík, M., & Schapire, R.E. (2004). A maximum entropy approach to species distribution modeling. *In Proceedings of the Twenty-first International Conference on Machine Learning*, Banff, Alta., July 2004. Edited by R. Greiner and D. Schuurmans. ACM Press, New York. 655-662 pp. https://doi.org/10.1145/1015330.1015412

Poulakakis, N., Lymberakis, P., Valakos, P. Pafilis, Zouros, E., & Mylonas, M. (2005a). Phylogeography of Balkan wall lizard (*Podarcis taurica*) and its relatives inferred from mitochondrial DNA sequences. *Molecular Ecology*, 14(8), 2433-2443. https://doi.org/10.1111/j.1365-294X.2005.02588.x

Poulakakis, N., Lymberakis, P., Valakos, E., Zouros, E., & Mylonas, M. (2005b). Phylogenetic relationships and biogeography of *Podarcis* species from the Balkan Peninsula, by bayesian and maximum likelihood analyses of mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution*, 37(3), 845-857. https://doi.org/10.1016/j.ympev.2005.06.005

Promnun, P., Tandavanitj, N., Kongrit, C., Kongsatree, K., Kongpraphan, P., Dongkumfu, W., Kumsuan, D., & Khudamrongsawat, J. (2021). Phylogeography and ecological niche modeling reveal evolutionary

history of *Leiolepis ocellata* (Squamata, Leiolepidae). *Ecology and Evolution*, 11(5), 2221-2233. https://doi.org/10.1002/ece3.7186

Psonis, N., Antoniou, A., Karameta, E., Leaché, A.D., Kotsakiozi, P., Darriba, D., Kozlov, A., & Poulakakis, N. (2018). Resolving complex phylogeographic patterns in the Balkan Peninsula using closely related wall-lizard species as a model system. *Molecular Phylogenetics and Evolution*, 125, 100-115. https://doi.org/10.1016/j.ympev.2018.03.021

Psonis, N., Antoniou, A., Kukushkin, O., Jablonski, D., Petrov, B., Crnobrnja-Isailovi´C, J., Sotiropoulos, K., & Poulakakis, N. (2017). Hidden diversity in the *Podarcis tauricus* (Sauria, Lacertidae) species subgroup in the light of multilocus phylogeny and species delimitation. *Molecular Phylogenetics and Evolution*, 106(6), 6-17. https://doi.org/10.1016/j.ympev.2016.09.007

Pulliam, H.R. (2000). On the relationship between niche and distribution. *Ecology Letters*, 3(4), 349-361. https://doi.org/10.1046/j.1461-0248.2000.00143.x

Rehák, I., Fischer, D., Kratochvíl, L., & Rovatsos, M. (2022). Origin and haplotype diversity of the northernmost population of *Podarcis tauricus* (Squamata, Lacertidae): Do lizards respond to climate change and go north?. *Biodiversity Data Journal*, 10, e82156. https://doi.org/10.3897/BDJ.10.e82156

Roubicek, A.J., VanDerWal, J., Beaumont, L.J., Pitman, A.J., Wilson, P., & Hughes, L. (2010). Does the choice of climate baseline matter in ecological niche modelling? *Ecological Modelling*, 221(19), 2280-2286. https://doi.org/10.1016/j.ecolmodel.2010.06.021

Rudnick, D.A., Ryan, S.J., Beier, P., Cushman, S.A., Dieffenbach, F., Epps, C.W., …, & Trombulak, S.C. (2012). The Role of Landscape Connectivity in Planning and Implementing Conservation and Restoration Priorities. *Issues in Ecology*, Report No. 16, Washington, DC, Ecological Society of America, 20 pp. Retrieved from https://scholars.unh.edu/geog_facpub/

Sagonas, K., Poulakakis, N., Lymberakis, P., Parmakelis, A., Pafilis, P., & Valakos, E.D. (2014). Molecular systematics and historical biogeography of the green lizards (Lacerta) in Greece: Insights from mitochondrial and nuclear DNA. *Molecular Phylogenetics and Evolution*, 76, 144 154. http://doi.org/10.1016/j.ympev.2014.03.013

Sillero, N., & Carretero, M.A. (2013). Modelling the past and future distribution of contracting species. The Iberian lizard *Podarcis carbonelli* (Squamata: Lacertidae) as a case study. *Zoologischer Anzeiger A Journal of Comparative Zoology*, 252(3), 289-298. https://doi.org/10.1016/j.jcz.2012.08.004

Sindaco, R., & Jeremcenko, V.K. (2008). The reptiles of the Western Palearctic. Vol. 1: Annotated Checklist and Distributional Atlas of the Turtles, Crocodiles, Amphisbaenians and Lizards of Europe, North Africa, Middle East and Central Asia, Edizioni Belvedere, Italy, 580 pp.

Smith, M.A., & Green, D.M. (2005). Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? *Ecography*, 28(1), 110-128. https://doi.org/10.1111/j.0906-7590.2005.04042.x

Soberón, J. (2007). Grinnellian and Eltonian niches and geographic distributions of species. *Ecology Letters*, 10(12), 1115-1123. https://doi.org/10.1111/j.1461-0248.2007.01107.x

Soberón, J., & Peterson, A.T. (2005). Interpretation of models of fundamental ecological niches and species' distributional areas. *Biodiversity Informatics*, 2, 1-10. https://doi.org/10.17161/bi.v2i0.4

Sofaer, H.R., Jarnevich, C.S., Pearse, I.S., Smyht, R.L., Auer, S., Cook, G.L., …, & Hamilton, H. (2019). Development and Delivery of Species Distribution Models to Inform Decision-Making. *BioScience*, 69(7), 544-557. https://doi.org/10.1093/biosci/biz045

Sokolov, L.V. (2019). Data on the Distribution of the Crimean Wall Lizard, *Podarcis tauricus* (Pallas, 1814) (Sauria: Lacertidae), in the North-Western Black Sea Region (Ukraine). *Current Studies in Herpetology*, 19(3-4), 132-146 (in Russian). https://doi.org/10.18500/1814-6090-2019-19-3-4-132-146

Stănescu, F., Tudor, T., Székely, D., & Losif, R. (2013). Diversity and distribution of reptiles in Romania. *ZooKeys*, 341, 49-76. https://doi.org/10.3897/zookeys.341.5502

Stewart, J.R., Lister, A.M., Barnes, I., & Dalén, L. (2010). Refugia revisited: individualistic responses of species in space and time. *Proceedings of the Royal Society B: Biological Sciences*, 277, 661-671. https://doi.org/10.1098/rspb.2009.1272

Sudhaus, W., Kiontke, K., Fürst von Lieven, A., Manegold, A., & Seitz, V. (1997). Speziation in Mitteleuropa im Gefolge der Eiszeiten. *Sitzungsberichte der Gesellschaft Naturforschender Freunde zu Berlin (N.F.)*, 36, 143-175.

Taberlet, P., Fumagalli, L., Wust-Saucy, A.G., & Cosson, J.F. (1998). Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology*, 7(4), 453-464. https://doi.org/10.1046/j.1365-294x.1998.00289.x

The Bern Convention. (1979). Convention on the conservation of European wildlife and natural habitats, Bern. Appendix II: Strictly protected fauna species, Page 14.

The European Union Habitats Directive. (1992). Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. Annex IV: 4.2 Reptiles and amphibians, Page 41.

Thomas, C.D. (2010). Climate, climate change and range boundaries. *Diversity and Distributions*, 16(3), 488-495. https://doi.org/10.1111/j.1472-4642.2010.00642.x

Tok, C.V., & Çiçek, K. (2014). Amphibians and reptiles in the province of Çanakkale (Marmara Region, Turkey). *Herpetozoa*, 27(1-2), 65-76.

Tomovic, L., Timotijevic, M., Aitic, R., Krizmanic, İ., & Labus, N. (2018). Contribution to the herpetofauna of Serbia – Distribution of reptiles in Kosovo and Metohija Province. *The University Thought - Publication in Natural Sciences*, 2(8), 1-6. https://doi.org/10.5937/univtho8-16981

Uetz, P., & Hallermann, J. (2022). The reptile database, Zoological Museum Hamburg, Germany. Retrieved from https://reptile-database.reptarium.cz/species?genus=Podarcis&species=tauricus

Urošević, A., Ljubisavljević, K., Tomović, L., Krizmanić, I., Ajtić, R., Simović, A., Labus, N., & Dzukić, G. (2015). Contribution to the knowledge of distribution and diversity of lacertid lizards in Serbia. *Ecologica Montenegrina*, 2(3), 197-227. https://doi.org/10.37828/em.2015.2.26

Varela, S., Lima-Riberio, M.S., & Terribile, L.C. (2015). A short Guide to the Climatic Variables of the Last Glacial Maximum for Biogeographers. *Plos One*, 10(6), e0129037. https://doi.org/10.1371/journal.pone.0129037

Vicenzi, N., Corbalán, V., Miles, D., Sinervo, B., & Ibargüengoytía N. (2017). Range increment or range detriment? Predicting potential changes in distribution caused by climate change for the endemic high-Andean lizard *Phymaturus palluma*. *Biological Conservation*, 206, 151–160. http://doi.org/10.1016/j.biocon.2016.12.030

Waltari, E., Hijmans, R.J., Peterson, A.T., Nyári, A.S., Perkins, S.L., & Guralnick, R.P. (2007). Locating Pleistocene refugia: comparing phylogeographic and ecological niche model predictions. *Plos One*, 2(7), e563. https://doi.org/10.1371/journal.pone.0000563

Watanabe, S., Hajima, T., Sudo, K., Nagashima, T., Takemura, T., Okajima, H., Nozawa, T., …, & Kawamiya, M. (2011). MIROC-ESM 2010: model description and basic results of CMIP5-20c3m experiments. *Geoscientific Model Development*, 4(4), 845-872. https://doi.org/10.5194/gmd-4-845-2011

Wilson, R.J., Gutierrez, D., Gutierrez, J., & Monserrat, V.J. (2007). An elevational shift in butterfly species richness and composition accompanying recent climate change. *Global Change Biology*, 13(9), 1873-1887. https://doi.org/10.1111/j.1365-2486.2007.01418.x

Yang, W., Feiner, N., Pinho, C., While, G.M., Kaliontzopoulou, A., Harris, D.J., Salvi, D., & Uller, T. (2021). Extensive introgression and mosaic genomes of Mediterranean endemic lizards. *Nature Communications*, 12, 2762. https://doi.org/10.1038/s41467-021-22949-9

Yousefkhani, S.S.H., Rastegar-Pouyani, E., & Aliabadian, M. (2016). Ecological niche differentiation and taxonomic distinction between *Eremias strauchi strauchi* and *Eremias strauchi* kopetdaghica (Squamata: Lacertidae) on the Iranian Plateau based on ecological niche modeling. *Italian Journal of Zoology*, 83(3), 408–416. http://doi.org/10.1080/11250003.2016.1209581

Yu, H., Zhang, Y., Liu, L., Qi, W., Li, S., & Hu, Z. (2015). Combining the least cost path method with population genetic data and species distribution models to identify landscape connectivity during the late Quaternary in Himalayan hemlock. *Ecology and Evolution*, 5(24), 5781-5791. https://doi.org/10.1002/ece3.1840

Zakkak, S., Halley, J.M., Akriotis, T. & Kati, V. (2015). Lizards along an agricultural land abandonment gradient in Pindos Mountains, Greece. *Amphibia-Reptilia*, 36(3), 253-264. https://doi.org/10.1163/15685381-00003002

Zhang, Y., Clauzel, C., Li, J., Xue, Y., Zhang, Y., Wu, G., Giraudoux, P., & Li, D. (2019). Identifying refugia and corridors under climate change conditions for the Sichuan snub-nosed monkey (*Rhinopithecus roxellana*) in Hubei Province, China. *Ecology and Evolution*, 9(4), 1680-1690. https://doi.org/10.1002/ece3.4815

Supplemental Tables

Supplemental Table 1. Bioclimatic variables. Highlighted variables were used to create four sets of non-correlated variables.

Short name	Description	Unit
BIO1	Annual Mean Temperature	^{0}C
BIO2	Mean Diurnal Range (Mean of monthly max temp - min temp)	^{0}C
BIO3	Isothermality (BIO2/BIO7) (×100)	%
BIO4	Temperature Seasonality (standard deviation ×100)	^{0}C
BIO 5	Max Temperature of Warmest Month	^{0}C
BIO6	Min Temperature of Coldest Month	^{0}C
BIO7	Temperature Annual Range	^{0}C
BIO8	Mean Temperature of Wettest Quarter	^{0}C
BIO9	Mean Temperature of Driest Quarter	^{0}C
BIO10	Mean Temperature of Warmest Quarter	^{0}C
BIO11	Mean Temperature of Coldest Quarter	^{0}C
BIO12	Annual Precipitation	mm
BIO13	Precipitation of Wettest Month	mm
BIO14	Precipitation of Driest Month	mm
BIO15	Precipitation Seasonality (Coefficient of Variation)	%
BIO16	Precipitation of Wettest Quarter	mm
BIO17	Precipitation of Driest Quarter	mm
BIO18	Precipitation of Warmest Quarter	mm
BIO19	Precipitation of Coldest Quarter	mm

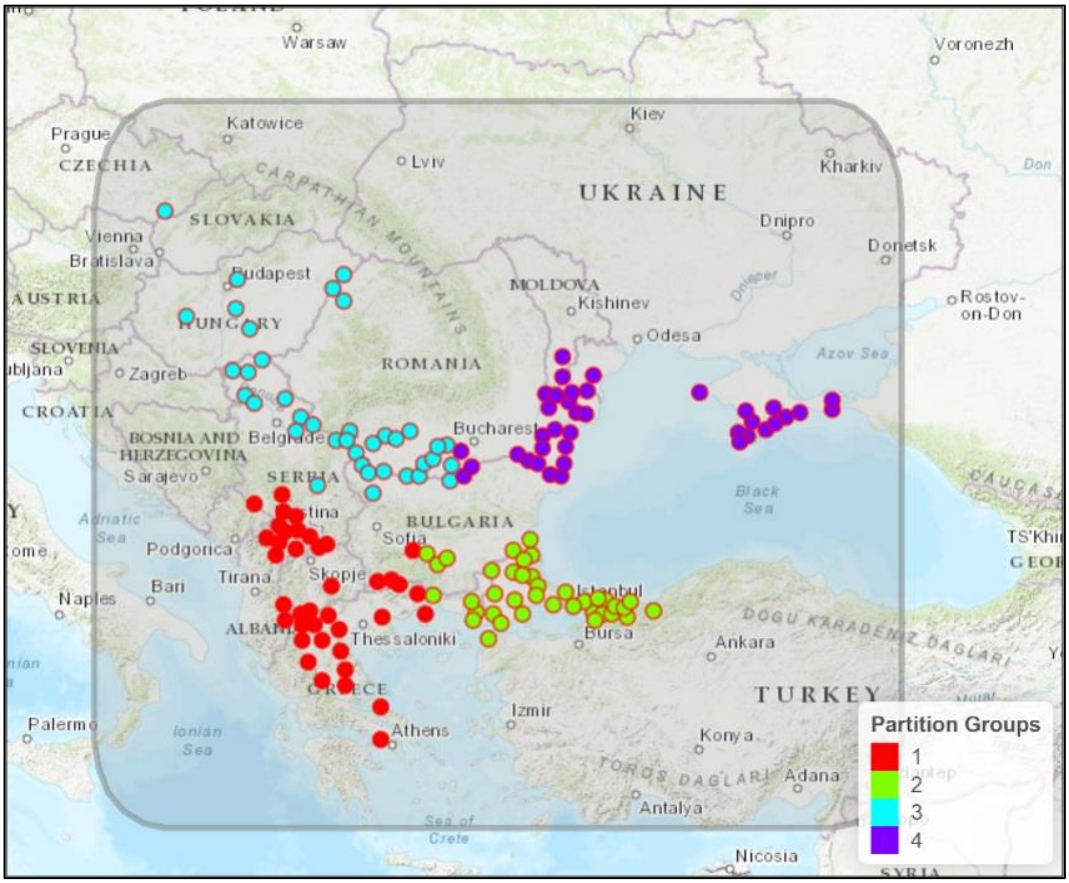
Supplemental Table 2. Correlation Matrix of Bioclimatic variables

	BIO1	BIO2	BIO3	BIO4	BIO10	BIO11	BIO12	BIO15	BIO16	BIO17
BIO1	1	0.15831	0.46289	0.49141	**0.90417**	**0.95238**	0.00596	0.47657	0.22899	0.45993
BIO2		1	0.76516	0.16014	0.08889	0.15429	0.17890	0.33977	0.05153	0.45400
BIO3			1	0.74147	0.16099	0.60212	0.18693	0.52871	0.33357	0.38805
BIO4				1	0.07716	0.72711	0.46817	0.42953	0.53480	0.10247
BIO10					1	0.73979	0.21423	0.32895	0.00378	0.47045
BIO11						1	0.17171	0.51910	0.36864	0.39375
BIO12							1	0.09192	**0.85977**	0.55185
BIO15								1	0.55066	0.72152
BIO16									1	0.11176
BIO17										1

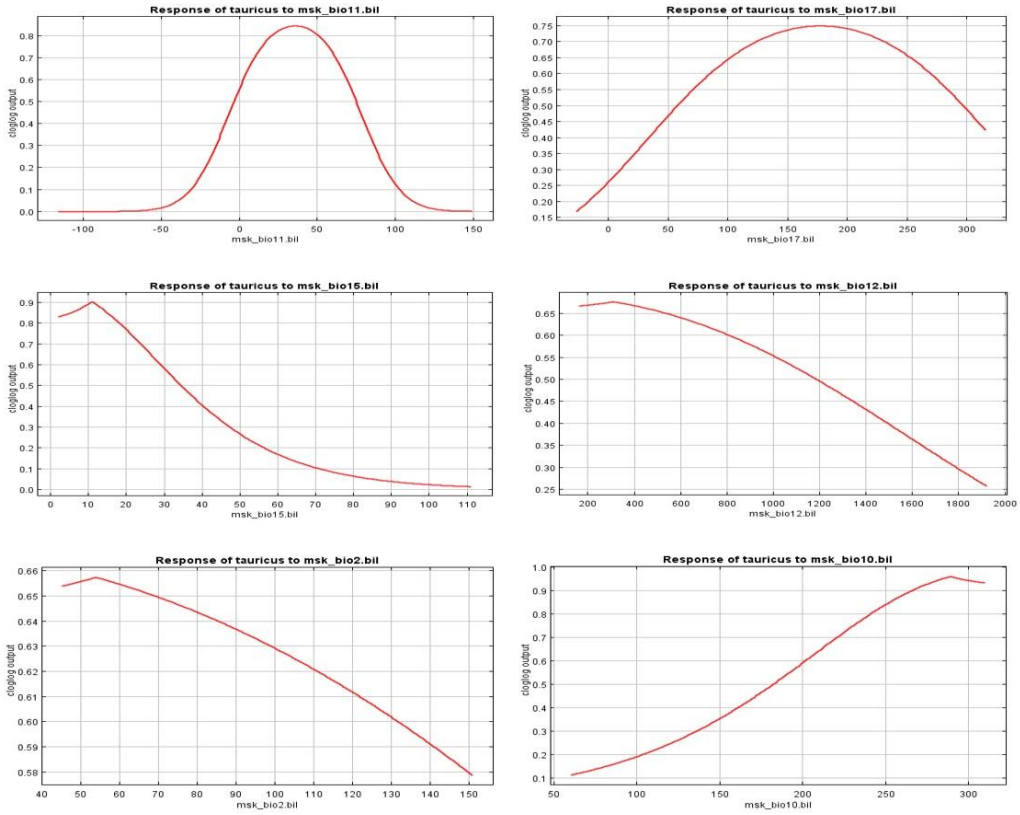
Supplemental Table 3. Four sets of non-correlated (r ≤|0.80|) bioclimatic variables.

Bioclimatic Sets	1	2	3	4
Bioclimatic variables	BIO1	BIO1	BIO2	BIO2
	BIO2	BIO2	BIO3	BIO3
	BIO3	BIO3	BIO4	BIO4
	BIO4	BIO4	BIO10	BIO10
	BIO12	BIO15	BIO11	BIO11
	BIO15	BIO16	BIO12	BIO15
	BIO17	BIO17	BIO15	BIO16
			BIO17	BIO17

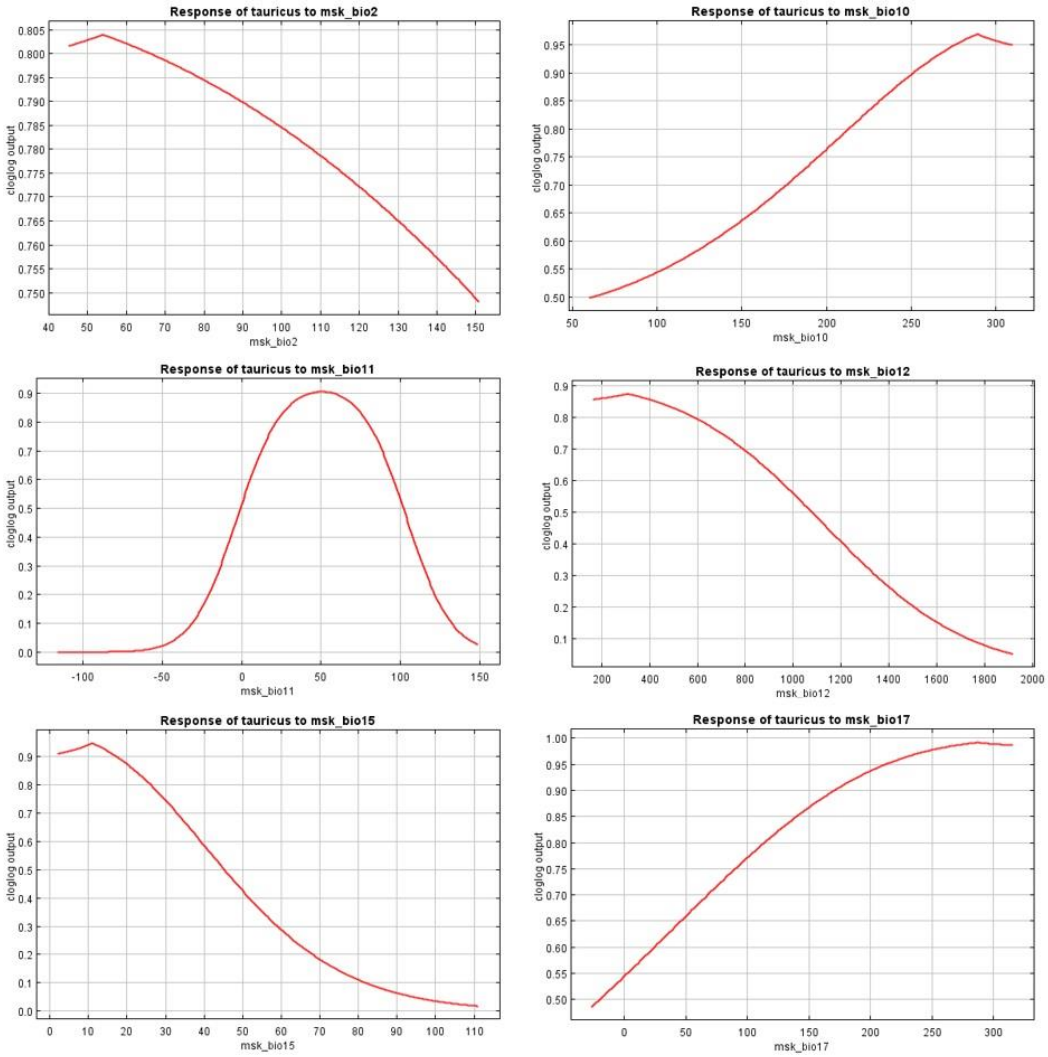
Supplemental Figures



Supplemental Figure 1. The bounding box polygon from the presence records (circles) to which a 2-degree buffer (corresponds to 15.25° to 38.33° E and 36.08° to 50.92° N) was applied and the presence data partitioned into four bins of equal numbers.



Supplemental Figure 2. The univariate response curves.



Supplemental Figure 3. The marginal response curves.

Supplemental Figure 4. The results of the jackknife test of variable importance.

Maxent Lambdas File

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[2] "bio11.bil, 10.39136031319209, -94.0, 127.0"
[3] "bio12.bil, 0.0, 309.0, 1773.0"
[4] "bio15.bil, -3.185050864237784, 11.0, 102.0"
[5] "bio17.bil, 1.7986466238465624, 2.0, 287.0"
[6] "bio2.bil, 0.0, 54.0, 142.0"
[7] "bio3.bil, 0.0, 19.0, 43.0"
[8] "bio4.bil, 0.0, 4730.0, 10226.0"
[9] "bio10.bil^2, 1.2888186807473745, 6561.0, 83521.0"
[10] "bio11.bil^2, -7.4885622396314755, 0.0, 16129.0"
[11] "bio12.bil^2, -3.1424326881260245, 95481.0, 3143529.0"
[12] "bio15.bil^2, -1.289763940255604, 121.0, 10404.0"
[13] "bio2.bil^2, -0.1389729264813303, 2916.0, 20164.0"
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[15] "densityNormalizer, 4615.114023936132"
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[17] "entropy, 9.835222031250549"
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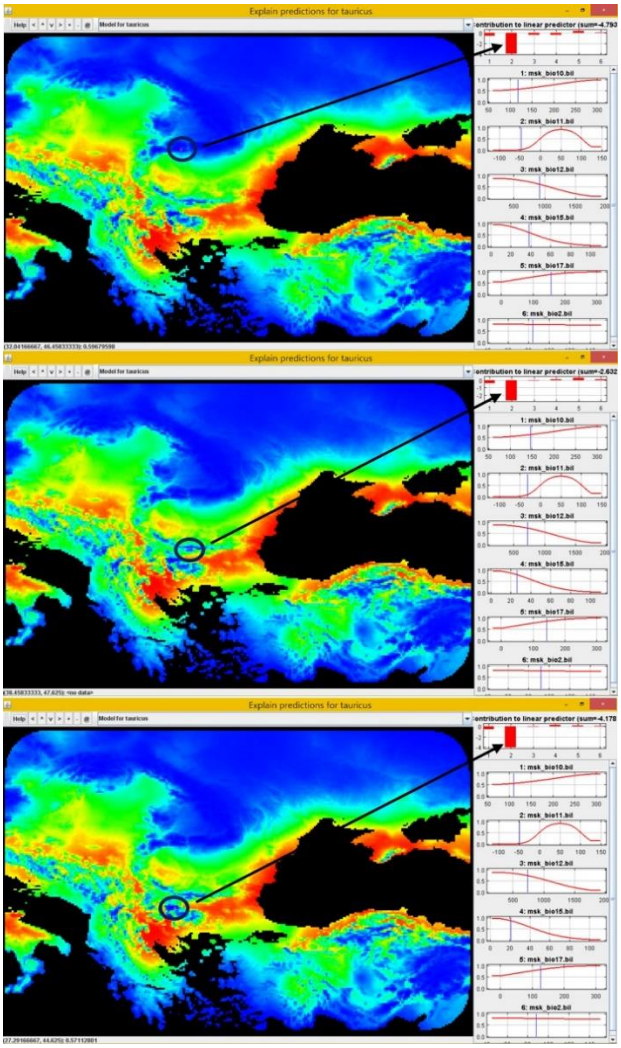
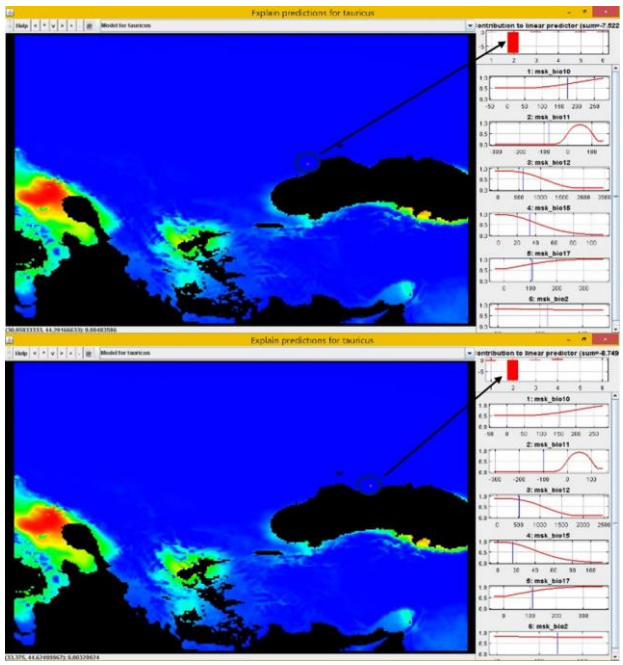
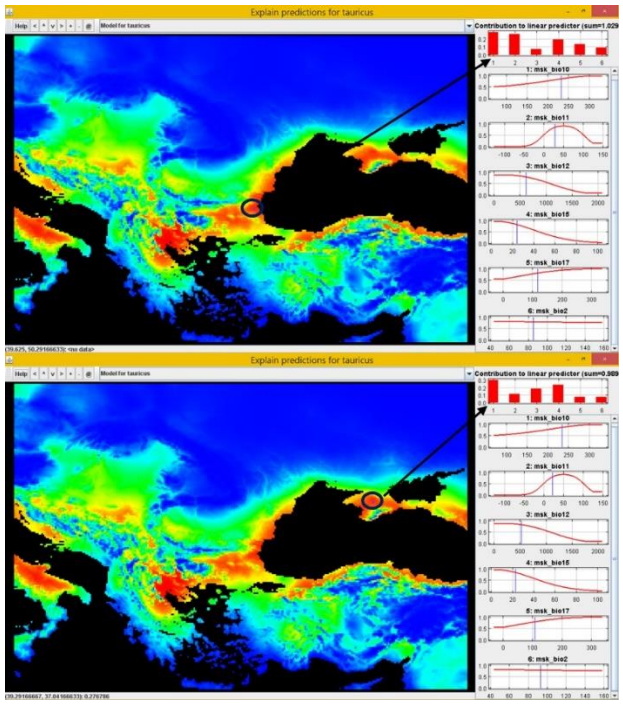
Supplemental Figure 5. The coefficients of the final model.



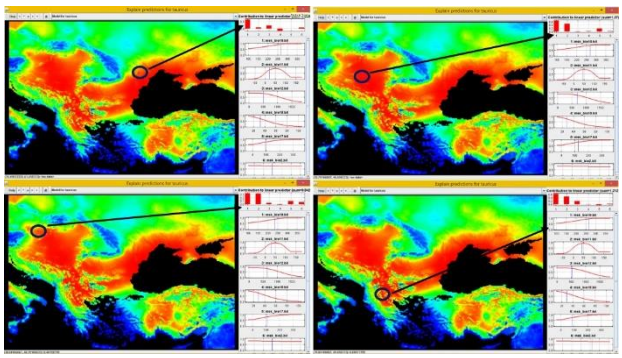
Supplemental Figure 6. Explain tool from Maxent. Warm map colors represent high and cold colors represent low habitat suitability for the Balkan wall lizard (*Podarcis tauricus*) under present conditions. The effect of variables is explored at point locations in Eastern Europe. Very low suitabilities in these regions were driven by BIO11.



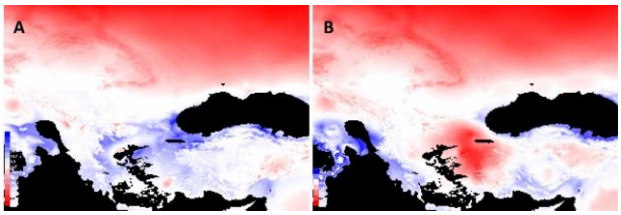
Supplemental Figure 7. Explain tool from Maxent. Warm map colors represent high and cold colors represent low habitat suitability for the Balkan wall lizard (*Podarcis tauricus*) under LGM conditions (CCSM4 model). The effect of variables is explored at northern locations. Very low suitabilities in these regions were driven mainly by BIO11.



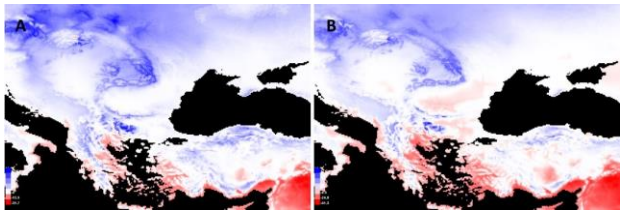
Supplemental Figure 8. Explain tool from Maxent. Warm map colors represent high and cold colors represent low habitat suitability for the Balkan wall lizard (*Podarcis tauricus*) under Mid-Holocene conditions (CCSM4 model). The bioclimatic suitability has shown more northern latitudes due to the positive effects of the bioclimatic variables with changing contribution from one pixel to the other.



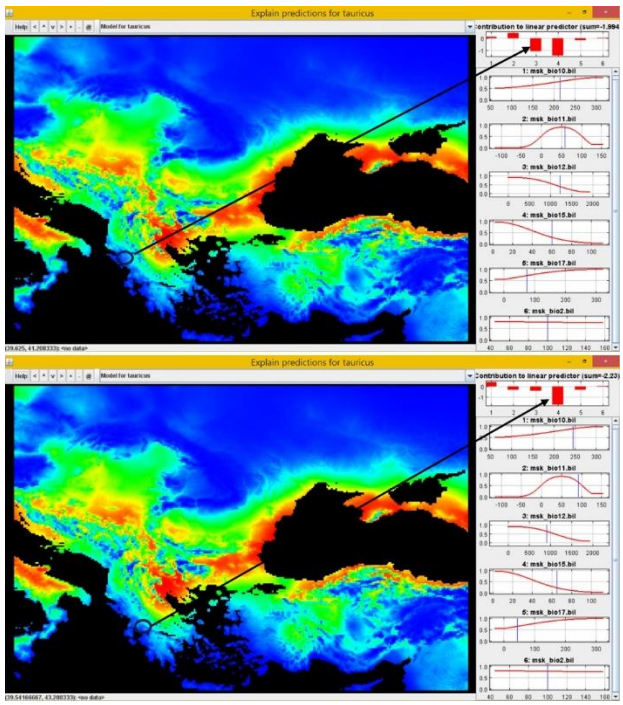
Supplemental Figure 9. Explain tool from Maxent. Warm map colors represent high and cold colors represent low habitat suitability for the Balkan wall lizard (*Podarcis tauricus*) under future conditions (MIROC-ESM, RCP8.5, 2070). The effect of variables is explored at northern locations. Very high suitabilities were driven mainly by BIO10 and BIO11.



Supplemental Figure 10. Multivariate environmental similarity surface (MESS) analysis for LGM for the Balkan wall lizard (*Podarcis tauricus*), A: for CCSM4 and B: for MIROC-ESM models. Cells shown in red indicate areas for at least one environmental variable value occurs outside the range of values in the training region. In both, some pixels in pink around Thessaloniki were highly predicted.



Supplemental Figure 11. Multivariate environmental similarity surface (MESS) analysis for 2070 for the Balkan wall lizard (*Podarcis tauricus*), A: for RCP4.5 MIROC-ESM and B: for RCP8.5 MIROC-ESM models. Cells shown in red indicate areas for at least one environmental variable value occurs outside the range of values in the training region. In both, some pixels in pink around Thessaloniki were highly predicted.



Supplemental Figure 12. Explain tool from Maxent. Warm map colors represent high and cold colors represent low habitat suitability for the Balkan wall lizard (*Podarcis tauricus*) under present conditions. Very low suitability on the western part of Pindus Mountains were driven mainly by BIO12 and BIO15.

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Research Article/Araştırma Makalesi

Helminth Fauna of Dwarf Lizards, *Parvilacerta parva* (Boulenger, 1887), Collected from Sivas and Van Provinces, Türkiye

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Abstract: In this study, Dwarf Lizard (*Parvilacerta parva*) is collected in June 2016, 30 (6 females, 24 males) from Ulaş district of Sivas province and 12 (6 females, 6 males) from Çaldıran district of Van province in May 2011 and August 2012 were investigated for helminth parasites. A total of 17 *Oochoristica tuberculata* (Cestoda) specimens were found in 8 of 42 dwarf lizards. *O. tuberculata* is a common cestode species in lizards.

It is the second helminth study carried out on dwarf lizards in our country and *O. tuberculata* is a new record for the host Dwarf Lizard (*P. parva).*

Keywords: Türkiye, parasite, cestoda, *Oochoristica tuberculata*.

Sivas ve Van'dan Toplanan Cüce Kertenkelelerin (*Parvilacerta parva*) Boulenger, 1887 Helmint Faunası

Öz: Müze materyali 42 Cüce Kertenkele Haziran 2016 Sivas ili Ulaş ilçesinden 30 adet (6 dişi, 24 erkek), Mayıs 2011 ve Ağustos 2012 tarihinde Van ili Çaldıran ilçesinden 12 adet (6 dişi, 6 erkek) helmint parazitleri açısından incelenmiştir. Cüce kertenkelelerin 8'inde toplam 17 *Oochoristica tuberculata* (Cestoda) örneği tespit edilmiştir. *O. tuberculata* kertenkelelerde yaygın bulunan bir cestod türüdür.

Yurdumuzda Cüce Kertenkeleler üzerinde gerçekleştirilen 2. helmint çalışması olup, *Oochoristica tuberculata* konak kertenkele için yeni kayıttır.

Anahtar kelimeler: Türkiye, parazit, cestod, *Oochoristica tuberculata.*

1. Giriş

Parvilacerta parva (Cüce Kertenkele) ilk kez 1887 yılında Boulenger tarafından tanımlanmıştır. Türkiye'de İç Anadolu ve Doğu Anadolu'da yayılış gösterirken, ülkemiz dışında ise Ortadoğu'da görülmektedir. *Lacertidae* familyasına ait olan *P. parva,* taşlık, kayalık ve bozkır alanlarda yaşamayı tercih eder. Nisan–Eylül ayları arasında faaliyet gösterir (Baran et al., 2012).

Parazitik yaşam, parazit yaşayan organizmaların birbirleriyle ve çevreleriyle olan bağlantılarını gözler önüne seren bir yaşam biçimidir. İşte bu nedenle ülkemizde ve dünyanın birçok yerinde ekonomik olarak değeri olan ya da olmayan tüm hayvanların parazitleri üzerine çalışmalar yapılmıştır. Sürüngen helmintleri üzerine yapılan araştırmalarda, helmintlerin gösterdikleri büyük çeşitlilik, bu tür çalışmalar için teşvik edici olmaktadır (Gupta et al., 2009).

Türkiye'de 66 kertenkele türü bulunmaktadır (Baran et al., 2012; Uetz et al., 2015). Bunlardan sadece 32 tür helmintolojik açıdan incelenmiştir. Konakların bu geniş çeşitliliği arasında, Türkiye'nin bazı bölgelerinde kertenkelelerin endoparazitleri hakkında daha az bilgi bulunmaktadır. Bu durum, bu parazitler ile konakları arasındaki ilişkiyi anlamayı güçleştirmektedir.

Türkiye'de, Lacertidae ailesine ait 40 kertenkele türü yaşamaktadır. Helmint faunası açısından incelenen türler; *Acanthocdatylus harranensis, A. schreiberi, Mesalina brevirostris* (Düşen et al., 2016), *Anatololacerta anatolica* (Yıldırımhan et al., 2020a), *A. danfordi* (Gürelli et al., 2007), *Apathya cappadocica* (Birlik et al., 2015), *D. clarkorum, D. raddei, D. parvula, D. valentini, D. armeniaca, D. unisexualis* (Roca et al., 2016), *D. valentini* (Birlik et al., 2018a), *D. rudis* (Roca et al., 2015a; Birlik et al., 2018b; Yıldırımhan et al., 2020a), *D. uzzelli, D. bendimahiensis, D. sapphirina* (Roca et al., 2015b), *Eremias pleskei, E. strauchi, E. suphani* (Düşen et al., 2013), *Iranolacerta brandtii* (Birlik et al., 2017), *Lacerta trilineata* (Yıldırımhan et al., 2011), *L. viridis* (Schad et al., 1960; Yıldırımhan et al., 2020b), *Ophisops elegans* (Yıldırımhan & Sümer, 2019), *Parvilacerta parva* (Saygı & Olgun, 1993), *Phoenicolacerta laevis* (Birlik et al., 2016), *Podarcis muralis* (Yıldırımhan & Sümer, 2019), *P. siculus* (Yıldırımhan & Sümer, 2019) ve *P. tauricus* (Schad et al., 1960).

Yapılan literatür taramasında, günümüze kadar Cüce Kertenkele endoparazitleri hakkında yapılmış tek bir çalışma olduğu tespit edilmiştir. Saygı ve Olgun (1993) tarafından yapılan çalışmada Sivas'tan toplanan 25 *Parvilacerta parva* örneği incelenmiştir. Bunların 20 tanesinin *Spauligodon* türü helmintler tarafından enfekte olduğu tespit edilmiştir. Tespit edilen bu parazit türü, Türkiye'den ilk kez rapor edilmiştir.

Ülkemizdeki kertenkelelerin helmint faunasının tam anlamıyla ortaya konmamış olmasından dolayı ve

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parazitolojik çalışmaların az yapıldığı Cüce Kertenkelenin helmintlerinin çalışılmasına karar verilmiştir.

Bu çalışma ile *Parvilacerta parva*'da bulunan helmint tür veya türlerinin belirlenmesi, Türkiye faunasına katkı sağlanması ve ileride yapılacak olan diğer çalışmalara referans olunması amaçlanmıştır.

2. Materyal ve Metot

2.1. Materyal

Parvilacerta parva, (BOULENGER, 1887) (Cüce Kertenkele)

P. parva'ya ait 30 birey (6 ♀♀, 24 ♂♂) Sivas'ın Ulaş ilçesinden, 12 birey Van'ın Çaldıran (6 ♀♀, 6 ♂♂) ilçesinden toplanmıştır. Adıyaman Üniversitesi, Zooloji Müzesi'ne kayıtlı örnekler Uludağ Üniversitesi Fen Edebiyat Fakültesi Biyoloji Bölümü Parazitoloji Laboratuvarına getirilmiş ve dissekte edilmişlerdir. Örneklerle ilgili bilgiler disseksiyon öncesinde kaydedilmiştir.

2.2. Genel Bilgiler

Cüce Kertenkele, vücut boyu 10-14 cm olan küçük bir kertenkeledir. Sırt taraf grimsi veya açık kahverengi, siyah beyaz lekelidir. Gövde yanlarında da benzer şekilde lekeler bulunur. Alt taraf erkeklerde sarı veya beyaz, dış ventral üzerinde genellikle mavi veya yeşil lekeler bulunur. Kuyruk veya bacak altları pembemsidir. Dişilerde alt taraf genellikle beyaz, bazen sarı renkli, yanlarda mavi lekeler bulunmaz (Şekil 1).

Az bitkili ve kurak yüksek steplerde, taşlık ve toprak zeminli kısımlarda yaşar. Taş yığınlarının aralarında gizlenir. Besinlerini böcekler, solucanlar ve örümcekler oluşturur. Senede 3 kez yumurta bırakabilir. Bir dişi tek seferde 2- 5 yumurta bırakabilir. Bu tür Türkiye ile Ermenistan arasında yayılmıştır. Vertikal dağılışı 800-2000 metre arasında değişir. Türkiye'de Orta ve Doğu Anadolu Bölgesi'nde bulunur (Baran et al., 2012).

Şekil 1. *Parvilacerta parva*'nın genel görünüşü (Fotoğraf: M. Z. YILDIZ)

Figure 1. General view of *Parvilacerta parva* (Photo: M. Z. YILDIZ)

2.3. Yöntem

Aktif oldukları dönemde Sivas ve Van'dan toplanan kertenkeleler uygun büyüklükteki bez torbalar içerisinde uygun nem ortamı sağlanarak laboratuvar ortamına aktarılmıştır. Adıyaman Üniversitesi, Zooloji Müzesine kayıtlı örneklerimiz Araştırma döneminde müzeden alınarak çalışılmıştır. Diseksiyon işlemine başlamadan önce anteriorden anüs kısmına kadar olan standart uzunluk ve anteriorden kuyruk ucuna kadar olan total boy uzunluğu ölçüleri alınmıştır. Mumlu petriye alınan kertenkele ventral tarafı yukarı gelecek şekilde yerleştirilmiştir. Ardından anüs açıklığından anteriore kadar kesilerek iç organlar çıkarılmıştır. Gastrointestinal sistem incelenmek üzere ayrılmış ve diğer iç organlarda ayrı ayrı petrilere alınarak stereo mikroskop altında incelenmiştir. Ayrılan gastrointestinal sistem organları mumlu petride açılarak gergin biçimde petriye iğnelenmiş ve stereo mikroskop altında incelenmiştir. Yapılan incelemeler sırasında sadece cestoda sınıfından bulunan parazitler ve bulundukları organlar not edilmiştir. Bunlardan alınan iyi örnekler demirli asetokarmin ile boyanmış, entallan ile kapatılmıştır (Georgiev et al., 1986).

Parazitlerin teşhisleri Skrjabin (1951), Yamaguti (1959) ve Khalil et al. (1994)'e göre yapılmıştır.

Bu çalışma için canlı kertenkele kullanılmamış, müze örnekleri incelenmiştir. Bu sebeple etik kurul iznine gerek yoktur. Bu çalışma Kübra KIRIM'ın yüksek lisans tezinden hazırlanmıştır.

3. Bulgular

Helmintolojik araştırmamız Sivas ili Ulaş ilçesinden toplanmış 30 adet ve Van ili Çaldıran ilçesinden toplanmış 12 adet Cüce Kertenkele üzerinde yapılmıştır. 8 bireyde toplam 17 adet Cestoda sınıfına ait *O. tuberculata* türüne rastlanmıştır. 34 kertenkelede parazite rastlanmamıştır.

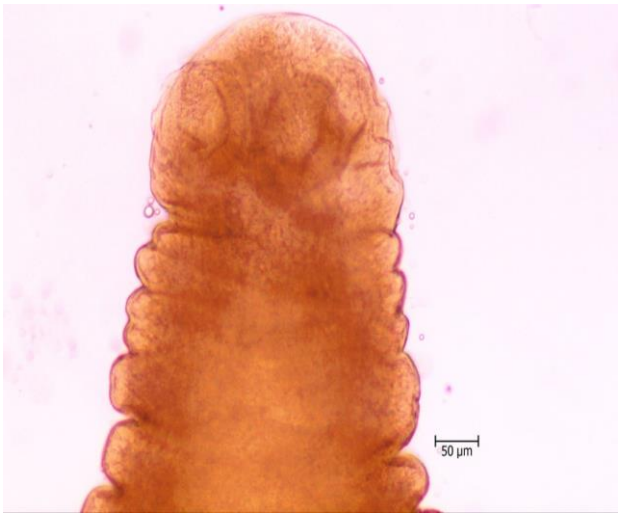
3.1. CESTODA

3.1.1. *Oochoristica tuberculata* (Rudolphi, 1819) Lühe, 1898

Sinonimleri: *Taeina tuberculata* Rudolphi, 1819; *T. rotundata* Molin, 1859; *T. pseudopodis* Krabbe, 1879.

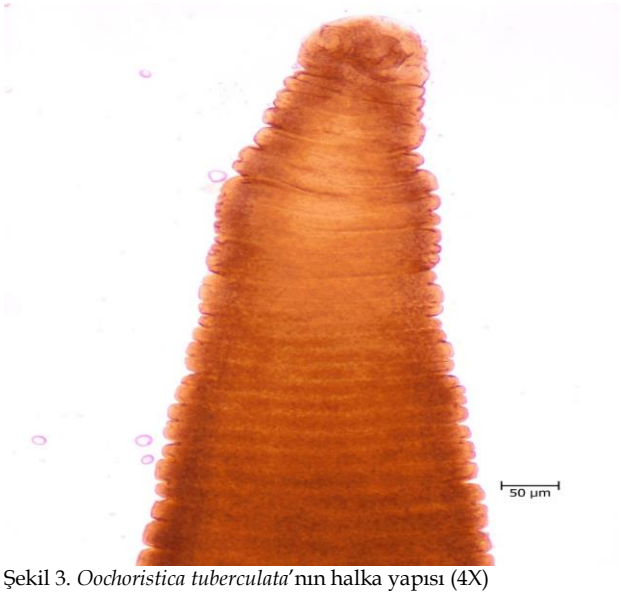
3.1.2. *Oochoristica tuberculata*'nın Morfolojik ve Anatomik Özellikleri

Vücut skoleks (Şekil 2), boyun ve halkalar (strobila)'dan oluşmuştur. Vücudun uzunluğu 2.5 – 20 cm'dir. Ön kısım dile benzer ve skoleksin gerisinde boynun bölgesinde daralma yoktur (Şekil 3). Vantuz seviyesinde skoleksin genişliği 304–424 (360) µm. Vantuz boyutları 116–148 (130) x 114–136 (124) µm'dir. Boynun uzunluğu 1040–2200 (1710) µm'dir.



Şekil 2. *Oochoristica tuberculata*'nın ön kısmı (10X)

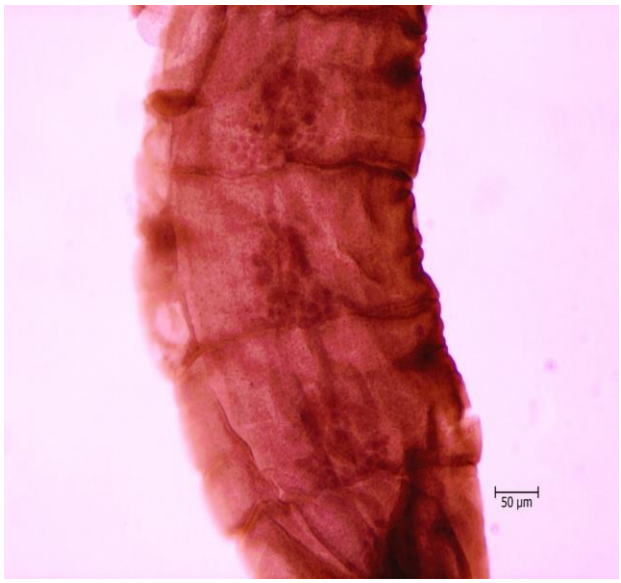
Figure 2. Anterior of *Oochoristica tuberculata* (10X)



Şekil 3. *Oochoristica tuberculata*'nın halka yapısı (4X)

Figure 3. Strobila of *Oochoristica tuberculata* (4X)

Boynun gerisinde başlayan halkalar boyuna yakın bölgelerde kare, posterior uç kısımlarda ise enine genişlemiş durumdadır (Şekil 4). Eşeysel açıklık sağlı ve sollu sıralanmış durumdadır. Testisler aynı büyüklükte ve vitellojen bezinin arkasında yerleşmiştirler. Sayıları 35–65 arasında değişmektedir. Eşeysel açıklık halkanın ilk 1/3'lük kısmında ve kas lifleri ile çevrelenmiştir. Gelişmiş olan ovaryum iki parçalı, poral kısım aporal kısımla hemen hemen aynı büyüklüktedir. Vitellojen bezi ovaryumun arkasında iki parçanın arasında yer almaktadır. Sirrus kesesi 200–280 (233) µm uzunluktadır. Ergin halkanın genişliği 584–1180 (840) µm'dir.



Şekil 4. *Oochoristica tuberculata* olgun halka yapısı (4X)

Figure 4. Mature strobila of *Oochoristica tuberculata* (4X)

4. Tartışma ve Sonuç

Günümüze kadar *Oochoristica* cinsine ait 85 tür tanımlanmıştır. Bu cinsin türleri arasındaki morfolojik varyasyonlar hakkında az şey bilinmektedir. Türe ait metrik parametrelerin, fiksasyon tekniklerine ve konakta bulunma yoğunluğuna bağlı olarak oldukça değişken olabileceği ifade edilmektedir (Schuster, 2011).

Oochoristica cinsine ait türleri tanımlamada kullanılan en temel özellikler; Skoleks yapısı, halkaların sayısı ve şekli, testislerin sayısı ve dağılımı ile yumurtalıkların pozisyonudur. Bu özellikleri Skrjabin (1951), Yamaguti (1959),ve Khalil et al. (1994),Groschaft ve Moravec (1983)'in *O. tuberculata* tanımlaması ile uyumluluk göstermektedir.

Kertenkelelerin bağırsaklarında yaşayan bu helminth türü kertenkeleler için zorunlu parazittir. Tip türü *Lacerta lepida*, (Rudolphi, 1819)'dır. Bulunduğu diğer türler *Acanthodactylus erythrurus* (Dollfus, 1958; Busack & Jaksic, 1982), *Agama agama* (Joyeux & Baer, 1928; Della Santa, 1956), *Chalcides ocellatus* (Della Santa, 1956; Groschaft & Moravec, 1983), *C. sexlineatus* (Lamas et al., 1985), *C. viridanus* (Roca et al., 1987), *Eumeces schneideri* (Baer, 1928), *Lacerta agilis* (Ivanitzky, 1940; Sharpilo et al., 2001), *L. ocellata* (Luhe, 1898), *L. viridis* (Della Santa, 1956), *Laudakia tuberculata* (Raina et al., 1975), *Mabuya carinata* (Della Santa, 1956), *Podarcis hispanicus* (Della Santa, 1956), *P. muralis* (Joyeux & Baer, 1936; Della Santa, 1956), *Psammodromus algirus* (Della Santa, 1956), *Pseudopus apodus* (Vakker et al., 1985), *Scincuss cincus* (Groschaft & Moravec, 1983), *Tarentola delalandii* (Roca et al., 1987), *Trapelus sanguinolenta* (Della Santa, 1956), *Uromastyx acanthinura* (Della Santa, 1956), *Varanus griseus* (Della Santa, 1956), *Cerastes vipera* (Dollfus, 1932), *Malpolon monspessulanus* (Joyeux & Gaud, 1945), *Psammophis sibilans* (Joyeux & Baer, 1928).

Türün Coğrafik dağılışı Avrupa, Kuzey Afrika ve Orta Asya bölgesidir (Dollfus, 1954; Della Santa, 1956).

Ülkemizde 7 tür kertenkelenin bağırsaklarında rastlanmıştır. Bunlar *Laudakia caucasia* (Yıldırımhan et al., 2006), *Lacerta trilineata* (Yıldırımhan et al., 2011), *Chalcides ocellatus* (İncedoğan et al., 2014), *Apathya cappadocica* (Birlik et al., 2015), *Acanthodactylus harrenensis* (Düşen et al., 2016), *Phoenicolacerta laevis* (Birlik et al., 2016), *Darevskia valentini* (Birlik et al., 2018)'dir. Cüce Kertenkelede ise ilk defa tespit edilmiştir.

Daha önce aynı bölgede Cüce Kertenkele üzerinde yapılan parazitolojik çalışmada 1 Nematod türüne rastlanmıştır. Spauligodon cinsine ait nematod örneklerin tür tanımlaması yapılamamış cins seviyesinde bırakılmıştır (Saygı ve ark. 1993). *Spauligodon* cinsi kertenkelelerde yaygın bulunan nematoddur. Ancak ne Sivas ne de Van örneklerimizde bu cinse rastlanmamıştır. *Oochoristica tuberculata* için Cüce Kertenkele yeni konak kaydıdır.

Çalışmamız sadece Ermenistan ve Türkiye'de yayılış gösteren Cüce Kertenkele'nin helminth faunasını ortaya çıkarmaya yöneliktir. Açılan örnek sayısının artırılması ve daha farklı alanlardan yeni örneklerin toplanması durumda farklı helmint türlerine de rastlamak muhtemeldir.

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Kaynaklar

Baer, J.G. (1928). Contribution A` La Faune Helminthologique De La Suisse. *Revue Suisse De Zoologie*, 35, 27–41.

Baran, I., Ilgaz, Ç., Avcı, A., Kumlutaş, Y., & Olgun, K. (2012). Türkiye Amfibi ve Sürüngenleri. Ankara, Tübitak Yayınları, 204 pp.

Birlik, S., Yıldırımhan, H.S., Sümer, N., Kumlutas, Y., Ilgaz, C., Guclu, O., & Durmus, S.H. (2015). The Helminth Fauna of *Apathya cappadocica* (Werner, 1902) (Anatolian Lizard) From Turkey. *Helminthologia*, 52(4), 310–315. https://doi.org/10.1515/helmin-2015-0049

Birlik, S., Yıldırımhan, H.S., Sümer, N., Kumlutaş, Y., Ilgaz, Ç., Durmuş, H., …, & Candan, K. (2016). Helminth Fauna of Lebanon Lizard, *Phoenicolacerta laevis* (Gray, 1838), (Squamata: Lacertidae) From Southern Turkey. *Helmintologia*, 53(3), 262-269. https://doi.org/10.1515/helmin-2016-0016

Birlik, S., Yıldırımhan, H.S., Kumlutaş, Y., Candan, K., & Ilgaz, Ç. (2017). The first Helminth Study on Brandt's Persian Lizard *Iranolacerta brandtii* (De Filippi, 1863) (Squamata: Lacertidae) from Van Province, Turkey. *Helminthologia*, 54(2), 174–178. https://doi.org/10.1515/Helm-2017-0021

Birlik, S., Yıldırımhan, H.S., Ilgaz, Ç., & Kumlutaş, Y. (2018a). Helminth Fauna of Valentin's Lizard *Darevskia valentini* (Boettger, 1892) (Squamata: Lacertidae) Collected From Central and Eastern Anatolia, Turkey. *Helminthologia*, 55(2), 134-139. https://doi.org/10.2478/helm-2018-0005

Birlik, S., Yıldırımhan, H.S., Ilgaz, Ç., & Kumlutaş, Y. (2018b). Helminth Fauna of Spiny Tailed Lizard, *Darevskia rudis* (Bedriaga, 1886) (Sauria: Lacertidae) From Turkey. *Helminthologia*, 55(1), 45-51. https://doi.org/10.1515/helm-2017-0057

Busack, S.D., & Jaksic, F.M. (1982). Autecological Observations of *Acanthodactylus erythrurus* (Sauria: Lacertidae) in Southern Spain. *Amphibia-Reptilia*, 3, 237–255.

Della Santa, E. (1956). Revision Du Genre *Oochoristica* Luhe (Cestodes). *Revue Suisse De Zoologie*, 63, 1–113. https://doi.org/10.5962/bhl.part.75445

Dollfus, R.P. (1932). Mission Saharienne Augie´Ras Draper 1927–1928. Cestodes De Reptiles. *Bulletin Du Museum National D'histoire Naturelle*, 4, 539–554.

Dollfus, R.P. (1954). Miscellanea Helminthologica Maroccana XVIII. Quelques Cestodes Du Groupe *Oochoristica auctorum* Re´Colte´S Au Maroc, Avec Une Liste Des Cestodes Des He´Rissons (Erinaceidae) Et Une Liste Des Sauriens Et Ophidians (Exclus. Ame´Rique Et Australie) Ou` Ont E´Te´ Trouve´S Des Oochoristica. *Archives Institut De Parasitologie*, 4, 654–714.

Dollfus, R.P. (1958). Deux Nouvelles Varietes *Oochoristica* Chez Les Sauriens D'afrique Du Nord (Cestoda, Linstowiidae). *Archives Institut Pasteur Du Alge*, 36, 32–40.

Düşen, S., Kumlutaş, Y., Ilgaz, C., Yaka, H., & Karadayı, F. (2013). Helminth Parasites of The Three Racerunner Lizards: *Eremias pleskei* Nikolsky, 1905 (Pleske's Racerunner-Transcaucasian Racerunner), *Eremias strauchi* Kessler, 1878 (Strauch's Racerunner) and *Eremias suphani* Basoglu and Hellmich, 1968 (Suphan Racerunner) Collected from Eastern Part of Turkey. *Helminthologia*, 50(2), 108–111. https://doi.org/10.2478/S11687-013-0117-3

Düşen, S., Kumlutaş,Y., Ilgaz, C., & Yakagul, C. (2016). A Helminthological Research on Three Lacertid Lizards Species: *Acanthodactylus harranensis* Baran et al., 2005, *Acanthodactylus schreiberi* Boulenger, 1878 and *Mesalina brevirostris* Blanford, 1874, Collected from South and South-Eastern Regions of Turkey. *Helminthologia*, 53(2), 200–206. https://doi.org/10.1515/Helmin-2016-0010

Georgiev, B.B., Biserkov, V.Y., & Genov, T. (1986). In toto staining method for cestodes with iron acetocarmine. *Helminthologia*, 23, 279–281.

Groschaft, J., & Moravec, F. (1983). Some Trematodes and Cestodes from Amphibians and Reptiles in Egypt. *Vestnik Ceskoslovenske Spollecnosti Zoologicke*, 47, 241–249.

Gupta, N., Bhaskar, M., & Gupta, D.K. (2009). Gastro-Intestinal Invasion in *Hemidactylus flaviviridis*with a New Species of *Parapharyngodon* (Oxyyuroidea: Pharyngodonidae). *Zootaxa*, 2165, 39-51. https://doi.org/10.11646/zootaxa.2165.1.3

Gürelli, G., Göçmen, B., Çetin-Doğan, T., & Alpagut-Keskin, N. (2007). First Record of *Mesocestoides* spp. Vaillant, 1863 Tetrathyridia (Cestoidea: Cyclophyllidae) in Anatolian Lizard, *Anatololacerta danfordi* (Günther, 1876) İn Turkey. *North Western Journal of Zoology*, 3(2), 96–104.

Ivanitzky, S.V. (1940). On The Helminth Fauna of Vertebrates in The Ukraine (Cestoda, Nematoda and Acanthocephala). *Sbornik Trudov*, 19, 129–155.

İncedogan, S., Yıldırımhan, H.S., & Bursey, C.R. (2014). Helminth Parasites of The Ocellated Skink, *Chalcides ocellatus* (Forskal,1775) (Scincidae) From Turkey. *Comparative Parasitology*, 81(2), 260–269. https://doi.org/10.1654/4708

Joyeux, C., & Baer, J.E. (1928). Cestodes. In: Joyeux, C., Gendre, E.and Baer, J.G. (ed) Recherches Sur Les Helminthes De L'afrique Occidentale Franc¸ Aise. Collection De La Socie´Te´ De Pathologie Exotique, 17–54.

Joyeux, C., & Baer, J.E. (1936). Cestodes. *Faune De France*, 30, 1–613.

Joyeux, C., & Gaud, J. (1945). Recherches Helminthologiques Marocaines. *Archives De L'institut Pasteur Du Maroc*, 3, 111–143.

Khalil, L.F., Jones, A., & Bray, R.A.(1994). Keys to the Cestode Parasite of Vertebrates. USA, CAB international, 309-751 pp.Lamas, A.M.,Pez-Orge, R.H., Gonza Lez-Lama, Z.,Zapatero Ramos, L.,&Martınez-Fernandez, A.R. (1985). *Chalcides sexlineatus* Boettger, 1914, Nuevo Hospedador De *Oochoristica Tuberculata* LuHe, 1896. *Revista Ibe´Rica De Parasitology*,47, 263–270.

Lühe, M. (1898). *Oochoristica*nov. gen. *Taeniadarum* (Vorläufige Mittheilung). *Zoologischer Anzeiger*, 21, 650–652.

Raina, M.K., Chisti, M.Z., & Kaul, R.K. (1975). A New Report of *Oochoristica tuberculata* (Rudolphi, 1819) LuHe, 1898 from the İntestine of *Agama tuberculata*in Kashmir, with Remarks on its Synonyms. *Indian Journal of Helminthology*, 27, 1–4.

Roca, V., Garcia-Adell, G., Lopez, E., & Zapatero-Ramos, L.M. (1987). Algunas Formas Adultas Larvarias De Platelmintos De Reptiles De Las Islas Canarias. *Revista Ibe´Rica De Parasitology*, 47, 263–270.

Roca, V., Jorge, F., Ilgaz, Ç., Kumlutaş, Y., Durmuş, S.H., &Carretero, M.A. (2015a). The İntestinal Helminth Community of The Spiny-Tailed Lizard *Darevskia rudis* (Squamata, Lacertidae) From Northern Turkey. *Journal of Helminthology*, 90, 144-151. https://doi.org/10.1017/S0022149X14000911

Roca, V. Jorge F., Ilgaz, C., Kumlutas, Y., Durmus, S.H., & Carretero, M.H. (2015b). Are The Helminth Communities From Unisexual and Bisexual Lizards Different Evidence From Gastrointestinal Parasites of *Darevskia* spp. in Turkey. *Acta Zoologica Academiae Scientiarum Hungarie*, 61(3), 279-288. https://doi.org/10.17109/AZH.61.3.6.2015

Roca, V. Jorge F., Ilgaz, C., Kumlutas, Y., Durmus, S.H., & Carretero, M.H. (2016). Intestinal Parasites of Unisexual and Bisexual Lizards *Darevskia* spp. (Lacertidae) from Northeastern Anatolia. *Helminthologia*, 53(3), 298–303. https://doi.org/10.1515/Helmin-2016-0021

Rudolphi, C. (1819). *Entozoorum synopsis*, Cui Accedunt Mantissa Duplex Et Indices Locupletissimi. Berlin, Germany, Augusti Rucker, 811 pp.

Saygı, G., & Olgun, K. (1993). Sivas'ta Cüce Kertenkelelerde (*Lacerta parva)* Bulduğumuz Nematod: *Spauligodon* Cinsi. *Türkiye Parazitoloji Dergi*si, 17(1), 40-45.

Schad, G.A., Kuntz, R.E. & Wells, W.H. (1960). Nematode Parasites from Turkish Vertebrates. An Annotated List. *Canadian Journal of Zoology*, 38, 949-963. https://doi.org/10.1139/z60-101

Schuster, R.K. (2011). *Oochoristica chalcidesi* n. sp. (Eucestoda: Linstowiidae) from the ocellated skink, *Chalcides ocellatus* (Forskal, 1775) in the United Arab Emirates. *Journal of Helminthology*, 85, 468–471.

Sharpilo, V.P., Bıserkov, V., Kostadınova, A., Behnke, J.M., & Kuzmın, Y.I. (2001). Helminths of the Sand Lizard, *Lacerta agilis* (Reptilia, Lacetidae), in the Palaearctic: Faunal Diversity and Spatial Patterns of Variation in the Composition and Structure of Component Communities. *Parasitology*, 123(4), 389-400. https://doi.org/10.1017/S0031182001008587

Skrjabin, K.I. (1951). Essentials of Cestodology. Moscow, The Academy of Sciences of the USSR, 783 pp.

Uetz, P., Freed, P., & Hosek, J. (Eds) (2015). The Reptile Database. Retrieved from http://www. Reptile-Database.org (Erişim tarihi: 02.04. 2017)

Vakker, V.G., Brushko, Z.K., & Kolbintsev, V.G. (1985). Parasite Fauna of *Ophisaurus apodus*in the Kazakh S.S.R. Izvesiya Akademii Nauk Kazakhskoi S.S.R. *Seriya Biologichskaya*, 4, 36–39.

Yamaguti, S. (1959). Systema Helminthum: The Cestodes of Vertebrates. Vol. II,. London, England, İnterscıences Publishers, 166-332 pp.

Yıldırımhan, H.S., Goldberg, S.R., & Bursey, C.R. (2006). Helminth Parasites of the Caucasian Agama, *Laudakia caucasia*, and the Roughtail Rock Agama, *Laudakia stellio* (Squamata: Agamidae) from Turkey. *Comparative Parasitology*, 73(2), 257-262. https://doi.org/10.1654/4205.1

Yıldırımhan, H.S., Bursey, C.R., & Altunel, F.N. (2011). Helminth Parasites of the Balkan Green Lizard, *Lacerta trilineata* Bedriaga 1886, from Bursa, Turkey. *Turkish Journal of Zoology*, 35(4), 519-535. https://doi.org/10.3906/zoo-0910-1

Yıldırımhan, H.S., & Sümer, N. (2019). Studies on gastrointestinal helminth of three Lacertid Lizard species, *Podarcis muralis, Podarcis siculus* and *Ophisops elegans* (Sauria: Lacertidae) from Bursa, North-Western Turkey. *Helminthologia*, 56(4), 310-318. https://doi.org/10.2478/helm-2019-0030

Yıldırımhan, H.S., Sümer, N., & Bursey, C.R. (2020a). Helminth parasites of two Lacertid species, *Anatolacerta anatolica* (Werner, 1902) and *Darevskia rudis* (Bedriaga, 1886)(Sauria: Lacertidae) from Bursa Province, North-Western Turkey. *Acta Zoologica Bulgarica*, 72(2), 315-320.

Yıldırımhan, H.S., Karaman D., & Bursey, C.R. (2020b). Helminth Fauna of the European Green Lizard, *Lacerta viridis* (Laurenti, 1768), from Bursa, Turkey. *Comparative Parasitology*, 87(1), 56–67. https://doi.org/10.1654/1525-2647-87.1.56

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Research Article/Araştırma Makalesi

Determination of Botanical Origin and Mineral Content of Propolis Samples from Balveren (Şırnak) Beekeepers Accommodation Areas

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Abstract: Researches on bee products have become popular in recent years. In fact, the content and component of bee products varies depending on many ecological and floristic factors and its nutritional and therapeutic properties are directly related to its content. Balveren (Şırnak province) beekeepers place their hives in locations with different geographical structure, floristic and topographic characteristics. This variability not only affects the quality of honey but also changes the properties of propolis. Studies on propolis, known as bee glue, have gained importance in recent years. As with other bee products, the propolis content also depends on the floristic characteristics of the region. In this study, propolis samples were collected from the regions where Balveren beekeepers stayed and their botanical origins, wax ratios, phenolic content, and mineral substance contents were analyzed. In the microscopic analysis, pollen grains belonging to 14 different families used by bees were determined. It was determined that the total phenolic and mineral contents of propolis vary completely depending on the location. With this study, the propolis properties of the hives in the region were tried to be revealed and it was aimed that this study would help the region's propolis to be used for technological and therapeutic purposes.

Keywords: Bee, pollen, total phenolic, wax, ICP OES.

Balveren (Şırnak) Arıcılarının Konaklama Alanlarındaki Propolis Örneklerinin Botanik Kökeni ve Mineral İçeriğinin Belirlenmesi

Öz Arı ürünleri ile ilgili çalışmalar gün geçtikçe artmaktadır. Aslında arı ürünlerinin içeriği, bileşeni; ekolojik ve floristik birçok faktöre bağlı olarak değişkenlik göstermekte, besleyici ve tedavi edici özelliği ise içeriği ile doğrudan ilişkilidir. Balveren beldesi (Şırnak) arıcıları kovanlarını; coğrafik yapısı, floristik ve topografik özellikleri farklı lokasyonlara yerleştirmektedirler. Bu değişkenlik balın kalitesini etkilediği gibi propolis özelliklerini de değiştirmektedir. Kovan yapıştırıcısı olarak bilinen propolis ile ilgili çalışmalar son yıllarda önem kazanmaktadır. Diğer arı ürünlerinde olduğu gibi, propolis içeriği de bölgenin floristik özelliklere bağlıdır. Bu çalışmada Balveren arıcılarının konakladıkları bölgelerden propolis örnekleri toplanmış, botanik orijinleri, mum oranları, fenolik madde içerikleri ve mineral madde miktarları analiz edilmiştir. Yapılan mikroskobik analizlerde arıların kullandığı 14 farklı familyaya ait polen taneleri tespit edilmiştir. Fenolik madde ve mineral madde içeriklerinin ise tamamen propolis örneği alınan lokasyona bağlı olarak değişkenlik gösterdiği tespit edilmiştir. Bu çalışma ile bölgede bulunan kovanların propolis özellikleri ortaya konulmaya çalışılmış ve yapılan çalışmanın bölge propolislerinin teknolojik ve tedavi edici amaçlar ile kullanılmasına yardımcı olması hedeflenmiştir.

Anahtar kelimeler: Arı, Polen, toplam fenolik, balmumu, ICP OES.

1. Introduction

Anatolia has a rich vegetation due to the factors such as its geographical structure, climatic characteristics, extreme microclimate area, and topographic structure. In addition, with its plant diversity/biological richness, it is a natural habitat for many living species (Davis, 1971). In this context, due to its natural structure and depending on its geographical diversity, beekeeping in Anatolia has been practiced by the people using traditional and modern techniques for many years (Üreten, 2011; Şenoğlu Fenerci, 2021).

Bees generally collect products for honey from different parts of the plants. These attractants are usually lipophilic substances found in flowers, leaves, leaf buds, mucus, gums, resins, and similar substances (Crane, 1999; Bankova et al., 2014). Propolis is known as a resinous, fragrant mixture obtained by bees by mixing flowers, pollen, buds, and other plant products with their own salivary enzymes and metabolites (Anjum et al., 2019; Sforcin, 2016). In addition, propolis is a part of the protection mechanisms of the hives and is a mixture of plant resin and wax. This product enables bees to reduce disease and/or parasite effects due to its antimicrobial and antiseptic properties (Simone-Finstrom et al., 2017; Saelao et al., 2020). Propolis is also known as bee glue, which is used to mummify dead bees and to eliminate a potential source of microbial infection (Guzmán-Gutiérrez et al., 2018).

The content of propolis can originate from different plant species; thus, the type and amount of the content

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vary widely around the world. The specificity of the flora determines the chemical composition of propolis (Bankova et al., 2014). Volatile components give propolis a uniquely pleasant aromatic odor and contribute to its biological activity. Generally, raw propolis consists of 50% resin, 30% beeswax, 10% essential oils-balsams, 5% pollen, and 5% other organic compounds and minerals (Anjum et al., 2019). Propolis contains more than 500 components, including phenolic compounds (flavonoids, phenolic acids, and esters), fatty acids, sugars, minerals, and terpenoids (Kurek Gorecka et al., 2014; Kasote et al., 2017). There are many studies on the therapeutic properties of propolis as antibacterial (Sforcin et al., 2000), antifungal (Ota et al., 2001; Herrera et al., 2010), anti-inflammatory (Borrelli et al., 2002), anticancer (Sawicka et al., 2012), and antitumor (Oršolić & Bašić, 2003; Sobočanec et al., 2011; Bagatir et al., 2022).

Within the scope of this study, appropriate propolis sampling was made and samples were collected from beekeepers registered in Balveren Town (Şırnak province-Turkey). The collected samples were examined in two stages: microscopic and chemical analysis. The vegetative origins of the pollen samples were determined by examining the propolis samples with a light microscope. In addition, propolis surfaces were examined by electron microscopy. For chemical analyses, the varying total phenolic contents of propolis samples were revealed. In addition, the wax ratios of the samples were obtained by obtaining propolis extracts and the mineral content and ratios in the samples were determined by using the ICP-OES device. Thus; in this study, it was aimed to determine the propolis characteristics and chemical properties in the region and also the plant families used as a source material.

2. Material and Methods

2.1. Collection of Propolis Samples and Determination of Botanical Origins

Propolis samples were taken from the hives with the support of beekeepers who have been dealing with beekeeping for many years in and around Balveren. For this purpose, propolis samples were collected on 27-30 August 2021, usually in the morning (08:00-10:00) from 12 different locations (Table 1). Approximately 300 g propolis samples were collected from each locality, labeled, and brought to the laboratory environment and analysis processes were started.

Table 1. Location of propolis samples

Sample	Location
1.	Kaval valley (Hakkari province)
2.	Balveren (Şırnak province)
3.	Kaval valley (Hakkari)
4.	Beytuşşsebap Gökce village (Şırnak province)
5.	Balveren (Şırnak province)
6.	Kaval valley (Hakkari province)
7.	Kaval valley (Hakkari province)
8.	Beytuşşsebap Gökce village (Şırnak province)
9.	Kaval valley (Hakkari province)
10.	Beytuşşsebap Dönmezler village (Şırnak province)
11.	Feraşin Valley (Şırnak province)
12.	Feraşin Valley (Şırnak)

2.2. Electron Microscopy Analysis

By forming extraction of propolis by ethanol, the colloidal mixture of propolis particles was dried in an oven at 40°C. The resulting dry extract was then suspended in ultrapure water to a concentration of about 1% w/v. The mixture was sonicated for 10 minutes to obtain a homogeneous suspension. Then, the dimensions and morphological properties of propolis particles were investigated using scanning electron microscopy (SEM) (Abdullah et al., 2019).

2.3. Preparation of Propolis Extracts

Pure propolis and wax (wax, fatty acid) were obtained by extraction for each propolis sample. The extraction method was carried out by modifying the methods of Cunha et al., 2004 and Negri et al., 2000. 3 g of each crude propolis sample was weighed and wrapped in filter paper, transferred to a 500 mL Soxhlet extractor (60°C). In the Soxhlet extractor, 750 mL of pure n-hexane (for wax extraction) and 750 mL of pure ethanol (for propolis extraction) were used. Extraction continued for 6 hours for each solvent. Filtering was done using Whatman filter papers. The resulting solution was evaporated on an evaporator to remove the solvent and the same procedure was repeated for each sample to yield pure propolis and wax samples. The initial weight and final weight of each sample were compared to determine the wax ratio.

2.4. Total Phenolic Substance Analysis

Folin-Ciocalteu (FCR) method was used for phenolic content. 1 mL of FCR reagent was added to the propolis extracts and incubated at room temperature for 3 minutes. Then, 1 mL of saturated Na_2CO_3 (7%) was added. After 90 minutes of incubation at room temperature in the dark, absorbance was taken at 760 nm wavelength. It was prepared with solutions of different concentrations of Gallic acid (0.05-1 mg/mL) as a standard and the results were calculated as gallic acid equivalents (Su et al., 2007).

2.5. Elemental Analysis of Propolis Samples

For the elemental analysis of the propolis samples, 0.6-1.0 g of the samples were weighed and solubilized with the help of a microwave. For this, the weighed samples were transferred to pressure-resistant polytetrafluoroethylene (PTFE) containers and after adding HNO_3/H_2O_2 (10.0/2.0) acid mixture, the digesting process was carried out in the Speedwave MWS-3 Berghof brand microwave oven under the conditions specified by Yüksel (2017). After the necessary procedures, elemental analysis was performed with Model Optima ™ 7000 DV ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer) (Perkin Elmer, Inc., Shelton, CT, USA).

3. Results and Discussion

3.1. Detection of Botanical Origins

Microscopic images of propolis from different localities and pollen grains found in plants were compared with each other. It was found to be compatible with pollens belonging to the families of Asteraceae, Fabaceae, Hypericaceae, Salicaceae, Anacardiaceae, Lamiaceae, Apiaceae, Brassicaceae, Juglandaceae, Malvaceae, Asteraceae, Boraginaceae, Asparagaceae, Caryophyllaceae, and Euphorbiaceae (Fig. 1). It has also been stated in previous

studies that Asteraceae, Fabaceae, Lamiaceae are the families most visited by honey bees (Perveen & Qaiser 2003; Özhatay et al., 2012; Özaltan & Kocyigit, 2022).

Pollen grains belonging to 14 different families were detected in propolis samples. The pollen analysis study actually reveals the vegetation and diversity of the region. In pollen studies, identification is a very difficult and complex process on the basis of species identification, although it is clear on the basis of families. For this reason, only family-based determination was made in pollen samples obtained from propolis samples. Depending on the biodiversity and the region of origin of the natural substance, propolis has a different chemical composition.

3.2. Electron Microscope Images

Electron microscope images were obtained in order to determine whether there were differences in pattern and texture in the propolis samples. As a result of the electron microscopy analysis, no significant structure, pattern or texture difference could be detected in the images of the propolis samples (Fig. 2).

There were no unusual compounds found during visual or electron microscopic investigation. Rugged surfaces hidden by wax and extractive layers can be seen in every shot. The properties of Bulgarian propolis samples were found to be similar (Tylkowski et al., 2010).

3.3. Wax Ratios of Propolis Samples

It is known that balsam, active ingredient, and wax contents of propolis vary considerably. Researchers found high amounts of balsam that is found to be caused by high amounts of phenolic compounds and low amounts of wax. Indeed, Popova et al. (2017) stated that raw propolis contains between 40% and 60% balsam (Popova et al., 2007). It is also reported to be between 27.7% (Bonvehí & Gutierrez, 2011). It is reported that the amount of wax found in raw propolis in different Portuguese propolis samples varies between 4.8% and 16.0% (Dias et al., 2012). In a study conducted on ethanolic extracts of crude propolis samples collected from Brazil, China, and Uruguay, it was stated that while there was no wax in Uruguay propolis extract, the amount of wax for other regions varied between 2.40% and 30.60% (Bonvehí & Coll, 1994).

In our study, wax rates varied between 19% and 63% (Table 2). This situation is considered to be caused by the plant origin and phenolic and tannin substances in its content.

In the wax analysis, it was determined that the lowest rate was in the propolis of the Feraşin region and the highest wax rate was from the village of Beytüşşebap Dönmezler. It is considered that this situation occurs depending on the plant diversity.

3.4. Total Phenolic Substance Contents

As a result of the total phenolic analysis, it was detected that one of the active substances that gives the functional properties of propolis is phenolic compounds. The phenolic content of the collected samples varies considerably.

As a result of our study, the highest three values among the phenolic values belonging to 12 different regions were 4. Locality; 99.46 µg /mL, Locality 9; 1 11.3 µg /mL, and 7th Locality; 77.46 µg /mL. These values seem to be significant as they are quite high compared to other studies (Fig. 3). Ethanol, water and olive oil were used for extraction of Lithuania propolis samples and according to phenolic analysis; 12.7 /1.6 and 0.5 mg/mL GAE values were obtained respectively (Maden, 2013). Bonvehí & Gutierrez (2011) found that ethanol and propylene glycol extracts had total phenol content of 21 to 34 g/100 g and 20 to 30.3 g/100 g, respectively. It is estimated that the difference between the total phenolic compounds of propolis samples in 70 different places may be due to the geographical location and climatic characteristics.

The biological activities of propolis, such as its antioxidant and antibacterial properties, are dependent on its phenolic compounds. Numerous studies have shown that propolis type, origin, raw materials, and extraction techniques all affect changes in the chemical composition of propolis. It has been reported that the total amount of phenolic in Anatolian propolis ranges from 10.6-178 mg GAE/g and the amount of total phenolic increases as the amount of balsam increases (Keskin & Kolaylı, 2018). Aliyazicioğlu et al. (2013) reported that the total phenolic content for different Turkish propolis samples ranged from 115 to 210 mg GAE/g (Li et al., 2008). The total phenolic content of chestnut propolis was reported to range from 1.2 to 15.6 mg/g (Sarıkaya et al., 2009). It is clear that the total phenolic content of propolis samples obtained from different regions of Turkey varies in a wide range.

In the phenolic substance analysis, it was determined that there was variability in the samples taken from the Kaval valley. It was seen that the highest rates belonged to the Gökçe village of Beytüşşebap. This situation is considered to be caused by the source that the bees preferred for propolis.

3.5. Mineral Substance Contents

The presence of minerals, which are a natural part of terrestrial systems, can significantly affect the pharmacotherapeutic properties of derived products. It is important to know the essential mineral content of propolis that has a nutritional supplement or healing effect. The role of macroelements in development is well known. In addition, the mineral content in propolis gives more specific results regarding the condition of the region because pollen and propolis are much less processed by bees than beeswax and honey and more precisely reflect environmental contamination (Formicki et al., 2013). In this context, mineral substance values in our study vary considerably depending on the location. These variations occur especially in Ca, Mg, and Zn values (Table 3).

There is a close relationship between the level of heavy metals accumulated in the soil and plants and their content in bee products (Kabata-Pendias, 2011). Propolis is much more contaminated with toxic elements than polyfloral honey but both can be used as a bioindicator to assess the extent of environmental pollution by determining the level of accumulated toxic elements (Roman et al., 2011). Such elements, even in low concentrations, can cause many diseases and abnormalities in the functioning of the human organism.

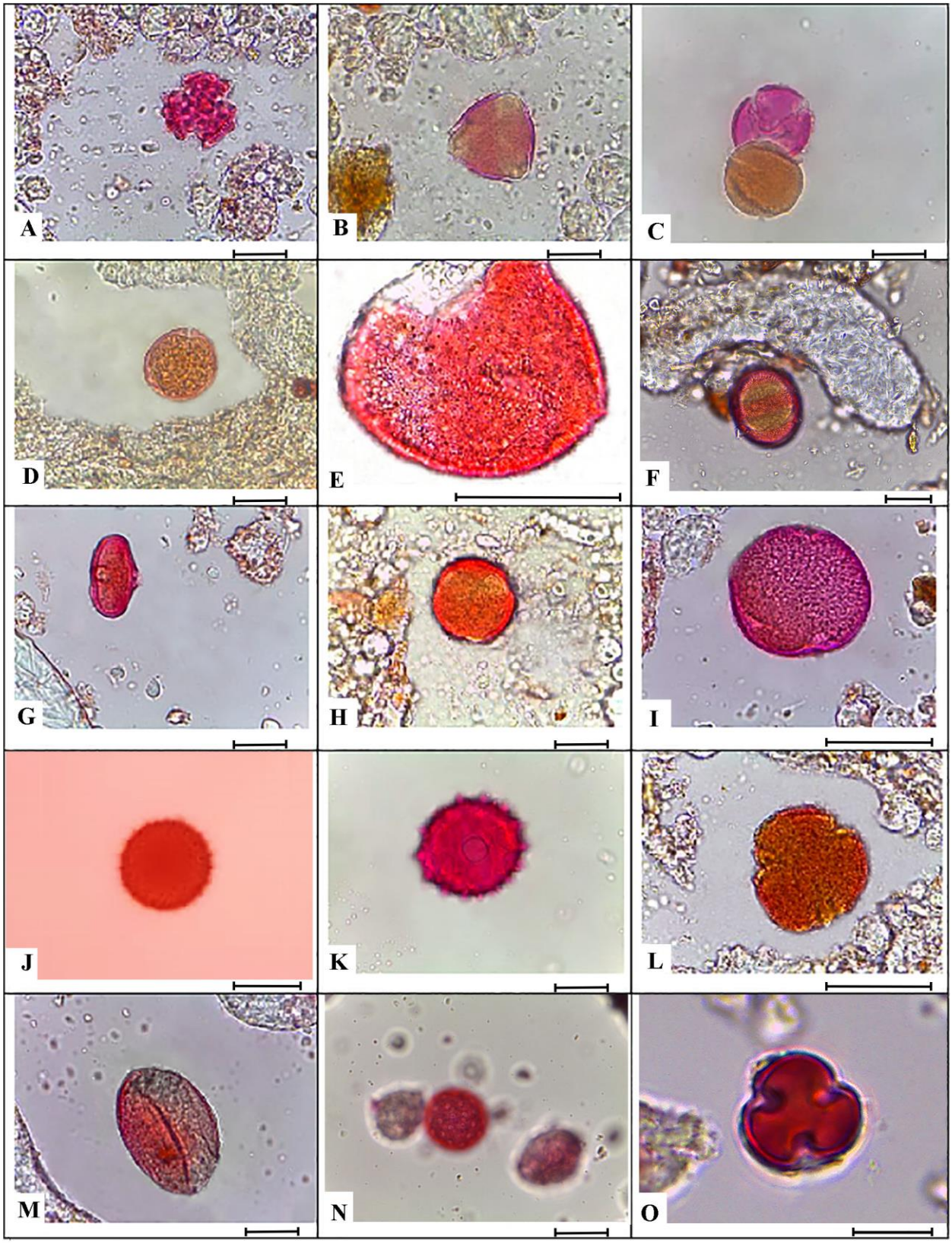

Figure 1. Pollen grains found in propolis samples (Bar : 20 µm)

A- Asteraceae, B- Fabaceae, C- Hypericaceae, D- Salicaceae, E- Anacardiaceae, F- Lamiaceae, G- Apiaceae, H- Brassicaceae, I- Juglandaceae, J- Malvaceae, K- Asteraceae, L- Boraginaceae, M- Asparagaceae, N- Caryophyllaceae, O- Euphorbiaceae.

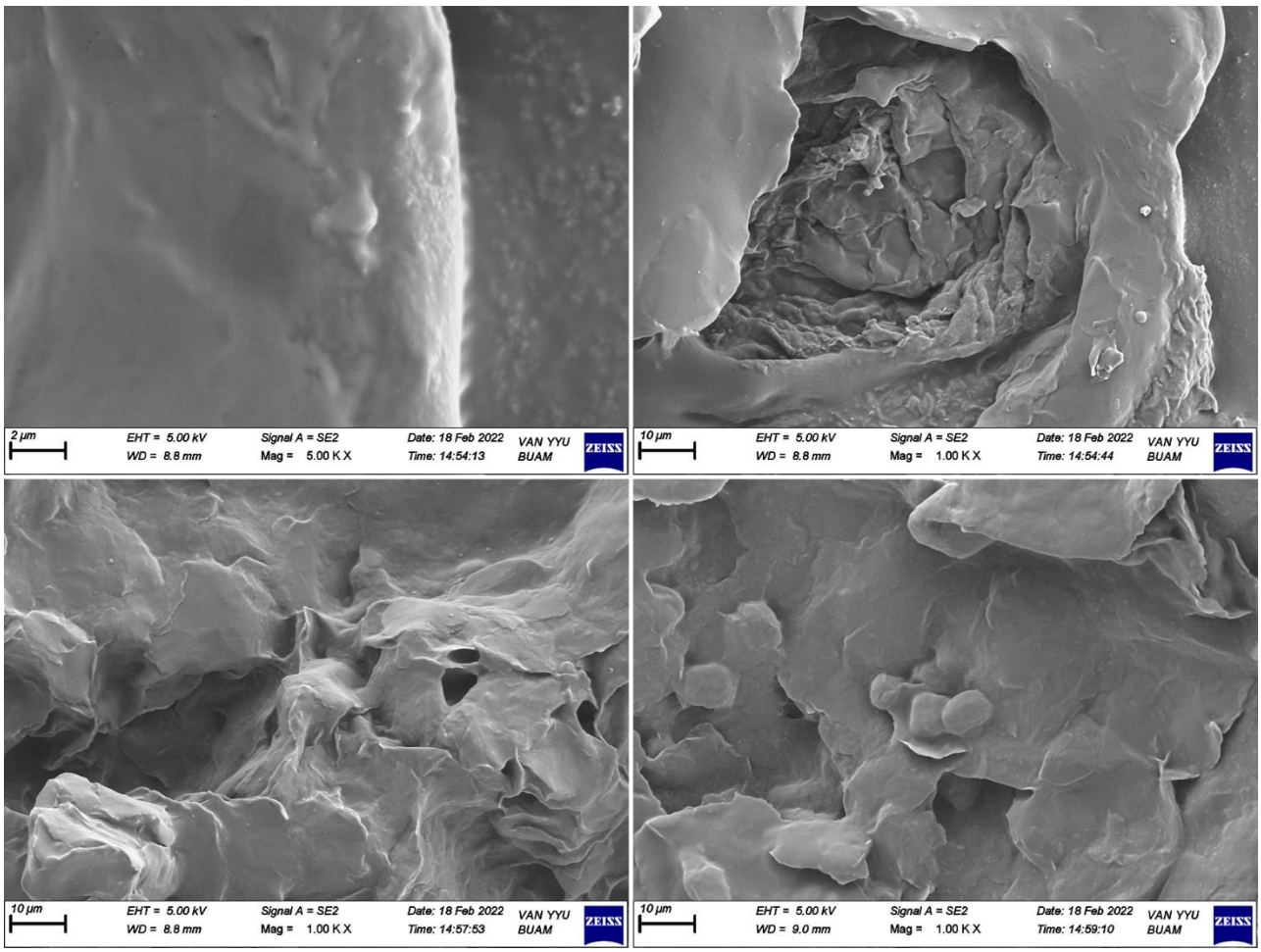

Figure 2. SEM images of propolis samples

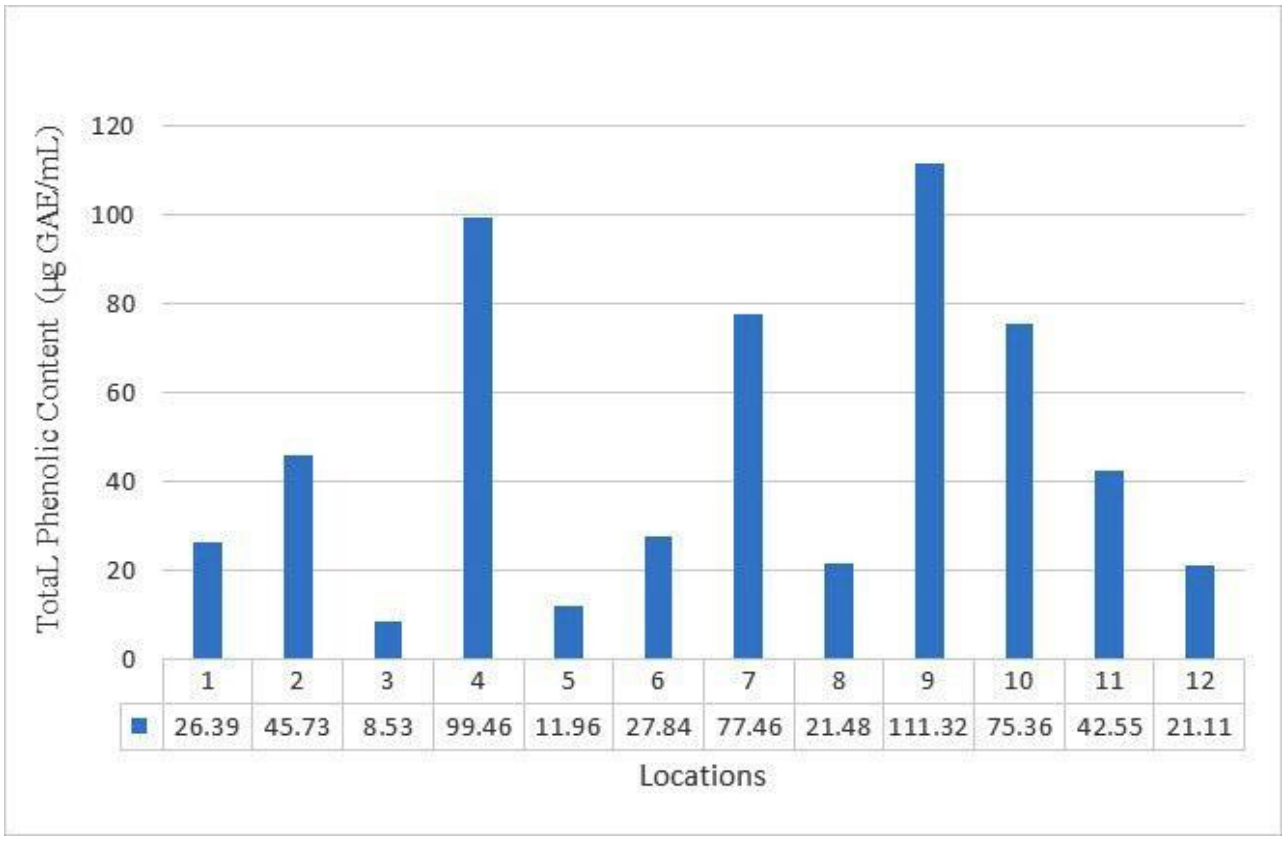

Figure 3. The total amount of phenolic substances (µg GAE/mL) contained in the propolis samples

Table 2 Analyzing the Wax content of propolis samples

Sample	Wax amount (g)	Pure propolis (g)	Wax (%)	Pure propolis (%)
1	5.76	7.57	43.21	56.80
2	6.46	4.98	56.50	43.50
3	8.57	5.95	59.06	40.94
4	6.88	4.09	62.73	37.27
5	5.54	5.25	51.37	48.63
6	4.06	2.37	63.18	36.82
7	7.59	7.76	49.47	50.53
8	6.08	4.54	57.27	42.73
9	6.94	6.97	49.92	50.08
10	10.28	8.79	53.94	46.06
11	4.23	16.66	20.25	79.75
12	3.87	16.49	19.04	80.96

Table 3. Mineral content of propolis samples (µg/kg)

Sample	Ca	Cu	Fe	Pb	Mg	Mn	Ni	P	Zn
1.	464.34	2.81	11.69	2.89	10.82	1.86	6.78	49.8	10.08
2.	398.88	1.903	7.12	5.25	52.87	2.10	4.61	59.3	25.76
3.	255.56	9.01	4.34	7.68	35.31	2.44	8.59	45.6	12.07
4.	377.12	5.44	29.23	7.64	19.99	2.63	1.38	26.5	29.11
5.	358.88	6.74	15.66	2.66	10.95	1.96	1.09	11.1	74.94
6.	786.81	1.739	49.24	1.55	78.22	3.39	2.56	32.4	12.50
7.	331.61	5.08	14.94	4.62	14.87	3.37	5.42	86.9	4.32
8.	168.35	1.47	21.68	5.66	25.38	2.19	1.49	13.6	9.88
9.	468.91	2.17	75.39	1.08	20.59	2.16	3.35	11.2	9.67
10.	707.36	3.20	14.49	1.57	13.02	1.96	1.99	32.6	10.64
11.	506.87	7.76	19.71	2.98	91.44	5.73	2.62	53.7	49.53
12.	486.32	2.49	14.76	2.43	48.72	4.39	3.44	39.3	85.04

Differences in the content of elements in individual mineral substances are also present in many other studies. Soil type and parameters, mobile metals, botanical origin of the samples, and weather conditions may cause differences in the mineral profile of the investigated propolis obtained from different locations. These facts can be used in some distant and different grouping of mineral substances in the same cluster or subset.

Finally, this study found that, especially due to Türkiye's rich biodiversity, the propolis sample taken from the Balveren beekeepers has an average level of phenolic and botanical origin and it can also be utilized as a natural source in the food and medicinal industries. Additionally, the identification of fully active components in Balveren propolis allows it to be regarded as a significant source of natural antioxidant chemicals.

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References

Abdullah, N.A., Ja'afar, F., Yasin, H.M., Taha, H., Petalcorin, M.I., Mamit, M.H., ..., & Usman, A. (2019). Physicochemical analyses, antioxidant, antibacterial, and toxicity of propolis particles produced by stingless bee *Heterotrigona itama* found in Brunei Darussalam. *Heliyon*, 5(9), 1-8. https://doi.org/10.1016/j.heliyon.2019.e02476

Aliyazıcıoglu, R., Sahin, H., Erturk, O., Ulusoy, E., & Kolayli, S. (2013). Properties of phenolic composition and biological activity of propolis from Turkey. *International Journal of Food Properties*, 16(2), 277-287. https://doi.org/10.1080/10942912.2010.551312

Anjum, S.I., Ullah, A., Khan, K.A., Attaullah, M., Khan, H., Ali, H., ..., & Dash, C.K. (2019). Composition and functional properties of propolis (bee glue): a review. *Saudi Journal of Biological Sciences*, 26(7), 1695-1703. https://doi.org/10.1016/j.sjbs.2018.08.013

Bagatir, G., Kaya, M., Suer, I., Cefle, K., Palanduz, A., Palanduz, S., Becerir, H. B., Koçyigit, M., & Ozturk, S. (2022). The effect of Anzer honey on X-ray induced genotoxicity in human lymphocytes: An in vitro study. *Microscopy Research and Technique*, 1–10. https://doi.org/10.1002/jemt.24081

Bankova, V., Pappaova M., & Trusheva B. (2014). Propolis volatile compounds: Chemical diversity and biological activity: a review. *Chemistry Central Journal*, 8, 28. https://doi.org/10.1186/1752-153X-8-28

Bankova, V., Trusheva, B., & Popova, M. (2021). Propolis extraction methods: a review. *Journal of Apicultural Research*, 60(5), 734-743. https://doi.org/10.1080/00218839.2021.1901426

Bonvehí, J.S., & Gutiérrez, A.L. (2011). Antioxidant activity and total phenolics of propolis from the Basque Country (Northeastern Spain). *Journal of the American oil chemists' society*, 88(9), 1387-1395. https://doi.org/10.1007/s11746-011-1792-1

Bonvehí, J.S., & Coll, F.V. (1994). Phenolic composition of propolis from China and from South America. *Zeitschrift für Naturforschung c*, 49(11-12), 712-718. https://doi.org/10.1515/znc-1994-11-1204

Borrelli, F., Maffia, P., Pinto, L., Ianaro, A., Russo, A., Capasso, F., & Ialenti, A. (2002). Phytochemical compounds involved in the anti-inflammatory effect of propolis extract. *Fitoterapia*, 73, S53-S63. https://doi.org/10.1016/S0367-326X(02)00191-0

Crane, E. (1999). History of other products from bees In: Crane, B. (ed) The world history of beekeeping and honey hunting, Routledge; New York; 545-553.

Cunha, I.B.S., Sawaya, A.C.H.F., Caetano, F.M., Shimizu, M.T., Marcucci, M.C., Drezza, F.T., Povia, G.S., & Carvalho, P. (2004). Factors that influence the yield and composition of Brazilian propolis extracts. *Journal of the Brazilian Chemical Society*, 15(6), 964-970.

Davis, P.H. (1971). Distribution patterns in Anatolia with particular reference to endemism. In Davis, P.H., Harper, P.C & Hedge, I.C. (eds) Plant Life of South-West Asia. Botanical Society of Edinburgh, Edinburgh. 15-27.

Dias, L.G., Pereira, A.P., & Estevinho, L.M. (2012). Comparative study of different Portuguese samples of propolis: pollinic, sensorial, physicochemical, microbiological characterization and antibacterial activity. *Food and Chemical Toxicology*, 50(12), 4246-4253. https://doi.org/10.1016/j.fct.2012.08.056

Formicki, G., Greń, A., Stawarz, R., Zyśk, B., & Gał, A. (2013). Metal content in honey, propolis, wax, and bee pollen and implications for metal pollution monitoring. *Polish Journal of Environmental Studies*, 22(1), 99-106.

Guzmán-Gutiérrez, S.L., Nieto-Camacho, A., Castillo-Arellano, J.I., Huerta-Salazar, E., Hernández-Pasteur, G., Silva-Miranda, M., ..., & Reyes-Chilpa, R. (2018). Mexican propolis: a source of antioxidants and anti-inflammatory compounds, and isolation of a novel chalcone and ε-caprolactone derivative. *Molecules*, 23(2), 334. https://doi.org/10.3390/molecules23020334

Herrera, C.L., Alvear, M., Barrientos, L., Montenegro, G., & Salazar, L.A. (2010). The antifungal effect of six commercial extracts of Chilean propolis on *Candida* spp. *Ciencia e investigación agraria*, 37(1), 75-84. http://doi.org/10.4067/S0718-16202010000100007

Kabata-Pendias, A. (2011). Trace elements in soils and plants, 4th edn. Boca Rato, CRC Press, 548 pp.

Kasote, D.M. (2017). Propolis: a neglected product of value in the Indian beekeeping sector. *Bee World*, 94(3), 80-83. https://doi.org/10.1080/0005772X.2017.1345223

Keskin, M., & Kolaylı, S. (2018). Standardization of propolis, Is it possible. *Uludag bee journal*, 18(2), 101-110. https://doi.org/10.31467/uluaricilik.485080

Kurek-Górecka, A., Rzepecka-Stojko, A., Górecki, M., Stojko, J., Sosada, M., & Świerczek-Zięba, G. (2014). Structure and antioxidant activity of polyphenols derived from propolis. *Molecules*, 19(1), 78-101. https://doi.org/10.3390/molecules19010078

Li, F., Awale, S., Tezuka, Y., & Kadota, S. (2008). Cytotoxic constituents from Brazilian red propolis and their structure–activity relationship. *Bioorganic & Medicinal Chemistry*, 16(10), 5434-5440. https://doi.org/10.1016/j.bmc.2008.04.016

Maden Çalışkol, M. (2013). Azerbaycan yöresine ait propolis örneklerinin antioksidan özelliklerinin belirlenmesi. Yüksek Lisans Tezi (332135). Retrieved from https://tez.yok.gov.tr/UlusalTezMerkezi/tezSorguSonucYeni.jsp

Negri, G., Marcucci, M.C., Salatino, A., & Salatino, M.L.F. (2000). Comb and propolis waxes from Brazil (states of São Paulo and Paraná). *Journal of the Brazilian Chemical Society*, 11, 453-457.

Oršolić, N., & Bašić, I. (2003). Immunomodulation by water-soluble derivative of propolis: a factor of antitumor reactivity. *Journal of Ethnopharmacology*, 84(2-3), 265-273. https://doi.org/10.1016/S0378-8741(02)00329-X

Ota, C., Unterkircher, C., Fantinato, V., & Shimizu, M.T. (2001). Antifungal activity of propolis on different species of *Candida*. *Mycoses*, 44(9-10), 375-378. https://doi.org/10.1046/j.1439-0507.2001.00671.x

Özaltan, Z., & Koçyiğit, M. (2022). Pollen morphology of some taxa in the family Lamiaceae (Labiatae) from Turkey. *EMU Journal of Pharmaceutical Sciences*, 5(1), 11-20.

Özhatay, N., Kocyigit, M., & Bona, M. (2012). Istanbul's Honey Plants. Istanbul. Turkey.

Perveen, A., & Qaiser, M. (2003). Pollen Morphology of the Family Labiatae from Pakistan. *Pakistan Journal of Botany*, 35(5), 671–693.

Popova, M.P., Bankova, V.S., Bogdanov, S., Tsvetkova, I., Naydenski, C., Marcazzan, G.L., & Sabatini, A.G. (2007). Chemical characteristics of poplar type propolis of different geographic origin. *Apidologie*, 38(3), 306-311. https://doi.org/10.1051/apido:2007013

Popova, M., Trusheva, B., & Bankova, V. (2017). Content of biologically active compounds in Bulgarian propolis: a basis for its standardization. *Bulgarian Chemical Communications*, 49(2017), 115-120.

Roman, A., Madras-Majewska, B., & Popiela-Pleban, E. (2011). Comparative study of selected toxic elements in propolis and honey. *Journal of Apicultural Science*, 55(2), 97-106.

Saelao, P., Borba, R.S., Ricigliano, V., Spivak, M., & Simone-Finstrom, M. (2020). Honeybee microbiome is stabilized in the presence of propolis. *Biology letters*, 16(5), 1-5. https://doi.org/10.1098/rsbl.2020.0003

Sarıkaya, A.O., Ulusoy, E., Öztürk, N., Tuncel, M., & Kolayli, S. (2009). Antioxidant activity and phenolic acid constituents of chestnut (*Castania sativa* Mill.) honey and propolis. *Journal of food biochemistry*, 33(4), 470-481. https://doi.org/10.1111/j.1745-4514.2009.00231.x

Sawicka, D., Car, H., Borawska, M.H., & Nikliński, J. (2012). The anticancer activity of propolis. *Folia Histochemica et Cytobiologica*, 50(1), 25-37.

Sforcin, J.M., Fernandes J.A., Lopes, C.A.M., Bankova, V., & Funari, S.R.C. (2000). Seasonal effect on Brazilian propolis antibacterial activity. *Journal of ethnopharmacology*, 73(1-2), 243-249. https://doi.org/10.1016/S0378-8741(00)00320-2

Sforcin, J.M. (2016). Biological properties and the rapeuticapplications of propolis. *Phytotherapy Research*, 30(6), 894-905. https://doi.org/10.1002/ptr.5605

Simone-Finstrom, M., Borba, R.S., Wilson, M., & Spivak, M. (2017). Propolis counteracts some threats to honey bee health. *Insects*, 8(2), 46. https://doi.org/10.3390/insects8020046

Sobočanec, S., Balog, T., Šarić, A., Mačak-Šafranko, Ž., Štroser, M., Žarković, K., ..., & Marotti, T. (2011). Antitumor effect of Croatian propolis as a consequence of diverse sex-related dihydropyrimidine dehydrogenase (DPD) protein expression. *Phytomedicine*, 18(10), 852-858. https://doi.org/10.1016/j.phymed.2011.01.015

Su, L., Yin, J.J., Charles, D., Zhou, K., Moore, J., & Yu, L.L. (2007). Total phenolic contents, chelating capacities, and radical-scavenging properties of black peppercorn, nutmeg, rosehip, cinnamon and oregano leaf. *Food chemistry*, 100(3), 990-997. https://doi.org/10.1016/j.foodchem.2005.10.058

Şenoğlu Fenerci B. (2021). Eskiçağ'da bal. *Masrop E-Dergi*, 15(1), 62-74.

Tylkowski, B., Trusheva, B., Bankova, V., Giamberini, M., Peev, G., & Nikolova, A. (2010). Extraction of biologically active compounds from propolis and concentration of extract by nanofiltration. Journal of Membrane Science, 348(1–2), 124–130. http://doi.org/10.1016/j.memsci.2009.10.049

Yüksel, U., Tegin, I., & Ziyadanogullari, R. (2017). Recovery of copper and cobalt from copper slags as selective. *Journal of Environmental Science and Engineering A*, 6(8), 388-394.

Üreten, H., (2011). Eski Anadolu'da arı ve bal. *International journal of history*, 3(3), 363-382.

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Research Article/Araştırma Makalesi

A New Approach for Dye Removal with a Polymer: Removal of Acid Orange 12 from Aqueous Solution with Shrimp Chitin

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Abstract: Chitin, a naturally abundant mucopolysaccharide, is the supporting material of crustaceans, insects, and etc. Chitin and its main derivative chitosan have various applications in medicine, pharmacy, biotechnology, environment, and food engineering because of their nontoxicity, biodegradability, biocompatibility, antimicrobial, and antioxidant properties. Here, research was conducted on the removal of Acid Orange 12, which is among the most used azo dyes in textiles, from aqueous solutions using shrimp chitin, a polymer. To determine the most suitable conditions, different parameters (pH degrees, amount of chitin, amount of dye, contact time) were studied. Chitin was determined to be the most efficient in removing Acid Orange 12 using pH 5 conditions. The adsorption of dye onto chitin followed the Langmuir isotherm and pseudo-second-order kinetic model.

Keywords: Arthropod, biopolymer, water, cleaning, environment.

Polimerle Boya Gideriminde Yeni Bir Yaklaşım: Karides Kitini İle Sulu Çözeltiden Asit Portakal 12'nin Giderimi

Öz Kitin, doğal olarak bol miktarda bulunan bir mukopolisakkarit olup kabukluların, böceklerin vb. canlıların destekleyici malzemesidir. Kitin ve ana türevi kitosan, toksik olmamaları, biyolojik olarak parçalanabilirlikleri, biyouyumlulukları, antimikrobiyal ve antioksidan özelliklerinden dolayı tıp, eczacılık, biyoteknoloji, çevre ve gıda mühendisliğinde çeşitli uygulamalara sahiptir. Burada, tekstilde en çok kullanılan azo boya maddelerden biri olan Asit Portakal 12'nin bir polimer olan karides kitini vasıtasıyla sulu çözeltilerden uzaklaştırılması üzerine araştırma yapılmıştır. En uygun koşulları belirlemek için farklı parametreler (pH dereceleri, kitin miktarı, boya miktarı, temas süresi) çalışılmıştır. Kitin, pH 5 koşullarında kullanılarak Asit Portakal 12'nin uzaklaştırılmasında en verimli olarak tespit edilmiştir. Boyanın kitin üzerine adsorpsiyonu Langmuir izotermine ve yalancı-ikinci-dereceden kinetik modele uymuştur.

Anahtar kelimeler: Eklembacaklı, biyopolimer, su, temizleme, çevre.

1. Introduction

Chitin is a naturally abundant mucopolysaccharide and the supporting material of arthropods. It is known to contain 2-acetamido-2-deoxy-(3-D-glucose) via a β-(1→4) linkage that is cleaved by chitinase. Despite the presence of nitrogen, its immunogenicity is extremely low. It is a highly insoluble material similar to cellulose with its solubility and low chemical reactivity. It can be considered as cellulose with hydroxyl at the C-2 position replaced by an acetamido group. Like cellulose, it naturally functions as a structural polysaccharide (Kumar, 2000). Chitin and its derivative chitosan have various implications in pharmacy, medicine, biotechnology, the environment, and the food industry due to their non-toxicity, biocompatibility, biodegradability, and antimicrobial and antioxidant properties (Kaya et al., 2016). Water pollution is at the top of the list of pollution in the world. Water resources are polluted with different substances such as organic and inorganic, heavy metals, pesticides, detergents, reactive substances, phenol, antibiotics, aromatic hydrocarbons, plastics, and dyes (Goswami et al., 2021). Water-containing pollutants adversely affect the living life in the aquatic ecosystem with the formation of odor, color change, decrease in light transmittance, and decrease in oxygen content. Pollution and odor occur not only underwater but also on the water (Sen et al., 2016). Industrial establishments using dyestuffs somehow generate wastewater with dye waste. The textile industry comes first among the industrial establishments in forming dyed water that plays a major role in reducing light transmittance. This ranking is followed by the paper, leather, and paint production industries (Katheresan et al., 2018). According to the literature, Cellulose (Liu et al., 2015; Boran, 2022) and Chitin (McKay et al., 1982) are important tools for removing dyes from water systems.

Dyes are classified differently according to their areas of use and chemical structures. Some of the dye types are acid, basic, solvent, azo, reactive, metal, indigo, natural dyes, and pigments. Azo dyes are formed by combining reactive dyes with different reactive groups and are commonly used dyes in the industry. The most distinctive feature of these dyes is that they contain one or more azo groups (such as ─N=N─, ─C≡N─) and are bound to textile fibers by covalent bonds. Azo dyes are known as the most preferred dye group with their usage of 65-70% in the world. Azo dyes used in the textile industry cause allergic

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reactions and increased hyperactivity in children. It is very important to remove these chemicals in wastewater. Acid Orange 12 dye is a kind of industrial dye that is among the azo dyes (Yadav et al., 2012; Benkhaya et al., 2020; Ajaz et al., 2020; Hameed et al., 2022; Zhou et al., 2011).

Azo dyes are recalcitrant molecules that are highly stable against oxidizing agents and resistant to aerobic decay. Therefore, they are difficult to remove from wastewater. Three different methods, physical, chemical, and biological, can be used to remove this type of paint from water. Processes such as membrane separation, aerobic/anaerobic digestion, flocculation, ozonolysis, and electrodialysis among these methods have different disadvantages such as low removal efficiency, high energy consumption, or sludge formation. On the other hand, dye removal by adsorption is a positive alternative. Different materials such as activated sludge-activated carbon, chitin, and chitosan can be used in dye removal by adsorption (Desbrières & Guibal, 2018; Millicent Mabel et al., 2019; Maderova et al., 2016; Herrera-González et al., 2019; Joshi et al., 2004; Stingley et al., 2010; Cho et al., 2015).

Our study aims to remove Acid Orange 12, an azo dye, using chitin. In the study, pH, contact time and kinetics, initial dye concentration, and chitin dose parameters were tested and the suitable conditions for adsorption were determined (Fig. 1.).

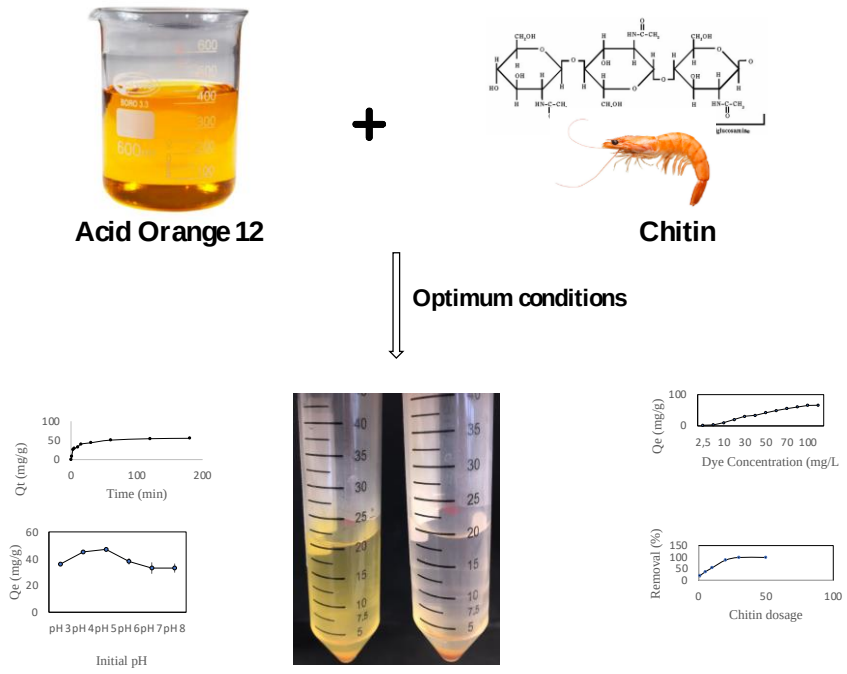

Figure 1. The removal of Acid Orange 12 from an aqueous solution by chitin

2. Material and Methods

2.1. Materials

Acid Orange 12 ($C_{16}H_{11}N_2NaO_4S$), chitin from shrimp shells, and all of the chemicals used in the study were purchased from Sigma-Aldrich (Merck).

2.2. Adsorption process

2.2.1. Determination of pH value on Acid Orange 12 adsorption

To determine the optimum pH value for dye adsorption, solutions were prepared with 20 mg of chitin and 20 mL of dye solution (50 mg/dm³). The pH of these solutions was adjusted individually between 3.0 and 8.0. The samples were kept in a rotary shaker (150 rpm) (Mikrotest, Turkey) at 25°C for 24 hours.

2.2.2. Contact time and kinetics on Acid Orange 12 adsorption

The concentration of Acid Orange 12 in the aqueous solutions was determined using the UV–Vis spectrophotometer (Shimadzu UV-1700, Kyoto, Japan) after a certain period at 1, 3, 5, 10, 15, 30, 60, 120, 180 min.

The measurement of the dye adsorption of the polymer includes adsorption kinetics in which the reaction sequence and rate constants are evaluated. The reactions of the pseudo-first-order model and pseudo-second-order models are used to describe the dye adsorption kinetics (Hosseini et al., 2016; Aljeboree et al., 2017). In our study, the reaction rate constants were calculated with the Lagergren equation which consists of the correlation analysis between the time of Acid Orange 12 adsorbed by 1 g of chitin. The equation for a so-called pseudo-first-order kinetic model reaction is:

$$ln\ (q_e - q_t) = ln(qe) - K_1 t \quad \text{(Eq.1)}$$

The model of pseudo-second-order kinetic model reaction is:

$$t / q_t = 1 / (K_2 q_e^2) + t / q_e \quad \text{(Eq.2)}$$

2.2.3. Effect of initial dye concentration on adsorption and isotherms

The determination of the initial Orange 12 dye concentrations was studied by varying the initial dye concentration from 2.5 mg/L to 130 mg/L and by keeping the other parameters constant.

The Langmuir and Freundlich isotherm model, which is the most widely used to explain the adsorption mechanism, was used in the study. Langmuir and Freundlich's equations for these models are as follows (Huang et al., 2016):

Langmuir → $C_e/Q_e = (Q_e/Q_{max}) + (1/Q_{max}*K_L)$ (Eq.3)

Freundlich → $\ln Q_e = 1/n \ln C_e + \ln K_F$ (Eq.4)

2.2.4. Determination of chitin dose on removal rate

To determine the effect of the amount of chitin on the removal of Acid Orange 12, the amount of chitin was changed between 1 and 50 mg while keeping the pH and volume of the aqueous solution constant.

3. Results

3.1. Effect of medium pH on dye removal

The pH of the medium is the most significant parameter for removal processes. Dye adsorption was carried out in media prepared between pH 3 and 8 to monitor the effect of the initial pH of the environment where the adsorption takes place on Orange 12 (Fig. 2). Statistically, a significant difference was observed (Kruskal Wallis p-value = 0.022, Dunn's test p-value = 0.0381). As depicted in Figure 2, maximum dye adsorption occurred at low pH values (36.76-47.08 mg/g). Above pH 5, the adsorption efficiency started to decrease and the lowest efficiency was observed at pH 8. The adsorbent surface attained a positive charge under acidic conditions (Ergene et al., 2009). A previous study reported that protonated amino groups play a key role in adsorption of acidic dye with nano-chitosan particles and due to the insufficient hydrogen ion concentration, the adsorbent surface is less protonated at pH 6 value (Cheung et al., 2009).

As a result, it is considered that the electrostatic interactions between the positively charged adsorbent and the dye directly affect the adsorption capacity.

3.2. Effect of contact time and kinetics

As seen in Figure 3, adsorption takes place very quickly in the first 15 minutes and almost 75% of the biosorption capacity is reached within 30 minutes. The rapid adsorption in the first 30 minutes can be attributed to the empty binding sites on the biosorbent surface. As the contact time increases, the binding sites begins to saturate and the amount of adsorption slows down. In this study, in which chitin was used as an adsorbent, equilibrium was reached at 120 minutes.

The data obtained from the change in adsorption capacity versus time were applied to the pseudo-first-order and pseudo-second-order kinetic models. When evaluated according to the correlation coefficient, it was found that the pseudo-second-order kinetic model explained the removal of Acidic Orange 12 with chitin better than the pseudo-first-order kinetic model (Table 1).

Table 1. Kinetic constants

Pseudo-first order			Pseudo-second order		
Q_e (mg/g)	k_1 (min^{-1})	R^2	Q_e (mg/g)	k_2 (g/mg/min)	R^2
31.68	0.00025	0.971	56.49	0.0029	0.998

A previous study showed that Reactive Red 120 dye adsorption with different adsorbents followed a pseudo-second-order kinetic model (Arica & Bayramoğlu, 2007).

3.3. Effect of initial dye concentration

The dye biosorption capacity increased with the increase in the amount of Orange 12 dye in the medium and reached equilibrium at 100 mg/L dye concentration (Fig. 4). Dye adsorption capacity at equilibrium was found to be 65.75 mg/g. The increase in adsorption capacity with an increase in initial dye concentration is based on the increase in driving force for mass transfer with increasing dye concentration. However, the vacant active binding sites begin to fill and adsorption reaches equilibrium (Bayramoğlu & Yılmaz, 2018).

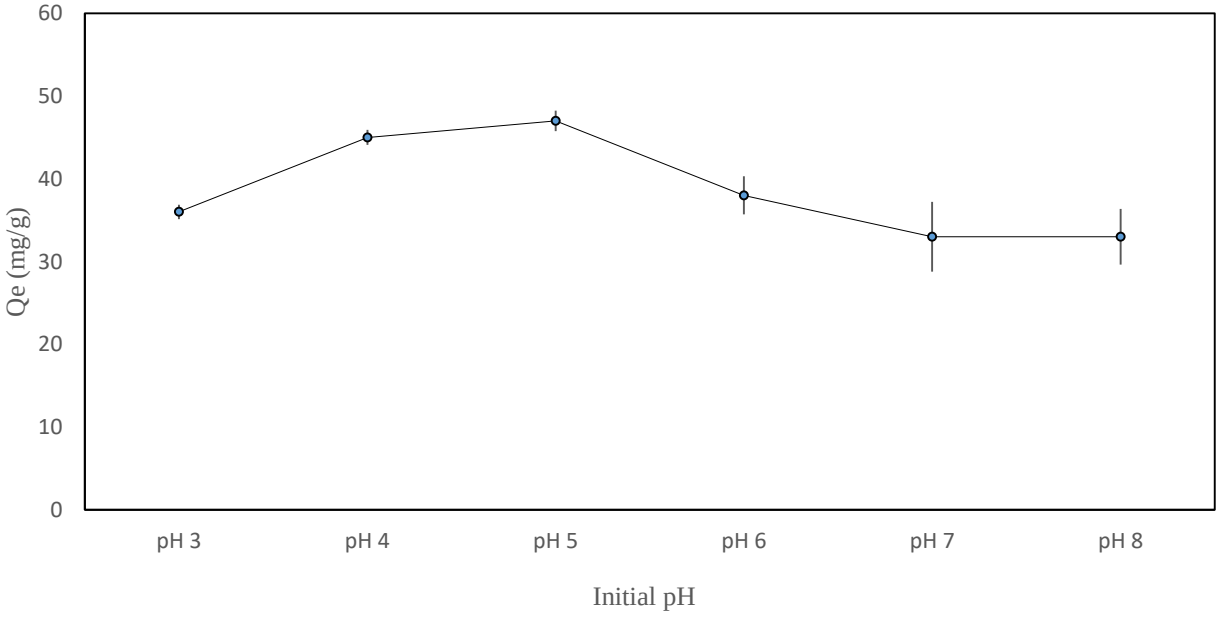

Figure 2. Effect of medium pH on dye adsorption (V: 20 ml, Biomass dosage: 20 mg, Dye concentration: 50 mg/L, Temperature: 25ºC, Contact time: 24 h).

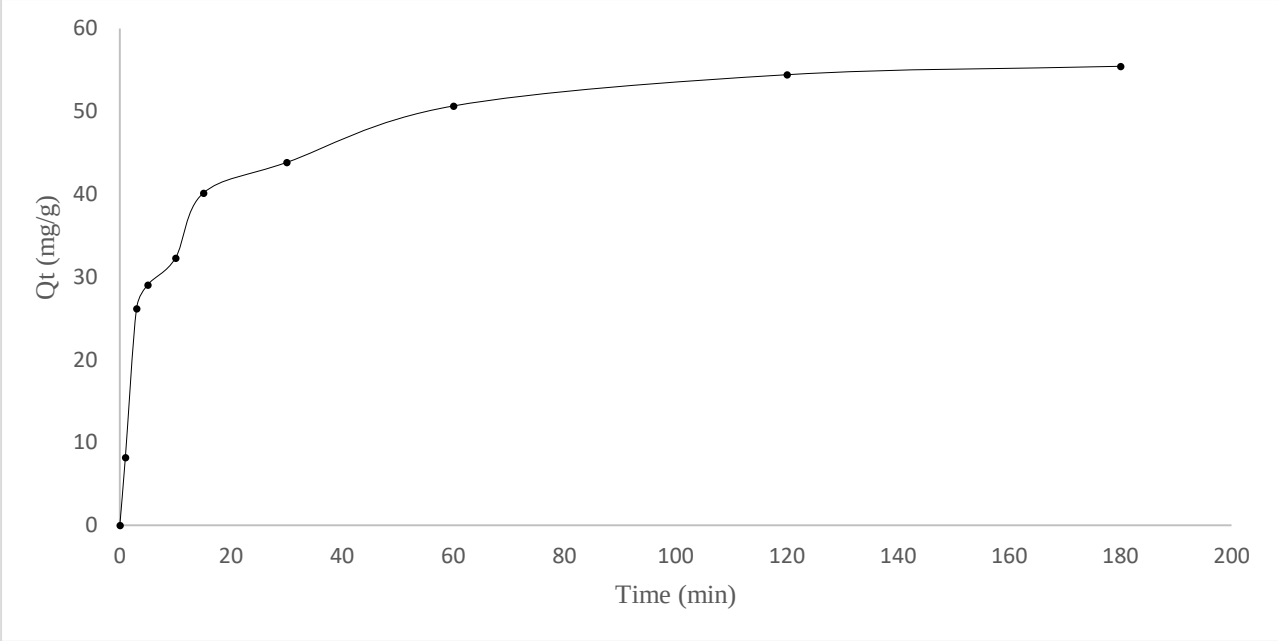

Figure 3. Effect of contact time on dye adsorption

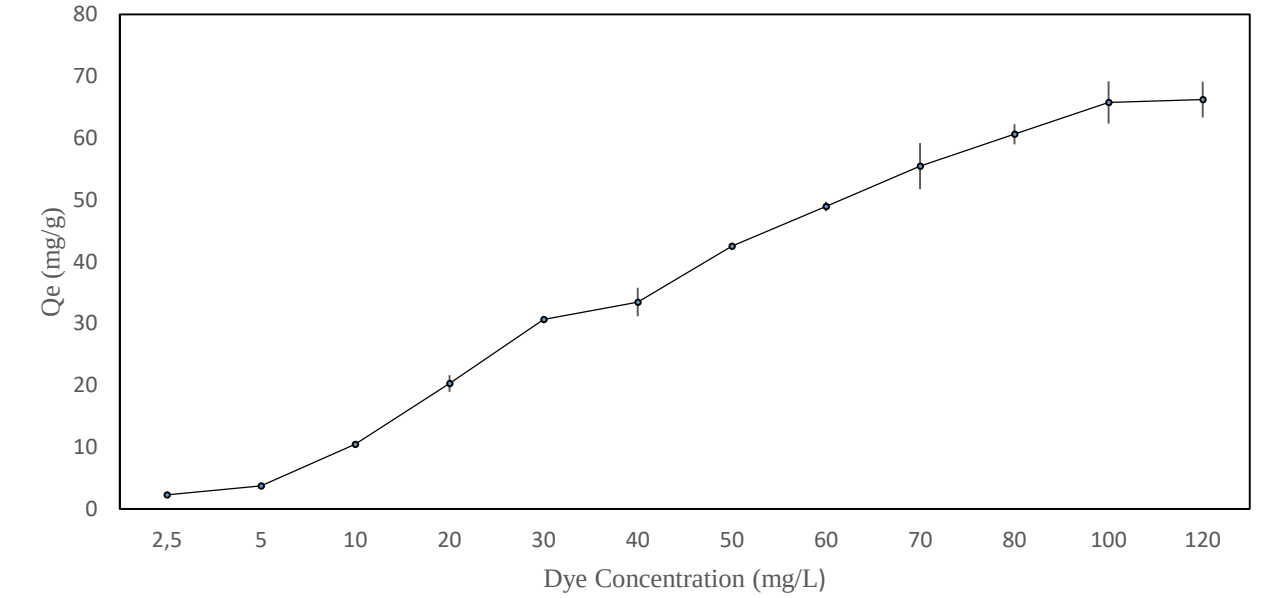

Figure 4. Effect of initial dye concentration on adsorption (V: 20 ml, Biomass dosage:20 mg, pH: 5, Temperature: 25º C, Contact time: 24 h)

3.4. Isotherms

Adsorption isotherms explain the relationship between adsorbate and adsorbent and; therefore, have great importance for the efficient use of adsorbent (Wong et al., 2003). According to our results, adsorption of Orange 12 onto chitin better fit Langmuir isotherm rather than Freundlich isotherm (Table 2). Langmuir isotherm assumes that the adsorbent has a limited number of binding sites, the binding sites are homogeneous, there is no interaction between adsorbates, and the adsorption is monolayer (Hussain et al., 2021).

The calculated Q_{max} value was found as 67.57 mg/g and the experimental value was found as 65.75 mg/g. The fact that the theoretical and experimental Qmax values are very close to each other shows the power of the isotherm model.

Table 2. The constants of isotherms

Langmuir constants			Freundlich constants		
Q_{max} (mg/g)	K_L (L/mg)	R^2	K_F (L/g)	n	R^2
67.57	0.58	0.994	3.74	2.51	0.765

3.5. Effect of chitin dose on removal rate

The amount of chitin in the adsorption environment directly affected the percent removal rate. As seen in Figure 5, 98% percent dye removal was achieved with 30 mg/L chitin.

As the amount of adsorbent in the solution increases, the areas that bind the dye will increase and; as a result, the removal will increase (Ratnamala et al., 2012).

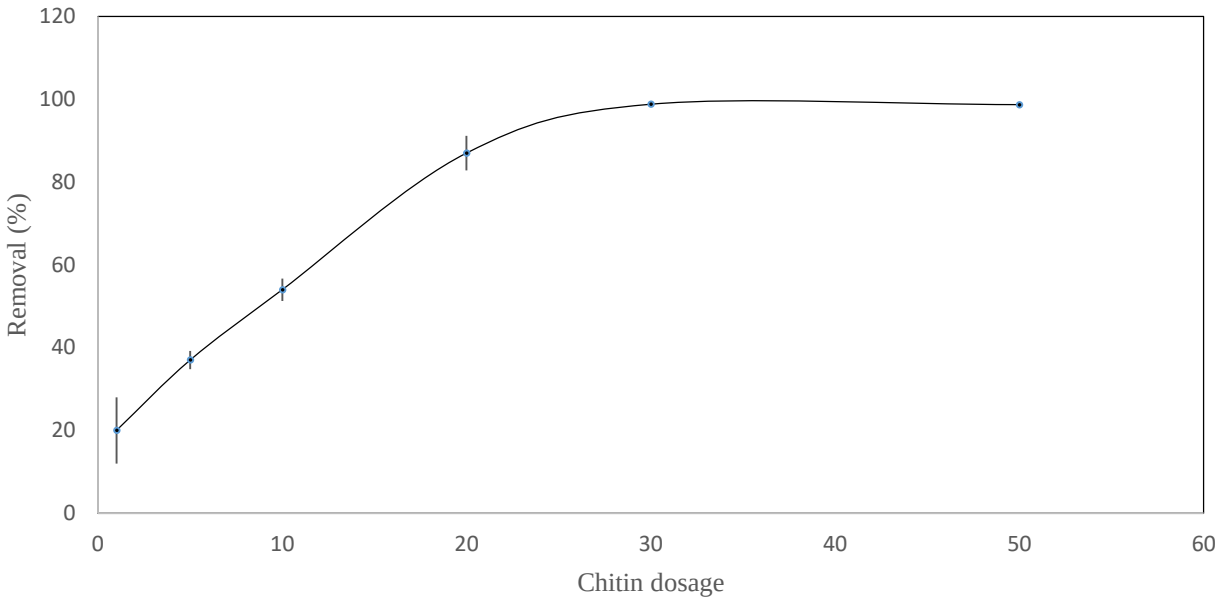

Figure 5. Effect of chitin amount on dye removal (adsorption (V: 20 ml, Dye concentration: 80 mg/L, Temperature: 25°C, Contact time: 24 h)

4. Conclusion

In summary, this study is aimed removing dye contamination from water with chitin which is a waste obtained from shrimp shells. At different optimization conditions, Acid Orange 12 dye was removed with chitin. The most efficient condition was measured at pH 5. Equilibrium was reached in 120 minutes after rapid removal in the first 30 minutes. The dye-holding capacity of chitin increased with the increase of dye concentration in the aqueous solution and the adsorption capacity at equilibrium was measured as 65.75 mg/g. With the amount of 30 mg/L chitin, most of the dye (98%) could be removed from the aqueous solution.

We have reported for the first time the usability of chitin, which is generated as a waste but whose value is increasing day by day, in cleaning water contaminated with Acid Orange 12 dye.

Ethics committee approval: Ethics committee approval is not required for this study.

Conflict of interest: The authors declared that there is no conflict of interest.

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References

Ajaz, M., Shakeel, S., & Rehman A. (2020). Microbial use for azo dye degradation—a strategy for dye bioremediation. *International Microbiology*, 23, 149–159. https://doi.org/10.1007/s10123-019-00103-2

Aljeboree, A.M., Alshirifi, A.N., & Alkaim, A.F. (2017). Kinetics and equilibrium study for the adsorption of textile dyes on coconut shell activated carbon. *Arabian Journal of Chemistry*, 10(2), 3381-3393. https://doi.org/10.1016/j.arabjc.2014.01.020

Arica, M.Y., & Bayramoğlu, G. (2007). Biosorption of Reactive Red-120 dye from aqueous solution by native and modified fungus biomass preparations of Lentinus sajor-caju. *Journal of Hazardous Materials*, 149(2), 499-507. https://doi.org/10.1016/j.jhazmat.2007.04.021

Bayramoğlu, G., & Yilmaz, M. (2018). Azo dye removal using free and immobilized fungal biomasses: Isotherms, Kinetics and thermodynamic studies. *Fibers and Polymers*, 19, 877–886. https://doi.org/10.1007/s12221-018-7875-y

Benkhaya, S., M'rabet, S., & El Harfi, A. (2020). Classifications, properties, recent synthesis and applications of azo dyes. *Heliyon*, 6(1), e03271. https://doi.org/10.1016/j.heliyon.2020.e03271

Boran, F. (2022). Dye Removing with Dry and Wet Forms of Pure Bacterial Cellulose Produced by Gluconacetobacter xylinus. *Commagene Journal of Biology*, 6(1), 1-5. https://doi.org/10.31594/commagene.1037538

Cheung, W.H., Szeto, Y.S., & McKay, G. (2009). Enhancing the adsorption capacities of acid dyes by chitosan nano particles. *Bioresource Technology*, 100(3), 1143-1148. https://doi.org/10.1016/j.biortech.2008.07.071

Cho, D.W., Jeon, B.H., Chon, C.M., Schwartz, F.W., Jeong, Y., & Song, H. (2015). Magnetic chitosan composite for adsorption of cationic and anionic dyes in aqueous solution. *Journal of Industrial and Engineering Chemistry*, 28, 60-66. https://doi.org/10.1016/j.jiec.2015.01.023

Desbrières, J., & Guibal, E. (2018). Chitosan for wastewater treatment. *Polymer International*, 67(1), 7-14. https://doi.org/10.1002/pi.5464

Ergene, A., Ada, K., Tan, S., & Katircioğlu, H. (2009). Removal of Remazol Brilliant Blue R dye from aqueous solutions by adsorption onto immobilized Scenedesmus quadricauda: Equilibrium and kinetic modeling studies. *Desalination*, 249(3), 1308-1314. https://doi.org/10.1016/j.desal.2009.06.027

Goswami, R.K., Mehariya, S., Verma, P., Lavecchia, R., & Zuorro A. (2021). Microalgae-based biorefineries for sustainable resource recovery from wastewater. *Journal of Water Process Engineering*, 40, 101747. https://doi.org/10.1016/j.jwpe.2020.101747

Hameed, A.B., Dekhyl, A.B., & Alabdraba, W.M.S. (2022). Removing the Acid Orange 12 Azo Dye from Aqueous Solution Using Sodium Hypochlorite, A Kinetic and Thermodynamic Study. *IOP Conference Series: Earth and Environmental Science*, 961, 012056. https://doi.org/10.1088/1755-1315/961/1/012056

Herrera-González, A.M., Caldera-Villalobos, M., & Peláez-Cid, A.A. (2019). Adsorption of textile dyes using an activated carbon and crosslinked polyvinyl phosphonic acid composite. *Journal of Environmental Management*, 234, 237-244. https://doi.org/10.1016/j.jenvman.2019.01.012

Hosseini, F., Sadighian, S., Hosseini-Monfared, H., & Mahmoodi, N.M. (2016). Dye removal and kinetics of adsorption by magnetic chitosan nanoparticles. *Desalination and Water Treatment*, 57, 51. https://doi.org/10.1080/19443994.2016.1143879

Huang, J., Liu, D., Lu, J., Wang, H., Wei, X., & Liu, J. (2016). Biosorption of reactive black 5 by modified *Aspergillus versicolor* biomass: Kinetics, capacity and mechanism studies. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 492, 242-248. https://doi.org/10.1016/j.colsurfa.2015.11.071

Hussain, S., Kamran, M., Khan, S.A., Shaheen, K., Shah, Z., Suo, H., …, & Ghani, U. (2021). Adsorption, kinetics and thermodynamics studies of methyl orange dye sequestration through chitosan composites films. *International Journal of Biological Macromolecules*, 168, 383-394. https://doi.org/10.1016/j.ijbiomac.2020.12.054

Joshi, M,, Bansal, R., & Purwar, R. (2004). Colour removal from textile effluents. *International Journal of Fibre and Textile Research*, 29, 239-259.

Katheresan, V., Kansedo, J., & Lau, S.Y. (2018). Efficiency of various recent wastewater dye removal methods: A review. *Journal of Environmental Chemical Engineering*, 6(4), 4676-4697. https://doi.org/10.1016/j.jece.2018.06.060

Kaya, M., Baublys, V., Sargin, I., Šatkauskienė, I., Paulauskas, A., Akyuz, B., …, & Yurtmen, H. (2016). How taxonomic relations affect the physicochemical properties of chitin. *Food Biophysics*, 11, 10-19. https://doi.org/10.1007/s11483-015-9404-5

Kumar, R.M.N.V. (2000). A review of chitin and chitosan applications. *Reactive and Functional Polymers*, 46 1–27. http://doi.org/10.1016/S1381-5148(00)00038-9

Liu, L., Gao, Z.Y., Su, X.P., Chen, X., Jiang, L., & Yao, J.M. (2015). Adsorption Removal of Dyes from Single and Binary Solutions Using a Cellulose-based Bioadsorbent. *Acs Sustainable Chemistry & Engineering*, 3, 432-442. https://doi.org/10.1021/sc500848m

Maderova, Z., Baldikova, E., Pospiskova, K., Safarik, I., & Safarikova, M. (2016). Removal of dyes by adsorption on magnetically modified activated sludge. *International Journal of Environmental Science and Technology*, 13, 1653–1664. https://doi.org/10.1007/s13762-016-1001-8

McKay, G., Blair, H.S., & Gardner, J. R. (1982). Adsorption of dyes on chitin. I. Equilibrium studies. *Journal of Applied Polymer Science*, 27, 3043-3057. https://doi.org/10.1002/app.1982.070270827

Millicent Mabel, M., Sundararaman, T.R., Parthasarathy, N., & Rajkumar, J. (2019). Chitin beads from peneaus sp. shells as a biosorbent for methylene blue dye removal. *Polish Journal of Environmental Studies*, 28(4), 2253-2259. https://doi.org/10.15244/pjoes/90359

Ratnamala, G.M., Shetty, K.V., & Srinikethan, G. (2012). Removal of remazol brilliant blue dye from dye-contaminated water by adsorption using red mud: Equilibrium, kinetic, and thermodynamic studies. *Water, Air, & Soil Pollution*, 223, 6187-6199. https://doi.org/10.1007/s11270-012-1349-4

Sen, S.K., Raut, S., Bandyopadhyay, P., & Raut, S. (2016). Fungal decolouration and degradation of azo dyes: A review. *Fungal Biology Reviews*, 30(3), 112-133. https://doi.org/10.1016/j.fbr.2016.06.003

Stingley, R.L., Zou, W., Heinze, T.M., Chen, H., & Cerniglia, C.E. (2010). Metabolism of azo dyes by human skin microbiota. *Journal of Medical Microbiology*, 59(1), 108-114. https://doi.org/10.1099/jmm.0.012617-0

Wong, Y.C., Szeto, Y.S., Cheung, W.H., & McKay, G. (2003). Equilibrium studies for acid dye adsorption onto chitosan. *Langmuir*, 19, 7888-7894. https://doi.org/10.1021/la030064y

Yadav, A.K., Jena, S., Acharya, B.C., & Mishra, B.K. (2012). Removal of azo dye in innovative constructed wetlands: Influence of iron scrap and sulfate reducing bacterial enrichment. *Ecological Engineering*, 49, 53-58. https://doi.org/10.1016/j.ecoleng.2012.08.032

Zhou, L., Jin, J., Liu, Z., Liang, X., & Shang, C. (2011). Adsorption of acid dyes from aqueous solutions by the ethylenediamine-modified magnetic chitosan nanoparticles. *Journal of Hazardous Materials*, 185(2-3), 1045-1052. https://doi.org/10.1016/j.jhazmat.2010.10.012

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Research Article/Araştırma Makalesi

Assessment of The Effect of *Thymbra capitata* Ethanolic Extract on *Galleria mellonella* Hemolymph Antioxidant Enzymes

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Abstract: Conehead thyme (*Thymbra capitata*) is widely distributed in the countries of the Mediterranean region and used due to its medical properties. The antibacterial, antifungal, and strong antioxidant properties of *T. capitata* are known. The model organism *Galleria mellonella* is mostly preferred for immunological studies and for the study of human pathogens. The aim of the study was to determine the effect of the ethanolic extract of *T. capitata* on the antioxidant defense of the hemolymph in *G. mellonella* larva. Solutions prepared with Phosphate-Buffered Saline (PBS) from the dry matter obtained from ethanolic extract at doses between 2 mg mL^{-1} and 20 mg mL^{-1} were injected into *G. mellonella* larvae. According to our findings, *T. capitata* extract had no effect on malondialdehyde (MDA) levels. However, it was determined that all doses between 10 to 20 mg mL^{-1} significantly reduced superoxide dismutase (SOD) and catalase (CAT) activities compared to the control groups. According to the results of our study, high doses of *T. capitata* extract had negative effects on *G. mellonella* antioxidant defense.

Keywords: Malondialdehyde, superoxide dismutase, catalase, total protein.

Thymbra capitata Etanolik Ekstraktının *Galleria mellonella* Hemolenf Antioksidan Enzimleri Üzerine Etkisinin Değerlendirilmesi

Öz: Acı kekik (*Thymbra capitata*), Akdeniz bölgesi ülkelerinde geniş yayılış göstermektedir ve medikal özelliklerinden dolayı kullanımı yaygındır. *T. capitata*'nın antibakteriyel, antifungal ve güçlü antioksidan özellikleri bilinmektedir. Model organizma *Galleria mellonella* bağışıklık araştırmaları için ve insan patojenlerinin araştırılmasında çoğunlukla tercih edilmektedir. Çalışmanın amacı, *T. capitata*'nın etanolik ekstraktının *G. mellonella* larvasında hemolenf antioksidan savunması üzerindeki etkisinin belirlenmesidir. Etanolik ekstraktan elde edilen kuru maddeden Fosfat Tampon Tuzu (PBS) ile 2 mg mL^{-1} ile 20 mg mL^{-1} arasındaki dozlarda hazırlanan çözeltiler *G. mellonella* larvalarına enjekte edilmiştir. Bulgularımıza göre *T. capitata* ekstraktının malondialdehit (MDA) düzeylerine etkisi yoktur. Ancak 10 - 20 mg mL^{-1} arasındaki dozların hepsinin kontrol gruplarına kıyasla süperoksit dismutaz (SOD) ve katalaz (CAT) aktivitelerini önemli ölçüde azalttığı belirlendi. Çalışmamızın sonuçlarına göre *T. capitata* ekstrakt yüksek dozları *G. mellonella* antioksidan savunması üzerinde olumsuz etkilere sahiptir.

Anahtar kelimeler: Malondialdehit, süperoksit dismutaz, katalaz, toplam protein.

1. Introduction

Conehead thyme (*Thymbra capitata*) is a medicinal and aromatic plant species that grows in the Mediterranean region and has important pharmacological properties related to its essential oil (Gagliano Candela et al., 2019). It is used as a source of antioxidants in the food industry (Blanco-Salas et al., 2010). Some medicinal properties of *T. capitata* extracts is antifungal (Palmeira-de-Oliveira et al., 2012), antispasmodic (Al-Qura'n, 2009), anti-inflammatory (Albano & Miguel, 2011), antiprotozoan (Machado et al., 2010), antibacterial (Marinelli et al., 2018), and antioxidant (Hortigón-Vinagre et al., 2014) capacity. It is the result of another study in which essential oils of *T. capitata* showed nematicidal activity against Pinewood nematode, *Bursaphelenchus xylophilus* (Barbosa et al., 2010). In the study of Vila (2002), *T. capitata* contained over 70% carvacrol (Faleiro et al., 2005; Saija et al., 2016) and thymol as a secondary important component and its antioxidant capacity was mainly due to these two contents (Gagliano Candela et al., 2019). Carvacrol (5-isopropyl-2-methyl phenol) is also a natural compound that occurs in the leaves of many herbs and plants including wild bergamot, thyme, and black pepper (Marchese et al., 2018). Studies on broiler chickens revealed that carvacrol and thymol increased antioxidant activity and supported immunity and growth (Acamovic & Brooker, 2005; Hashemipour et al., 2013; Silveira et al., 2013; Du et al.,2016).

Model organisms are generally defined as non-human species that have been extensively studied to understand a range of biological phenomena (Leonelli & Ankeny, 2013). The model organism is selected according to the biological phenomenon and the characteristics to be studied. In recent years, the importance of invertebrates among model organisms has increased when ethical concerns, the cost of growing specimens, the growth rate of specimens, and the physical conditions required for the cultivation of specimens are evaluated. Invertebrate model organisms include *G. mellonella* (Lepidoptera): among other things, its ability to withstand high temperatures offers unique opportunities for the evaluation of human pathogens. This strain is also the most preferred strain for immune studies (Eguchi & Iwabuchi, 2006; Mukherjee et al., 2010; Cook & McArthur, 2013).

The aim of the study was to determine the effect of *T. capitata* leaf extract on the antioxidant enzymes of the

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model organism *G. mellonella* hemolymph. *T. capitata* is consumed as a spice and for its therapeutic effects. The role of *T. capitata* in the regulation of antioxidant defense system, which is an important part of natural immunity in animals, was clarified with this study.

2. Material and Methods

2.1. Insect Rearing

Galleria mellonella specimens were grown in the Department of Biology, Faculty of Sciences, Çanakkale Onsekiz Mart University, Türkiye under equal photoperiod (12:12 light:dark) conditions under 25±2 °C and 65±5% relative humidity conditions. When the first larvae were seen in the jars created for the experiment, 10 gr of artificial food (Sak et al., 2006) was added to the medium and fed. The last stage larvae (0.18±0.02 gr) grown in these jars were chosen.

2.2. Plant material and extraction

The aerial parts of the *T. capitata* specimens, which grow naturally around the central district of Çanakkale province, were collected during the flowering season and dried naturally in a dark and airy environment in the Insect Physiology Laboratory of the Department of Biology of Çanakkale Onsekiz Mart University. 50 gr of these collected dry leaves were extracted by soxhlet extractor. At the end of this period, the ethanol was removed by rotary evaporator (OMNILab, China). The remaining 2 gr dry matter was dissolved in 100 mL phosphate buffer saline (PBS - Sigma, Germany pH: 7.4). The highest solubility levels of the dry material were determined as a 2 gr 100 mL^{-1}.

2.3. Injection

The Control (Untreated) and PBS injected groups were used as the control groups. As for the dose groups, 2-20 mg mL^{-1} doses prepared in PBS were injected into the body cavity from the last proleg of the *G. mellonella* larvae with the help of a 5 µl microinjector (Hamilton, USA). This procedure was performed at four different times (four repetitions) and using 4 larvae in each repetition (n=16). Both control and injected larvae were subjected to a 24-hour waiting period in glass petri dishes under the same temperature, humidity, and light conditions as the main colony-rearing conditions.

2.4. Hemolymph collection

24 hours after the injection, 20 µl of the hemolymph leaking from the larvae was taken and placed in microcentrifuge tubes containing 180 µl of phosphate buffer solution. This prepared hemolymph/phosphate buffer mixture was centrifuged for five minutes at 12 000 rpm at +4 °C. Afterwards, the cell-free supernatant was collected and stored at -20 °C until the experiments were performed.

2.5. Enzyme assays

All the studies for the determination of enzyme activities were carried out in a microplate reader (Thermo Scientific Multiskan GO Microplate Spectrophotometer, Finland) at the appropriate wavelength. In our study, for each dose and the control groups, a total of 16 larval hemolymph were collected in four independent time periods. The enzyme activity and total protein (TP) measurement were performed in four independent times after all four larval hemolymph was collected each time.

The TP determination in the study was carried out using the Bradford (1976) method. A 5 µl sample taken from the prepared hemolymph-phosphate buffer mixture was placed in the well of the 96-well F base microplate. A 155 µl of distilled water and 40 µl of Bradford reagent were added to the sample. After the prepared microplate was incubated for 30 minutes at room temperature, measurement was performed at 595 nm absorbance in the microplate reader. The results were determined as mg protein mL^{-1}.

While determining the Superoxide Dismutase (SOD) activity, the method of Flöhe and Ötting (1984) was used. The prepared hemolymph/phosphate buffer mixture was incubated for 20 minutes under bright light at room temperature by adding 3.5 µl of xanthine oxidase and 190 µl of SOD reagent on the 6.5 µl sample. After incubation, 6.5 µl of $CuCl_2$ (0.8 mM) was added to each well. The microplate prepared after the procedures was measured at 560 nm absorbance. The results obtained were calculated as unit mg $protein^{-1}$.

The Aebi (1984) method was used to determine the Catalase (CAT) activity. According to this, 60 µl of phosphate buffer (50 mM, pH=7.2) and 133.5 µl of H_2O_2 (30 mM) were added to the 6.5 µl sample of hemolymph/phosphate buffer mixture and kinetic measurements were taken at 240 nm absorbance for two minutes with 10-second intervals. The results were determined as mmol min^{-1} mg $protein^{-1}$.

While determining malondialdehyde (MDA) levels, the Buege and Aust (1978) method was used. While applying the method, 150 µl of TBA-TCA mixture was added to the 75 µl sample of hemolymph/phosphate buffer mixture and incubated at 90 °C for 20 minutes. After incubation, the measurement was made at 532 nm absorbance. The results were presented as nmol mg $protein^{-1}$.

2.6. Statistics

The obtained data were evaluated with one-way-ANOVA ($p<0.05$) and Tukey's HSD test in the SPSS statistical program.

3. Results

3.1. Hemolymph TP

The TP levels determined in the *G. mellonella* larval hemolymph after extract injection are presented in Figure 1. Accordingly, there was no significant difference between the groups in terms of TP. The highest TP value was determined in the 20 mg mL^{-1} dose group as 1.07 mg protein mL^{-1}. In the control group, it was determined as 1.025 mg protein mL^{-1}.

3.2. Hemolymph SOD activity

The data obtained as a result of the experiments of the study for the determination of SOD activity are shown in Figure 2. Accordingly, no significant difference was found between the control and PBS injection groups and the 2-8 mg mL^{-1} injection groups ($p>0.05$). However, a significant difference was determined between these groups and the 10-20 mg mL^{-1} injection groups ($p<0.05$). At the same time,

the difference between the 10-20 mg mL^{-1} injection groups was found to be insignificant (p>0.05).

3.3. Hemolymph CAT activity

The changes in CAT activity as a result of the injection of *T. capitata* doses in *G. mellonella* larval hemolymph are presented in Figure 3. The results obtained are similar to the results of SOD activity. No significant difference was found between the control groups and the 2-8 mg mL^{-1} injection groups (p>0.05). At the same time, the difference between the 10-20 mg mL^{-1} injection groups was found to be insignificant (p>0.05). However, a significant difference was found between these groups and the 10-20 mg mL^{-1} injection groups (p<0.05).

3.4. Hemolymph MDA levels

The MDA level results of the study are shown in Figure 4. According to the experimental results, the injection of *T. capitata* doses did not cause significant changes in the MDA level in *G. mellonella* larval hemolymph (p>0.05).

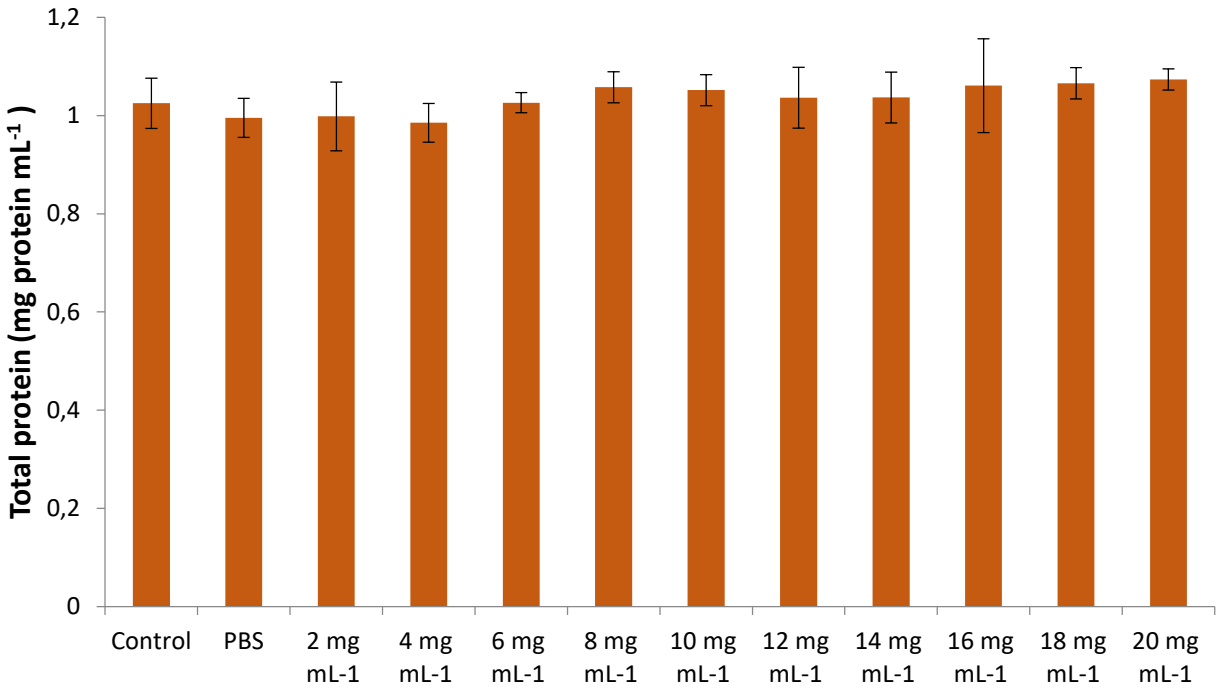

Figure 1. Total protein levels in *G. mellonella* larval hemolymph exposed to *T. capitata* extract. Each column represents the mean of 16 samples.

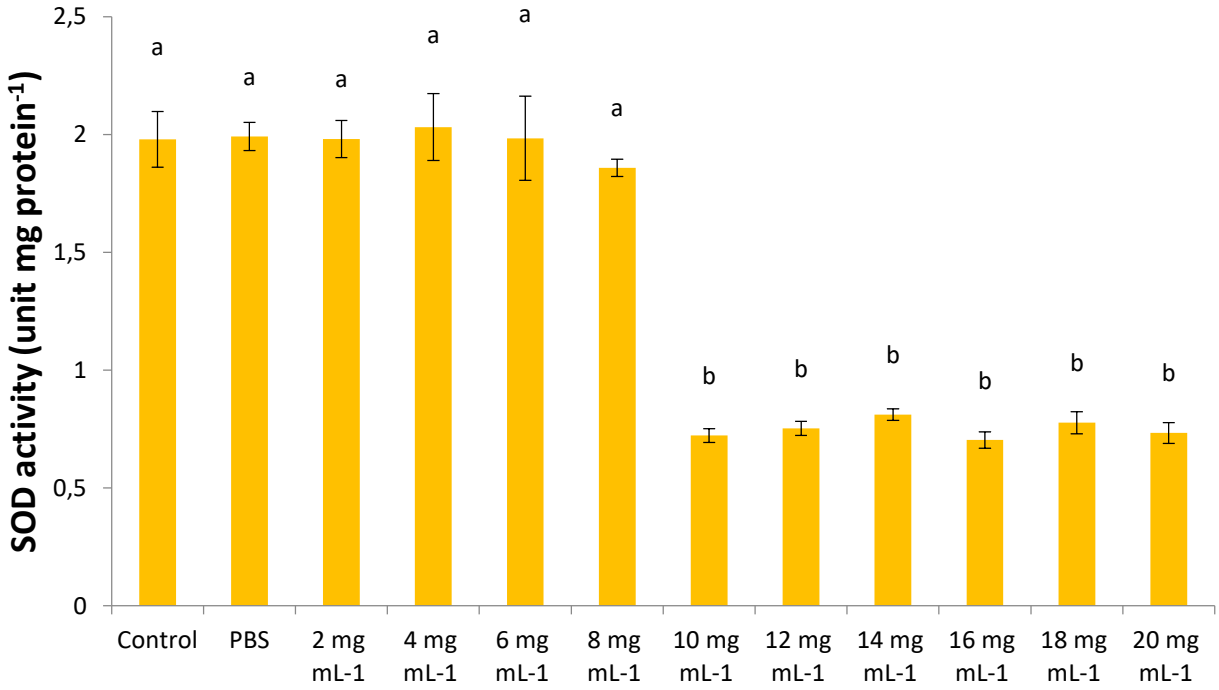

Figure 2. SOD activity changes in *G. mellonella* larval hemolymph exposed to *T. capitata* extract. Each column represents the mean of 16 samples. The difference between groups with different letters (a-b) was statistically significant (p<0.05).

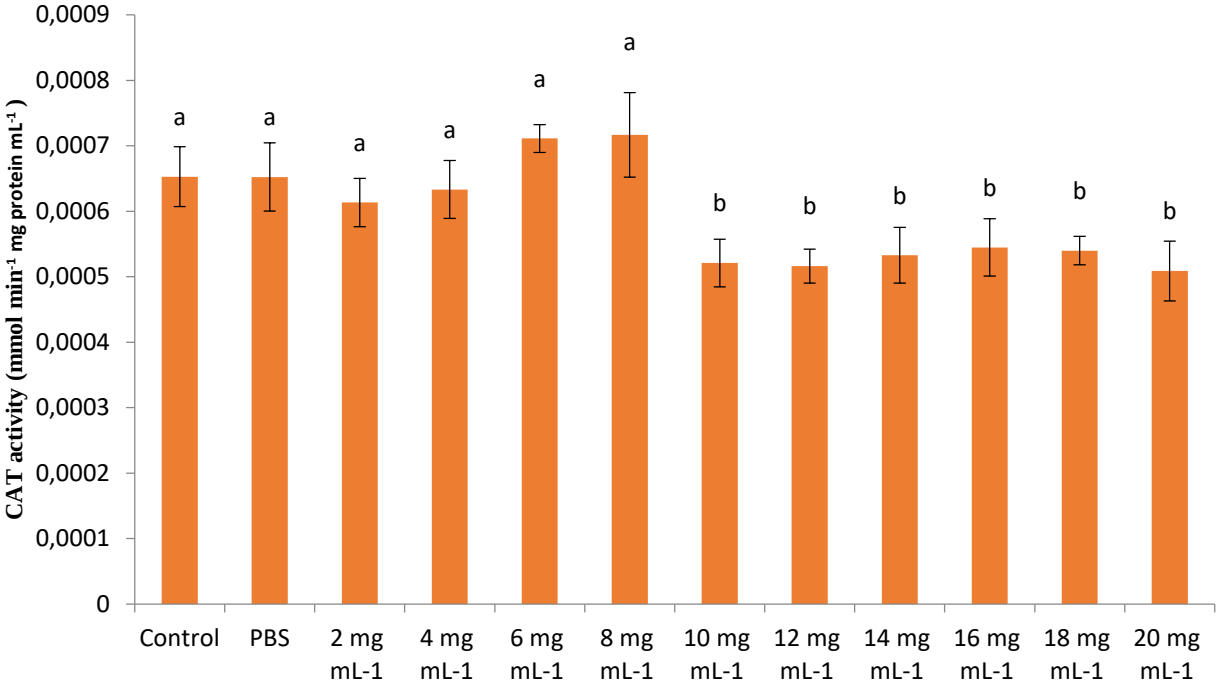

Figure 3. CAT activity changes in *G. mellonella* larval hemolymph exposed to *T. capitata* extract. Each column represents the mean of 16 samples. The difference between groups with different letters (a-b) was statistically significant ($p<0.05$).

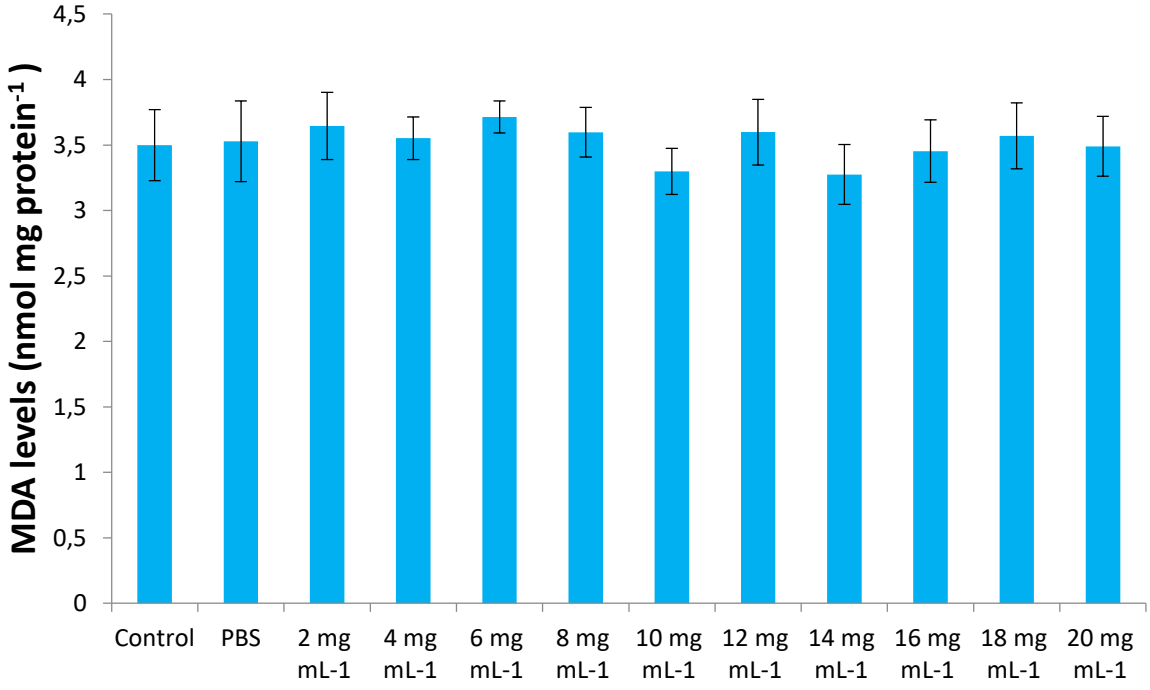

Figure 4. MDA levels changes in *G. mellonella* larval hemolymph exposed to *T. capitata* extract. Each column represents the mean of 16 samples.

4. Discussion

It is of vital importance to remove the oxygen radicals that occur as a result of normal body functions from the body. Hemolymph antioxidants are considered an important marker in immune studies as they support immunity in insects.

Studies reported that extracts of *T. capitata* showed strong antioxidant properties (Faleiro et al., 2005; Saija et al., 2016). In a study with Gilthead seabream (*Sparus aurata* L. - Sparidae), it was determined that the addition of *T. capitata* extract to the food caused lower indices of oxidation (Álvarez et al., 2012). In a study conducted with New Zealand white rabbits, it was determined that thymol reduced oxidative stress by showing antioxidant activity and could suppress the progression of hyperlipidemia and atherosclerosis due to high-fat diet by attenuating inflammatory responses (Yu et al., 2016).

There are many studies examining the effects of various natural or chemical substances on the antioxidant enzymes of the model organism *G. mellonella*. Lozinskaya

et al. (2004) found that free radical formation decreased due to suppression of the prophenoloxidase system and increased antioxidant activity in *G. mellonella* hemolymph in the sprogonium stage of microspores. It was determined that the antioxidant enzyme activity of *G. mellonella* larvae was not affected by increasing concentrations of boric acid (Hyršl et al., 2007). Dubovskiy et al. (2008) reported that *Bacillus thuringiensis* ssp. *galleriae* infection of *G. mellonella* increased the oxidative stress level in the larval midgut and that oxidative damage contributed to cell death in the midgut during infection in their study. Dubovskii et al. (2010) revealed a statistically significant increase in reactive oxygen species (ROS) formation in lymph and a decrease in enzymatic antioxidant activities in insect hemocytes after foreign body injection. In another study, researchers found that eicosanoids mediated enzymatic responses to insect antioxidants and dietary pro-oxidants (Büyükgüzel et al., 2010). In another study on *G. mellonella* with boric acid, it was found that catalase, superoxide dismutase, glutathione S-transferase, and glutathione peroxidase activities increased in relation to lipid peroxidation (Büyükgüzel et al., 2013). Büyükgüzel and Kayaoğlu (2014) found in their study that niclosamide had negative effects on the antioxidant enzymes of *G. mellonella*. Dere et al. (2015) examined the effects of azadirachtin on the antioxidant enzymes of *G. mellonella* in their study and found that it increased oxidative stress in this species depending on the dose. It was found that heavy metals increased the oxidative stress of *G. mellonella* (Wu & Yi, 2015). Zorlu et al. (2018) found that titanium dioxide nanoparticles included in the *G. mellonella* diet had a dose-dependent toxic effect and increased resistance to oxidative stress at low concentrations. Increasing doses of Cucurbitacin-E essential oil caused decreasing in SOD, CAT, GST GPx, GR, and AChE activities and increasing in MDA levels (Ercan et al., 2022).

As a result of a study examining the toxic effects of different plant extracts on *G. mellonella* larvae, the high variability of Lethal Dose (LD) values from one plant to another indicates that *G. mellonella* is highly sensitive to plant extracts and that *G. mellonella* can be used as a reliable system model for the evaluation of the toxicity of medicinal plants (Mbarga et al., 2021).

When evaluated in terms of the results of our study, it is seen that *T. capitata* extract does not affect lipid peroxidation and does not change the amount of TP in *G. mellonella* larval hemolymph. In the literature, it is seen that some substances have no effect on *G. mellonella* antioxidant enzymes, and some of them decrease antioxidant enzyme activities depending on the dose. In the results of our study, it was determined that a high rate (10 mg mL^{-1}) of *T. capitata* extract decreased SOD and CAT activities depending on the dose. These results are compatible with the literature.

Different effects on immunity are expected as different plants have different toxic effects on *G. mellonella* larvae (Mbarga et al., 2021). There are limited studies on the effects of medicinal plant extracts on *G. mellonella* immunity. Some plant extracts cause a decrease in the antioxidant enzyme activity of *G. mellonella* (Ercan et al., 2022), and this decrease is probably due to the effect of plant secondary metabolites. It was determined that *Helichrysum arenarium* increased cell-mediated immunity at low doses in *G. mellonella* larvae at certain doses and had no effect outside this range (Kaya et al., 2021). According to the results of the study with *Olea europea* leaf extract, immune responses were strengthened at the lowest dose in *G. mellonella* larvae (Kaya & Demir, 2020).

It is thought that the most important reason why *T. capitata*, which has strong antioxidant properties, cannot show these effects in the living body is the dilution of the enzyme rate when it enters the living body and the lack of optimum enzyme activity environment. It was also determined that Carvacrol nanoemulsion caused apoptosis in human lung adenocarcinoma (A549) cells by inducing the production of ROS (Khan et al., 2018).

According to our findings, the injection of *T. capitata* significantly reduced antioxidant enzyme activity in the model organism above a certain dose (10 mg mL^{-1}). This result shows that the use or consumption of this plant species, which is also preferred as a spice, in high doses reduces antioxidant enzyme activities which are critical for immunity. The results of our study support the results of Khan et al. (2018) study. Therefore, it is thought that the consumption of *T. capitata* should be within certain limits.

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References

Acamovic, T., & Brooker, J.D. (2005). Biochemistry of plant secondary metabolites and their effects in animals. *Proceedings of the Nutrition Society*, 64(3), 403-412. https://doi.org/10.1079/PNS2005449

Aebi, H. (1984). Catalase in vitro. *Methods of Enzymology*, 105, 121-126. https://doi.org/10.1016/S0076-6879(84)05016-3

Albano, S.M., & Miguel, M.G. (2011). Biological activities of extracts of plants grown in Portugal. *Industrial Crops and Products*, 33(2), 338-343. https://doi.org/10.1016/j.indcrop.2010.11.012

Al-Qura'n, S. (2009). Ethnopharmacological survey of wild medicinal plants in Showbak, Jordan. *Journal of Ethnopharmacology*, 123(1), 45-50. https://doi.org/10.1016/j.jep.2009.02.031

Álvarez, A., García, B.G., Jordán, M.J., Martínez-Conesa, C., & Hernández, M.D. (2012). The effect of diets supplemented with thyme essential oils and rosemary extract on the deterioration of farmed gilthead seabream (*Sparus aurata*) during storage on ice. *Food Chemistry*, 132(3), 1395-1405. https://doi.org/10.1016/j.foodchem.2011.11.127

Barbosa, P., Lima, A.S., Vieira, P., Dias, L.S., Tinoco, M. T., Barroso, J. G., ... & Mota, M. (2010). Nematicidal activity of essential oils and volatiles derived from Portuguese aromatic flora against the pinewood nematode, *Bursaphelenchus xylophilus*. *Journal of Nematology*, 42(1), 8.

Blanco-Salas, J., Téllez, T.R., Alonso, M.J.P., Pardo, F.M.V., de los Ángeles Cases Capdevila, M., & Gervasini Rodríguez, C. (2010). Chemical composition and antioxidant activity of the essential oil of *Thymbra capitata* (L.) Cav. in Spain. *Acta Botanica Gallica*, 157 (1), 55-63. https://doi.org/10.1080/12538078.2010.10516189

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*, 72(1-2), 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

Buege J.A., & Aust S.D. (1978). Microsomal Lipid Peroxidation, *Methods in Enzymology*, 52: 302-310. https://doi.org/10.1016/S0076-6879(78)52032-6

Buÿuĸgüzel, E., Buÿuĸgüzel, K., Snela, M., Erdem, M., Radtke, K., Ziemnicki, K., & Adamski, Z. (2013). Effect of boric acid on antioxidant enzyme activity, lipid peroxidation, and ultrastructure of midgut and

fat body of *Galleria mellonella*. *Cell biology and toxicology*, 29(2), 117-129. https://doi.org/10.1007/s10565-013-9240-7

Büyükgüzel, E., Hyršl, P., & Büyükgüzel, K. (2010). Eicosanoids mediate hemolymph oxidative and antioxidative response in larvae of *Galleria mellonella* L. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 156(2), 176-183. https://doi.org/10.1016/j.cbpa.2010.01.020

Büyükgüzel E., & Kayaoğlu S. (2014). The effect of niclosamide on some biological and physiological aspects of *Galleria mellonella* L. (Lepidoptera: Pyralidae). *Türkiye Entomoloji Dergisi*, 38(1), 83-100. https://doi.org/10.16970/ted.87976

Cook S.M., & McArthur J.D. (2013). Developing *Galleria mellonella* as a model host for human pathogens. *Virulence*, 4, 350-353. https://doi.org/10.4161/viru.25240

Dere, B., Altuntaş, H., & Nurullahoğlu, Z.U. (2015). Insecticidal and oxidative effects of azadirachtin on the model organism *Galleria mellonella* L. (Lepidoptera: Pyralidae). *Archives of insect biochemistry and physiology*, 89(3), 138-152. https://doi.org/10.1002/arch.21231

Du E, Wang W, Gan L, Li Z, Guo S., & Guo Y. (2016). Effects of thymol and carvacrol supplementation on intestinal integrity and immune responses of broiler chickens challenged with *Clostridium perfringens*. *Journal of animal science and biotechnology*. 7: Article number: 19. https://doi.org/10.1186/s40104-016-0079-7

Dubovskii, I.M., Grizanova, E.V., Chertkova, E.A., Slepneva, I.A., Komarov, D.A., Vorontsova, Y.L., & Glupov, V.V. (2010). Generation of reactive oxygen species and activity of antioxidants in hemolymph of the moth larvae *Galleria mellonella* (L.) (Lepidoptera: Piralidae) at development of the process of encapsulation. *Journal of Evolutionary Biochemistry and Physiology*, 46(1), 35-43. https://doi.org/10.1134/S0022093010010044

Dubovskiy, I.M., Martemyanov, V.V., Vorontsova, Y.L., Rantala, M.J., Gryzanova, E.V., & Glupov, V.V. (2008). Effect of bacterial infection on antioxidant activity and lipid peroxidation in the midgut of *Galleria mellonella* L. larvae (Lepidoptera, Pyralidae). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 148(1), 1-5. https://doi.org/10.1016/j.cbpc.2008.02.003

Eguchi D., & Iwabuchi K. (2006). A new cell line from the wax moth *Galleria mellonella* Linne (Lepidoptera: Pyralididae). *In Vitro Cellular & Developmental Biology-Animal*, 42, 1-3. https://doi.org/10.1007/s11626-006-0002-1

Ercan, F.S., Baş, H., & Azarkan, S Y. (2022). In silico detection of Cucurbitacin-E on antioxidant enzymes of model organism *Galleria mellonella* L.(Lepidoptera: Pyralidae) and variation of antioxidant enzyme activities and lipid peroxidation in treated larvae. *Beni-Suef University Journal of Basic and Applied Sciences*, 11(1), 1-17. https://doi.org/10.1186/s43088-022-00310-3

Faleiro, L., Miguel, G., Gomes, S., Costa, L., Venâncio, F., Teixeira, A., Figueiredo, A.C., Barroso J.G., & Pedro, L. G. (2005). Antibacterial and antioxidant activities of essential oils isolated from *Thymbra capitata* L.(Cav.) and *Origanum vulgare* L. *Journal of Agricultural and Food Chemistry*, 53(21), 8162-8168. https://doi.org/10.1021/jf0510079

Flöhe L., & Ötting F. (1984). Superoxide Dismutase Assays. Methods of Enzymology, 105, 93-104. https://doi.org/10.1016/S0076-6879(84)05013-8

Gagliano Candela, R., Maggi, F., Lazzara, G., Rosselli, S., & Bruno, M. (2019). The Essential Oil of *Thymbra capitata* and its Application as A Biocide on Stone and Derived Surfaces. *Plants*, 8(9), 300. https://doi.org/10.3390/plants8090300

Hashemipour H, Kermanshahi H, Golian A, & Veldkamp T. (2013). Effect of thymol and carvacrol feed supplementation on performance, antioxidant enzyme activities, fatty acid composition, digestive enzyme activities, and immune response in broiler chickens. *Poultry science*, 92, 2059-2069. https://doi.org/10.3382/ps.2012-02685

Hortigón-Vinagre, M.P., Blanco, J., Ruiz, T., & Henao, F. (2014). *Thymbra capitata* essential oil prevents cell death induced by 4-hydroxy-2-nonenal in neonatal rat cardiac myocytes. *Planta medica*, 80(15), 1284-1290. https://doi.org/10.1055/s-0034-1383051

Hyršl, P., Büyükgüzel, E., & Büyükgüzel, K. (2007). The effects of boric acid-induced oxidative stress on antioxidant enzymes and survivorship in *Galleria mellonella*. Archives of Insect Biochemistry and Physiology: Published in Collaboration with the *Entomological Society of America*, 66(1), 23-31. https://doi.org/10.1002/arch.20194

Kaya, S., Akkuş, G., Türkdoğan, S., & Gündüz, B. (2021). Influence of *Helichrysum arenarium* on hemocyte-mediated immune responses and phenoloxidase enzyme activity of model organism *Galleria mellonella* (L.). *International Journal of Tropical Insect Science*, 41(4), 2521-2528. https://doi.org/10.1007/s42690-021-00432-8

Kaya, S., & Demir, N. (2020). Zeytin (*Olea europaea*) Yaprağı Ekstraktının Model Organizma *Galleria mellonella* Hemosit Aracılı Bağışıklık Tepkileri Uzerine Etkileri. *Türk Tarım ve Doğa Bilimleri Dergisi*, 7(3), 646-653. https://doi.org/10.30910/turkjans.691138

Khan, I., Bahuguna, A., Kumar, P., Bajpai, V.K., & Kang, S.C. (2018). In vitro and in vivo antitumor potential of carvacrol nanoemulsion against human lung adenocarcinoma A549 cells via mitochondrial mediated apoptosis. *Scientific Reports*, 8(1), 144. https://doi.org/10.1038/s41598-017-18644-9

Leonelli, S., & Ankeny, R.A. (2013). What makes a model organism? *Endeavour*, 37(4), 209-212. https://doi.org/10.1016/j.endeavour.2013.06.001

Lozinskaya, Y.L., Slepneva, I.A., Khramtsov, V.V., & Glupov, V.V. (2004). Changes of the antioxidant status and system of generation of free radicals in hemolymph of *Galleria mellonella* larvae at microsporidiosis. *Journal of Evolutionary Biochemistry and Physiology*, 40(2):119-125. https://doi.org/10.1023/B:JOEY.0000033802.97996.65

Machado, M., Dinis, A. M., Salgueiro, L., Cavaleiro, C., Custódio, J. B., & do Céu Sousa, M. (2010). Anti-Giardia activity of phenolic-rich essential oils: effects of *Thymbra capitata*, *Origanum virens*, *Thymus zygis* subsp. *sylvestris*, and *Lippia graveolens* on trophozoites growth, viability, adherence, and ultrastructure. *Parasitology Research*, 106(5), 1205-1215. https://doi.org/10.1007/s00436-010-1800-7

Marchese, A., Arciola, C.R., Coppo, E., Barbieri R., Barreca D., & Chebaibi S. (2018). The natural plant compound carvacrol as an antimicrobial and anti-biofilm agent: mechanisms, synergies and bio-inspired anti-infective materials. *Biofouling*. 34, 630-56. https://doi.org/10.1080/08927014.2018.1480756

Marinelli, L., Di Stefano, A., & Cacciatore, I. (2018). Carvacrol and its derivatives as antibacterial agents. *Phytochemistry Reviews*, 17(4), 903-921. https://doi.org/10.1007/s11101-018-9569-x

Mbarga, M. J. A., Podoprigora, I. V., & Anyutoulou, K. L. D. (2021). *Galleria mellonella* (greater wax moth) as an eco-friendly in vivo approach for the assessment of the acute toxicity of medicinal plants: Application to some plants from Cameroon. *Open Veterinary Journal*, 11(4), 651-661. https://doi.org/10.5455/OVJ.2021.v11.i4.15

Mukherjee, K., Altincicek, B., Hain, T., Domann, E., Vilcinskas, A., & Chakraborty, T. (2010). *Galleria mellonella* as a Model System for Studying Listeria Pathogenesis. *Applied And Environmental Microbiology*, 76 (1), 310–317. https://doi.org/10.1128/AEM.01301-09

Palmeira-de-Oliveira, A., Gaspar, C., Palmeira-de-Oliveira, R., Silva-Dias, A., Salgueiro, L., Cavaleiro, C. & Rodrigues, A.G. (2012). The anti-Candida activity of *Thymbra capitata* essential oil: effect upon pre-formed biofilm. *Journal of Ethnopharmacology*, 140(2), 379-383. https://doi.org/10.1016/j.jep.2012.01.029

Saija, A., Speciale, A., Trombetta, D., Leto, C., Tuttolomondo, T., La Bella, S., & Napoli, E. (2016). Phytochemical, Ecological and Antioxidant Evaluation of Wild Sicilian Thyme: *Thymbra capitata* (L.) Cav. *Chemistry & Biodiversity*, 13(12), 1641-1655. https://doi.org/10.1002/cbdv.201600072

Sak O., Uçkan F., & Ergin E., (2006). Effects of cypermethrin on total body weight, glycogen, protein, and lipid contents of *Pimpla turionellae* (L.) (Hymenoptera: Ichneumonidae), *Belgian Journal of Zoology*, 136, 53-58.

Silveira S.R.C., Andrade L.N., & Sousa D.P. (2013). A Review on Anti-Inflammatory Activity of Monoterpenes. *Molecules*. 18, 1227-1254. https://doi.org/10.3390/molecules18011227

Vila, R. (2002). Flavonoids and further polyphenols in the genus Thymus (pp. 144-176). in ed: Stahl- Biskup, E., & Sáez, F. *Thyme: the genus Thymus*. CRC Press. Taylor & Francis: New York.

Wu, G., & Yi, Y. (2015). Effects of dietary heavy metals on the immune and antioxidant systems of *Galleria mellonella* larvae. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 167, 131-139. https://doi.org/10.1016/j.cbpc.2014.10.004

Yu, Y.M., Chao, T.Y., Chang, W.C., Chang, M.J., & Lee, M.F. (2016). Thymol reduces oxidative stress, aortic intimal thickening, and inflammation-related gene expression in hyperlipidemic rabbits. *Journal of Food and Drug Analysis*, 24(3), 556-563. https://doi.org/10.1016/j.jfda.2016.02.004

Zorlu, T., Nurullahoğlu, Z.U., & Altuntaş, H. (2018). Influence of dietary titanium dioxide nanoparticles on the biology and antioxidant system of model insect, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae). *Journal of the Entomological Research Society*, 20(3), 89-103.

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Research Article/Araştırma Makalesi

The Morphology of the Sensilla on the Proboscis of *Aporia crataegi* (Linnaeus, 1758) (Lepidoptera: Pieridae)

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Abstract: Proboscis structure and sensilla types are important morphological characters for the systematic analysis of Lepidoptera families. There is no study on proboscis structure and sensilla types of *Aporia crataegi* (Linnaeus, 1758) (Lepidoptera: Pieridae) despite the fact that it is an important pest. For this purpose, the sensilla types and proboscis structure of *A. crataegi* were investigated by using stereomicroscope and scanning electron microscope in detail. The results show that the proboscis of *A. crataegi* has three sensillum types (sensilla basiconica, sensilla trichodea, and sensilla styloconica). Sensilla basiconica consists of a sensory cone with a single terminal pore surrounded by a shallow socket and has a flat surface. Sensilla trichodea (chaetica) is bristle-shaped. The bristles of sensilla trichodea are poreless and smooth. Sensilla styloconicum has a smooth stylus, blunt tip, and long peg. In this study, the proboscis structure and sensilla types of *A. crataegi* were discussed with morphological similarities and differences of the other lepidopteran species' proboscis structure and sensilla types. Thus, they contribute to the understanding of proboscis structure and sensilla types in Lepidoptera including Pieridae.

Keywords: Sensilla basiconica, Sensilla trichodea, Sensilla styloconica, scanning electron microscope.

Aporia crataegi (Linnaeus, 1758) (Lepidoptera: Pieridae) Emme Hortumundaki Sensilla Morfolojisi

Öz: Lepidoptera familyalarının sistematik analizinde emme hortumu yapısı ve sensilla tipleri önemli morfolojik karakterlerdir. Zararlı bir tür olmasına rağmen *Aporia crataegi* (Linnaeus, 1758) (Lepidoptera: Pieridae)'nin emme hortumu yapısı ve sensilla tipleri üzerine bir çalışma bulunmamaktadır. Bu amaçla *A. crataegi*'nin sensilla tipleri ve hortum yapısı stereomikroskop ve taramalı elektron mikroskobu kullanılarak detaylı bir şekilde incelenmiştir. Sonuçlar, *A. crataegi*'nin emme hortumunun üç sensilla tipine (sensilla basiconica, sensilla trichodea ve sensilla styloconica) sahip olduğunu göstermektedir. Sensilla basiconica, sığ bir yuva ile çevrili tek bir terminal gözenek ve düz bir yüzeye sahip duyusal bir koniden oluşur. Sensilla trichodea (chaetica) kıl şeklindedir. Sensilla trichodea'nın kılları gözeneksiz ve pürüzsüzdür. Sensilla styloconicum'un kör uçlu, düz bir stilusu ve uzun bir peg kısmı vardır. Bu çalışmada A. crataegi'nin emme hortumu yapısı ve sensilla tipleri ile diğer lepidoptera türlerinin emme hortumu yapısı ve sensilla tiplerinin morfolojik benzerlikleri ve farklılıkları tartışılmıştır. Böylece, Pieridae dahil Lepidoptera'daki emme hortumu yapısı ve sensilla tiplerinin anlaşılmasına katkıda bulunulmuştur.

Anahtar kelimeler: Sensilla basiconica, Sensilla trichodea, Sensilla styloconica, taramalı elektron mikroskobu.

1. Introduction

Lepidoptera consists of approximately 150,000 species and constitutes the second largest class of insects (Bibi et al., 2022). The Black-veined White *Aporia crataegi* L. (Lepidoptera: Pieridae) is a trans-Palearctic species with high migratory activity, a pest of various fruit and berry crops, with population outbreaks causing complete deforestation (Ilyinskiy & Tropin, 1965; Tolman & Lewington, 2008; Maximov & Marushchak, 2012).

Most adult Lepidoptera proboscis obtain their nutrients by sucking only liquid with their sucking mouthparts in the form of rostrum. Liquid food is drawn into the mouth by the sibarium or pharynx muscles. The proboscis of butterflies is highly elongated, composed of thin maxillary galea, and this proboscis can be elongated by an increase in hemolymph pressure (Gullan & Cranston, 2014). The proboscis is equipped with microtrichia, a row of lamellata, several porous sensilla, and a small muscle attached to the tubules along with the bristle-shaped sensilla (Krenn & Kristensen, 2004). Sensilla play important roles in feeding behavior and host site (Zaspel et al., 2013). The outer surface of the Lepidopteran proboscis can bear six morphological sensilla types: basiconicum, chaeticum, styloconicum, filiformium, coeloconicum, and campaniformium. The first three types are the most common. There are four types of pore systems: porous, single-pore, multi-porous, and single-pore-multi-porous (Faucheux, 2013). Sensillum styloconicum has a long stylus and a shorter peg (Paulus & Krenn, 1996). Sensillum basiconicum has a shorter stylus than peg. This sensilla is considered as a chemo or mechanical sensor (Krenn, 1998). Sensillum trichodeum is a hair-like sensory organ. It is considered mechanically sensitive (Krenn, 1998). The morphology and sensilla distribution of the proboscis differ depending on their feeding habits (Faucheux, 2013). Types, sizes, and shapes of sensilla on proboscis differ interspecifically.

This study contributed to the studies of all Lepidoptera species including Pideridae by describing the variation of sensilla types on proboscis composition in *Aporia crataegi* and comparing them with other lepidopteran species.

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2. Material and Methods

Ten adult *Aporia crataegi* were collected from Ödek Village, Sivas province, Türkiye in June 2019. The proboscises from *A. crataegi* were cleaned and photographed under an Olympus SZX7 stereomicroscope (SM). Then, they were dehydrated in ethanol series (from 70 to 99%). Next, the proboscises were transferred to hexamethyldisilazane (HMDS) and air dried (Nation, 1983). After drying in air, samples were sprayed with gold (Polaron SC 502). Proboscises were placed in the stubs. Then, they were examined by scanning under electron microscope (SEM) (JEOL JSM 6060 LV) at 10 kV.

3. Results

The proboscis is a tubular structure which consists of the two elongated proboscis (galea) (Figs. 1-3). Proboscis of *Aporia crataegi* is long and is characterized by 3 different types of sensilla (basiconica, trichodea, and styloconica) according to their external morphology (Figs. 4-8). At rest, the proboscis of *A. crataegi* forms 5–6 spirals in a convoluted state (Figs. 2a, b). The proboscis was about 8 mm long. The surface of the proboscis showed a spiny appearance. The spines (microtrichia) extend from the proximal point to the tip of the proboscis in all areas of the galea (Figs. 3a, b). Dorsal legulae form overlapping double rows of finger-like projections (Fig. 3b). Various sensilla types are found along the entire proboscis. Three morphological types can be found on the exterior of the proboscis as sensilla basiconica, sensilla trichodea, and sensilla styloconica (Figs. 4-8). Sensilla basiconica consists of a cone-shaped sensory cone with a single terminal pore surrounded by a shallow socket and has a flat surface (Figs. 4a, b). Sensilla basiconica is 3 µm long and 2µm wide. There are numerous bristle-shaped sensilla trichodea (chaetica) of varying lengths from proximal to distal to the galea. Sensilla trichodea are arranged in irregular rows on the surface of the galea. Sensilla trichodea of *A. crataegi* vary 2.77–22.5 µm long. The bristles of sensilla trichodea are poreless and smooth (Figs. 5-6). The galea is tuberculous toward its distal end (Fig. 7b). Sensilla styloconica is located distal to the galea (Figs. 7a, b). Sensilla styloconicum has a smooth stylus, blunt tip, and long peg (Figs. 6-8). Peg is 5 µm long 2 µm wide.

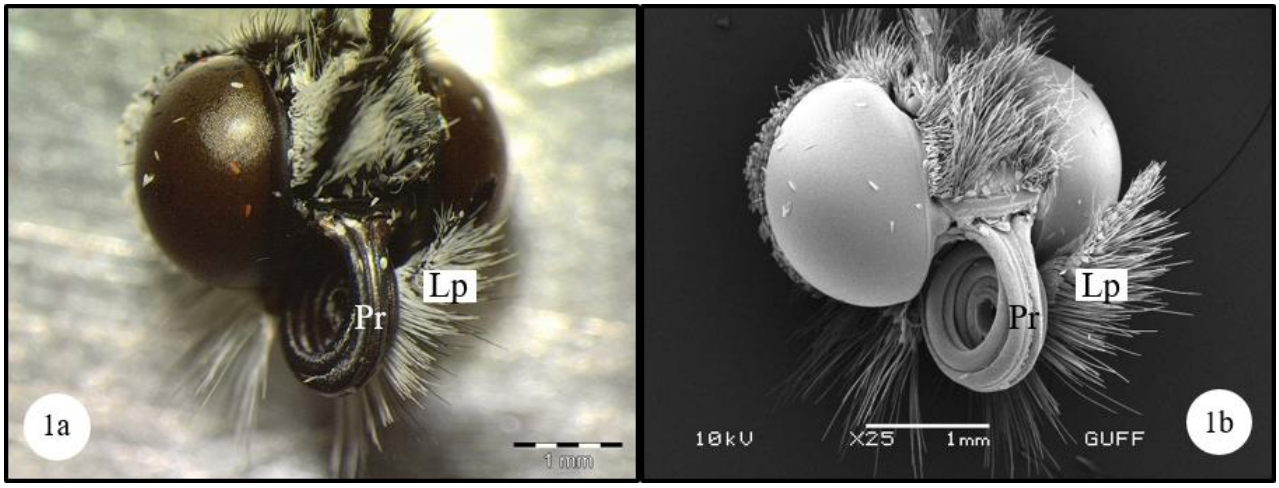

Figure 1. a, b. The general view of proboscis (SM, SEM). Pr-proboscis, Lp-labial palp.

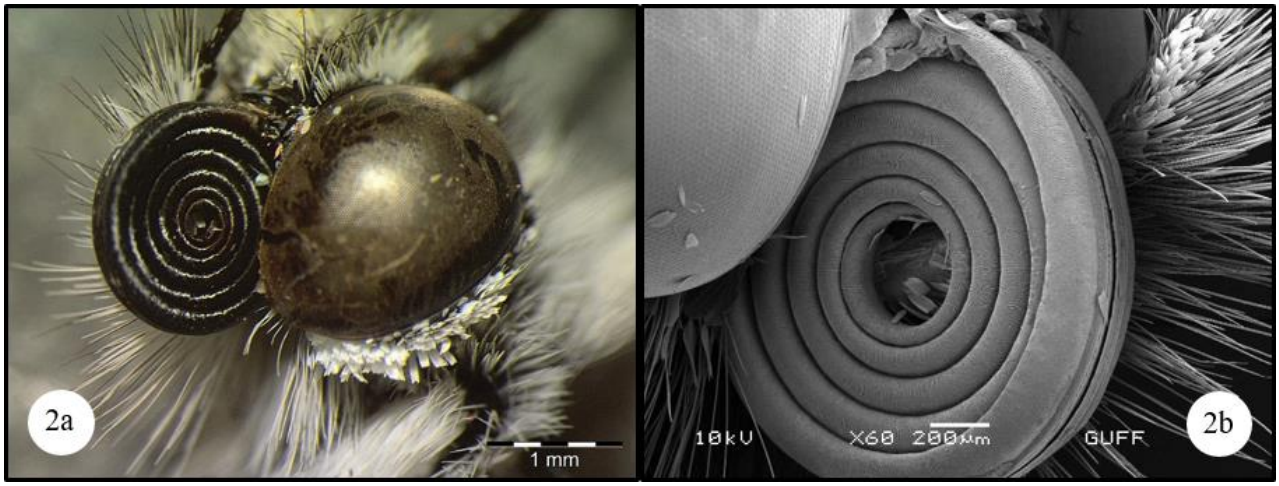

Figure 2. a, b. The lateral view of proboscis (SM, SEM).

4. Discussion

We identified three types of sensilla on the proboscis of adult *A. crataegi*. The morphology of these sensilla was compared with the other Lepidopteran species. Proboscis lengths vary according to the nutrient the butterfly is fed. There has been a process of evolution during which the length of the proboscis to receive pollen from flowers with long spurs has been increasing. It is seen that the helix number of proboscis of *A. crataegi* is 5–6. In *Pieris rapae* (Linnaeus, 1758) (Pieridae) and *Inachis nio* (Linnaeus, 1758) (Nymphalidae), the number of coils varies from 3.5 to 7

coils (Krenn, 1990). The proboscis coil number of *Helicoverpa armigera* (Hübner, 1805) (Noctuidae), *Mythimna separate* Walker, 1865 (Noctuidae), *Scotogramma trifolii* (Hufnagel, 1766) (Noctuidae) and *Protoschinia scutosa* (Denis & Schiffermüller, 1775) (Noctuidae) is 4-5 (Chen et al., 2019; Zhang et al., 2021).

The length of the proboscis varied. The proboscis of *A. crataegi* is about 8 mm long. The proboscis of *Tuta absoluta* (Meyrick, 1917) (Gelechiidae) is 1.5 mm long (Abd El-Ghany & Faucheux, 2022). The proboscis lengths of *H. armigera* (Noctuidae) and *M. separate* (Noctuidae) are ~11.38 mm and ~10 mm (Chen et al., 2019). The proboscis length in *Pieris brassicae* (L. 1758) (Pieridae) is 16 mm, in *Macroglossum stellatarum* (L. 1758) (Sphingidae) has 25–28 mm, in *Sphinx ligustri* (L. 1758) (Sphingidae) has 37–42 mm (Amsel, 1938). The proboscis of *Vanessa cardui* (Linnaeus, 1758) (Nymphalidae) is 11.5–15.5 mm long (Krenn, 1998).

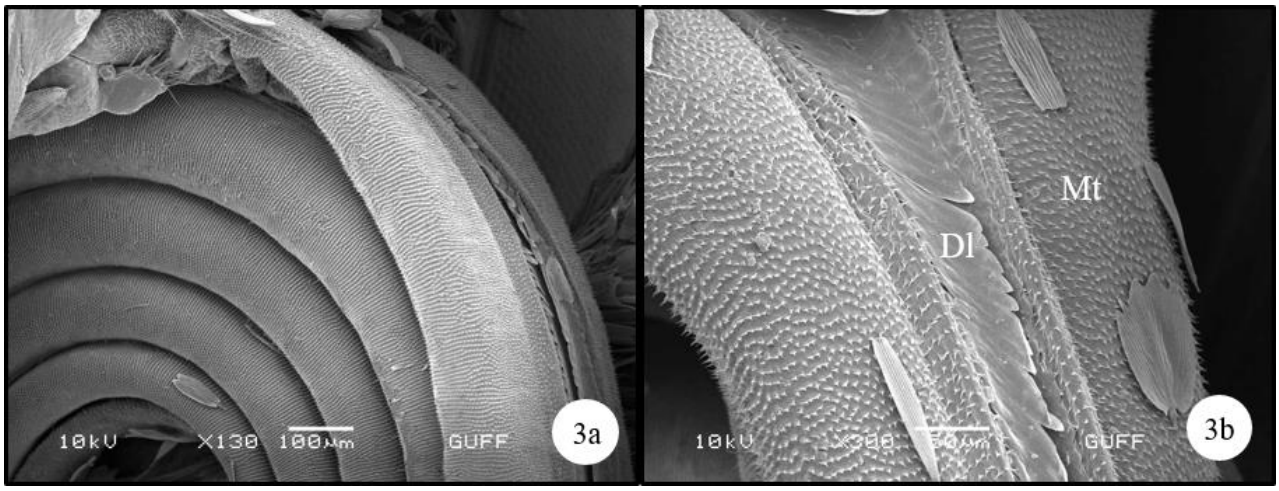

Figure 3. a, b. The spines (microtrichia) extend from the proboscis surface (SEM). Mt- microtrichia, Dl-Dorsal ligulae.

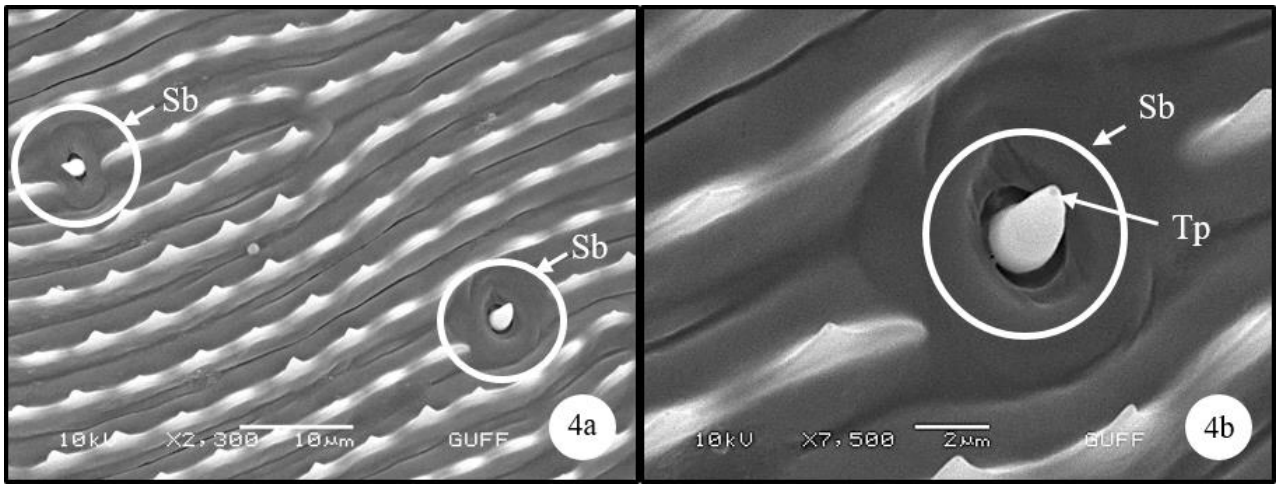

Figure 4. a, b. Sensilla basiconica (Sb) which is composed of a sensory cone with a smooth surface and a single terminal pore (Tp) (SEM).

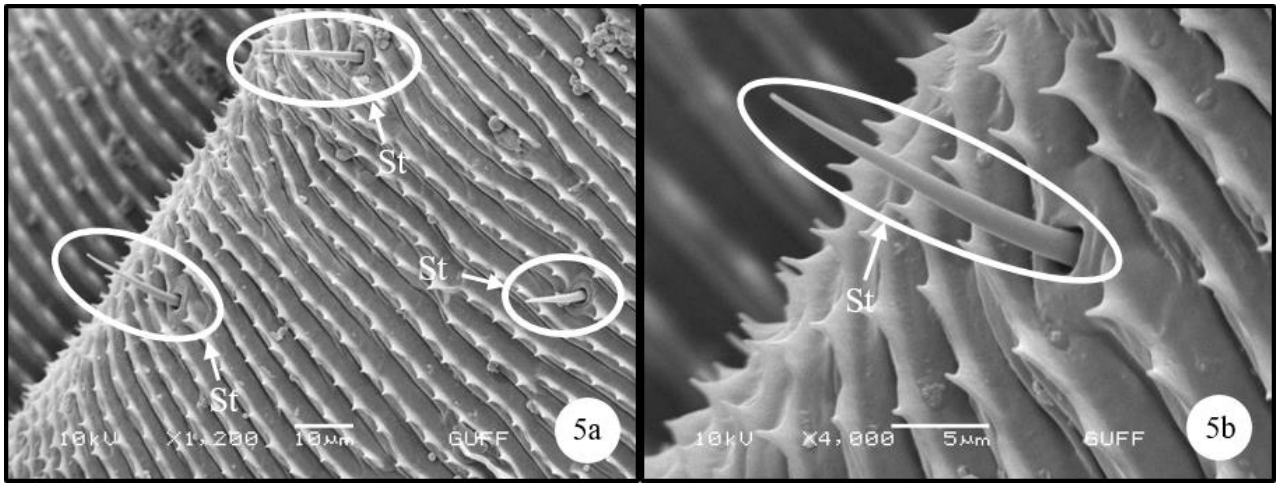

Figure 5. a, b. Bristle-shaped sensilla trichodea (St) which varied in lengths (SEM).

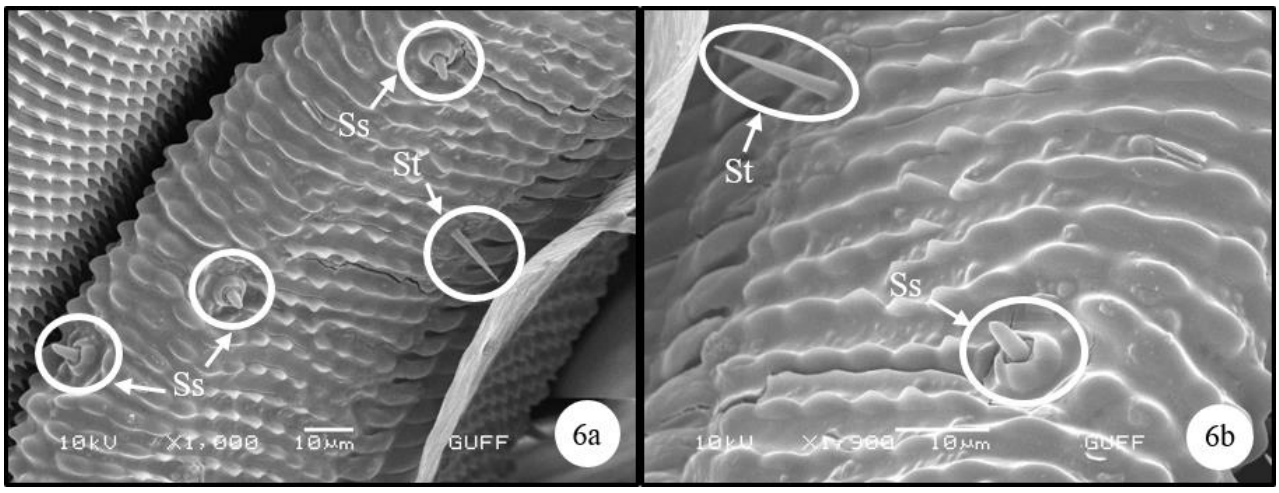

Figure 6. a, b. Sensilla trichodea (St) and sensilla styloconica (Ss) (SEM).

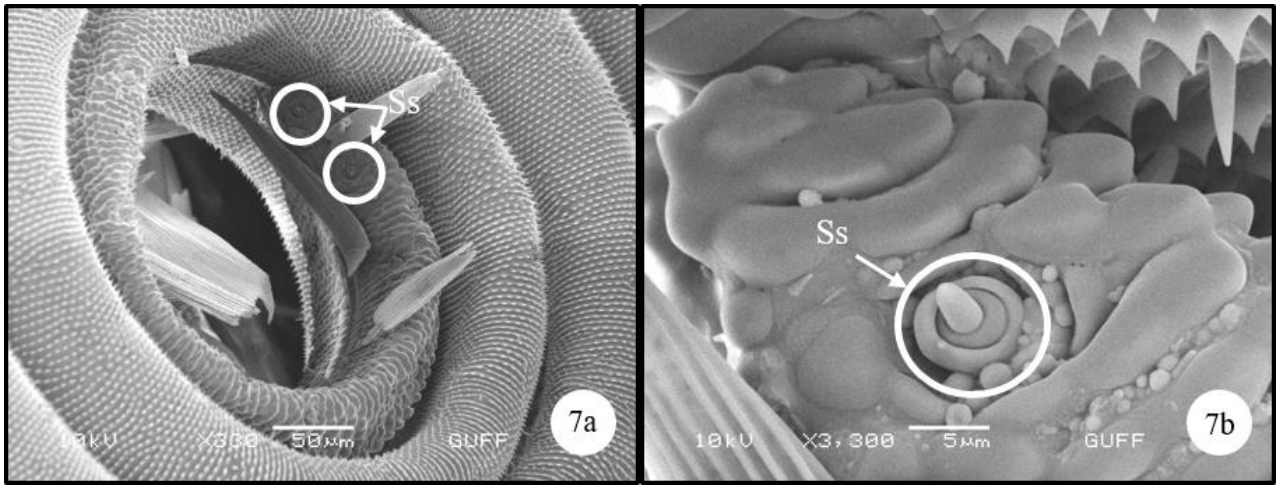

Figure 7. a, b. Sensilla styloconica (Ss) which is located distal to the proboscis (SEM).

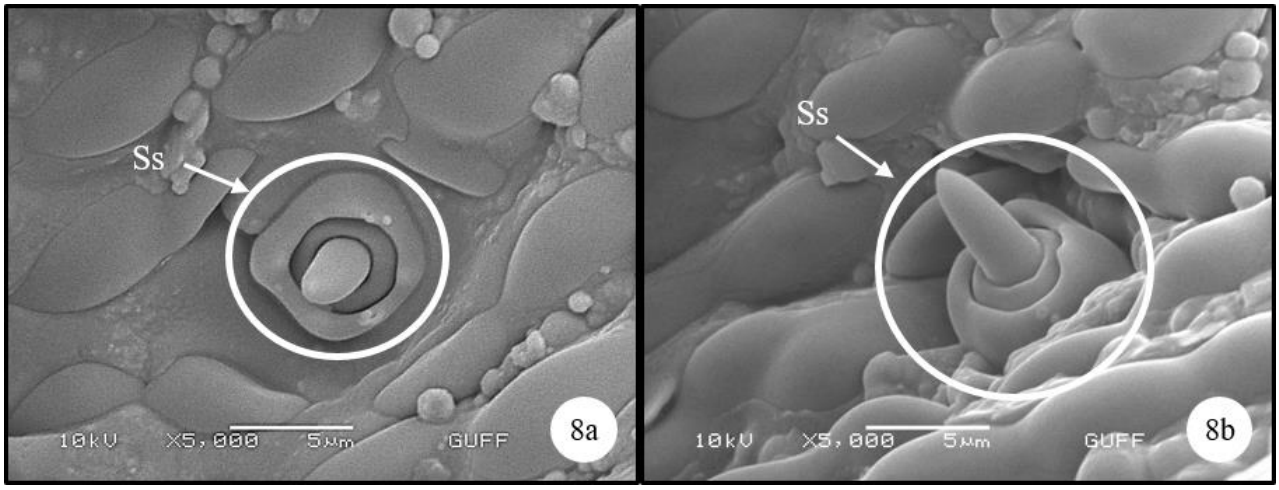

Figure 8. a, b. Sensilla styloconica (Ss)which have a smooth stylus, blunt tip, and long peg (SEM).

Sensilla are classified according to the presence or absence of pores and the morphology of the sensilla (basonica, chaetica, styloconica, filiformia, coeloconica, and campaniformia) (Faucheux, 2013). In *A. crataegi* proboscis, there are a total of three major types of sensilla as in *Parara guttata* (Bremer & Grey, 1852) (Hesperiidae), *Colias fieldii* Ménétriés, 1855 (Pieridae), *Celastrina oreas* (Leech, [1893]) (Lycaenidae), *Sasakia charonda* (Hewitson, 1863) (Nymphalidae), *Acraea issoria* (Hübner, 1819) (Nymphalidae), *Stichophthalma neumogeni* Leech, [1892] (Nymphalidae), *Callerebia suroia* Tytler, 1914 (Nymphalidae), *Libythea celtis* (Laicharting, 1782) (Nymphalidae), *Scotogramma trifolii* Rottemberg (Noctuidae), and *Protoschinia scutosa* (Denis & Schiffermüller, 1775) (Noctuidae), (Ma et al., 2019; Zhang et al.,2021). *Helicoverpa armigera* (Hübner, 1805) (Noctuidae) has three major types of sensilla, including nine subtypes as sensilla basiconica (esb1, esb2, esb3, isb1, and isb2), sensilla chaetica (sch1 and sch2), and sensilla styloconica (ss1 and ss2) (Guo et al.,2018). The types of

proboscis sensilla of *Iphiclides podalirius* (Linnaeus, 1758) (Papilionidae) and *Tirumala limniace* (Cramer, [1775]) (Nymphalidae) are sensilla chaetica, sensilla coeloconica, and sensilla basiconica (Ma et al., 2019). *T. absoluta* (Gelechiidae) has four sensillum types, that are aporous sensilla chaetica, uniporous sensilla chaetica, uniporous sensilla styloconica, and aporous sensilla squamiformia (Abd El-Ghany & Faucheux, 2022).

The length of the sensilla differs from species to species. Sensilla basiconica of *A. crataegi* is 3 µm long. The cone measures 1.6 µm in length in *Homoeosoma electellum* (Hulst, 1887) (Pyralidae), 4 µm in *Monopis crocicapitella* (Clemens, 1859) (Tineidae), and 10 µm in *Tineola bisselliella* (Hummel, 1823) (Tineidae) (Faucheux, 2013).

Sensilla trichodea of *A. crataegi* vary 2.77–22.5 µm long. Sensilla trichodea lengths are ~4 µm in *T. limniace* (Nymphalidae) and ~6 µm in *I. podalirius* (Papilionidae) (Ma et al., 2019). Sensilla trichodea measures are 6–9 µm in *Homoeosoma* sp. (Pyralidae), 8–15 µm in *Adela reaumurella* (L. 1758) (Adelidae) (Faucheux 1991, 1995, 2005, 2009). In *V. cardui* (Nymphalidae), sensilla trichodea greatly varies in length (8–48 µm) (Krenn, 1998).

Sensilla styloconica of *Aporia crataegi* (Linnaeus, 1758) (Pieridae) has one type. Sensilla styloconica length is 5 µm. Sensilla styloconica in *Avatha bubo* (Geyer, 1832) (Erebidae) has two different types: the measure of first type 7.8–9.5 µm in length and the second type is 5.9–7.4 µm in length (Faucheux, 2013). Sensilla styloconica lengths are ~10 µm in *P. guttata* (Hesperiidae), ~11 µm in *Co. fieldii* (Pieridae), ~14 µm in *Ce. oreas* (Lycaenidae), ~15 µm in *A. issoria* (Nymphalidae), ~27 µm in *Ca. suroia* (Nymphalidae), ~44 µm in *L. celtis* (Nymphalidae) (Ma et al., 2019). Reinwald et al. (2022) defined sensilla styloconica length as 28.8 µm in *Protambulyx strigilis* (Linnaeus, 1771) (Sphingidae), 33.3 µm in *Neococytius cluentius* (Cramer, 1776) (Sphingidae), 25.4 µm in *Sphinx pinastri* Linnaeus, 1758 (Sphingidae), 28.3 in *Euryglottis aper* (Walker, 1856) (Sphingidae) and *Xylophanes pyrrhus* Rothschild & Jordan, 1906 (Sphingidae), and 31 µm in *Manduca scutata* (Rothschild & Jordan, 1903) (Sphingidae). Microtrichia observed on the surface of the galea are common on the proboscis and are likely to act as mechanical receptors (Molleman et al., 2005). They also help collect and fix the pollen (Gilbert, 1972).

Proboscis structure and sensilla types are important morphological characters in the systematic analysis of Lepidoptera families.

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Conflict of interest: The authors declared that there is no conflict of interest.

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References

Amsel, H.G. (1938). *Amphimoea walkeri* Bsd. Der Schwarmer mit dem längsten Rüssel. *Entomologische Rundschau*, 55, 165-167.

Bibi, M., Bibi, S., Akhtar, N., Ullah, Z., Khan, M. F., & Qureshi, I. Z. (2022). Butterfly (Order: Lepidoptera) species Richness, diversity and distribution in different localities of Battagram, Pakistan. *Saudi Journal of Biological Sciences*, 29, 1853-1857. https://doi.org/10.1016/j.sjbs.2021.10.039

Chen, Q.X., Li, W.L., Chen, Y.W., Chen, J., & Song, Y.Q. (2019). Morphological comparison of proboscides and associated sensilla of *Helicoverpa armigera* and *Mythimna separate* (Lepidoptera: Noctuidae). *Arthropod Structure & Development*, 49, 119-127. https://doi.org/10.1016/j.asd.2018.12.001

Faucheux, M.J. (1991). Morphology and distribution of sensilla on the cephalic appendages, tarsi and ovipositor of the European sunflower moth, *Homoeosoma nebulella* Den. & Schiff. (Lepidoptera: Pyralidae). *International Journal of Insect Morphology and Embryology*, 20, 291-307. https://doi.org/10.1016/0020-7322(91)90018-5

Faucheux, M.J. (1995). Sensilla on the antennae, mouthparts, tarsi and ovipositor of the sunflower moth, *Homoeosoma electellum* (Hulster) (Lepidoptera: Pyralidae): a scanning electron microscopic study. *Annales des Sciences Naturelles (Zoologie, Biologie animale)*, 16, 121-136.

Faucheux, M.J. (2005). *Heterobathmia pseuderiocrania* (Heterobathmiina): mouthparts, mouthpart sensilla, and comparison with other non-glossatan suborders (Insecta, Lepidoptera). *Revista del Museo Argentino de Ciencias Naturales*, nueva serie 7, 57-65.

Faucheux, M.J. (2009). Deux types de sensilles basiconiques à pores pariétaux sur la trompe de l'Adèle de Réaumur, *Adela reaumurella* (Linnaeus, 1758) (Lepidoptera: Adelidae): Considérations morpho-fonctionnelles et implications phylogénétiques. *Bulletin de la Société des Sciences Naturelles de l'Ouest de la France (n.s.)*, 31, 84-94.

Faucheux, M.J. (2013). Sensillum types on the proboscis of the Lepidoptera: a review. *Annales de la Société Entomologique de France*, 49, 1, 73-90. https://doi.org/10.1080/00379271.2013.767514

Gilbert, L. E. (1972). Pollen feeding and reproductive biology of *Heliconius* butterflies. Proceedings of the National Academy of Sciences, 69(6), 1403-1407.

Gullan, P.J., & Cranston, P.S. (2014). The insects: an outline of entomology. John Wiley & Sons Ltd, USA.

Guo, M., Chen, Q., Liu, Y., Wang, G., & Han, Z. (2018). Chemoreception of mouthparts: sensilla morphology and discovery of chemosensory genes in proboscis and labial palps of adult *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Frontiers in Physiology*, 9, 970. https://doi.org/10.3389/fphys.2018.00970

Ilyinskiy, A.I. & Tropin, I.V. (1965). Surveillance, registration, and prognosis of mass growth of needle and leaf eating insects in the forests of the USSR. Moscow, Lesnaya Promyshlennost, 525 pp. (In Russian).

Krenn H.W. (1990). Functional morphology and movements of the proboscis of Lepidoptera (Insecta). *Zoomorphology*, 110,105-114.

Krenn, H.W. (1998). Proboscis sensilla in *Vanessa cardui* (Nymphalidae, Lepidoptera): functional morphology and significance in flower-probing. *Zoomorphology*, 118, 23-30.

Krenn H.W. & Kristensen N.P. (2004). Evolution of proboscis musculature in Lepidoptera. *European Journal of Entomology*, 101, 565-575.

Ma, L., Hu, K., Li, P., Liu, J., & Yuan, X. (2019). Ultrastructure of the proboscis sensilla of ten species of butterflies (Insecta: Lepidoptera). *Plos One*, 14(3), e0214658. https://doi.org/10.1371/journal.pone.0214658

Maximov, S.A. & Marushchak, V.N. (2012). Towards reasons of outbreaks of the Black-veined White in the Ural. *Agrarnyy Vestnik Urala*, 11(103), 28-30 (In Russian).

Molleman, F., Krenn, H.W., Van Alphen, M. E., Brakefield, P.M., DeVries, P.J., & Zwaan, B.J. (2005). Food intake of fruit-feeding butterflies: evidence for adaptive variation in proboscis morphology. *The Biological Journal of the Linnean Society*, 86, 333-343. https://doi.org/10.1111/j.1095-8312.2005.00533.x

Nation, J.L. (1983). A new method using hexamethyldisilazane for preparation of soft insect tissues for scanning electron microscopy. *Stain Technology*, 58(6), 347–351.

Paulus, H.F., & Krenn, H.W. (1996). Vergleichende morphologie des schmetterlingsrüssels und seiner sensillen-ein beitrag zur phylogenetischen systematik der Papilionoidea (Insecta, Lepidoptera). *Journal of Zoological Systematics and Evolutionary Research*, 34(4), 203-216.

Reinwald, C., Bauder, J.A.S., Karolyi, F., Neulinger, M., Jaros, S., Metscher, B., & Krenn, H.W. (2022). Evolutionary functional morphology of the proboscis and feeding apparatus of hawk moths (Sphingidae: Lepidoptera). *Journal of Morphology*, 283(11), 1390-1410. https://doi.org/10.1002/jmor.21510

Tolman, T. & Lewington, R. (2008). Collins butterfly guide. Harper Collins Publishers Ltd., London, pp 384.

Zaspel, J.M., Coy, S., Habanek, K., & Weller, S.J. (2013). Presence and distribution of sensory structures on the mouthparts of self-medicating moths. *Zoologischer Anzeiger*, 253(1), 6-10. https://doi.org/10.1016/j.jcz.2013.06.001

Zhang, C. M., Niu, Y., Hu, G. L., & Lu, J. Q. (2021). Ultramorphological comparison of proboscis and associated sensilla of *Scotogramma trifolii* and *Protoschinia scutosa* (Lepidoptera: Noctuidae). *Insects*, 12(11), 992. https://doi.org/10.3390/insects12110992

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DOI: 10.31594/commagene.1197055

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Research Article/Araştırma Makalesi

Laccase Production of Newly Isolated *Trametes versicolor* under Batch, Repeated-Batch, and Solid-State Fermentation Processes

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Abstract: In this study, the laccase production ability of the newly isolated *Trametes versicolor* strain was investigated in three different fermentation processes. In all three fermentation processes, the fungus was able to produce the laccase enzyme. During the solid-state fermentation process 13.21 U/mL laccase activity was detected on the 20th day in the 10 mM copper-containing medium, while this value reached to 27.30 U/mL in the medium containing 0.5 mM ABTS+10 mM copper. During the liquid batch fermentation process, laccase activity was significantly induced in the medium containing 1 mM copper and the laccase activities reached 2.25, 19.83 and 24.57 U/mL compared to the medium without copper on the 3rd, 6th, and 9th days, respectively. ABTS and xylidine induced the laccase production of this strain at a much lower level than copper. The liquid repeated-batch process also significantly induced the laccase production. While low level of enzyme activities were detected in a copper-free medium, laccase activities were induced in the copper-containing medium and the activity increased from 0.66 U/mL to 9.87 U/mL at the 6th use of the pellets. Copper was detected as an effective inducer for laccase production in all fermentation processes and activity staining after native polyacrylamide gel electrophoresis clearly showed the active laccase bands. The results revealed that this strain is a good laccase producer and the laccase production yield varies depending on the fermentation process, production time, and inducer used.

Keywords: Copper, enzyme, incubation, inducer, white rot fungus, zymogram.

Yeni İzole Edilen *Trametes versicolor*'un Kesikli, Tekrarlı-Kesikli ve Katı-Faz Fermentasyon Süreçlerinde Lakkaz Üretimi

Öz: Bu çalışmada yeni izole edilmiş *Trametes versicolor* suşunun lakkaz üretim yeteneği üç farklı fermentasyon sürecinde araştırılmıştır. Üç fermentasyon sürecinde de fungus lakkaz enzimini üretebilmiştir. Katı-faz fermentasyonu sürecinde 10 mM bakır içeren ortamda üretimin 20. gününde 13.21 U/mL lakkaz aktivitesi izlenmiştir, bu değer 0.5 mM ABTS+10 mM bakır içeren ortamda ise 27.30 U/mL'ye ulaşmıştır. Sıvı kesikli fermentasyon sürecinde ise 1 mM bakır içeren ortamda bakır içermeyen ortama göre lakkaz aktivitesi önemli oranda indüklenmiş ve lakkaz aktiviteleri 3, 6 ve 9. günlerde sırasıyla 2.25, 19.83 ve 24.57 U/mL'ye ulaşmıştır. ABTS ve ksilidin bu suşun lakkaz üretimini bakıra göre çok daha düşük düzeyde indüklemiştir. Sıvı tekrarlı-kesikli süreç de lakkaz üretimini önemli oranda indüklemiştir. Bakır içermeyen ortamda düşük düzeyde enzim aktiviteleri saptanırken, bakır içeren ortamda lakkaz aktiviteleri indüklenmiş ve peletlerin 6. kullanımında aktivite 0.66 U/mL'den 9.87 U/mL'ye ulaşmıştır. Tüm fermentasyon süreçlerinde, bakırın lakkaz üretimi için önemli bir indükleyici olduğu gözlenmiştir ve doğal poliakrilamid jel elektroforezi sonrası yapılan aktivite boyamaları aktif lakkaz bantlarını net olarak göstermiştir. Sonuçlar, bu suşun iyi bir lakkaz üreticisi olduğunu ve fermentasyon sürecine, üretim zamanına ve kullanılan indükleyiciye bağlı olarak lakkaz üretim veriminin değiştiğini ortaya koymuştur.

Anahtar kelimeler: Bakır, enzim, inkübasyon, indükleyici, beyaz çürükçül fungus, zimogram.

1. Introduction

Laccase enzyme was first discovered by Yoshida in 1883 in the secretions of the Japanese lacquer tree called *Rhus vernicifera* and was described as a metal-containing oxidase (Thurston, 1994; Mayer & Staples, 2002). White rot fungi, bacteria, plants, and insects can produce laccase enzyme. This enzyme, which has low substrate specificity, is commonly found in white rot fungi (Eggert et al., 1996; Upadhyay et al,. 2016). Laccases (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) are multi-copper oxidases with phenol oxidase activity and due to their low substrate specificity, they can oxidize many different substrates (Yeşilada et al., 2014; Agrawal et al., 2018; Moreno et al., 2020). They can be used in different areas and processes (Yeşilada et al., 2014, Forootanfar & Faramarzi, 2015; Rodríguez-Delgado et al., 2016; Dhull et al., 2020;).

Laccase can be produced by different fermentation processes such as solid-state fermentation (SSF), liquid batch fermentation (LBF), and liquid repeated-batch fermentation (LRBF). SSF is a medium in which there is no free liquid in the medium. That is, organisms can grow and carry out their metabolic reactions using moistened solid substrates (Pandey et al., 2000; Krishna, 2005). Lignocellulose substrates are abundant in nature and some of them are in the form of waste materials. White rot fungi can use lignocellulose raw materials as substrate. These fungi can degrade cellulose, hemicellulose, and lignin in lignocellulosic substrates thanks to their enzymes such as laccase, ligninase, cellulase, pectinase, and xylanase (Pandey et al., 2000; Papinutti et al., 2007; Boran & Yeşilada, 2011). SSF is a preferred application in the production of important products as it resembles the

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natural habitats of the filamentous fungi. Bacteria, yeasts, and filamentous fungi can be grown on solid substrates. However, the most suitable microorganisms for SSF processes are fungi that grow well in humid environments (Manan & Webb, 2017). Many different substrates can be used in SSF. These substrates include wheat bran, straw, various fruit peels, plant leaves, and sawdust (Rosales et al 2007; Elisashvili et al 2008; Levin et al 2008; Sharma & Arora, 2010; Boran & Yeşilada, 2011). The batch process is a production process in which no fresh substrate is added to or removed from the culture until the end of production, after the microorganism has been inoculated into a growth medium. In the repeated-batch process after inoculation and incubation, a certain amount of the culture liquid is removed and the same amount of fresh medium is added. Microorganisms remain in the environment throughout the process and the process is repeated as many times as desired. In this process, microorganisms can be used repeatedly for a long time (Birhanli & Yesilada, 2010).

In this study, the laccase production ability of the newly isolated *Trametes versicolor* strain was investigated under different fermentation processes and the effect of various inducers on laccase production ability of this strain was tested.

2. Material and Methods

2.1. White Rot Fungus Used

Trametes versicolor, a white rot fungus belonging to the Basidiomycetes class, was used in the study. This fungus was collected from Hatay by Dr. Özfer Yeşilada and identified after being isolated as a pure culture. The fungus used in the study is maintained as a pure culture in the Biotechnology Laboratory of the Department of Biology at İnönü University.

2.2. Macroscopic Determination of Laccase Enzyme Production of *Trametes versicolor*

Laccase production ability of this fungus was detected on SDA plates containing 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS). Firstly, the fungus inoculated on this plate and then the culture was statically incubated at 30°C. The color change due to ABTS oxidation shows the laccase production ability of this fungus (Boran & Yeşilada, 2022).

2.3. Preparation of Stock Inoculum Culture

Firstly, the fungus inoculated on slant SDA medium was incubated at 30 °C for 5 days. Then, distilled water was added onto this culture and mycelial suspension was obtained. After that, 4 mL of this suspension was inoculated into 100 mL Sabouraud dextrose broth (SDB)/250 mL flask and it was incubated at 30 °C and 150 rpm for 7 days. After incubation, the obtained liquid culture was homogenized at low speed with a homogenizer under sterile conditions and 4 mL of this homogenized culture was inoculated into 100 mL SDB/250 mL flasks. This culture was incubated at 30 °C and 150 rpm for 7 days. After production, this liquid culture was gently homogenized at low speed and used as stock inoculum culture (Yeşilada et al 2014).

2.4. Preparation of Fungal Pellets for Repeated-Batch Fermentation

In order to obtain the fungal pellets, 600 mL SDB/1000 mL flask was prepared first and it was autoclaved at 121 °C for 20 minutes. 7 mL of the homogenized stock inoculum culture prepared as stated in Section 2.3, was inoculated into this broth. The culture was incubated for 7 days at 30 °C and 150 rpm for the formation of fungal pellets. Then, the pellets were filtered under sterile conditions, washed with sterile distilled water, and the fungal pellets obtained were used for repeated-batch studies.

2.5. The Fermentation Methods Used

2.5.1. Batch fermentation

Batch fermentation (BF) studies were carried out in 50 mL SDB/250 mL flasks. The effect of various inducers on laccase production was also tested in batch studies. For this purpose, the media containing 1 mM copper, 1 mM xylidine, 0.05 mM 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 1 mM xylidine+1 mM copper, and 0.05 mM ABTS+1 mM copper (final concentrations) and the media without an inducer were prepared. These media were inoculated with 1 mL of the homogenized stock inoculum culture and they were incubated at 30°C and 150 rpm for 9 days.

2.5.2. Repeated-batch fermentation

Fungal pellets obtained as stated in Section 2.4 were used in the repeated-batch fermentation (RBF). 30 grams of pellets were transferred to 50 mL SDB/250 mL flasks with and without inducer under sterile conditions. After the cultures were incubated for 24 hours at 30 °C and 150 rpm, the culture liquids were filtered and fresh sterile media were added to these flasks containing pellets and incubated for 24 hours in the same way (Birhanlı & Yeşilada, 2010). These studies were carried out with medium without inducer and also in media containing 1 mM copper, 1 mM xylidine, 0.05 mM ABTS, 1 mM xylidine+1 mM copper, and 0.05 mM ABTS+1 mM copper separately.

2.5.3. Solid-state fermentation

For the preparation of solid-state medium, 3.5 g wheat bran+1.5 g soy flour were transferred into 250 mL flasks and these solid media were moistened with 15 mL of moistening liquid (sterile distilled water or sterile distilled water containing the appropriate inducer). Prepared solid media were autoclaved at 121°C for 20 minutes. Then, 4 mL of homogenized stock culture prepared as specified in Section 2.3 was inoculated into these solid media and they were statically incubated at 30°C. After incubation, 40 mL of sterile distilled water was added to each solid-state cultures and the cultures were mixed with sterile sticks. Then, the cultures were shaken at 30°C and 150 rpm for 2 hours. After shaking, the cultures were filtered and the obtained filtrates were centrifuged twice for 10 minutes at 7000 rpm. Laccase enzyme activity was determined in the supernatants obtained after centrifugation (Boran & Yeşilada, 2011).

2.6. Determination of Laccase Activity

For the determination of laccase (EC 1.10.3.2) activity, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)

was used as a substrate and laccase activity was determined depending on ABTS oxidation by laccase enzyme. The measurement of laccase activity was carried out at 40 °C and the reaction mixture was prepared with sodium acetate buffer (100 mM, pH 4.0), ABTS (5 mM), and the appropriate amount of supernatant. As a result of the oxidation of ABTS, the absorbance change occurring in 1 minute at 420 nm was determined and the amount of enzyme that converts 1 µmol of substrate to product in 1 minute at 40°C was expressed as 1 unit (Yeşilada et al., 2014). All laccase activity values are given as the mean of at least three studies.

2.7. Native Gel Electrophoresis

Native gel electrophoresis was applied to show the presence of laccase enzyme. TGX gels were used. The crude enzymes were loaded into the wells and were run at 40 mA. After the electrophoresis, the gel was incubated in pH 4.0 acetate buffer containing ABTS and activity staining was performed (Yeşilada et al., 2014).

3. Results and Discussion

White rot fungus *Trametes versicolor* is a biotechnologically important fungus. It is also a medicinal fungus (Kılıç & Yeşilada, 2013; Winder et al., 2021). *T. versicolor* was also the effective producer of the laccase enzyme. Since the laccase enzyme can be used in many applications, it is important to produce this enzyme in high amounts. The laccase production potential of white rot fungi may vary depending on the species used and even on the strain. Therefore, in this study, the laccase production ability of *Trametes versicolor*, a white rot fungus isolated from Hatay province, was investigated.

3.1. Detection of Laccase on Sabouraud Dextrose Agar Media containing ABTS

T. versicolor was cultivated on SDA media with and without ABTS. If the fungus is able to oxidize ABTS, the color of the medium changes to green-purple. As shown in Figure 1 the fungus was able to oxidize ABTS and a purple color was formed. This is an indication that this fungus is a producer of laccase.

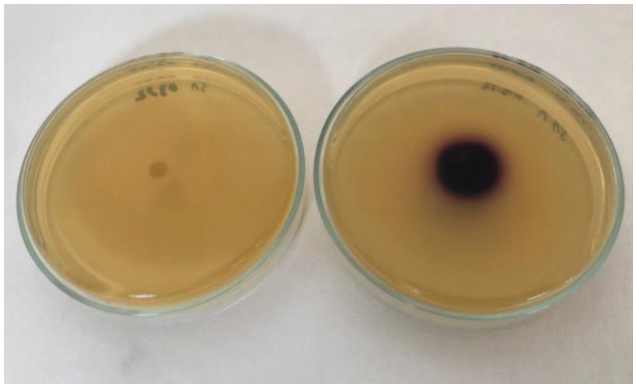

Figure 1. Color change in SDA media due to ABTS oxidation. a) ABTS-free medium, b) ABTS-containing medium.

3.2. Laccase Production of *T. versicolor* Under Different Fermentation Processes

The laccase production potential of white rot fungi could vary depending on the fermentation processes (solid-state fermentation, liquid batch fermentation, and liquid repeated-batch fermentation).

3.2.1. Laccase production during solid-state fermentation process

During the SSF, wheat bran+soy flour (3.5 g+1.5 g) was used as solid substrate. After this solid substrate was prepared, it was moistened with distilled water and sterilized in an autoclave (Boran & Yeşilada, 2011). After sterilization, the fungus was inoculated and they were statically incubated at 30°C for 20 days. Firstly, laccase production ability of this fungus was monitored by adding ABTS to the solid culture. After ABTS addition, the formation of green color due to the oxidation of ABTS showed the laccase production of this fungus under SSF (Fig. 2).

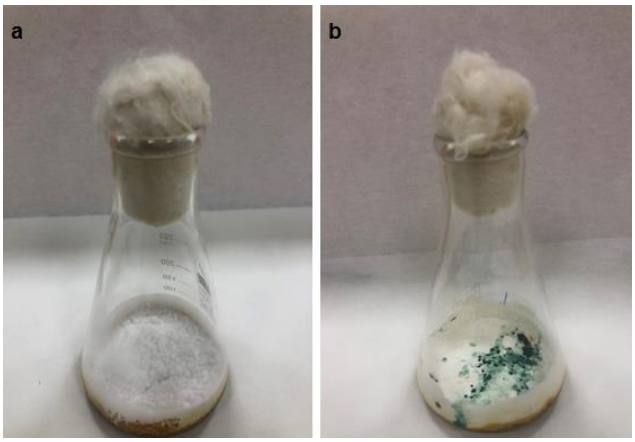

Figure 2. The macroscopic images of *T. versicolor* incubated on (a) solid-state medium and (b) solid-state medium treated with ABTS after incubation.

After the macroscopic observation, the effect of incubation time (5, 10, 15 and 20 days) on laccase production of this fungus was tested. As seen in Figure 3, while the laccase activity was determined as 0.17 U/mL on the 5th day, the laccase activity increased with time and reached 10.70 U/mL on the 20th day. The laccase production potential of white rot fungi under SSF was reported in other studies (Boran and Yeşilada, 2011).

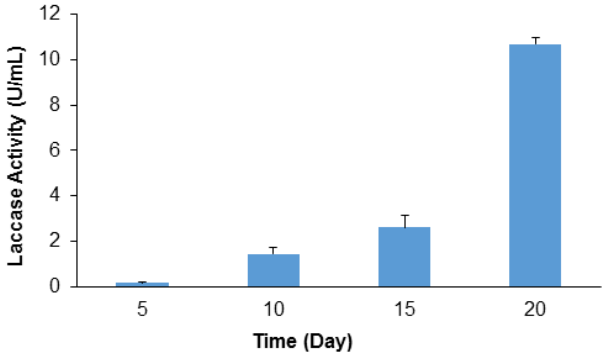

Figure 3. Time-dependent laccase production during SSF.

3.2.2. Effect of inducers on laccase production during solid-state fermentation

It has been reported in various studies that inducers affect the laccase production of white rot fungi (Collins & Dobson, 1997; Boran & Yeşilada, 2011; Birhanlı & Yeşilada, 2017). Therefore, the effect of inducers such as copper, xylidine, and ABTS on laccase production during solid-

state fermentation was tested. In addition, the combined effect of copper+xylidine and copper+ABTS was also investigated. It was reported that copper is a good inducer for laccase production (Birhanlı & Yeşilada, 2006). The addition of copper to the medium increased the laccase activity. While the laccase activity detected on the 20th day was 13.21 U/mL in the copper-containing medium, it was 10.70 U/mL in the copper-free medium. Laccase activities detected on solid media moistened with distilled water containing 10 mM copper were 0.74, 3.87, 6.79 and 13.21 U/mL on the 5th, 10th, 15th, and 20th days, respectively (Fig. 4).

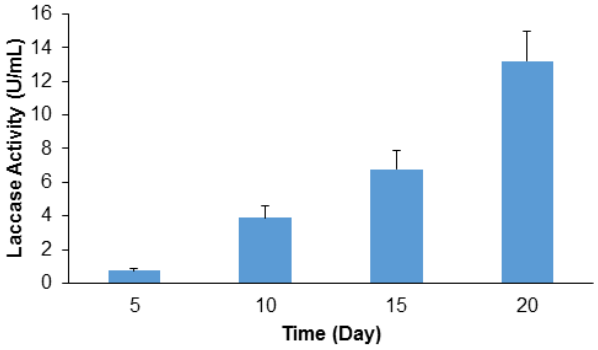

Figure 4. Time-dependent laccase production in 10mM copper-containing medium during SSF.

The effect of ABTS and xylidine on laccase production of this fungus was also tested. In addition, the combined effect of ABTS+copper and xylidin+copper was also tested in order to test their possible synergistic effect with copper. For this purpose, the fungus was incubated the solid media containing these inducers for 20 days and laccase activities were measured. While 5.60 U/mL laccase activity was obtained in 0.5 mM ABTS medium, 27.30 U/mL laccase activity was detected in 0.5 mM ABTS+10 mM copper medium (Fig. 5). Furthermore, while 5.53 U/mL laccase activity was detected on the 20th day in the medium containing 10 mM xylidine, 22.59 U/mL enzyme activity was determined in the medium containing 10 mM xylidine+10 mM copper (Fig. 6).

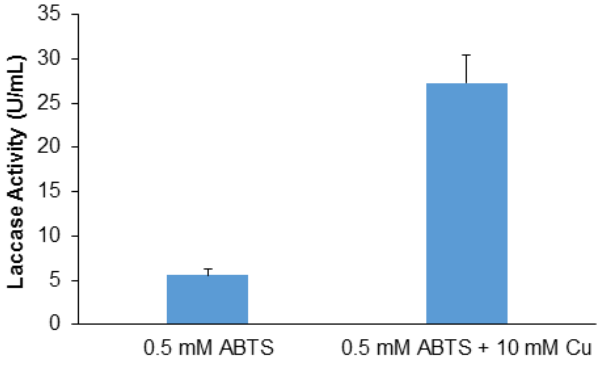

Figure 5. Time-dependent laccase production in 0.5 mM ABTS and 0.5 mM ABTS+10mM copper-containing media during SSF.

3.2.3. Laccase production under liquid phase fermentation

Laccase production potential of *T. versicolor* was investigated during the liquid phase fermentation processes (batch and repeated-batch processes).

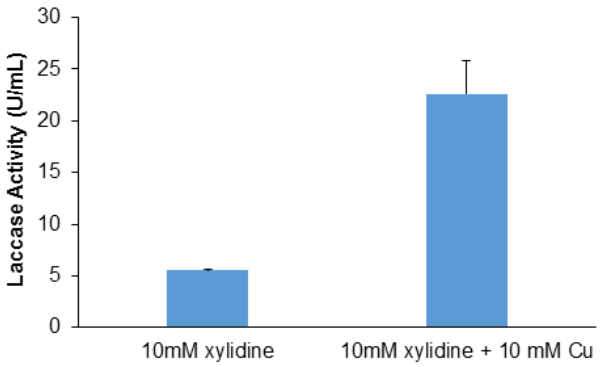

Figure 6. Time-dependent laccase production in 10mM xylidine and 10 mM xylidine+10mM copper-containing media during SSF.

3.2.3.1. Laccase production during batch fermentation

Time-dependent laccase production monitored for 9 days. As seen in Figure 7, laccase activities were significantly lower in the SDB medium without any inducer. The fungus produced 0.033, 0.026, and 0.013 U/mL laccase enzymes after incubation for 3, 6, and 9 days in copper-free SDB media, respectively. On the other hand, laccase production was significantly induced in the media containing 1mM copper and laccase activities reached 2.25, 19.83 and 24.57 U/mL on the same days (Fig. 8). The laccase activity increased 68 times on the 3rd day, 762 times on the 6th day and 1890 times on the 9th day with the addition of copper compared to the cultures growth in copper-free media. This result shows that copper plays an important role as an inducer of laccases during batch fermentation (Lorenzo et al., 2006; Cordi et al., 2007).

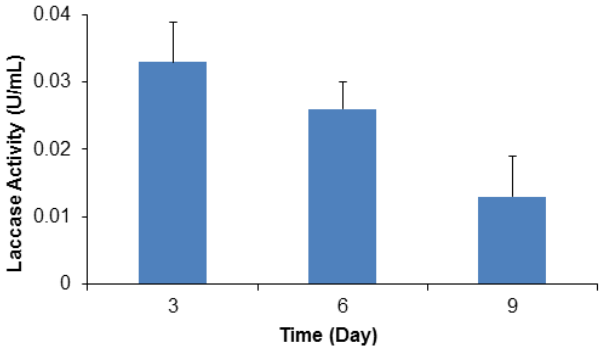

Figure 7. Time-dependent laccase production in SDB medium without inducer during BF.

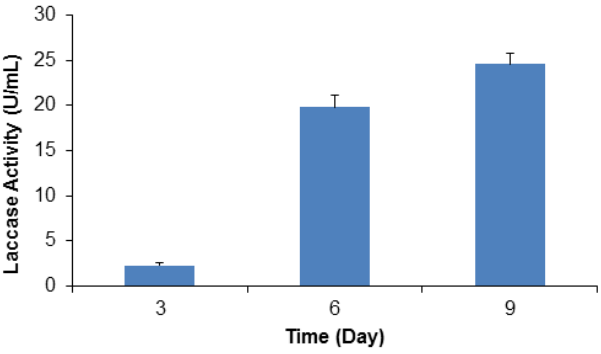

Figure 8. Time-dependent laccase production in 1 mM copper-containing SDB medium during BF.

In addition to copper, the effect of ABTS and xylidine on laccase production after 6 days of incubation was tested. In SDB medium containing 0.05 mM ABTS, 0.17 U/mL laccase activity was detected. On the other hand, this value was 0.16 U/mL in SDB medium containing 1 mM xylidine (Fig. 9). These results showed that these inducers slightly induce the laccase production activity of this fungus under BF condition. Copper was detected as the best inducer for this fermentation process (Fig. 7-Fig. 9).

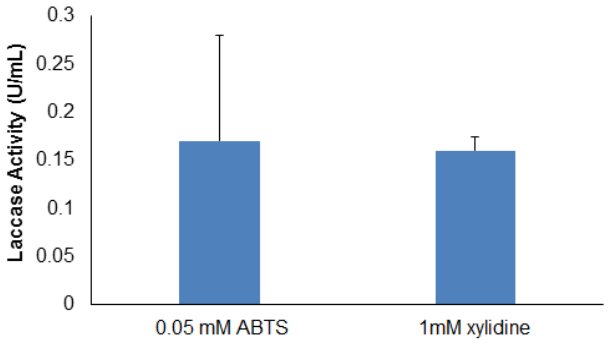

Figure 9. Time-dependent laccase production in SDB media containing 0.05 mM ABTS and 1 mM xylidine during BF.

Laccase production ability of this fungus was tested in media containing 0.05mM ABTS+1mM copper and also media containing 1mM xylidin+1mM copper. While 10.50 U/mL laccase activity was detected on the 6th day in the medium containing 0.05 mM ABTS+1 mM copper, 10.69 U/mL laccase activity was obtained in the medium containing 1 mM xylidin+1 mM copper.

3.2.3.2. Laccase production during repeated-batch fermentation

During the RBF, firstly, the effect of copper as an inducer was tested. As can be seen from Figure 10, low-level enzyme activities were detected in the copper-free medium. However, laccase production was significantly induced in the copper-containing medium. Laccase production increased from the 1st use of the pellets until the 6th use, in a copper-containing medium. Laccase production ability of the pellets decreased after the 6th use. However, the laccase activity was high even at the 8th use. This shows that these self-immobilized pellets can be used for repeatedly and long-term production of laccase could be achieved. This is consistent with studies reporting the positive effect of copper on laccase production during repeated-batch process (Birhanlı & Yeşilada, 2017).

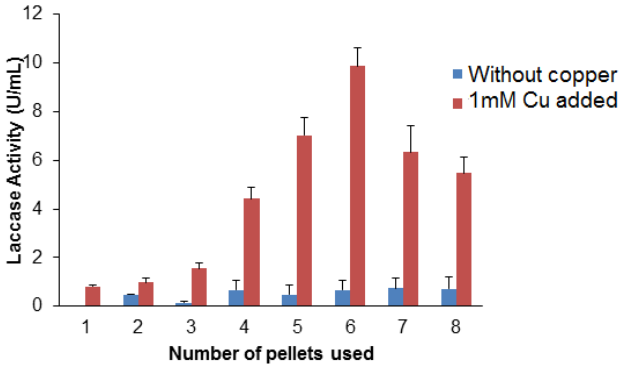

Figure 10. Time-dependent laccase production in 1 mM copper-containing and copper-free SDB media during RBF.

The effect of ABTS and xylidine, as inducers on laccase production, during the repeated-batch process was also investigated. 0.05 mM ABTS showed no positive effect on laccase production. Although xylidine has a positive effect on laccase production, this effect is negligible compared to copper (Fig. 11).

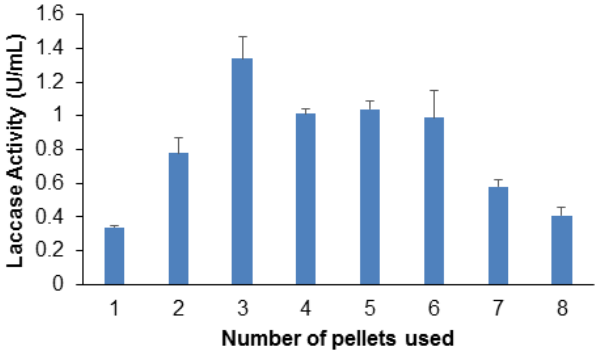

Figure 11. Time-dependent laccase production in 1 mM xylidine-containing medium during RBF.

The possible synergistic effect of 0.05 mM ABTS+1 mM copper and 1 mM xylidine+1 mM copper was also tested. No significant increase was observed in the medium containing 0.05 mM ABTS+1 mM copper compared to the medium containing only copper (Fig. 12). As can be seen in Figure 13, laccase activity in the medium containing 1 mM xylidine+1 mM copper increased during the first 5 uses and after that, the activity decreased. In previous studies, a synergistic effect of inducers such as ABTS+copper or xylidine+copper on laccase production on fungi was reported (Birhanlı & Yeşilada, 2017).

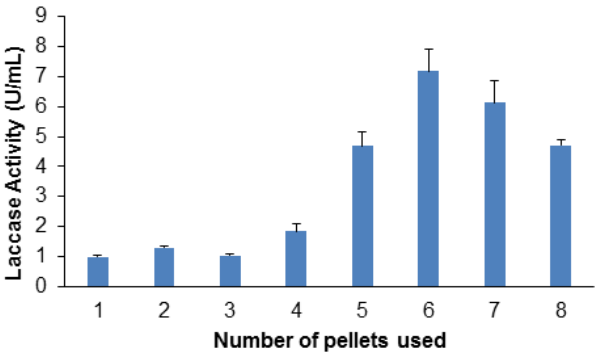

Figure 12. Time-dependent laccase production in SDB medium containing 0.05 mM ABTS+1 mM copper during RBF.

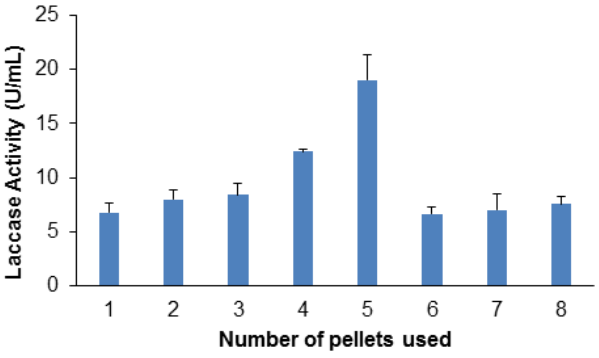

Figure 13. Time-dependent laccase production in SDB medium containing 1 mM xylidine+1 mM copper during RBF.

3.3. Zymogram Studies

Zymogram applications of enzyme sources obtained from SSF, BF, and RBF processes were made. After the electrophoresis, the activity staining was done using ABTS and the gel was photographed. As can be seen from Figure 14, active laccase bands were observed in all fermentation conditions.

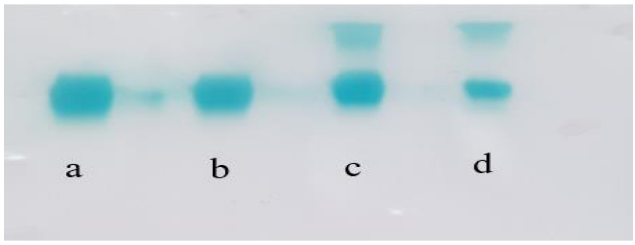

Figure 14. Detection of laccase on native PAGE gels (a) Enzyme source from copper-free 20th day culture of SSF (b) Enzyme source from 10 mM copper-containing 20th day solid-state culture (c) Enzyme source from 1 mM copper-containing 9th day batch culture (d) Enzyme source from 1 mM copper-containing repeated-batch culture (at the 6th use of pellets).

4. Conclusions

The fermentation process, time, and inducer selection significantly affected the laccase production potential of this *T. versicolor* strain. In all three fermentation processes, this fungus was able to produce the laccase enzyme. Copper was detected as an effective inducer for laccase production in all fermentation processes. The highest laccase activity was determined in SSF medium with 0.5 mM ABTS + 10 mM Cu. The high level of biotechnologically important laccase enzyme production with this strain shows that this strain could be used as an effective laccase producer by selecting the proper inducer and fermentation mode.

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Ethics committee approval: Ethics committee approval is not required for this study.

Conflict of interest: The author declared that there is no conflict of interest.

Author Contributions: Conception – Ö.Y.; Design – Ö.Y., T.T.; Supervision – Ö.Y.; Fund – Ö.Y.; Materials – Ö.Y., T.T.; Data Collection or Processing – Ö.Y., F.B.; Analysis Interpretation – T.T., Ö.Y., F.B.; Literature Review – T.T., Ö.Y., F.B.; Writing – F.B., Ö.Y.; Critical Review – Ö.Y., F.B.

References

Agrawal, K., Chaturvedi, V., & Verma, P. (2018). Fungal laccase discovered but yet undiscovered, *Bioresources and Bioprocessing*, 5(1), 1-12. https://doi.org/10.1186/s40643-018-0190-z

Birhanli, E., & Yesilada, O. (2006). Increased production of laccase by pellets of *Funalia trogii* ATCC 200800 and *Trametes versicolor* ATCC 200801 in repeated-batch mode. *Enzyme and Microbial Technology*, 39(6), 1286-1293. https://doi.org/10.1016/j.enzmictec.2006.03.015

Birhanli, E., & Yesilada, O. (2010). Enhanced production of laccase in repeated-batch cultures of *Funalia trogii* and *Trametes versicolor*. *Biochemical Engineering Journal*, 52(1), 33-37. https://doi.org/10.1016/j.bej.2010.06.019

Birhanli, E., & Yeşilada, Ö. (2017). The effect of various inducers and their combinations with copper on laccase production of *Trametes versicolor* pellets in a repeated-batch process, *Turkish Journal of Biology*, 41(4), 587-599. https://doi.org/10.3906/biy-1608-44

Boran, F., & Yesilada, O. (2011). Enhanced production of laccase by fungi under solid substrate fermentation condition. *BioResources*, 6(4), 4404-4416.

Boran, F., & Yesilada, O. (2022). Laccase production by newly isolated *Ganoderma lucidum* with solid state fermentation conditions and its using for dye decolorization. *Adıyaman Üniversitesi Mühendislik Bilimleri Dergisi*, 9(17) 458-470. https://doi.org/10.54365/adyumbd.1107682

Collins, P.J., & Dobson, A. (1997). Regulation of laccase gene transcription in *Trametes versicolor*. *Applied and Environmental Microbiology*, 63(9), 3444-3450.

Cordi, L., Minussi, R.C., Freire, R.S., & Durán, N. (2007). Fungal laccase: copper induction, semi- purification, immobilization, phenolic effluent treatment and electrochemical measurement. *African Journal of Biotechnology*, 6(10), 1255-1259.

Dhull, N., Michael, M., Simran, P., Gokak, V.R., & Venkatanagaraju, E. (2020). Production and Purification strategies for laccase. *International Journal of Pharmaceutical Sciences and Research*, 11(6),2617-2625. https://doi.org/10.13040/IJPSR.0975-8232.11(6).2617-25

Eggert, C., Temp, U., & Eriksson, K.E. (1996). The ligninolytic system of the white rot fungus *Pycnoporus cinnabarinus*: purification and characterization of the laccase. *Applied and Environmental Microbiology*, 62(4), 1151-1158.

Elisashvili, V., Penninckx, M., Kachlishvili, E., Tsiklauri, N., Metreveli, E., Kharziani, T. & Kvesitadze, G. (2008). *Lentinus edodes* and *Pleurotus* species lignocellulolytic enzymes activity in submerged and solid-state fermentation of lignocellulosic wastes of different composition. *Bioresource Technology*, 99(3), 457-462. https://doi.org/10.1016/j.biortech.2007.01.011

Forootanfar, H., & Faramarzi, M.A. (2015). Insights into laccase producing organisms, fermentation states, purification strategies, and biotechnological applications. *Biotechnology Progress*, 31(6), 1443-1463. https://doi.org/10.1002/btpr.2173

Kılıç, A., & Yeşilada, E. (2013). Preliminary results on antigenotoxic effects of dried mycelia of two medicinal mushrooms in *Drosophila melanogaster* somatic mutation and recombination test. *International Journal of Medicinal Mushrooms*, 15(4) 415-421. https://doi.org/10.1615/intjmedmushr.v15.i4.90

Krishna, C. (2005). Solid-state fermentation systems—an overview. *Critical Reviews in Biotechnology*, 25(1-2), 1-30. https://doi.org/10.1080/07388550590925383

Levin, L., Herrmann, C., & Papinutti, V. L. (2008). Optimization of lignocellulolytic enzyme production by the white-rot fungus *Trametes trogii* in solid-state fermentation using response surface methodology. *Biochemical Engineering Journal*, 39(1), 207-214. https://doi.org/10.1016/j.bej.2007.09.004

Lorenzo, M., Moldes, D., & Sanromán, M.Á. (2006). Effect of heavy metals on the production of several laccase isoenzymes by *Trametes versicolor* and on their ability to decolourise dyes. *Chemosphere*, 63(6), 912-917. https://doi.org/10.1016/j.chemosphere.2005.09.046

Manan, M.A., & Webb, C. (2017). Design aspects of solid state fermentation as applied to microbial bioprocessing. *Journal of Applied Biotechnology and Bioengineering*, 4(1), 91. https://doi.org/10.15406/jabb.2017.04.00094

Mayer, A.M., & Staples, R.C. (2002). Laccase: new functions for an old enzyme. *Phytochemistry*, 60(6), 551-565. https://doi.org/10.1016/S0031-9422(02)00171-1

Moreno, A.D., Ibarra, D., Eugenio, M.E., & Tomás-Pejó, E. (2020). Laccases as versatile enzymes: from industrial uses to novel applications. *Journal of Chemical Technology & Biotechnology*, 95(3), 481-494. https://doi.org/10.1002/jctb.6224

Pandey, A., Soccol, C. R., Nigam, P., & Soccol, V. T. (2000). Biotechnological potential of agro-industrial residues. I: sugarcane bagasse. *Bioresource Technology*, 74(1), 69-80. https://doi.org/10.1016/S0960-8524(99)00142-X

Papinutti, V.L., & Forchiassin, F. (2007). Lignocellulolytic enzymes from Fomes sclerodermeus growing in solid-state fermentation. *Journal of Food Engineering*, 81(1), 54-59. https://doi.org/10.1016/j.jfoodeng.2006.10.006

Rodríguez-Delgado, M., Orona-Navar, C., García-Morales, R., Hernandez-Luna, C., Parra, R., Mahlknecht, J., & Ornelas-Soto, N. (2016). Biotransformation kinetics of pharmaceutical and industrial micropollutants in groundwaters by a laccase cocktail from *Pycnoporus sanguineus* CS43 fungi. *International Biodeterioration & Biodegradation*, 108, 34-41. https://doi.org/10.1016/j.ibiod.2015.12.003

Rosales, E., Couto, S.R., & Sanromán, M.A. (2007). Increased laccase production by *Trametes hirsuta* grown on ground orange peelings. *Enzyme and Microbial Technology*, 40(5), 1286-1290. https://doi.org/10.1016/j.enzmictec.2006.09.015

Sharma, R.K., & Arora, D.S. (2010). Production of lignocellulolytic enzymes and enhancement of in vitro digestibility during solid state fermentation of wheat straw by *Phlebia floridensis*. *Bioresource Technology*, 101(23), 9248-9253. https://doi.org/10.1016/j.biortech.2010.07.042

Thurston, C.F. (1994). The structure and function of fungal laccases. *Microbiology*, 140(1), 19-26. http://dx.doi.org/10.1099/13500872-140-1-19

Upadhyay, P., Shrivastava, R., & Agrawal, P.K. (2016). Bioprospecting and biotechnological applications of fungal laccase 3. *Biotech*, 6(1), 1-15. https://doi.org/10.1007/s13205-015-0316-3

Winder, M., Bulska-Bedkowska, W., & Chudek, J. (2021). The use of *Hericium erinaceus* and *Trametes versicolor* extracts in supportive treatment in oncology. *Acta Pharmaceutica*, 71, 1–16 https://doi.org/10.2478/acph-2021-0007

Yeşilada, Ö., Birhanli, E., Ercan, S., & Özmen, N. (2014). Reactive dye decolorization activity of crude laccase enzyme from repeated-batch culture of *Funalia trogii*. *Turkish Journal of Biology*, 38(1), 103-110. https://doi.org/10.3906/biy-1308-38

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DOI: 10.31594/commagene.1203263

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Research Article/Araştırma Makalesi

Investigation of Some Bioactivities and Odor Components of *Jasminum officinale* Linn. (Oleaceae): A Valuable Tool for Cosmetic Product Design

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Abstract: In this study, researches were carried out on the protease enzyme activity of *Jasminum officinale* Linn. flower which grows naturally in Muğla and its surroundings. In addition, fragrance components in the content of jasmine flower were determined. It was aimed to be used in perfume making based on the harmony of white jasmine flower with other flowers and the concept of note. Protease enzyme was purified from *J. officinale* flower using TPP (Three Phase Partitioning) method. Optimal pH and optimal temperature for the enzyme, K_m and V_{max} values for casein, azokazaein, gelatin, hemoglobin, and azoalbumin substrates were determined. SDS-PAGE was used to check the purity of the protease enzyme purified from the *J. officinale*. The molecular weight of the enzyme was calculated as 21.386 kDa using gel filtration chromatography. The phenolic content was also determined. It has been determined that the content of jasmine flower can be used in perfume design which is the most prestigious product of the cosmetic industry.

Keywords: Cosmetics, Jasmine flower, protease enzyme, three phase system.

Jasminum officinale Linn.'nin (Oleaceae) Bazı Biyoaktiviteleri ve Koku Bileşenlerinin Araştırılması: Kozmetik Ürün Tasarımı için Değerli bir Araç

Öz: Bu çalışmada Muğla ve çevresinde doğal olarak yetişen *Jasminum officinale* Linn. çiçeğinin proteaz enzim aktivitesi üzerine araştırmalar yapılmıştır. Ayrıca yasemin çiçeğinin içeriğindeki koku bileşenleri tespit edilmiştir. Yasemin çiçeğinin diğer çiçeklerle olan uyumu ve nota kavramı esas alınarak parfüm yapımında kullanılması amaçlanmıştır. Proteaz enzimi, *J. officinale* çiçeğinden ÜFA (Üç fazlı sistem) yöntemi kullanılarak saflaştırıldı. Enzim için optimum pH ve optimum sıcaklık, kazein, azokazaein, jelatin, hemoglobin, azoalbümin substratları için K_m ve V_{max} değerleri belirlendi. *J. officinale* çiçeğinden saflaştırılan proteaz enziminin saflığını kontrol etmek için SDS-PAGE kullanıldı. Enzimin moleküler ağırlığı jel filtrasyon kromatografisi kullanılarak 21.386 kDa olarak hesaplandı. Fenolik içeriği belirlendi. Kozmetik sektörünün en prestijli ürünü olan parfüm tasarımında yasemin çiçeğinin içeriğinin kullanılabileceği belirlendi.

Anahtar kelimeler: Kozmetik, Yasemin çiçeği, proteaz enzimi, üç fazlı sistem.

1. Introduction

Historically, it is considered that people have benefited from the healing and beautification features of plants and oils for hundreds of thousands of years. It is seen that the understanding of natural life has come to this day by being transferred from generation to generation and has become a lifestyle today. Consuming natural products, doing our personal care with natural cosmetic materials, living by taking vitamins and minerals that we cannot get with food supplements, and many similar examples are important elements for a natural and healthy life (Aşık, 2017). NC (natural cosmetics) aim at the care and prettiness of the human body with the help of active constituents in nature. This can only be achieved with raw materials that are harmless to the skin and the environment. Natural cosmetics help our natural skin functions and rejuvenate the skin. It provides a soft and natural care; and thus, it can be said that it helps the skin of all ages to stay healthy. It is thought that natural cosmetics make the body-spirit harmony more alive. Nature, the source of life, offers us everything necessary to lead a healthy and long life and indigenous people are collecting herbaceous and ligneous species from the wild and other commercial and home gardens to treat a wide range of diseases and satisfy other social conditions. As a result, it is important to be aware of plant availability and use them accordingly (Aşık, 2017; Dossou-Yovo et al., 2021; Dossou-Yovo et al., 2022a; Dossou-Yovo et al., 2022b).

The Oleaceae family is represented by 29 genus and 600 species as trees, shrubs, and rarely climbers in tropical and temperate regions of the World (Yaltırık, 1978; Wallander & Albert, 2000). There are 7 genus (*Ligustrum* L., *Jasminum* L., *Osmanthus* Lour., *Fraxinus* L., *Fontanesia* Labill., *Olea* L., *Phillyrea* L.) belonging to the Oleaceae family in the flora of Turkey. Species belonging to the Oleaceae botanical family have important therapeutical, economic, and horticultural importance and are used in many fields and ways. Among the concerned species, for instance, some are cultivated for adornment and their flowers are extracted (Huang et al., 2019), some are treated as therapeutical wine for the process of joint pain and dried flowers are used as recreational tea (Sõukand et al., 2017). Moreover, some are exploited as antidiabetic (Bai et al., 2010), for treating lowering cholesterol and diarrhea

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(De Feo et al.,1992) and for antioxidant and anticancer effects (Fares et al., 2011). They also have the functions of expelling phlegm and cleaning the eye (Chinese Pharmacopoeia, 2015). Jasminum (*Oleaceae* genus) which consists of more than 2000 plant species all over the world, is one of the important genus of this botanical family (Green, 2003). Jasmine flowers are described by a very beautiful aroma and are of great importance in medical, industrial, and food implementations (Kunhachan et al., 2012). Notably, ethanolic *Jasminum* flower extracts include, CG (cardiac glycosides), phenolics, flavonoids, essential oil, antioxidants, saponins, and coumarins steroids (Reshma et al., 2021). However, it was reported that *Jasminum* contains substances such as Iridoids, secoiridoids, phenolics, essential oils, flavonoids and tannins as its main components, with the isolation and characterization of chemical components (Reshma et al.,2021). Due to their various phytochemical contents, they are the main provenance of raw materials especially for the production of quality perfumes (Joshi, 2000). In general, *Jasminum* has pharmacological features such as angiotensin converting enzyme (Ace) inhibitor, antiulcer, spasmolytic activity, wound healing, anti-acne, anti-inflammatory, antimicrobial, vasodilation, aromatherapy, antioxidant and antiaging, gastroprotective, anti-lipid peroxidative, hepatoprotective, and cytoprotective (Reshma et al.,2021).

There are *J. officinale* L. and *J. fructicans* L. species belonging to the genus *Jasminum* in Turkey. Among the ethnobotanical uses of this genus in Turkey, the essential oil is especially used in the cosmetic industry. The infusion (5%) prepared from its flowers is used as a chest softener, nerve sedative, and constipation. The flowers are also added to tea to give flavor. In addition, infusion (5%) prepared from flowering branches is used as diuretic and worm reducer (Baytop, 1999). In addition to these, the plant *J. officinale*, which constitutes the biological material of our research, has shown various pharmacological actions, anti-viral, anti-spasmodic, and anti-microbial, cytotoxic in addition to wound support (Elhawary et al., 2020). The leaves are responsible for their therapeutic properties such as anti-diabetic, antioxidant, antiseptic, antispasmodic and wound healing (Prachee et al., 2019). The root part is believed to expedite the healing of fractures and is also known to be used to treat headaches and insomnia (Alrashdi et al., 2012). *J. officinale* flowers are conventionally used as a sedative, a mild anesthetic, depressant, and astringent. Its flowers contain a variety of volatile compounds, including farnecene, benzyl benzoate, nerolidol, benzyl alcohol, benzyl acetate, hexenyl benzoate, linalool, jasmine lactone, indole, and jasmine and its usefulness is thought to be due to such phytochemicals (Sahu et al., 2022).

The purpose of the present was to investigate some bioactivities of *J. officinale* flower that has limited studies in Turkey and to conduct an analysis on the purification of the protease enzyme as well as to investigate the use of the plant in industry by designing a perfume. To the best of our knowledge, there is no such a detailed research and we believe that this study will not only be informative but it will also motivate many other investigations in many areas serving the industrial sectors.

2. Material and Methods

2.1. Plant material

Specimen of *J. officinale* used in the study was collected from Muğla rural areas (in June and September 2016). The plant was authenticated by Dr. Alevcan Kaplan and given a voucher specimen Muğla/2016/01 before being deposited at the Muğla Sıtkı Koçman University. Since the flowers of the plant would be used in the study, they were separated from the plant and gathered together. It was stored in a deep freezer at -80°C until the experiments were undertaken.

2.2. Extraction of protease enzyme

10 g of *J. officinale* flowers stored in the freezer were weighed first, finely chopped, then thoroughly crushed and homogenized by joining 100 mL of pH 7.05 M sodium phosphate buffer. It was spotted in a -80 ^{o}C cooler and taken out after a few hours and waited to dissolve. This step was performed three times. The samples removed from -80 ^{o}C were thawed and the homogenate was separated from the pulp by filtering with a 3-layer cheesecloth and centrifuged at 6.000 rpm for 25 min and supernatant was gathered carefully. The resulting supernatant was used as the crude enzyme extract (Rawdkuen et al., 2010).

2.3. TPP method

Compared to the traditional protein purification methods; TPP is a fast and effective procedure applied in purification. An important advantage of the TPP technique is that it can be applied to large and small-scale studies. Another reason of TPP importance is the low molecular weight structures, removal of lipids and phenolic structures. The basis of the method is the addition of a high concentration of ammonium sulfate (0.8-2.4M) and a water-restricted aliphatic alcohol (usually *t*-butanol) to the crude enzyme solution. Although alcohols such as 1-propanol, 2-propanol, methanol, *t*-butanol, and ethanol dissolve in water, they do not dissolve in cosmotropic salts thus, two separate liquid phases (alcohol phase at the top and salty aqueous phase at the bottom) are formed (Dennison & Lovrein, 1997). *t*-butanol was used in this study. The relationship between TPP and *t*-butanol can be explained as follows: *t*-butanol imparts buoyancy to the proteins that precipitate during TPP. By redissolving the intermediate phase in the buffer, the specific and total activity is recovered and sometimes increased. To calculate the TPP of *J. officinale* flowers protease enzyme, methods of Rawdkuen et al. (2010) were applied. Since the purification ratio of the middle phase and the activity profit are a high match to the upper and bottom phases, optimization studies were carried out following the middle phase (Rawdkuen et al., 2010).

2.4. SDS-PAGE analysis

Sodium dodecyl sulfate (SDS)-Polyacrylamide gel electrophoresis (PAGE) of the enzyme was implemented according to the method of Laemmli (1970).

2.5. Determination of protease activity

The PA (Protease activity) was determined by using casein as the substrate and determining the amount of protein (casein) that the enzyme breaks down (Fadıloğlu, 2001). 1

EU was determined as the amount of µg of enzyme-cleaved protein per min. 1 g of casein was added to 95 mL (0.05) M phosphate buffer with a pH 7 and the temperature was gradually increased while stirring in a magnetic stirrer. The substrate solution was prepared by stirring for 10 min at a temperature range of 95 °C. The reaction was started by joining 500 µL of pure enzyme solution to 1 mL of casein solution and adding 1 mL buffer, making the total volume to 2.5 mL. This reaction mixture was incubated at 40 °C for 20 min and the reaction was stopped by adding 3 mL of 5% trichloroacetic acid (TCA). Non-degraded proteins were allowed to settle for 30 min and then centrifuged at 6.000 rpm for 20 min. The amount of protein cleaved by the enzyme remaining without precipitation in the supernatant was defined by the Bradford method and the enzyme activity was determined (Bradford, 1976).

2.6. Efficacy of pH and temperature

Optimal pH for *J. officinale*'s flowers protease activity was determined using different buffer system. For measurements, acetate buffer (for pH:4-5), phosphate buffer (for pH:6-7), Tris-HCl buffer (for pH:8-9), and borate buffer was used (for pH:10). The Protease enzyme activity was evaluated at temperature values ranging from 0 to 90 °C to define the optimal temperature of *J. officinale*'s flowers protease activity. The temperature effect was defined by heating the substrate solution in the buffer to the suitable temperature in a water bath; then, the reaction was initiated by adding the protease enzyme extract and incubated for 20 min at each temperature step. Afterwards, by joining 3 mL of 5% TCA, the reaction was stopped and the proteins were allowed to precipitate for 30 min. After this time, centrifuge itself for 15 min at 6.000 rpm. The amount of cleaved products in the supernatant was defined by the Bradford (1976).

2.7. Molecular mass estimation by gel filtration chromatography

140 mL suspension Sepharose 4B was dissolved in ddH_2O (distilled water) and allowed to swell overnight at RT. The gel (1x30) was then fraughted onto the column. Equilibration was performed with 0.05 M Na_3PO_4/1mM dithioerythretol buffer (pH: 7.0) until no absorbance was observed in the column at 280 nm. lysozyme (14.3 kDa), BSA (66 kDa), ß- amylase (200 kDa), albumin EGG (45 kDa), ß-lactalbumin (18.4 kDa), and standard solutions were loaded to be 0.2 mg/mL and eluted with 0.05 M Na_3PO_4/1mM DTT buffer. The standard graphic was drawn. The flow rate of the column was setted to 20 mL/h with the aid of a peristaltic pump. Eluates were collected at 4 mL in each tube. The molecular mass of the enzyme was defined with the aid of the standard graph created (Fig. 5).

2.8. Substrate specificity and enzyme kinetics

Substrate specificity study was performed to define which substrates the enzyme can transform into the product and against which it has higher affinity. The activity of the enzyme against five different (Casein, hemoglobin, azoalbumin, gelatin, azocasein) substrates was determined. In order to determine the enzyme's activity, 100- 800 µL of 1% hemoglobin, gelatin, azoalbumin, and azokazein solutions were taken and the volume was completed to 1 mL using ddH_2O. 0.5 mL of enzyme solution was joined to each prepared tube, and buffer was joined so that the final volume was 2.5 mL. The experiment continued according to the procedure for determining the normal protease enzyme activity and the reaction was terminated. After the supernatant was filtered, the amount of fragmented products in the supernatant was defined by the Bradford method (Bradford, 1976). K_m and V_{max} were calculated by plotting 1/V versus 1/[S] Lineweaver Burk plot. The PA for the enzyme breaks down µg protein/mL calculated in min (Pauling et al., 1973).

2.9. Obtaining Essential Oils

2.9.1. Obtaining EOs (essential oils) by hydrodistillation

Hydrodistillation procedure was implemented 3h using Clevenger. Approximately, 150 g of plant flowers were used in assay. After 3h, the water and EOs mixture harvested was partitioned by liquid-liquid extraction using hexane. Na_2SO_4 was joined to remove any water that may have remained on the EOs. Ultimately, the EOs obtained by lifting the solvent under pressurized nitrogen gas was used in perfume desingning.

2.10. Cosmetic product formulation development with obtained biological materials

In order to appraise the use of flowers of some plants such as jasmine, honeysuckle, lotus, chamomile, lily, primrose, and cedar/sandalwood in the area of cosmetics formulations were prepared with EOs obtained by hydrodistillation procedure and cosmetic product formulations containing protease enzymes were improved. The perfume desingning is shown in Table 1 and Figure 1. Perfume is an odorous liquid obtained by mixing natural or synthetic scented oils (raw materials), water, and alcohol in various proportions (Can et al., 2015).

2.11. Determination of aromatic volatile organic compounds of *J. officinale* flowers by Headspace GC/MSD

The aromatic volatile organic compounds of *J. officinale* flowers were determined by Headspace GC/MSD according to Daşdemir (2017). Fragmented fresh jasmine flowers were weighed at 5.00g into a 20 mL headspace vial. Then, $MgSO_4$ (anhydrous magnesium sulfate) was joined and it was completely mixed with the magnetic. The vial was placed in the headspace sampler and the extraction process was started, that would take 30 min at 90°C. After 30 min, the volatile components at the top of the vial were transferred by the headspace sampler with a GC Split/Splitless inlet transferline for 1 min with helium gas. Headspace GC/MSD instrument analysis parameters are shown in Table 2.

Figure 1. Perfume studies

Table 1. Perfume formulation

Formulation	Percentage of substances in perfume (%)
EtOH	65
Plant extract	10
Natural essential oil	5
Pure water	15
Odor stabilizer	5

Table 2. Headspace GC/MSD instrument analysis parameters

Device Parameters	
Balancing Time	2 min
Maximum Temperature	300°C
Device Program	60°C for 1min 10°C/min; 100°C for 1min 10°C/min; 260°C for 8 min
Operation time	30 min
MMI Input Parameters	
Method	Divide
Heater	250°C
Thermal Aux (Tranfer Line)	
Heater	On
Temperature	250°C
Column	
Name	Agilent J&W 19091S-431UI HP-5MS UI (15µmx250µmx0.25µm)
Pressure	21.801 psi
Flow	1.8 mL/min
MS Acquire Parameter	
Acquisition Mode	View
EM voltage	1200
Low mass	35.0
High mass	400.0
Threshold	150
MS Source	230°C max 250°C
MS quadrupole	150°C max 200°C
GC-MSD Parameters	
Device Temperature	95°C
Cycle Temperature	110°C
Transfer Line Temperature	120°C
Bottle Balance	30 min
Injection Time	1 min
GC Turnover Time	40 min
Bottle Size	20 mL
Fill Mode and Pressure	Pressure / 14psi
Cooldown	0.5 min
Extraction Method	Multiple extraction

2.12. Determination of phenolic components

2.12.1. Extraction conditions of samples

For sample analysis, MeOH extracts were arranged at RT for 24 h using a magnetic stirrer, and the solutions were filtered with the help of blue band filter paper in order to get rid of possible solid particles and dirtiness and to ensure further homogeneity. After defining the final concentration of the obtained extracts, the extract solvent was lifted in a RE at 60°C and the residue was dissolved in 10 mL of distilled water with a pH of 2. Thereafter, diethylether and then ethylacetate extraction of 5 mL was performed 3 times each. The extracts obtained at the end of the extraction treat were taken from evaporator balloons and their solvents were removed in a RE at 60°C. The phenolic component analyzes of the extracts, the contents of which were dissolved with 2 mL of MeOH, were performed by HPLC-UV (Can et al., 2015).

2.12.2. Definition of phenolic compounds by HPLC-UV

HPLC-UV study was carried out on an HPLC system equipped with a UV-Vis detector (Elite LaChrom Hitachi, Japan) at 280 nm. Gradient program with 2% acetic acid (pure water) in A reservoir and 70-30% acetonitrile-pure water in B reservoir is given in Table 3. Additionally, working optimization was achieved by adjusting the injection volume of samples and standards to 25 µL, the mobile phase flow rate to 1.2 mL.min^{-1} and the column temperature to 30 °C in the column furnace (Can et al., 2015).

Table 3. RP-HPLC-UV gradient program

Time (min)	A (2% acetic acid in pure water)	B (70-30% acetonitrile-pure water)
0.01	95.00	5.00
3.00	95.00	5.00
8.00	85.00	15.00
10.00	80.00	20.00
12.00	75.00	25.00
20.00	60.00	40.00
30.00	20.00	80.00
35.00	95.00	5.00
50.00	95.00	5.00

3. Results and Discussion

The world of cosmetics, which has reached the present day from the prehistoric times and now is a sector, has attracted the attention of people in every period. The emergence of cosmetics on the stage of history begins with the point where the perception of beauty and aesthetics begins; that is, almost with the birth of humanity. Along with the rapid developments in the chemical industry, the production of synthetic chemicals from the end of the 19th to the beginning of the 20th century has also positively affected the issue of odor (Aşık, 2017). On the other hand, the rapid and great increase in the world population and the increase in the cultural level have led to the production and consumption of a wide variety of personal cleaning, cosmetic products, and domestic and industrial cleaning products. Parallel to this increase, the need for fragrance substances has increased (Aşık, 2017). According to the literature review, no study has been conducted in which protease enzyme is purified and characterized from *J. officinale* flowers and these parameters are combined with perfume design, which is the most prestigious product in the cosmetic world.

The protease enzyme was purified by TPP, one of the biodegradation techniques. In the TPP method used in this study, ammonium sulfate saturation 30% (w/v) and

homogenate: *t*-butanol rate 1:1.5, the protease enzyme remained predominantly in the middle phase. Therefore, identification was started using these ratios. Purification results are summed up Table 4. From Table 4, it can be shown that, the protease enzyme was purified with a purification fold of 1.052 and a yield of 50.30%. SDS-PAGE was performed to check the purity of the protease enzyme purified from *J. officinale* flowers (Laemli, 1976) (Fig. 2). Rawdkuen et al. (2010) reported that they purified *Calotropis procera* (Aiton) Dryand. latex protease enzyme 6.92 times with 132% efficiency using TPP systems. Chaiwut et al. (2010) performed the extraction of proteases from papaya peels by TPP method and noted that they achieved a recovery rate of 10.1 times with a recovery of about 89.4%. Then, these authors also performed the optimization by joining up to 55% $(NH4)_2SO_4$ at the lower phase of the first step. These results were generally consistent with our findings. In the TPP system, the phase in which the enzyme will be collected differs depending on the characteristics of the amino acids contained in the enzyme and their isoelectric points. For this reason, the interphase distributions of the same enzyme may vary in extracts obtained from different sources (Kat & Yılmazer Keskin, 2013). In the current study, it has been shown that TPP which is a simple, cost-effective, bioseparation technique that can be used in large volumes and can be performed at room temperature compared to other multi-step, costly chromatographic purification techniques can be efficiently used for the extraction of proteases from *J. officinale* flowers. Thus, we can suggest to used it effectively in industrial purposes.

Table 4. Purification results of *J.officinale* flower's protease enzyme by TPP method

Samples	Activity (EU/mL)	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification (folds)	Yield (%)
Homogenate	0.66	66	78.5	0.841	1	100
Medium phase	0.332	33.2	37.5	0.885	1.052	50.30

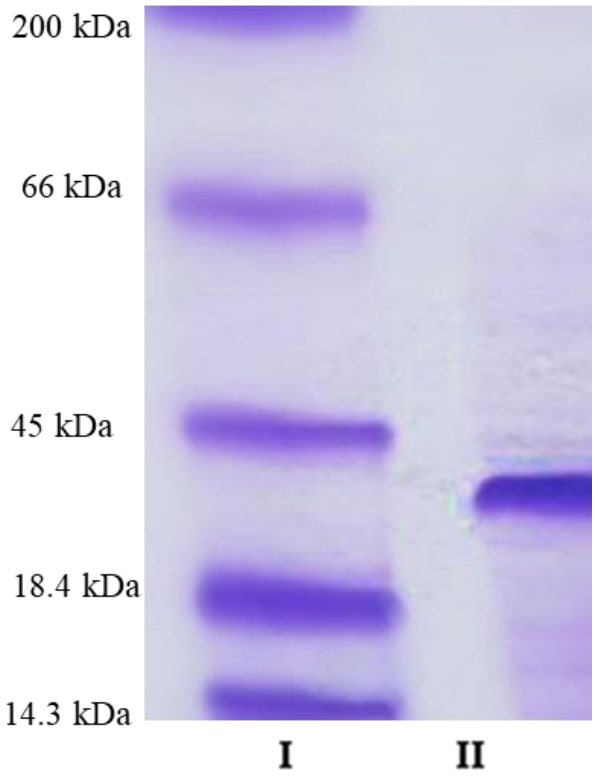

Figure 2. SDS-PAGE image of the purified *J. officinale* flower's protease enzyme (I, standard protein mix: BSA (66 kDa), Albumin EGG (45 kDa), ß-Amylase (200 kDa), ß-lactalbumin (18.4 kDa), Lysozyme (14.3 kDa); II, Protease enzyme purified from *J. officinale* flower)

In order to define the optimal pH of the protease enzyme purified from the *J. officinale* flower, activity measurements were made by various buffer systems. The amount of proteolytic activity against pH change for the protease enzyme is depicted in Figure 3. It was defined that the optimal pH of the enzyme was 5 and it showed activity in the pH:4-9 range. To define the optimal temperature of the protease enzyme purified from *J. officinale* flowers, activity measurements were made at 10-100°C. Activity - temperature graphic is given in Figure 4. It was defined that the optimal temperature was 30°C and the enzyme was active in the range of 20-60°C. Thermal factors such as increase in temperature or prolongation of the incubation period cause denaturation in the three-dimensional structure of the enzyme in the protein structure, leading to results such as failure to ensure the enzyme-substrate relationship, structural deterioration of the active center and failure to function and leading to losses in activity. Hereby, in industrial applications temperature ranges in which the studied enzyme can maintain its stability are preferred and it is important to define this range. The stability of the enzyme, that is, its ability to maintain its activity over time, increases its applicability in processes and significantly affects the cost (Karkaş, 2009). In addition, Banik et al. (2018) determined the optimal pH and temperature of the protease enzyme obtained from the leaves of *Moringa oleifera* L. as 8 and 37 °C, respectively. In another study, Prabhu et al. (2018), determined the pH of the protease enzyme obtained from pod, seed, and leaf samples of *Vicia faba* L. as 6. Parlak et al. (2008) determined that the optimal pH of the protease enzyme purified from the flowers of Anatolian orchid (*Orchis anatolica* Boiss.) is 6 and the pH range in which it is active is 4-9. The temperature range at which the enzyme is active was determined as 10-70°C and the temperature at which it showed maximum activity was determined as 60°C. In Atrooz & Alomari's (2020) study on the protease enzyme of *Mentha piperita* L. and *Thymus capitatus* (L.) Hoffmanns. & Link plants, the pH of *Thymus* was determined as 2.0 and 3.5, the maximum relative activity was 65%, between pH 6.0-8.0, the maximum relative activity was 100%, Mentha's was 100% and 70%, respectively. They calculated that it has two optimal pH at 3.0 and 9.0 with relative activity. In the same work, the calculated maximum relative activity for the protease enzyme for *Thymus* and *Mentha* to be 40°C (100%) and 35°C (100%), respectively. Considering the importance of the enzyme, it is clear that the protease enzyme has been broadly studied in different plant and food products by various researchers (Cheng et al., 2016; Mandujano - González et al., 2016; Niemer et al., 2016; Sequeiros et al.,

2016). It is thought that the enzyme purified in the present study will also be advantageous as a preferable source for cosmetic products to be active at the appropriate temperature and pH.

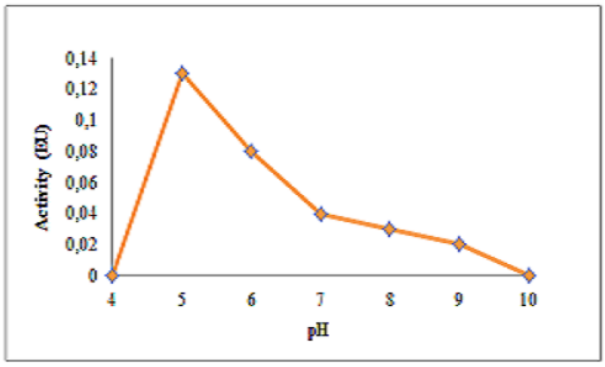

Figure 3. The efficacy of pH on the activity of enzyme

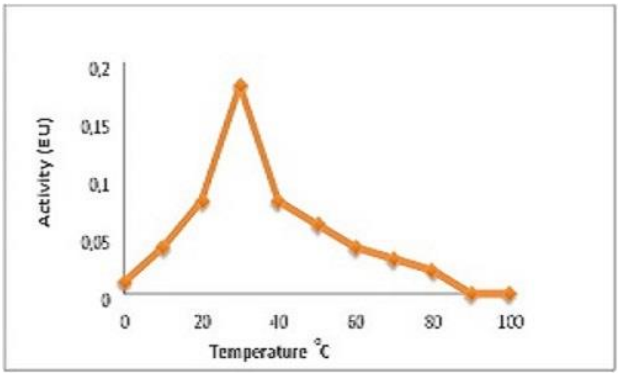

Figure 4. The efficacy of temperature on the activity of enzyme

The molecular mass of the enzyme was defined by GFC (gel filtration chromatography). The values of Ve/Vo and LnMW were calculated by determining which tube the standard proteins were in according to their molecular mass (Table 5). Based on the data calculated in Table 5, the standard protein graph to be used in the molecular mass calculation was created and given in Figure 5. The absorbance evaluation at 280 nm of the eluted proteins were taken as a result of gel filtration chromatography of the purified enzyme. Tubes with protein were identified. The enzyme activity was determined in these tubes and the molecular mass was calculated by determining which tube the protease enzyme in the white jasmine flower was in (Table 6). The molecular mass of the protease enzyme purified from the flowers of *J. officinale* was defined as 21.386 kDa using gel filtration chromatography. Prabhu et al. (2018) determined the molecular weight of protease enzyme from pod, seed and leaf samples of *V. faba* L. leaf supernatant (100kDa), leaf pellet (100kDa), seed supernatant (60kDa, seed, pellet (60 kDa), pod supernatant. (85 kDa), and pod pellet (100 kDa). Jinka et al. (2009) prufied Cysteine protease from *Horse gram*. Asif-Ullah et al. (2006) isolated Serine protease from the *Cucumis trigonas* Roxb. In the study by Parlak et al. (2008), the molecular mass of the protease enzyme purified from *O. anatolica* flowers was calculated as 8.4 kDa using SDS-polyacrylamide gel electrophoresis and sephadex G-100 gelfiltration chromatography. Previous literature studies have noted the presence of proteases in different plant sources. The protease enzyme can be classified into four types, namely serine, aspartate, metallo, and cysteine proteases, respectively. Among these groups, cysteine proteases are predominantly found in plant sources (Domsalla & Melzig, 2008; Rawlings et al., 2010; González et al., 2011). It was observed that the molecular mass of jasmine flower has a value among the molecular weights of other plants.

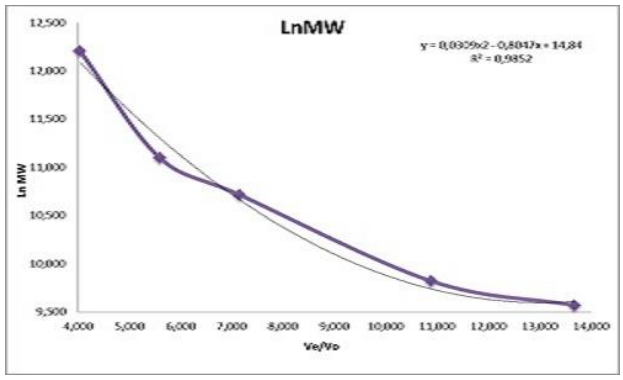

Figure 5. Gel filtration chromatography standard curve

The PA of the enzyme was used to define the V_{max} and K_m values of the enzyme. The substrate specificity of the enzyme purified from *J. officinale* flowers was evaluated according to the K_m and V_{max} results obtained from the Lineweaver burk plot using different substrates and are given in Table 7. It shows that, the substrate affinity of the enzyme was determined as casein (K_m and V_{max} values; 1.16 µM and 1.27 µg/mL.min)>, azoalbumin (K_m and V_{max} values; 1.90 µM and 0.70 µg/mL.min)>, hemoglobin (K_m and V_{max} values; 2.14 µM and 1.42 µg/mL.min)>, gelatin (K_m and V_{max} values; 2.82 µM and 1.03 µg/mL.min, respectively. It was defined that the enzyme did not show affinity for azocasein substrate. Parlak et al. (2008), investigated the substrate specificity of the protease enzyme purified from the flowers of *O. anatolica* and found that it hydrolysed hemoglobin, albumin, azoalbumin and casein, but did not hydrolyze gelatin. The same researcher, using the casein of the purified protease enzyme purified from the flowers of *O. anatolica*, plotted the Linewear-Burk plot and calculated the V_{max} and K_m values as 0.16 µg/L.min and $2.8x10^{-3}$g/mL, respectively. Atrooz & Alomari (2020) determined that the most suitable substrate for the protease enzyme obtained from *M. piperita* and *T. capitatus* plants was egg albumin. It was found that the egg albumin has more affinity toward *Mentha* proteases (K_m 1.7 mg/mL) than *Thymus* proteases (K_m 3.33 mg/mL). Working on the kinetics of an enzyme is considered to be of crucial value in determining the appropriate affinity and conditions for optimal activity, especially if these enzymes are to be used for industrial and biochemical applications (Atrooz & Alomari 2020).

Aroma substances of *J. officinale* flowers were determined using Headspace GC/MSD (Table 8 and Fig. 6). The dominant compound was determined to be 64.87 % Linalool. Linalool ($C_{10}H_{18}O$) is a monoterpene naturally found in more than 200 oils derived from different parts of plants (Peana & Moretti, 2008). Linalool is defined as a light and refreshing, floral-woody, specific fragrance with a slight citrus note (Arctander, 1994). Linalool is also the main ingradient of many essential oils known to exhibit a variety of biological abilities such as antiplasmodial and antibacterial effects (Van Zyl et al., 2006). Further, the

antihyperalgesic anti-inflammatory and antinociceptive efficacy of linalool have been studied by various researchers by establishing experimental setups to examine the effects in different animal models (Peana et al., 2002; Peana et al., 2006a; Peana et al., 2006b). Roughly, 95% of synthetic linalool, and practically all by volume from natural sources, is used in formulations to add a specific scent to cosmetics, soaps, perfumes, and household cleaning products, while only about 1% is joined to flavor and flavor foods and beverages (Kamatou & Viljoen, 2008). Before the 1950s, nearly all linalool used in perfumery was isolated from EOs, especially rosewood oil (Kamatou & Viljoen, 2008). Nowadays, with the expansion of the range of products in the sectors and the increase in demand, the tendency to seek new sources has increased. The high amount of this component in our study shows us that the *J. officinale* flower can be used in perfume design. Moreover, the perfume products we obtained in our studies were produced very successfully (Fig. 1).

Phenolic components of *J. officinale* flowers were defined by HPLC-UV and are detailed in Figure 7 and Table 9. As a result of the phenolic component study, it was observed that the amount of *p*-OH benzoic acid and protocatechuic acid was high. These components are the ones with high antioxidant capacity. Coumaric acid, coumarin, and their derivatives are widely found in nature. Many natural and synthetic coumarin derivatives are components that can be used in different applications in the fields of chemistry, biology, medicine, and physics. Coumarin and its derivatives are components used in food, perfume, cosmetics, and pharmaceuticals (Angelescu et al., 2006). It can be said that the coumaric acid found in the *J. officinale* flower is an important phenolic component for perfume. Ferulic acid is a property that is exceedingly helpful in creating anti-aging cosmetics products. Ferulic acid is one of the most powerful natural antioxidants. Ferulic acid is a phenolic component that neutralizes free radicals such as superoxide and nitric oxide that damage a cell wall and DNA. Ultraviolet rays can increase the antioxidant effect of ferulic acid. Ferulic acid can be included in the content of products that prevent aging in the field of cosmetics (Aşık, 2017). In addition to the use of essential oil from the flowers of *J. officinale*, it is important to report the phenolic components giving an opportunity to use the species in medicine. Such a medicinal exploitation will no doubt positively impact the country's economy.

Table 5. Calculations for the Standard Protein chart

Standard protein mixture	MW(Dalton)	Tube sequence	Ve/Vo	LnMW
Lysozyme	14300	44	13.681	9.568
ß-Lactoglobuline	18400	35	10.882	9.820
Albumin, EGG	45000	23	7.151	10.714
Albumin, Bovine	66000	18	5.597	11.097
ß- Amylase	200000	13	4.042	12.206

Table 6. Calculations for molecular mass determination of protease enzyme purified from *J. officinale* flowers by GFC

Proteins	MW (Dalton)	Tube sequence	Ve/Vo	LnMW
1. Protein	559591	7	2.1765	13.235
2. Protein	178478	13	4.0420	12.092
3. Protein	109262	16	4.9748	11.602
4. Protein	81164	18	5.5967	11.304
5. Protein	61751	20	6.2185	11.031
6. Protein	24256	29	9.0169	10.096
7. Protein	21386	53	16.4791	9.970
8. Protein	49537	62	19.2774	10.810
9. Protein	156906	70	21.7648	11.963

Table 7. Substrate specificity results of purified protease enzyme

Substrate	Km (µM)	Vmax (µg/mL.min)
Casein	1.16	1.27
Hemoglobin	2.14	1.42
Azoalbumin	1.90	0.70
Gelatin	2.82	1.03
Azocasein	nd	nd

*nd: not determined

Table 8. Percentage of aromatic volatile organic compounds of *J. officinale* flowers

No	Component Name	Percentage (%)
1	Linalol	64.87

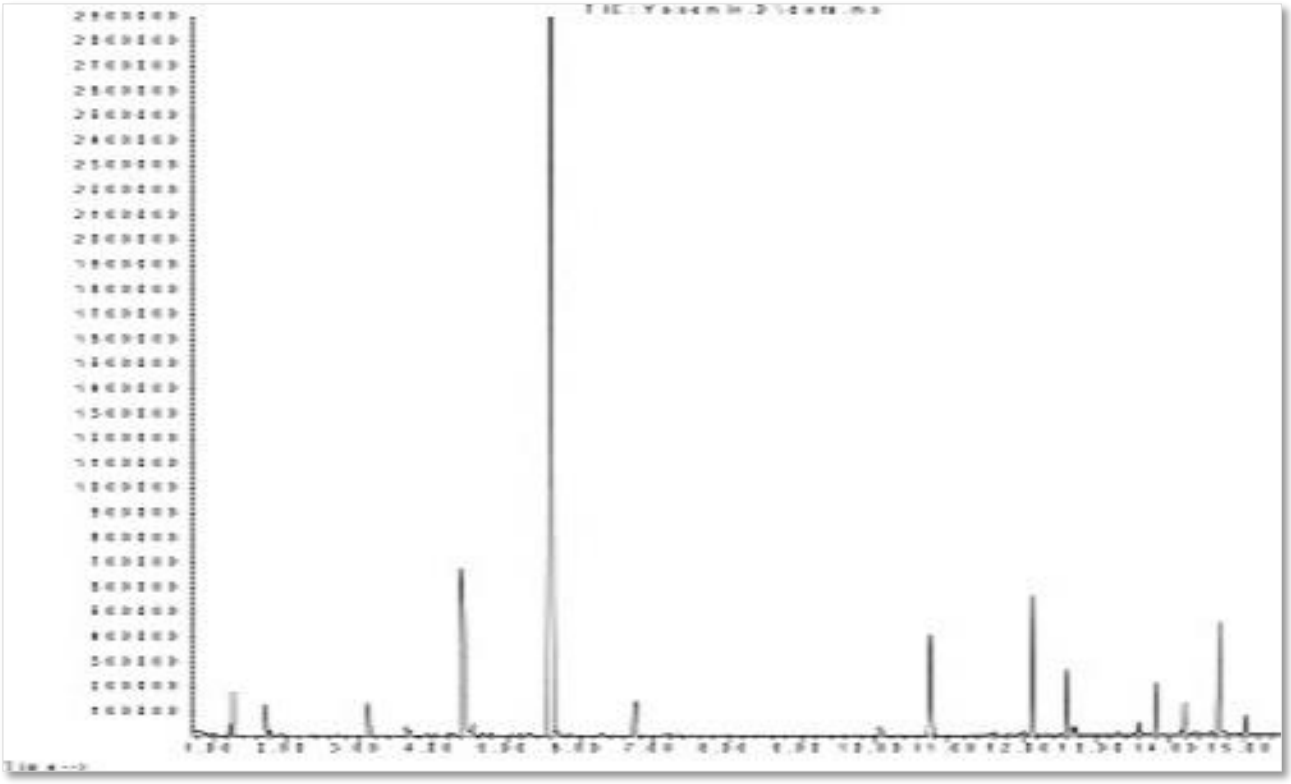

Figure 6. Agilent 7890 GC/ 5975C MSD device measurement results by using Head-space solid-phase microextraction (HS-SPME) methods for flavoring volatile organic compounds of *J. officinale* flowers

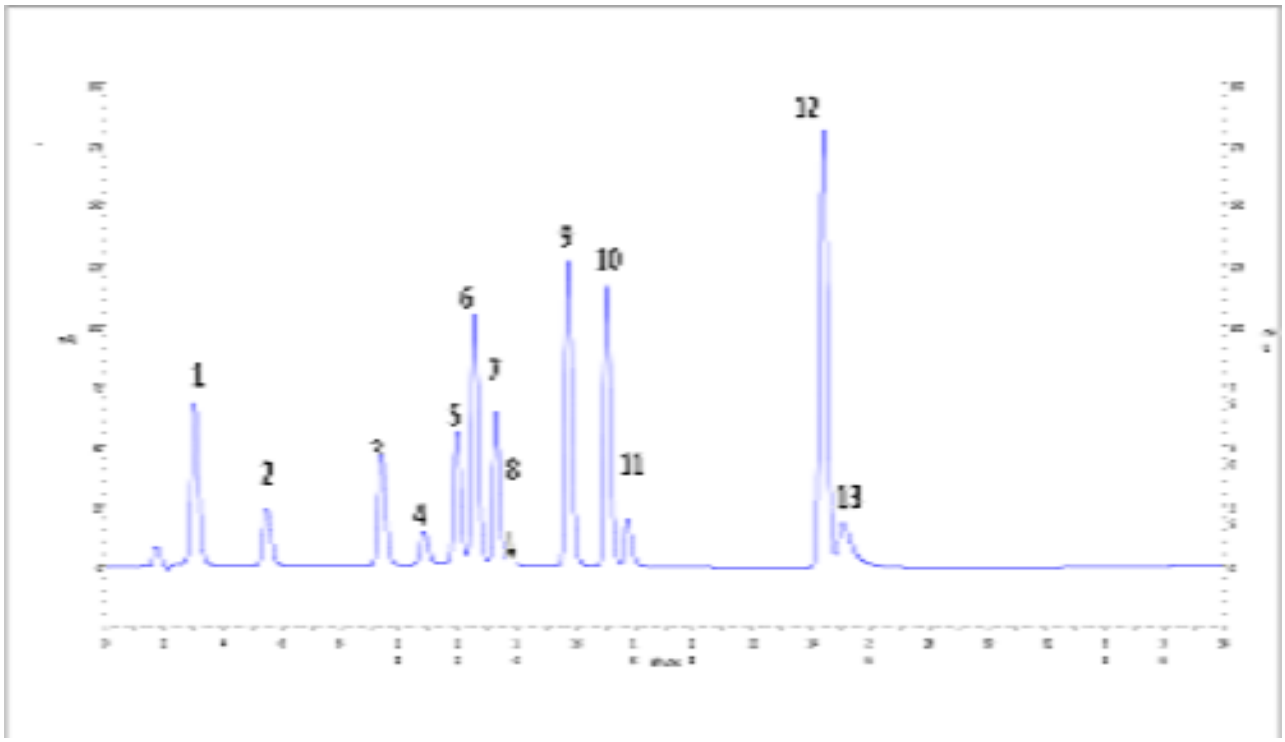

Figure 7. Phenolic acid standard chromatogram 1. Gallic acid, 2. Protocatechuic acid, 3. *p*-OH benzoic acid, 4. Catechin, 5. Vanillic acid, 6. Caffeic acid, 7. Syringic acid, 8. Epicatechin, 9. *p*-Coumaric acid, 10. Ferulic acid, 11. Rutin, 12. Daizein, 13. *t*-Cinnamic acid, 14. Luteolin

Table 9. Phenolic ingradients of *J. officinale* flowers

No	Standarts	Samples (µgextract/g sample)
1	Gallic acid	T.E
2	Protocatechuic Acid	108.92±0.31
3	*p*-OH Benzoic Acid	73.97±0.21
4	Catechin	T.E

Table 9. (Continued)

No	Standarts	Samples (µgextract/g sample)
5	Vanillic Acid	43.81±0.17
6	Caffeic Acid	5.69±0.02
7	Syringic Acid	8.39±0.05
8	Epicatechin	T.E
9	*p*-Coumaric Acid	5.76±0.02
10	Ferulic Acid	1.42±0.01
11	Rutin	38.15±0.11
12	Daidzein	T.E
13	*t*-Cinnamic Acid	T.E
14	Luteolin	T.E

*T.E: It means that it is below the analysis limit

4. Conclusions

To summarize the present work, different properties of the *J. officinale* plant have been investigated and its use in the cosmetics and pharmaceutical sectors has been examined and given in detail. The effect of the raw materials used in perfume making, the characteristic of the smell and its naturalness can be taken into consideration. The harmony of fragrances with flowers, fruits or spices can be taken into account. An idea can be presented based on the scent characteristics of flowers. In this study, perfume formulations, the most prestigious product of the cosmetic factor, were developed and fragrant perfumes were designed and it was predicted that the flower of *J. officinale* could be used in the perfume industry.

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References

Alrashdi, A.S., Salama, S.M., Alkiyumi, S.S., Abdulla, M.A., Hadi, A.H., Abdelwahab, S.I., Taha, M.M., Hussiani, J., & Asykin, N. (2012). Mechanisms of Gastroprotective Effects of Ethanolic Leaf Extract of *Jasminum sambac* against HCl/Ethanol-Induced Gastric Mucosal Injury in Rats. *Evidence-Based Complementary and Alternative Medicine*, 2012, 786426, 1-15. https://doi.org/10.1155/2012/786426

Angelescu, E., Pavel, O.D., Bırjega, R., Zavoianu, R., Costentin, G., & Che, M. (2006). Solid base catalysts obtained from hydrotalcite precursors, for Knoevenagel synthesis of cinamic acid and coumarin derivatives. *Applied Catalysis A: General*, 308, 13-18. https://doi.org/10.1016/j.apcata.2006.04.011

Arctander, S. (1994). Perfume and Flavor Chemicals (Aroma Chemicals). Allured Publishing Corporation; Carol Stream, Illinois.

Asif-Ullah, M., Kim, K.S., & Yu, Y.G. (2006). Purification and characterization of a serine protease from *Cucumis trigonus* Roxburghi. *Phytochemistry*, 67(9), 870-875.

Aşık, S. (2017). Beyaz Yasemin Çiçeğinin (*Jasminum officinale*) Bazı Biyoaktiviteleri ile Koku Bileşenlerinin Araştırılması ve Parfüm Tasarımında Kullanılması. Retrieved from: https://tez.yok.gov.tr/UlusalTezMerkezi/tezDetay.jsp?id=YMYx1FtU5Irj-u1RU7QajQ&no=sHf1HfMghp84EAgyB0b6HQ

Atrooz, O.M., & Alomari, F.N. (2020). Determination of the activity and kinetics parameters of proteases in the crude plant extracts of *Mentha piperita* L. and *Thymus capitatus* L. *Journal of Applied Biology & Biotechnology*, 8(6), 33-37. https://doi.org/10.7324/JABB.2020.80606

Bai, N., He, K., Ibarra, A., Bily, A., Roller, M., Chen, X., & Rühl, R. (2010). Iridoids from *Fraxinus excelsior* with adipocyte differentiation-inhibitory and PPARα activation activity. *Journal of Natural Products*, 73, 2-6. https://doi.org/10.1021/np9003118

Balkrishna, A., Sharma, G., Sharma, N., Rawat, N., Kumar, A., & Arya, V. (2022). Transition of Indian Agriculture from Glorious Past to Challenging Future: A Serious Concern. *Indian Journal of Ecology*, 49(3), 977-986. https://doi.org/10.55362/IJE/2022/3625.

Banik, S., Biswas, S., & Karmakar, S. (2018). Extraction, purification, and activity of protease from the leaves of *Moringa oleifera* [version 1; referees: 2 approved, 1 approved with reservations]. *F1000Research*, 7, 1151. https://doi.org/10.12688/f1000research.15642.1

Baytop, T. (1999). *Türkiye'de Bitkiler İle Tedavi*, Baskı II, Nobel Tıp Kitabevleri LTD., İstanbul, 234 ss.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72, 248-254. https://doi.org/10.1006/abio.1976.9999

Can, Z., Yildiz, O., Sahin, H., Turumtay, E.A., Silici, S., & Kolayli, S. (2015). An investigation of Turkish honeys: Their physico-chemical properties, antioxidant capacities and phenolic profiles. Food Chemistry,180, 133-141. https://doi.org/10.1016/j.foodchem.2015.02.024

Chaiwut, P., Pintathong, P., & Rawdkuen, S. (2010). Extraction and three-phase partitioning behavior of proteases from papaya peels. *Process Biochemistry*, 45, 1172-1175. https://doi.org/10.1016/j.procbio.2010.03.019

Chaturverdi, A.P, Kumar, M. & Tripathi, Y.B. (2013). Efficacy of *Jasminum grandiflorum* L. Leaf extract od dermal wound healing in rats. *International Wound Journal*, 10(6), 675-82. https://doi.org/10.1111/j.1742-481X.2012.01043.x

Cheng, Z., Li, J.F., Niu, Y., Zhang, X.C., Woody, O.Z., Xiong, Y., Djonović, S., Millet, Y., Bush, J. & McConkey, B.J. (2015). Pathogen-secreted proteases activate a novel plant immune pathway. *Nature*, 521(7551), 213-216.

Chinese Pharmacopoeia Commission. (2015). Pharmacopoeia of the People's Republic of China, vol. 1, China Medical Science Publisher, Beijing, 45-46 (&271).

Daşdemir, S.N. (2017). Müge Çiçeğinin (*Convallaria majalis*) Bazı Biyoaktivitelerinin Belirlenmesi Ve Diğer Bazı Çiçeklerle Birlikte Parfüm Tasarımında Kullanılabilirliğinin Araştırılması. Retrieved from https://tez.yok.gov.tr/UlusalTezMerkezi/tezDetay.jsp?id=4bBx1zmuywZwb8LkKyUN-w&no=0EfeptaMkhl8uE62XxAb4A

De Feo, V., Aquino, R., Menghini, A., Ramundo, E. & Senatore, F. (1992). Traditional Phytotherapy in the peninsula Sorrentina, Campania, Southern Italy. *Journal of Ethnopharmacology*, 36, 113-125. https://doi.org/10.1016/0378-8741(92)90010-O

Dennison, C., & Lovrein, R. (1997). Three phase partitioning: concentration and purification of proteins. *Protein Expression and Purification*, 11, 149-161. https://doi.org/10.1006/prep.1997.0779

Domsalla, A., & Melzig, M.F. (2008). Occurrence and properties of proteases in plant latices. *Planta Medica*, 74(07), 699-711. https://doi.org/10.1055/s-2008-1074530

Dossou-Yovo, H.O., Kindomihou, V., Vodouhè Fifanou, G., & Sinsin, B. (2021). Assessment of the diversity of medico-magic knowledge on four herbaceous species in Benin. *The Scientific World Journal*, 6650704, 1- 11. https://doi.org/10.1155/2021/6650704

Dossou-Yovo, H.O., Kindomihou, V., Vodouhè Fifanou, G., & Sinsin, B. (2022a). Investigating the use profile of *Kigelia africana* (Lam.) Benth. through market in Benin. *Conservation*, 2(2), 275-285. https://doi.org/10.3390/conservation2020019

Dossou-Yovo, H.O., Vodouhè Fifanou, G., Kaplan, A., & Sinsin, B., (2022b). Application of ethnobotanical indices in the utilization of five medicinal herbaceous plant species in Benin, West Africa. *Diversity*, 14, 612. https://doi.org/10.3390/d14080612

Elhawary, S., EL-Hefnawy, H., & Mokhtar, F.A. (2020). Green synthesis of silver nanoparticles using extract of *Jasminum officinale* L. leaves and evaluation of cytotoxic activity towards bladder (5637) and breast cancer (MCF-7) cell lines. *International Journal of Nanomedicine*, 15, 9771-9781. https://doi.org/10.2147/IJN.S269880

Fadıloğlu, S. (2001). Immobilization and characterization of ficin. *Nahrung/Food*, 45 (2), 143-146. https://doi.org/10.1002/1521-3803(20010401)45:2<143::AID-FOOD143>3.0.CO;2-8

Fares, R., Bazzi, S., Baydoun, S.E. & Abdel-Massih, R.M. (2011). The antioxidant and antiproliferative activity of the Lebanese *Olea europaea* extract. *Plant Foods for Human Nutrition*, 66, 58-63. https://doi.org/10.1007/s11130-011-0213-9

González, R.N., Badillo, J.A., Aranda, A.J.S., & Carmen O.S.M. (2011). Production of plant proteases *in vivo* and *in vitro*-a review. *Biotechnology Advances*, 29(6), 983-996. https://doi.org/10.1016/j.biotechadv.2011.08.017

Green, P.S. (2003). Synopsis of the Oleaceae from the Indian sub-continent. *Kew Bulletin*, 58(2), 257-295. https://doi.org/10.2307/4120616

Huang, Yan-Li., Oppong, M.B., Guo, Y., Wang, Li-Zhi., Fang, Shi-Ming., Deng, Yan-Ru., & Gao, Xiu-Mei. (2019). The Oleaceae family: A source of secoiridoids with multiple biological activities. *Fitoterapia*, 136,104155. https://doi.org/10.1016/j.fitote.2019.04.010

Jinka, R., Ramakrishna, V., Rao, S.K., & Rao, R.P. (2009). Purification and characterization of cysteine protease from germinating cotyledons of horse gram. *BMC Biochemistry*, 10(1), 1-11. https://doi.org/10.1186/1471-2091-10-28

Joshi, M.C., Raju, A., Arulanandham, A., & Saraswathy, G.R. (2008). Hepatoprotcetive activity of *Jasminum angustifolium* Linn against CCl_4 induced hepatic injury in rat. *Pharmacologyonline*, 3, 197-205.

Kamatou, G.P.P., & Viljoen A.M. (2008). Linalool - A Review of a Biologically Active Compound of Commercial Importance. *Natural Product Communications*, 3(7), 1183-1192. https://doi.org/10.1177/1934578X0800300727

Karkaş, T. (2009). İnvertaz Enziminin Ekstraksiyonu ve Saflaştırılması için Sulu İkili-Faz Afinite Sistemlerinin Geliştirilmesi. Retrieved from https://tez.yok.gov.tr/UlusalTezMerkezi/tezDetay.jsp?id=H8tgyFc-BcxsxgrIA0Gz2g&no=7EkHfFxCHwM0PT6-p1vR-Q

Kat, B., Yılmazer Keskin S. (2013). Üçlü faz sistemi ile saflaştırılan invertaz enziminin termal kararlılığının incelenmesi. *Sakarya Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, 17 (2), 291-294.

Kunhachan, P., Banchonglikitkul, C., Kajsongkram, T., Khayungarnnawee A., & Leelamanit, W. (2012). Chemical Composition, Toxicity and Vasodilatation Effect of the Flowers Extract of *Jasminum sambac* (L.) Ait. *Evidence-Based Complementary and Alternative Medicine*, 2012, 471312, 1-7. https://doi.org/10.1155/2012/471312

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4, *Nature*, 227, 680-85.

Mandujano Gonzalez, V., Téllez, J.A., Anducho, R.M.A., Arana, C.A., & Mercado, F.Y. (2016). Purification and characterization of the extracellular aspartyl protease APSm1 from the phytopathogen fungus *Stenocarpella maydis*. *Protein Expression and Purification*, 117,1-5. https://doi.org/10.1016/j.pep.2015.09.017

Niemer, M., Mehofer, U., Verdianz, M., Porodko, A., Schähs, P., Kracher, D., Lenarcic, B., Novinec, M., & Mach, L. (2016). *Nicotiana benthamiana* cathepsin B displays distinct enzymatic features which differ from its human relative and aleurain-like protease. *Biochimie*, 12(2), 119-125. https://doi.org/10.1016/j.biochi.2015.06.017

Parlak, B., Güngör, A. A., Demir, Y., & Demir, N. (2008). *Anadolu Orkidesi (Orchis anatolica) Çiçeklerinden Proteaz Enziminin Saflaştırılması ve Peynir Üretimindeki Kullanılabilirliğinin Araştırılması*. Türkiye 10. Gıda Kongresi, Mayıs 21-23, Erzurum, Türkiye.

Pauling, L. (1973). Die Natur der chemischen Bindung, Chemie-Varlag, Weinheim, 523 pp.

Peana, T.A., & Moretti, M.D.L. (2008). Linalool in Essential Plant Oils: Pharmacological Effects. Botanical medicine in clinical practice, 716-724, CAB International, Wallingford, UK. https://doi.org/10.1079/9781845934132.0716

Peana, A.T., D'Aquila, P.S., Panin, F., Serra, G., Pippia, P., & Moretti, M.D.L. (2002). Anti-inflammatory activity of linalool and linalyl acetate constituents of essential oils. *Phytomedicine*, 9, 721-726. https://doi.org/10.1078/094471102321621322

Peana, A.T., Marzocco, S., Popolo, A., & Pinto, A. (2006a). (−)-Linalool inhibits in vitro NO formation: Probable involvement in the antinociceptive activity of this monoterpene compound. *Life Sciences*, 78, 719-723. https://doi.org/10.1016/j.lfs.2005.05.065

Peana, A.T., Rubattu, P., Piga, G.G., Fumagalli, S., Boatto, G., Pippia, P., & De Montis, M.G. (2006b). Involvement of adenosine A1 and A2A receptors in (−)-linalool-induced antinociception. *Life Sciences*, 78, 2471-2474. https://doi.org/10.1016/j.lfs.2005.10.025

Prabhu, S., D., Apoorva, S., Nandita, J., Palani, C., & Devi Rajeswari, V. (2018). Purification of protease enzyme from the leaf, seed and pod samples of *Vicia faba* L. *International Food Research Journal*, 25(5), 1904-1911.

Prachee, D., Ayushi, T., Praveen, T., Shikha, A., A.K, R., & Watal, G. (2019). Phytochemical and Phytoelemental Profile of *J. officinale*. *International Journal of Pharmacognosy and Phytochemical Research*, 11(1), 5-9.

Rawdkuen, S., Chawut, P., Pntathong, P., & Benjakul, S. (2010). Three-Phase Partitioning of Protease from *Calotropis procera* latex. *Biochemical Engineering Journal*, 50, 145-149.

Rawlings, N.D., Barrett, A.J., & Bateman, A. (2010). MEROPS: The peptidase database. *Nucleic Acids Research*, 38(1), 227-233. https://doi.org/10.1093/nar/gkp971

Reshma, D., Anitha, C.T., & Tharakan, S.T. (2021). Phytochemical and Pharmacological Properties of Five Different Species of *Jasminum*. *Plant Archives*, 21(2), 126-136. https://doi.org/10.51470/PLANTARCHIVES.2021.v21.no2.022

Sabharwal, S., Aggarwal, S., Vats, M., & Sardana, S. (2012). Preliminary phytochemical investigation and wound healing activity of *Jasminum sambac* (linn) ait. (Oleaceae) Leaves. *International Journal of Pharmacognosy and Phytochemical Research*, 4(3), 146-150.

Sahu, R., Arya, V., Joshi, R., Kaushik, P., & Chauhan, M. (2022). Pharmacological and Therapeutic Properties of *Jasminum officinale*. L: A Review. *Indian Journal of Ecology*, 49(3) (SI), 1122-1128. https://doi.org/10.55362/IJE/2022/3640

Sequeiros, C., Torres, M.J., Nievas, M.L., Caffini, N.O., Natalucci, C.L., López, L.M., & Trejo, S.A. (2016). The Proteolytic Activity of *Philibertia gilliesii* Latex. Purification of Philibertain g II. *Applied Biochemistry and Biotechnology*, 179(2), 322-346. https://doi.org/10.1007/s12010-016-1997-8

Sõukand, R., Hrynevich, Y., Vasilyeva, I., Prakofjewa, J., Vnukovich, Y., Paciupa, J., Hlushko, A., Knureva, Y., Litvinava, Y., Vyskvarka, S., Silivonchyk, H., Paulava, A., Kõiva, M., & Kalle, R. (2017). Multi-functionality of the few: current and past uses of wild plants for food and healing in Liubań region, Belarus. *Journal of Ethnobiology and Ethnomedicine*, 13(10), https://doi.org/10.1186/s13002-017-0139-x

Van Zyl, R.L., Seatlholo, S.T., van Vuuren, S.F., &Viljoen, A.M. (2006). The biological activities of 20 nature identical essential oil. *Journal of Essential Oil Research*, 18, 129-133. https://doi.org/10.1080/10412905.2006.12067134

Wallander, E., & Albert, V.A. (2000). Phylogeny and Classification of Oleaceae Based on rps 16 and trnL-F Sequence Data, *American Journal of Botany*, 87(12),1827-1841. https://doi.org/10.2307/2656836

Yaltırık, F. (1978). *Olea* L., In: Flora of Turkey and East Aegean Islands (Ed: P.H. Davis), Vol 6, Edinburgh, 155-156.

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Research Article/Araştırma Makalesi

Breeding and Migratory Bird Diversity in Iğdır Province (Eastern Anatolia)

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Abstract: The aim of this study was to contribute to the knowledge of avian diversity and breeding species in Türkiye. Observations were carried out in Iğdır Province within a total of 40 days spread throughout the migration and breeding periods of 2017-2018. Regional status and breeding codes were determined for each species. Some winter visitors have also been observed during the early stages of the spring migration. In the study, 192 species were identified belonging to 50 families from 20 orders and 58 residents, 83 summer visitors, 11 winter visitors, and 40 transit migratory birds. According to the result of the recording breeding behavior, 52 bird species were classified as confirmed breeders, 36 as probable breeders, and 70 as possible breeders. According to IUCN Red List, 10 globally threatened species (*Haematopus ostralegus, Vanellus vanellus, Numenius arquata, Gallinago media, Gypaetus barbatus, Aegypius monachus, Circus macrourus, Aythya ferina, Streptopelia turtur, Neophron percnopterus*) were observed. Aras Valley and Aralık-Karasu Wetlands are the most important areas for birds. The conservation of these areas is of high importance in the region for migratory birds.

Keywords: Avifauna, migratory birds, breeding birds, Aras Valley, stopover ecology.

Iğdır İlinde (Doğu Anadolu) Üreyen ve Göçmen Kuş Çeşitliliği

Öz: Bu çalışmanın amacı, Türkiye'deki kuş çeşitliliğine ve üreyen kuşlara dair bilgiye katkıda bulunmaktır. Iğdır ilinde 2017-2018 yıllarının göç ve üreme dönemlerinde 40 gün boyunca gözlemler yapılmıştır. Tespit edilen her tür için bölgesel durum ve üreme kodları belirlenmiştir. 20 takım ve 50 familyaya ait 192 kuş türü tespit edilmiştir. Bu türlerden 58'i yerli, 83'ü yaz ziyaretçisi, 11'i kış ziyaretçisi ve 40'ı transit göçmendir. Türlerin 52'si ilde kesin üreyen, 36'sı kuvvetle olası üreyen ve 70'i olası üreyen olarak sınıflandırılmıştır. Küresel olarak tehdit altındaki 10 tür (*Haematopus ostralegus, Vanellus vanellus, Numenius arquata, Gallinago media, Gypaetus barbatus, Aegypius monachus, Circus macrourus, Aythya ferina, Streptopelia turtur, Neophron percnopterus*) Iğdır ilinde gözlemlenmiştir. Aras Vadisi ve Aralık-Karasu Sulak Alanları kuşlar için en önemli alanlardır. Bu bölgenin korunması, göçmen kuşlar için büyük önem taşımaktadır.

Anahtar kelimeler: Avifauna, göçmen kuşlar, üreyen kuşlar, Aras Vadisi, konaklama ekolojisi.

1. Introduction

Birds are among the most remarkable groups in biodiversity and are relatively easy to observe directly than other vertebrate groups. However, detailed information is far from complete for most species and regions (Bibby et al., 1998). Birds are highly sensitive indicators of ecosystem quality (Smits & Fernie, 2013). Therefore, it is advantageous to monitor, count, and record them, even the most common ones, to better understand and follow the populations and communities (Lovette & Fitzpatrick, 2016).

Türkiye has one of the richest avifauna in the Western Palearctic due to different types of habitats, various climate regimes, a high degree of variable topography, location on the important migration routes, and being on the joint of several different biogeographic regions (Barış, 2000; Bilgin, 2004; Barış, 2012). A total of 491 bird species belonging to 76 families of 25 orders were listed in Türkiye and 313 of them breed in the country (Boyla et al., 2019; Furtun et al., 2021). Due to the increasing number of ornithology research and contributions to citizen science through bird watching in recent years, avifauna studies have become widespread in Türkiye. However, all breeding distributions and regional migration status even for the common species have been not revealed clearly. Therefore, there is still an information gap to determine the national-level population sizes for the assessments of national red list categories of the species. For this reason, the main objective of this study is to contribute to the knowledge of the avifauna of the province and also Türkiye by determining the breeding bird species.

2. Material and Methods

2.1. Study area

Iğdır Province is located in Eastern Anatolia. The Aras River constitutes the northern and northeastern border of the province. The province is surrounded by the Aras River and Armenia border in the north and northeast, Nakhichevan Autonomous Republic and Iran in the east and southeast, Ağrı Province in the south, and Kars Province in the west and northwest (Kaya, 2015). Mountainous and rugged terrain lands cover 74% of the province and the rest of the province is covered by Iğdır Plain. The average altitude of the plain is about 850 m (Parin & Gürbüz, 2022). Mount Ağrı, the highest mountain in the province is an ice-capped dormant compound volcano and the highest peak in Türkiye with an elevation of 5,137 m (Azzoni et al., 2017). General views of the study area were presented in Figure 1.

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2.2. Method

During the migration and breeding periods of 2017 and 2018, a total of 40 days of field study was carried out. Field studies were carried out for both years as April-May (pre-breeding migration period), June-July (late breeding period), and August-September (post-breeding migration period). The second half of the pre-breeding period were also considered as the early breeding period for the region and the coding of breeding behaviors began in this period.

The study was carried out in an area divided into 44 plots of a map with a scale of 1/25000 taken from the General Directorate of Mapping (Fig. 2). Binoculars, camera, and lens were used during the study. During observations, "point count" and "line transect" methods were used (Bibby et al., 2000). The locations for both methods were selected during field studies to cover different habitats in at least 10% of each plot. Field studies could not be carried out only in the region where Ağrı is located in the south of the province due to security reasons.

Figure 1. General views from the study area

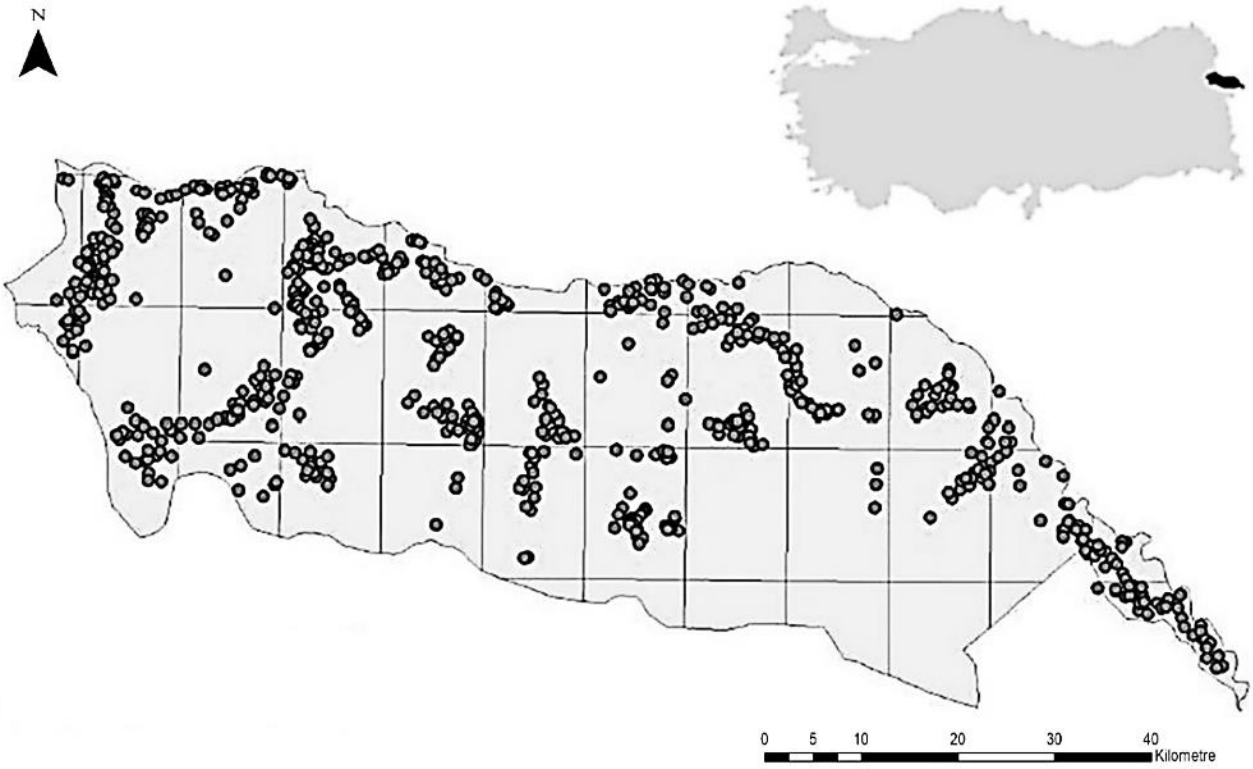

Figure 2. The map showing the plots and observation points, and the location of Iğdır province in Türkiye

The taxonomic list of bird species is arranged according to Gill & Donsker (2022). While determining the migration status of the species, the species seen throughout the year were categorized as "resident (R)" and those found during their breeding periods as "summer visitor (S)". In addition, some species recorded at the beginning of the spring migration were evaluated as winter visitors in the study area as a result of the regional assessment. Therefore, besides the breeding and transit migratory species targeted in the study, these species were categorized as "winter visitors (W)". The species observed only durissage or stopover during the migration periods were also classified as "transit migratory (T)". Since different populations of migratory bird species may have different migration behaviors, more than one migration status has been expressed for some species. The priority migration status is indicated by capital letters and the periods when the species are recorded in a smaller number are indicated by lowercase letters.

In order to identify breeding bird species, 16 codes system based on breeding behaviors suggested by Hagemeijer & Blair (1997) was used during breeding periods. In cases where more than one breeding behavior is coded, the highest breeding code was accepted.

3. Results

A total of 192 bird species were recorded in this study. The regional migration status were as follows: 58 residents (R/r), 83 summer visitors (S/s), 11 winter visitors (W/w), and 40 transit migrantories (T, t). They belong to 50 families of 20 orders (Table 1). Order Passeriformes (Passerines; 93 species) were the most diversified order followed by Charadriiformes (Shorebirds and relatives; 25), Accipitriformes (Raptors; 18), Pelecaniformes (Ibises, herons, pelicans; 9), and Anseriformes (Waterfowls; 7). 15 other orders cover 20.8% of all species (Fig. 3). The families Accipitridae (Raptors; 18) and Muscicapidae (Old World Flycatchers; 18 species) were the richest families followed by Scolopacidae (Sandpipers and Snipes; 11), Fringillidae (Finches; 10), and Ardeidae (Herons and bitterns; 8) (Fig. 3). The highest number of species (122 species) have been recorded in the Aras Valley.

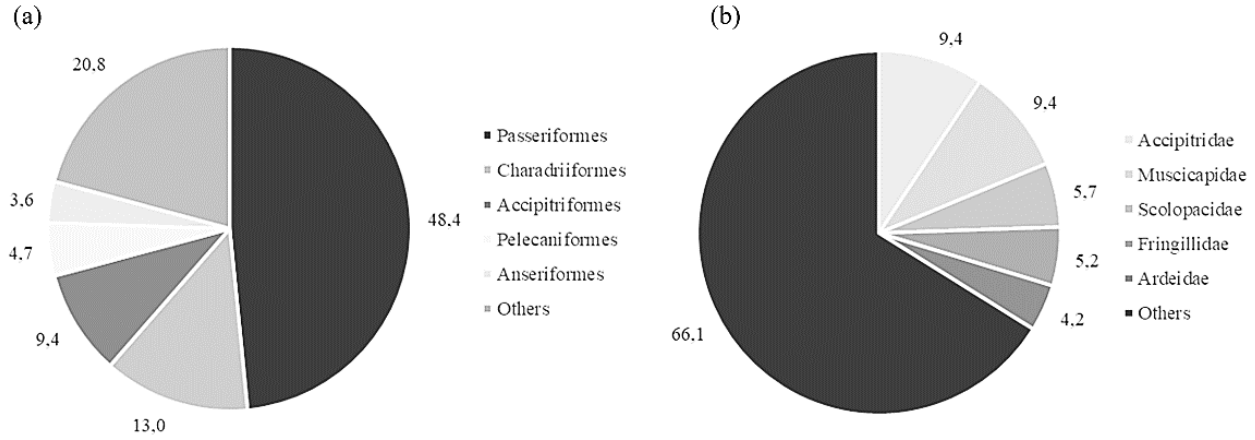

Figure 3. Percentage representation of the orders (a) and families (b) with the most species

The most frequently observed species are *Merops apiaster* (35 times) followed by *Ciconia Ciconia* (34), *Upupa epops* (28), *Corvus frugilegus* (27), *Coracias garrulus* (25), and *Larus armenicus* (25). The highest number of recorded species during a point count are *Hirundo rustica* (500 individuals), *Chlidonias leucopterus* (220), *Merops persicus* (150), *Larus armenicus* (102), and *Tadorna ferruginea* (90) (Table 1).

Breeding behaviors of 158 species were coded; 52 were classified as confirmed breeders, 36 as probable breeders, and 70 possible breeders. The other 34 species are thought not to breed within the provincial borders. The highest breeding codes were given for every species in Table 1.

According to the IUCN criteria, 10 globally threatened species including "Near Threatened species – NT" were recorded. *Haematopus ostralegus*, *Vanellus vanellus*, *Numenius arquata*, *Gallinago media*, *Gypaetus barbatus*, *Aegypius monachus*, and *Circus macrourus* are listed as "Near Threatened (NT)"; *Aythya ferina* and *Streptopelia turtur* are listed as "Vulnerable (VU)"; and *Neophron percnopterus* is listed as "Endangered (EN)" category.

Table 1. Recorded bird species in Iğdır province, regional status, breeding codes (0: non-breeders, A1-2: possible breeders, B3-9: probable breeders, C10-16: confirmed breeders), and the maximum numbers

Scientific Name & Order	Common English Name & Family	Regional Status	Breeding Codes	Highest Recorded Number
Anseriformes	Anatidae			
Tadorna ferruginea	Ruddy Shelduck	R, W	C12	90
Spatula querquedula	Garganey	t	B3	4
Spatula clypeata	Northern Shoveler	w	0	4
Mareca strepera	Gadwall	w, t	B3	3
Anas platyrhynchos	Mallard	R, W	C12	14
Aythya ferina	Common Pochard	t, s	B3	16
Aythya fuligula	Tufted Duck	w, t	B3	2
Galliformes	Phasianidae			
Alectoris chukar	Chukar Partridge	R	C12	11
Tetraogallus caspius	Caspian Snowcock	r	A1	-
Coturnix coturnix	Common Quail	S, T	A2	4

Table 1. (Continued)

Scientific Name & Order	Common English Name & Family	Regional Status	Breeding Codes	Highest Recorded Number
Caprimulgiformes	Caprimulgidae			
Caprimulgus europaeus	European Nightjar	S, T	B3	2
Apodiformes	Apodidae			
Tachymarptis melba	Alpine Swift	S, T	A1	17
Apus apus	Common Swift	S, T	C13	22
Cuculiformes	Cuculidae			
Cuculus canorus	Common Cuckoo	S, T	B3	3
Pterocliformes	Pteroclidae			
Pterocles orientalis	Black-bellied Sandgrouse	S, t	B3	11
Columbiformes	Columbidae			
Columba livia	Rock Dove	R	C12	21
Columba palumbus	Common Wood Pigeon	S, T	B3	16
Streptopelia turtur	European Turtle Dove	s, T	A2	2
Streptopelia decaocto	Eurasian Collared Dove	R	C13	4
Spilopelia senegalensis	Laughing Dove	r	A1	1
Gruiformes	Rallidae			
Rallus aquaticus	Water Rail	r	A1	1
Porzana parva	Little Crake	t	0	1
Porzana porzana	Spotted Crake	t	0	1
Gallinula chloropus	Common Moorhen	S	C12	4
Fulica atra	Eurasian Coot	R, W	C12	16
Podicipediformes	Podicipedidae			
Tachybaptus ruficollis	Little Grebe	W, t	A1	4
Podiceps grisegena	Red-necked Grebe	t, s	B3	1
Podiceps cristatus	Great Crested Grebe	w	A1	1
Podiceps nigricollis	Black-necked Grebe	s, T	B3	3
Charadriiformes	Haematopodidae			
Haematopus ostralegus	Eurasian Oystercatcher	S	B7	8
Charadriiformes	Recurvirostridae			
Himantopus himantopus	Black-winged Stilt	S, T	C12	12
Recurvirostra avosetta	Pied Avocet	t	0	1
Charadriiformes	Charadriidae			
Vanellus vanellus	Northern Lapwing	R	C12	20
Charadrius dubius	Little Ringed Plover	S	A1	7
Charadrius alexandrinus	Kentish Plover	t	0	1
Charadriiformes	Scolopacidae			
Numenius arquata	Eurasian Curlew	t	0	1
Calidris pugnax	Ruff	T, s	0	42
Calidris minuta	Little stint	t	0	7
Gallinago media	Great Snipe	t	0	1
Gallinago gallinago	Common Snipe	t, W	0	2
Actitis hypoleucos	Common Sandpiper	S, T	B3	6
Tringa ochropus	Green Sandpiper	T, W	0	4
Tringa totanus	Common Redshank	S	B7	13
Tringa glareola	Wood Sandpiper	T	0	2
Tringa erythropus	Spotted Redshank	T	0	1
Tringa nebularia	Common Greenshank	T, W	0	10

Table 1. (Continued)

Scientific Name & Order	Common English Name & Family	Regional Status	Breeding Codes	Highest Recorded Number
Charadriiformes	Glareolidae			
Glareola pratincola	Collared Pratincole	t	A1	5
Charadriiformes	Lariidae			
Chroicocephalus ridibundus	Black-headed Gull	r, W	A1	3
Larus armenicus	Armenian Gull	R, W	B9	102
Gelochelidon nilotica	Gull-billed Tern	t	0	1
Sternula albifrons	Little Tern	s, T	A1	2
Sterna hirundo	Common Tern	s	A1	4
Chlidonias leucopterus	White-winged Tern	s, T	C12	220
Chlidonias niger	Black Tern	t	0	2
Ciconiiformes	Ciconiidae			
Ciconia nigra	Black Stork	s, t	B3	2
Ciconia ciconia	White Stork	S, T, w	C16	39
Suliformes	Phalacrocoracidae			
Microcarbo pygmeus	Pygmy Cormorant	r, w	A1	21
Phalacrocorax carbo	Great Cormorant	r, w	0	4
Pelecaniformes	Threskiornithidae			
Plegadis falcinellus	Glossy Ibis	s, T	A1	12
Pelecaniformes	Ardeidae			
Ixobrychus minutus	Little Bittern	s	A1	2
Nycticorax nycticorax	Black-crowned Night Heron	s, t	A1	4
Ardeola ralloides	Squacco Heron	s, t	0	1
Bubulcus ibis	Western Cattle Egret	s, T	0	6
Ardea cinerea	Grey Heron	R, W	C16	4
Ardea purpurea	Purple Heron	S, T	A1	2
Ardea alba	Great Egret	W, t	0	2
Egretta garzetta	Little Egret	s, T	0	3
Accipitriformes	Accipitridae			
Gypaetus barbatus	Bearded Vulture	R	B6	2
Neophron percnopterus	Egyptian Vulture	S	A1	3
Pernis apivorus	European Honey Buzzard	T	0	29
Gyps fulvus	Griffon Vulture	R	A1	7
Aegypius monachus	Cinereous Vulture	t	0	1
Circaetus gallicus	Short-toed Snake Eagle	s, t	A1	1
Clanga pomarina	Lesser Spotted Eagle	T	0	4
Hieraaetus pennatus	Booted Eagle	s, t	A1	1
Aquila chrysaetos	Golden Eagle	R	B3	2
Accipiter brevipes	Levant Sparrowhawk	s, T	A1	11
Accipiter nisus	Eurasian Sparrowhawk	T, r	A1	3
Circus aeruginosus	Western Marsh Harrier	R, T	B9	4
Circus cyaneus	Hen Harrier	W, t	0	1
Circus macrourus	Pallid Harrier	t	0	2
Circus pygargus	Montagu's Harrier	S, T	B3	3
Milvus migrans	Black Kite	T	0	14
Buteo rufinus	Long-legged Buzzard	R	C14	12
Buteo buteo	Common Buzzard	T, r	A1	25

Table 1. (Continued)

Scientific Name & Order	Common English Name & Family	Regional Status	Breeding Codes	Highest Recorded Number
Strigiformes	Strigidae			
Otus scops	Eurasian Scops Owl	s, T	A2	1
Athene noctua	Little Owl	R	C13	2
Asio otus	Long-eared Owl	r	A1	1
Bucerotiformes	Upupidae			
Upupa epops	Eurasian Hoopoe	S, T	C14	4
Coraciiformes	Coraciidae			
Coracias garrulus	European Roller	S, T	C13	4
Coraciiformes	Alcedinidae			
Alcedo atthis	Common Kingfisher	T, r	A1	2
Coraciiformes	Meropidae			
Merops persicus	Blue-cheeked Bee-eater	S, T	C14	30
Merops apiaster	European Bee-eater	S, T	C14	150
Piciformes	Picidae			
Jynx torquilla	Eurasian Wryneck	T	0	1
Dendrocopos syriacus	Syrian Woodpecker	R	B3	3
Falconiformes	Falconidae			
Falco naumanni	Lesser Kestrel	t	A1	3
Falco tinnunculus	Common Kestrel	R, T	C13	4
Falco subbuteo	Eurasian Hobby	S, T	A1	1
Falco peregrinus	Peregrine Falcon	r	B3	2
Passeriformes	Laniidae			
Lanius collurio	Red-backed Shrike	S, T	C12	10
Lanius minor	Lesser Grey Shrike	s, T	C12	4
Lanius senator	Woodchat Shrike	s, t	A1	1
Passeriformes	Oriolidae			
Oriolus oriolus	Eurasian Golden Oriole	s, T	A2	10
Passeriformes	Corvidae			
Pica pica	Eurasian Magpie	R	C12	19
Pyrrhocorax pyrrhocorax	Red-billed Chough	R	B9	14
Coloeus monedula	Western Jackdaw	R	C12	30
Corvus frugilegus	Rook	R	C13	80
Corvus cornix	Hooded Crow	R	C13	20
Corvus corax	Northern Raven	R	A1	4
Passeriformes	Paridae			
Cyanistes caeruleus	Eurasian Blue Tit	r	A1	3
Parus major	Great Tit	R	A2	10
Passeriformes	Remizidae			
Remiz pendulinus	Eurasian Penduline Tit	R	C12	3
Passeriformes	Panuridae			
Panurus biarmicus	Bearded Reedling	r	A1	4
Passeriformes	Alaudidae			
Alauda arvensis	Eurasian Skylark	R	C12	10
Galerida cristata	Crested Lark	R	C12	12
Eremophila alpestris	Horned Lark	R	C12	4
Calandrella brachydactyla	Greater Short-toed Lark	T	A2	6
Alaudala rufescens	Lesser Short-toed Lark	T	A2	2

Table 1. (Continued)

Scientific Name & Order	Common English Name & Family	Regional Status	Breeding Codes	Highest Recorded Number
Passeriformes	Hirundinidae			
Riparia riparia	Sand Martin	S, T	C13	50
Hirundo rustica	Barn Swallow	S, T	C13	500
Ptyonoprogne rupestris	Eurasian Crag Martin	S	C13	30
Delichon urbicum	Common House Martin	S, T	C13	20
Passeriformes	Cettiidae			
Cettia cetti	Cetti's Warbler	R	A2	8
Passeriformes	Phylloscopidae			
Phylloscopus trochilus	Willow Warbler	T	0	10
Phylloscopus sindianus	Mountain Chiffchaff	T	A2	2
Phylloscopus collybita	Common Chiffchaff	s, T	A2	4
Passeriformes	Acrocephalidae			
Acrocephalus arundinaceus	Great Reed Warbler	s, T	C14	3
Acrocephalus schoenobaenus	Sedge Warbler	t	A1	1
Acrocephalus agricola	Paddyfield Warbler	s, t	A2	2
Acrocephalus scirpaceus	Marsh Warbler	s, T	A2	3
Acrocephalus palustris	Eurasian Reed Warbler	T	A2	1
Iduna pallida	Eastern Olivaceous Warbler	S, T	A2	2
Hippolais languida	Upcher's Warbler	s	B3	2
Passeriformes	Locustellidae			
Locustella luscinioides	Savi's Warbler	t	A2	1
Passeriformes	Sylviidae			
Sylvia atricapilla	Eurasian Blackcap	T	A1	4
Sylvia borin	Garden Warbler	T	0	5
Curruca nisoria	Barred Warbler	T	A2	2
Curruca curruca	Lesser Whitethroat	T	C12	2
Curruca communis	Common Whitethroat	S	A2	5
Curruca mystacea	Menetries's Warbler	S	C12	2
Passeriformes	Sittidae			
Sitta neumayer	Western Rock Nuthatch	R	C13	6
Sitta tephronota	Eastern Rock Nuthatch	r	B3	2
Passeriformes	Sturnidae			
Pastor roseus	Rosy Starling	s, T	A2	75
Sturnus vulgaris	Common Starling	R, w	C13	30
Passeriformes	Turdidae			
Turdus merula	Common Blackbird	T	A1	2
Passeriformes	Muscicapidae			
Cercotrichas galactotes	Rufous-tailed Scrub Robin	s, t	C13	10
Muscicapa striata	Spotted Flycatcher	s, T	A1	1
Luscinia svecica	Bluethroat	T	A1	
Luscinia luscinia	Thrush Nightingale	T	0	2
Luscinia megarhynchos	Common Nightingale	s, T	A2	2
Ficedula parva	Red-breasted Flycatcher	T	0	2
Ficedula hypoleuca	European Pied Flycatcher	T	0	1
Ficedula albicollis	Collared Flycatcher	T	0	1
Phoenicurus ochruros	Black Redstart	S, T	C11	3
Phoenicurus phoenicurus	Common Redstart	s, T	B3	3

Table 1. (Continued)

Scientific Name & Order	Common English Name & Family	Regional Status	Breeding Codes	Highest Recorded Number
Monticola saxatilis	Common Rock Thrush	t	B3	1
Monticola solitarius	Blue Rock Thrush	s, T	C14	3
Saxicola rubetra	Whinchat	T	A1	3
Saxicola maurus	Siberian Stonechat	T	A1	2
Oenanthe oenanthe	Northern Wheatear	S, T	C12	8
Oenanthe isabellina	Isabelline Wheatear	S, T	C12	20
Oenanthe hispanica	Western Black-eared Wheatear	S, T	C12	4
Oenanthe finschii	Finsch's Wheatear	S, T	C12	9
Passeriformes	Cinclidae			
Cinclus cinclus	White-throated Dipper	r	A1	2
Passeriformes	Passeridae			
Passer domesticus	House Sparrow	R	C12	35
Passer hispaniolensis	Spanish Sparrow	s, T	A1	20
Passer montanus	Eurasian Tree Sparrow	R	B6	*8*
Carpospiza brachydactyla	Pale Rockfinch	s	A2	2
Petronia petronia	Rock Sparrow	R	C14	10
Montifringilla nivalis	White-winged Snowfinch	R	B3	25
Passeriformes	Prunellidae			
Prunella collaris	Alpine Accentor	r	A2	1
Passeriformes	Motacillidae			
Motacilla flava	Western Yellow Wagtail	S, T	B3	7
Motacilla cinerea	Grey Wagtail	S, T	B3	3
Motacilla alba	White Wagtail	r, S, T	C12	6
Anthus campestris	Tawny Pipit	S, T	A1	4
Anthus trivialis	Tree Pipit	T	0	1
Anthus spinoletta	Water Pipit	r, T	A1	3
Passeriformes	Fringillidae			
Fringilla coelebs	Common Chaffinch	W, t	C14	10
Rhodopechys sanguineus	Asian Crimson-winged Finch	s	A1	3
Bucanetes githagineus	Trumpeter Finch	s	B5	7
Bucanetes mongolicus	Mongolian Finch	s	A1	1
Carpodacus erythrinus	Common Rosefinch	s, T	B3	3
Chloris chloris	European Greenfinch	w, T	A2	4
Linaria flavirostris	Twite	S, t	A2	12
Linaria cannabina	Common Linnet	R, S	A2	14
Carduelis carduelis	European Goldfinch	R	B6	10
Serinus pusillus	Red-fronted Serin	r	A1	2
Passeriformes	Emberizidae			
Emberiza calandra	Corn Bunting	S, r	B3	10
Emberiza cia	Rock Bunting	R	C14	2
Emberiza buchanani	Grey-necked Bunting	s	B5	4
Emberiza hortulana	Ortolan Bunting	S, T	B5	6
Emberiza melanocephala	Black-headed Bunting	s, T	C12	3

4. Discussion

This study reports the breeding and transit migratory bird species, their regional status, and the highest breeding codes in Iğdır Province. A total of 192 species were recorded in the study area. Approximately 39% of the avifauna of Türkiye (Furtun et al., 2021) was recorded in this research. According to the literature review, 322 bird species were identified in Iğdır province (eBird, 2022;

Kirwan et al., 2010; Türkoğlu & Şekercioğlu, 2018). Approximately 60% of this number was reported in this study. About 900 species was recorded in the Western Palearctic (Beaman & Madge, 2010), 21.2% of this number was recorded in this study. There are two main reasons for the variety of birds on a regional scale being so high: (1) the location of the province on the African-Eurasian bird migration routes, (2) due to various habitat types, the existence of suitable breeding, wintering, and stopover sites for birds. Species such as *Circus pygargus*, *Pterocles orientalis*, and *Buteo rufinus* can be seen frequently in steppe habitats and such habitats are common throughout the province. The species with limited distribution in Türkiye such as *Bucanetes githagineus* and *Bucanetes mongolicus* breed in rocky areas in the steppes of Iğdır. *Tetraogallus caspius*, *Gypaetus barbatus*, and *Prunella collaris* are remarkable species of alpine and sub-alpine habitats. All waterfowl species observed during the study and a high number of other migratory species use the wetlands as suitable stopover and breeding sites in the province.

The number of confirmed breeders were 52. According to Boyla et al. (2019) 313 bird species breed regularly in Türkiye. 16.6% of all breeding bird species in Türkiye were recorded as confirmed breeders in Iğdır. However, this number is thought to be higher than the results of the study show. The reasons for this are the cryptic behaviors of some species and the difficulty of reaching their breeding habitats. No study presenting the breeding birds in the study area was found in the literature. There is only one published study with the list of recorded species in the province (Türkoğlu & Şekercioğlu, 2018). Therefore, this study is the first published one focused on Iğdır province about breeding birds.

The highest number of the species was recorded in Aras Valley (63.55% of all species). Different habitat types such as steep cliffs, deep valleys, floodplains, rivers, agricultural fields, reedbeds, sandy, and graveled islets, scrub areas, orchards, and mountain steppes through the valley offer suitable stopover and breeding areas for the species with different ecological demands (Eken et al., 2006; Türkoğlu & Şekercioğlu, 2018; Neate-Clegg et al., 2019). The area meets 4 Ramsar criteria (Neate-Clegg et al., 2019; Ramsar, 2020) and is a Key Biodiversity Area due to hosting fourteen globally and/or regionally endangered plant species (Eken et al., 2006). Among 10 globally threatened bird species reported in this study, 7 of them were recorded in the valley. Of these, *Haematopus ostralegus* is a possible breeder on the islets or graveled or sandy riverside. According to del Hoyo et al. (1996), the global population of the nominate subspecies *ostralegus* which breeds also in Türkiye declined at a rate of over 40% after the 1990s (BirdLife International, 2019). Although no active threat to this species in the valley was identified, a dam to be established in the river in the future may have potentially negative effects on its breeding habitat. *Neophron percnopterus* was also observed in the valley. The global population of this spectacular species has declined dramatically over the past few decades (Velevski et al., 2015) and it is listed as "Endangered" (BirdLife International, 2021). The largest European breeding population of *Neophron percnopterus* was reported from Türkiye (Iñigo et al. 2008). Therefore, determining the breeding pairs in the area would be essential for conservation and monitoring studies for the future of the species. A rubbish dump located very close to the Aras valley plays an important role as a feeding area for this species as well as 3 other vulture species. *Aegypius monachus* and *Gyps fulvus* died due to feeding on a poisoned animal carcass at this rubbish dump in 2015 (Buechley et al., 2018). Such a risk of poisoning is a fatal threat to the *Neophron percnopterus*. In order to prevent the use of poisonous carcasses against feral dogs, red foxes, and grey wolfs, it is of vital importance to raise the awareness of the local people, to have criminal sanctions in place, and to make regular inspections in the rubbish dump. The valley is also an important transit flyway for many raptors that follow the Northeast Anatolian route during their migration periods.

A significant part of Aras Valley is located in the Iğdır Plain which is an Important Bird Area (hereafter: IBA). There are 5 IBA trigger species in this area (BirdLife International, 2020). Only two of these were recorded in this study: *Microcarbo pygmaeus* and *Gyps fulvus*. *Microcarbo pygmaeus* and other many water birds use Karasu and Aralık Wetlands in the plain for stopover and wintering. These wetlands are the biggest wetlands with slow-flowing and standing freshwaters in the province. The ponds, reeds, and seasonal wetlands in the area are suitable stopover sites for a large number of waders especially during migration periods. *Merops persicus* and *Curruca mystacea*, species have limited distribution in Türkiye (Kirwan et al., 2010; Boyla et al., 2019) and breed in/around these areas. One or two pairs of *Marmaronetta angustirostris* listed as "Vulnerable" globally have been reported from these wetlands in 1999 (IUCN, 2022). This species was also an IBA trigger species for the plain. The other IBA trigger species *Vanellus gregarious* listed as "Critically Endangered" globally have been reported these areas in the past from (IUCN, 2022). These two threatened bird species have not been recorded in this study. The last pair of *Marmaronetta angustirostris* was recorded in Göksu Delta in Türkiye between 2011-2013 but, as of 2014, all breeding pairs in Anatolia have disappeared (Kuş Araştırmaları Derneği, 2010). For this reason, it was expected that it was not recorded in the Aras Valley. In a study in which the migration routes of this highly site-faithful *Vanellus gregarious* were determined by attaching a satellite transmitter, none of the documented stopover sites were in the Aras Valley (Donald et al., 2021). However, since the migration route includes a corridor that also includes the Aras Valley, it is likely to be seen occasional. *Vanellus vanellus* is another threatened species around Aralık Wetland. Overgrazing and agriculture are potential threats to this species in hatching areas. Iğdır Plain is also one of only a few regular wintering sites of *Ciconia ciconia* in Türkiye and there is a great breeding colony throughout the area. Pollution caused by solid domestic waste has been observed as a common problem in the area. Using these materials in the nest by *Ciconia ciconia* is the most noticeable threat. Using artificial materials such as string, foil, fabric, and such for nesting material may result in the mortality or injuries of fledglings (Jagiello et al., 2018). A juvenile *Ciconia ciconia* killed by this type of material by getting tangled around its neck was observed on the Aralık Plain. Throughout the

study period, *Tadorna ferruginea* and *Larus armenicus* were regularly observed in large numbers in all wetlands in Iğdır. *Larus armenicus* use wetlands for feeding and resting. *Tadorna ferruginea* is the most common anatid breeder in wetlands of Iğdır Plain and also in Aras Valley and Abbasgöl Pond (border of Ağrı Province).

Tetraogallus caspius is one of the IBA trigger species. An important part of Mount Ağrı has potential breeding areas of this species. Unfortunately, the area was visited only once, due to safety, and the habitat of this species could not have been reached. No breeding record of the species was found in the available literature (Kirwan et al., 2010; eBird, 2022). In the oral interviews with ornithologists, bird watchers active in the region and the locals, it was learned that this species breeds in Mount Tekaltı. However, no confirmed breeding behavior was recorded during the observations. Therefore, the breeding status of this species is unclear, and it needs further research. It was noted that it was under the threat of illegal hunting. Mount Ağrı isolates the province from the harsh continental climate of the Eastern Anatolian region during the winter and causes a unique microclimate, making the province a more temperate shelter for birds. Nevertheless, the area which lays between the slopes of Mount Ağrı and northeast of Iğdır plain (Aralık District) is among the highest-risk areas of desertification in Türkiye (Türkeş et al., 2020). This habitat degradation will undoubtedly affect the species composition and abundance in the area in the future.

In conclusion, the province is important to have different habitats for birds such as the high mountains, alpine meadows, plains, riparian habitats, arid and semi-arid steppe, and standing freshwaters. It is clear that the riverine habitats along Aras River represent a hotspot for avian diversity in Asia Minor. Many migratory bird species with high numbers in different groups such as raptors, passerines, and waterfowls use the area as breeding, stopover, and wintering sites. The bird ringing station running in the area is a very important ornithological study that reveals the importance of the area in terms of bird migration and the continuity of its activities has a very high value. Determining the breeding population of *Neophron percnopterus* in the area and constant monitoring studies are vital for the species. The conservation of Aralık and Karasu Wetlands, which are very suitable stopover sites for many songbirds and waders during migration periods, is a high priority. The high ecotourism potential of the province for bird watching and wildlife photography was emphasized by Çelik et al. (2021). If bird watching and photography can be combined with the potential of other ecotourism activities in the region, Iğdır province may evolve into a major tourist destination for both Türkiye and Eastern Anatolia.

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References

Azzoni, R.S., Zerboni, A., Pelfini, M., Garzonio, C.A., Cioni, R., Meraldi, E., …& Diolaiuti, G.A. (2017). Geomorphology of Mount Ararat/Ağri Dağı (Ağri Dağı Milli Parki, Eastern Anatolia, Türkiye). *Journal of Maps*, 13(2), 182-190. https://doi.org/10.1080/17445647.2017.1279084

Barış, S. (2000). Kuşların otoyolu. *Yeşil Atlas,* 3, 81-83.

Barış, S. (2012). Türkiye kuş faunası. kuş halkalama temel eğitimi ders notları. Ankara, Orman ve Su İşleri Bakanlığı, 129 pp.

Beaman, M., & Madge, S. (2010). The Handbook of bird identification for Europe and the Western Palearctic. London, Christopher Helm Publishers, 868 pp.

Bibby, C.J., Jones, M., & Marsden, S. (1998). Bird surveys. Expedition Advisory Centre, London, 137 pp.

Bibby, C.J, Burgess N.D., Hill D.A., & Mustoe S.H. (2000). Bird census techniques (2nd ed.). London, Elsevier Academic Press, 302 pp.

Bilgin, C.C. (2004). Kuşların gizemli yolculuğu: göç. *Bilim ve Teknik Dergisi, Yeni Ufuklar Eki*, 438, 3-5.

BirdLife International (2019). *Haematopus ostralegus*. The IUCN Red List of Threatened Species 2019: e.T22693613A154998347. https://dx.doi.org/10.2305/IUCN.UK.2019-3.RLTS.T22693613A154998347.en.

BirdLife International (2020). Important Bird Areas factsheet: Iğdır Plain. Retrieved from http://www.birdlife.org

BirdLife International (2021). *Neophron percnopterus*. The IUCN Red List of Threatened Species 2021: e.T22695180A205187871. https://dx.doi.org/10.2305/IUCN.UK.2021-3.RLTS.T22695180A205187871.en

Boyla, K.A., Sinav, L., & Dizdaroğlu D.E. (2019). Türkiye Üreyen Kuş Atlası. WWF-Türkiye, Doğal Hayatı Koruma Vakfı, İstanbul.

Buechley, E.R., McGrady, M. J., Çoban, E., & Şekercioğlu, Ç.H. (2018). Satellite tracking a wide-ranging endangered vulture species to target conservation actions in the Middle East and East Africa. *Biodiversity and Conservation*, 27(9), 2293-2310. https://doi.org/10.1007/s10531-018-1538-6

Çelik, E., Durmuş, A., & Türkoğlu, M. (2021). Iğdır İli ve Yakın Çevresinin Ornito-Turizm Perspektifinde Değerlendirilmesi. *Journal of Academic Tourism Studies*, 2(1), 32-44. http://dx.doi.org/10.29228/jatos.51950

del Hoyo, J., Elliott, A., & Sargatal, J. (1996). Handbook of the birds of the world. Vol. 3. Lynx Edicions, Barcelona, 821 pp.

Donald, P.F., Kamp, J., Green, R.E., Urazaliyev, R., Koshkin, M., & Sheldon, R.D. (2021). Migration strategy, site fidelity and population size of the globally threatened Sociable Lapwing *Vanellus gregarius*. *Journal of Ornithology*, 162(2), 349-367. https://doi.org/10.1007/s10336-020-01844-y

eBird (2022). eBird. Retrieved from https://ebird.org/Türkiye/

Eken, G., Bozdoğan, M., İsfendiyaroğlu, S., Kılıç, D.T., & Lise, Y., (eds) (2006). Türkiye'nin Önemli Doğa Alanları. Doğa Derneği, Ankara, Türkiye, 639 pp.

Furtun, Ö.L., Erciyas Yavuz, K. & Karataş A. (2021). Trakuş Türkiye'nin Kuşları. Türkiye İstanbul, İş Bankası Kültür Yayınları, 414 pp.

Gill, F., Donsker, D., & Rasmussen, P. (2022). IOC World Bird List (v12.2). https://doi.org/10.14344/IOC.ML.7.1

Hagemeijer, W.J., & Blair, M.J. (1997). The EBCC atlas of European Breeding Birds. Poyser, London, 479.

HGM (2020). İl ve İlçe Yüzölçümleri. Retrieved from https://www.harita.gov.tr/il-ve-ilce-yuzolcumleri,

Iñigo, A., Barov, B., Orhun, C., & Gallo-Orsi, U. (2008) Action plan for the Egyptian Vulture *Neophron percnopterus* in the European Union. Brussels, Belgium: BirdLife International and European Commission Brussels.

IUCN (2022). The IUCN Red List of Threatened Species. Version 2022-1.

Jagiello, Z.A., Dylewski, Ł., Winiarska, D., Zolnierowicz, K.M., & Tobolka, M. (2018). Factors determining the occurrence of anthropogenic materials in nests of the white stork *Ciconia ciconia*. *Environmental Science and Pollution Research*, 25(15), 14726-14733. https://doi.org/10.1007/s11356-018-1626-x

Kaya, F. (2015). Iğdir İli'nin idari coğrafya analizi. *Journal of International Social Research*, 8(49), 703-716.

Kirwan, G.M., Boyla K.A., Castell, P., Demirci, B., Özen, M., Welch, H., & Marlow, T. (2010). The birds of Türkiye. London: Christopher Helm, 512 pp.

Kuş Araştırmaları Derneği 2010. Göksu Deltası Özel Çevre Koruma Bölgesi Tür ile Habitat Koruma ve İzleme Projesi. Rapor. Ankara.

Lovette, I.J., & J.W. Fitzpatrick (Eds.). (2016). Handbook of Bird Biology. John Wiley & Sons, UK, 716 pp.

Neate-Clegg, M.H., Román, J.R., Demir, B., & Şekercioğlu, Ç.H. (2019). Endangered Basra Reed-warbler (*Acrocephalus griseldis*) recorded for the first time in Turkey (Aves: Acrocephalidae). *Turkish Journal of Zoology*, 43(2), 250-253. https://doi.org/10.3906/zoo-1808-16

Parin, E., & Gürbüz, R. (2022). Determination of weeds flora in apple orchards of Iğdır province and approaches of farmers to solve the problems. *Commagene Journal of Biology*, 6(1), 85-93. https://doi.org/10.31594/commagene.1106320

Ramsar (2020). The Ramsar Sites Criteria. Retrieved from https://www.ramsar.org/sites/default/files/documents/library/ramsarsites_criteria_eng.pdf

Smits, J. E., & Fernie, K. J. (2013). Avian wildlife as sentinels of ecosystem health. Comparative Immunology. *Microbiology and Infectious Diseases*, 36(3), 333-342. https://doi.org/10.1016/j.cimid.2012.11.007

Türkeş, M., Öztaş, T., Tercan, E., Erpul, G., Karagöz, A., Dengiz, O., ... & Avcıoğlu, B. (2020). Desertification vulnerability and risk assessment for Turkey via an analytical hierarchy process model. *Land Degradation & Development*, 31(2), 205-214. https://doi.org/10.1002/ldr.3441

Türkoğlu, M., & Şekercioğlu, Ç.H. (2018). Iğdır'ın kuşları (2nd Edition). Republic of Türkiye Ministry of Forestry and Water Affairs, General Directorate of Nature Protection and National Parks, Ankara, 222 pp.

Velevski, M., Nikolov, S.C., Hallmann, B., Dobrev, V., Sidiropoulos, L., Saravia, V., …& Oppel, S. (2015). Population decline and range contraction of the Egyptian vulture *Neophron percnopterus* in the Balkan Peninsula. *Bird Conservation International*, 25(4), 440–450. https://doi.org/10.1017/S0959270914000343

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Research Article/Araştırma Makalesi

An Ethnobotanical Study in Ceylanlı Village (Kırıkhan/Hatay-Türkiye)

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Abstract: This study was carried out to determine the ethnobotanical characteristics of some plants distributed in Ceylanlı village (Kırıkhan/Hatay) on the slopes of Amanos Mountain. As a result of the field studies carried out between 2019 and 2021 and the interviews with the local people, it was determined that 75 taxa belonging to 41 families were used by the local people in the research area. The local names of the plants, the harvest season, the parts used, the usage purposes, and forms were determined by face-to-face interviews with different local people in their houses, farms, gardens or natural areas. The families having the highest number of using taxa in the area were determined to be Asteraceae and Lamiaceae (8 taxa each), Rosaceae (6 taxa), Amaryllidaceae, Brassicaceae, and Fabaceae (4 taxa each). In total 116 different uses belonging to the plants in the study area were determined, including uses for medical purposes such as wounds, respiratory tract, and stomachic diseases (47 taxa), food (20 taxa), daily items (6 taxa), spice (4 taxa), firewood (3 taxa), and for other different purposes (14 taxa). The obtained results were discussed by comparing them with the relevant literature. Our results show that most of the plants identified were collected by local people from their natural habitats. The local community in our study area was informed to reduce the collection of natural medicinal plants unconsciously and about the importance of cultivating medicinal and aromatic plants. It is believed that this study will contribute to all relevant studies at national and international scales.

Keywords: Ethnobotany, folk remedies, traditional knowledge, Amanos Mountain, East Mediterranean, Türkiye.

Ceylanlı Köyü'nde (Kırıkhan/Hatay-Türkiye) Etnobotanik Bir Araştırma

Öz: Bu çalışma Amanos Dağı eteklerindeki Ceylanlı köyü (Kırıkhan/Hatay)'nde yayılış gösteren bazı bitkilerin etnobotanik özelliklerini belirlemek amacıyla yapılmıştır. 2019-2021 yılları arasında gerçekleştirilen arazi çalışmaları ve bölge halkıyla yapılan görüşmeler sonucu araştırma alanında 41 familyaya ait 75 bitki taksonunun yöre halkı tarafından kullanıldığı tespit edilmiştir. Bitkilerin yöresel adları, toplanma mevsimleri, kullanılan kısımları, kullanım amaçları ve şekilleri yöre halkıyla evlerinde, çiftliklerinde, bahçelerinde veya doğal alanlarında yüz yüze görüşülerek tespit edilmiştir. Kullanılan bitkilerde takson sayıları bakımından en büyük familyalar sırasıyla Asteraceae ve Lamiaceae (8'er takson), Rosaceae (6 takson), Amaryllidaceae, Brassicaceae ve Fabaceae (4'er takson) şeklinde belirlenmiştir. Çalışma alanında bitkilere ait; tıbbi amaçla (47 takson), gıda olarak (20 takson), günlük eşya yapımı (6 takson), baharat (4 takson), yakacak odun (3 takson) ve diğer farklı amaçlar (14 takson) için olmak üzere toplam 116 farklı kullanım şekli belirlenmiştir. Elde edilen sonuçlar ilgili literatürler ile karşılaştırılarak tartışılmıştır. Sonuçlarımız, tespit edilen bitkilerin çoğunun yerel halk tarafından doğal yaşam alanlarından toplandığını göstermektedir. Çalışma alanımızdaki yerel halk bilinçsizce doğal şifalı bitki toplamanın azaltılması ve tıbbi bitkilerin kültüre alınmasının önemi konusunda bilgilendirilmiştir. Bu çalışmanın ulusal ve uluslararası ölçekte ilgili tüm çalışmalara katkı sağlayacağı düşünülmektedir.

Anahtar kelimeler: Etnobotanik, halk ilaçları, geleneksel bilgi, Amanos Dağı, Doğu Akdeniz, Türkiye.

1. Introduction

Many societies and cultures have used plants as food, medicine, clothing, ornaments, hunting, construction, agriculture, musical instruments, household appliances and similar tools, shade and shelter, superstitious/religious uses, against natural disasters such as floods, drought and soil erosion throughout history. (Altay et al., 2015; Öztürk et al., 2012). According to the latest archaeological evidence in the Sierra de Atapuerca (northern Spain), the relationship between humans and plants dates back to 1.2 million years. Chemical analyzes on human tooth finds show that 70-80% of daily calories were obtained from herbal products at that time (Hardy et al., 2017).

The relationship between humans and plants has been acquired through trial and error and has reached the present day by being transferred from generation to generation in a long period of time. This strong relationship between humans and plants led to the birth of the ethnobotanical discipline whose importance is recognized by the whole world today and in which serious researches are carried out (Koçyiğit & Özhatay, 2006; Kendir & Güvenç, 2010; Altay & Çelik, 2011; Altay & Karahan, 2012; Yesilada, 2013).

Recently, the use of plants as herbal or natural health products beneficial to health has increased worldwide, especially in developed countries. Although the use of plants for medicinal purposes in traditional treatment methods in history has lost its former value due to technological developments, the demand for medicinal plants has increased in recent years, especially due to the chemical side effects of drugs. It is known that 80% of the world population benefits from medicinal plants against diseases and more than 80.000 plant species are used for medicinal purposes (Karahan, 2022). For this reason, the

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investigation and conservation of these plants used in traditional medicine and the determination of their economic value as natural resources contribute to sustainable development in both developed and developing countries (Öztürk et al., 2018a, b, c; Malik et al., 2021).

Many ethnobotanical studies have been carried out in Hatay, which has hosted numerous cultures throughout history and has a rich ethnobotanical heritage, contributing to the efforts to define and protect this natural and cultural heritage (Karahan, 2022). Kırıkhan, chosen as the study area, is one of the most developed districts of Hatay province after Iskenderun and Antakya. The district is host to many different ethnic cultures and communities (Altay et al., 2015).

With this study, some ethnobotanical characteristics of the plants that are distributed in Ceylanlı village (Kırıkhan) established on the slopes of Amanos Mountain were tried to be determined.

The aims of this study are:

•making inventory and records regarding the use of medicinal and aromatic plants by the local community,

•determining which parts of the plants are used by local people and for what purposes,

•identifying the most common plants used in the region, and

•contributing to the studies to be carried out in our country and nearby geography in the future by collecting data on the preparation procedures for herbal medicines.

2. Material and Methods

2.1. Study area

The region chosen as the research area is Ceylanlı village of Kırıkhan district of Hatay province. This region is 52 km away from Hatay city center and 7 km away from Kırıkhan district and is located on the eastern slopes of the Nur Mountains (Fig. 1). It is surrounded by the Amik Plain to the east and the Nur Mountains to the west, south, and north. The name of the village "Ceylanlı" was named after "Ceylan Osman", one of the notables of the village, and the mountain gazelles (Dağ Ceylanı in Turkish) that still exist in the region.

The study area has geomorphologically diverse surface forms (hills, wet and dry streams, mountains, and mountain slopes). The study area has a semi-arid Mediterranean climate type with an annual average precipitation of 557 mm and a medium temperature of 19.3°C (Mesothermal). While annual precipitation is maximum in winter, it decreases in spring. Average minimum and maximum temperatures were measured as 8.2 and 30.4°C in January and July, respectively (Altay et al., 2016; Topuz et al., 2016). The humid rainy air mass coming from the Mediterranean Sea rises over the Amanos Mountains, which extend parallel to the sea from the Iskenderun Bay, and causes heavy precipitation. On the other hand, humid air masses coming from Samandağ and terrestrial air masses coming from the north also meet in the eastern parts of Amanos Mountains. Due to this climate and geographical richness, Kırıkhan and its surroundings are very rich in plant diversity (Altay et al., 2015).

Figure 1. The study area

2.2. Plant material

A total of 75 plant taxa were collected in Ceylanlı village (Kırıkhan) and its surroundings during different vegetation periods between 2019 and2021. The plant samples were identified by Dr. Faruk Karahan according to the relevant literature (Davis, 1965-1988) and voucher specimens were stored at the Herbarium of Hatay Mustafa Kemal University (Hatay, Türkiye). The local names of the plants, the harvest season, the parts used, the usage purposes, and forms were determined by reportages. Face-to-face interviews were conducted at least four times in Turkish with different local people in their houses, farms, gardens or natural areas in accordance with the ethical rules. Especially, elderly people, middle-aged women, and men are chosen and it is aimed to transfer traditional knowledge to the present day correctly. The total number of people from whom information was obtained is 24. 10 of these resource persons are men and 14 of them are women, the average age of women is 58 and the average age of men is 55. In terms of the educational status, 2 of the local people are uneducated while 14 are primary school, 5 high school, and 1 university graduates (Fig. 2).

3. Results

Within the scope of this study, a total of 116 local uses belonging to 75 taxa in 41 families were determined in the research area. These plants are locally used for medicine (47) followed by used as food (20), as spice (4), as fuel (3), and other 14 for different purposes. Ethnobotanical characteristics of these plant taxa are alphabetically listed with their botanical and local names, part used, purpose of use, usage, recorded literature uses, and collector numbers (Table 1). Asteraceae and Lamiaceae (8 taxa each), Rosaceae (5 taxa), Brassicaceae, and Fabaceae (4 taxa each) are the families with the highest number of species in the study. 12 taxa of studied samples are cultivated plants, 2 taxa are exotic, and the others are natural plants (Table 1).

Table 1. Useful plants in Ceylanlı village and the other ethnobotanical properties in the related literature

No	Plant taxa	Local name	Plant part used	Uses	Aim of uses	Recorded literature uses
	AMARYLLIDACEAE					
1	*Allium ampeloprasum* L. F. Karahan 1421	Körmen	Whole plant	As a food	Cooked with bulgur wheat	Visual impairment, diabetes. Eaten as a pastry and salad (Sargın et al., 2013; Kerar & Akan, 2019; Yeşil et al., 2019)
2	****Allium cepa*** L. F. Karahan 1422	Soğan	Bulbs	Medicinal, As a food	Poisonous animal bite, ear infection, blood stopper, eaten raw	Gastrointestinal diseases, renal colic, menstrual cramps, aphrodisiac, antiseptic, diuretic, diabetes treatment, antiparasitic (Al-Qura'n, 2008; Sargın et al., 2013; Owfi, 2021).
3	****Allium sativum*** L. F. Karahan 1425	Sarımsak	Bulbs	Medicinal, cosmetic	Poisonous bite, hair and beard care	Edible, colds, cataracts, skin reactions, oxidative stress, mouth sores, fatigue, constipation, against hair loss, disinfectant, anti-parasitic, appetizing, blood pressure lowering, kidney stone lowering (Karahan & İlçim, 2017; Owfi, 2021; Kültür et al., 2021).
4	****Narcissus tazetta*** L. F. Karahan 1432	Nergis	Bulbs	Medicinal	The bulbs is pounded and crushed and used against the inflamed wound, and then a turmeric leaf is immediately put to relieve the pain and burning.	Eaten as pastry, medicinally for sore throat, wounds, skin disease, also ornamental (Gürdal & Kültür, 2013; Kerar & Akan, 2019)
	ANACARDIACEAE					
5	*Pistacia terebinthus* L. subsp. *palaestina* (Boiss.) Engler F. Karahan 1438	Menengiç	Leaves, Seeds	As a food	It is fried with onions and eaten as menemen. Salad is made.	Respiratory diseases (flu, cough, bronchitis, asthma), stomachache, joint, muscle and stomach aches, constipation, expectorant, diuretic, antiseptic (Honda et al., 1996; Karahan et al., 2020)
6	*Rhus coriaria* L. F. Karahan 1437	Sumak	Fruits, Leaves, Stems	Medicinal, as a spice	It is used as a spice. It is mixed with the Dardagan plant and cooked on the stove and used against the heel nail. When the hands are burned while chopping peppers, if the leaf is fresh, it is applied directly or if the water is boiled and the water is applied, the burning of the hand goes away. Sumac and pine stem are boiled together and used as a mouthwash against mouth sores.	Mouth and skin sores, cut wounds, common cold, toothache and food (Honda et al., 1996; Kocabaş & Gedik, 2016; Sargin & Büyükcengiz, 2019; Özçelik, 2022)
	APIACEAE					
7	*Eryngium creticum* Lam. F. Karahan 1423	Devetabanı	Whole plant	Medicinal	Crushed and used against wounds and athlete's foot	Consumed as a snack after peeling (Yeşil et al., 2019)
	ARACEAE					
8	*Arum dioscoridis* Sm. var. *luschanii* R. Mill. F. Karahan 1462	Dağ pancarı	Leaves	As a food	Soap made by boiling (low temperature/long time)	Leaves cooked as vegetable, soap made by boiling, pie made by roasting the leaves, digestive disorders, diuretic, antitussive, tranquilizer, gastritis, intestinal parasites, hemorrhoids (Akbulut, 2015; Altay et al., 2015; Sargın, 2015; Güneş et al., 2018; Kerar & Akan, 2019)
	ASPARAGACEAE					
9	*Asparagus acutifolius* L. F. Karahan 1424	Kaplan bıyığı	Whole plant	Medicinal	As a tea against stomach wounds	Diabetes, diuretic, analgesic, kidney inflammation desiccant, antipyretic, rheumatism, flu (Fakir et al., 2009; Öztürk et al., 2017)

No	Plant taxa	Local name	Plant part used	Uses	Aim of uses	Recorded literature uses
	ASTERACEAE					
10	*Anthemis haussknechtii* Boiss. & Reuter F. Karahan 1463	Yoğurtlama	Capitula	Medicinal	As a tea against colds and pains.	Hair loss, indigestion, menstrual regulator and tranquilizer (Fakir et al., 2009; Kerar & Akan, 2019)
11	*Centaurea iberica* Trev ex Sprengel F. Karahan 1430	Çakırdikeni	Flowers	Medicinal	It is crushed into pill form and drunk to get rid of dirty water and air while going to the plateau. Also headaches and gallstones	Antipyretic, wound healing, diabetes and stomach ailments (Çakılcıoğlu & Türkoğlu, 2010; Güzel et al., 2015)
12	*Cota tinctoria* (L.) J. Gay. F. Karahan 1442	Boyacı papatyası	Capitula	Cosmetic	Flowers in boiled water to dye hair yellow	Diabetes, throat diseases (Çakılcıoğlu & Türkoğlu, 2010)
13	*Inula viscosa* (L.) Aiton F. Karahan 1433	Çakalotu	Whole plant	Medicinal	Plant in boiled water and sit in its steam against cold	Eye diseases, stomach ailments, wounds, ulcers, pain, respiratory tract infection, hemorrhoids, bone fractures, diabetes, backaches, skin fungus, loss of appetite, dysentery, muscle aches, infertility, lung disorders, skin and joint diseases (Öztürk et al., 2017; Özyigit et al., 2022)
14	*Matricaria chamomilla* L. F. Karahan 1426	Mayıs papatyası	Whole plant, capitula	Medicinal	Used as a tea against pains	Stomachache, cough, cold, bronchitis, malaria, carminative, insomnia, headache, appetite stimulant, depression, antipruritic, hemorrhoids, menstrual problem, laxative, digestive, spasm, diuretic, laxative, aphrodisiac, obesity, stimulant, fever lowering, anti-inflammatory, kidney stone, sedative, ear and toothache, cardiovascular disease, eye disease, mastitis, constipation, female, respiratory, nervous and skin diseases (Gürdal ve Kültür, 2013)
15	*Silybum marianum* (L.) Gaertner F. Karahan 1429	Kangal, Kenger	Whole plant	As food	Eaten raw	Asthma, liver diseases, eaten fresh (Sargin et al., 2015; Sargin & Büyükcengiz, 2019; Sargin, 2019)
16	*Sonchus asper* (L.) Hill. F. Karahan 1431	Eşek marulu	Leaves	Medicinal	Used for wound healing	Food, insect bites, mouth sores (Fakir et al., 2009)
17	*Taraxacum microcephaloides* Soest F. Karahan 1441	Karahindibağı	Whole plant	Medicinal, as food	It is used as a tea against cancer. Consumed as salad.	Diabetes, malaria, ulcers, stomach pain, constipation, eczema, warts and calluses (Fakir et al., 2009)
	BRASSICACEAE					
18	***Brassica oleracea* L. F. Karahan 1443	Lahana	Leaves	Medicinal, as a food	Consumed as sarma food. It is boiled in water and consumed as a stew. Cabbage is boiled and wrapped in knee pain	Edible, scurvy, inflamed wounds, constipation problems (Karahan & İlçim, 2017; Owfi, 2021; Özçelik, 2022)
19	*Capsella bursa-pastoris* (L.) Medik. F. Karahan 1434	Çobançantası	Whole plant	As a food	Eaten as a pastry	Uterine bleeding, malignant ulcers, stomach cancer, kidney stones, dysentery, gastritis, tuberculosis and eye diseases, diabetes, tooth and nose bleeding, burn treatment, constipation, intestinal spasm, rheumatism (Bağcı et al., 2006; Sargın et al., 2013; Zaurov et al., 2013)
20	*Isatis* sp. F. Karahan 1451	Meyana	Whole plant	Medicinal	It is used as a tea against constipation in children. It is dried and powdered with parsley and figs and given the form of pills by adding real honey. It is drunk on an empty stomach in the morning against stomach diseases and hemorrhoids.	Eaten as a meal and salad (Kerar & Akan, 2019)
21	*Nasturtium officinale* W.T. Aiton F. Karahan 1427	Ispatan	Whole plant	Medicinal	It is mixed with flour and roasted on the stove and used against abdominal swelling in children.	Medicinally against goiter, neck swelling; thyroid gland diseases, also eaten as a meal and salad (Altay & Karahan, 2012; Kerar & Akan, 2019; Özçelik, 2022)

No	Plant taxa	Local name	Plant part used	Uses	Aim of uses	Recorded literature uses
	CACTACEAE					
22	***Opuntia ficus-indica* (L.) Miller F. Karahan 1439	Papuç inciri, Frenk inciri	Stem, Fruits	Medicinal	The stems are crushed and used against knee pain.	Pain, rheumatism, insect bites, abdominal pain, kidney stones, respiratory diseases, hematomas, diarrhea, edema, skin, liver, and musculoskeletal disorders, sedative, diuretic, antispasmodic (Gürdal & Kültür, 2013)
	CAPPARACEAE					
23	*Capparis spinosa* L. F. Karahan 1436	Kemer kökü	Seeds, buds	Medicinal, As a food	Antibiotics, eaten as pickles	Wounds, asthma and gastrointestinal diseases, hepatitis, hemorrhoids, toothaches, diarrhea cataract, skin reactions, oxidative stress, analgesic and vermifuge, also, pickles are appetizing. (Zaurov et al., 2013; Karahan & İlçim, 2017; Kerar & Akan, 2019)
	CHENOPODIACEAE					
24	****Beta vulgaris* L. var. *cicla* (L.) K. Koch F. Karahan 1428	Pancar, Pazı	Whole plant	Medicinal	It is cooked with pomegranate syrup and used against intestinal parasites	As food, detox (Altay et al., 2015; Kocabaş & Gedik, 2016)
	ELAEAGNACEAE					
25	*Elaeagnus angustifolia* L. F. Karahan 1435	İğde	Woods	Firewood	Used as firewood	Breath freshener, food, necklace, rosary making (Kocabaş & Gedik, 2016)
	ERICACEAE					
26	*Erica manipuliflora* Salisb. F. Karahan 1452	Puren	Stem	Tool making	Broom is made	Slimming, diuretic, constipation, arthritis (Öztürk et al., 2017)
	EUPHORBIACEAE					
27	*Euphorbia* spp. F. Karahan 1440	Sütloğan, Sütleğen	Stem	Medicinal	Stem milk is applied to temra wounds in livestock	Eczema, hemorrhoids, constipation, rheumatism, warts, diuretick (Güzel et al., 2015; Öztürk et al., 2017)
	FABACEAE					
28	*Cercis siliquastrum* L. subsp. *siliquastrum* F. Karahan 1487	Erguvan	Flowers	Medicinal	As a tea against cough	Malaria, burn treatment (Güzel et al., 2015; Kerar & Akan, 2019)
29	*Spartium junceum* L. F. Karahan 1490	Boruk, Katırtırnağı	Whole plant	Tool making	Figs and molasses are dried on it. Also broom is made	Stomachic, kidney stones, anesthetic, diuretic (Senkardes & Tuzlacı, 2014; Kültür et al., 2021)
30	*Trifolium* spp. F. Karahan 1491	Üçgül	Whole plant	Medicinal	It is used as a tea against atherosclerosis.	Kidney pains, animal feed (Ünver, 2019; Kültür et al., 2021)
31	*Trifolium pilulare* Boiss. F. Karahan 1492	Sancı otu	Whole plant	Medicinal	It is used as a tea for pains	Animal feed (Kerar & Akan, 2019)
	GERANIACEAE					
32	**Erodium amanum* Boiss. & Kotschy F. Karahan 1489	İğnelik	Whole plant	As a food	It is cooked by frying with onions and olive oil	Chronic and acute rheumatism (Karahan, 2022)
	HYPERICACEAE					
33	*Hypericum perforatum* L. F. Karahan 1453	Kantaron	Flowers	Medicinal	As a tea against stomach wound, externally as a wound healer.	Hemorrhoid, prostate, diabetes, hypertension, urinary tract infections, diaper rash, rheumatism, osteoporosis, skin lesion, sunburn, antiseptic, antispasmodic, constipation, ulcer, sedative, arthritis, depression, insomnia, expectorant, jaundice, tuberculosis, asthma, stomach and abdominal pains, rheumatism, hemorrhoids, skin burns, inflammation and wounds, diarrhea, mastitis (for animal), enteritis, ulcer (Polat & Satıl, 2012; Karahan & İlçim, 2017; Öztürk et al., 2017)
	JUGLANDACEAE					
34	*Juglans regia* L. F. Karahan 1461	Ceviz	Fruits, stem barks	Tool making, dye plants	It is used to prevent the dye from flowing when women dye their hair or to make the henna dye red and bright. Daily kitchen utensils made such as breadboard, spoon, ladle, rolling pin (also local names such as tokaç,	Fruits for hair loss and diabetes, wood is used in carpentry and for furniture, household utensils, and musical instruments (Kocabaş & Gedik, 2016; GC et al., 2021)

No	Plant taxa	Local name	Plant part used	Uses	Aim of uses	Recorded literature uses
					kernep, astım, evraç).	
	LAMIACEAE					
35	*Ajuga chamaepitys* (L.) Schreber F. Karahan 1454	Kızlarleylimi	Whole plant	Medicinal	As a tea against stomach ailments, kidney and gallstones.	Tonic, antipyretic, hemorrhoidal, diuretic, wound healer, pain reliever (Terzioğlu & Coşkunçelebi, 2021)
36	*Lavandula stoechas* L. F. Karahan 1444	Karabaş otu	Whole plant	Medicinal, tool making	It is used as a tea against cardiovascular diseases, shortness of breath, skin wounds, it is a slimming tea and germicidal. The broom is made.	Stomach and headaches, cancer, urinary tract diseases, antiseptic, ulcer, nervous disease, asthma, cardiovascular disease, diabetes, cholesterol, cough, cold, bronchitis, smoking cessation, sedative, insomnia, epilepsy, obesity digestive, carminative, kidney stone, injury, rheumatism, skin diseases, musculoskeletal disease, respiratory disease, menstrual disorders, bad breath (Gürdal & Kültür, 2013)
37	*Melissa officinalis* L. F. Karahan 1445	Melisa	Whole plant, flowers	Medicinal	Relaxation	Brain stimulant, cold, high fever, stomach pain and spasms, cardiovascular diseases, insomnia, headache, migraine, weakness, hyperthyroidism, ear ache, relaxing (Korkmaz & Karakurt, 2014; Özyigit et al., 2022)
38	*Mentha longifolia* (L.) Hudson F. Karahan 1446	Nane, Yabani nane, Yarpuz	Whole plant	Medicinal, as a spice, fragrant	It is used as a tea against cold and flu. It is used as a blood stopper and as a spice in meals. In addition, tarhana is dried on it because it gives a nice smell	Cold, flu, bronchitis, asthma, cough, flu, stomachache, menstrual pain, stomach, headache, lung ailments, diarrhea, hemorrhoids, sunstroke (Altundağ & Özturk 2011)
39	***Ocimum basilicum* L. F. Karahan 1447	Mor reyhan, Reyhan	Whole plant	Medicinal, as a spice	It is used as a spice and a tea against cough	Upper respiratory tract infections (cough, bronchitis, laryngitis, pharyngitis, etc.), chronic gastritis, enterocolitis, food poisoning, nausea and spasm, dysentery, cramps, diuretic, appetizing, sedative, relaxing (Özyiğit et al., 2018; Sharopov & Setzer, 2018)
40	*Rosmarinus officinalis* L. F. Karahan 1448	Biberiye	Above plant	Medicinal	It is used as a tea against indigestion, against blood pressure, and as a sedative.	Hypertension, asthma, obesity, anorexia, diuretic (Sağıroğlu et al., 2013; Sargin & Büyükcengiz, 2019)
41	*Teucrium polium* L. F. Karahan 1449	Peryavşan	Whole plant	Medicinal	It is used as a tea against headache and diabetes. It is put in the shower water and is good for fatigue.	Cold, cough, sore throat, back and foot pain due to fatigue, rheumatic pain, stomach cold, against motion sickness toothache (Fakir et al., 2009; Kocabaş & Gedik, 2016; Özçelik, 2022)
42	*Thymus kotschyanus* Boiss. & Hohen subsp. *kotschyanus* F. Karahan 1450	Kekik	Whole plant	Medicinal, as a spice, superstitious and religious beliefs	After being boiled in water and cooled, mouthwash is made and used against intra-oral wounds and inflammations. It is used as a spice. In addition, it is fried with salt in a pan and it is believed that it is good for the eyes and evil eye by drawing salawat in its smoke.	As a spice, colds (Kocabaş & Gedik, 2016)
	LAURACEAE					
43	*Laurus nobilis* L. F. Karahan 1455	Defne, Har, Gar	Leaves, seeds	Medicinal, cosmetic	It is used as a tea against cough, laurel and olive oil are boiled together to make soap. The laurel soap is chopped into the egg white and whisked. This mixture is used as a	Spices, hair care (Kocabaş & Gedik, 2016)

No	Plant taxa	Local name	Plant part used	Uses	Aim of uses	Recorded literature uses
					plaster for fractures in a cloth.	
44	****Persea americana** Mill. F. Karahan 1456	Avakado	Leaves, Fruits	Cosmetic	It is boiled and used as a hair nourisher.	Antihypertensive, exfoliative, vasorelaxation, analgesia, anti-inflammatory activity, anticonvulsant activity, antiviral, wound healing, hepatoprotective, antioxidant and hypoglycemic (Saleem et al., 2019)
	MALVACEAE					
45	*Malva sylvestris* L. F. Karahan 1482	Ebegümeci, Kömeç	Whole plant	Medicinal, as a food, superstitious/ religious beliefs	It is fried with oil and onions and eaten. It is thrown into the water where the clothes are washed and provides a nice foaming. It is roasted with flour and used externally against boils on the feet. It is crushed in a mortar and added to the water in which the deceased was washed and it is believed to produce urine.	Diuretic, sedative, expectorant, eczema, bee sting, insect sting, burn, abscess boil, acne and sores, laxative, stomachache, bronchitis, cold, rheumatism, tonsillitis, gastritis and ulcer, cancer, sedative, hoarseness, edema, headache, cardiovascular and gastrointestinal diseases, asthma, kidney stones, miscarriage, menstrual problem, dislocation, weight loss, toothache, sore throat, galactagogue, constipation, gynecological diseases, prostatitis, stomatitis, lung diseases, cholesterol, hemorrhoids, constipation, neurosis (Gürdal & Kültür, 2013)
	MELIACEAE					
46	*Melia azedarach* L. F. Karahan 1483	Tespih ağacı	Seeds	Medicinal	Its seeds are pounded together with hibiscus, mixed with flour and white onion and rubbed into bruised wounds. If the wound is very bruised, sumac syrup is also added.	Hemorrhoids, psoriasis, rheumatism, headache, intestinal parasites (Al-Qura'n 2008; Güzel et al., 2015; Ekren & Çorbacı, 2021)
	MORACEAE					
47	*Ficus carica* L. subsp. *carica* F. Karahan 1484	İncir, Yemiş	Fruits	As a food	It is eaten as a snack, and the milk of its fruit is used as rennet (Teleme).	Medically, cancer, flu, wart treatment, callus treatment, constipation, analgesic, bee and insect bites are also eaten wet or dry, jam is made, and it is also used as yeast in cheese (teleme) production (Sargın et al., 2013; Güzel et al., 2015; Kerar & Akan, 2019)
48	*Morus alba* L. F. Karahan 1486	Dut	Fruits	Medicinal	Dried and eaten, it is used against kidney stones, constipation and also accelerates metabolism.	Food, indigestion, milk enhancer, abscess, stomach ailments, stomach ulcer (Altundağ & Özturk, 2011; Kocabaş ve Gedik 2016)
	MYRTACEAE					
49	*****Myrtus communis** L. F. Karahan 1485	Hambeles	Whole plant, fruits	Superstitious and religious beliefs	The fruits are prayed and put in the shroud of the dead. It is also used against calcification by boiling the above-ground parts and sitting in the water. Broom is made	Food, high cholesterol, diabetes, cough, weight loss, mouth sores, constipation, astringent, antiseptic, laxative, hypoglycemic, analgesic, hemostatic, hair tonic, stimulant, stomachic, appetizing, hemorrhagic and wound healing (Özçelik, 2022; Özyiğit et al., 2022)
	OLEACEAE					
50	*Fraxinus excelsior* L. F. Karahan 1493	Dişbudak	Leaves	Dye plants	Leaves in boiled water for dyeing of woolen yarns with yellow	Medically, migraine pain, kidney stones, constipation, rheumatism, antipyretic, expectorant, astringent, diuretic, laxative, diarrhea, rheumatism, gout, slimming tea, boat and pulley are made from wood (Korkmaz & Karakurt, 2014, Öztürk et al., 2018a)
51	*****Olea europaea** L. F. Karahan 1494	Zeytin	Fruits, seeds, leaves	Medicinal, as a food, cosmetic, as a fuel	It is consumed as olives, olive salad, and olive oil. The leaves are chewed against mouth	Cardiovascular, skin and eye diseases diabetes tissue repair, inflammation, blood pressure, constipation, antipyretic, appetizing (Gürdal & Kültür, 2013; Karahan & İlçim, 2017).

No	Plant taxa	Local name	Plant part used	Uses	Aim of uses	Recorded literature uses
					sores, olive oil is applied to the hair, eyebrows and beard, and soap is made from the seed oil. Its pulp is used as a fuel in the form of pomace.	
52	*Phillyrea latifolia* L. F. Karahan 1457	Akçakesme	Woods	Firewood	Used as firewood	Animal feed (Kerar ve Akan 2019)
	PAPAVERACEAE					
53	*Papaver rhoeas* L. F. Karahan 1488	Gelincik	Whole plant, flowers	Medicinal, as a food	It is used as a tea against insomnia, it is also roasted with rush broom and pastry is made.	Antistress, burn, wound, insomnia, sedative, anthelmintic cough, aphthae, anemia, tonic, hemorrhoids, rheumatism, immunotoxic, epistaxis, galactagogue, eye disease, nervous disease, baldness, nervous disease, digestive, respiratory disease (Gürdal & Kültür, 2013)
	PEDALIACEAE					
54	*Sesamum indicum* L. F. Karahan 1459	Küncü	Fruits, Seeds	Medicinal	It is mixed with crushed and roasted milk and used against inflamed wounds.	Burns and wounds, skin and hair care, backaches (Sargin, 2015; Güneş et al., 2018)
	PINACEAE					
55	*Pinus brutia* Ten. F. Karahan 1458	Çam ağacı	Cones, flowers, resin and stem barks	Medicinal, as a food, toys	Jams are made from cones and toys for children are made from stem shells. The flowers are used as a tea against cough. Its resin is used in pill form against liver diseases	Cough, bronchitis, asthma, bad breath, teeth cleaning, stomachache and ulcer, diabetes, appetizer, skin sores, scabies, anti-acarides (Karahan et al., 2020)
	PLANTAGINACEAE					
56	*Plantago lanceolata* L. F. Karahan 1464	Damar otu	Whole plant	Medicinal	Used as a tea for atherosclerosis	Abscess, respiratory problems, visceral wounds, cough, wounds, insect bites, acne ulcers, stomachaches, hemorrhoids, embolism, gynecological diseases, urinary tract infections, shortness of breath, cardiovascular diseases, lung protective, also antiparasitic, expectorant, slimming and anti- inflammatory (Karahan, 2022; Özçelik, 2022)
	PLATANACEAE					
57	*Platanus orientalis* L. F. Karahan 1481	Çınar	Leaves, Fruits	Medicinal	Especially 5-part leaves are used as tea against joint calcification.	Calcification, joint pain, menstrual regulator (Bulut & Tuzlaci, 2013; Kerar & Akan, 2019)
	POLYGONACEAE					
58	*Rumex acetosella* L. F. Karahan 1480	Ebelik, Evelik	Stem, Seeds	Medicinal	It is used as a tea against diarrhea and stomachache. It is used against itching by boiling the stem and seeds.	Raw "ekşimen salatası" and roasted "Kilime pilavı" (Özer & Türkmen, 2019; Sargın, 2019
	PORTULACEAE					
59	*Portulaca oleracea* L. F. Karahan 1460	Semiz otu, Soğukluk	Whole plant	As a food	It is consumed as vegetables, salad, tzatziki and pastry alongside meals and it is also fed to cool the animals in hot weather.	Eaten as a meal and salad, animal feed (Kerar & Akan, 2019)
	PUNICAEAE					
60	***Punica granatum* L. F. Karahan 1465	Nar	Fruits, Flowers	Medicinal, as food, dye plants	It is used as a tea against intestinal disorders. The pomegranate syrup is made. Dye is obtained from its flowers.	Diabetes, cholesterol, abdominal pain, digestive, cardiovascular and kidney diseases, constipation problems, diuretic, diarrhea, cough, pain, prostate, mouth sores (Gürdal & Kültür, 2013; Kocabaş & Gedik, 2016; Özçelik, 2022

No	Plant taxa	Local name	Plant part used	Uses	Aim of uses	Recorded literature uses
	PTERIDACEAE					
61	*Adianthum capillus-veneris* L. F. Karahan 1478	Erefe otu, baldırıkara	Whole plant	Medicinal	Kidney stones	Expectorant, kidney stone, heartburn, menstrual regulation (Fakir et al., 2009; Kocabaş & Gedik, 2016)
	RHAMNACEAE					
62	*Paliurus spina-christi* P. Mill. F. Karahan 1466	Karadal	Whole plant	Dye plants	A yellow color thread is obtained by boiling and a brown color thread is obtained by mixing with ash.	For muscle cramps, joint pain, warts, kidney stones and inflammation, constipation, kidney stones, hepatitis, lung inflammation, stomachache, dysentery, diuretic (Kocabaş & Gedik, 2016; Karahan et al., 2022).
63	*Zizyphus lotus* (L.) Lam. F. Karahan 1479	Gülnap, Hannep	Fruits	As a food	The fruits are used for regulating blood sugar	Medicinally for gastric ulcers and spasms, also its stem is used to make garden fencing (İlçim, 2014; Kerar & Akan, 2019)
	ROSACEAE					
64	***Prunus avium* L. F. Karahan 1468	Kiraz	Fruits, Leaves	Medicinal, As a food	The jam is made. It is used as a tea against skin wounds, acne, psoriasis.	Inflammation, acne, obesity, diuretic, kidney stones, also eaten as jam (Metin, 2009; Sargın, 2015)
65	***Prunus domestica* L. F. Karahan 1469	Erik ağacı	Stems, Fruits	Tool making	Daily kitchen utensils made such as breadboard, spoon, ladle, rolling pin (also local names such as tokaç, kernep, astım, evraç).	Anticoagulant, analgesic, stomachic, also eaten raw or jam, music instrument "Mey" made (Al-Qura'n, 2008; Akaydın et al., 2013; Karahan et al., 2015)
66	*Rosa* spp. F. Karahan 1473	Kafirin, Gül	Flowers	Superstitious and religious beliefs	It is put in the shroud because it gives a nice smell.	Diabetes, flu, diarrhea, shortness of breath (Kocabaş & Gedik, 2016; Güneş et al., 2018)
67	*Rubus idaeus* L. F. Karahan 1467	Ahududu	Fruits	Medicinal	Used against mouth sores by chewing	Diabetes, sugar, toothache, gum disease, high fever, burning and allergic itching, also eaten as syrup, jam and marmalade (Metin, 2009; Ünver, 2019)
68	*Rubus sanctus* Schreber F. Karahan 1476	Yabani böğürtlen	Whole plant	Medicinal	Used as a tea against high blood pressure	Rheumatism, eczema, hemorrhoids, kidney stones, diabetes, tonsillitis, stomachache, antipyretic, cancer, constipation, birthmark, infertility, ulcer, diuretic, respiratory disease, toothache, liver diseases, hemostatic, musculoskeletal disease, stomach, wound, biliary bladder disease (Gürdal & Kültür, 2013)
69	*Sanguisorba minor* Scop. F. Karahan 1471	Dardağan otu	Whole plant	Medicinal	It is used as an ointment against wounds, especially heel wounds.	Kidney stone, hernia (Kültür et al., 2021)
	RUTACEAE					
70	*Citrus sinensis* (L.) Osbeck F. Karahan 1474	Portakal	Leaves	Medicinal	As a tea against constipation, to lose weight	Antiseptic, nervous disease, constipation, cardiovascular and respiratory diseases, sedative, digestive, abdominal pain, wart (Gürdal & Kültür, 2013)
	SALICACEAE					
71	*Salix triandra* L. F. Karahan 1477	Söğüt ağacı	Leaves	Medicinal	Tea is used against malaria and to strengthen immunity.	Energizing, antipyretic, relieving constipation, rheumatic pains (Saday, 2009)
	SOLANAECAE					
72	*Solanum tuberosum* L. F. Karahan 1470	Patates	Tubers	Medicinal	Used against eye inflammation and swelling	Abscesses, headache (Senkardes & Tuzlacı, 2014; Karakaya et al., 2020)
	TILIACEAE					
73	*Tilia argentea* Desf. ex DC. F. Karahan 1475	Ihlamur	Above plant, Stems, Flowers	Medicinal, tool making	As a tea, it is good for flu and cold. Daily kitchen items made such as breadboard, spoon, ladle, rolling pin (also local names such as tokaç, kernep, astım, evraç)	Pain reliever, diuretic, antipyretic, sore throat, nausea, insomnia, sedative, sedative, expectorant, antioxidant (Akbulut, 2015; Öztürk et al., 2017)
	URTICACEAE					
74	*Urtica dioica* L. F. Karahan 1472	Isırgan otu	Whole plant	Medicinal, as a food	It is used as a blood purifier as tea, and also as a meal.	Medicinally as a diuretic, blood purifier, anemia, rheumatic pains, inflammations, hair care, also as a pastry filling, roasted or

No	Plant taxa	Local name	Plant part used	Uses	Aim of uses	Recorded literature uses
						as a salad. (Saday, 2009; Kocabaş & Gedik, 2016)
	VITACEAE					
75	*Vitis vinifera* L. F. Karahan 1495	Üzüm	Fruits, Leaves	Medicinal, as a food	Molasses is made for treating anemia. Tea boiled with olive oil is used against cough. Used for wrap food	Tonsillitis, anemia, bleeding, skin and hair care, bruises, bruises, puerperal women mix molasses and black pepper for their immune system and drink it, antipyretic. (Metin, 2009; Polat & Satıl, 2012)

*Endemic, **: Exotic, ***: Cultured plants

Figure 2. The field and survey studies (Ceylanlı village/Kırıkhan)

When evaluated in terms of the usage purposes, it was determined that the identified plants were generally used for medicinal purposes (50%) and as food (21%) as shown in Figure 3. If the usage purposes are to be classified in detail, the plants are mostly used as food (13 taxa), for wound healing (8 taxa), cosmetics (7 taxa), stomach ailments and making daily goods (6 taxa), kidney-gallstone reducer, cough suppressant, and dye plant (5 taxa each) (Table 2).

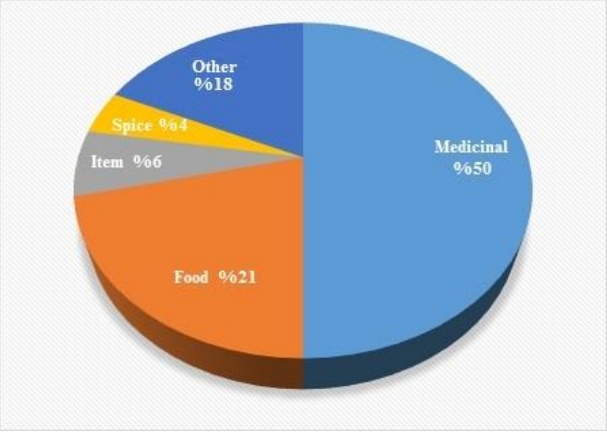

Figure 3. Purpose of use of studied plants (%)

Generally, the plants in the study area are medicinally used by the local people for upper respiratory tract infections (cold, flu, cold, bronchitis, etc.), asthma, skin diseases, wound healing, hemorrhoids, painkillers, kidney and gallstones, mouth sores, high cholesterol, and knee pain. A majority of these plants are used for the treatment of respiratory disorders (common cold, cough, etc.) (12%), wounds (10.7%), stomachic problems (8%), and the other purposes (Fig. 4). It is used in the treatment of many diseases such as joint diseases. Plants used for medicinal purposes generally belong to the Asteraceae and Lamiacae families which contain many medicinal aromatic plants and are widely used for medicinal purposes in different regions (Table 1).

Plants consumed in the study area as food (fresh, pastry, meal, salad, pickle, spice, etc.) are: *Allium ampeloprasum* (Körmen), *Arum dioscoridis* (Dağ pancarı), *Brassica oleracea* (Lahana), *Capparis spinosa* (Kemer kökü), *Capsella bursa-pastoris* (Çobançantası), *Erodium amanum* (İğnelik), *Ficus carica* (İncir, Yemiş), *Malva sylvestris* (Ebegümeci, kömeç), *Olea europaea* (Zeytin), *Papaver rhoeas* (Gelincik), *Pistacia terebinthus* (Menengiç), *Portulaca oleracea* (Semizotu, Soğukluk), *Prunus avium* (Kiraz), *Prunus* × *domestica* (Erik), *Punica granatum* (Nar), *Rhus coriaria* (Sumak), *Silybum marianum* (Kangal, Kenger), *Taraxacum microcephaloides* (Karahindibağı), *Thymus kotschyanus* subsp. *kotschyanus* (Kekik), *Urtica dioica* (Isırgan otu), and *Vitis vinifera* (Üzüm) taxa (Table 1).

The parts of the plants whose ethnobotanical characteristics are determined change according to the way they are used. Local people most commonly use above-ground parts (34 taxa), fruits (14 taxa), leaves (13 taxa), flowers (11 taxa), stems (8 taxa), seeds (6 taxa), and other parts such as cones and resin of the plants (8 taxa each) in the study area (Fig. 5).

Previously, the ethnobotanical uses of the plants sold in the neighborhood markets and herbalists by the Kırıkhan district of Ceylanlı village, which is our research area, were examined and it was determined that 70 plant taxa belonging to 32 families are sold in herbalists and a total of 37 different plant taxa belonging to 23 families are sold in neighborhood markets (Altay et al 2015). It was also determined that *Arum dioscoridis* (Dağ pancarı), *Beta vulgaris* (Pancar, Pazı), *Portulaca oleracea* (Semizotu), *Spartium junceum* (Boruk çiçeği), *Narcissus tazetta* (Nergiz), *Rhus coriaria* (Sumak), *Allium cepa* (Zambık), *Malva sylvestris* (Kömeç), *Pistacia terebinthus* subsp. *palaestina* (Işkın), *Nasturtium officinale* (Ispatan), and *Teucrium polium* (Peryavşan) are similarly sold in herbalists and neighborhood markets for commercial purposes (Table 1).

Table 2. Purpose of use of studied plants

Purpose of usage	Number of taxa
as a food	13
wound healer	8
cosmetic	7
stomachic	6
item making	6
kidney and gallstone	5
cough	5
as dye plants	5
mouth sores	4
common cold	4
skin diseases	4
spice	4
superstitious/religious beliefs	4
constipation	3
as firewood	3
pains	2
animal poisoning	2
knee joint diseases	2
antibiotic	2
headaches	2
animal diseases	2
slimming tea	2
blood pressure	2
diabetes	2
athlete's foot	1
blood stopper	1
interference	1
bowel	1
diarrhea	1
weakness	1
indigestion	1
hemorrhoids	1
shortness of breath	1
relaxing tea	1
eye inflammation and swelling	1
cancer	1
blood purifier	1
anemia	1
as rennet	1
fragrant	1
toy making	1
	116

4. Discussion

Compared to the previous ethnobotanical studies, although the species, local names, parts used, and usage patterns of the plants in this study are similar to nearby regions, especially the purposes of benefiting from plants may vary between local societies. For instance, while the *Eryngium creticum* (Devetabanı) species in our study is used against wounds and foot fungus by beating and crushing, it is known as "Beektire/Ekkeyde" in a study carried out in Mardin province and is eaten as a snack (Yeşil et al., 2019). Fruits of *Ficus carica* (incir) is eaten fresh/dry or as jam. In addition, cheese (teleme) is made by dripping the milk of the raw fruit into cow, goat or sheep milk. This usage pattern is similar to the ethnobotanical usage in the nearby Aktepe and Zeytinoba villages (Hassa/Hatay) (Kerar & Akan, 2019). *Rumex acetocella*, known as "ebelik/evelik" in the research area and used medically against diarrhea, stomachache, and skin itching, is known with the local name "ekşimen" in Gaziantep province and eaten as salad (Özer & Türkmen, 2019). Also, it is known that " Kuzukulağı, ekşimek and ekşikulak" and "Kilime pilavı" is made by roasting with bulgur in Bozyazı district (Mersin province) (Sargın, 2019). These differences confirm that Anatolian geography has a very rich cultural and ethnobotanical heritage.

When compared in terms of ethnobotanical uses, many ethnobotanical studies have reported that some plants studied have different ethnobotanical uses in other countries. For instance, bulbs of *A. cepa* are used as wound healing, diuretic, and for vagina washing while bulbs of *Allium sativum* are used for the treatment of hypertension, acne, wasp bites, and wounds, to protect against evil eye, and as antihelmintic in Italy (Cornara et al., 2009). *Capsella bursa-pastoris* have been used as haemostatic, hypotensive, alimentary, and astringent in Jordan and Italy (Leporatti & Guarrera, 2007; Al-Quran, 2008). In Iran, it was reported that *Elaeagnus angustifolia* has been used as anti-diarrheal and hepatoprotective and also against gastric pains and rheumatoid arthritis (Karimi et al., 2010; Ghasemi et al., 2013). Latex of *Ficus carica* are medicinally used to heal warts and calluses and also fruits are eaten raw and used to make jams in Marche region, Italy (Lucchetti et al., 2019). *Hypericum perforatum* have been used as antidepressant, choleretic, wound healing (human and veterinary use), anti-diarrheal, and antimicrobial in Romania and Italy (Ghasemi et al., 2013; Marinescu et al., 2020). *Laurus nobilis* has been used medicinally as antirheumatic, digestive, antiscabies, stomach ache, gases, and cough and its leaves are used to flavor meat and fish, and cosmetically it is used in bath water to relax in Italy, Spain, and Jordan (Guarrera et al., 2005; Al-Quran, 2008; Benítez et al., 2010; Lucchetti et al., 2019).

The other taxa *Myrtus communis* is useful traditionally for mouthwash in gingivitis, stomachic, chronic bronchitis and epilepsy in Italy while it is used for dysentery, diarrhea, and rheumatism in Pakistan (Leporatti & Guarrera, 2007; Ahmad et al., 2021). *Opuntia ficus-indica* is used in the treatment of diabetes in Morocco; against burns, kidney pains, clean ailments, and to bring good luck in Bolivia; and as antispasmodic, skin emollient, and diuretic in Liguria region of Italy (Jouad et al., 2001; Macía et al., 2005; Passalacqua et al., 2007). *Tilia argentea* is one of the most important medicinally plants used for the sore throat, bronchitis, kidney disorders, common colds, inhalation, sedative in Bulgaria and it also used against migraine, ingestion problems, liver and gall bladder disorders, nervous tension, and ingestion problems in Central Italy (Koleva et al., 2015; Frezza et al., 2020). Another ethnobotanical study reported that *Urtica dioica* has been used for hypertension, sedative, blood sugar, and

digestive in north of Iran (Mirdeilami et al., 2011). As a result, all these reports demonstrate that the ethnobotanical uses of plants in different societies and cultures are various.

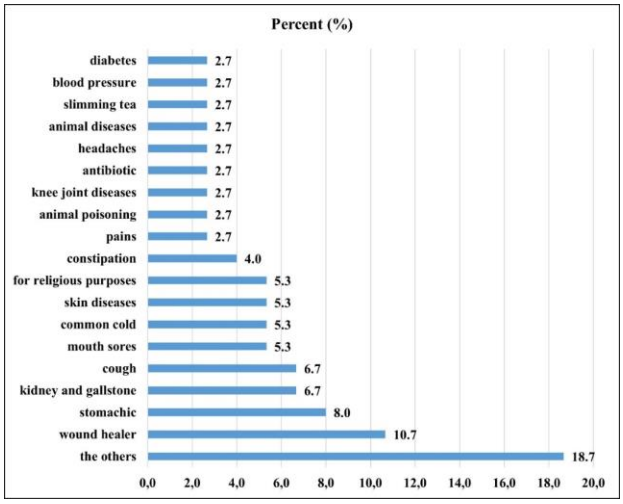

Figure 4. Therapeutic uses of the medicinal and aromatic plant taxa on Percentage Basis.

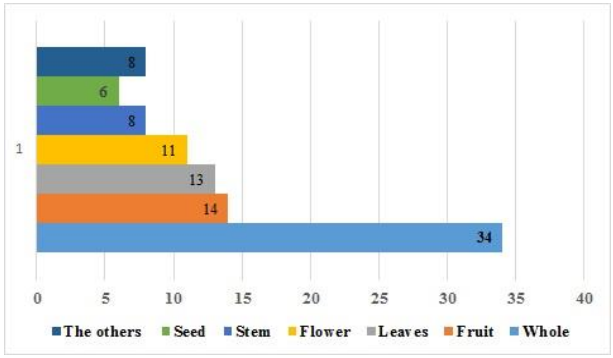

Figure 5. Used parts of the studied plants

Most of the plants identified in our study area are collected from nature. Very few of them are cultivated plants. The unconscious collection of the medicinal plants, especially the endemic *Erodium amanum* (İğnelik otu) taxa which takes its name from the Amanos Mountains and is used by the people of the region, causes a significant decrease in the populations in nature. This situation once again reveals the importance of cultivating medicinal and aromatic plants.

When collecting medicinal plants from nature, especially during the appropriate vegetation period, they should be collected from relatively clean rural areas far from urbanization, traffic, mining sites, agricultural lands, and cement and stone quarries. Their habitats should be away from heavy metal pollution, which causes many serious health problems in the human body, especially when exposed. Finally, awareness of the local people should be raised through academic studies to be carried out in coordination with different disciplines such as medicine, pharmacy, phytochemistry, and ethnology about the plant species in the region.

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References

Ahmad, L., Riaz, M., Jan, H.A., Semotiuk, A.J., Ahmad, I., Khan, I., ... & Bussmann, R.W. (2021). An ethnobotanical survey of wild food plants used by the local communities of Kumrat Valley in District Upper Dir, Pakistan. *Ethnobotany Research and Applications*, 22, 1-13.

Akaydin, G., Şimşek, I., Arituluk, Z.C., & Yeşilada, E. (2013). An ethnobotanical survey in selected towns of the Mediterranean subregion (Turkey). *Turkish Journal of Biology*, 37(2), 230-247. https://doi.org/10.3906/biy-1010-139

Akbulut, S. (2015). Differences in the traditional use of wild plants between rural and urban areas: the sample of Adana. *Studies on Ethno-Medicine*, 9(2), 141-150. https://doi.org/10.1080/09735070.2015.11905430

Al-Quran, S. (2008). Taxonomical and pharmacological survey of therapeutic plants in Jordan. *Journal of Natural Products*, 1(1), 10-26.

Altay, V., & Çelik, O. (2011). Antakya semt pazarlarındaki bazı doğal bitkilerin etnobotanik yönden araştırılması. *Biyoloji Bilimleri Araştırma Dergisi*, 4(2), 137-139

Altay, V., & Karahan, F. (2012). Tayfur Sökmen Kampüsü (Antakya-Hatay) ve çevresinde bulunan bitkiler üzerine etnobotanik bir araştırma. *Karadeniz Fen Bilimleri Dergisi*, 3(2), 13-28.

Altay, V., Karahan, F., Öztürk, M., Hakeem, K. R., Ilhan, E., & Erayman, M. (2016). Molecular and ecological investigations on the wild populations of *Glycyrrhiza* L. taxa distributed in the East Mediterranean area of Turkey. *Journal of Plant Research*, 129(6), 1021-1032. https://doi.org/10.1007/s10265-016-0864-6

Altay, V., Karahan, F., Sarcan, Y.B., & İlçim, A. (2015). An ethnobotanical research on wild plants sold in Kırıkhan district Hatay/Turkey herbalists and local markets. *Biyolojik Çeşitlilik ve Koruma*, 8(2), 81-91.

Altundag, E., & Öztürk, M. (2011). Ethnomedicinal studies on the plant resources of east Anatolia, Turkey. *Procedia-Social and Behavioral Sciences*, 19, 756-777. https://doi.org/10.1016/j.sbspro.2011.05.195

Bağcı, Y., Savran, A., & Dural, H. (2006). Pozantı (Adana) ve çevresindeki bazı bitkilerin yerel adları ve etnobotanik özellikleri. *Selçuk Üniversitesi Fen Fakültesi Dergisi*, 2(27), 77-82.

Benítez, G., González-Tejero, M. R., & Molero-Mesa, J. (2010). Pharmaceutical ethnobotany in the western part of Granada province (southern Spain): Ethnopharmacological synthesis. *Journal of Ethnopharmacology*, 129(1), 87-105.

Bulut, G., & Tuzlaci, E. (2013). An ethnobotanical study of medicinal plants in Turgutlu (Manisa-Turkey). *Journal of Ethnopharmacology*, 149(3), 633-647. https://doi.org/10.1016/j.jep.2013.07.016

Çakılcıoğlu, U., & Türkoğlu, I. (2010). An ethnobotanical survey of medicinal plants in Sivrice (Elazığ-Turkey). *Journal of Ethnopharmacology*, 132(1), 165-175. https://doi.org/10.1016/j.jep.2010.08.017

Cornara, L., La Rocca, A., Marsili, S., & Mariotti, M. G. (2009). Traditional uses of plants in the Eastern Riviera (Liguria, Italy). *Journal of Ethnopharmacology*, 125(1), 16-30.

Davis, P.H. (1965–1988). Flora of Turkey and the East Aegean Islands Vol. 1–10, University Press, Edinburgh

Ekren, E., & Çorbacı, Ö.L. (2021). Etnobotanik kullanım olanakları üzerine bir araştırma: Rize kentsel açık yeşil alanlar örneği. *Journal of Anatolian Environmental and Animal Sciences*, 6(4), 487-497.

Fakir, H., Korkmaz, M., & Güller, B. (2009). Medicinal plant diversity of western Mediterrenean region in Turkey. *Journal of Applied Biological Sciences*, *3*(2), 33-43.

Frezza, C., De Vita, D., Spinaci, G., Sarandrea, M., Venditti, A., & Bianco, A. (2020). Secondary metabolites of Tilia tomentosa Moench inflorescences collected in Central Italy: chemotaxonomy relevance and phytochemical rationale of traditional use. *Natural Product research*, 34(8), 1167-1174.

GC, D.B., Bhandari J., Gautam, D., Jan, H.A., Abbasi, A.M., Bussmann, R.W. and Paniagua-Zambrana N.Y. (2021). *Juglans regia* L. JUGLANDACEAE. In: Kunwar, R.M., Sher, H., Bussmann, R.W. (eds) Ethnobotany of the Himalayas. Ethnobotany of Mountain Regions.

Springer, Cham. pp 1123-1139. https://doi.org/10.1007/978-3-030-57408-6_132

Ghasemi, P.A., Momeni, M., & Bahmani, M. (2013). Ethnobotanical study of medicinal plants used by Kurd tribe in Dehloran and Abdanan districts, Ilam province, Iran. *African Journal of Traditional, Complementary and Alternative Medicines*, 10(2), 368-385.

Guarrera, P.M., Forti, G., & Marignoli, S. (2005). Ethnobotanical and ethnomedicinal uses of plants in the district of Acquapendente (Latium, Central Italy). *Journal of Ethnopharmacology*, 96(3), 429-444.

Güneş, S., Savran, A., Paksoy, M.Y., & Çakılcıoğlu, U. (2018). Survey of wild food plants for human consumption in Karaisalı (Adana-Turkey). *Indian Journal of Traditional Knowledge,* 17(2), 290-298.

Gürdal, B., & Kültür, Ş. (2013). An ethnobotanical study of medicinal plants in Marmaris (Muğla, Turkey). *Journal of Ethnopharmacology*, 146(1), 113-126.

Güzel, Y., Güzelşemme, M., & Miski, M. (2015). Ethnobotany of medicinal plants used in Antakya: a multicultural district in Hatay Province of Turkey. *Journal of Ethnopharmacology*, 174, 118-152. https://doi.org/10.1016/j.jep.2015.07.042

Hardy, K., Radini, A., Buckley, S., Blasco, R., Copeland, L., Burjachs, F., ... & Bermúdez de Castro, J.M. (2017). Diet and environment 1.2 million years ago revealed through analysis of dental calculus from Europe's oldest hominin at Sima del Elefante, Spain. *The Science of Nature*, 104(1), 2. https://doi.org/10.1007/s00114-016-1420-x

Honda, G., Yeşilada, E., Tabata, M., Sezik, E., Fujita, T., Takeda, Y., ... & Tanaka, T. (1996). Traditional medicine in Turkey VI. Folk medicine in West Anatolia: Afyon, Kütahya, Denizli, Muğla, Aydin provinces. *Journal of Ethnopharmacology*, 53(2), 75-87.

İlçim, A. (2014). Hatay'in Sessiz Güzelleri 900 Yabani Çiçek, Hatay Bitki Envanteri. Hatay Valiliği yayınları, Hatay, 996 pp.

Jouad, H., Haloui, M., Rhiouani, H., El Hilaly, J., & Eddouks, M. (2001). Ethnobotanical survey of medicinal plants used for the treatment of diabetes, cardiac and renal diseases in the North centre region of Morocco (Fez–Boulemane). *Journal of Ethnopharmacology*, 77(2-3), 175-182.

Karahan, F. (2022). Evaluation of Trace Element and Heavy Metal Levels of Some Ethnobotanically Important Medicinal Plants Used as Remedies in Southern Turkey in Terms of Human Health Risk. *Biological Trace Element Research*, https://doi.org/10.1007/s12011-022-03299-z

Karahan, F., & İlçim, A. (2017). The potential benefits of medicinal and aromatic plants in cancer patients undergoing radiotherapy. *Biological Diversity and Conservation*, 10(2), 51-61.

Karahan, F., Altay, V., & Keskin, M. (2015). An ethnobotanical study on woody plants benefits from handicrafts in Antakya District (Hatay-Turkey). *International Journal of Scientific and Technological Research*, 1(1), 1-18.

Karahan, F., Ozyigit, I. I., Saracoglu, I. A., Yalcin, I. E., Ozyigit, A. H., & Ilcim, A. (2020). Heavy metal levels and mineral nutrient status in different parts of various medicinal plants collected from eastern Mediterranean region of Turkey. *Biological Trace Element Research*, 197(1), 316-329. https://doi.org/10.1007/s12011-019-01974-2

Karakaya, S., Polat, A., Aksakal, Ö., Sümbüllü, Y. Z., & İncekara, Ü. (2020). Ethnobotanical study of medicinal plants in aziziye district (Erzurum, Turkey). *Turkish Journal of Pharmaceutical Sciences*, 17(2), 211-220. https://doi.org/10.4274%2Ftjps.galenos.2019.24392

Karimi, G., Hosseinzadeh, H., Rassoulzadeh, M., Razavi, B.M. & Taghiabadi, E. (2010). Antinociceptive effect of Elaeagnus angustifolia fruits on sciatic nerve ligated mice. *Iranian Journal of Basic Medical Sciences*, 13, 97-101.

Kendir, G., & Güvenç, A. (2010). Etnobotanik ve Türkiye'de Yapılmış Etnobotanik Çalışmalara Genel Bir Bakış, *Hacettepe Üniversitesi Eczacılık Fakültesi Dergisi*, 1, 49-80.

Kerar, B.A., & Hasan, A. (2019). Aktepe ve Zeytinoba köyleri (hassa/hatay-türkiye) arasında kalan bölgenin florası ve etnobotaniği üzerine bir araştırma. *Bağbahçe Bilim Dergisi*, 6(3), 76-96.

Kocabaş, Y. Z., & Gedik, O. (2016). Kahramanmaraş il merkezi semt pazarlarında satılan bitkiler hakkında etnobotanik araştırmalar. *Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, 6(4), 41-50.

Koçyiğit, M., & Özhatay, N. (2006). Wild plants used as medicinal purpose in Yalova (Northwest Turkey). *Turkish Journal of Pharmaceutical Sciences*, 3 (2), 91-103.

Koleva, V., Dragoeva, A., Nanova, Z., Koynova, T., & Dashev, G. (2015). An ethnobotanical study on current status of some medicinal plants used in Bulgaria. *International Journal of Current Microbiology and Applied Sciences*, 4(4), 297-305.

Korkmaz, M., & Karakurt, E. (2014). Kelkit (Gümüşhane) aktarlarında satılan tıbbi bitkilerin etnobotanik özellikleri. *Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, 18(3), 60-80.

Kültür, Ş., Gürdal, B., Sari, A., & Melikoğlu, G. (2021). Traditional herbal remedies used in kidney diseases in Turkey: an overview. *Turkish Journal of Botany*, 45(4), 269-287. https://doi.org/10.3906/bot-2011-32

Leporatti, M.L., & Guarrera, P.M. (2007). Ethnobotanical remarks in Capitanata and Salento areas (Puglia, southern Italy). *Etnobiología*, 5(1), 51-64.

Lucchetti, L., Zitti, S., & Taffetani, F. (2019). Ethnobotanical uses in the Ancona district (Marche region, Central Italy). *Journal of Ethnobiology and Ethnomedicine*, 15(1), 1-33.

Macía, M.J., García, E., & Vidaurre, P.J. (2005). An ethnobotanical survey of medicinal plants commercialized in the markets of La Paz and El Alto, Bolivia. *Journal of Ethnopharmacology*, 97(2), 337-350.

Malik, K., Ahmad, M., Öztürk, M., Altay, V., Zafar, M., & Sultana, S. (2021). Herbals of Asia-Prevalent Diseases and Their Treatments. Springer International Publishing, Switzerland, 519 pp.

Marinescu, E., Elisei, A.M., Aprotosoaie, A.C., Cioancă, O., Trifan, A., Miron, A., & Hăncianu, M. (2020). Assessment of heavy metals content in some medicinal plants and spices commonly used in Romania. *Farmacia*, 68, 1099-1105.

Metin, A. (2009). Mut ve Çevresinde Yetişen Bitkilerin (Mersin) Etnobotanik Özellikleri. Retrived from https://tez.yok.gov.tr/UlusalTezMerkezi/tezDetay.jsp?id=TrDgY0Kelo4WMHrSFhJaQw&no=V81Ogwmp5iX3STFElhKK8g

Mirdeilami, S.Z., Barani, H., Mazandarani, M., & Heshmati, G.A. (2011). Ethnopharmacological survey of medicinal plants in Maraveh Tappeh region, north of Iran. *Iranian Journal of Plant Physiology,* 2(1), 325-336.

Owfi, R.E. (2021). An Overview of Important Endemic Plants and Their Products in Iran. In: Cooper R, Deakin JJ (eds) Natural Products of Silk Road Plants, CRC Press, 171-200.

Özçelik, H. (2022). Folk physician applications in everyday life in Turkey. *Bio Science Research Bulletin*, 38(1), 1-20.

Özer, H., & Türkmen, N. (2019). Investigation of plants with ethnobotanical use in Gaziantep province (Turkey). *GSC Biological and Pharmaceutical Sciences*, 7(2), 71-78.

Öztürk, M., Altay, V., & Gönenç, T.M. (2017). Herbal from high mountains in the East Mediterranean. In: Bhojraj S et al (eds) Drug Discovery from Herbs-Approaches and Applications. DAYA Publishing House, New Delhi, 327-367.

Öztürk, M., Altay, V., Latiff, A., Asad Ziaee, M., Iqbal Choudhry, M., Shaheen, F., & Durmuşkahya, C. (2018b). A comparative analysis of the medicinal plants used for diabetes mellitus in the traditional medicine in Turkey, Pakistan, and Malaysia. In: Öztürk M, Hakeem KR (eds) Plant and Human Health, Vol. 1, Springer, Switzerland, 409-461.

Öztürk, M., Altay, V., Latiff, A., Shareef, S., Shaheen, F., & Iqbal Choudhry, M. (2018c). Potential medicinal plants used in the hypertension in Turkey, Pakistan, and Malaysia. In: Öztürk M, Hakeem KR (eds) Plant and Human Health, Vol. 1, Springer, Switzerland, 595-618.

Öztürk, M., Altundağ, E., Ibadullayeva, S. J., Altay, V., & Aslanipour, B. (2018a). A comparative analysis of medicinal and aromatic plants used in the traditional medicine of Iğdır (Turkey), Nakhchivan (Azerbaijan), and Tabriz (Iran). *Pakistan Journal of Botany,* 50(1), 337-343.

Öztürk, M., Gücel, S., Altay, V., & Altundağ, E. (2012). Alliums, an underutilized genetic resource in the East Mediterranean. Proc. 6th IS on Edible Alliaceae. *Acta Horticulturae*, 969, 303-309. https://doi.org/10.17660/ActaHortic.2012.969.39.

Özyigit, I.I., Karahan, F., Yalcin, I.E., Hocaoglu-Ozyigit, A., & İlcim, A. (2022). Heavy metals and trace elements detected in the leaves of medicinal plants collected in the southeast part of Turkey. *Arabian Journal of Geosciences* 15, 27. https://doi.org/10.1007/s12517-021-09264-9

Özyiğit, I.I., Yalcin, B., Turan, S., Saracoglu, I.A., Karadeniz, S., Yalcin, I.E., & Demir, G. (2018). Investigation of heavy metal level and mineral nutrient status in widely used medicinal plants' leaves in Turkey: Insights into health implications. *Biological Trace Element Research*, 182(2), 387-406. https://doi.org/10.1007/s12011-017-1070-7

Passalacqua, N.G., Guarrera, P.M., & De Fine, G. (2007). Contribution to the knowledge of the folk plant medicine in Calabria region (Southern Italy). *Fitoterapia*, 78(1), 52-68.

Polat, R., & Satıl, F. (2012). An ethnobotanical survey of medicinal plants in Edremit Gulf (Balıkesir–Turkey). *Journal of Ethnopharmacology*, 139(2), 626-641. https://doi.org/10.1016/j.jep.2011.12.004

Saday, H. (2009). Güzeloluk Köyü ve Çevresinin (Erdemli/Mersin) Etnobotanik Özellikleri. Retrived from https://tez.yok.gov.tr/UlusalTezMerkezi/tezSorguSonucYeni.jsp

Sağıroğlu, M., Topuz, T., Ceylan, K., & Turna, M. (2013). An ethnobotanical survey from Yahyalı (Kayseri) And Tarsus (Mersin). *SAÜ Fen Edebiyat Dergisi*, (2013-II), 13-37.

Saleem, S., Khan, R., Kazmi, I., & Afzal, M. (2019). Medicinal plants in the treatment of arthritis. In: Öztürk, M., Hakeem, KR (eds) Plant and Human Health, Vol. 3 Springer, Switzerland, 101-137.

Sargın, S.A. (2015). Ethnobotanical survey of medicinal plants in Bozyazı district of Mersin, Turkey. *Journal of Ethnopharmacology*, 173, 105-126. https://doi.org/10.1016/j.jep.2015.07.009

Sargın, S.A. (2019). Mersin'in Bozyazı ilçesinde gıda olarak tüketilen yabani bitkiler. *Yüzüncü Yıl Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, 24(3), 152-169.

Sargın, S.A., Akçiçek, E., & Selvi, S. (2013). An ethnobotanical study of medicinal plants used by the local people of Alaşehir (Manisa) in Turkey. *Journal of Ethnopharmacology*, 150(3), 860-874. https://doi.org/10.1016/j.jep.2013.09.040

Şenkardeş, İ., & Tuzlacı, E. (2014). Some ethnobotanical notes from Gündoğmuş district (Antalya/Turkey). *Clinical and Experimental Health Sciences*, 4(2), 63-75.

Sharopov, F., & Setzer, W.N. (2018). Medicinal plants of Tajikistan. In: Egamberdieva K, Öztürk M (eds) Vegetation of Central Asia and Environs. Springer, Switzerland, 163-209.

Terzioğlu, S., & Coşkunçelebi, K. (2021). Medicinal plants of Northeast Anatolia. In: Öztürk M, Altay V, Efe R (eds) Biodiversity, conservation and sustainability in Asia, Springer, Switzerland, 275–337.

Topuz, M. (2016). Trend analysis of precipitation in Hatay. *Journal of International Social Research*, 9, 1186–1186.

Ünver, A. (2019). Lamas Çayı Çevresindeki Köylerde (Erdemli, Silifke/Mersin) Etnobotanik Araştırmalar. Retrived from https://tez.yok.gov.tr/UlusalTezMerkezi/tezSorguSonucYeni.jsp

Yeşil, Y., Çelik, M., & Yılmaz, B. (2019). Wild edible plants in Yeşilli (Mardin-Turkey), a multicultural area. *Journal of Ethnobiology and Ethnomedicine*, 15, 52. https://doi.org/10.1186/s13002-019-0327-y.

Yesilada, E. (2013). An overview of Turkish folk medicine; past and present. *Current Drug Delivery*, 10(1), 92-95.

Zaurov, D.E., Belolipov, I.V., Kurmukov, A.G., Sodombekov, I.S., Akimaliev, A.A., & Eisenman, S.W. (2013). The medicinal plants of Uzbekistan and Kyrgyzstan. In: Eisenman, S., Zaurov, D., Struwe, L. (eds) Medicinal Plants of Central Asia: Uzbekistan and Kyrgyzstan, Springer, New York, 15-273.

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Research Article/Araştırma Makalesi

Breeding Biology of the Yellow-legged Gull (*Larus michahellis*, Naumann, 1840): a Small Island Population in Southwestern Türkiye

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Abstract: Urbanization and fisheries affected a rapid increase in seagull populations in western Mediterranean populations of the yellow-legged gull. The main reason is the increased food resources such as fisheries, big ports, and dumps. In this study, we aimed to understand and to compare the breeding biology of the yellow-legged gull in eastern Mediterranean. We studied on the small island in southwestern Türkiye between 2013 and 2015. All nesting sites were determined, marked, and monitored once a week. The breeding season has started in late February, nesting and laying eggs have started at the beginning of March, and they left the island in the second half of June after fledging. The incubation period is 29.17±2.85 days, only one clutch in a season and 2 or 3 eggs were laid in the nests. There was a slight increase in the number of pairs and nests between years but there is no significant difference in all parameters such as laying date, clutch size, hatching and fledging success in Pırasalı Island population and the rate of both hatching and fledging success are lower than western Mediterranean populations. While predation is the weakest factor for lower breeding success, intraspecific predation may be the factor but the main effect is the lower food abundance for the hatchlings.

Keywords: Breeding, fledgling, food, nesting, yellow-legged gull.

Türkiye'nin Güneybatısında Küçük Bir Ada Populasyonu örneğinde Gümüş Martı *(Larus michahellis*, Naumann, 1840) Üreme Biyolojisi

Öz: Kentleşme ve balıkçılık Gümüş martı populasyonlarının Batı Akdeniz'de hızla artmasında etkili olmuştur. Balıkçılık, büyük limanlar ve çöplükler gibi artan gıda kaynakları bu artışın ana sebepleridir. Bu çalışmada, Doğu Akdeniz'de kuluçkaya yatan Gümüş martı üreme biyolojisini belirlemek ve karşılaştırma yapmak amaçlanmıştır. Türkiye'nin güneybatısında yer alan küçük bir ada popülasyonu 2013-2015 yıllarında çalışılmıştır. Tüm yuva alanları tespit edilmiş, işaretlenmiş ve haftada bir gün izlenmiştir. Üreme dönemi Şubat ayı sonlarında başlamış, yuva yapımı ve yumurta bırakma mart ayı başlarında gerçekleşmiş ve yavruların uçmasıyla birlikte tüm popülasyon Haziran ayının ikinci yarısında adadan ayrılmıştır. Kuluçka süresi 29.17±2.85 gün, üreme döneminde sadece bir kuluçka gerçekleştirilmiş ve yuvalara 2 veya 3 yumurta bırakılmıştır. Pırasalı Adası popülasyonunda yıllar arasında çift sayıları ve yuva sayıları açısından küçük bir artış olmasına rağmen, yumurta bırakma tarihi, kuluçka sayısı, yumurtadan çıkan yavru başarısı ve yuvadan uçan yavru başarısı parametrelerinde istatistiksel anlamda bir farklılık bulunmamıştır. Yumurtadan çıkan yavru başarısı ve yuvadan uçan yavru başarısı oranları Batı Akdeniz popülasyonlarından daha düşüktür. Predasyon düşük üreme başarısı için düşük bir faktörken, tür içi predasyon belki etkili olabilir, ancak yumurtadan çıkan yavrular için düşük besin bolluğu temel etkidir.

Anahtar kelimeler: Üreme başarısı, yavru, besin, kuluçka, Gümüşi martı.

1. Introduction

The Yellow-legged gull (*Larus michahellis*) is one of the common species and widely distributed throughout the Mediterranean (Vidal et al., 1998; Bosch et al., 2000). It is the most common gull species in the Mediterranean, Marmara Sea, and Black Sea in Türkiye (Kiziroğlu, 2009; Svensson et al., 2009). The Yellow-legged gull is a large sized gull (800-1500 g) among other species (Liebers et al., 2001) nesting in colonies on rocks or cliffs (Cramp, 1998).

In the last 30-40 years, there has been a great increase in seagull populations in Europe, North America and Australia due to the increasing food abundance as a result of human activities (Blokpoel and Spans, 1991; Pons, 1992; Smith and Carlile, 1993). Similar increases were observed in the populations of the Yellow-legged gull in the Mediterranean (Vidal et al., 1998). However, with this increase, negative effects have emerged in airports, cities, reservoirs, agricultural areas, and fisheries (Monaghan et al., 1995; Dolbeer et al., 1997).

The availability of food is a determining factor in the reproductive success and population dynamics of the species (Oro et al., 2006). In recent years, increasing the amount of waste with the human population has caused an increase in the population of the Yellow-legged gull (Pons, 1992; Kilpi and Öst, 1998). This situation has been influential to conduct numerous studies on the population dynamics, diet, and reproductive biology in the Mediterranean basin (Bosch et al., 2000; Perez et al., 2006; Arizaga et al., 2008; Ramos et al., 2009). Studies have been conducted on the breeding of the Herring gull (*Larus argentatus*) (Ayvaz, 1988) and heavy metal concentration in the eggshells of Audouin's gull (*Larus audouinii*) (Ayaş,

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Çelikkan and Aksu, 2008) but no comprehensive studies have been found in Türkiye, except for the distribution areas with the Yellow-legged gull: although, it is one of the most common seagulls (Tabur, 2002; Tabur and Ayvaz, 2005; Ekmekçi, 2011). We aimed with this research to understand the breeding biology such as incubation phenology, clutch size, hatching, and fledging success of the Yellow-legged gull population breeding on the small island in the Mediterranean coast of Southwestern Türkiye.

2. Material and Methods

This study was carried out during the breeding season (March-July) in 2013, 2014, and 2015. The field work was started at the beginning of March to determine the breeding biology of the Yellow-legged gull and research was carried out once a week until the end of July.

2.1. Study Area

The study area, Pırasalı Island, is a small rocky island about 1.5-2 ha, located 3.5 miles from the coast of Adrasan and 250 m from the mainland (Fig. 1). Pırasalı Island is located within the Beydağları Olympos National Park.

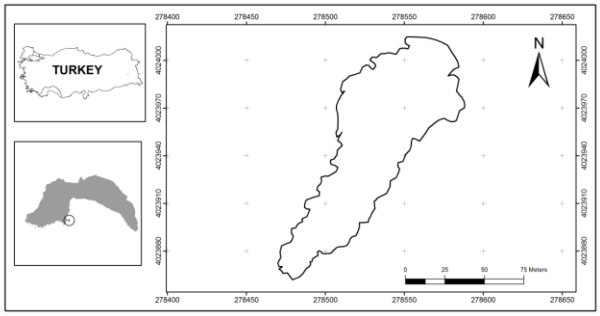

Figure 1. The location of the study area, Pırasalı Island.

2.2. Nest Monitoring

Nest monitoring studies started at the beginning of March each year and nest locations were determined. Nests are built on the ground between rocks and were easy to find by observing. Coordinates were recorded by using GPS (Garmin etrex 20) and by using small wood pieces marked and left close to the nests (Fig. 2). Nest building, laying dates, incubation period, and hatching dates were recorded by checking the nests once a week. Distribution maps of the nest locations on the island were prepared for each year.

Figure 2. One of the marked nests of the Yellow-legged gull on the island.

2.3. Statistical Analysis

The incubation period was calculated by using the data between the first hatching and the last laying egg dates. Hatching success was calculated by the proportion of the clutch that hatched and fledgling success was calculated by the proportion of the fledglings that left the island. The differences of egg number, hatchlings, fledglings, and dead hatchlings between years were tested by using one-way ANOVA (Dytham, 2011).

3. Results

In this study, 26 nests in 2013, 34 nests in 2014, and 38 nests in 2015 were found and monitored during the breeding seasons. The fact that the island is small and has very little vegetation provided the opportunity to determine the entire population nests on the island.

3.1. Nest Distribution and Breeding Phenology

The nests are almost located in the northern part where there is a small number of annual vegetation: although, the whole island is rocky. Only one nest was found in the southern part in 2014 and 2015 (Fig. 3). Nests were mostly built on this poor vegetation, with very few found to be built cliffs with few nesting materials, and in these nests eggs were laid directly on the rock. Although it is very difficult to tell whether the same pairs used the same nesting sites in subsequent years, it was evaluated that the pairs mostly used the old nesting sites (Fig. 3).

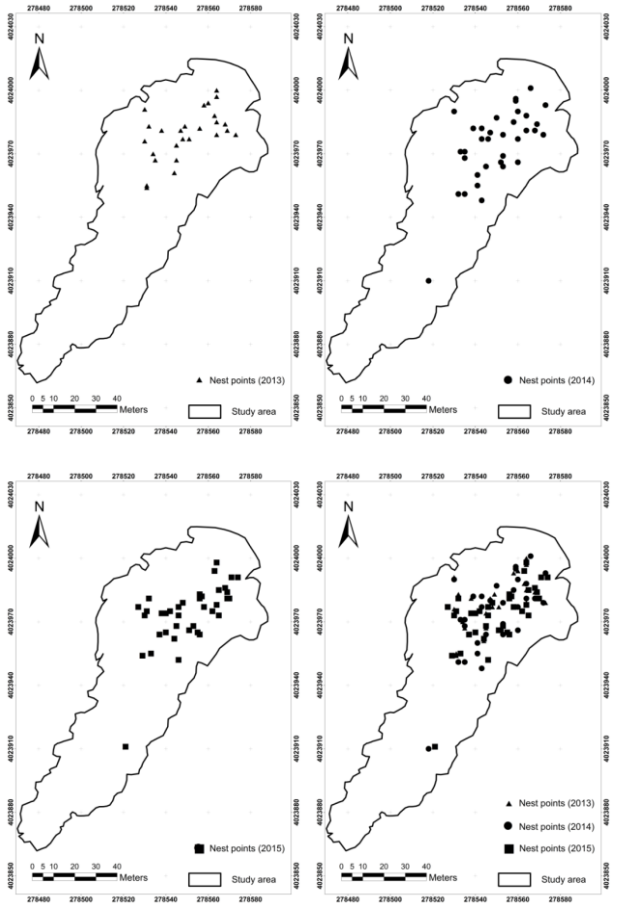

Figure 3. Distribution maps of all nests by year (Upper left 2013, upper right 2014, down left 2015 and downright all years).

The Yellow-legged gulls started to arrive the island in late February and started to lay eggs from the beginning of March. Laying the first egg in the nest was on 10th March (n=17, med. 15th March) in 2013, on 11th March (n=22, med. 21st March) in 2014, and on 11th March (n=25, med. 17th March) in 2015, respectively (Fig. 4). Hatching started on 13th April (n=17, med. 18th April) in 2013, on 12th April (n=22, med. 23rd April) in 2014, and on 13th April (n=25, med. 18th April) in 2015, respectively (Fig. 4). The breeding density in the colony was one pair within about 10-20 m^2 surface area with varying nesting locations ranging from 5 meters above sea level up to about 20 m.

3.2. Incubation Period and Breeding Success

The Yellow-legged gull has a single brood during the breeding season. There were unsuccessful broods in all the studied years: 9 nests in 2013, 12 nests in 2014, and 13 nests in 2015, respectively. These unsuccessful broods were excluded for the calculation of the incubation period and it is 29.17±2.85 days (n=64 nests) (Table 1). According to the age of hatchlings, the hatching dates were estimated. Although 2 or 3 eggs were laid in the nests, all of these eggs were not hatched. Three hatchlings were observed in only two 3-egg nests but one of the hatchlings died in a very short time in both nests. In addition, there was no statistically significant difference between years in terms of incubation period, brood length, number of eggs laid, hatchlings, dead hatchlings, hatchling success, fledglings, and fledgling success (Table 2).

4. Discussion

This study was carried out in 2013-2015 to investigate the breeding biology of the Yellow-legged gull (*Larus michahellis*) population on Pırasalı Island in southwestern Türkiye. About the breeding population on Kızkalesi, which is an ancient building, in Beyşehir Lake, Ekmekçi (2011) identified 270 individuals and 38 nests, 25 of which were active. Studies conducted on different populations in Spain (Ramos et al., 2009) and Italy (Rubolini et al., 2005) indicate that almost all of the pairs in the colony were nesting (between 300 and 1500 pairs). In this study, all observed adult birds in all seasons were incubating at the island. There were also non-breed individuals but these few individuals were immatures. Ekmekçi (2011) did not give information in detail about the number and proportions of immature and adult birds. However, in our study nesting of 30% individuals is a very low rate compared to other populations (Rubolini et al., 2005; Ramos et al., 2009).

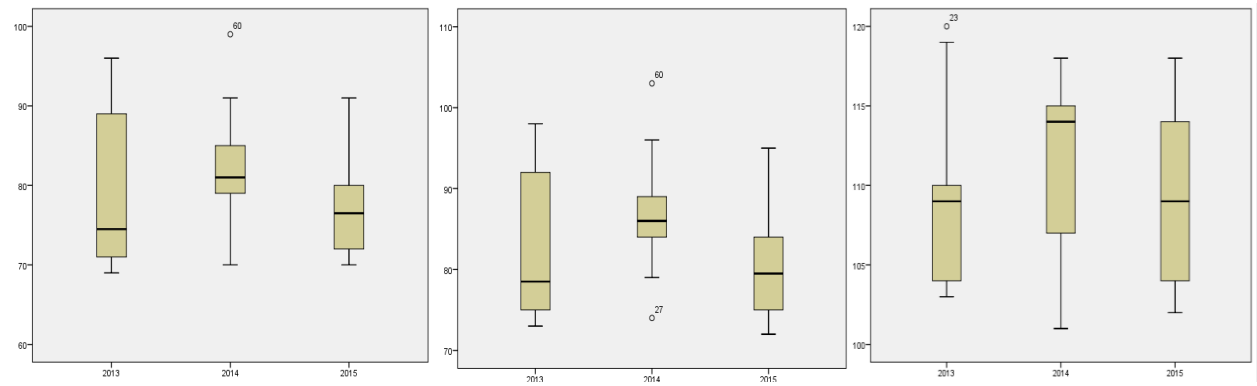

Figure 4. According to the years, the date distribution of laying first egg (left), laying last egg (middle), and hatching days (right). X axis shows the years and y axis shows the Julian date.

Table 1. Incubation time, number of eggs, number of hatchlings, number of fledglings, and death hatchlings of the yellow-legged gull according to the years.

		2013	2014	2015	3-years Mean
Incubation Period (day)	N	17	22	25	21.3
	Mean±SD	29.71±2.89	26.82±2.40	30.88±1.56	29.17±2.85
	Min-Max	25-35	23-33	29-34	23-35
Number of eggs	N	17	22	25	21.3
	Mean±SD	2.81±0.40	2.82±0.39	2.79±0.41	2.81±0.40
	Min-Max	2-3	2-3	2-3	2-3
Number of Hatchlings	N	35	41	47	41
	Mean±SD	1.35±1.06	1.21±1.12	1.24±1.02	1.26±1.06
	Min-Max	0-3	0-3	0-3	0-3
Number of Fledglings	N	26	29	34	29.6
	Mean±SD	1.00±0.94	0.85±1.05	0.90±0.80	0.91±0.92
	Min-Max	0-3	0-3	0-2	0-3
Number of Death Hatchlings	N	9	12	10	10.3
	Mean±SD	0.39±0.64	0.35±0.65	0.26±0.45	0.33±0.57
	Min-Max	0-2	0-2	0-1	0-2
Hatching Success		47.9%	42.7%	44.3%	44.7%
Fledging Success		35.9%	30.2%	32.7%	32.4%

Table 2. Differences between years about incubation time, number of laid eggs, hatchlings, fledglings, death hatchlings, hatchling success, and fledgling success.

	Years	Clutch size	N	Mean ± SD	ANOVA (between years)	P
Number of egg	2013	26	73	2.81±0.40		
	2014	34	96	2.82±0.39	0.937	>0.05
	2015	38	106	2.79±0.41		
Number of hatchling	2013	26	35	1.35±1.06		
	2014	34	41	1.21±1.12	0.873	>0.05
	2015	38	47	1.24±1.03		
Number of fledgling	2013	26	26	1.00±0.94		
	2014	34	29	0.85±1.05	0.826	>0.05
	2015	38	34	0.90±0.80		
Number of death hatchling	2013	26	9	0.39±0.64		
	2014	34	12	0.35±0.65	0.671	>0.05
	2015	38	10	0.26±0.45		

In Türkiye, there are not enough studies on the population biology of not only yellow-legged gull but also of many species. However, the populations of this species have increased in recent years (Bosch et al., 2000; Rubolini et al., 2005; Ramos et al., 2009; Arizaga et al., 2010). Although there are no statistically significant differences between years in Pırasalı Island population (see Table 2), there is a slight increase in the number of pairs and nests during the breeding season. Breeding data for Pırasalı Island population such as nest building, laying egg, and hatching dates are similar to other breeding populations in the Mediterranean (Bosch et al., 2000; Arizaga et al., 2010; Rubolini et al., 2011). Although the clutch size is 2 or 3, mostly 1 or 2 eggs were hatched in this study. Only in two nests all three eggs were hatched but one of these hatchlings died in a few days in both nests. According to Arizaga et al. (2010), the first hatchlings have an advantage over the others and the surviving offspring are generally these first hatchlings. Similarly, Perez et al. (2006) found out that hatchings from the first and second eggs have higher survival rate than third eggs. We did not mark and ring the hatchlings: thus, it is not possible to say the advantage of hatching priority.

Food competition and predation are important factors affecting hatching and fledging success. The survival rate of offspring is affected by food abundance, food competition, and predation (Arizaga et al., 2010). In our results, hatching and fledging success (see Table 1) are lower than western Mediterranean populations (between 60% and 65%) (Bosch et al., 2000; Rubolini et al., 2005; Ramos et al., 2009; Arizaga et al., 2010). Intraspecific predation might affect the hatching and fledging success (Martinez-Abrain et al., 2002). A small population of 40 pairs of our colony has also hatchlings mortality. We do not have any observation of intraspecific attacks but according to Arizaga et al. (2010), intraspecific predation may also cause these deaths in our population, too. However, there is not a big port and fisheries around the study area and this may be the most important factor as a low amount of nutrients resulting in having low hatching and fledging success. Gulls use pelagic prey, refuse dumps, brackish, and freshwater ecosystems to feed their offspring (Ramos et al., 2009) and big ports and fisheries provide abundant food (Bosch et al., 1994; Belant, 1997), the Yellow-legged gull interactions rate is very high (90.5%) in many aquaculture in Aegean Sea (Ceyhan and Akyol, 2020) and this adequate amount of food gives advantage to the survival rate of the hatchlings (Duhem et al., 2007). Moreover, the dumps also provide one of the best food abundance for many species including gulls. There is not any dump close to the breeding site. On the other hand, some predators such as Peregrine falcon (*Falco peregrinus*) and Eleonora's falcon (*Falco eleonorae*) breed on the cliffs of the mainland (Karaardıç, 2020) and many attacks (some of them successful) were observed against the Yellow-legged gulls and breeding population of Alpine swifts (*Apus melba*) (Karaardıç et al., 2013; Meier et al., 2018; Meier et al., 2020) on the island. Nevertheless, observed dead hatchlings mostly were not predated. This may help to explain the reason of the death as the low food abundance may decrease both hatching and fledging success.

5. Conclusion

The increase in urbanization and fisheries affected to a rapid increase in seagull populations in Europe, North America, and Australia due to the increasing food abundance as a result of human activities. Similar increases were observed in the populations of the Yellow-legged gull in the western Mediterranean, the reasons of food resources such as low fisheries, dumps, and others does not affect having the same increase in eastern Mediterranean populations. In Aegean Sea, Marmara Sea, and Black Sea regions, although the individual numbers of seagulls have increased, it cannot be expressed that there is a similar increase due to the lack of information on reproductive biology of the Yellow-legged gulls. However, according to this study, the population size is stable between years in the southwestern coastal breeding populations in Türkiye.

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References

Arizaga, J., Aldalur, A., Herrero, A., & Galicia, D. (2008). Sex differentiation of Yellow-legged Gull (*Larus michahellis lusitanius*): the use of Biometrics, bill morphometrics and wing tip coloration. *Waterbirds*, 31 (2), 211-219. https://doi.org/10.1675/1524-4695(2008)31[211:SDOYGL]2.0.CO;2

Arizaga, J., Herrero, A., Galarza, A., Hidalgo, J., Aldalur, A., Cuadrado, J.F., & Ocio, G. (2010). First-year movements of Yellow-legged Gull (*Larus michahellis lusitanius*) from the Southeastern Bay of Biscay. *Waterbirds*, 33 (4), 444-450. https://doi.org/10.1675/063.033.0403

Ayaş, Z., Çelikkan, H., & Aksu, M.L. (2008). Lead and Cupper concentration in the egg shells of Audouin's gull (*Larus audouinii*) in Türkiye. *Turkish Journal of Zoology*, 32 (4), 379-384. https://journals.tubitak.gov.tr/zoology/vol32/iss4/3/

Ayvaz, Y. (1988). The breeding of the herring gull (*Larus argentatus*). *Journal of Fırat University*, 3, 53-60.

Belant, J.L. (1997). Gulls in urban environments: landscape-level management to reduce conflict. *Landscape and Urban Planning*, 38, 245-258. https://doi.org/10.1016/S0169-2046(97)00037-6

Blokpoel, H., & Spaans, L. (1991). Superabundance in gulls: causes, problems and solutions. Acta XX Congressus Internationalis Ornithologici (ed. BD Bell), pp 2361-2364.

Bosch, M., Oro, D., & Ruiz, X. (1994). Dependence of yellow-legged Gulls (*Larus cachinnans*) on food from human activity in two western Mediterranean colonies. *Avocetta*, 18, 135-139.

Bosch, M., Oro, D., Cantos, F.J., & Zabala, M. (2000). Short-term effects of culling on the ecology and the population dynamics of the Yellow-legged gull. *Journal of Applied Ecology*, 37, 369-385.

Ceyhan, T. & Akyol, O. (2020). Some interactions between coastal fisheries and sea birds in Aegean Sea. *Ege Journal of Fisheries and Aquatic Sciences*, 37(2), 139-147. https://doi.org/10.12714/egejfas.37.2.04

Cramp, S. (1998). The complete birds of the western Palearctic on CD-Rom. Oxford Uni. Press, Oxford.

Dolbeer, R.A., Belant, J.L., & Bernhardt, G.E. (1997). Aerial photography techniques to estimate populations of Laughing Gull nests in Jamaica Bay, New York, 1992-1995. *Colonial Waterbirds*, 20(1), 8-13. https://doi.org/10.2307/1521758

Duhem, C., Roche, P., Vidal, E., & Tatoni, T. (2007). Effects of antropogenic food resources on yellow-legged gull colony size on mediterranean islands. *Population Ecology*, 50, 91-100.

Dytham, C. (2011). Choosing and using statistics: a biologist's guide. London, Wiley & Sons.

Ekmekci, A.İ. (2011). Bio-ecology of Yellow-legged gull (*Larus cachinnans*) population on Kızkalesi Island (Beyşehir Lake, Isparta). Retrieved from https://tez.yok.gov.tr/UlusalTezMerkezi/tezSorguSonucYeni.jsp

Karaardıç, H., Köse, F., & Kocakahya, V. (2013). Pırasalı Island: Paradise of Alpine swift (*Apus melba*), Adrasan, Kumluca, Antalya. *Nature and Man, June* 2013, 3-10.

Karaardıç, H. (2020). New breeding site of Eleonora's falcon (*Falco eleonorae*) and Peregrine falcon (*Falco peregrinus*) in Southern Türkiye. Adıyaman University Journal of Science, 10(1) 22-28. https://doi.org/10.37094/adyujsci.684417

Kilpi, M. & Öst, M. (1998). Reduced availability of refuse and breeding output in a herring gull (*Larus argentatus*) colony. *Annales Zoologici Fennici*, 35(1), 37-42. https://www.jstor.org/stable/23735584

Kiziroğlu, İ. (2009). Birds of Türkiye. Ankamat matbaası, Ankara, 564 pp.

Liebers, D., Helbig, A.J., & De Knijff, P. (2001). Genetic differentiation and phylogeography of gulls in the *Larus cachinnans-fuscus* group (Aves, Charadriiformes). *Mol. Ecol*. 10, 2447-2462.

Martinez-Abrain, A., Gonzales-Solis, J., Pedrocchi, V., Genovart, M., Abella, J.C., Ruiz, X., …, & Oro, D. (2003). Kleptoparasitism, disturbance and predation of yellow-legged gulls in three colonies of the western *Mediterranean Scientia Marina*, 67, 89-94. https://doi.org/10.3989/scimar.2003.67s289

Meier, C.M., Karaardıç, H., Aymi, R., Peev, S.G., Baechler, E., Weber, R., …, & Liechti, F. (2018). What makes Alpine swift ascend at twilight? Novel geolocators reveal year-round flight behaviour. *Behavioral Ecology and Sociobiology*, 72-45. https://doi.org/10.1007/s00265-017-2438-6

Meier, C.M., Karaardıç, H., Aymi, R., Peev, S.G., Witvliet, W., & Liechti, F. (2020). Population-specific adjustment of the annual cycle in a super-swift trans-Saharan migrant. *Journal of Avian Biology*, e02515. https://doi.org/10.1111/jav.02515

Monaghan, P., Shedden, C.D., Ensor, K., Fricker, C.R., & Girdwood, R.W.A. (1985). Salmonella carriage by herring gulls in the Clyde area of Scotland in relation to their feeding ecology. Journal of Applied Ecology, 22, 669-680. https://doi.org/10.2307/2403220

Perez, C., Velando, A., & Dominguez, J. (2006). Parental food conditions affect sex-specific embryo mortality in the Yellow-legged Gull (*Larus michahellis*). *Journal of Ornithology*, 147, 513-519. https://doi.org/10.1007/s10336-006-0074-4

Pons, J.M. (1992). Effects of changes in the availability of human refuse on breeding parameters in a herring gull *Larus argentatus* population in Brittany, France. *Ardea*, 80, 143-150. https://avibirds.com/wp-content/uploads/pdf/zilvermeeuw7.pdf

Ramos, R., Tamirez, F., Sanpera, C., Jover, L., & Ruiz, X. (2009). Diet of yellow-legged gull (*Larus michahellis*) chicks along the Spanish western Mediterranean coast: The relevance of the refuse dumps. *Journal of Ornithology*, 150, 265-272. https://doi.org/10.1007/s10336-008-0346-2

Rubolini, D., Romano, M., Boncoraglio, G., Ferrari, R.P., Martinelli, R., Galeotti, P, …, & Saino, N. (2005). Effects of elevated egg corticosterone levels on behavior, growth and immunity of Yellow-legged Gull (*Larus michahellis*) chicks. *Hormones and Behavior*, 47, 592-605. https://doi.org/10.1016/j.yhbeh.2005.01.006

Rubolini, D., Romano, M., Navara, K.J., Karadas, F., Ambrosini, R., Caprioli, M., & Saino, N. (2011). Maternal effects mediated by egg quality in the Yellow-legged Gull Larus michahellis in relation to laying order and embryo sex. *Frontiers in Zoology*, 8, 24-38. https://doi.org/10.1186/1742-9994-8-24

Oro, D., Martinez-Abrain, A., Paracuellos, M., Nevado, J.C., & Genovart, M. (2006). Influence of density dependence on predator-prey seabird interactions at large spatio-temporal scales. Proceeding of The Royal Society B, 273, 379-383. https://doi.org/10.1098/rspb.2005.3287

Smith, G.C., & Carlile, N. (1993). Methods for population control within a silver gull colony. *Wildlife Research*, 20, 219-226. https://doi.org/10.1071/WR9930219

Svensson, L., Mullarney, K., Zetterström, D., & Grant, P.J. (2009). Collins Bird Guide. Collins, 448 s.

Tabur, M.A. (2002). Göller Bölgesi (Gölhisar Gölü, Burdur Gölü, Eğirdir Gölü, Kovada Gölü, Beyşehir Gölü) kuşlarının biyoekolojisi. Retrieved from https://tez.yok.gov.tr/UlusalTezMerkezi/tezSorguSonucYeni.jsp

Tabur, M.A. & Ayvaz, Y. (2005). Birds of lake Beyşehir (Isparta-Konya). *Turkish Journal of Zoology*, 29 (4), 361-369. https://journals.tubitak.gov.tr/zoology/vol29/iss4/12

Vidal, E., Medail, F., & Tatoni, T. (1998). Is the yellow-legged gull a superabundant bird species in the Mediterranean? Impact on fauna and flora, conservation measures and research priorities. *Biodiversity and Conservation*, 7, 1013-1026. https://doi.org/10.1023/A:1008805030578

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A New Record for Spider Fauna of Cyprus: *Porrhoclubiona genevensis* (L. Koch, 1866) (Araneae, Clubionidae)

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Abstract: In this study, *Porrhoclubiona genevensis* is reported as a new record for the spider fauna of Cyprus based on the male and female individuals collected from the northern part of Cyprus. In the article, the general appearance and photos of the copulatory organs of the species are given and the morphological resemblance of *P. genevensis* with *P. vegeta* (Simon, 1918), which is distributed on the island, is also discussed.

Keywords: Cymbial setae, distribution, Mediterranean, sac spiders.

Kıbrıs'ın Örümcek Faunası İçin Yeni Bir Kayıt: *Porrhoclubiona genevensis* (L. Koch, 1866) (Araneae, Clubionidae)

Öz: Bu çalışmada Kıbrıs adasının kuzeyinden toplanan erkek ve dişi bireyler üzerinden *Porrhoclubiona genevensis* türü, Kıbrıs'ın örümcek faunası için yeni bir kayıt olarak rapor edilmektedir. Makale içerisinde türün genel görünüş ile eşeysel organlarının fotoğrafları verilmiş ve ayrıca *P. genevensis* türünün adada dağılım gösteren *P. vegeta* (Simon, 1918) ile olan yapısal benzerliği de tartışılmıştır.

Anahtar kelimeler: Simbiyal kıllar, dağılım, Akdeniz, kese örümcekleri.

Kese örümcekleri olarak anılan Clubionidae familyası üyeleri; yoğun perçemlerle donanmış bir çift tarsal tırnaklı, küçük ve orta boylu araneomorf örümceklerdir. Serbest yaşayıp geceleri avlanan kese örümcekleri gündüzleri genelde bitki yapraklarının üzerlerine ördükleri kese şeklindeki ağdan sığınaklarından dolayı bu isimle anılmaktadırlar. Bu familya üyeleri entelejin ve ekribellat olup dört çift gözleri vardır (Jocqué & Dippenaar-Schoeman, 2007).

Clubionidae familyasının dünya genelinde dağılım gösteren 19 cinse ait 662 yaşayan türü bulunmaktadır. Bunlardan 11'i, yakın zamana kadar *Clubiona* cinsinin *genevensis* tür grubu altında değerlendirilen *Porrhoclubiona* Lohmander 1944 cinsine aittir (WSC, 2022).

Porrhoclubiona, erkek palplerinin yumurtamsı şekli ve simbiyumlarının retrolateralinde yer alan koyu renkli özelleşmiş kıllar; dişilerinin ise birisi kitinize bir diğeri hiyalinimsi yuvarlak reseptakulumları ve geniş atriyumlu vulvaları dolayısıyla Marusik ve Omelko (2018) tarafından cins seviyesine yükseltilmiştir.

Bu kısa makalenin amacı son yıllarda araknolojik çalışmaların hız kazandığı Kıbrıs'tan *Porrhoclubiona genevensis* türü kese örümceğinin yeni bir tür kaydını vermektir. Makale içerisinde ayrıca türe ait genel görünüş ile taksonomik karakterleri olan erkek ve dişi üreme organlarının yapısını ortaya koyan fotoğraflar verilmiştir.

Çalışmada değerlendirilen örnekler, Kıbrıs'ın kuzeyindeki Girne ilçesinde yer alan Lapta kasabasından el aspiratörü vasıtasıyla toplanmıştır. Tür teşhisi Leica M125 marka stereomikroskop vasıtası ile yapılmış; betimlemede kullanılan vücut ölçümlerinde, üreme organlarının kısımlarının isimlendirilmesinde ve tür teşhisinde Bosmans et al. (2017), Danışman et al. (2018) ve Marusik ve Omelko (2018) kaynak alınmıştır. Örnekler %70'lik etil alkol içerisinde CHNM (Kıbrıs Herbaryum ve Doğa Tarihi Müzesi, Yakın Doğu Üniversitesi, Lefkoşa)'da saklanmaktadır. Metin içerisinde verilen tüm rakamsal birimler milimetre cinsindendir.

Familya: Clubionidae

Cins: *Porrhoclubiona* Lohmander, 1944

Porrhoclubiona genevensis (L. Koch, 1866) (Şekil 1-3)

İncelenen örnekler. 3 ♂♂, 5 ♀♀, Kıbrıs, Girne, Lapta (35°20'19.00"K, 33°10'51.00"D), 18 Kasım 2017, toplayan Ö. Özden.

Vücut Ölçümleri (♂ / ♀). Toplam uzunluk 4.40-5.90 Karapaks uzunluğu 2.00-1.90, genişliği 1.50-1.40 Abdomen uzunluğu 2.40-4.00, genişliği 1.40-2.30, göz bölgesi uzunluğu 0.90-0.85; Keliser uzunluğu 1.10-0.85, genişliği 0.55-0.45 Sternumun uzunluğu 1.00-1.00, genişliği 0.80-0.70.

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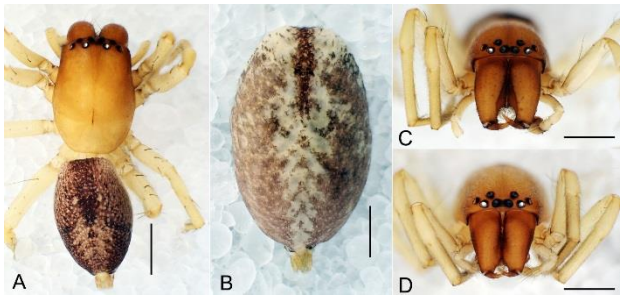
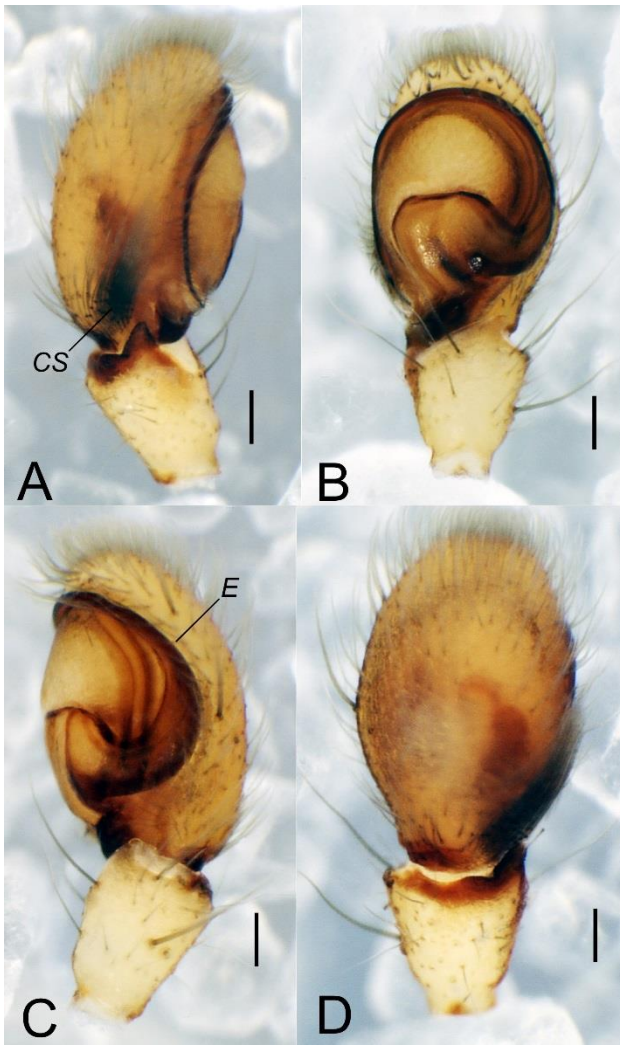


Şekil 1. *P. genevensis*. A. Genel görünüş, erkek, B. Abdomen, dişi, C ve D. Ön vücut, sırasıyla erkek ve dişi. Ölçüm çizgileri: 1 mm.

Figure 1. *P. genevensis*. A. Habitus, male, B. Abdomen, female, C and D. Prosoma, male and female respectively. Scale lines: 1 mm.

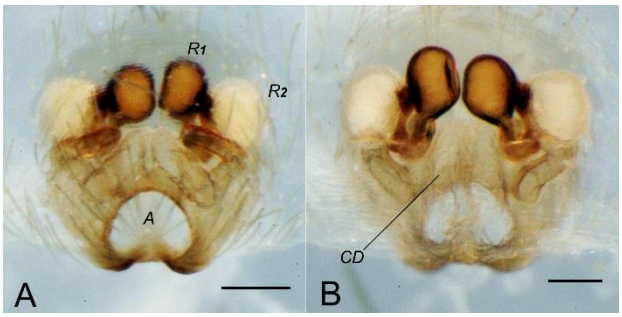


Şekil 2. *P. genevensis*, erkek palpi. A. retrolateral görünüş, B. ventral görünüş, C. prolateral görünüş, D. dorsal görünüş. Kısaltmalar: *CS*, Simbiyal kıllar *E*, Embolus. Ölçüm çizgileri: 0.1 mm.

Figure 2. *P. genevensis*, male palp. A. retrolateral view, B. ventral view, C. prolateral view, D. dorsal view. Abbreviations: *CS*, Cymbial setae *E*, Embolus. Scale lines: 0.1 mm.

Betimleme. Erkek ve dişi bireyler arasında genel görünüş ve renklenme bakımından önemli bir farklılık bulunmamaktadır. Karapaks kahverengi. Göz bölgesi ile göğüs bölgesi arasında belirgin bir ton farkı var. Göğüs çukuru (fovea) belirgin, kahverengi (Şekil 1A). Keliserler, labium ve gnathokoksa kahverengi. Bunların içerisinde labium ton olarak en koyu olanı. Gnathokoksanın labiuma ve palpal koksaya bakan kenarları kuvvetlice kitinize. Sternum sarımsı kahverengi, kenarları koyu kahverengi. Bacaklar sarımsı, kahverengi. Bacakların metatarsus ve tarsusları diğer üyelerine göre daha koyu. Bacak formülü: 4213. Ayrıntılı bacak ölçümleri Tablo 1'de verilmiştir.

Abdomenin dorsali morumsu kahverengi, dişilerde nispeten daha açık renkte, ön orta kısmında ucu arkaya yönelik ok başı şeklinde belirgin bir desen ve onu takiben altı adet ters v işareti (kevron) bulunmaktadır (Şekil 1A-B). Abdomenin ventrali sarımsı kahverengi.



Şekil 3. *P. genevensis*, epijin. A. ventral görünüş, B. dorsal görünüş. Kısaltmalar: A, Atriyum CD, Boyuna çiftleşme kanalları R_1, Reseptakulum$_1$ R_2, Reseptakulum$_2$. Ölçüm çizgileri: 0.2 mm.

Figure 3. *P. genevensis*, epigyne. A. ventral view, B. dorsal view. Abbreviations: A, Atrium CD, longitudinal copulatory duct R_1, Receptaculum$_1$ R_2, Receptaculum$_2$. Scale lines: 0.2 mm.

Palp. Palp şeklen yumurtamsı, retrolateral tibial apofiz kısa, üçgenimsi, ucu yuvarlak ve küttür. Bunun arka hizasında, simbiyumun retrolateral alt kenarında, hemen hemen simbiyumun yarısına kadar uzanan bir tutam simbiyal kıl (*CS*) demeti bulunmaktadır (Şekil 2A). Tegular apofiz ve kondüktör gelişmemiş, embolus; tegulumun orta prolateral kenarından köken almakta, ince ve uzundur (Şekil 2B-C).

Epijin. Atriyum ters kalp şeklinde ve geniş, epigastrik yarığa bakan kenarları kitinize, bir tanesi kuvvetli kitinize olmuş (R_1), diğeri hiyalinimsi (R_2) birer çift uç konumlu reseptakulum (spermateka veya sperm depolama haznesi) bulunmaktadır. Reseptakulumların bağlantı noktaları kuvvetli kitinize olmuştur (Şekil 3A).

Atriyum kısa bir septuma (bölmeye) sahip; atriyumdan içeriye yönelen bir çift boyuna çiftleşme kanalı döngüler yaparak reseptakulumlara bağlanır (Şekil 3B).

Avrupa'dan, Güney Sibirya ve Orta Asya'ya kadar son derece geniş dağılıma sahip olan *Porrhoclubiona genevensis* türünün Akdeniz adaları içerisinde Balear Adaları ile Sardinya Adası'ndan kaydı bulunmaktadır (WSC, 2022; Helsdingen, 2021). Bosmans et al. (2019), 449 tür kaydettikleri "Kıbrıs Örümcekleri Kontrol Listesinde" adadan Clubionidae familyasına ait iki tür rapor etmişlerdir: *Clubiona pseudoneglecta* Wunderlich 1994 ve *P. vegeta* Simon 1918. Bununla birlikte *P. genevensis* türünün dağılım özelliklerinden dolayı Kıbrıs adasından kaydı şaşırtıcı olmamıştır.

Porrhoclubiona genevensis ve *P. vegeta* kardeş türler olup özellikle dişi üreme organlarının yapısal özelliklerinin birbirlerine çok benzedikleri daha önceleri de ifade edilmiştir (Helsdingen, 1979). İki türü birbirlerinden ayırt edebilmenin yanılgı oranı en düşük yolu abdominal desenleri dikkatle incelemekle ve erkek

palplerinin retrolateral tibial apofizlerini şeklen karşılaştırmakla olacaktır. *P. vegeta* türünde bu apofizin ucu bariz bir şekilde daha sivri, *P. genevensis*'te ise yuvarlağımsıdır (Helsdingen, 1979).

Sonuç olarak bu çalışma ile kese örümceklerine ait Kıbrıs adasında temsil edilen tür sayısı üçe yükselmiştir. Gelecekte yapılacak faunistik tespit çalışmaları ile bu sayının yükseleceği ve familyaya ait çeşitliliğin artacağı düşünülmektedir.

Tablo 1. *Porrhoclubiona genevensis*, Bacak kısımlarının uzunlukları (♂ / ♀), Kısaltmalar: Fe: Femur, Pa: Patella, Ti: Tibia, Me: Metatarsus, Ta: Tarsus.

Table 1. *Porrhoclubiona genevensis*, Leg measurements (♂ / ♀), Abbrevations; Fe: Femur, Pa: Patella, Ti: Tibia, Me: Metatarsus, Ta: Tarsus.

Bacak	Fe	Pa	Ti	Me	Ta	Toplam
I	1.25/1.00	0.75/0.50	1.10/0.80	0.80/0.55	0.45/0.45	4.35/3.30
II	1.40/1.15	0.70/0.55	1.35/0.90	0.90/0.65	0.50/0.40	4.85/3.65
III	1.10/1.00	0.55/0.50	0.85/0.60	0.90/0.70	0.40/0.40	3.80/3.20
IV	1.50/1.50	0.65/0.65	1.15/1.10	1.40/1.30	0.45/0.50	5.15/5.05

Etik kurul onayı: Bu çalışma için etik kurul onayı alınmasına gerek yoktur.

Çıkar çatışması: Yazarlar, çıkar çatışması olmadığını beyan etmiştir.

Yazar katkısı: Fikir/Kavram – K.B.K., Ö.Ö., R.S.Ö.; Tasarım – K.B.K., Ö.Ö., R.S.Ö.; Denetleme/Danışmanlık – K.B.K., Ö.Ö., R.S.Ö.; Kaynaklar/Fon Sağlama – K.B.K., Ö.Ö., R.S.Ö.; Materyaller – K.B.K., Ö.Ö.; – Veri Toplama veya İşleme – K.B.K., Ö.Ö., R.S.Ö.; Analiz Yorumlama – M.C.; Kaynak Taraması – K.B.K., Ö.Ö., R.S.Ö.; Makalenin Yazımı – K.B.K., Ö.Ö., R.S.Ö.; Eleştirel İnceleme – K.B.K., Ö.Ö., R.S.Ö.

Kaynaklar

Bosmans, R., Henrard, A., Benhalima, S., & Kherbouche-Abrous, O. (2017). The genus *Clubiona* Latreille, 1904 (Araneae: Clubionidae) in the Maghreb, with notes on the *genevensis* group and new records from the Mediterranean Region. *Zootaxa*, 4353(1), 1-28. https://doi.org/10.11646/zootaxa.4353.1.1

Bosmans, R., Van Keer, J., Russell-Smith, A., Hadjiconstantis, M., Komnenov, M., Bosselaers, J., Huber, S., McCowan, D., Snazell, R., Decae, A., Zoumides, C., Kielhorn, K.-H., & Oger, P. (2019). Spiders of Cyprus (Araneae). A catalogue of all currently known species from Cyprus. *Newsletter of the Belgian arachnological Society*, 34(Supplement), 1-173.

Danışman, T., Coşar, İ., & Özgen, İ. (2018). Three new *Clubiona* records for the spider fauna of Turkey (Araneae: Clubionidae). *Serket*, 16(2), 90-95.

Helsdingen, P.J. van (1979). Remarks concerning Clubionidae. *Bulletin of the British Arachnological Society*, 4, 298–302.

Helsdingen, P.J. van (2021). Fauna Europaea: spiders. Version 2021.2. Retrieved from https://fauna-eu.org

Jocqué, R., & Dippenaar-Schoeman, A.S. (2007). Spider families of the world (2nd ed). Musée royal de l'Afrique centrale; ARC-PPRI.

Marusik, Y.M., & Omelko, M.M. (2018). A survey of the *Porrhoclubiona* Lohmander, 1944 from Central Asia (Araneae, Clubionidae [sic]). *ZooKeys*, 802, 19-38. https://doi.org/10.3897/zookeys.802.30236

World Spider Catalog (2022). World Spider Catalog. Version 23.0. Natural History Museum Bern. Retrieved from http://wsc.nmbe.ch. https://doi.org/10.24436/2

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Prodidomus rufus Hentz, 1847 (Arachnida: Araneae), Redescription of Female and New Record of Male from South of Iraq

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Abstract: The female of the species *Prodidomus rufus* Hentz 1847, which was previously recorded from Al-Najaf province in central Iraq, was described inaccurately. Therefore, we re-described it as the males were first recorded in Al-Gharraf district, north of Thi Qar province- southern Iraq, depending on the copulatory organs (Palp and Epigynum) illustrated in the figures and have a role in distinguishing between species. The habitus of males and females, leg parts measurements of males and females, and a map of specimen's locations are also presented.

Keywords: Al-Gharraf, Prodidomidae, spiders, Thi Qar.

Prodidomus rufus Hentz, 1847 (Arachnida: Araneae), Irak'ın Güneyinden Dişinin Yeniden Tanımlanması ve Yeni Erkek Kaydı

Öz: Daha önce Al-Necef ilinden (Merkez, Irak) kaydedilen *Prodidomus rufus* Hentz 1847 dişisi, hatalı olarak tanımlanmıştır. Bu nedenle, türün erkek bireyleri Irak'ın güneyindeki Thi Qar ilinin kuzeyindeki Al-Gharraf ilçesinde ilk kez kaydedilerek yeniden tanımlanmıştır. Türleri ayırt etmede taksonomik karakter olan çiftleşme organları (Palp ve Epigynum) gösterilmiştir. Türün habitatı, erkek ve dişi bireylerin bacak parçaları ölçümleri ve bir dağılış haritası da sunulmuştur.

Anahtar kelimeler: Al-Gharraf, Prodidomidae, örümcekler, Thi Qar.

Prodidomidae Simon, 1884 is a widespread family, including 23 genera and 191 species distributed worldwide. Among them, species belonging to genus *Prodidomus* Hentz, 1847, which has almost worldwide distribution, includes ten known species from the Near East and Middle East regions (World Spider Catalog, 2022). In Iraq, previously, only one species of this genus was recorded: the female *P. redikorzevi* Spassky, 1940 by Al-Khazali (2021) in Thi Qar province. Afterwards, Mizhir (2021) recorded the female *P. rufus* Hentz, 1847 in Al-Najaf province without explaining the exact details of the epigynum. In the current study, the male species *P. rufus* Hentz, 1847 was first recorded in Thi Qar province, south of Iraq and the female was re-described based on the genitalia. *P. rufus* has been recorded from many countries including Argentina, Chile, China, Cuba, Japan, New Caledonia, and the United States (World Spider Catalog, 2022).

Specimens were collected by hand from agricultural lands, near palm trees in the city of Al-Gharraf city, north of Thi Qar Province (Fig. 1) in December 2021. The specimens were preserved in 70% ethanol and were photographed using a Nikon Z50 camera on a Krüss stereomicroscope in the Entomology Laboratory of the Biology Department, College of Science, University of Basrah. The identification was done according to Cooke (1964); Platnick and Baehr (2006); Ferrández and Carrillo (2018). Measurements are given for the segments of the legs (femur, patella, tibia, metatarsus, tarsus) and all measurements are given in millimeters.

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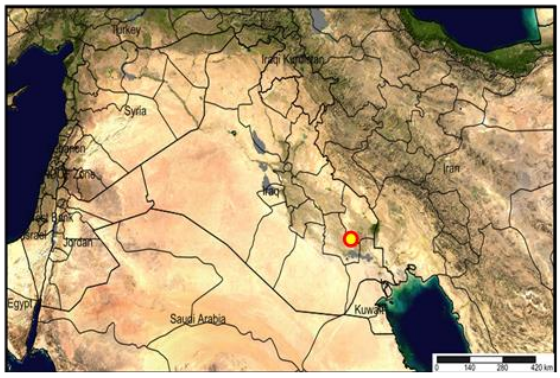

Figure 1. Map of Iraq showing specimens collection location: Thi Qar Province, Al-Gharraf city, Iraq (circle).

Prodidomus rufus Hentz, 1847 as in Figs. 2 and 3, the materials examined were 1 ♂ and 2♀♀ from Al-Gharraf city, north of Thi Qar province, south of Iraq, coordinates (31º184`34.252'N, 46º12`57.211`E) in December 2021 (G. A. A. Al-Yacoub). *Prodidomus* Hentz, 1847 can be distinguished from other genera of Prodidomidae by inferior spinnerets with only short fusuli, superior spinnerets are obliquely truncated (Fig. 2C), sternum oval, while the species can be easily distinguished from other members of the genus by their widely divergent, geniculate chelicerae (Fig. 2D) and very different genitalia. Anterior eyes straight, posterior eyes procurved, forming a triangle.

Male and female are similar but there are differences

in the measurements of the body parts. In the dorsal view, the cephalothorax is brownish-yellow, the abdomen is light pink with gray setae, both without patterns. The eyes are eight, each three of them on one side and their color is silver, anterior median eyes are surrounded with black. The legs are light yellow with two claws, tibia and metatarsus III and IV with apical ventral spines. In the ventral view, Sternum is oval, pale yellowish-brown. Abdomen is light colored. Fangs are long and thin.

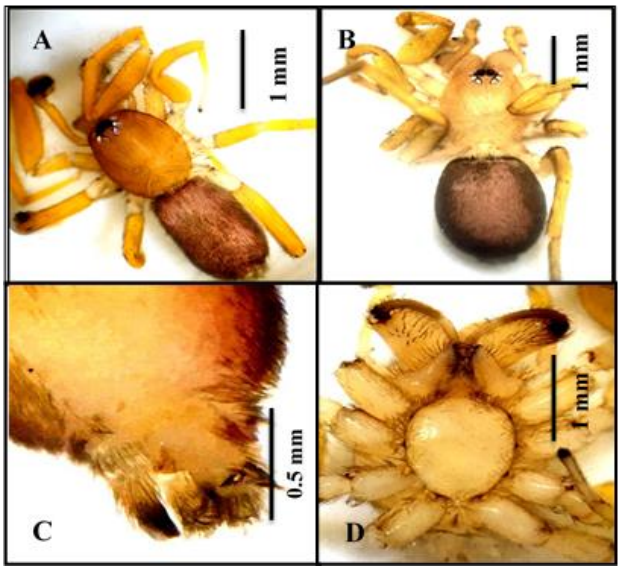

Figure 2. *Prodidomus rufus* Hentz, 1847 ♂♀. A: Habitus of male; B: Habitus of female; C: Spinnerets; D: Sternum and Chelicerae.

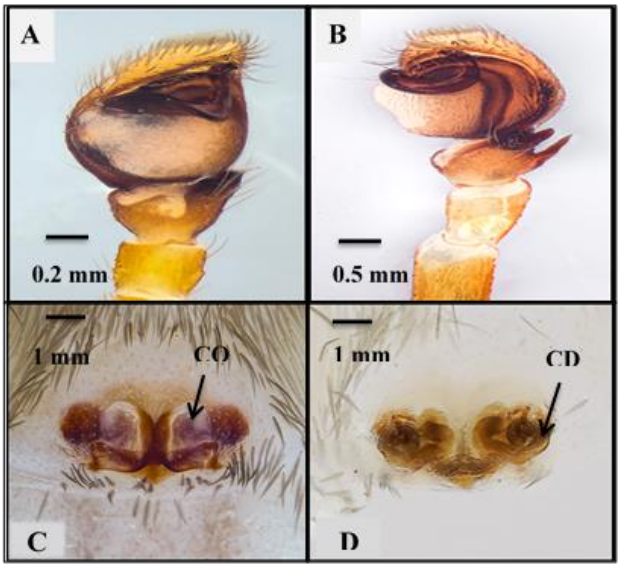

Figure 3. *Prodidomus rufus* Hentz, 1847 ♂♀. A, Ventral view of left palp; B, retrolateral left palps; C, ventral view of epigynum; D, dorsal view of epigynum. Abbreviations: CO: copulatory openings; CD: copulatory duct.

Habitus of male as in Fig. 2A. Total length 2.9, cephalothorax 1.34 length and 0.9 width. Opisthosoma 1.56 length. Leg measurements: I: 5.6 (1.6, 0.9, 1.3, 1, 0.8), II: 4.4 (1.2, 0.8, 1, 0.8, 0.6), III: 3.7 (1, 0.4, 0.9, 0.8, 0.6), IV: 5.9 (1.6, 0.9, 1.4, 1.1, 0.9). Habitus of female as in Fig. 2B. Total length 4.5, cephalothorax 2 length and 1.5 width. Opisthosoma 2.5 length. Leg measurements: I: 6.1 (1.8, 1, 1.4, 1, 0.9), II: 5 (1.4, 0.9, 1.1, 0.9, 0.7), III: 4.2 (1.1, 0.5, 1, 0.9, 0.7), IV: 6.7 (1.9, 1, 1.6, 1.2, 1).

Palp of male as in Fig. 3A and B is characterized by its cymbium straight retrolaterally, sperm duct semicircular, embolus thickened sinuous, retrolateral tibia with long, divided and sharp pointed apophysis. Epigynum of female as in Fig. 3C and D with two large inverted U shaped copulatory openings, epigynal ducts wide and irregularly curved, spermathecae widely separated.

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References

Al-Khazali, A.M. (2021). *Prodidomus redikorzevi* Spassky, 1940 (Araneae: Gnaphosidae: Prodidominae), the first record of the subfamily in Iraq. *Serket*, 18(1), 36-39.

Cooke, J.A.L. (1964). A revisionary study of some spiders of the rare family Prodidomidae. *Proceedings of the Zoological Society of London*, 142(2), 257-305. https://doi.org/10.1111/j.1469-7998.1964.tb04625.x

Ferrández, M.-Á. & Carrillo, J. (2018). *Prodidomus* cfr *rufus* Hentz, 1847 (Araneae: Prodidomidae), una especie "enigmática". *Revista Ibérica de Aracnología*, 32, 123-126.

Mizhir, A.H. (2021). New Species of Spiders *Prodidomus rufus* Hentz, 1847 (Araneae: Gnaphosidae) in Iraq. *International Journal of Animal Biotechnology and Applications*, 7(2), 6-9.

Platnick, N.I. & Baehr, B.C. (2006). A revision of the Australasian ground spiders of the family Prodidomidae (Araneae, Gnaphosoidea). *Bulletin of the American Museum of Natural History*, 298, 1–287. https://doi.org/10.1206/0003-0090(2006)298[1:AROTAG]2.0.CO;2

World Spider Catalog (2022). World Spider Catalog. Version 23.0. Natural History Museum Bern. Retrieved from http://wsc.nmbe.ch

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Review / Derleme

Cold Hardiness in Animals: The Cryobiology of Amphibians

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Abstract: Organisms adapt to abiotic environmental conditions in order to survive. Especially environmental temperature changes are effective on their feeding, reproduction, development, and morphology. Extreme temperature changes can be fatal, especially for ectothermic animals. Terrestrial ectotherms have developed some special behavioral, physiological, and biochemical strategies to survive in freezing temperatures in nature. Some species avoid freezing temperatures by migrating and hibernating under water or soil. Others have to spend the winter exposed to freezing conditions. In general, cold hardiness depends on freeze avoidance (supercooling) and freeze tolerance strategies. In the case of freeze avoidance, the liquid form of body fluids is preserved at temperatures below the freezing point, while the freezing of more than 50% of the total water in their bodies can be tolerated in animals using the freeze tolerance strategy. The freeze tolerance strategy, which has also been found in some amphibian and reptile groups from terrestrial hibernator animals, enables them to survive in freezing winter conditions. These special species are protected from the deadly effects of freezing by the cryoprotective mechanisms. These animals, whose vital activities are completely stopped during freezing, return to normal life in a short time after thawing. The research of this miraculous mechanism not only explains the complex adaptation of animals but also provides resources for tissue and cell cryopreservation technology. This review will contribute to those who want to do research on this subject, which has not yet been studied enough, by providing information on the freeze tolerance strategies of amphibians.

Keywords: Ectoterm hibernation, freeze tolerance, freeze avoidance, Cryoprotectants, antifreeze proteins, antioxidant defense.

Hayvanlarda Soğuğa Dayanıklılık: Çift Yaşarların Kriyobiyolojisi

Öz: Organizmalar yaşamlarını devam ettirebilmek için abiyotik çevresel koşullara uyum sağlarlar. Özellikle ortam sıcaklığındaki değişimler; canlıların beslenme, üreme, gelişim ve morfolojileri üzerinde etkilidir. Sıra dışı sıcaklık değişimleri özellikle ektotermik hayvanlar için ölümcül olabilir. Karasal ektotermler. doğada donma noktasının altındaki sıcaklıklarda hayatta kalabilmek için davranışsal, fizyolojik ve biyokimyasal bazı özel stratejiler geliştirmişlerdir. Bazı türler göç ederek su ya da toprak altında kış uykusuna yatmak suretiyle dondurucu sıcaklıklardan kaçınırlar. Bazıları ise donma koşullarına maruz kalarak kışı geçirmek zorundadırlar. Genel olarak dondurucu soğuğa dayanıklılık donmadan kaçınma (süper soğuma) ve donma toleransı stratejilerine bağlıdır. Donmadan kaçınma durumunda vücut sıvılarının donma noktasının altındaki sıcaklıklarda sıvı formu korunurken donma toleransı stratejisini kullanan canlılarda ise vücutlarındaki toplam suyun %50'sinden fazlasının donması tolere edilebilir. Karasal hibernatör hayvanlardan bazı amfibi ve sürüngen gruplarında da tespit edilen donma toleransı stratejisi onların dondurucu kış koşullarında hayatta kalmalarını sağlamaktadır. Bu özel türler kriyoprotektif mekanizmaları ile donmanın ölümcül etkilerinden korunurlar. Donma süresince yaşamsal faaliyetleri tamamen duran bu hayvanlar çözündükten sonra kısa bir süre içerisinde de normal yaşama dönerler. Bu mucizevi mekanizmanın araştırılması yalnızca hayvanların karmaşık adaptasyonunu açıklamakla kalmaz, aynı zamanda doku ve hücre kriyoprezervasyon teknolojisine de kaynak sağlar. Bu derleme amfibilerin donma toleransı stratejilerine dair bilgiler sunarak henüz yeterince çalışılmamış bu konuda araştırma yapmak isteyenlere katkı sağlayacaktır.

Anahtar kelimeler: Ektoterm kış uykusu, donma toleransı, donmadan kaçınma, Kriyoprotektanlar, antifriz proteinler, antioksidan savunma.

1. Giriş

Ilıman iklimde yaşayan canlılar için kış döneminin dondurucu soğuk koşulları hayati sorunlar teşkil etmektedir. Bu koşullarda, bazı endotermik hayvanlar yüksek metabolik hızları ve homeotermik yapılarının gereksinimi olan yiyeceği temin edemedikleri için ya göç ederek ya da metabolik hızlarının azaldığı kış uykusu (hibernasyon) gibi fizyolojik ve davranışsal adaptasyonlar ile hayatta kalabilirler (Geiser, 2004; Geiser, 2020). Dondurucu sıcaklıklar heterotermik yapıları nedeniyle ektotermik hayvanlar için daha tehlikelidir. Birçok soğukkanlı hayvan için göç davranışı enerji ve zaman maliyeti açısından mümkün olmayan bir seçenektir. Bu sebepten dolayı bu organizmalar uzun süreli kış koşullarında kış uykusu, donmadan kaçınma (süper soğuma) ya da donma toleransı gibi adaptasyon mekanizmalarını kullanarak hayatta kalabilirler. Bu stratejilerin temel ortak noktaları ise metabolik hızın düşürülmesidir. Hipometabolizmada birçok ekofizyolojik ve biyokimyasal mekanizma canlıların donma koşullarında hayatta kalma başarısını artırır (Donoso et al., 2013; Ultsch, 2006).

Dondurucu soğuğa maruz kalan hayvanlar için temelde üç ana stres ortaya çıkar. Bunlar; hücre ve dokulardaki suyun donması ile oluşan fiziksel hasar, kan akışının durmasına bağlı olarak anoksi/iskemi oluşumu ve suyun hücreler arası boşluklara çıkışından kaynaklı fizyolojik dehidrasyon stresleridir. Donma toleranslı

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omurgalılar donma, anoksi ve dehidrasyon koşullarında glikoz, gliserol, üre gibi kriyoprotektan moleküller, bazı protein ve antioksidanlar sayesinde buz formunun kontrolü, anaerobik enerji optimizasyonu, yüksek asidozis ve anhidrobiyozis ile başa çıkmak gibi birçok metabolik süreci kontrol ederek doku ve hücrelerini korumayı başarırlar (Costanzo et al., 2013; Storey & Storey, 2017).

Donma koşullarına maruz kalmamak için böcekler çeşitli mekanizmalar geliştirmişlerdir. Örneğin bal arıları termoregülasyon ile kovan sıcaklığını korumaya çalışırlar, çeşitli yusufçuk ve kelebek türleri kış dönemini donmayan sularda ya da toprak derinliklerinde larva olarak geçirirler, *Phylloscopus trochilus*, *Catharus ustulatus* ve *Danaus plexippus* gibi bazı böcek türleri de göç yolunu tercih edebilir (Duman & Newton, 2020; Merlin & Liedvogel, 2019; Storey & Storey, 2012). Donma koşullarına maruz kalmaktan kaçınamayan birçok böcek türü kriyoprotektan metabolitler (gliserol gibi) ve antifriz proteinler sayesinde vücut sıvılarının donma noktasını aşağıya çekebilirler ve donmadan kaçınma (süper soğuma) adı verilen bu adaptasyon mekanizması ile uzun süre hayatta kalabilirler (Storey & Storey, 2012).

Birçok su kurbağası (*Pelophylax ridibundus*, *Rana pipiens ve R. catesbeiana* gibi) ve tatlı su kaplumbağası (örneğin, *Trachemys scripta elegans*) gibi soğukkanlı omurgalılar kışı sulak alanların dip kısmlarında geçirirler (Storey & Storey, 2017; Wijenayake & Storey, 2016). Bu ortamlarda yüzey tamamen buz tutsa da dip bölge donma sıcaklıklarına ulaşmadığı için donmadan fiziksel olarak korunmuş olurlar (Storey et al., 2021; Tattersall & Ultsch, 2008). Karasal soğukkanlı bazı omurgalılar da korunaklı yuva yaparak ve yer altı sığınaklarına çekilerek donmadan korunabilirler. Bunların dışında vücut suyunun %65'ine kadar donmasını tolere edebilen türlerin kullandığı donma toleransı stratejisi de bir diğer ekofizyolojik adaptasyon mekanizmasıdır. Bu strateji gel git bölgelerindeki deniz yumuşakçaları, birçok böcek türü, karada kış uykusuna yatan bazı sürüngen ve amfibi türleri için özel bir adaptasyondur (Costanzo, 1995; Storey & Storey, 2017).

Soğukkanlı omurgalılardan birçok sürüngen türü kısa süreli donma periyodunda hayatta kalabilirken bazı kaplumbağa ve kertenkele türlerinin ise uzun süreli donma maruziyetine karşı tolerans gösterebildiği bilinmektedir (Costanzo et al., 2008; Ultsch, 2006). Kışı yüzeye yakın toprak ya da yaprak katmanları arasında geçiren bazı amfibiler donma koşulları ile başa çıkabilmek için donma toleransına sahiptirler. Örneğin, *Pseudacris crucifer* ve *Rana sylvatica* gibi bazı türler vücutlarındaki suyun %65'inin donmasını tolere edebilirler (McNally et al., 2003). Anadolu türleri üzerinde gerçekleştirilen çalışmalarda da endemik *Pelophylax caralitanus*, *Rana holtzi* ve *R. macrocnemis*'in de belirli düzeylerde donma toleransına sahip olduğu son dönemde ortaya çıkarılmıştır (Güleç, 2019; Yoldaş, 2021; Yoldas & Erismis, 2021).

Bu derlemenin amacı, doğada dondurucu çevresel sıcaklıklara maruz kalan ve bu süreçte hayatta kalabilen hayvanlardan özellikle bazı amfibi türlerinin hayatta kalma stratejilerine dair mevcut çalışmaları bir araya getirmek ve Türkiye'de henüz çok az çalışılmış bu konuda daha kapsamlı çalışmalar tasarlanması için araştırmacılara rehberlik etmesidir.

2. Dondurucu Soğukta Hayatta Kalma

Kış mevsiminin dondurucu koşulları ılıman iklimlerde yaşayan canlılar için ölümcül olabilir. Birçok küçük memeli ve *Phalaenoptilus nuttallii* gibi bazı kuş türleri de göç sürecindeki mesafeye bağlı enerji kullanımı açısından dezavantajlı oldukları için hibernasyon davranışı sergilerler (Geiser, 2020; Woods et al., 2019). Hibernatif memeliler torporda kısmi heterotermik özellik gösterirler, yani sabit vücut sıcaklığı korunamaz (Kart Gür et al., 2014).

Ektotermlerin hayvanların donma koşullarında hayatta kalabilmeleri metabolik hızın düşürülmesinin yanında ilave stratejileri de gerektirir. Termal açıdan korunaklı yuvaya sahip olmayan ve donma sıcaklığı altındaki koşullardan kaçınamayan türler donmanın sonuçlarından korunabilmek için donmadan kaçınma veya donma toleransı stratejilerini kullanmalıdır (Donoso et al., 2013). *Zootoca vivipara* (*Lacerta vivipara*) ve *Chrysemys picta* gibi bazı sürüngen türleri her iki stratejiyi de kullanabilmektedir. Bu durum, türlerin coğrafi olarak çok geniş alanlara uyum sağlayabilmelerini sağlamıştır (Costanzo & Lee, 2013). Her iki adaptasyonu da kullanabilen bazı böcek türleri ise kriyoprotektif dehidrasyon ve vitrifikasyon gibi ek stratejiler de kullanarak inanılmaz düşük sıcaklıklara dayanabilmektedirler. Örneğin *Cucujus clavipes puniceus* (Alaska kırmızı yassı odun böceği) larvalarının -100°C'ye maruz kaldıktan sonra hayatta kaldığı bilinmektedir (Sformo et al., 2010; Storey & Storey, 2012).

Donmadan kaçınma ve donma toleransı adaptasyonlarını kullanan canlılar hücresel dehidrasyon, enerji ihtiyacı, oksidatif hasar, iskemi, ozmo-iyonik dengesizlik, hücre hacminde azalma, yapısal hasar ve protein denatürasyonu gibi farklı birçok stresle başa çıkmak zorundadırlar (Costanzo & Lee, 2013). Donmadan kaçınan ve donma toleranslı canlılar mükemmel adaptasyon mekanizmaları ile kendilerini hem donma, anoksi ve dehidrasyonun getirdiği fiziksel hasarlarından korurlar hem de koşullar normalleştiğinde metabolizmalarının ve davranışlarının da hızlıca normale dönmesini sağlarlar.

2.1. Donmadan Kaçınma (Süper Soğuma)

Donmadan kaçınma ya da kurtulma olarak nitelendirilen bu adaptasyon davranışı, vücut sıvılarının donma noktasını çevresel sıcaklığın altına indirilmesi prensibine dayanır. Bu stratejiyi kullanan canlılar yüksek miktarda kriyoprotektan madde birikimi ve buz oluşumunu engelleyici proteinlerin yardımı ile 0°C'nin altındaki sıcaklıklarda donmaktan kaçınabilirler. Birçok böcek ve eklembacaklılar yaygın olarak bu stratejiyi kullanır ve -40°C gibi sıra dışı soğuklarda bile donmadan kalabilirler (Costanzo & Lee, 2013; Duman & Newton, 2020).

Donmadan kaçınma adaptasyonunda sulu çözeltilerin kolligatif özelliği ve süper soğuma fenomeni sayesinde donma noktası aşağıya çekilir. Bu davranışı sergileyen canlılar birçok polihidrik alkol ve şekerleri antifriz madde olarak kullanırlar. Bu konuda en yaygın biriktirilen madde gliserol olarak tespit edilmiştir. Çok yüksek oranda kriyoprotektan birikimi ve dehidrasyon ile birlikte sulu vücut sıvıları süper soğuma fenomeni göstererek donma gerçekleşmeden 0°C'nin altındaki

sıcaklıklarda formunu koruyabilmektedir. Bu koşullarda vücuttaki suyun donmaya başladığı sıcaklık noktası süper soğuma noktası (SSN, SCP, "*supercooling point*") olarak adlandırılır (Kelleher et al., 1987; Storey & Storey, 2012).

Sıra dışı soğuk çevre koşullarına maruz bırakılan omurgalı ektoterm su kaplumbağalarından *Chrysemys picta* -20°C ve *Graptemys geographica* -14.8°C'ye kadar donmadan hayatta kalabilmektedirler (Baker et al., 2003; Costanzo et al., 2013). Asya ve Avrupa'da oldukça geniş bir alanda dağılış gösteren *Z. vivipara* kertenkele türünün de glisemik indeksini dört kat arttırarak 3 hafta -3.5°C'de donmadan hayatta kalabildiği bildirilmiştir (Costanzo, Grenot, et al., 1995). Bunlara ek olarak *Podarcis sicula* ve *P. muralis* duvar kertenkeleleri, *Vipera berus* ve *Thamnophis sirtalis* gibi yılan türlerinin de donmadan kaçınma stratejisini kullandığı rapor edilmiştir (Voituron et al., 2003). Böceklerde ise bu stratejinin kullanımı omurgalılara göre çok daha yaygındır. Lepidoptera, Diptera, Hymenoptera ve Coleoptera grubuna ait birçok böcek türü genellikle gliserol ve sorbitolü kriyoprotektan olarak kullanarak kolaylıkla -10 ile -30°C civarında SSN'ye sahip olabilir. Arktik bölge böceklerinden *Pytho deplanatus*'un donma noktası -55°C olarak kaydedilmiştir (Duman, 2001).

2.2. Donma Toleransı

Donma noktasının altındaki sıcaklıklara maruz kalan birçok canlı vücut sıvılarının donmasını engellemeye çalışırken bazı türler vücutlarındaki suyun %65'ine kadarının buza dönüşmesini tolere edebilirler. Donma toleranslı türler olarak adlandırılan bu canlılarda aylarca sürebilen uzun kış dönemine bağlı vücut suyunun %50 sinden fazlası buz formuna dönüşür; solunum, dolaşım ve sinirsel iletim gibi tüm hayati fonksiyonlar durur. Ancak, donma toleranslı türler geliştirdikleri özel adaptasyon stratejisi sayesinde çevresel koşulların normale dönmesi ile birlikte tüm bu hayati fonksiyonların tekrar geri kazanımı ile normalleşebilirler. Bu özel canlılar donma ve çözünme evrelerinde ölümcül olabilecek birçok fiziksel ve biyokimyasal stresle başa çıkabilirler (Biggar et al., 2013; Layne & Lee, 1987).

Donma toleransı adaptasyon mekanizması temelde iç organların ve hücrelerin dehidrasyonu, anoksi ile mücadele, ölümcül hücre içi buzlanmayı engellemek için yoğun miktarda metabolit biriktirilmesi, vücuttaki buzlanmanın yeri ve şekillendirilmesinin kontrolü, hipometabolizma ve antioksidan savunma gibi birçok stratejiyi barındırır (Costanzo et al., 2013; Storey & Storey, 2017). Bu adaptasyon mekanizmasının bugüne kadar hayvanlar âleminden eklembacaklılar, karından bacaklılar ve böcekler gibi omurgasızların yanında amfibiler ve sürüngenler gibi omurgalılarda da kullanıldığı belirlenmiştir (Costanzo & Lee, 2013).

Donma toleransı kendi içerisinde üç temel strese karşı dayanıklılığı gerektirir. Bu dayanıklılıklar anoksi toleransı, dehidrasyon toleransı ve soğuk (buzlanma derecesindeki) toleransı olarak bilinirler ve mekanizmaları enerjinin minimum kullanımına karşılık maksimum yaşam süresi elde etmeyi destekleyen metabolizmanın baskılanması (hipometabolizma) temeline dayanır (Storey & Storey, 2017). Donma esnasında oluşan buz kristallerinin hücre ve dokulara fiziki hasarı, hücre içi buzlanmayı önlemek için hücredeki suyun hücreler arası boşluklara itilmesinden kaynaklı hücresel dehidrasyon, akciğer, kalp ve kan akışının durması sonucu besin ve oksijenin taşınamaması sonucu anoksi ve iskemi oluşumu, sinirsel iletimin durmasından kaynaklı tüm hayati süreçlerin durması gibi baskılar birçok canlı için geri dönülmez ölümcül sonuçlar doğururken, donma toleranslı hayvanların doğal olarak başa çıkabildikleri koşullardır. Donma toleransı adaptasyonu bunlara ek olarak çözünme sürecinde buzun erimesi sonucu ortaya çıkan suyun hücrelere girme çabasının getirdiği ozmotik basınç ve oksijen içeren kanın ani reperfüzyonu ile birlikte reaktif oksijen türleri (ROS) ile de mücadeleyi gerektirir (McNally et al., 2003; Storey et al., 2021). Tüm bu streslere karşı başarılı adaptasyon sağlanabilmesi canlıların ekosistemdeki yaşam döngüsü, beslenme ve üreme davranışlarındaki başarısına da katkı sağlamaktadır.

Poikiloterm omurgalılardan *Terrapene ornata*, *T. carolina*, *C. picta* ve *Z. vivipara* bilinen güçlü donma toleransına sahip sürüngen türleridir (Storey & Storey, 2017; Voituron et al., 2004). Karasal hibernatör olan anur türlerinden ise *R. sylvatica*, *P. triseriata*, *P. crucifer*, *Dryophytes chrysoscelis* ve *D. versicolor* (*Hyla versicolor*) yüksek donma toleransına sahiptirler (Costanzo et al., 2013; Storey & Storey, 2004). Bunların yanında hibernasyon evresini toprağın ya da suyun derin ve donmayan bölgelerinde geçiren sucul kurbağaların daha zayıf donma toleransı gösterdiği laboratuvar ortamı testlerinde tespit edilmiştir (Costanzo et al., 1993). Semenderlerdeki donma toleransının yaygın olmadığı düşünülse de yeterli araştırmalar yapılmamıştır. Bu kapsamda en bilinen örnekler Sibirya ve Schrenck semenderleri (*Salamandrella keyserlingii, Salamandrella schrenckii*)'dir. Bu iki türün de -30°C ye kadar sıra dışı dondurucu koşullarda hayatta kalabildikleri bilinmektedir (Berman & Meshcheryakova, 2012; Niu et al., 2018). Günümüzde giderek artan çalışmalar neticesinde donma toleranslı amfibi sayısı giderek artmaktadır. Literatürde donma toleransı açısından değerlendirilen bazı amfibi türleri Tablo 1'de örneklendirilmiştir.

3. Donma Süreci

Bilindiği üzere sıcaklık 0°C ve altına düştüğünde su buza dönüşür. Soğukkanlı hayvanların da bu donma sıcaklıklarına maruz kalmaları esnasında oluşabilecek patofizyolojik sorunlardan biri de hücre içindeki suyun kristalleşmesidir. Hücre içi buzlanmanın gerçekleşmesi hemen hemen tüm canlılar için (bazı nematod türleri hücre içi buzlanmayı tolere edebilir) tamir edilemez ölümcül hasarlara sebep olur. Bu sebeple donma toleransında yalnızca hücre dışı ve vasküler boşluklardaki buz oluşumu ve bunun oluşturacağı mekanik hasara dayanılmasından söz edilebilir (Roy & Goswami, 2019; Storey & Storey, 2017).

Güçlü donma toleransına sahip *R. sylvatica* ile yapılan çalışmalarda, donmanın nefes alma, kalp atışı, kan dolaşımı, uzuv hareketleri ve refleksler gibi normal fizyolojik ve fiziksel faaliyetleri kesintiye uğrattığı tespit edilmiştir (Costanzo & Lee, 2013). Yoldaş (2021) çalışmasında elde edilen *Rana macrocnemis*'e ait donma ve çözünme süreçleri boyunca gerçekleşen fiziksel gözlemler, sıcaklık kayıt verileri ve fizyolojik değişimler Şekil 1'de gösterilmiştir. Kan akışının durması iskemi ve anoksiyi; hücre dışı boşluklarda, deri altı ve karın boşluğundaki buzlanma da hücresel dehidrasyonu meydana getirir

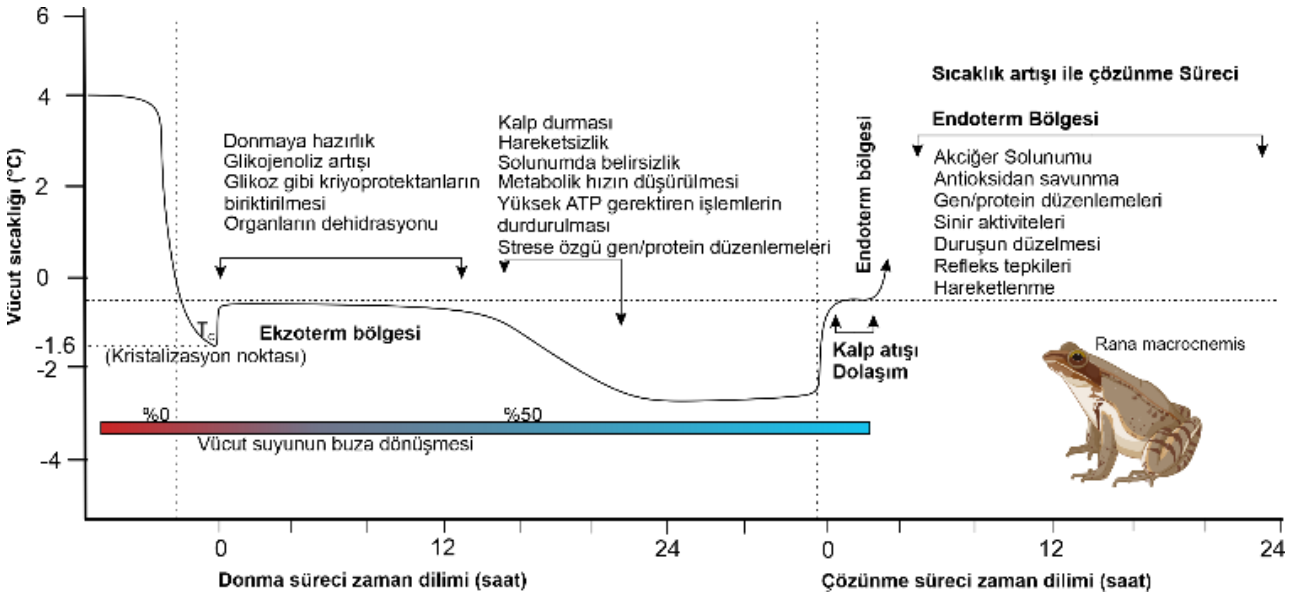
(Sullivan et al., 2015).

Donma toleranslı hayvanlarda buz oluşumu genellikle vücut sıvılarının donma noktasının 2-3°C daha altında başlar. Donmanın başladığı bu sıcaklığa "süper soğuma noktası, SSN" adı verilir (Berman et al., 2020). Buzlanmanın bu şekilde başlatılması hayvanlara hem hayatta kalmaları için gerekli düzenlemeleri yapmaları için zaman kazandırır hem de ani buzlanma dalgasının önüne geçilmesini sağlar. Buz çekirdeklenmesi yani suyun buz kristali olarak sıvı fazdan katı faza dönüşmesi için gerekli ilk adım; vücut sıvılarının cilt yüzeyi gibi bir epitel tabakadan çevresel buza teması, spesifik olmayan buz kaynaklarının (deri ya da bağırsak bakterileri gibi) epitel dokuya teması ya da özel "buz çekirdekleyici proteinler" (BÇP, en: INP, *ice nucleating protein*) vasıtası ile başlatılır (Duman, 2015; Storey & Storey, 2017).

Tablo 1. Güçlü ve zayıf donma toleransına sahip bazı amfibiler.

Table 1. Some amphibians with low & well-developed freeze tolerance.

Tür Adı	Test Edilen Koşulları/Hayatta Kalma Durumu	Referanslar
Donma Toleranslı Olduğu Belirlenmiş Semenderler		
Salamandrella keyserlingi	-10°C ile +5°C arası döngüde 130 gün	(Berman & Meshcheryakova, 2012)
Salamandrella schrenckii	-20°C'de 40 günden fazla	(Berman et al., 2010)
Güçlü Donma Toleranslı Kurbağalar		
Rana sylvatica	-2.5 ile -4°C'de 2 aya kadar, -16°C'de de 14 güne kadar	(Costanzo et al., 2013)
Rana arvalis	-2.5°C'de 2 gün %100, -3.5°C'de 3 günde %50 yaşam	(Voituron et al., 2009)
Pseudacris crucifer	-6.0°C'de 5 güne kadar hayatta kalma	(Storey & Storey, 1986)
Pseudacris triseriata	-3.0°C'de 3 gün, -2.5°C'de 4 günden fazla hayatta kalma	(Storey & Storey, 1987)
Dryophytes versicolor	-6.0°C'de 6 gün, -2.5°C'de 14 güne kadar hayatta kalma	(Layne & Lee, 1989)
Dryophytes chrysoscelis	-2.5 ile -2.9°C arasında 20-42 sa. sürelerde hayatta kalma	(Irwin & Lee, 2003)
Zayıf (Donma koşullarına kısa süreli dayanabilen) Donma Toleranslı Kurbağalar		
Hyla regilla ve H. japonica	-6.0°C'de 6 sa. / -4.0°C'de 6 sa. %80 hayatta kalma	(Croes & Thomas, 2000; Hirota et al., 2015)
Rana ridibunda	-2.0 ile -3.0°C'de 8-36 sa. dayanabilme	(Voituron et al., 2003)
Rana lessonae ve *Rana esculenta*	-2.5°C'de 9-13 sa. donmaya dayanabilme	(Voituron et al., 2005)
Rana dalmatina ve Rana temporaria	-2.0°C'de 8 sa. donma sonunda %50 hayatta kalma	(Voituron, 2009)
Rana amurensis	-2.5°C'de 3 gün ve -1.5°C'de 10 gün hayatta kalma.	(Berman et al., 2017)
Pelophylax caralitanus	-4 ve -20°C'de %0, -3°C'de 24 sa. %100 hayatta kalma	(Güleç, 2019)
Rana macrocnemis	Dağıstan (~1100-1800m) hayvanlar -1°C'de 5 günde %5;	(Bulakhova et al., 2020)
	Türkiye (>2000m) hayvanları -2.5°C 24 sa. %100 yaşam	(Yoldas & Erismis, 2021)



Şekil 1. *Rana macrocnemis*'in donma ve çözünme döngüsünün şematik gösterimi.

Figure 1. Illustration of the freezing and thawing cycle of *Rana macrocnemis*.

Suyun hücre dışı buza dönüşmesi sırasında içerisinde çözünen maddeler matrisden uzaklaştırılır ve saf su olarak kristalleşir. Böylece donmadan kalan hücre dışı sıvının ozmolalitesi daha fazla yükselir. Buradaki yüksek ozmoz hücre içi suyun dışarı çekilmesinde de etkilidir. Hücre içinde kalan az miktarda sıvının donma

noktası vücut sıcaklığına eşitlenene kadar ozmolalite yükselir. Böylece hücresel sıvı daha fazla soğutulamayacağından hücre içi buzlanma riski de en aza indirgenmiş olur. Vücuttaki buz ve su dengesi çevresel sıcaklıktan etkilense de donma süreci boyunca devam eder (Storey & Storey, 2013). Suyun hücre dışına çıkmasıyla oluşan dehidrasyon hücre hacmini azaltır, doku da küçülür. Bu hacimsel azalışlar kritik seviyenin altına indiğinde aşırı sıkışma ile plazma membranının çift katmanlı yapısının hasar görmesine, bu da çözünme esnasında işlevsiz bir hücre iskeleti olmasına yol açar (Storey, 2017).

Dehidrasyonun yanında donma sürecinin bir diğer ana sonucu da anoksidir (Storey & Storey, 2013). Gelişmiş anoksi toleransına sahip karasal hibernatör canlılar (kara kaplumbağaları, bazı kurbağalar ve kertenkeleler gibi) donduklarında tüm dolaşımları durmasına rağmen oluşan iskemik/anoksik koşullarda hayatta kalabilirler. Anoksik koşullarla başa çıkabilmek için asidozun azaltılması, anaerobik ATP kullanımı optimizasyonu ve ilgili gen/protein düzenlemelerinin yapılması gerekmektedir (Storey & Storey, 2017; Sullivan & Storey, 2012).

Hücre dışı buzlanmada vasküler genişleme ve damarların lümeninde buzlanma görülmektedir. Vasküler genişleme kontrol edilemediğinde damarların yapısal bütünlüğü bozulur ve çözünme sonrası kanın damar dışına çıkarak iç kanama ve ölüme yol açabilir (Storey & Storey, 2001). Donma koşullarının uzun süre var olması durumunda buz bağlayıcı proteinler (BBP, IBP, *ice binding protein*) ya da antifriz proteinler (AFP, *antifreeze protein*) adı verilen proteinler küçük buz kristallerine bağlanarak onların büyümesini ve rekristalizasyonu engellerler. Böylelikle buz kristallerinin doku ve hücrelere zarar vermesinin önüne geçilir (Storey & Storey, 2017).

3.1. Donma Sürecinin Kontrolü - Hipometabolizma

Metabolizmanın baskılanması durumu (hipometabolizma) birçok organizma tarafından çeşitli çevresel streslere karşı kullanılan bir yanıt mekanizmasıdır. Metabolik hızın %30 altına indirilmesi torpor, hibernasyon, estivasyon, diyapoz, anhidrobiyoz, anaerobiyoz ve donma toleransı gibi birçok koşulda hayat kurtarıcıdır (Kart Gür & Gür, 2017; Storey & Storey, 2011). Örneğin *C. picta* su sıcaklığının 10°C olduğu koşullarda metabolik hızını %10'a düşürerek su altında 3-4 ay boyunca hayatta kalabilir (Jackson, 2002). Kurbağalardan *R. sylvatica*'nın da metabolik hızı ve biyolojik süreçlerin sıcaklığa duyarlılık ölçüsü olan Q_{10} değerinin sıcaklığın düşmesi ile doğru orantılı olarak azaldığı bildirilmiştir (Sinclair et al., 2013).

Hayatta kalmak için kritik öneme sahip hipometabolizmanın kontrolünde ise epigenetik faktörler ve transkripsiyonel kontrol, hücre sinyal düzenlenmesi ve enerji kullanımı yüksek metabolik olayların baskılanması, hücre döngüsü yavaşlatılması ve anti apoptoz mekanizmaları rol oynar (Al-attar et al., 2020; Storey & Storey, 2007). Hipometabolik evreye geçişte hücre döngüsü, moleküler adaptasyonlar, enzim aktiviteleri, protein ve RNA sentezi, iyon kanallarında taşıma işlemleri gibi süreçlerin baskılanması büyük oranda ATP kullanımını azaltarak yeni bir homeostaz oluşumunu sağlar. Örneğin, kış uykusundaki yer sincabı (*Spermophilus lateralis*)'nın birçok dokusunda ATPaz aktivitesinin %40'a kadar düştüğü belirlenmiştir (MacDonald & Storey, 1999).

Hipometabolizmayı etkileyen mekanizmalardan epigenetik faktörler de donma toleransında nesiller boyu aktarılabilecek özelliklerin oluşmasını sağlayabilir. Epigenetik varyasyonlar, çevresel strese adaptasyonda DNA dizilerindeki varyasyonlardan daha yüksek bir oranda oluşabilir. Örneğin Storey & Storey (2017)'de son buzullaşmanın ardından kuzeye doğru genişleyen *R. sylvatica* popülasyonlarında hayatta kalma limitlerinin gelişmesindeki kilit unsurun epigenetik kalıtım olabileceğini belirtmiştir. Bu türün hayatta kalma limitleri Ontario bölgesi hayvanlarında -5°C, Alaska hayvanlarında ise -16°C olarak kaydedilmiştir (Costanzo et al., 2013). Çevresel streslere karşı hipometabolizmayı düzenleyici yanıtlardan bir diğeri de miRNA'lardır. *R. sylvatica* ve *D. chrysoscelis* ile yapılan çalışmalar donmaya yanıt olarak karaciğer ve kas dokularında hücre döngüsünün yavaşlatılmasında ve apoptozisin engellenmesinde rol alan miR16, miR2 ve miR30'un ekspresyonlarında artış olduğunu göstermiştir (Amaral et al., 2020; Biggar et al., 2009). Bunların dışındaki birçok miRNA'nın ekspresyonun enerji yönetimi ve nöroprotektif etki amaçlı olarak azaldığı düşünülmektedir. Hücre döngüsü kontrolünde aktif rol oynayan bazı siklin bağlı kinazların (Cdk) *R. sylvatica*'da donma, anoksi ve dehidrasyon koşullarında %50-60 oranında azalarak, Cdk inhibe eden proteinlerin fosforilasyonunun arttığı tespit edilmiştir (Storey & Storey, 2017; Tan et al., 2013).

Solunum, dolaşım, sinyal iletimi ve kas hareketleri gibi yaşamsal faaliyetlerin durduğu donma sırasında hayatta kalabilmek için ihtiyaç duyulan enerji eldesi anaerobik enerji üretim yöntemi ile sağlanır. Yeterli glikan depolama ve gelişmiş glikolitik kapasite vasıtasıyla uzun süre ATP sentezi sağlanabilir. Glikoz, gliserol ve ürenin donma toleranslı amfibilerde enerji üretiminde ve kriyoprotektan olarak sıklıkla kullanıldığı bilinmektedir (Yoldas & Erismis, 2021).

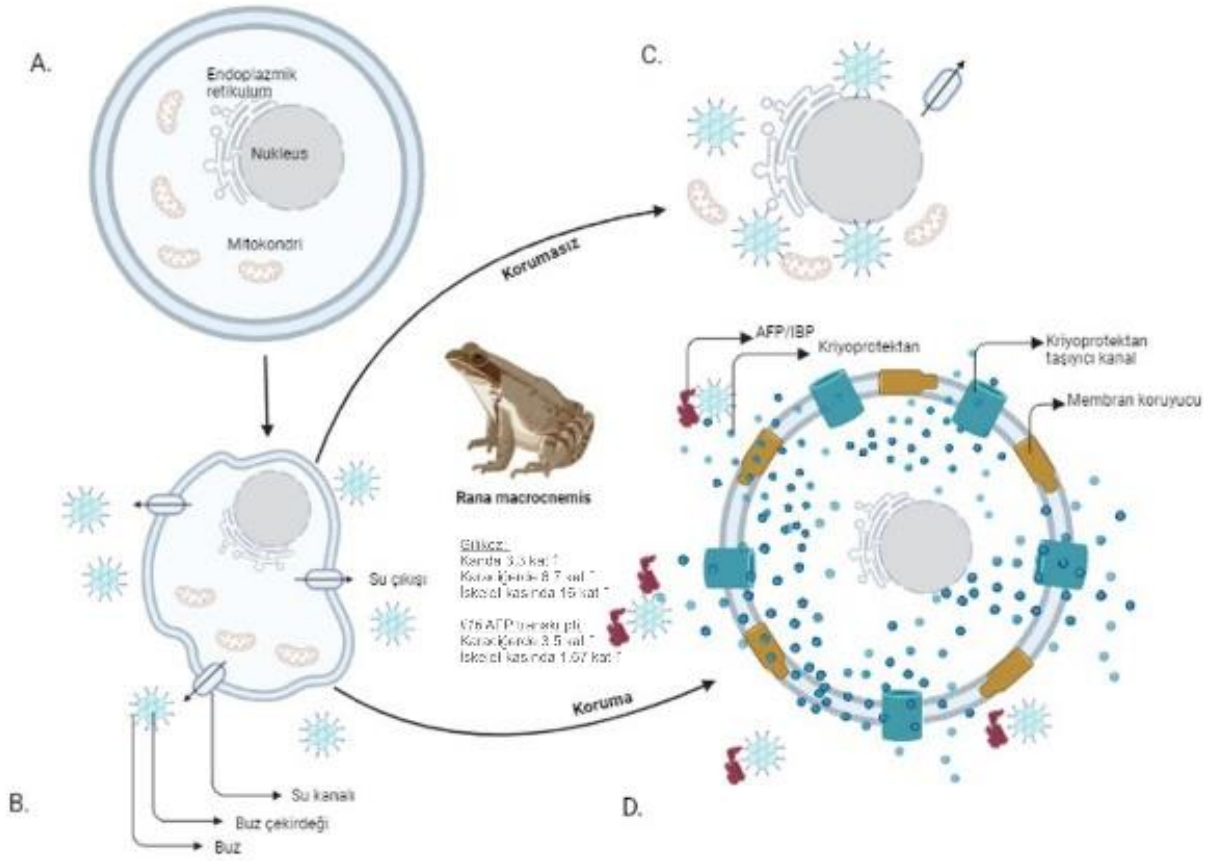
4. Kriyoprotektanlar

Donma ve donmaya bağlı streslere maruz kalan ektotermik canlıların kullandığı en önemli strateji yüksek miktarlarda metabolit biriktirmektir. Kriyoprotektan olarak adlandırılan bu metabolitler, hücre iç ve dış bölümlerinde yer alarak ozmolalitenin yükselmesine dolayısı ile de donma noktasının düşmesine sebep olurlar ve böylece hücreyi buzlanmanın etkilerinden korurlar (Şekil 2). Kriyoprotektan madde olarak karbonhidratlar, proteinler (IBP, BÇP, AFP'ler gibi) üre ve antioksidan maddeler kullanılabilir.

Glikoz, gliserol ve üre en yaygın kullanılan kriyoprotektanlar olarak tespit edilmiştir. Kurbağalarda da oldukça yaygın olan bu kriyoprotektanlardan gliserol ve üre *D. versicolor* ve *D. chrysoscelis*'de, glikoz da *R. sylvatica*, *Pseudacris triseriata*, *P. maculata* ve *P. crucifer* gibi birçok türde baskın kriyoprotektan metabolit olarak rol aldığı tespit edilmiştir (Amaral et al., 2018; Costanzo et al., 2015).

Donma sürecinde rol aldığı tespit edilerek buz çekirdekleyici (BÇP), buz şekillendirici (BŞP, en: ISP, *ice structuring protein*) ve antifriz proteinler (AFP) olarak isimlendirilen özel protein grupları buzlanmanın tetiklenmesi, baskılanması ya da şekillendirilmesini

sağlayarak donma sürecinin kontrolünü sağlarlar. BÇP'ler özellikle böcekler tarafından kullanılan, hemolenf sıvısının ozmolalitesinin arttırılarak hücrelerin dehidrasyonunu sağlayan, AFP'ler ise çoğunlukla donma toleranslı türlerde buz kristallerinin büyümesini engellemede etkin proteinlerdir (Duman, 2001; Olijve et al., 2016; Storey & Storey, 2017).



Şekil 2. Donma koşullarında korunan ve korunmayan hücrelerin durumu.

Figure 2. Illustration of cryoprotected and unprotected cells under freezing conditions.

Canlılarda kriyobiyolojik çalışmalar devam ettikçe donma ve çözünme süreçlerinde rol alan yeni moleküller ve bu moleküllerin detaylı işlevleri ortaya konmaya devam edecektir. Dondurucu kış dönemlerinde kullanılan tüm bu stratejiler ile ektotermik hayvanlar hücre içi buz oluşumunu engeller, anoksi ve dehidrasyon ile başa çıkabilir ve çözünme sürecindeki oksidatif stresleri bertaraf ederek zorlu koşullarda habitatlarında var olmaya devam edebilirler.

4.1. Glikoz, Gliserol ve Üre

Hücrelerde kriyoprotektanların biriktirilmesi donmaya dayanıklılıkta en önemli faktörlerden biridir. Sıklıkla omurgasız hayvanlar tarafından kullanılan teraloz ve prolin gibi hücre membranı ile etkileşimde olanlar donma sürecinde hacmi azalan hücrenin membranındaki çift katlı yapının stabil kalmasına destek olurlar. Daha düşük molekül ağırlıklı glikanlardan oluşan glikoz, gliserol, laktat gibi moleküllerin yüksek konsantrasyonlarda biriktirilmesi hücredeki su kaybını sınırlayarak kolligatif bir yapı ile minimum hücre hacminin korunmasını sağlar (Storey et al., 2021; Storey et al., 1997). Karasal omurgasızlar donmadan kaçınma ve donma toleransında kriyoprotektan olarak fazla miktarda gliserol ve/veya sorbitol, ribitol, ve etilen glikol gibi diğer polihidrik alkolleri biriktirirler (Duman et al., 1991). Omurgalılardan donma toleranslı kurbağaların kullandığı en yaygın kriyoprotektanların ise glikoz, gliserol ve üre olduğu belirlenmiştir (Amaral et al., 2020; Costanzo, 2005; Niu et al., 2018).

Çoğu organizma temel enerji eldesini glikolizis ile sağlar ve kullanabileceğinden fazla glikoz ve diğer glikanları da glikojenez ile glikojene dönüştürerek depolar. Omurgalı hayvanlarda karbonhidratların depolanma formu olan glikojen, önemli bir enerji deposu olmasının yanı sıra donmadan korunmada da önemli bir kaynak rolündedir. Glikojen kurbağalarda aktif dönemde enerji kaynağı olmanın yanında pasif dönemde de bazal metabolizmayı destekleyici unsurdur. Kış döneminde donma toleranslı kurbağalar için çok önemli bir kriyoprotektan kaynağıdır (Dieni et al., 2012; Sinclair et al., 2013). Kurbağalardaki glikojen rezervleri üreme dönemi ile en alt seviyeye ve kış öncesi dönemde de en üst seviyeye ulaşır. Kış dönemi öncesi *R. sylvatica* karaciğer ağırlığının %18'i kadar yüksek seviyelerde glikojen depoladığı kaydedilmiştir (Storey et al., 1997). Bunun dışında çevresel şartların glikojen rezervlerini etkilediği bilinmektedir. Örneğin Alaska bölgesindeki *R. sylvatica* bireylerinde Ohio'dakilere göre daha yüksek glikojen sentaz aktivitesi tespit edilmiştir (Costanzo et al., 2013).

R. sylvatica ile yapılan çalışmalarda donma başladığında en fazla tepki veren kriyoprotektanın glikoz

olduğu, 5 mM'den 250 mM (Alaska bölgesi hayvanlarında 800 mM'a kadar) seviyelerine yükseldiği belirtilmiştir (Costanzoet al., 2013; Costanzo, 2019; Hawkins et al., 2019). Glikoz donmanın yanı sıra anoksi ve dehidrasyon koşullarında da artmaktadır. Örneğin dehidrasyon koşullarında donma toleransı bulunmayan *R. pipiens*'in karaciğer dokusundaki glikoz seviyesi kontrole göre 24 kat artış göstermiştir. Bu oran aynı koşullara maruz bırakılan donma toleranslı türlerin (*R. sylvatica* ve *P. crucifer*) tepkisinden bile fazla olarak kaydedilmiştir (Churchill & Storey, 1994, 1995). Donma toleranslı kurbağalardaki başarı donma ile tetiklenen glisemik cevaba bağlıdır. Glikojenoliz ile karaciğerde sentezlenen glikoz, dolaşım durmadan önce diğer dokulara hızlıca taşınır (Hawkins et al., 2019; Storey & Storey, 2004). Bu süreçte plazma membranlarındaki glikoz taşıyıcıların da sayısı artarak organlardaki glikoz birikimi de hızlandırılır (Roy & Goswami, 2019).

Soğuğa karşı cevap stratejilerinde çoğunlukla omurgasızların kullandığı kriyoprotektan metabolit ise gliseroldür (Duman & Newton, 2020; Storey & Storey, 2012). Amfibilerden *Salamandrella keyserlingii* ve *Hylid* türü kurbağaların da gliserolü donma toleransında donmadan koruyucu molekül olarak kullandığı kaydedilmiştir. *Dryophytes chrysoscelis* ve *D. versicolor* (*Hyla versicolor*) türlerinde donma maruziyeti sonrası gliserol seviyesinin 300 - 425 mM seviyelerine yükseldiği bildirilmiştir (Layne & Lee, 1989; Stogsdill et al., 2017).

Hücre hasarı açısından gliserolün sentezi ve kullanımı glikoza göre daha avantajlı olsa da gliserolün üretimi enerji maliyeti açısından ve daha kompleks yolaklar gerektirmesi yönüyle dezavantajlıdır. Amfibilerde kriyoprotektan olarak kullanılacak karbonhidratın seçimi evrimsel bir sıçrama olabileceği gibi semenderlerin gliserolü kullanması maruz kalınan aşırı soğuk çevre koşullarına da bağlı olabilir (Layne & Stapleton, 2009; Storey & Storey, 2017).

Kriyoprotektan olarak kullanılan bir diğer önemli molekülün üre olduğu bilinmektedir. Üre, özellikle hücre hacmindeki aşırı azalmayı engellemek için kolligatif direnç sağlayan ve ozmolalitenin artışına büyük katkı sağlayan bir metabolittir (Costanzo et al., 2013; Costanzo & Lee, 2008). Hayvanlarda yıl boyunca vücuttaki hidrasyona bağlı değişikliklere ve kış uykusu döneminde çevrenin kuraklaşmasına yanıt olarak etki gösteren üre, nadiren de olsa donma toleranslı kurbağalar tarafından donmaya yanıt olarak da kullanılmaktadır (Costanzo, 2005; Hawkins et al., 2019). Alaska bölgesindeki *R. sylvatica*'da soğuğa tepki olarak plazma üre seviyesi 8.6 kat artmıştır. Aynı türün dehidrasyona maruziyetinde ise bu artış 18.7 kata kadar çıkmıştır (Costanzo et al., 2013). *D. chrysoscelis* gliserolün yanında üreyi de kriyoprotektan olarak kullanmaktadır. Bunların dışında Tibet platasonda yayılış gösteren *Nanorana pleskei* ve *N. parkeri*'nin de donma maruziyetlerinde üre, maltotrioz ve melezitoz moleküllerinin kriyoprotektan olarak kullanılabileceği belirtilmiştir (Niu et al., 2018, 2021). Karasal kurbağalarda ortamdaki suyun azalmasına bağlı olarak biriken üre yüksek ozmolalite ile vücuttan su kaybını geciktirir ve su alımını teşvik eder. Neredeyse tüm kurbağalarda dehidrasyon, estivasyon sürecindeki kuraklık ya da aşırı tuzlu şartlara karşı bir savunma aracı olarak kullanılmaktadır (Hillman et al., 2008; Storey & Storey, 2017).

4.2. Antifriz Proteinler

Buzlanmanın kontrol edilmesinde buz çekirdekleyici, buz bağlayıcı ya da antifriz proteinler olarak adlandırılan protein grubpları tespit edilmiştir (Duman, 2015; Duman & Newton, 2020).

Donmanın başlaması buzlanma yapılanmasını kontrol eden proteinler (BÇP, buz çekirdekleyici protein ve BŞP, buz şekillendirici protein) tarafından da gerçekleşebilir. BÇP'ler hücre dışında kristallenmeyi artırarak buzun ulaşmadığı hücrelerin çevresindeki ozmolalitenin artmasını sağlar. Böylelikle bu bölgelerdeki hücrelerin buzlanması ya da aşırı soğuması engellenir (Duman & Newton, 2020; Bektaş & Altıntaş, 2007; Storey et al., 2021). Antifriz proteinler (AFP) buz kristallerinin büyümesini kontrol ederek buzun rekristalizasyonunu yani daha tehlikeli boyutlara ulaşmasını engellerler (Storey & Storey, 2017).

Buzlanmanın artmasını ve rekristalizasyonunu önleyen AFP'ler Antartktik teleost balıklarında (Notothenioidei) keşfedilmiştir (Devries, 1971). Normal koşullardaki teleost balıklarda kan dokusunun donma noktası -0.5°C iken, Antartik ve subantartik bölgelerde yaşayan Nototheniodei alttakımına ait balıklarda -1.9°C'ye kadar düştüğü kaydedilmiştir (Costanzo et al., 1995). Sonraki çalışmalarda AFP'lerin soğuk su balıklarında ve donmadan kaçınma stratejisi ile kışı atlatan böceklerde de bulunarak AFP 1-4 ve antifriz glikoprotein (AFGP) olarak sınıflandırılmıştır (Eskandari et al., 2020).

Günümüzdeki çalışmalar tıp, veteriner ve gıda endüstrisi alanlarındaki araştırmalarda yaygın araştırma ve kullanım alanına sahip olan AFP'lerin psikrofil mikroorganizmalar, bitkiler, nematotlar, böcekler ve ektoterm omurgalılar gibi birçok canlı grubunda var olduğunu göstermiştir (Naing & Kim, 2019). Adsorbsiyon-inhibisyon mekanizmasına sahip olan AFP'ler buz yüzeyine adsorbe olurlar ve termal histerezis etkileri ile rekristalizasyonu ve buzlanmanın artmasını kısıtlarlar. Bu özellikler AFP'lerin donmuş gıda endüstrisinde gıdalarda raf ömrünün uzatılmasında, tıp alanında da organ nakli, trombositlerin korunması ve habis tümörlerin yok edilmesi gibi alanlarda kullanım avantajı bulmasını sağlamaktadır (Storey & Storey, 2017; Tejo et al., 2020). Bunların dışında AFP'lerin sert kış koşullarına sahip bölgelerdeki ekinlerin korunması, sıcak su balıklarının daha soğuk ortamlarda yetiştirilmesi, transplant ve transfüzyon dokularının daha iyi ve uzun süre saklanması, kriyocerrahinin geliştirilmesi ve hipotermi tedavisi gibi farklı alanlarda da kullanılabileceği belirtilmiştir (Bektaş & Altıntaş, 2007).

Omurgalılardaki donma toleransı çalışmalarındaki model organizma statüsünde olan *R. sylvatica* karaciğer cDNA kütüphanesi ile gerçekleştirilen geçmiş dönem çalışmalarda donma ile ilişkili olarak bazı genlere odaklanılmıştır. Bunlardan bazıları daha önceden tanımlanmış fibrinojen alt üniteleri, ADP/ATP translokaz ve mitokondri fosfat taşıyıcılardan sorumlu genler iken üç adet yeni protein ve sorumlu genleri tanımlanmıştır. Amfibilere özgü FR10, FR47 ve Li16 olarak adlandırılan bu proteinlerin membranlarla ilişkili hidrofobik bölgelerinin bulunduğu, farklı sinyal yolakları ile donma, anoksi ve

dehidrasyon streslerinde görev alabildikleri ve farklı dokularda farklı ekspresyon seviyesi gösterebildikleri bildirilmiştir (Cai & Storey, 1997; McNally et al., 2002, 2003; Storey, 2004). Bu proteinlerle ilişkili olan genler (*fr10*, *fr47* ve *li16*) de farklı dokularda farklı seviyelerde ifade olmakla birlikte her üçünün de donmadan sorumlu olduğu gösterilmiştir (Storey, 2004; McNally et al., 2003).

İlk olarak *R. sylvatica* cDNA kütüphanesinden ortaya konulan *fr10* geni protein kodlayan bölgesinin mRNA dizisinin 550 bp olduğu, dizinin 90 amino asitlik (aa) ve 10 kDa büyüklüğünde FR10 proteininin kodlandığı bir açık okuma bölgesine sahip olduğu tespit edilmiştir (Cai & Storey, 1997). Donma toleransında rol alan, 2002 yılında tanımlanan yeni bir protein olan Li16 (115 aa)'nın sentezinden sorumlu olan *li16* geni transkripti 446 bp uzunluğa sahip olup, Li16 proteinin hesaplanan kütlesi 12.8 kDa iken western blot çalışmaları proteinin 15 kDa civarında olduğunu göstermiştir (McNally et al., 2002). Yine *R. sylvatica* çalışmalarında tespit edilen ve en büyük AFP'lerden biri olma özelliğine sahip FR47 proteinine ait *fr47* geni 3678 bp uzunluğundadır (McNally et al., 2003). Yapılan teorik hesaplamalar ve western blot analizleri proteinin yaklaşık 47 kDa ağırlığa sahip olduğunu göstermiştir. Protein yapısındaki belirli bölgelerin güçlü hidrofobik yapıda olması proteine muhtemelen transmembran özellik kazandırdığı bildirilmiştir (Storey & Storey, 2004; Sullivan et al., 2015).

R. sylvatica'nın donmaya (24 saat -2.5°C) maruz bırakılması sonrası *fr10* mRNA seviyesinin karaciğer ve bağırsaklarda artış gösterdiği; kalp, akciğer, mesane ve beyin dokularında orta seviyede yükseldiği ve böbrek dokusunda ise azalış gösterdiği tespit edilmiştir. İskelet kasında ise gen ekspresyon seviyesi stabil kalmıştır (Cai & Storey, 1997; Storey & Storey, 2001). Yine *R. sylvatica*'nın 24 saat donma maruziyeti sonrası *li16* transkript seviyesinin karaciğerde 3.7 kat arttığı kaydedilmiştir. Li16 protein seviyesinin de transkript seviyesine paralel olarak 2.4 kat arttığı tespit edilmiştir. Karaciğer dokusundaki gen ve protein ekspresyon seviyelerinin anoksi maruziyetinde de artış yönünde tetiklenirken dehidrasyon stresine tepkisi çok fazla olmamıştır. Ekspresyon seviyeleri anoksi sonrası normalleşme sürecinde ise hızla azalış göstermiştir. Bu sonuçlar doğrultusunda Li16 proteinin donma esnasındaki iskemi durumuna direnç göstermede rol oynayabileceği belirtilmiştir (McNally et al., 2002; Sullivan & Storey, 2012). Donma toleransından sorumlu genler üzerine yapılan araştırmalarda *fr47* geni mRNA'sının donma toleranslı *R. sylvatica*, *H. versicolor* ve *P. crucifer* türlerinde ifade edilirken *R. pipiens* ve *Scaphiopus couchii* gibi donma toleranslı olmayan türlerde olmadığı tespit edilmiştir. *R. sylvatica* karaciğer dokusunda *fr47* ekspresyon seviyesi 24 saat donma sonrası 5.1 kat, 24 saat anoksi sonrası 6.4 kat ve dehidrasyon sonrası 2.7 kat arttığı tespit edilmiştir. Yapılan immunoblotlama çalışmaları da donma ve çözünme sürecinde protein seviyesinin arttığını, anoksi ve dehidrasyon süreçlerinde de bir miktar azalış olduğunu göstermiştir (McNally et al., 2003; Sullivan et al., 2015).

Anadolu dağ kurbağalarından *Rana macrocnemis* ve endemik *R. holtzi* ile yapılan bir çalışmada donma, anoksi, dehidrasyon stresleri ve stres sonrası normalleşme süreçlerindeki *fr10*, *fr47* ve *li16* gen ekspresyon seviyeleri araştırılmıştır. Doğu Anadolu bölgesinden 2000 m ve üzeri rakımdan toplanan *R. macrocnemis* karaciğer dokusunda donma maruziyeti (24 saat -2.5°C) *li16* transkript seviyesini yaklaşık 3.5 kat, anoksi stresi ise *fr47* mRNA ekspresyonunu 2.7 kat arttırmıştır. Bolkar dağlarında endemik olarak kabul edilen ve 2500 m rakımdan toplanan *R. holtzi* karaciğer dokusunda donma, anoksi ve dehidrasyon koşullarının hepsi *fr10* gen ekspresyon seviyesinin artış göstermesine sebep olmuştur (Yoldaş, 2021).

4.3. Antioksidanlar

Organizmalar birçok strese karşı antioksidan savunma sistemi ile cevap oluştururlar (Kültz, 2005). Donma toleranslı hayvanlarda oksijen seviyesine bağlı oluşan hipoksi ya da anoksi streslerine tepki olarak antioksidan savunma sistemi rol oynar (Lima & Savin, 2002; Storey & Storey, 2017). Metabolik hızın düşük olduğu kış döneminde maruz kalınan donma, ultraviyole radyasyon ve oksijene erişememe gibi abiyotik faktörler ile donma ve çözünme döngüsündeki oksijen seviyesi ROS oluşumunu arttırır. Bu ROS seviyesine bağlı olarak iskemi - reperfüzyonu hasarlarının felç ve kalp krizi ile ilişkili olduğu bilinmektedir (Storey et al., 2021; Storey & Storey, 2013, 2017).

Donmuş bir hayvan donma, anoksi ve dehidrasyon koşulları ortadan kalktığında başlayan normalleşme sırasında sisteme oksijenin hızlı girmesi sonucu oluşan reaktif oksijen türleri (ROS)'nin saldırısına uğrar. ROS'lar hücresel düzeyde protein, lipit ve hatta DNA yapılarında hasara sebep olabilirler (Storey & Storey, 2012). Donma toleranslı kurbağalardan *R. sylvatica*'nın normalleşme sürecinde antioksidan rolü olan glutatyon peroksidaz enziminin böbrek, kalp ve iskelet kasında neredeyse iki katına çıktığı bildirilmiştir (Joanisse & Storey, 1996). Benzer çalışmalarda antioksidan savunmada temel olarak süperoksit dismutaz, glutatyon peroksidaz, glutatyon redüktaz, ve glutatyon S-transferaz enzimlerinin rol aldığı belirlenmiştir (Lima & Savin, 2002; Storey & Storey, 2017).

Anoksi toleranslı kaplumbağalar ve kış uykusundaki memeliler gibi hareket kapasitesi ve oksijene erişimi daha fazla olan hayvanların yapısal antioksidan savunmalarının ve mevsimsel değişken antioksidan kapasitelerinin yüksek olduğu belirtilmiştir (Lima & Savin, 2002; Storey & Storey, 2017). Gelgit bölgelerindeki yumuşakçalar ve böcekler de dahil birçok donma toleranslı türün incelenmesi sonucu antioksidan savunmanın başarılı bir donma toleransında önemli faktörlerden biri olduğu gösterilmiştir (Storey & Storey, 2017; Tang et al., 2021).

5. Tartışma ve Sonuç

Donma toleranslı türler; doğal ortamlarında haftalarca boyunca hareketsiz, bir buz parçası formunda hayatta kalabilirler ve tekrar normale dönebilirler. Anadolu dağ kurbağalarından *R. macrocnemis* de laboratuvar koşullarında donduktan sonra tekrar hayatta kalabilen türler arasına girmiştir (Şekil 3). Bunu sağlayan hayvanların sahip oldukları biyokimyasal ve ekofizyolojik adaptasyonlardır. Genel olarak organlardaki dehidrasyon, hücre içi buzlanmayı önlemek için metabolit biriktirilmesi, anaerobik enerji üretimi, buzlanmanın kontrol edilmesi ve oksidatif streslerin bertarafı gibi stratejilerin sistematik olarak kullanılması donma sürecinde hayatta kalmayı

sağlamaktadır. Böylece bu ektotermik özel türler zorlu iklimsel koşullara rağmen habitatlarında varlıklarını devam ettirebilirler.

Şekil 3. Donma sonrası *Rana macrocnemis*'e ait genel görünüş (Yoldaş, 2021).

Figure 3. General view of *Rana macrocnemis* after freezing (Yoldaş, 2021).

AFP'lerin tıp, gıda ve tarım endüstrilerinin yanında birçok farklı alanlarda kullanım avantajı sağlayabileceği öngörülmektedir. Son yıllarda en çok ilgi çeken konulardan birisi de insanların dondurulmasıdır. Uluslararası bazı araştırma enstitüleri ve özel şirketler hastaların klinik ölümünden sonra kriyoprotektanlar kullanılarak dondurulması konusunu araştırmaktadır. Fakat teoride vitrifikasyonun sinir hücreleri ve beyin dokusuna çok fazla hasar vereceğinden dolayı tekrar hayata dönmenin mümkün olmayacağı düşünülmektedir. Bunun dışında yasal sorunlar ve etik açıdan uygun görülmemesi sebebi ile insanlar üzerinde kriyonik uygulamalar büyük oranda sekteye uğramaktadır.

Zorlu çevre koşullarında poikiloterm canlıların hayatta kalma stratejilerinin aydınlatılması biyoçeşitliliğin korunmasına da katkı sağlayabilir. Türlerin çevreye bağlı hayatta kalma sınırlarının belirlenmesi koruma planlarının yapılmasında, bölgedeki tür çeşitliliğin arttırılmasında, üreme verimliliği çalışmalarında ve istilacı türler ya da patojen organizmalar ile mücadele gibi konularda doğru stratejilerin belirlenmesini sağlayabilir.

Sonuç olarak AFP'lerin ve kriyoprotektif maddelerin birçok alanda kullanılma potansiyeli; hayatı kolaylaştırma, gıda problemleri, tedavi ve sıra dışı koşullarda hayatta kalma gibi konularda insanlığa avantajlar sağlayacaktır. Bu kapsamda ilerleyebilmek için kriyobiyolojik çalışmalara daha fazla önem verilmesi, hangi türlerde hangi kriyoprotektanların kullanıldığı ve kullanım stratejilerinin ne olduğunun daha iyi anlaşılması gerekmektedir.

Etik kurul onayı: Bu çalışma için etik kurul onayı alınmasına gerek yoktur.

Çıkar çatışması: Yazarlar, çıkar çatışması olmadığını beyan etmiştir.

Yazar katkısı: Fikir/Kavram – T.Y., U.C.E.; Tasarım – T.Y.; Denetleme/Danışmanlık – T.Y.; Kaynaklar/Fon Sağlama – U.C.E.; Materyaller – T.Y., U.C.E.; Veri Toplama veya İşleme – T.Y.; Analiz Yorumlama – U.C.E.; Kaynak Taraması – T.Y.; Makalenin Yazımı – T.Y.; Eleştirel İnceleme – T.Y., U.C.E.

Kaynaklar

Al-attar, R., Wu, C.-W., Biggar, K. K., & Storey, K. B. (2020). Carb-Loading: Freeze-Induced Activation of the Glucose-Responsive ChREBP Transcriptional Network in Wood Frogs. *Physiological and Biochemical Zoology*, *93*(1), 49–61. https://doi.org/10.1086/706463

Amaral, M. C. F. do, Frisbie, J., Crum, R. J., Goldstein, D. L., & Krane, C. M. (2020). Hepatic Transcriptome of the Freeze-Tolerant Cope's Gray Treefrog, *Dryophytes chrysoscelis* : Responses to Cold Acclimation and Freezing. *BMC Genomics*, 21(1), 1–18. https://doi.org/10.1186/s12864-020-6602-4

Amaral, M. C. F. do, Frisbie, J., Goldstein, D. L., & Krane, C. M. (2018). The Cryoprotectant System of Cope's Gray Treefrog, *Dryophytes chrysoscelis*: Responses to Cold Acclimation, Freezing, And Thawing. *Journal of*

Comparative Physiology B, 188(4), 611–621. https://doi.org/10.1007/s00360-018-1153-6

Baker, P. J., Costanzo, J. P., Iverson, J. B., & Lee, R. E. (2003). Adaptations to terrestrial overwintering of hatchling northern map turtles, *Graptemys geographica*. *Journal of Comparative Physiology B*, 173(8), 643–651. https://doi.org/10.1007/s00360-003-0373-5

Bektaş, G. I., & Altıntaş, A. (2007). Antifriz Proteinler. *Etlik Veteriner Mikrobiyoloji Dergisi*, 18, 27–32.

Berman, D. I., Bulakhova, N. A., & Meshcheryakova, E. N. (2017). Adaptive strategies of brown frogs (amphibia, anura, Rana) in relation to winter temperatures in the northern Palaearctic. *Zoologicheskii Zhurnal*, 96(11), 1392–1403. https://doi.org/10.7868/S0044513417110034

Berman, D. I., Bulakhova, N. A., Meshcheryakova, E. N., & Shekhovtsov, S. V. (2020). Overwintering and cold tolerance in the Moor Frog (*Rana arvalis*) across its range. *Canadian Journal of Zoology*, 98(11), 705–714. https://doi.org/10.1139/cjz-2019-0179

Berman, D. I., Leirikh, A. N., & Meshcheryakova, E. N. (2010). The Schrenck Newt (*Salamandrella schrenckii*, Amphibia, Caudata, Hynobiidae) is The Second Amphibian That Withstands Extremely Low Temperatures. *Doklady Biological Sciences*, 431(1), 131–134. https://doi.org/10.1134/S0012496610020171

Berman, D. I., & Meshcheryakova, E. N. (2012). Is the western boundary of the Siberian salamander (*Salamandrella keyserlingii*, Amphibia, Caudata, Hynobiidae) range determined by the specific features of its wintering? *Doklady Biological Sciences*, 443(1), 97–100. https://doi.org/10.1134/S0012496612020068

Biggar, K. K., Dubuc, A., & Storey, K. (2009). MicroRNA Regulation Below Zero: Differential Expression of miRNA-21 and miRNA-16 During Freezing in Wood Frogs. *Cryobiology*, 59(3), 317–321. https://doi.org/10.1016/j.cryobiol.2009.08.009

Biggar, K. K., Kotani, E., Furusawa, T., & Storey, K. B. (2013). Expression of freeze-responsive proteins, Fr10 and Li16, from freeze-tolerant frogs enhances freezing survival of BmN insect cells. *FASEB Journal*, 27(8), 3376–3383. https://doi.org/10.1096/fj.13-230573

Bulakhova, N. A., Mazanaeva, L. F., Meshcheryakova, E. N., & Berman, D. I. (2020). Resistance of the Iranian long-legged wood frog (*Rana macrocnemis* Boulenger, 1885) (Amphibia, Anura) to negative temperatures on land and to hypoxia in water during overwintering. *Herpetology Notes*, 13(December), 1079–1086.

Cai, Q., & Storey, K. B. (1997). Upregulation of a Novel Gene by Freezing Exposure in The Freeze-Tolerant Wood Frog (*Rana sylvatica*). *Gene*, 198(1–2), 305–312. https://doi.org/10.1016/S0378-1119(97)00332-6

Churchill, T. A., & Storey, K. B. (1994). Effects of Dehydration on Organ Metabolism In The Frog Pseudacris crucifer: Hyperglycemic Responses to Dehydration Mimic Freezing-Induced Cryoprotectant Production. *Journal of Comparative Physiology B*, 164(6), 492–498. https://doi.org/10.1007/BF00714587

Churchill, T. A., & Storey, K. B. (1995). Metabolic Effects of Dehydration on An Aquatic Frog, *Rana pipiens*. *The Journal of Experimental Biology*, 198(Pt 1), 147–154. http://www.ncbi.nlm.nih.gov/pubmed/7891032

Costanzo, J. P. (2005). Cryoprotection By Urea In A Terrestrially Hibernating Frog. *Journal of Experimental Biology*, 208(21), 4079–4089. https://doi.org/10.1242/jeb.01859

Costanzo, J. P. (2019). Overwintering adaptations and extreme freeze tolerance in a subarctic population of the wood frog, *Rana sylvatica*. *Journal of Comparative Physiology B*, 189(1), 1–15. https://doi.org/10.1007/s00360-018-1189-7

Costanzo, J. P., Amaral, M. C. F. do, Rosendale, A. J., & Lee, R. E. (2013). Hibernation Physiology, Freezing Adaptation and Extreme Freeze Tolerance In A Northern Population of The Wood Frog. *Journal of Experimental Biology*, 216(18), 3461–3473. https://doi.org/10.1242/jeb.089342

Costanzo, J. P., Grenot, C., & Lee, R. E. (1995). Supercooling, ice inoculation and freeze tolerance in the European common lizard, *Lacerta vivipara*. *Journal of Comparative Physiology B*, 165(3), 238–244. https://doi.org/10.1007/BF00260815

Costanzo, J. P., & Lee, R. E. (2008). Urea Loading Enhances Freezing Survival and Postfreeze Recovery In A Terrestrially Hibernating Frog. *Journal of Experimental Biology*, 211(18), 2969–2975. https://doi.org/10.1242/jeb.019695

Costanzo, J. P., & Lee, R. E. (2013). Avoidance and Tolerance of Freezing In Ectothermic Vertebrates. *Journal of Experimental Biology*, 216(11), 1961–1967. https://doi.org/10.1242/jeb.070268

Costanzo, J. P., Lee, R. E., DeVries, A. L., Wang, T., & Layne, J. R. (1995). Survival mechanisms of vertebrate ectotherms at subfreezing temperatures: applications in cryomedicine. *The FASEB Journal*, 9(5), 351–352. https://doi.org/10.1096/fasebj.9.5.7896003

Costanzo, J. P., Lee, R. E., & Lortz, P. H. (1993). Physiological responses of freeze-tolerant and -intolerant frogs: clues to evolution of anuran freeze tolerance. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 265(4), R721–R725. https://doi.org/10.1152/ajpregu.1993.265.4.R721

Costanzo, J. P., Lee, R. E., & Ultsch, G. R. (2008). Physiological ecology of overwintering in hatchling turtles. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, 309A(6), 297–379. https://doi.org/10.1002/jez.460

Costanzo, J. P., Reynolds, A. M., Amaral, M. C. F. do, Rosendale, A. J., & Lee, R. E. (2015). Cryoprotectants and Extreme Freeze Tolerance In A Subarctic Population of The Wood Frog. *PLOS ONE*, 10(2), e0117234. https://doi.org/10.1371/journal.pone.0117234

Croes, S. A., & Thomas, R. E. (2000). Freeze tolerance and cryoprotectant synthesis of the Pacific Tree Frog *Hyla regilla*. *Copeia*, *3*, 863–868. https://doi.org/10.1643/0045-8511(2000)000[0863:FTACSO]2.0.CO;2

Devries, A. L. (1971). Glycoproteins As Biological Antifreeze Agents In Antarctic Fishes. *Science*, 172(3988), 1152–1155. https://doi.org/10.1126/science.172.3988.1152

Dieni, C. A., Bouffard, M. C., & Storey, K. B. (2012). Glycogen Synthase Kinase-3: Cryoprotection and Glycogen Metabolism In The Freeze-Tolerant Wood Frog. *Journal of Experimental Biology*, 215(3), 543–551. https://doi.org/10.1242/jeb.065961

Donoso, D. P., Bauer, A. M., Meiri, S., & Uetz, P. (2013). Global Taxonomic Diversity of Living Reptiles. *PLoS ONE*, 8(3), e59741. https://doi.org/10.1371/journal.pone.0059741

Duman, J. G. (2001). Antifreeze and Ice Nucleator Proteins in Terrestrial Arthropods. *Annual Review of Physiology*, 63(1), 327–357. https://doi.org/10.1146/annurev.physiol.63.1.327

Duman, J. G. (2015). Animal Ice-Binding (Antifreeze) Proteins And Glycolipids: An Overview With Emphasis on Physiological Function. *Journal of Experimental Biology*, 218(12), 1846–1855. https://doi.org/10.1242/jeb.116905

Duman, J. G., & Newton, S. S. (2020). Insect Antifreeze Proteins. In H. Ramløv & D. S. Friis (Eds.), *Antifreeze Proteins Volume 1* (Vol. 1, pp. 131–187). Springer International Publishing. https://doi.org/10.1007/978-3-030-41929-5_6

Duman, J. G., Wu D. W., Xu L., Tursman D., Olsen T. M. (1991). Adaptations of Insects to Subzero Temperatures, *The Quarterly Review of Biology*, 66(4), 387-410. https://doi.org/10.1086/417337

Eskandari, A., Leow, T. C., Rahman, M. B. A., & Oslan, S. N. (2020). Antifreeze Proteins and Their Practical Utilization in Industry, Medicine, and Agriculture. *Biomolecules*, 10(12), 1649. https://doi.org/10.3390/biom10121649

Geiser, F. (2004). Metabolic Rate and Body Temperature Reduction During Hibernation and Daily Torpor. *Annual Review of Physiology*, 66(1), 239–274. https://doi.org/10.1146/annurev.physiol.66.032102.115105

Geiser, F. (2020). Seasonal Expression of Avian and Mammalian Daily Torpor and Hibernation: Not a Simple Summer-Winter Affair†. *Frontiers in Physiology*, 11(May), 1–19. https://doi.org/10.3389/fphys.2020.00436

Güleç, S. (2019). Hibernasyonda 'Pelophylax caralitanus' (Amphibia: Anura)'da DNA Hasarının Araştırılması (536228). Retrieved from https://tez.yok.gov.tr/UlusalTezMerkezi/giris.jsp.

Hawkins, L. J., Wang, M., Zhang, B., Xiao, Q., Wang, H., & Storey, K. B. (2019). Glucose and urea metabolic enzymes are differentially phosphorylated during freezing, anoxia, and dehydration exposures in a freeze tolerant frog. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, *30*(December 2018), 1–13. https://doi.org/10.1016/j.cbd.2019.01.009

Hillman, S. S., Withers, P. C., Drewes, R. C., & Hillyard, S. D. (2008). Ecological and Environmental Physiology of Amphibians. In *Ecological and Environmental Physiology of Amphibians*. Oxford University Press. https://doi.org/10.1093/acprof:oso/9780198570325.001.0001

Hirota, A., Takiya, Y., Sakamoto, J., Shiojiri, N., Suzuki, M., Tanaka, S., & Okada, R. (2015). Molecular Cloning of cDNA Encoding an Aquaglyceroporin, AQP-h9, in the Japanese Tree Frog, *Hyla japonica*: Possible Roles of AQP-h9 in Freeze Tolerance. *Zoological Science*, 32(3), 296–306. https://doi.org/10.2108/zs140246

Irwin, J. T., & Lee, J. R. E. (2003). Geographic Variation In Energy Storage and Physiological Responses to Freezing In The Gray Treefrogs *Hyla versicolor* and *H. chrysoscelis*. *Journal of Experimental Biology*, 206(16), 2859–2867. https://doi.org/10.1242/jeb.00500

Jackson, D. C. (2002). Hibernating without Oxygen: Physiological Adaptations of the Painted Turtle. *The Journal of Physiology*, 543(3), 731–737. https://doi.org/10.1113/jphysiol.2002.024729

Joanisse, D. R., & Storey, K. B. (1996). Oxidative Damage And Antioxidants In *Rana sylvatica*, The Freeze-Tolerant Wood Frog. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 271(3), R545–R553. https://doi.org/10.1152/ajpregu.1996.271.3.r545

Kart Gür, M., Bulut, Ş., Gür, H., & Refinetti, R. (2014). Body Temperature Patterns And Use of Torpor In An Alpine Glirid Species, Woolly Dormouse. *Acta Theriologica*, 59(2), 299–309. https://doi.org/10.1007/s13364-013-0154-9

Kart Gür, M., & Gür, H. (2017). Küçük Bir Memeli Türünün Ekofizyolojisi ve Evrimsel Coğrafyası: Anadolu Yer Sincabı. *Kebikec: Insan Bilimleri Icin Kaynak Arastirmali Dergisi*, 43, 189–198.

Kelleher, M. J., Rickards, J., & Storey, K. B. (1987). Strategies of Freeze Avoidance In Larvae of The Goldenrod Gall Moth, *Epiblema scudderiana*: Laboratory Investigations of Temperature Cues In The Regulation of Cold Hardiness. *Journal of Insect Physiology*, 33(8), 581–586. https://doi.org/10.1016/0022-1910(87)90073-4

Kültz, D. (2005). Molecular and Evolutionary Basis of the Cellular Stress Response. *Annual Review of Physiology*, 67(1), 225–257. https://doi.org/10.1146/annurev.physiol.67.040403.103635

Layne, J. R., & Lee, R. E. (1987). Freeze tolerance and the dynamics of ice formation in wood frogs (*Rana sylvatica*) from southern Ohio. *Canadian Journal of Zoology*, 65(8), 2062–2065. https://doi.org/10.1139/z87-315

Layne, J. R., & Lee, R. E. (1989). Seasonal variation in freeze tolerance and ice content of the tree frog *Hyla versicolor*. *The Journal of Experimental Zoology*, 249(2), 133–137. https://doi.org/10.1002/jez.1402490203

Layne, J. R., & Stapleton, M. G. (2009). Annual Variation In Glycerol Mobilization and Effect of Freeze Rigor on Post-Thaw Locomotion In The Freeze-Tolerant Frog *Hyla versicolor*. *Journal of Comparative Physiology B*, 179(2), 215. https://doi.org/10.1007/s00360-008-0304-6

Lima, M. H., & Savin, T. Z. (2002). Animal Response to Drastic Changes In Oxygen Availability and Physiological Oxidative Stress. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 133(4), 537–556. https://doi.org/10.1016/S1532-0456(02)00080-7

MacDonald, J. A., & Storey, K. B. (1999). Regulation of Ground Squirrel Na+K+-ATPase Activity by Reversible Phosphorylation During Hibernation. *Biochemical and Biophysical Research Communications*, 254(2), 424–429. https://doi.org/10.1006/bbrc.1998.9960

Merlin, C., & Liedvogel, M. (2019). The Genetics and Epigenetics of Animal Migration and Orientation: Birds, Butterflies and Beyond. *Journal of Experimental Biology*, 222(Pt Suppl 1), jeb191890. https://doi.org/10.1242/jeb.191890

McNally, J. D., Sturgeon, C. M., & Storey, K. B. (2003). Freeze-Induced Expression of a Novel Gene, *fr47*, In The Liver of The Freeze-Tolerant Wood Frog, *Rana sylvatica*. *Biochimica et Biophysica Acta (BBA) - Gene Structure and Expression*, 1625(2), 183–191. https://doi.org/10.1016/S0167-4781(02)00603-6

McNally, J. D., Wu, S.-B., Sturgeon, C. M. C. M., & Storey, K. B. (2002). Identification and Characterization of a Novel Freezing Inducible Gene, *li16*, In The Wood Frog *Rana sylvatica*. *The FASEB Journal*, 16(8), 902–904. https://doi.org/10.1096/fj.02-0017fje

Naing, A. H., & Kim, C. K. (2019). A brief review of applications of antifreeze proteins in cryopreservation and metabolic genetic engineering. *3 Biotech*, 9(9), 329. https://doi.org/10.1007/s13205-019-1861-y

Niu, Y., Cao, W., Wang, J., He, J., Storey, K. B., Ding, L., Tang, X., & Chen, Q. (2021). Freeze tolerance and the underlying metabolite responses in the Xizang plateau frog, *Nanorana parkeri*. *Journal of Comparative Physiology B*, 191(1), 173–184. https://doi.org/10.1007/s00360-020-01314-0

Niu, Y., Wang, J., Men, S., Zhao, Y., Lu, S., Tang, X., & Chen, Q. (2018). Urea and Plasma Ice-Nucleating Pproteins Ppromoted The Modest Freeze Tolerance In Pleske's High Altitude Frog *Nanorana pleskei*. *Journal of Comparative Physiology B*, 188(4), 599–610. https://doi.org/10.1007/s00360-018-1159-0

Olijve, L. L. C., Meister, K., DeVries, A. L., Duman, J. G., Guo, S., Bakker, H. J., Voetsa, I. K., & Voets, I. K. (2016). Blocking Rapid Ice Crystal Growth Through Nonbasal Plane Adsorption of Antifreeze Proteins. *Proceedings of the National Academy of Sciences*, 113(14), 3740–3745. https://doi.org/10.1073/pnas.1524109113

Roy, P., & Goswami, P. (2019). Freeze tolerance in wood frogs. *Journal of Investigative Genomics*, 6(1), 1–4. https://doi.org/10.15406/jig.2019.06.00078

Sformo, T., Walters, K., Jeannet, K., Wowk, B., Fahy, G. M., Barnes, B. M., & Duman, J. G. (2010). Deep supercooling, vitrification and limited survival to -100°C in the Alaskan beetle *Cucujus clavipes puniceus* (Coleoptera: Cucujidae) larvae. *Journal of Experimental Biology*, 213(3), 502–509. https://doi.org/10.1242/jeb.035758

Sinclair, B. J., Stinziano, J. R., Williams, C. M., MacMillan, H. A., Marshall, K. E., & Storey, K. B. (2013). Real-time measurement of metabolic rate during freezing and thawing of the wood frog, *Rana sylvatica*: implications for overwinter energy use. *Journal of Experimental Biology*, 216(2), 292–302. https://doi.org/10.1242/jeb.076331

Stogsdill, B., Frisbie, J., Krane, C. M., & Goldstein, D. L. (2017). Expression of the aquaglyceroporin HC-9 in a freeze-tolerant amphibian that accumulates glycerol seasonally. *Physiological Reports*, 5(15). https://doi.org/10.14814/phy2.13331

Storey, J. M., Wu, S., & Storey, K. B. (2021). Mitochondria and the Frozen Frog. *Antioxidants*, 10(4), 543. https://doi.org/10.3390/antiox10040543

Storey, K. B. (2004). Strategies for exploration of freeze responsive gene expression: advances in vertebrate freeze tolerance. *Cryobiology*, 48(2), 134–145. https://doi.org/10.1016/j.cryobiol.2003.10.008

Storey, K. B., & Storey, J. M. (1986). Freeze tolerance and intolerance as strategies of winter survival in terrestrially-hibernating amphibians. *Comparative Biochemistry and Physiology Part A: Physiology*, 83(4), 613–617. https://doi.org/10.1016/0300-9629(86)90699-7

Storey, K. B., & Storey, J. M. (1987). Persistence of Freeze Tolerance in Terrestrially Hibernating Frogs after Spring Emergence. *Copeia*, 1987(3), 720. https://doi.org/10.2307/1445665

Storey, K. B., & Storey, J. M. (2001). Signal Transduction and Gene Expression In The Regulation of Natural Freezing Survival. *Cell and Molecular Response to Stress*, 2(613), 1–19. https://doi.org/10.1016/S1568-1254(01)80003-6

Storey, K. B., & Storey, J. M. (2004). Physiology, Biochemistry, and Molecular Biology of Vertebrate Freeze Tolerance: The Wood Frog. In E. Benson, B. Fuller, & N. Lane (Eds.), *Life in the Frozen State* (Vol. 274, pp. 243–274). CRC Press. https://doi.org/10.1201/9780203647073.ch7

Storey, K. B., & Storey, J. M. (2007). Tribute to P. L. Lutz: Putting Life on `Pause' - Molecular Regulation of Hypometabolism. *Journal of Experimental Biology*, 210(10), 1700–1714. https://doi.org/10.1242/jeb.02716

Storey, K. B., & Storey, J. M. (2012). Insect cold hardiness: metabolic, gene, and protein adaptation. *Canadian Journal of Zoology*, 90(4), 456–475. https://doi.org/10.1139/z2012-011

Storey, K. B., & Storey, J. M. (2013). Molecular Biology of Freezing Tolerance. *Comprehensive Physiology*, 3(3), 1283–1308. https://doi.org/10.1002/cphy.c130007

Storey, K. B., & Storey, J. M. (2017). Molecular Physiology of Freeze Tolerance In Vertebrates. *Physiological Reviews*, 97(2), 623–665. https://doi.org/10.1152/physrev.00016.2016

Storey, K. B., Storey, J. M., & Churchill, T. A. (1997). De Novo Protein Biosynthesis Responses to Water Stresses In Wood Frogs: Freeze–Thaw and Dehydration–Rehydration. *Cryobiology*, 34(3), 200–213. https://doi.org/10.1006/cryo.1997.2001

Storey, K. B.., & Storey. (2011). Heat shock proteins and hypometabolism: adaptive strategy for proteome preservation. *Research and Reports in Biology*, 57. https://doi.org/10.2147/RRB.S13351

Sullivan, K. J., Biggar, K. K., & Storey, K. B. (2015). Expression and Characterization of the Novel Gene *fr47* During Freezing In The Wood Frog, *Rana sylvatica*. *Biochemistry Research International*, 2015, 1–8. https://doi.org/10.1155/2015/363912

Sullivan, K. J., & Storey, K. B. (2012). Environmental Stress Responsive Expression of The Gene *li16* In *Rana sylvatica*, The Freeze Tolerant Wood Frog. *Cryobiology*, 64(3), 192–200. https://doi.org/10.1016/j.cryobiol.2012.01.008

Tan, Y. J., Xiong, Y., Ding, G. L., Zhang, D., Meng, Y., Huang, H. F., & Sheng, J. Z. (2013). Cryoprotectants up-regulate expression of mouse oocyte AQP7, which facilitates water diffusion during cryopreservation. *Fertility and Sterility*, 99(5), 1428–1435. https://doi.org/10.1016/j.fertnstert.2012.11.049

Tang, Z., Chen, B., & Niu, C. (2021). Antioxidant defense response during hibernation and arousal in Chinese soft-shelled turtle *Pelodiscus sinensis* juveniles. *Cryobiology*, 99(January), 46–54. https://doi.org/10.1016/j.cryobiol.2021.01.015

Tattersall, G. J., & Ultsch, G. R. (2008). Physiological Ecology of Aquatic Overwintering in Ranid Frogs. *Biological Reviews*, 83(2), 119–140. https://doi.org/10.1111/j.1469-185X.2008.00035.x

Tejo, B. A., Asmawi, A. A., & Rahman, M. B. A. (2020). Antifreeze Proteins: Characteristics and Potential Applications Bimo. *Makara Journal of Science*, 24(1), 58–64. https://doi.org/10.7454/mss.v24i1.11728

Ultsch, G. R. (2006). The Ecology of Overwintering Among Turtles: Where Turtles Overwinter and Its Consequences. *Biological Reviews*, 81(03), 339. https://doi.org/10.1017/S1464793106007032

Voituron, Y., Barré, H., Ramløv, H., & Douady, C. J. (2009). Freeze Tolerance Evolution Among Anurans: Frequency and Timing of Appearance. *Cryobiology*, 58(3), 241–247. https://doi.org/10.1016/j.cryobiol.2009.01.001

Voituron, Y., Eugene, M., & Barré, H. (2003). Survival and Metabolic Responses to Freezing by The Water Frog (*Rana ridibunda*). *Journal of Experimental Zoology Part A: Comparative Experimental Biology*, 299A(2), 118–126. https://doi.org/10.1002/jez.a.10285

Voituron, Y., Heulin, B., & Surget-Groba, Y. (2004). Comparison of the cold hardiness capacities of the oviparous and viviparous forms of *Lacerta vivipara*. *Journal of Experimental Zoology*, 301A(4), 367–373. https://doi.org/10.1002/jez.a.20042

Voituron, Y., Joly, P., Eugène, M., & Barré, H. (2005). Freezing Tolerance of The European Water Frogs: The Good, The Bad, and The Ugly. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 288(6), R1563–R1570. https://doi.org/10.1152/ajpregu.00711.2004

Voituron, Y., Paaschburg, L., Holmstrup, M., Barré, H., & Ramløv, H. (2009). Survival and Metabolism of *Rana arvalis* During Freezing. *Journal of Comparative Physiology B*, 179(2), 223–230. https://doi.org/10.1007/s00360-008-0307-3

Wijeneyaka, S., Storey, K. (2016) The Role of DNA Methylation During Anoxia Tolerance in a Freshwater Turtle (*Trachemys scripta elegans*). *Journal of Comparative Physiology B.*, 186(3), 333-342. https://doi.org/10.1007/s00360-016-0960-x

Woods, C. P., Czenze, Z. J., & Brigham, R. M. (2019). The avian "hibernation" enigma: thermoregulatory patterns and roost choice of the common poorwill. *Oecologia*, 189(1), 47–53. https://doi.org/10.1007/s00442-018-4306-0

Yoldaş, T. (2021). Anadolu Dağ Kurbağalarının Kriyobiyoloji Üzerine Araştırmalar (689754). Retrieved from https://tez.yok.gov.tr/UlusalTezMerkezi/giris.jsp.

Yoldas, T., & Erismis, U. C. (2021). Response of Anatolian mountain frogs (*Rana macrocnemis* and *Rana holtzi*) to freezing, anoxia, and dehydration: Glucose as a cryoprotectant. *Cryobiology*, 98(November 2020), 96–102. https://doi.org/10.1016/j.cryobiol.2020.11.019