



Yuzuncu Yil University
Journal of Agricultural Sciences
(Yüzüncü Yil Üniversitesi Tarım Bilimleri Dergisi)

<https://dergipark.org.tr/en/pub/yyutbd>



ISSN: 1308-7576

e-ISSN: 1308-7584

Research Article

Distribution and DNA Barcoding of Anomalini Beetles (Coleoptera: Scarabaeidae: Rutelinae) in Wheat Fields of Van, Türkiye

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Article Info

Received: 12.12.2023

Accepted: 25.03.2024

Online published: 15.06.2024

DOI: 10.29133/yyutbd.1403342

Keywords

Anomalini beetles,
DNA barcoding,
Fauna,
Van Province and district,
Wheat pests

Abstract: Anomalini beetles (Coleoptera: Scarabaeidae: Rutelinae) constitute an important group of pests causing significant crop losses in wheat cultivation areas worldwide, including Türkiye. The aim of this study was to comprehensively evaluate the phylogeny, diversity, abundance, and distribution of Anomalini beetles in wheat fields of Van province, Türkiye. Surveys were conducted between April and August 2021, involving monthly sample collection at predetermined locations within six districts: Başkale, Çaldıran, Erciş, Gevaş, İpekyolu, and Tuşba. A Standard sweepnet with a diameter of 35 cm was used to collect samplings. In molecular studies, the mitochondrial COI gene region has been amplified and sequenced using universal primers. Anomalini beetles were detected in all sampling areas except Çatak district. Seven species were identified: *Anisoplia austriaca*, *A. signata*, *A. lata*, *Brancoplia leucaspis*, *Blitopertha nigripennis*, *Chaetopteroptia segetum*, and an unidentified *Anisoplia* sp. *Chaetopteroptia segetum* emerged as the most prevalent and abundant species across all districts. Notably, all identified Anomalini species represent the first records for Van province and its environs. While Anomalini beetles were present in the region, their population densities were not considered high enough to cause economic damage.

To Cite: Özgökçe, M S, Göksugüzel, G, Kara, H, Rişvanlı M R, Doğaç, M, 2024. Distribution and DNA Barcoding of Anomalini Beetles (Coleoptera: Scarabaeidae: Rutelinae) in Wheat Fields of Van, Türkiye. *Yuzuncu Yil University Journal of Agricultural Sciences*, 34(2): 271-285. DOI: <https://doi.org/10.29133/yyutbd.1403342>

1. Introduction

The tribe Anomalini are ecologically and agriculturally important beetles that wide-range around the world, contain one of the largest genera in the animal kingdom (genus *Anomala* with approximately 1000 species), and have over 2000 species (Jameson et al., 2003; Morón and Ramírez-Ponce, 2012). The life history of species of the tribe Anomalini, some of which are agricultural pests, is highly variable (Jameson et al., 2003). The larvae of many species feed on the subsoil parts of a wide

variety of plants. While the adults of some species feed little or not at all, the adults of others feed on the leaves, flower parts, and fruits of angiosperms and gymnosperms, causing severe damage to fruits and leaves (Jameson et al., 2003; Filippini et al., 2016; Vittum, 2020).

In 1918, Ohaus established a comprehensive worldwide classification for the Anomalini tribe, which involved the division of the tribe into four subtribes, namely the Anisopliina, Anomalina, Popilliina, and Isopliina (Jameson et al., 2007). The Anisopliina subtribe (Scarabaeidae: Rutelinae: Anomalini) inhabits a large geographical area spanning the Palaearctic, Oriental, Ethiopian, Nearctic, and Neotropical biogeographical regions, with about 100 species belonging to nine different genera (Jameson et al., 2007). Anisopliinae beetles, also known as grain beetles or grass-feeding beetles, feed on grass pollen and seeds as adults and grassroots as larvae. This dietary preference has shaped their life cycle and ecological niche within grassland ecosystems. Their adults exhibit a diversified feeding strategy, consuming a wide range of non-cultivated grasses as adults, with some species also adapted to feed on cultivated plant species such as oats, wheat, rye, and corn (Hurpin, 1962; Mico et al., 2001; Jameson et al., 2007).

In the Old World, where Anisopliina's species richness is highest, species inhabit a wide variety of grassy habitats, including scrub forests, grasslands, meadows, riparian areas, and roadsides. New World species of Anisopliina are distributed in the dry, desert zone of southern Arizona and northwestern Mexico, the pine-oak forests of central Mexico, and the tropical oak and deciduous forests of central Mexico (Morón et al., 1996). Although larvae of many Anisopliina species are recognized as pests of various crops (Bogachev, 1946), little is known about larval morphology or adult biology. The genus *Anisoplia* is the most abundant and widespread genus of Anisopliina, with more than 50 species (Machatschke, 1961). In various regions of Türkiye, 28 different *Anisoplia* species have been identified (Lodos, 1989). *Anisoplia syriaca*, *A. austriaca* and *A. segetum* are important *Anisoplia* species in Türkiye (Lodos, 1989). The species of the genus *Anisoplia* found in Türkiye are *Anisoplia (Anisoplia) agnata* Reitter., *A. lata* Erichson., *A. agricola* Poda, *A. lanuginosa* Erichson., *A. aprica* Erichson., *Brancoptia leucaspis* (Castelnau, 1840) ., *A. austriaca* (Herbst), *A. mülleri* Pilleri., *A. (Anisoplia) clypealis* Reitter. (*Anisoplia*) *nohai* Petrovitz, *A. (Anisoplia) dispar* Erichson., *A. parva* Kraatz., *A. egregia* Petrovitz, *A. petrovitzi* Machatschke, *A. faldermanni* Reitter, *A. (Anisoplia) reitteriana* Semenov, *A. farraria farraria* Erichson., *Chaetopteroptia segetum* (Herbst, 1783), *A. flavipennis* Brullé, *A. (Anisoplia) signata* Faldermann, *A. hebes* Reitter, *Chaetopteroptia syriaca* (Burmeister, 1844) , *A. (Anisoplia) hirta* Zaitzev, *A. tenebralis* Burmeister., *A. imitatrix* Apfelbeck, *A. thessalica* Reitter, *Chaetopteroptia inculta* (Erichson, 1847) ., and *A. tritici* Kieswetter (Anonymous, 2021a). *Anisoplia* spp are widespread in areas where cereals are cultivated in Türkiye. *Anisoplia* larvae, which cause damage to many gramineas, especially wheat, barley, oats, and rye, cause the main damage by eating the root of the plant, and the adults cause the main damage by eating the wheat grains and the presence of 3-4 adult individuals per m² in the fields cause economic damage (TAGEM, 2008).

In this study, we determined the faunistic presence, diversity, density, and DNA Barcoding of a tribe of Anomalini beetles in wheat growing areas in and around Van province. No comprehensive phylogenetic analysis has been conducted, but the DNA sequences of the species were recorded by DNA barcoding. DNA barcoding has become an indispensable tool in faunistic studies of insects, primarily due to its capacity to offer precise species identification and assist in the detection of new species. The vast diversity of insects and their ecological and economic significance have positioned them as a primary focus for DNA barcoding (Jinbo et al., 2011; Wilson et al., 2017). This approach has been particularly beneficial in expediting species identification and description, particularly in scenarios where traditional taxonomic methods pose challenges. It has facilitated the identification of agriculturally significant insects, thereby contributing to pest management and control. Additionally, DNA barcoding has played a pivotal role in unveiling concealed biodiversity and delineating species within intricate taxa, such as parasitoid species (Jinbo et al., 2011; Ferreira et al., 2020; Li et al., 2021). With this study, the presence of the relatively weakly volatile Anomalini (Mico et al., 2001; Micó and Galante, 2002; Bekircan and Tosun, 2021) species in the study area will be important in terms of understanding the species adapted to the region and revealing the regional inventory by determining the DNA records.

2. Material and Methods

The study was conducted in the wheat-growing areas of Van province and its districts (Bahçesaray, Başkale, Çaldıran, Çatak, Edremit, Erciş, Gevaş, Gürpınar, İpekyolu, Muradiye, Özalp, Saray and Tuşba) from April to August 2021 (Figure 1). Surveys were carried out in each sampling district at two-week intervals starting from the tillering period of wheat plants. At least 3 fields were selected in each district and 200 samples were collected in each field using a standard sweepnet with a diameter of 35 cm. The location of each sampled field was marked on the map with the help of a GPS device (Garmin) and marked on the map (Figure 1).

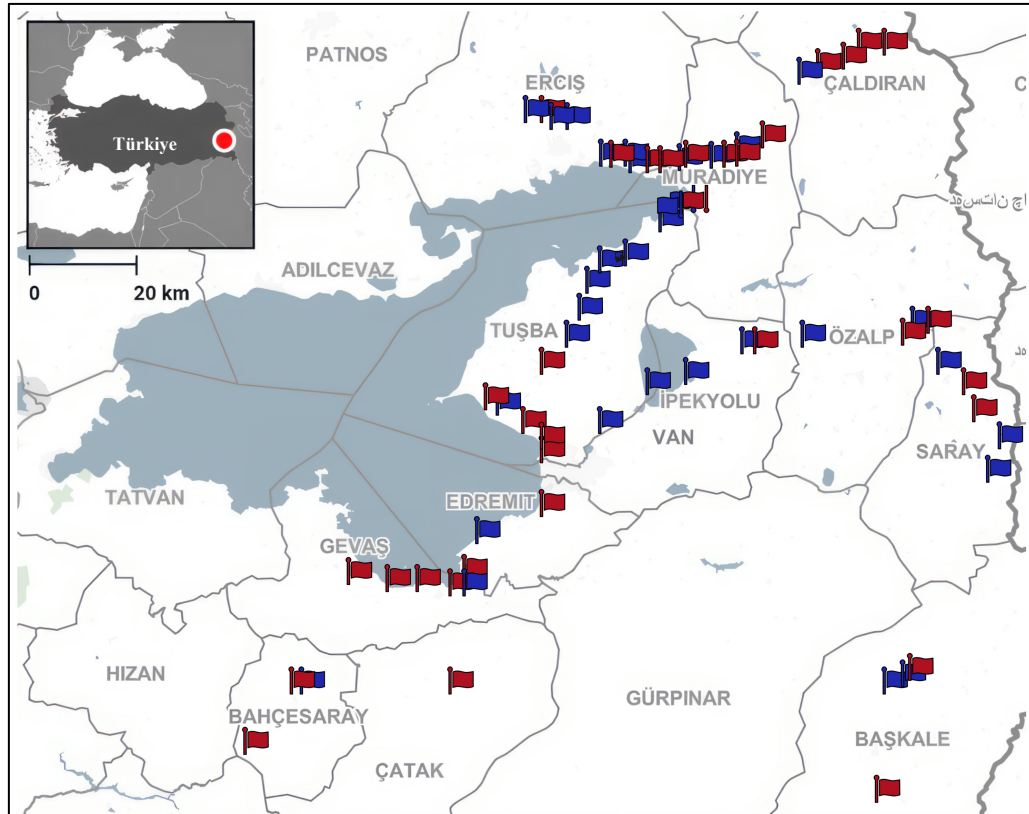


Figure 1. GPS locations of the sampled fields (blue and red flags), where insects were detected (red flag) (Garmin BaseCamp).

2.1. Morphologic identification

The collected individuals were separated according to their location and morphological characters, and the specimens that were very similar to each other were counted and recorded as the same species. Genital organ preparations of male individuals were made for species identification. For this purpose, specimens were soaked in 10% potassium hydroxide for 1-2 hours, and genital organs were removed. Identification keys were used for species identification and also confirmed with the opinions of Dr Denis Keith (Muséum d'Histoire Naturelle et de Préhistoire, France), an expert on these beetles.

2.2. Molecular studies

After the morphological identification procedures, DNA barcoding of the samples was carried out according to the following methods; thus, the genetic sequences of the species were obtained.

DNA isolation was carried out by removing the samples in 99% ethanol from their media stored at -20 °C, firstly the legs of each sample were dissected and washed with distilled water, and DNA isolation procedures were started. DNA isolation was performed using PureLink™ Genomic DNA Mini Kit (Invitrogen). For isolation, 180 µl Genomic Digestion Buffer and 20 µl Proteinase K were added to the insect legs, which were thoroughly crushed with metal pliers, and incubated at 55 °C for half an

hour. After centrifugation at the highest speed for 2-3 min, the pellet formed in the tube was removed and 20 µl RNase, 200 µl PureLink™ Genomic Lysis/Binding Buffer, and 200 µl 96-100% ethanol were added to the clear liquid and vortexed thoroughly to form a homogenous mixture. After these procedures, genomic DNA was obtained by washing twice.

Polymerase Chain Reaction (PCR)-Agarose Gel Electrophoresis The universal primers LCO1490 5'-GGTCAACAAACAAATCATAAAGATATATTGG-3' and HCO 2198 5'TAAACTTCAGGGTGACCAAAAAATCA-3' were used for the mitochondrial COI regions of the isolated DNAs. PCR reactions were prepared in a volume of 25 µl and tubes were prepared by adding 5 µl of Genomic-DNA, 2.5 µl of 10X reaction buffer (100 mm Tris-HCl, pH 8.8, 500 mm KCl, 0.8% Nonidet P40), 3 µl of 25 mM MgCl₂, 10 mM each dNTP (thermo scientific), 0.5 µl of each of the primers diluted to 10 pmol (Sentebiolab, Ankara). The PCR reaction was prepared by adding 0.625 U Taq DNA polymerase (Thermo Scientific) into the prepared 25 µl final volume reaction. The PCR process was carried out in a VWR brand PCR device with initial denaturation at 95°C for 3 min, followed by 35 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 30 s, and then 72°C for 5 min as the final elongation to amplify the targeted region. The amplicons obtained after the reaction were run on a 1% agarose gel at 100 volts for 60 min.

2.3. Phylogenetic analysis

COI sequences of 6 species were obtained from specimens collected in Van and its districts. Two taxa, *Phyllognathus dionysius* Fabricius (Scarabaeidae: Dynastinae: Pentodontini) and *Cyclocephala atripes* Bates (Scarabaeidae: Dynastinae: Cyclocephalini) with MZ664295.1 and KX298196.1 NCBI accession numbers, respectively, were used as outgroup. In addition, 4 species belonging to the subtribes Anisopliina and Anomalina with MF706436.1, AY090506.1, AY090507.1, and MH115532.1 NCBI accession numbers were used for comparison with the in-groups. COI sequences of the species selected as outgroups were retrieved from GenBank for phylogenetic analysis. A total of 654 bp nucleotide sequences of the mitochondrial COI region of all individuals (including the outgroup) were aligned and visually checked with ClustalW using MEGA 11 (Tamura et al., 2021). The evolutionary history of beetles was elucidated through the implementation of the Maximum Likelihood (ML) method and the Tamura-Nei (T92) model (Tamura and Nei, 1993). The optimal tree generated by this method is presented in the figure. The bootstrap test, which involved 1000 replicates, was employed to assess the robustness of the inferred tree topology.

3. Results and Discussion

Anomalini beetles were found in 42 of 85 sampling points in Van province, and a total of 149 individuals were collected. Among these specimens, a total of seven species were identified from wheat cultivation areas in Van province (Table 1). Six of the identified species (*Anisoplia (Autanisoplia) austriaca*, *Anisoplia (Anisoplia) lata*, *Anisoplia (Anisoplia) signata*, *Anisoplia* sp., *Brancoplia leucaspis*, *Chaetopteropia segetum*) belong to the Anisopliina subtribe of the Anomalini tribe, and one species (*Blitopertha nigripennis*) belongs to the Anomalina subtribe. All of the identified specimens are new records for Van Province.

In the study areas, *C. segetum* was the most common species (28%), followed by *Br. leucaspis* (24%), *A. austriaca* (21%), *Bl. nigripennis* (12%), *A. signata* (9%), *A. lata* and *Anisoplia* sp. (3%). The most abundant species was *C. segetum* 38%, the second most abundant species was *Br. leucaspis* 32%, followed by *A. austriaca* 18%, *Bl. nigripennis* 8%, *A. signata* 2%, and *A. lata* and *Anisoplia* sp. 1%. In previous studies, 28 different *Anisoplia* species were identified in various regions of Türkiye, and *A. syriaca*, *A. austriaca*, and *A. segetum* were reported as the most important species (Lodos, 1989).

Table 1. Anomalini beetles collected from wheat fields in Van and its districts

Subfamily	Tribe	Subtribe	Genus	Species	Location, number of individuals	
Rutelinae	Anomalini	Anisopliina	Anisoplia	<i>Anisoplia (Autanisoplia) austriaca</i>	1* (4**), 3 (6), 5 (3), 6 (3), 7 (2), 10 (1), 11 (2), 12 (29)	
				<i>Anisoplia (Anisoplia) lata</i>	2 (1)	
				<i>Anisoplia (Anisoplia) signata</i>	1 (1), 2 (1), 3 (1)	
		Anomalina	Blitopertha		<i>Anisoplia sp.</i>	9 (1)
					<i>Brancoplia leucaspis</i>	1 (10), 3 (1), 6 (2), 7 (3), 9 (3), 10 (2), 11 (1), 13 (5)
					<i>Chaetopteroptia segetum</i>	1 (23), 3 (4), 5 (16), 6 (1), 7 (3), 8 (3), 9 (1), 10 (1), 13 (4)
				<i>Blitopertha nigripennis</i>	2 (3), 3 (2), 10 (1), 11 (5)	

*Locations: 1: Bahçesaray, 2: Başkale, 3: Çaldıran, 4: Çatak, 5: Edremit, 6: Erciş, 7: Gevaş, 8: Gürpınar, 9: İpekyolu, 10: Muradiye, 11: Özalp, 12: Saray, 13: Tuşba. **Number of individuals.

3.1. The subtribe Anisopliina beetles in Van Province and its surroundings

3.1.1. *Anisoplia (Autanisoplia) austriaca* (Herbst, 1783)

The adults of *Anisoplia (Autanisoplia) austriaca*, also known as crop grain beetle or wheat grain beetle, cause damage to crops such as wheat, maize, rye, and oats (Hurpin, 1962). Regarding the morphological characteristics of the insect, Baraud (1991) reported that the body length is 13-20 mm, the head, and pronotum are sometimes green or bluish-black, and the elytra, which does not completely cover the body, is brown-yellow or brown-red. Baraud (1991) further adds that there is a rectangular black spot surrounding the scutellum, the body is almost glabrous or with a small amount of pseudo-pubescence, the large front claw of males is very long and slightly curved or not curved, and this shape of the claw is similar to the shape observed in *Brancoplia leucaspis* (Figure 2). Baraud (1991), noted another important feature: the metasternum has very long, very dense, and erect bristles in the shape of a brush (Schoonhoven et al., 1998; Schoonhoven et al., 2005). This observation is in line with the study by (Jones et al., 2016), which collected morphological data on insect wings, indicating the significance of bristles in insect morphology. Additionally, the study by Jameson et al. (2007) provides further context by categorizing *Brancoplia* within a specific clade, emphasizing the importance of understanding its unique morphological features. Furthermore, the work of Engels et al. (2021) discusses the concept of ptiloptery, which refers to wings with long bristles attached to a narrow membranous section, providing insights into the potential aerodynamic implications of such bristles.



Figure 2. *Anisoplia austriaca*, adult (a: dorsal, b: ventral).

Distribution in and around Van: *Anisoplia austriaca* was found in Bahçesaray (at N38° 08.940' E42° 49.202' and 1874 m, 1 individual; N38° 08.954' E42° 49.173' and 1880 m, 1 individual; N38° 08.906' E42° 49.520' and 1847 m, 2 individuals), Caldıran (N39° 08.091' E43° 56.024' and 2048 m, 4 individuals; N39° 07.296' E43° 52.714' and 2254 m, 2 individuals), Erciş (N39° 05.007' E43° 15.264' and 1755 m, 3 individuals), Gevaş (N38° 19.631' E42° 55.980' and 1667 m, 1 individual; N38° 18.302' E43° 08.754' and 1691 m, 1 individual), Muradiye (N38° 57.605' E43° 41.457' and 1673 m, 1 individual), Özalp (N38° 39.789' E44° 01.418' and 2024 m, 1 individual; N38° 39.807' E44° 05.587' and 2080 m, 1 individual), and Saray (N38° 35.701' E44° 13.212' and 2254 m, 9 individuals; N38° 37.966' E44° 11.461' and 2152 m, 20 individuals).

Distribution in Türkiye: Distribution to Ankara, Adana, Adıyaman, Antalya, Bayburt, Bilecik, Bitlis, Çankırı, Çorum, Denizli, Diyarbakır, Erzincan, Erzurum, Eskişehir, Isparta, İzmir, Kars, Konya, Muğla, Sivas, and Yozgat in Türkiye (Rezaei, 2015; Polat et al., 2018).

Distribution in Worldwide: It was recorded in Austria, Bulgaria, Czechoslovakia, South Germany, Hungary, Romania, Russia, Ukraine, Baraud (1991), cited by Porta (1932), Azerbaijan, Armenia, Georgia, Iraq, Iran, Israel, Switzerland, Lebanon, Syria, Türkiye, and Greece (Rezaei, 2015).

3.1.2. *Anisoplia lata* (Erichson, 1847)

Wilhelm Ferdinand Erichson described the beetle *A. lata* in 1847. *Anisoplia lata* is a member of the *Anisoplia* genus and the Rutelidae family, with the subspecies *A. l. lamiensis* also recognized (Anonymous, 2021b). Baraud (1991) noted that this species is represented by two forms: *Anisoplia lata lata* Erichson and *A. lata lamiensis* Apfelbeck. Baraud (1991) provided morphological details, stating that the body length ranges from 11-14 mm, the elytra are brownish-yellow, mostly black, and the pronotum is nearly hairless. Males have straight-sided pronotums, while females have pronotums parallel to the body's rear and sides curved forward from the base (Figure 3). Additionally, males have long, pointed, and highly curved front tarsi. Zazharska et al. (2019) described the beetle's head and pronotum as highly glossy, black, and greenish, with the elytra being uniformly brownish-red, brown, or black. They also noted that the setae near the scutellum are not prominent in males' elytra, and the abdominal setae are scattered. These details provide a comprehensive understanding of the taxonomy and morphological characteristics of *A. lata* and its subspecies.



Figure 3. *Anisoplia lata*, adult.

Distribution in and around Van: *Anisoplia lata* was collected in Başkale at N37° 50.640' E44° 06.646' and 1847 m 1 individual.

Distribution in Türkiye: Ankara, Çanakkale, Eskişehir (Rezaei, 2015).

Distribution in Worldwide: It was found in Albania, Austria, Hungary, Macedonia, Romania, Yugoslavia, and Greece (Baraud, 1991). It has been observed in Southern and Southeastern Europe, the European part of Russia, and Moldova (Mico et al., 2001).

3.1.3. *Anisoplia signata* (Faldermann, 1835)

Only 3 female individuals of this species were found. Since there were no male individuals, genital preparations could not be obtained, but Denis Keith reported that the specimens may belong to *A. signata* based on the morphological characters of the female individuals (Figure 4).

Distribution in and around Van: *Anisoplia signata* was found in Bahçesaray (at N38° 08.787' E42° 50.269' and 1891 m, 1 individual), Başkale (N37° 50.640' E44° 06.646' and 1847 m, 1 individual), and Çaldıran (N39° 07.293' E43° 52.717' and 2046 m, 1 individual).

Distribution in Türkiye: Ardahan, Artvin, Bingöl, Erzurum, Iğdır, Mersin, Rize, Trabzon (Baraud, 1991).

Distribution in Worldwide: Armenia, Siberia (Pilleri, 1954), Syria (Baraud, 1991).



Figure 4. *Anisoplia signata*, adult.

3.1.4. *Anisoplia* sp.

Only 1 specimen of this species belonging to the genus *Anisoplia* could be collected. Morphological measurements of the species are as follows: Total body length was 11.1 mm, elytra 6.57 mm, thorax 2.89 mm, head 1.64 mm. The width of the head was 2.35 mm, the width of the thorax was 4.48 mm, and the width of the elytra was 6.06 mm. The body is generally black, especially the abdomen and lower parts of the thorax are covered with dense, long yellow hairs. The upper part of the thorax is glossy black and glabrous, while the abdomen is mustard yellow or yellowish brown on a glossy black background (except around the scutellum and the outer edges of the elytra and where the two wings meet). Neither the thorax nor the upper part of the elytra have any hairs (Figure 5).

Distribution in and around Van: *Anisoplia* sp. was found in İpekyolu (at N38° 30.212' E43° 19.380' and 1657 m, 1 specimen).



Figure 5. *Anisoplia* sp., adult (a; dorsal, b; ventral).

3.1.5. *Brancoplia leucaspis* (Laporte, 1840)

According to current knowledge, *Brancoplia* has six species found exclusively or predominantly in the Middle East (Rössner, 2016). These species are *Br. leucaspis* (Laporte, 1840), *Br. umila* (Marseul, 1878), *Br. vseteckai* (Pilleri, 1951), *Br. mesopotamica* (Pilleri, 1954), *Br. klapperichi* (Petrovitz, 1971) and *Br. waitzbaueri* (Rössner, 2017; Rössner and Sabatinelli, 2020). Elytra are densely pubescent, male genitalia have a long and chitinized canal, and the parameres are absent or slightly S-shaped curved when viewed from the side (Anonymous, 2021c) (Figure 6). Two groups of species (*Br. leucaspis* group and *Br. pumila* group) can be distinguished based on external morphology and genital morphology (Rössner, 2016).

Distribution in and around Van: *Brancoplia leucaspis* was collected in Bahçesaray (at N38° 08.787' E42° 50.269' and 1891 m, 1 individual; N38° 08.940' E42° 49.202' and 1874 m, 5 individuals; N38° 08.906' E42° 49.520' and 1847 m, 1 individuals; N38° 06.051' E42° 45.073' and 1837 m, 3 individuals), Caldıran (N39° 08.583' E43° 57.412' and 2049 m, 1 individual), Erciş (N39° 00.131' E43° 29.005' and 1685 m, 2 individuals), Gevaş (N38° 19.851' E43° 11.378' and 1695 m, 1 individual; N38° 18.590' E43° 10.577' and 1704 m, 1 individual; N38° 18.467' E43° 00.078' and 1666 m, 1 individual), İpekyolu (N38° 30.961' E43° 18.945' and 1656 m, 3 individual), Muradiye (N38° 59.170' E43° 44.636' and 1688 m, 1 individual; N39° 05.206' E43° 47.939' and 1936 m, 1 individual), Özalp (N38° 39.453' E43° 44.702' and 1883 m, 1 individual), and Tuşba (N38° 34.625' E43° 16.335' and 1706 m, 3 individuals; N38° 40.126' E43° 18.236' and 1731 m, 1 individual; N38° 36.660' E43° 13.666' and 1729 m, 1 individual).



Figure 6. *Brancoplia leucaspis*, adult (a: dorsal, b: ventral).

Distribution in Türkiye: Adıyaman, Bingöl, Diyarbakır, Erzurum, Kars (Polat et al., 2018).

Distribution in Worldwide: The range of the *Brancoplia leucaspis* extends from the Crimean peninsula and the Caucasus in the north to Turkmenistan and south through the Zagros Mountains in Iran to northern Egypt (Zaitzev, 1917; Rössner, 2016). Azerbaijan, Georgia, Türkiye; (Medvedev, 1949): Iraq, Iran, Russia; (Pilleri, 1954): Azerbaijan, Armenia, Iran, Russia; (Rössner, 2016; Polat et al., 2018).

3.1.6. *Chaetopteropia segetum* (Erichson, 1847)

Harold (1869) initially named the beetle known as *Melolontha segetum* as *C. segetum*, and this name was later accepted by subsequent authors (Mulsant, 1871; Bedel, 1911). During the period between Fabricius' 1787 publication and Harold's 1869 publication, the names *M. fruticola* Fabricius 1787 and *M. segetum* Herbst 1783 were used for the species now known as *C. segetum* (Anonymous, 2021d). Regarding the morphological characteristics of the beetle, Machatschke (1961) reported that the body length was 9-13 mm, the body was broad and oval, the head and pronotum were blackish green or black covered with dense yellowish hairs, the elytra were brownish and the hairs were sparser (Figure 7). In addition, males had longer antennae and thickened front claws, while females had paler elytra.



Figure 7. *Chaetopteropia segetum*, adult.

Distribution in and around Van: *Chaetopteropia segetum* was found in Bahçesaray (at N38° 08.940' E42° 49.202' and 1874 m, 7 individuals; N38° 08.954' E42° 49.173' and 1880 m, 3 individuals; N38° 08.906' E42° 49.520' and 1847 m, 1 individual; N38° 06.051' E42° 45.073' and 1837 m, 11 individuals; N38° 08.744' E42° 50.310' and 1921 m, 1 individual), Çaldıran (N39° 07.293' E43° 52.717' and 2046 m, 1 individual; N39° 05.995' E43° 50.018' and 2012 m, 3 individuals), Edremit (N38° 25.572' E43° 15.787' and 1646 m, 16 individuals), Erciş (N39° 05.007' E43° 15.264' and 1755 m, 1 individual), Gevaş (N38° 19.851' E43° 11.378' and 1695 m, 1 individual; N38° 19.631' E42° 55.980' and 1667 m, 1 individual; N38° 18.585' E43° 03.427' and 1677 m, 1 individual), Gürpınar (N38° 19.198' E43° 20.110' and 1729 m, 1 individual; N38° 17.270' E43° 49.978' and 2030 m, 1 individual; N38° 19.084' E43° 24.731' and 1773 m, 1 individual), İpekyolu (N38° 30.961' E43° 18.945' and 1656 m, 1 individual), Muradiye (N38° 57.662' E43° 38.086' and 1646 m, 1 individual), and Tuşba (N38° 34.625' E43° 16.335' and 1706 m, 4 individuals).

Distribution in Türkiye: It was distributed to Konya (Venieraki et al., 2017), Adana, Afyonkarahisar, Ankara, Antalya, Artvin, Bitlis, Erzincan, Erzurum, Hatay, Iğdır, Kars, Mersin, Rize, and Trabzon (Polat et al., 2018).

Distribution in Worldwide: It was a widespread beetle that occurred in a broad area from eastern France to western Siberia (Baraud, 1992). Its distribution range includes Poland (Pawłowski et al., 2002), Anatolia, Belgium, central, eastern, and southeastern Europe, Greece, Syria, Siberia (Rezaei, 2015, Anonymous, 2021d), and Iran (Venieraki et al., 2017).

3.2. The subtribe Anomalina beetle in Van and its surroundings

3.2.1. *Blitopertha nigripennis* (Reitter 1888)

Blitopertha nigripennis, which is reported to be very dense and widespread in the regions where it was found in Türkiye (Yıldırım et al., 2018), was collected in four different regions in and around Van. The body length of *Bl. nigripennis* is 8-13 mm; head, pronotum, and scutellum are black; dorsum has 2 longitudinal black lines; elytra is yellow or light brown, upper wings are slightly hairy; pygidium is longer in males (Figure 8) (Rezaei, 2015).



Figure 8. *Blitopertha nigripennis*, adult.

Distribution in and around Van: Distributed to Başkale (at N38° 04.772' E44° 06.193' and 2099 m, 3 individuals), Çaldıran (N39° 08.091' E43° 56.024' and 2048 m, 2 individuals), Muradiye (N38° 57.531' E43° 38.272' and 1670 m, 1 individual), and Özalp (N38° 40.598' E44° 03.685' and 2076 m, 5 individuals).

Distribution in Türkiye: It was collected in Adana, Adıyaman, Ağrı, Antalya, Edirne, Eskişehir, Gaziantep, Hatay, İçel, Kahramanmaraş, Kastamonu, Kars, Kayseri, Osmaniye, Sakarya, Sinop, Yozgat (Lodos et al., 1999; Rozner and Rozner, 2009; Şenyüz and Şahin, 2009), Bingöl, Bursa, Erzurum (Polat et al., 2018).

Distribution in Worldwide: Azerbaijan, Armenia, Georgia, Iran, Israel, Cyprus, Russia: Southern Europe, Lebanon, Syria, Türkiye, Turkmenistan, Jordan (Löbl and Löbl, 2016).

3.3. Phylogenetic analyses

The phylogenetic tree was constructed using 12 sequences, two from the outgroup, four from the subtribes Anisopliina and Anomalina, and 6 from the ingroup. Sequences of species other than the inner group were obtained from GenBank (with species names and accession numbers given in Figure 9). Seven different species were identified in Van and its surroundings. Unfortunately, DNA isolation of *Anisoplia* sp. found just one in İpekyolu and not identified morphologically could not be performed

due to unsuitable waiting conditions. Therefore, sequences of only 6 species were used as ingroups in the phylogenetic tree.

The tree with the highest log likelihood (-3273.26) is shown. The percentage of trees in which the associated taxa clustered together is shown below the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G, parameter = 0.2268)]. This analysis involved 12 nucleotide sequences.

When the phylogenetic tree was analyzed, the internal groups were divided into two important clades. While 5 in-group species and 3 reference species belonging to the Anisopliina subtribe were clustered in the first clade, *Bl. nigripennis* and *Bl. lineolata* (reference species) belonging to the Anomalina subtribe was included in the other clade (Figure 9). The reference sequence of *Anisoplia austriaca* and the sequence obtained from this study were found to be on the same branch in the phylogenetic tree with a very high bootstrap rate (99%). The reference species belonging to the genus *Blitopertha* in the second clade and *Bl. nigripennis* morphologically identified in this study were located on the same branch with a bootstrap rate of 94%.

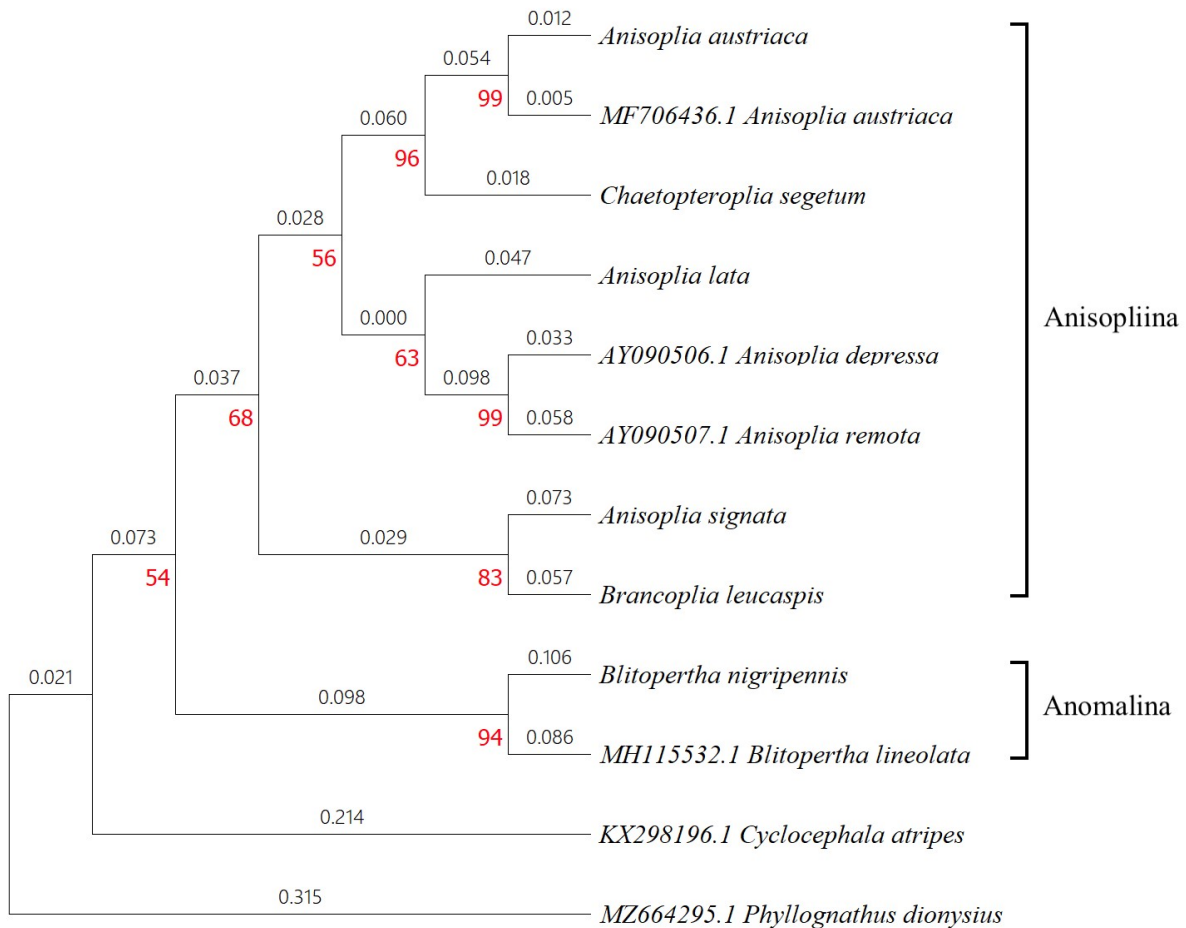


Figure 9. The phylogenetic tree of the Anomalini species in Van and surroundings was constructed by MEGA 11 using the Maximum Likelihood (ML) method and 1000 bootstrap replicates.

The study results conducted in the Van region provide valuable insights into the population dynamics and species composition of the Anomalini tribe in wheat fields. Species of the Anomalini tribe, which may cause economic losses in their high populations, were identified in wheat fields in Van and its surroundings at low densities. Among the collected species, 6 species (*Anisoplia (Autanisoplia) austriaca*, *Anisoplia (Anisoplia) lata*, *Anisoplia (Anisoplia) signata*, *Brancoplia leucaspis*,

Chaetopterolia segetum, and *Anisoplia* sp.) Anisopliina subtribe, while one species (*Blitopertha nigripennis*) belongs to the Anomalina subtribe. All of the detected species are the first records for the Van region.

When the sampling points where the tribe Anomalini beetles were collected were analyzed, the lowest altitude was 1646 m (Edremit-Muradiye) and the highest altitude was 2254 m (Çaldıran-Saray). Anomalini larvae started to pupate at the end of May and adults were collected towards the end of June. Towards the end of August, the adults started to disappear again. Individuals were observed to be denser at the edges of the wheat field than in the center. Crop bumblebees, which are described as weak fliers (Mico et al., 2001; Micó et al., 2003) are easily recognized by the eye due to their large body structure when disturbed and flown. Since adult individuals generally prefer to feed and mate on the stems, they were easily caught especially during the hot hours of the day. No individuals were found in the study areas between 22.05.2021 and 10.06.2021 when the surveys were carried out regularly. The first individuals, albeit at a very low density, started to be seen on 18.06.2021 and increased on 28.06.2021, and reached the highest population density on 20.07.2021. From this date onwards, the population density has decreased steadily until harvest.

Chaetopterolia segetum was found to be the most common and densely populated Anomalini species. It was observed that the insect population was denser in irrigated wheat fields than in non-irrigated wheat fields. However, it was concluded that the population densities of this species and other species were not economically significant. The main reasons for this may be the effectiveness of climatic factors or natural enemies. Agricultural product production in the region is carried out in limited areas due to climatic conditions and the use of chemical pesticides against disease and pest populations is almost non-existent. These results, the study highlights the prevalence of *C. segetum* and its population density variations in irrigated and non-irrigated wheat fields, shedding light on the impact of agricultural practices on insect populations.

In addition to the morphological identification of the collected species, the study undertook phylogenetic analyses of the Anomalini species. The importance of phylogenetic analyses in identifying, classifying, and ecologically characterizing insects has been better understood with research conducted in recent years. Phylogenetic studies underline their fundamental role in advancing our knowledge of evolutionary patterns, dynamics, ecological relationships, species diversity, gene expression, and gene and genome duplications among insects (Lewinsohn et al., 2005; Procheş et al., 2009; Yu et al., 2009; Trautwein et al., 2012; Moriyama et al., 2015; Li et al., 2018). The results of the phylogenetic analyses, particularly the sister branch relationships of the identified species with those registered in GenBank, provide valuable genetic insights into the evolutionary relationships and taxonomic affiliations of these insects. In the phylogenetic analyses of Anomalini species, the sequences of the species collected in this study and the closest species registered in GenBank were analyzed together as an ingroup. As a result of the analysis, the species *A. austriaca*, registered in GenBank with accession number MF706436.1, and the specimen morphologically identified with the same name in this study were in the sister branch with a 99% bootstrap ratio. In addition, the species diagnosed as *Bl. nigripennis* in this study and the species *Bl. lineolata* with accession number MH 115532.1 was in the sister branch with a 96% bootstrap ratio. In the analyses, the other species were included in the clade from which the subtribe they belonged was separated. However, the study also acknowledges the limitations posed by the unavailability of DNA sequences for all identified species in GenBank, emphasizing the need for comprehensive genetic reference databases. Since the DNA sequences of all species identified in this study were unavailable in GenBank, a complete comparison could not be made.

The findings of this study underscore the importance of integrating phylogenetic analyses, such as DNA barcoding, into entomological research. DNA barcoding has emerged as a powerful tool for species identification and discovery, particularly in cases where morphological identification may be challenging or inconclusive (Meyer and Paulay, 2005). Furthermore, the study's emphasis on the potential economic impact of the identified species and the influence of climatic factors and natural enemies on population densities underscores the practical implications of entomological research for agricultural management and pest control strategies. In conclusion, the entomological study in the Van region not only contributes to the knowledge of Anomalini species composition and population dynamics but also highlights the significance of phylogenetic analyses, such as DNA barcoding, in advancing entomological research and its practical applications in agricultural contexts.

Acknowledgements

This study was funded by Van Yüzüncü Yıl University Scientific Research Projects Coordination as project numbered FYL-2021-9437.

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