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e-mail: tujns@trakya.edu.tr
Tel: +90 284 2358230
Fax: +90 284 2358237

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CONTENTS

Research Article

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Research Article

INVESTIGATION OF THE GENETIC STRUCTURE OF SOME ANATOLIAN *Achillea* L. (ANTHEMIDEAE, ASTERACEAE) POPULATIONS USING THE ISSR MARKERS

Efkan BAĞDA

Cumhuriyet University, Faculty of Science, Department of Molecular Biology and Genetics, Sivas, TURKEY
e-mail: ebagda@cumhuriyet.edu.tr

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Abstract: Due to high level of hybridization and polyploidy, the perennial and allogamic *Achillea* L. genus with a complex phyletic structure has about 142 species widely distributed over in the Northern Hemisphere. The genus is widely distributed in Turkey with 48 species half of which are endemic. The gene and diversification center of the *Santolinoideae* section including 18 endemic species is thought to be in Anatolia. There exist no comprehensive molecular phylogenetic study on *Achillea* whose morphological revision in Turkey was completed in 2006.

In the study, phylogenetic analyzes were performed using 10 different oligonucleotides for amplification of ISSR bands based on 74 samples of 18 species from 3 sections of *Achillea* genus. All the oligonucleotides analyzed were found to be polymorphic. The total number of loci is 135 and 44 (33%) of them are parsimony informative. Serious topological differences showing that *Achillea* taxa include both monophyletic and polyphyletic lineages were revealed in phylogenetic trees obtained under UPGMA, MP and BI methods. Sections were not clearly separated in trees with clear species separations. The results of UPGMA and MP analyses showed that *A. vermicularis* Trin. was placed as the outgroup while *A. sipirokorensis* Hausskn. & Bornm. and *A. sintenisii* Hub.-Mor. formed the outgroup together in Bayesian Inference analysis (BI). The obtained clusters of PCA based on binary genetic distance values were consistent with the result of BI analysis. Molecular variation analysis showed that almost all of the molecular variation was completely resulted from variations within populations.

Key words: *Achillea*, ISSR, molecular phylogeny, Turkey.

Özet: Çok fazla hibridizasyon ve poliploidi görülmesinden dolayı karmaşık filetik yapıya sahip çok yıllık ve allogamik *Achillea* cinsi Kuzey Yarımküre üzerinde geniş yayılıma sahip 142 kadar türe sahiptir. Bunlardan 48 tanesi Türkiye'de geniş yayılış göstermektedir ve bu 48 türün yarısı endemiktir. 18 endemik türe sahip *Santolinoideae* seksiyonunun gen ve değişim merkezinin Anadolu olduğu düşünülmektedir. Türkiye taksonlarının morfolojik revizyonu 2006 yılında yapılmış olan bu cinsin, kapsamlı bir moleküler filogenetik çalışması halihazırda bulunmamaktadır.

Bu çalışmada, *Achillea* cinsinin 3 seksiyonundan, 18 türe ait 74 örnekle ISSR bantlarının amplifikasyonu için 10 farklı oligonükleotid kullanılarak filogenetik analizler yapılmıştır. Analiz edilen tüm oligonükleotidlerin polimorfik olduğu görülmüştür. Toplam lokus sayısı 135 olup, 44 (%33) tanesi parsinomik olarak bilgi vericidir. UPGMA, MP ve BI filogenetik analiz yöntemleri ile çizilen ağaçlardan *Achillea* taksonlarının monofiletik ve polifiletik olduğunu gösteren ciddi topolojik farklılıklar belirlenmiştir. Tür ayrımlarının olduğu ağaçlarda, seksiyonların net olarak ayrılmadığı belirlenmiştir. UPGMA ve MP analizi sonucunda *A. vermicularis* Trin., Bayesian çıkarsamalı analiz sonucunda ise *A. sipirokorensis* Hausskn. & Bornm. ile *A. sintenisii* Hub.-Mor. türlerinin birlikte dış grup olarak yerleştiği görülmüştür. İkili genetik uzaklık değerlerine dayalı PCA sonuçlarında görülen kümelenmeler Bayesiyan çıkarsamalı analiz sonuçlarıyla uyumlu gerçekleşmiştir. Moleküler varyasyon analiz sonuçları moleküler varyasyonun tamamına yakınının populasyonlar içinden kaynaklandığını göstermiştir.

Introduction

Turkey has a rich flora because of its geological features, soil types and climate conditions in addition to the fact that it is located at the intersection point of Asian, European and African continents. It has a moving geological structure formed by the closure of the Tethyan Sea and it played an important role during the glacial periods The presence of charecteristics of the Mediterranean, Euro-Siberian and Irano-Turanian plant geographical regions in the country is the most important factor increasing species diversity. The flora of Turkey includes about 9222 vascular plant species of which 138 are cultured. Turkey is also an important gene centre

(%33.27 with 2991 species) with a high endemism rate (Arabacı 2006). However, despite the floral richness and high endemism rate in the country thenumber of molecular phylogenetic studies on floral members is limited.

The first work on Turkey's flora is "Flora Orientalis" written by Geneva's famous botanist Edmond Boissier in 1867-1888, and the most comprehensive work, the book "Flora of Turkey and the East Aegean Islands", was written by P. H. Davis. This book consists of 10 volumes together with additional volume. A second additional volume with an increase in Turkish flora studies was also added to this book (Güner *et al*. 2000).

Studies on Anatolian flora have gained a pronounced acceleration recently and new taxa have been identified and/or the available taxonomic groups have been redesigned in studies carried out with numerous samples collected during intensive floristic studies. Revision studies have been increasingly carried out, particularly at genus level, to solve the existing taxonomic problems. The identification of new taxa, the determination of species boundaries and the rewriting of species keys are important consequences of these studies. On the other hand, complex phyletic relationships that are frequently encountered, especially due to high hybridization and polyploidy rates weaken the solving power of morphological revision studies. Molecular systematic approaches are often preferred to overcome these problems with the special aim of revealing evolutionary relationships.

Members of the family Asteraceae (Compositae) are composed of 24080 species distributed in 1545 genera belonging to 21 tribes and three subfamilies. Most of the species in the family are members of the subfamily Asteroideae in which 12 tribes, 1176 genera and about 17025 species are gathered (Arabacı 2006). In the Flora of Turkey, Asteraceae is represented by 11 tribes, 136 genera and 1195 species and is the richest family of flora in terms of both species and genus levels. The family also includes most of the endemic species (endemism rate is %37.3 with 446 species) of the country (Arabacı 2006).

The genus *Achillea* L. is represented with 142 species from the Anthemideae tribe of Asteroideae subfamily and is one of the most recently evolved genus of the family (Arabacı 2006, Rahimmalek *et al.* 2009). Members of the genus can grow in almost all habitat types, from the sea level up to altitudes of 3000m a.s.l., mainly in the temperate zone. It is characterized by perennial and allogamic plants adapted to various ecological environments ranging from deserts to sea shores, steady snowy hills and rocky habitats. (Guo *et al.* 2004). The genus is widely distributed in Europe and West Asia, but it is represented with several species in North America, Australia, New Zealand and North Africa (Rechinger 1963).

The total number of species of *Achillea* in Turkey is 48 (58 taxa) of which 25 are endemic for the country (Ehrendorfer & Guo 2005, Arabacı 2006, Çelik & Akpulat 2008, Arabacı & Budak 2009, Arabacı 2012, Aytaç *et al.* 2016), indicating the high endemism rate in Turkey (Arabacı 2006).

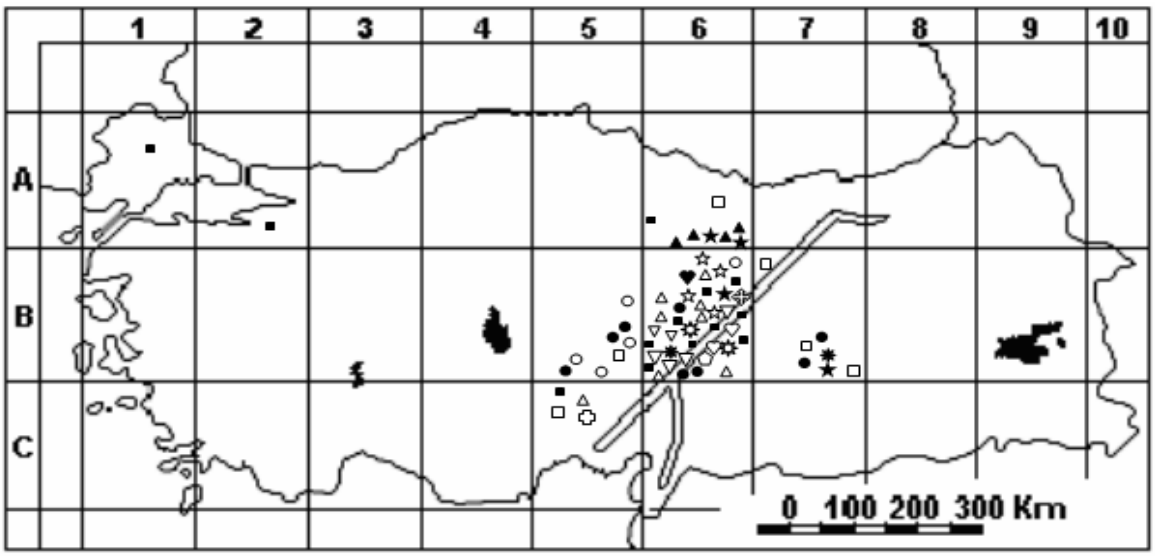
It is thought that the gene center of *Achillea* genus may be the area of the Asian and European continents coalesce. However, the *Santolinoideae* (DC.) Heimerl section of 38 varieties is represented by 26 species, 18 of which are endemic in Turkey, indicating that the genetic and diversification center of *Santolinoideae* section is in Anatolia (Ehrendorfer & Guo 2006, Aytaç *et al.* 2016). In addition, ITS and *trn*L-F analysis suggest that the ancestral section may be *Santolinoideae* based on the results of Guo *et al.* (2004). It has been reported by Arabacı (2006) that the *Achillea* species are mostly concentrated in the Anatolian diagonal, showing that the Anatolian diagonal is an important area in the evolution of the *Achillea* genus in Anatolia.

The phylogenetic relationships and various polyploidy species models in *Achillea* species are investigated with nuclear ribosomal ITS, ncp*GS*, *Pgi*C, *SBP*, chloroplast *trn*T, *trn*F, *trn*H-*psb*A, *trn*C-*ycf*6, *rp*L16 genes with the markers of isozyme electrophoresis, RAPD, AFLP, RFLP, microsatellite and ISSR (Purdy & Bayer 1996, Guo *et al.* 2004, Ehrendorfer & Guo 2005, Guo *et al.* 2005, Ehrendorfer & Guo 2006, Guo *et al.* 2006, Guo *et al.* 2008, Morsy 2007, Rahimmalek *et al.* 2009, Ma *et al.* 2010, Gharibi *et al.* 2011, Rawashdeh 2011, Rahimmalek *et al.* 2011, Ebrahimi *et al.* 2012 a b, Farajpour *et al.* 2012, Guo *et al.* 2012, Rahimmalek 2012, Badr *et al.* 2014, López-Vinyallonga *et al.* 2015, Inotai *et al.* 2016, Badr *et al.* 2017).

High biodiversity and natural hybrids in *Achillea* make it difficult to identify plant samples. Molecular markers are powerful alternative solutions especially for systematic problems which cannot be solved by morphological approaches. In this study, we aimed to contribute to the information about the genus systematic by investigating the biogenetic loci and genetic structures and phylogenetic relationships of 18 cultivars (10 endemic) of *Achillea* L (Asteraceae) genus spreading in and around Sivas using ISSR markers (Zietkiewicz 1994).

Materials and Methods

18 *Achillea* species collected during the period from 2011 to 2012 were used as the study material (Fig. 1, Table 1). All collected specimens are kept in the herbarium of the Cumhuriyet University Faculty of Science (CÜFH). Air dried samples were used for DNA isolation. The total DNA isolations were done using 74 different specimens of 18 species distributed in 59 populations and ISSR-PCR was run for all samples.



A. armenorum (⬠), *A. biebersteinii* (□),*A. cappadocica* (▼), *A. coarctata* (○), *A. cucullata* (✲), *A. kotschyi* (⯐), *A. lycaonica* (☆), *A. magnifica* (✸), *A. millefolium* (■), *A. nobilis* (●), *A. phyrgia* (✥), *A. schischkinii* (★), *A. sipikorensis* (♡), *A. sintenisii* (▲), *A. sivasica* (♥), *A. teretifolia* (▽), *A. wilhelmsii* (△), *A. vermicularis* (⊕)

Fig. 1. Distribution map of *Achillea* populations used in the study.

Table 1. Herbarium numbers (Hrb. No.) and localities of *Achillea* specimens used in the study.

No	Species	Hrb.No	Abbr.	Square	Localities
1	*A. armenorum* Boiss. & Hausskn.	15330	arm	C6	Kahramanmaraş: Göksun, Berit Mountain
2	*A. biebersteinii* Afan	15283	bieb 1	A6	Ordu: Mesudiye-Koyulhisar highway
3	*A. biebersteinii*	15293	bieb 2	B6	Sivas: Zara-Divriği highway, Çaypınar Village
4	*A. biebersteinii*	15367	bieb 3	B9	Between Tatvan-Gevas
5	*A. biebersteinii*	15309	bieb 4	B7	Malatya: Airport highway, Aksaray Village
6	*A. biebersteinii*	15370	bieb5	B5	Sarıkaya-Yozgat
7	*A. biebersteinii*	15373	bieb6	B6	Between Göksun- Kahramanmaraş
8	*A. biebersteinii*	15368	bieb 7	B9	7km to Bitlis
9	*A. cappadocica* Hausskn. & Bornm.	15201	cap1	B6	Yozgat, Between Çat-Güzelyayla
10	*A. cappadocica*	15204	cap2	B6	Yozgat, near Kızılcaova
11	*A. cappadocica*	15208	cap3	B6	Yozgat, near Bozhüyük
12	*A. coarctata* Poir	15295	coa 1	B6	Sivas: Zara-Divriği highway, Çaypınar Village
13	*A. coarctata*	15298	coa 2	B5	Kayseri: Hacılar-Develi highway, Erciyes Mountain
14	*A. coarctata*	15299	coa 3	B5	Kayseri: Sivas-Kayseri highway
15	*A. coarctata*	15304	coa 4	B5	Kayseri: Develi-Bakırdağı, Şahmelik Village
16	*A. coarctata*	15307	coa 5	B5	Kayseri: Hacılar-Develi highway, Hacılar out way
17	*A. cucullata* (Hausskn.) Bornm.	15226	cuc 1	B6	Sivas: Taşlıdere
18	*A. cucullata*	15241	cuc 2	B6	Sivas: Karaçayır highway
19	*A. kotschyi* Boiss.	15345	kot1	C5	Adana-Niğde highway, Niğde Entrance
20	*A. kotschyi*	15207	kot2	B6	Yozgat: Çayıralan out way
21	*A. lycaonica* Boiss. & Heldr.	15151	lyc 1	B6	Sivas: Ulaş, Tecer-Eskikarahisar Village
22	*A. lycaonica*	15153	lyc 2	B6	Sivas: Ulaş, Bostankaya Village
23	*A. lycaonica*	15354	lyc 3	B6	Sivas, Ulaş, Hacımirza Village
24	*A. lycaonica*	15262	lyc 4	B6	Sivas: Cemel-Altınyayla highway
25	*A. magnifica* Hub.-Mor.	15181	mag 1	B6	Sivas: Divriği-İliç highway, Gedikbaşı 8km
26	*A. magnifica*	15310	mag 2	B7	Malatya: arround airport
27	*A. millefolium* L.	15235	mil 1	B6	Sivas: Zara, Karabayır

No	Species	Hrb.No	Abbr.	Square	Localities
28	*A. millefolium*	15325	mil 2	B6	Kahramanmaraş: Göksun, Mehmetbey Village
29	*A. millefolium*	15340	mil 3	C5	Niğde: Çamardı-Yeniköy highway
30	*A. millefolium*	15365	mil 4	B9	Between Tatvan-Gevaş, 63km to Gevaş
31	*A. millefolium*	15275	mil 5	A6	Tokat: Çamlıbel, İhsaniye Village
32	*A. millefolium*	15321	mil 6	B6	Kayseri: Bünyan-Pınarbaşı highway, Erkek Village
33	*A. nobilis* L.	15215	nob 1	B6	Sivas: Ulaş, Hüyüktepe south hillside
34	*A. nobilis*	15270	nob 2	B7	Malatya: Arapkir-Kemaliye highway, 3km
35	*A. nobilis*	15324	nob 3	B5	Kayseri: Kayseri-Sarız, Sarız entrance
36	*A. nobilis*	15326	nob 4	B6	Kahramanmaraş: Mehmetbey Village
37	*A. phyrgia* Boiss. & Balansa	15164	phy	B6	Sivas: Between Gürün-Kangal, Kuşkayası
38	*A. schischkinii* Sosn.	15228	sch 1	A6	Sivas: Between Suşehri-Şerefiye, Karabayır
39	*A. schischkinii*	15146	sch 2	B6	Sivas: Hafik highway, Soğuk Çermik entrance
40	*A. schischkinii*	15279	sch 3	A6	Sivas: İmranlı-Karacaören, Bahadun detour
41	*A. schischkinii*	15274	sch 4	B7	Malatya: Arapgir-Kemaliye, 20km to Kemaliye
42	*A. sintenisii* Hub.-Mor	15154	sin 1	A6	Sivas: Ulaş, arround Bostankaya Village
43	*A. sintenisii*	15155	sin 2	A6	Sivas: Hafik highway, Soğuk Çermik detour
44	*A. sintenisii*	15187	sin 3	A6	Sivas: Between Hafik-Zara, Topçuyeniköy detour
45	*A. sintenisii*	15282	sin 4	A6	Sivas: İmranlı-Karacaören, Bahadun detour
46	*A. sipikorensis* Hausskn. & Bornm	15281	sip 1	B6	Sivas: İmranlı-Karacaören, Bahadun detour
47	*A. sipikorensis*	15268	sip 2	B6	Sivas: Çetinkaya-Divriği Çetinkaya detour
48	*A. sivasica* Çelik & Akpulat	15163	siv	B6	Sivas: Ulaş, Kovalı Village, arround Ziyarettepe
49	*A. teretifolia*	15236	ter 1	B6	Sivas: Ulaş, Baharözü, Düğnükkaya hill
50	*A. teretifolia*	15267	ter 2	B6	Sivas: Kangal, Höbek Village
51	*A. teretifolia*	15196	ter 3	B6	Sivas: Divriği-Gedikbaşı, Çayözü detour
52	*A. teretifolia*	15245	ter 4	B6	Sivas: Between Şarkışla-Altınyayla, Konakyazı Vill.
53	*A. teretifolia*	15364	ter 5	C3	Antalya-Elmalı-Güğübeli
54	*A vermicularis* Trin.	15369	ver	B9	Hakkari-Van detour
55	*A. wilhelmsii* C. Koch.	15162	wil 1	B6	Sivas: Ulaş, Kovalı Village
56	*A. wilhelmsii*	15261	wil 2	B6	Kayseri: Kaftangiyen-Taşlıgeçit Village
57	*A. wilhelmsii*	15344	wil 3	C5	Niğde: Maden Village
58	*A. wilhelmsii*	15220	wil 4	B6	Sivas: Kangal-Kazıklı bridge
59	*A. wilhelmsii*	15287	wil 5	B6	Sivas: İmranlı-Karacaören Village

Total Genomic DNA Isolation

Total genomic DNA isolations were done in equal amounts of tissue samples by modifying the CTAB procedure described by Doyle & Doyle (1987). Care was taken to select leaf samples whenever possible. 694 DNA isolations were performed in total from 294 different individuals. DNA samples were stored at +4°C by dissolving in 100µl 1xTE (10mM Tris-HCl, 1mM EDTA, pH 8.0). Total genomic DNA samples were checked by loading on 1% agarose gel.

Determination of Quality and Quantity of Genomic DNA

The quality and quantitation of DNA after isolation were determined by both agarose gel (1%) electrophoresis technique and spectrophotometric measurements at 260 and 280nm wavelengths. The quality and quantity of the DNA samples were estimated by electrophoresis band pattern and by comparing with DNA marker.

Amplification of ISSR Fragment by PCR

ISSR fragments were amplified by PCR using 10 different oligonucleotides (Table 2).

ISSR fragments, 4ng/µL of template DNA (100ng/µL), 1x *Taq* buffer [10x*Taq* Buffer; 100mM Tris-HCl (pH 8.8), 500mM KCl, 0.8% Nonidet P40], 1.5mM $MgCl_2$ (25mM), 0.1mM dNTP mix (each dATP, dTTP, dCTP, dGTP 0.5mM), 0.02U/µL *Taq* polimeraz (5U/µL), 0.2pmol/µL primer (25pmol/µL) were diluted to 25 µL with sterile distilled water and then amplified by PCR. For amplification; 94°C for 30sec, 65°C (Table 2) for 1min and 72°C for 1 min PCR temperature profile was applied over 35 cycles. PCR products were checked by loading on 1.5% agarose gel (Fig. 2).

Analysis of ISSR Data

The phylogenies of *Achillea* populations were investigated using different algorithms. Analyzes were

performed on three different approaches; DNA distance, maximum parsimony (MP) and Bayesian inference. Analyzes were evaluated according to the band profiles obtained from the ISSR markers. The presence of the bands is indicated by "1" and the absence of bands by "0". The presence of the band represents a dominant, non-existent recessive phenotype. Because ISSR markers are dominant, the genotype and allele frequencies can not be calculated since the alleles in the same locus can not be distinguished. Therefore, ISSR data is calculated based on the ratio of bands that are common to any locust to all bands. The ratios of existing bands (1) or non-existing bands (0) and common bands were used for the calculations. All these calculations are based on the assumption that the bands moving in the gel for the same distance (R_f), that is, of the same size, are similar. In fact, bands of similar length are directly proportional to the kinship grades of the compared individuals. So, we can say that individuals with more common bands are closer and those with less common bands are farther away. The obtained trees were drawn using FigTree v1.3.1 (Rambaut 2009).

Analysis of the obtained data was performed using a computer program called Popgene 32 (version 1.3.1) (Yeh *et al.* 1999). Assuming that the populations were in the Hardy-Weinberg equilibrium, the genetic distance (Fst) values between population pairs were calculated to determine population differentiation (Nei 1972).

The unweighted pair-group method with arithmetic mean (UPGMA) dendograms (10000 replicates) were generated by varying the Fst values of the Neighbor-Joining (Saitou & Nei 1987) procedure using the same analysis program. PCA (Principal Component Analysis) was used in the GenAlEx 6.3 package program (Peakall & Smouse 2006) to create a visual representation of the genetic relationship between populations.

Hierarchical analysis of the molecular variation (AMOVA) in the genetic construction of *Achillea* populations was performed using the Arlequin 3.11 (Excoffier *et al.* 2005) program using clusters obtained from biogeographic areas, phylogenetic and principal component analyzes. The significance ratings of fixation indices determined by AMOVA were determined by testing with 1000 proposed permutations given by Excoffier *et al.* (1992).

The MP analysis of the data sets was performed by applying the tree bisection-reconnection (TBR) branch swapping, random addition sequence replicates and 50% majority rule using PAUP * 4.0 beta 10 (Swofford 2002) with heuristic search algorithm. Character states were unordered and unweighted. The bootstrap values of the branches were investigated using heuristic search with 1000 bootstrap replicates.

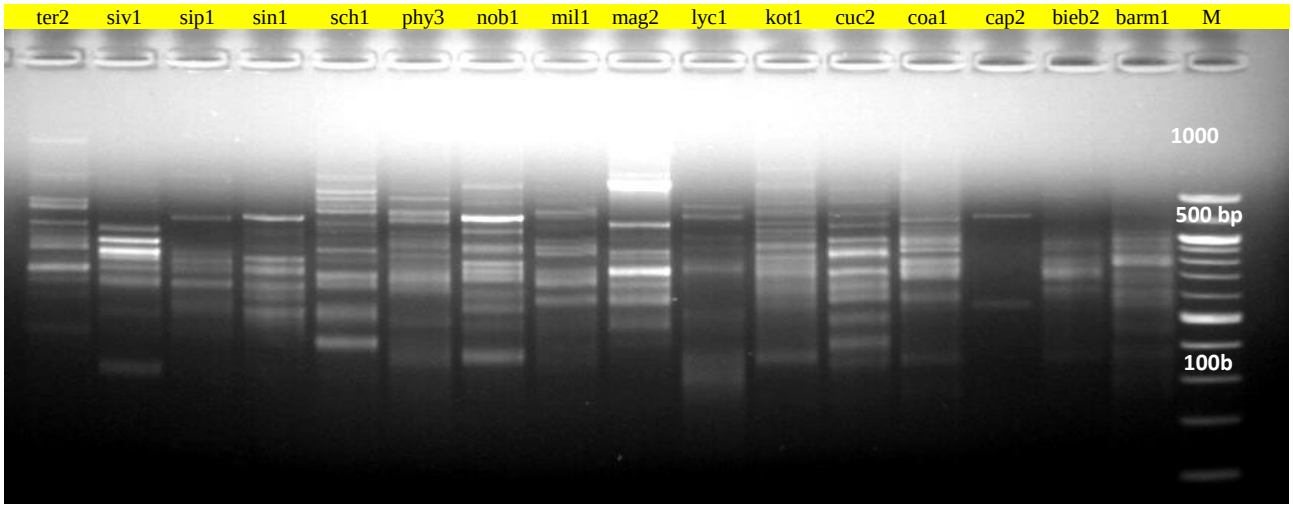


Fig. 2. DNA fragments amplified with ISSR-1 oligonucleotide. Marker (M): 100bp.

Table 2. Base sequences and annealing temperatures of the ISSR oligonucleotides used for amplification.

Name	Sequence	Repeat sequence	Anneling temperature
ISSR 1	CTCTCTCTCTCTCTCTG	$(CT)_8G$	53°C
ISSR 2	CACACACACACACACAG	$(CA)_8G$	53°C
ISSR 3	GAGAGAGAGAGAGAGAG	$(GA)_8G$	53°C
ISSR 4	GTGTGTGTGTGTGTGTC	$(GT)_8C$	58°C
ISSR 5	GTGTGTGTGTGTGTGTC	$(GT)_8C$	51°C
ISSR 6	CACACACACACACACAGAC	$(CA)_8GAC$	57°C
ISSR 7	GAGTCTCTCTCTCTCTCTC	$GAG(TC)_8$	57°C
ISSR 8	CACCACCACCACCACCACCACT	$(CAC)_7T$	61-67°C
ISSR 9	GTCACCACCACCACCACCACCAC	$(CAC)_7GT$	67°C
ISRR 10	TCTTCTTCTTCTTCTTCT	$(TCT)_6$	50°C

In MP analysis, *A. vermicularis* population was determined as the outgroup from UPGMA dendogram and consensus tree was formed. In this way, the most reliable tree was obtained by re-establishing the branches in the consensus tree. Genetic distance and parsinomic results were compared and common and non-common points were evaluated.

Phylogenetic analyses based on Bayesian and Markov Chain Monte Carlo (MCMC) were carried out using the program MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003). Analyzes were performed by presence or absence of markers in the populations (field analysis). In the ISSR analysis, "nst=1" and the rate is "rates=equal" was used. For the generation of 10^7, two independent runs of four chains (3 heated and 1 cold chain) were carried out and the trees were sampled every 1000 cycles. Convergence on stationary distribution was verified by checking whether the mean standard deviation of the separation frequencies is less than 0.05 between two independent executions. Bayesian posterior probabilities were estimated by constructing a Majority-Rule Consensus Tree among the last 750 sampled trees (25% of the samples, ie, 250 samples were pre-tested or burn-in removed).

Results

Amplifications of ISSR markers were performed using 10 oligonucleotides from 74 samples of 18 species distributed in 3 sections of the *Achillea* genus (Table 3).

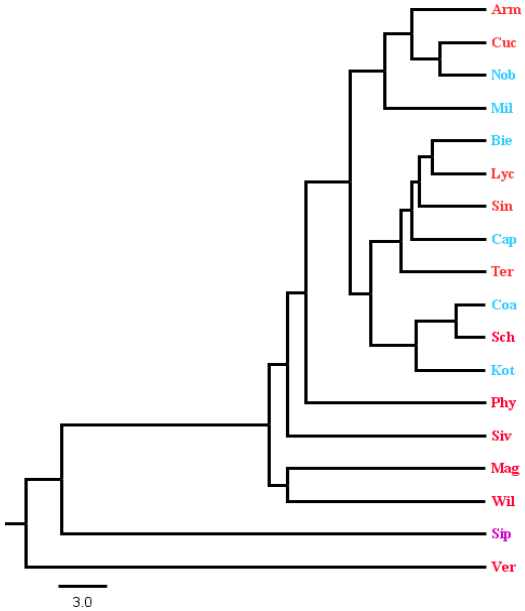


Fig. 3. UPGMA dendogram based on genetic distance data matrix between populations.

All of the oligonucleotides analyzed were found to be polymorphic. The total number of loci was 135 (at most 24, at least 9 bands) and it was determined that 50 of them were fixed, 41 of them were not informative and 44 of them were parsinomically informative.

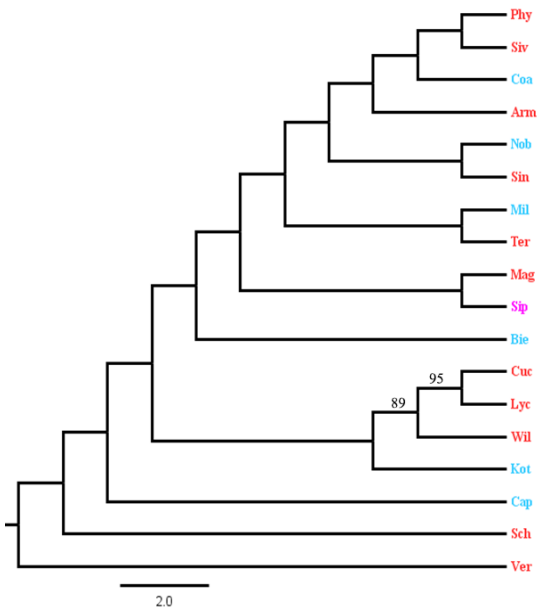


Fig. 4. Compatibility tree with 50% majority rule based on MP method. All branches except two branches (95% and 89%) were shown to support 100%. Colored groups stands for sections represented by populations.

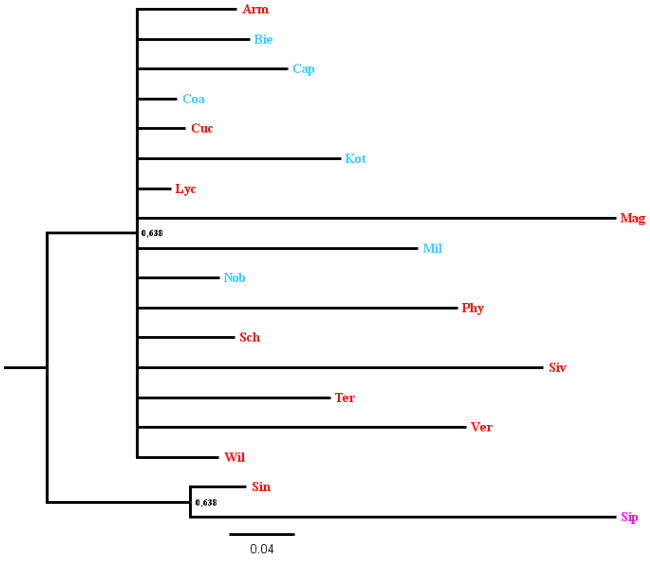
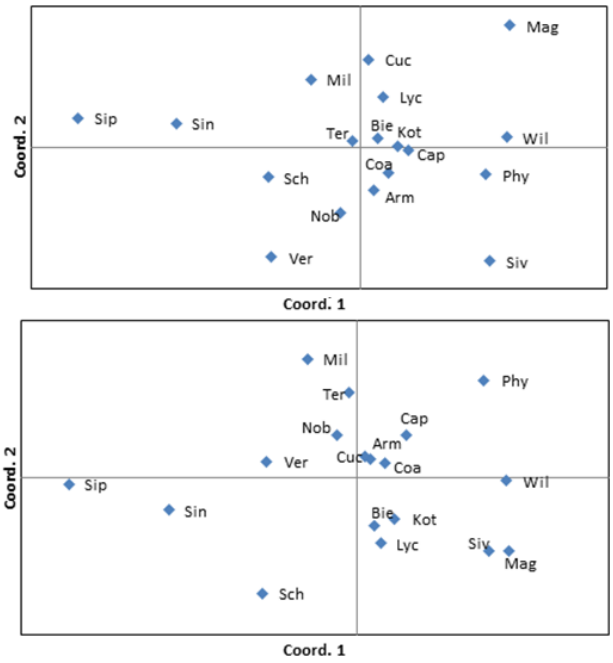


Fig. 5. Phylogenetic relationship between populations based on ISSR haplotypes. The cladogram is a majority-based compliance tree based on Bayesian analysis.

The smallest (0.0676) genetic distance was found between *A. schischkinii* and *A. coarctata* populations and the largest genetic distance (0.8149) was found (Nei 1972) between *A. sipikorensis* and *A. wilhelmsii* populations (Table 4).

It was determined from the UPGMA dendograms based on the genetic distance values between the populations (Fig. 3) that the Anatolian *Achillea* populations were monophyletic and *A. sipikorensis* was the sister group and *A. vermicularis* was the outgroup.



Percentage of variation explained by the first 3 axes			
Axes	**1**	**2**	**3**
%	21.62	19.92	17.17
Cum%	21.62	41.54	58.70

Fig. 6. PCA (Principal Component Analysis) results for the formation of phylogenetic relations between *Achillea* populations. A) Axes 1-2, b) Axes 2-3.

Maximum parsinomy analysis of *A. vermicularis*, which was located as an outgroup on the UPGMA dendogram, was assigned as an outgroup for 50% majority rule application. A consensus tree was constructed with 50% majority rule (CI 0.659; RI 0.476) (see Fig. 4) by using the most suitable 38 consecutive trees of 129 trees. Except two branches (95% and 89%), all branches were found to support 100%. It is found that, as in the UPGMA dendogram, *Achillea* populations are monophyletic in cladogram where *A. schischkinii* is a sister group. The branch settlements were very different and they were not similar except that they were monophyletic in both trees.

Table 3. The number of samples for which ISSR fragments were amplified.

No	Species	Number of Specimens
1	*A. armenorum*	3
2	*A. biebersteinii*	7
3	*A. cappodocica*	3
4	*A. coarctata*	5
5	*A. cucullata*	3
6	*A. kotschyi*	3
7	*A. lycaonica*	5
8	*A. magnifica*	4
9	*A. millefolium*	6
10	*A. nobilis*	4
11	*A. phyrgia*	3
12	*A. schischkinii*	4
13	*A. sintenisii*	4
14	*A. sipikorensis*	3
15	*A. sivasica*	3
16	*A. teretifolia*	6
17	*A. vermicularis*	3
18	*A. wilhelmsii*	5
	Total	74

Table 4. Genetic distance data matrix between populations (Nei 1972).

	Arm	Bie	Cap	Coa	Cuc	Kot	Lyc	Mag	Mil	Nob	Phy	Sch	Sin	Sip	Siv	Ter	Ver	Wil
Arm	0																	
Bie	0.2111	0																
Cap	0.1944	0.1094	0															
Coa	0.1394	0.1693	0.1485	0														
Cuc	0.123	0.1073	0.1434	0.1993	0													
Kot	0.1988	0.2104	0.2099	0.113	0.2248	0												
Lyc	0.1948	0.0861	0.1121	0.1415	0.1007	0.1447	0											
Mag	0.3134	0.2317	0.2392	0.2394	0.2583	0.293	0.2011	0										
Mil	0.2107	0.1896	0.1954	0.2684	0.0986	0.3329	0.2089	0.2854	0									
Nob	0.1132	0.1354	0.1491	0.1916	0.083	0.2676	0.1686	0.3101	0.1401	0								
Phy	0.1474	0.2961	0.3013	0.1845	0.2038	0.2451	0.2872	0.367	0.2147	0.2099	0							
Sch	0.167	0.159	0.161	**0.0676**	0.1953	0.118	0.1282	0.2815	0.2756	0.217	0.2674	0						
Sin	0.2262	0.1104	0.1258	0.1503	0.1545	0.2033	0.1045	0.2455	0.2188	0.1478	0.3343	0.1519	0					
Sip	0.3557	0.6165	0.607	0.4939	0.4718	0.4451	0.5856	0.6635	0.4848	0.4757	0.46	0.4908	0.5215	0				
Siv	0.256	0.2483	0.2481	0.3204	0.1725	0.3954	0.263	0.3239	0.2117	0.1854	0.2843	0.3296	0.2901	0.5196	0			
Ter	0.2206	0.1137	0.1471	0.1629	0.1519	0.2199	0.1322	0.2367	0.1993	0.173	0.2861	0.1753	0.122	0.597	0.2872	0		
Ver	0.6552	0.5948	0.6282	0.5739	0.6906	0.6407	0.603	0.644	0.5691	0.6143	0.5316	0.5167	0.5528	0.6739	0.5798	0.516	0	
Wil	0.4125	0.2383	0.3012	0.3146	0.3012	0.3677	0.2309	0.2624	0.2716	0.3522	0.2983	0.3204	0.2691	**0.8149**	0.3299	0.2407	0.3466	0

The population was found to be polyphyletic from the trees formed by Bayesian Inference analysis based on haplotype distribution. In addition, unlike the others, *A. sipikorensis* and *A. sintenisii*, which are monophyletic among themselves, co-existed as outgroups together (Fig. 5).

Molecular variation in the genetic construct (AMOVA) for *Achillea* population was carried out using clusters obtained from phylogenetic and basic component analyzes, as well as some biogeographic fields (Anatolian Diagonal considered). It has been shown that almost 100% of the molecular variation originated from within the populations (no results were given).

Two different clusters were observed according to PCA based on binary genetic distance values (Fig. 6). The first two axes of the major components bring out 51.16%

of the total genetic variation. The total variation ratio of the primary components to the first and third axes is 48.68%, and the total variation ratio of the axes 2 and 3 is 40.81%. While *A. sipikorensis* and *A. sintenisii* were located differently in both distributions, no significant distribution was observed in other populations. This distribution model is found compatible with the results of Bayesian analysis.

Discussion and Conclusion

Determination of the phylogenetic relationships of the genus *Achillea*, which has a complex phyletic structure due to hybrid and polyploidy frequency is problematic (Guo *et al.* 2004, Guo *et al.* 2012). Morphological revision of Turkey's *Achillea* taxa was conducted by Arabacı (2006) but molecular phylogenetic studies on the genus are limited and they were mostly based on inadequate sample size. Therefore, the present study is the most comprehensive molecular phylogenetic study so far on Anatolian *Achillea* species and aimed to contribute to the systematic information using ISSR markers of 18 species from three sections.

Turkey is an important evolutionary unit for the genus *Achillea* (ESU) considering the fact that 46 species of *Achillea* which constitute 1/3 of the total number of the species within the genus are found in Turkey and half of these species are endemic. Due to this evolutionary importance, species diversity needs to be well investigated. The *Santolinoideae* section has 38 species. Of these, 16 are endemic and 24 are located in Turkey. This indicates that the gene and the center of change of the *Santolinoideae* section is Anatolia Guo *et al.* (2004) suggested based on the results of ITS and *trn*L-F sequence analysis that the ancestral section may be *Santolinoideae*, providing an interesting detail for the origin of the genus. *A. teretifolia* and *A. wilhelmsii*, both belonging to the *Santolinoideae* section, were located in the ancestral clades in the study of Guo *et al.* (2004). The ISSR data obtained in the present study supports the *Santolinoideae* section as an outgroup, but *A. teretifolia* and *A. wilhelmsii* species were not found in the outgroup. UPGMA and MP support *A. vermicularis* (*Santolinoideae*) as an ancestral taxon, while BI results support the external group as *A. sintenisii* (*Santolinoideae*) and *A. sipikorensis* (*Arthrolepis*). It should be mentioned that, all sections are not included in the present study.

The phylogenetic tree patterns from the phylogenetic analysis of *Achillea* populations containing a large number of hybrids and polyploidy species/taxa are one of the most likely scenarios to be expected and this pattern has also been obtained from MP and BI cladograms generated from ISSR data. The data given by Guo *et al.* (2004) also supports a similar polyphyletic story based on analysis of ITS and *trn*L-F sequence data. Significant major differences were determined between the results of the alternative analysis, but *Achillea* populations were considered to be monophyletic by the analysis (without the choice of outgroup).

Some of the results obtained from the analysis were found to overlap with some information given in the Anatolian revision study of Arabacı (2006). *A. armenorum*, an endemic species unique to the Berit Mountain, has distributed in rocky areas above 2400m. There is no *Achillea* species that are similar or closely related to *A. armenorum* (Arabacı 2006).

BI cladogram and PCA, the pattern in which the monophyletic *A. sintenisii* and *A. sipikorensis* were evaluated together as an outgroup support the finding that two revised polyploidies are given in the revision.

A. sintenisii and *A. sipikorensis* which grow between 1200-2000m on gypsiferous peaks are endemic to Irano-Turanian region. They are distributed intensively in steppe and calcareous slopes in Sivas and its vicinity. The species commonly found in gypseous areas are close relatives and the spreading areas are the same or close together. There are significant differences between the populations of *A. sipikorensis* on the gypsifer bed rock and others (Arabacı 2006).

The ISSR data obtained from the study did not fully support the hypothesis that Anatolian Diagonal may have played an important role in the evolution of Anatolian *Achillea* populations. In addition to the sympatric speciation expected to be effective in the evolution of the *Achillea* taxa in Anatolia, the vicariance speciation model needs to be tested again using more extensive samplings. Thereby, looking at the nucleotide sequence variations, AFPL marker or chloroplast will increase the reliability of the result.

The present study is the first study on molecular phylogeny of non-endemic (*A. schischkinii, A. vermicularis*) and endemic (*A. armenorum, A. cappadocica, A. cucullata, A. kotschyi, A. lycaonica, A. magnifica, A. phyrgia, A. sintenisii, A. sipikorensis, A. sivasica*) *Achillea* species in Turkey. In addition, to the best of our knowledge, ISSR markers of 17 species (except *A. millefolium*) included in the study were obtained for the first time.

In conclusion, ISSR markers provided a comprehensive and significant contribution to the understanding of the genetic diversity and phylogenetic relationship of *Achillea* taxa in Turkey. The results obtained will help to understand the evolutionary dynamics of *Achillea* genus.

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References

1. Arabacı, T. 2006. Türkiye'de yetişen *Achillea L.* (Asteraceae) cinsinin revizyonu. İnönü Üni. Fen Bilimleri Enstitüsü, Doktora Tezi, 276 pp, Malatya.
2. Arabacı, T. & Budak, Ü. 2009. *Achillea hamzaoglui* (Asteraceae), a new species from Turkey. *Annales Botanici Fennici*, 46: 459-463.
3. Arabacı, T. 2012. *Achillea* L. In: Güner, A., Aslan, S., Ekim, T., Vural, M., Babaç, M.T., editors. Türkiye Bitkileri Listesi (Damarlı Bitkiler). İstanbul, Turkey: Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını, pp. 108-112.
4. Aytac, Z., Duman, H. & Ekici, M. 2016. Two new *Achillea* L. (Asteraceae) species from Turkey. *Turkish Journal of Botany*, 40: 373-379.
5. Badr, A., Ahmed, H.I.S., Hamouda, M. & El-Khateeb, E.A. 2014. Genetic diversity in Egyptian populations of *Achillea santolina* using morphological traits and ISSR markers. *Taeckholmia*, 34, 49-65.
6. Badr, A., El-Shazly, H.H., Ahmed, H.I.S., Hamouda, M., El-Khateeb, E. & Sakr, M. 2017. Genetic diversity of *Achillea* fragrantissima in Egypt inferred from phenotypic variations and ISSR markers associated with traits of plant size and seed yield. *Plant Genetic Resources*, 15(3): 239-247.
7. Çelik, N. & Akpulat, A. 2008. *Achillea sivasica* (Asteraceae: sect. Babounya (DC.) O. Hoffm.), a new species from Inner Anatolia, Turkey. *Kew Bulletin*, 63(3): 485-489.
8. Doyle, J.J. & Doyle, J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, 19: 11-15.
9. Ebrahimi, M. Farajpour, M. & Rahimmalek, M. 2012a. Inter- and intra-specific genetic diversity of Iranian yarrow species *Achillea santolina* and *Achillea tenuifolia* based on ISSR and RAPD markers. *Genetics and Molecular Research*, 11(3): 2855-2861.
10. Ebrahimi, M., Farajpour, M., Beigmohamadi, M. & Ebrahimi, M. 2012b. Genetic relationships among yarrow based on Random Amplified Polymorphic DNA markers. *E3 Journal of Biotechnology and Pharmaceutical Research*, 3(4): 69-73.
11. Ehrendorfer, F. & Guo, Y.-P. 2006. Multidisciplinary studies on *Achillea* sensu lato (Compositae-Anthemideae): new data on systematics and phylogeography. *Willdenowia*, 36: 69-87.
12. Ehrendorfer, F. & Guo, Y.-P. 2005: Changes in the circumscription of the genus *Achillea* (Compositae-Anthemideae) and its subdivision. *Willdenowia*, 35: 49-54.
13. Excoffier, L., Laval, G. & Schneider, S. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, 1: 47-50.
14. Excoffier, L., Smouse, P.E. & Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, 131: 479-491.
15. Farajpour, M., Ebrahimi, M., Amiri, R., Golzari, R. & Sanjari, S. 2012. Assessment of genetic diversity in *Achillea millefolium* accessions from Iran using ISSR marker. *Biochemical Systematics and Ecology*, 43: 73-79.
16. Gharibi, S., Rahimmalek, M., Mirlohi, A., Majidi, M.M. & Tabatabaei, B.E.S. 2011. Assessment of genetic diversity in *Achillea millefolium* subsp. *millefolium* and *Achillea millefolium* subsp. *elbursensis* using morphological and inter-simple sequence repeat (ISSR) markers. *Journal of Medicinal Plants Research*, 5(13): 2906-2916.
17. Guo, Y.P., Ehrendorfer, F. & Samuel, R. 2004. Phylogeny and systematics of *Achille* (Asteraceae-Anthemideae) inferre from nrITS and plastid trnL-F DNA sequences, *Taxon*, 53(3): 657-672.
18. Guo, Y.P., Vogl, C., Van Loo, M. & Ehrendorfer, F. 2006. Hybrid origin and differentiation of two tetraploid *Achillea* species in East Asia: molecular, morphological and ecogeographical evidence. *Molecular Ecology*, 15: 133-144.
19. Guo, Y.P., Wang, S.-Z., Vogl, C. & Ehrendorfer, F. 2012. Nuclear and Plastid Haplotypes Sugget Rapid Diploid and Plyploid Speciation in the N Hemisphere *Achillea millefolium* Complex (Asteraceae). *BMC Evolutionary Biology*, 12: 2.
20. Guo, Y.-P., Saukel, J. & Ehrendorfer, F. 2008. AFLP trees versus scatter plots: evolution and phylogeography of the polyploid complex *Achillea millefolium* agg. (Asteraceae). *Taxon*, 57: 1-17.
21. Guo, Y.-P., Saukel, J., Mittermayr, R. & Ehrendorfer, F. 2005. AFLP analyses demonstrate genetic divergence, hybridization, and multiple polyploidization in the evolution of *Achillea* (Asteraceae-Anthemideae). *New Phytologist*, 166: 273-290.
22. Güner, A., Özhatay, N., Ekim, T. & Başer, K.H.C. (Eds.) 2000. *Flora of Turkey and the East Aegean Islands, Vol.11 (supplement 2)*. Edinburg University Press, Edinburgh.
23. Huelsenbeck, J.P. & Ronquist, F. 2001. Mrbayes: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17, 754-755.
24. Inotai, K., György, Z., Kindlovits, S., Várady, G. & Zámboriné Németh, É. 2016. Evaluation of yarrow (*Achillea*) accessions by phytochemical and molecular genetic tools. *Journal of Applied Botany And Food Quality-Angewandte Botanik*, 89: 105-112.
25. López-Vinyallonga, S., Soriano, I. & Susanna, A. 2015. The polyploid series of the *Achillea millefolium* aggregate in the Iberian Peninsula investigated using microsatellites. *PLoS One*, 2015, 10(6): e0129861.
26. Ma, J.-X., Li, Y.-N., Vogl, C., Ehrendorfer, F. & Guo, Y-P. 2010. Allopolyploid speciation and ongoing backcrossing between diploid progenitor and tetraploid progeny lineages in the *Achillea millefolium* species complex: analyses of single-copy nuclear genes and genomic AFLP. *BMC Evolutionary Biology*, 10: 100.
27. Morsy, A.A. 2007. Molecular variations of *A. fragranissima* (Forssk.) SCH. BIP. Growing in five areas

of South Sinai. *International Journal of Agriculture and Biology*, 9: 11-17.

28. Nei, M. 1972. Genetic distance between populations. *American Naturalist*, 106: 283-292.
29. Peakall, R. & Smouse, P.E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, 6: 288-295.
30. Purdy, B.G., & Bayer, R.J. 1996. Genetic variation in populations of the endemic *Achillea millefolium* ssp. *megacephala* from the Athabasca sand dunes and the widespread ssp. *lanulosa* in western North America. *Canadian Journal of Botany*, 74: 1138-1146.
31. Rahimmalek, M., Tabatabaei, B.E.S., Arzani, A. & Khorrami, A. 2011. Development and characterization of microsatellite markers for genomic analysis of yarrow (*Achillea millefolium* L.). *Genes & Genomics*, 33: 475-482.
32. Rahimmalek, M. 2012. Genetic Relationships Among *Achillea tenuifolia* Accessions Using Molecular and Morphological Markers. *Plant Omics Journal*, 5(2): 128-135.
33. Rahimmalek, M., Tabatabaei, B.E.S., Arzani, A. & Etemadi, N. 2009. Assessment of genetic diversity among and within *Achillea* species using amplified fragment length polymorphism (AFLP). *Biochemical Systematics and Ecology*, 37: 354-361.
34. Rambaut, A. 2009. FigTree v1.3.1 Date accessed: Dec 2009: <http://tree.bio.ed.ac.uk/software/ FigTree/>.
35. Rawashdeh, İ.M. 2011. Genetic Diversity of *Achillea fragrantissima* (Forskal) Schultz BNip Populations Collected From Differnet Regions of Jordan Using RAPD Markers. *Jordan Journal of Biological Sciences*, 4: 21-28.
36. Rechinger, K.H. 1963. *Flora Iranica. No. 158.* Akademische Druke-U. Verlagsanstalt, Wien, Austria. pp. 49-71.
37. Ronquist, F. & Huelsenbeck, J.P. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19: 1572-1574.
38. Saitou, N. & Nei, M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4: 406-425.
39. Swofford, D.L. 2002. PAUP: Phylogenetic Analysis Using Parsimony, Version 4.0 beta Computer program distributed by Sinauer Associates, Sunderland.
40. Yeh, F. C., Yang, R. C., & Boyle, T. (1999). Popgene version 1.32: Microsoft Windows-based freeware for population genetic analysis. University of Alberta, Edmonton.
41. Zietkiewicz, E., Rafalski, A. & Labuda, D. 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics*, 20: 176-183.

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Research Article

CAPSULE, LEAF AND CYATHIAL GLAND MORPHOLOGY OF TURKISH PERENNIAL TAXA OF *Euphorbia* L. SECTION *Pithyusa* (Raf.) Lázaro

İlker GENÇ*, Şükran KÜLTÜR, Gülay ECEVİT-GENÇ

İstanbul University, Faculty of Pharmacy, Department of Pharmaceutical Botany, İstanbul, TURKEY
*Corresponding author: e-mail: ilkgenc@istanbul.edu.tr

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Abstract: Macro and micromorphology of cyathial glands, capsules and cauline leaf surfaces of 13 perennial species of *Euphorbia* L. subg. *Esula* Pers. sect. *Pithyusa* (Raf.) Lázaro in Turkey were studied. Cyathial gland structure, based on cyathial gland shapes, colour and appendages was examined from live material by direct field observations and investigations. Cauline leaves and capsule surface features were analysed with scanning electron microscopy (SEM). Capsule sizes, capsule shapes and capsule and cauline leaf surface ornamentations were also studied. The capsule shape was found to vary from subglobose to conical. In most species, cyathial gland colour ranged from greenish-yellow to yellowish-green but some species were distinguished by their reddish or purplish cyathial glands. Although the number of cyathial glands in all species was four, five cyathial glands were determined to exist in some specimens of *E. pestalozzae* Boiss. Three different cyathial gland shapes (elliptic, trapezoid-elliptic or elliptic-reniform) were observed. Capsule surface of more than half of the studied species (7 taxa) is covered with nipple-like projections (papillate-mammillate) and the rest are covered with tiny rounded or hillock-like ornamentation (colliculate). The cauline leaf surface is papillate-mammillate in the majority of the studied species, although, colliculate surface is also observed in two species (*E. seguieriana* Necker and *E. thessala* (Form.) Degen & Dörf.). In conlusion, macro and micromorphological structures of cyathial gland and capsule of *Euphorbia* sect. *Pithyusa* taxa appeared to be useful diagnostic characters for species identifications.

Key words: Leafy spurge, micromorphology, leaf surface, subg. *Esula*, Euphorbiaceae.

Özet: Bu çalışma *Euphorbia* L. subg. *Esula* Pers. sect. *Pithyusa* (Raf.) Lázaro çok yıllık türlerinin siatyum gland şekilleri, kapsula ve gövde yaprak yüzey mikromorfolojilerine dayanmaktadır. Siatyum gland yapılarının incelenmesi doğrudan arazi çalışmaları sırasında canlı örnekler üzerinden yapılmıştır. Gövde yaprağı ve kapsulanın yüzey özellikleri taramalı elektron mikroskobu (SEM) ile incelenmiştir. Ayrıca kapsula boyutu, kapsula şekli, kapsula ve gövde yaprak yüzey özellikleri de incelenmiştir. Çalışılan türlerin kapsula şekilleri hemen hemen küresel'den koniğe değişir. Araştırılan türlerden çoğunun gland rengi parlak sarı-yeşil veya yeşilimsi sarıdır. Bazı türlerde ise glandlar kırmızı veya mor renkli olabilir. Tüm türlerde gland sayıları *E. pestalozzae* Boiss. haricinde dörttür. Bu türün bazı bireylerinin 5 glandlı olduğu saptanmıştır. Üç farklı gland şekli (eliptik, trapezoid-eliptik veya böbreksi) gözlenmiştir. kapsula yüzeyleri, incelenen türlerin yarısından fazlasında (7 takson) meme başı benzeri çıkıntılarla (papillat-mammillat) kaplı iken, diğer türlerde küçük yuvarlak veya tepecik benzeri yükseltilerle (kollikulat) kaplıdır. İncelenen türlerin çoğunluğunda gövde yaprak yüzeyleri papillat-mammillattır. Kollikulat yüzeyler de iki türde görülmüştür (*E. seguieriana* Necker ve *E. thessala* (Form.) Degen & Dörf.) Sonuç olarak, *Euphorbia* sect. *Pithyusa* türlerine ait gland ve kapsula morfolojik özelliklerinin türlerin ayrımında ayırt edici karakterler olarak kullanılabileceği gösterilmiştir.

Introduction

Euphorbia L. is one of the largest genera of flowering plants with more than 2000 species distributed throughout the world. Life forms within the genus vary from tiny annuals to perennial herbs and trees (Bruyns *et al.* 2006, Horn *et al.* 012). Previous phylogenetic studies on *Euphorbia* yielded results used for separation of the genus into four main clades corresponding four subgenera as *Athymalus* Neck. ex. Rchb., *Chamaesyce* Raf., *Esula* Pers. and *Euphorbia* (Steinmann & Porter 2002, Bruyns *et al.* 2006, Zimmerman *et al.* 2010, Horn *et al.* 2012, Yang *et al.* 2012, Dorsey *et al.* 2013, Peirson *et al.* 2013, Riina *et al.* 2013). Genus *Euphorbia* is represented in Turkey by two subgenera *Chamaesyce* and *Esula* with a total of 120 taxa (Öztekin 2012, Genç & Kültür 2016).

Subgenus *Esula* comprises about 490 species most of which are annual or perennial herbs mostly distributed in the temperate regions of the northern hemisphere in the Old World (Geltman 2015, Pahlevani *et al.* 2017). Temperate Eurasia, particularly the Mediterranean and the Irano-Turanian regions are two most important diversity centres of *Euphorbia* subgenus *Esula* (Riina *et al.* 2013).

Turkish subgenus *Esula* has recently been embedded and classified, based on recent molecular studies, into 14 sections [*Arvales* (Geltman) Geltman, *Chylogala* (Fourr.) Prokh., *Esula* (Pers.) Dumort., *Exiguae* (Geltman) Riina & Molero, *Helioscopia* Dumort., *Lagascae* Lázaro, *Lathyris* Dumort., *Myrsiniteae* (Boiss.) Lojac., *Pachycladae* (Boiss.) Tutin, *Paralias* Dumort., *Patellares* (Prokh.) Frajman, *Pithyusa* (Raf.) Lázaro, *Szovitsiae* Geltman and *Tithymalus* (Gaertn.) Roep.] (Riina *et al.* 2013).

17 species of the 60 reported species of *Euphorbia* sect. *Pithyusa* occur in Turkey. Turkey is the second most species-rich country after Iran both in the number of species and endemics in sect. *Pithyusa* (Pahlevani 2017). The members of the section are mostly characterized by semisucculent and glaucous leaves, palmate leaf venation, papillose indumentum, usually entire leaf margin, conical or nearly so rounded capsules and elliptic or trapezoidal cyathial glands (Riina *et al.* 2013).

The only comprehensive study on the genus *Euphorbia* in Turkey was performed by Radcliffe-Smith (1982) and the last morphological study concerning the genus was made by Can & Küçüker (2015).

Seed, capsule and cyathial gland morphologies are useful parameters used in distinguishing taxa in *Euphorbia* (Pahlevani & Akhani 2011, Salmaki *et al.* 2011, Riina *et al.* 2013, Pahlevani *et al.* 2015). However, detailed studies are missing especially for most species distributed in Turkey, raising the need of comprehensive studies on seed, capsule and cyathial gland morphologies of Turkish *Euphorbia*. We, therefore, aimed in the present study to provide a detailed description and importance of capsula and cyathial gland morphology of perennial Turkish *Euphorbia* sect. *Pithyusa.* We also investigated the micromorphology of the cauline leaf surfaces after determining some variations on leaf surfaces of herbarium specimens included in the study.

Materials and Methods

Plant Material

The material included in the study was collected by the authors during field trips carried out between 2014 and 2017 in different parts of Turkey. Herbarium specimens are deposited in the Herbarium of the Faculty of Pharmacy of İstanbul University (ISTE). A list of the taxa included in the study was given in Table 1.

Table 1. List of voucher specimens of *Euphorbia* sect. P*ithyusa* studied in the study (* Endemic).

Species	Voucher specimens (ISTE)
E. cheiradenia Boiss. & Hohen.	**Şanlıurfa**, Tektek Mountain National Park, 460m, 18.vi.2015, *İ.Genç 2398, Ş.Kültür.*
E. erythrodon Boiss. & Heldr.	**Erzincan**, Sipikor mountain pass, 1400m, 25.vii.2015, *İ.Genç 2463, A. Kandemir.* **Antalya**, Bozburun mountain, 1600m, 27.vii.2017, *İ.Genç 2564, İ.G. Deniz, G. Ecevit Genç.*
E. glareosa Pall. ex Bieb.	**Kastamonu**, Kastamonu-Karabük, roadsides, 600m, 14.vii.2015, *İ.Genç 2418, G. Ecevit Genç.*
E. macroclada Boiss.	**Isparta**, Isparta-Eğirdir, roadside, 990m, 9.vii.2014, *İ.Genç 2210, S. Yüzbaşıoğlu.* **Sivas**, Tokat-Sivas road, near Kızıliniş, roadsides, 700m, 22.vii.2015, *İ.Genç 2445.* **Erzincan**-Erzurum road, roadsides, 1510m, 23.vii.2015, *İ.Genç 2448.* **Şanlıurfa**, Tektek Mountain National Park, 410m, 18.vi.2015, *İ.Genç 2392, Ş.Kültür.* **Van**-Erciş road, near Van Lake, 1750m, 24.vii.2015, *İ.Genç 2454.*
E. niciciana Borbás ex Novák	**Edirne**, Edirne-Uzunköprü, roadside, 70m, 6.vi.2015, *İ.Genç 2310, G. Ecevit Genç.* **Kırklareli**, Mahya Mountain, 720m, 17.viii.2014, *İ.Genç 2251, G. Ecevit Genç.* **Adapazarı**, Serindere valley, 760m, 11.vii.2014, *İ.Genç 2235, S. Yüzbaşıoğlu.*
E. pannonica Host	**Kırklareli**, Kırklareli-Edirne, roadside, 280m, 5.vi.2015, *İ.Genç 2306, G. Ecevit Genç.* **İstanbul**, Dağyenice-Kalfaköy, roadsides, 110m, 18.vii.2016, *İ.Genç 2477, G. Ecevit Genç.*
**E. pestalozzae* Boiss.	**Antalya**, Kuhu mountain, Çığlıkara, 1650m, 27.vi.2014, *İ.Genç 2168, İ.G.Deniz.* Kızlarsivrisi, 1650m, 26.vi.2014, *İ.Genç 2161, İ.G.Deniz.* Saklıkent, 1780m, 30.vii.2016, *İ.Genç 2499.*
E. petrophila C.A.Meyer	**Kastamonu**, Kastamonu-Sinop, roadsides, 720m, 14.vii.2015, *İ.Genç 2419, G. Ecevit Genç.* **Çankırı**, Ilgaz Mountain National Park, 1900m, 14.vii.2015, *İ.Genç 2435, G. Ecevit Genç.*
**E. pisidica* Hub.-Mor.&M.S.Khan	**Burdur**, Altınyayla-Gölhisar road, Dirmil pass, 1585m, 23.vi.2014, *İ.Genç 2114, G. Ecevit Genç.*
E. seguieriana Necker	**Iğdır**-Doğubeyazıt, roadsides, 1500m, 23.vii.2015, *İ.Genç 2451.* **Ağrı**, between Doğubeyazıt-Çaldıran, roadsides, 2600m, 24.vii.2015, *İ.Genç 2453.* **Van**, Erciş-Van roadsides, 1650m, 24.vii.2015, *İ.Genç 2456.*
E. smirnovii Geltman	**Erzincan**, Ergan mountain, 1510m, 25.vii.2015, *İ.Genç 2468, A. Kandemir.*
E. thessala (Form.) Degen & Dörf.	**Kırklareli**, Kırklareli-Pınarhisar, roadside, 190m, 5.vi.2015, *İ.Genç 2304, G. Ecevit Genç.*
**E. yildirimlii* Dinç	**Eskişehir**, Sivrihisar, Aşağıkepen village, gypsum slopes, 900m, 26.viii.2014, *İ.Genç 2267, G. Ecevit Genç.*

The classification based on the recent molecular phylogenetic study was used (Riina *et al.* 2013).

Morphological Investigations

Capsule, cauline leaf and cyathial gland morphologies were investigated as described below. The structures of the cyathial glands were examined using their photographs taken in the natural habitats of the plants or from herbarium materials when needed. Cyathial gland shapes and cyathial gland appendages were regarded as diagnostic characters for each taxon. To ensure stability and limit the range of variation in cyathial gland characters within taxa, several samples were investigated and photographed for each taxa (if available, because some of the studied samples were low in number).

The quantitative capsule characters were measured using different numbers of capsules depending on the material used. The number of capsules ranged from three in one population to a maximum of 24 in four populations because of the limited distribution of some taxa (*E. pisidica*, *E. smirnovii*, *E. thessala* and *E. yildirimlii*). For species with wider distributional ranges (e.g. *E. macroclada* and *E. niciciana*), populations from different parts of Turkey were selected. Only mature capsules were used for measurement. The capsules were examined in terms of size (length×width), shape, color and surface structures. Widths of the capsules were measured from the widest point. All measurement data of capsules were obtained under a stereomicroscope (Leica S8APO) with a camera attachment (Leica DFC295).

Sculpturing characteristics of capsule and cauline leaf surfaces were based on investigations of capsules and leaves mounted directly on stubs, attached with double adhesive tape and coated with a gold layer. Morphological observations concerning sculpturing were carried out with a FEI Quanta 450 FEG-EDS scanning electron microscope (SEM). All leaves were scanned from both surfaces. The terminology of macro- and micromorphology were based on Harris & Harris (1994) and Stearn (2004).

Results

The investigations of cyathial glands showed that they vary in shape and colour (see Fig. 1 for all cyathial gland visuals and Table 2 for cyathial gland shapes and the appendages). The majority of the cyathial glands are yellow to yellowish-green in colour while cyathial glands of *E. erythrodon*, *E. petrophila* and *E. smirnovii* are red to purplish (Figs. 1b, i, l). Reddish cyathial glands were also seen in *E. cheiradenia* which have normally yellowish cyathial glands (Fig. 1a).

There was no significant difference in cyathial gland size between the taxa. The cyathial gland number of all investigated species except *E. pestalozzae* was found to be four but some *E. pestalozzae* specimens with five cyathial glands were also determined (Fig. 1h). Cyathial gland shapes are elliptic, trapezoid-elliptic or reniform. Some species are hornless but truncate (*E. glareosa*, *E. niciciana*, *E. pannonica* and *E. seguieriana*) (Figs, 1c, f, g, k). Most species are characterized by their horned appendages. The horns are either short, as in *E. erythrodon*, *E. petrophila* and *E. smirnovii* (Figs. 1b, i, l), long as in *E. pisidica* (Fig. 1j) or lobate and denticulate as in *E. cheiradenia*, *E. macroclada* and *E. thessala* (Figs. 1a, d, m). *E. thessala* (Fig. 1m) has the longest horns among the studied species. Although it varied for some species, the number of cyathial gland horns is often two. Polymorphism has also been observed in some species, i.e. *E. cheiradenia* (Fig. 1a) and *E. thessala* (Fig. 1m) with two horns or denticulate ones.

The morphological characters related with capsules and cauline leaf surfaces of the studies species are presented in Table 2. Stereomicroscope images of the capsules are given in Fig. 2 and SEM micrographs of the studied species are provided in Figs. 3-4.

According to the morphometric measurements, the longest capsule (c. 6.7mm long) occurred in *E. macroclada* (Fig. 2d) followed by *E. cheiradenia* (Fig. 2a) and *E. pisidica* (Fig. 2i) with c. 5 and 4.75mm, respectively). The shortest capsule (c. 1.9mm long) was determined to be in *E. niciciana* (Fig. 2e). The widest capsule (c. 4.45-5.5mm wide) was seen in *E. macroclada* (Fig. 2d). The narrowest capsule (c. 2.25mm wide) was in *E. petrophila* (Fig. 2h), followed by *E. niciciana* and *E. seguieriana* with capsules about 2.4mm width (Figs. 2e, 2j).

The capsule shapes of the studied species vary from subglobose (*E. glareosa* and *E. niciciana*) to conical (Figs. 1, 2). Capsules of all taxa are trilobate, but the significance of the lobes varies from shallow to deep (Fig. 1, 2).

The micromorphological features of capsule surfaces are summarized in Table 2. The capsule surface is mostly covered with nipple-like projections (papillate-mammillate) (7 taxa) and others are covered with little rounded or hillock-like elevations (colliculate). The capsules of *E. erythrodon* (Fig. 3b), *E. glareosa* (Fig. 3c) and *E. macroclada* (Fig. 3d) are covered with long and nearly acute projections (papillate), while both projection types were observed in *E. pannonica*, *E. petrophila*, *E. smirnovii* and *E. yildirimlii*. The type of capsule indumentum varied from glabrous to villous (Fig. 1, 2).

The investigation of leaves showed that dorsal and ventral cauline leaf surface sculptures are correspondent, so only dorsal cauline leaf surface is discussed. Cauline leaf dorsal surface properties are summarized in Table 2. The cauline leaf surfaces are papillate-mammillate in most of the studied species. The colliculate surfaces are also observed in two species, *E. seguieriana* (Fig. 4j) and *E. thessala* (Fig. 4l).

Table 2. The morphological features of capsules, cauline leaf surfaces and cyathial glands of the examined *Euphorbia* species (*w*: width; *l*: length).

Taxa	Capsule size (*w*×*l*)	Capsule shape	Capsule surface	Cauline Leaf dorsal surface	Cyathial gland shape
E. cheiradenia	3-3.6×4.8-5	Conical, shallowly trilobate	Colliculate, glabrous	Colliculate-mammillate	Elliptic, two- or multi-horned
E. erythrodon	2.7-3.05×3.25-3.75	Ovoid-conical, trilobate	Papillate, glabrous to sparingly villose	Papillate	Trapezoid-elliptic, hornless or two short horned
E. glareosa	2.5-3.3×2.4-3.2	Subglobose-ovoid, shallowly trilobate	Papillate, glabrous to sparingly villose	Mammillate	Trapezoid-elliptic, hornless
E. macroclada	4.45-5.5×4.2-6.7	Subglobose-ovoid, shallowly trilobate	Papillate, glabrous to villose	Papillate	Elliptic, two lobate or simple horned to denticulate
E. niciciana	2.4-3×1.95-2.5	Subglobose-ovoid, deeply trilobate	Colliculate, glabrous	Mammillate	Trapezoid-elliptic, hornless
E. pannonica	2.6-3.35×2.95-3.75	Ovoid-conical, trilobate	Papillate-mammillate, glabrous to villose	Mammillate	Trapezoid, hornless
E. pestalozzae	3.55-3.65×4.25-4.4	Conical, shallowly trilobate	Colliculate, glabrous	Mammillate	Elliptic-truncate, two horned
E. petrophila	2.25-2.6×2.5-3	Ovoid-conical, shallowly trilobate	Papillate-mammillate, glabrous	Papillate	Truncate-reniform, two horned
E. pisidica	2.5-3.3×3.3-4.75	Conical, shallowly trilobate	Colliculate, glabrous	Mammillate	Elliptic, two- or multi- long horned
E. seguierana	2.4-3.2×2.6-3.2	Subglobose-ovoid, deeply trilobate	Colliculate, glabrous	Colliculate	Trapezoid-elliptic, hornless
E. smirnovii	2.6-3.7×3.6-3.75	Ovoid-conical, shallowly trilobate	Papillate-mammillate, glabrous	Papillate-mammillate	Trapezoid, two short horned
E. thessala	2.95-3.5×3.75-4.1	Conical, shallowly trilobate	Colliculate, glabrous	Colliculate	Trapezoid-elliptic, two horned
E. yildirimlii	3.05-4.1×2.3-4.2	Subglobose-ovoid, shallowly trilobate	Papillate-mammillate, glabrous to sparingly villose	Papillate-mammillate	Elliptic, two short horned

The density of the projections of cauline leaf surfaces decreased by aging of the plants. For example, the immature individuals of *E. cheiradenia* are covered with dense mammillate projections, whereas the mature ones are covered with colliculate ornamentations. With the exception of *E. pisidica* with pubescent leaves, the remained taxa are glabrous (Fig. 4i). Capsule and cauline leaf surfaces of all investigated taxa are covered with epicuticular wax secretion forming fine platelets. As the plant matures, surface secretions may be eroded (Figs. 3n, 4n).

Fig. 1. Photographs of cyathial glands of studied taxa: **a.** *E. cheiradenia*, **b.** *E. erythrodon*, **c.** *E. glareosa*, **d. & e.** *E. macroclada*, **f.** *E. niciciana*, **g.** *E. pannonica*, **h.** *E. pestalozzae*, **i.** *E. petrophila*, **j.** *E. pisidica*, **k.** *E. seguierana*, **l.** *E. smirnovii*, **m.** *E. thessala*, **n.** *E. yildirimlii*.

Fig. 2. Stereomicroscopic micrographs of capsules of studied taxa: **a.** *E. cheiradenia*, **b.** *E. erythrodon*, **c.** *E. glareosa*, **d.** *E. macroclada*, **e.** *E. niciciana*, **f.** *E. pannonica*, **g.** *E. pestalozzae*, **h.** *E. petrophila*, **i.** *E. pisidica*, **j.** *E. seguierana*, **k.** *E. smirnovii*, **l.** *E. thessala*, **m.** *E. yildirimlii*.

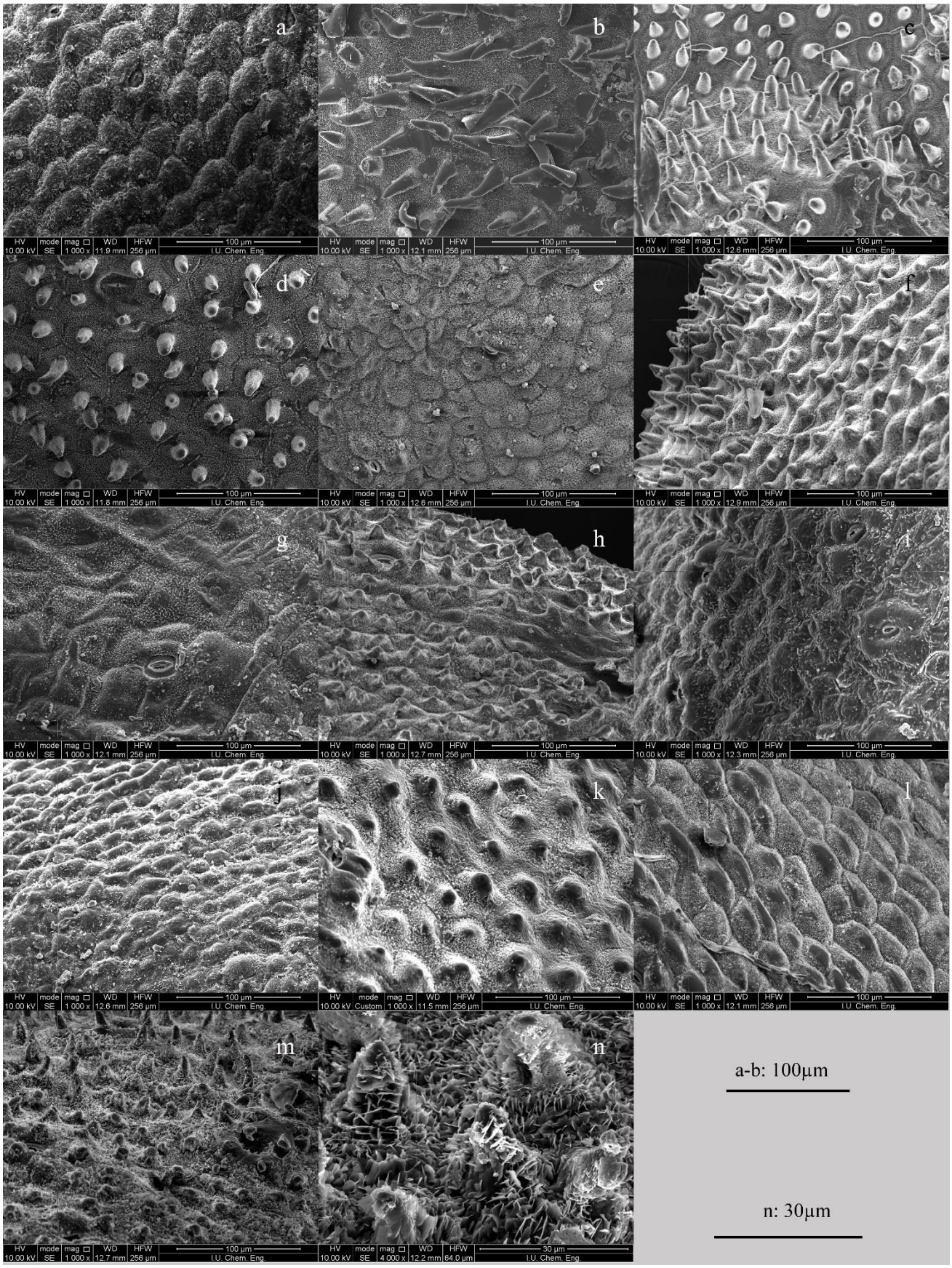

Fig. 3. Scanning electron micrographs of capsule surface of studied taxa: **a.** *E. cheiradenia*, **b.** *E. erythrodon*, **c.** *E. glareosa*, **d.** *E. macroclada*, **e.** *E. niciciana*, **f.** *E. pannonica*, **g.** *E. pestalozzae*, **h.** *E. petrophila*, **i.** *E. pisidica*, **j.** *E. seguierana*, **k.** *E. smirnovii*, **l.** *E. thessala*, **m.** *E. yildirimlii*, **n.** epicuticular wax.

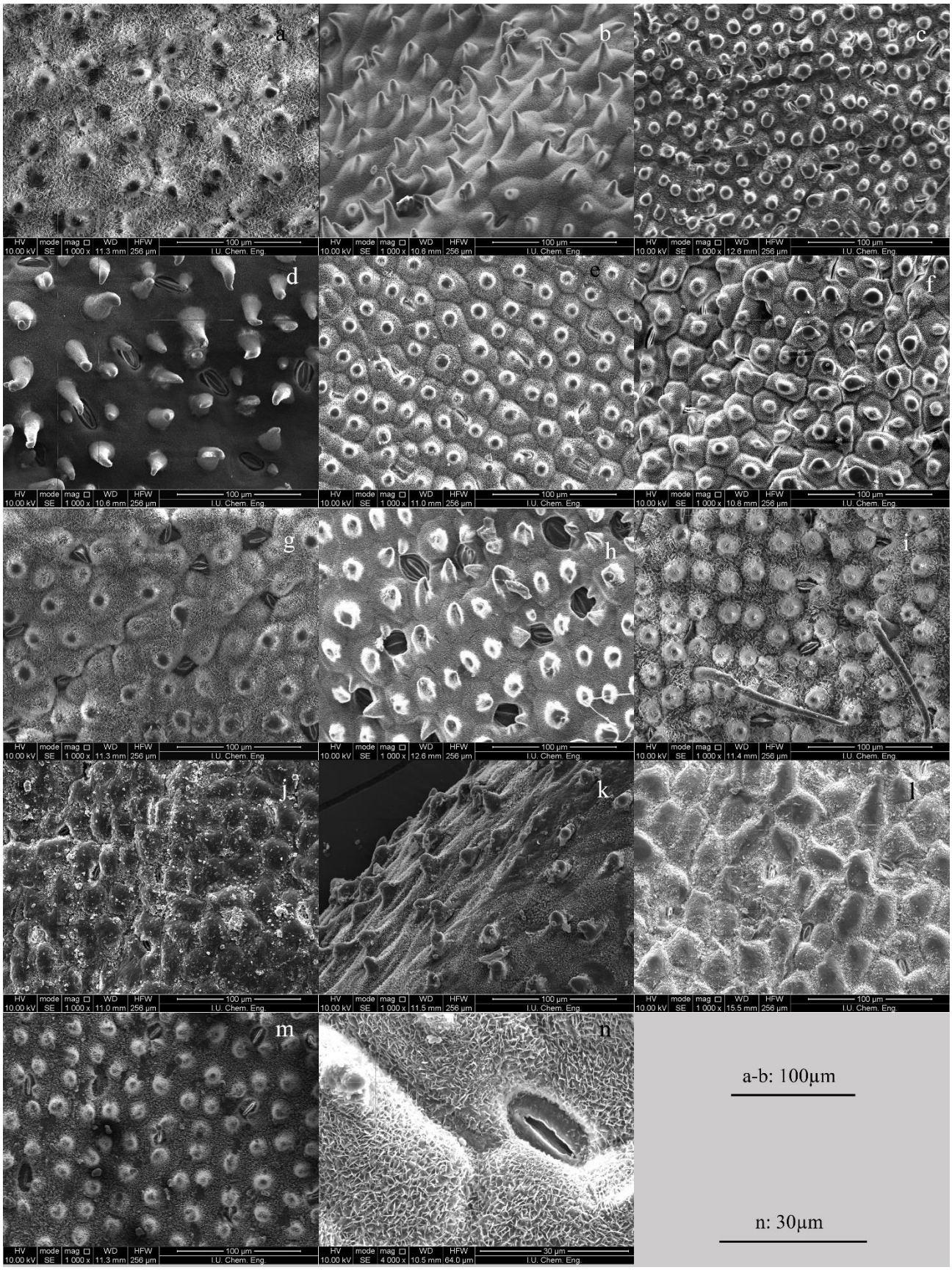

Fig. 4. Scanning electron micrographs of cauline leaf surface of studied taxa: **a.** *E. cheiradenia*, **b.** *E. erythrodon*, **c.** *E. glareosa*, **d.** *E. macroclada*, **e.** *E. niciciana*, **f.** *E. pannonica*, **g.** *E. pestalozzae*, **h.** *E. petrophila*, **i.** *E. pisidica*, **j.** *E. seguierana*, **k.** *E. smirnovii*, **l.** *E. thessala*, **m.** *E. yildirimlii*, **n.** epicuticular wax.

Discussion

Based on capsule micromorphology, two groups are recognized, one including species with colliculate capsule surface (*E. cheiradenia, E. niciciana, E. pestalozzae, E. pisidica, E. seguierana* and *E. thessala*) and the other with species with papillate-mammillate capsule surface. According to the result of this study, micromorphology of capsule surfaces is more useful as an identification character compared to the cauline leaf surfaces because the cauline leaf surface properties vary as the plant matures.

Members of section *Pithyusa* in Iran are characterized by a conical capsule shape (Pahlevani *et al.* 2015), however subglobose and ovoid capsule shapes are recorded in our present study.

Our results related to capsule sizes correspond to the results of Pahlevani *et al.* (2015) about the species with wider distribution ranges which also occur in Iran (*E. cheiradenia, E. glareosa, E. macroclada* and *E. seguieriana*). However, the capsule shape of *E. glareosa* and *E. seguieriana* differs from Iranian members. According to Pahlevani *et al.* (2015) these two species have conical shallowly trilobate capsules but the specimens in Turkey have subglobose clearly trilobate capsules.

The results show that capsule size may be used to determine some species morphologically close to each other. For example, *E. smirnovii* can be distinguished from *E. petrophila* with larger capsules as in the case of *E. macroclada* and *E. glareosa*. Morphology of cyathial glands shapes, their colour and appendages have been useful tools for identifications of some species. The cyathial gland shapes are elliptic or trapezoid-elliptic for all investigated taxa, except *E. petrophila*. The gland shape is truncate-reniform of this species (Fig. 1i).

In terms of cyathial gland appendage characteristics, intraspecific variation was most commonly seen in *E. macroclada*. The appendages of this species vary from not very distinct horns to lobate-multifid horns. No similar variation is observed in other species.

The number of cyathial glands of sect. *Pithyusa* is reported as four by Riina *et al.* (2013). On the other hand, it has been determined that some specimens of *E. pestalozzae* have five cyathial glands in this study.

The variation in the number of horns of *E. cheiradenia* cyathial glands has been previously reported by Salmaki *et al.* (2011). But no colour variation was reported. Reddish cyathial glands, in addition to yellow cyathial glands were also observed in some specimens of *E. cheiradenia* included in our present study.

The observed morphological characters of capsule and cyathial glands can be used as effective diagnostic characters to separate some close species (eg. *E. macroclada-E. yildirimlii*; *E. petrophila-E. smirnovii*; *E. pannonica-E. glareosa*) in the sect. *Pithyusa* of the genus *Euphorbia*.

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References

1. Bruyns, P.V., Mapaya, R.J. & Hedderson, T. 2006. A new subgeneric classification for *Euphorbia* (Euphorbiaceae) in southern Africa based on ITS and psbA-trnH sequence data. *Taxon*, 55: 397-420.
2. Can, L. & Küçüker, O. 2015. Seed morphology and surface microstructure of some *Euphorbia* (Euphorbiaceae) taxa distributed in Turkey-in-Europe. *Turkish Journal of Botany*, 39(3): 449-457.
3. Dorsey, B.L., Heavermans, T., Aubriot, X., Morawetz, J.J., Riina, R., Steinmann V.W. & Berry, P.E. 2013. Phylogenetics, morphological evolution, and classification of *Euphorbia* subgenus *Euphorbia*. *Taxon*, 62: 291-315.
4. Geltman, D.V. (2015). Phytogeographical analysis of *Euphorbia* subgenus *Esula* (Euphorbiaceae). *Polish Botanical Journal*, 60(2): 147-161.
5. Genç, İ. & Kültür, Ş. 2016. *Euphorbia akmanii* (Euphorbiaceae), a new species from Turkey. *Phytotaxa*, 265: 112-120.
6. Harris, J.G. & Harris, M.W. 1994. *Plant identification terminology: an illustrated glossary*. Spring Lake Publishing, Utah, 206 pp.
7. Horn, J.W., van Ee, B.W., Morawetz, J.J., Riina, R., Steinmann, V.W., Berry, P.E. & Wurdack, K.J. 2012. Phylogenetics and the evolution of major structural characters in the giant genus *Euphorbia* L. (Euphorbiaceae). *Molecular Phylogenetics and Evolution*, 63(2): 305-326.
8. Öztekin, M. 2012. *Euphorbia* L. pp. 414-424. *In:* Güner, A., Aslan, S., Ekim, T., Vural, M. & Babaç, M.T. (Eds.) *Türkiye Bitkileri Listesi (Damarlı Bitkiler)*. Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını, İstanbul, 1290 pp.
9. Pahlevani, A.H. & Akhani, H. 2011. Seed morphology of Iranian annual species of *Euphorbia* (Euphorbiaceae). *Botanical journal of the Linnean Society*, 167: 212-234.
10. Pahlevani, A.H., Liede-Schumann, S. & Akhani, H. 2015. Seed and capsule morphology of Iranian perennial species of *Euphorbia* (Euphorbiaceae) and its phylogenetic application. *Botanical journal of the Linnean Society*, 177: 335-377.
11. Pahlevani, A.H. 2017. Four new species of *Euphorbia* sect. *Pithyusa* (subg. *Esula*, Euphorbiaceae) from SW Asia. *Phytotaxa*, 312: 83-93.
12. Pahlevani, A.H., Feulner, M., Weig, A. & Liede-Schumann, S. 2017. Molecular and morphological studies disentangle species complex in *Euphorbia* sect. *Esula* (Euphorbiaceae) from Iran, including two new species. *Plant Systematics and Evolution*, 303: 139-164.

13. Peirson, J.A., Bruyns, P.V., Riina, R., Morawetz, J.J. & Berry, P.E. 2013. A molecular phylogeny and classification of the largely succulent and mainly African *Euphorbia* subg. *Athymalus* (Euphorbiaceae). *Taxon*, 62: 1178-1199.

14. Radcliffe-Smith, A. 1982. *Euphorbia* L. Pp. 571-630. *In:* Davis, P.H., Edmondson, J.R., Mill, R.R. & Kit Tan (Eds.) *Flora of Turkey and the East Aegean Islands, vol. 7*. Edinburgh University Press, Edinburgh, 947 pp.

15. Riina, R., Peirson, J.A., Geltman, D.V., Molero, J., Frajman, B., Pahlevani, A., Barres, L., Morawetz, J.J., Salmaki, Y., Zarre, S., Kryukov, A., Bruyns, P.V. & Berry, P.E. 2013. A worldwide molecular phylogeny and classification of the leafy spurges, *Euphorbia* subgenus *Esula* (Euphorbiaceae). *Taxon*, 62(2): 316-342.

16. Salmaki, Y., Zarre, S., Esser, H.-J. & Heubl, G. 2011. Seed and gland morphology in *Euphorbia* (Euphorbiaceae) with focus on their systematic and phylogenetic importance, a case study in Iranian highlands. *Flora*, 206(11): 957-973.

17. Steinmann, V.W. & Porter, J.M. 2002. Phylogenetic relationships in Euphorbieae (Euphorbiaceae) based on ITS and ndhF sequence data. *Annals of the Missouri Botanical Garden*, 89: 453-490.

18. Stearn, W.T. 2004. *Botanical Latin*. 1st paperback ed. Timber Press, Portland, 546 pp.

19. Yang, Y., Riina, R., Morawetz, J.J., Heavermans, T., Aubriot, X. & Berry, P.E. 2012. Molecular phylogenetics and classification of *Euphorbia* subgenus *Chamaesyce* (Euphorbiaceae). *Taxon*, 61: 764-789.

20. Zimmermann, N.F.A., Ritz, C.M. & Hellwig, F.H. 2010. Further support for the phylogenetic relationships within *Euphorbia* L. (Euphorbiaceae) from nrITS and *trnL-trnF* IGS sequence data. *Plant Systematics and Evolution*, 286: 39-58.

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Research Article

DAILY VERTICAL VARIATION IN PHYTOPLANKTON COMPOSITION OF A DRINKING WATER RESERVOIR (KADIKÖY RESERVOIR-EDİRNE) DURING SUMMER STRATIFICATION

Burak ÖTERLER

Trakya University, Faculty of Sciences, Department of Biology, 22030, Edirne, TURKEY
e-mail: burakoterler@trakya.edu.tr

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Abstract: This study is performed in August 2012 in Kadıköy Reservoir in Keşan district of Edirne. The deepest point of the reservoir when there is no water drown was chosen as the sampling station. Samples were taken from 6 different depths every three hours during a 24 hour period. 65 phytoplanktonic algae taxa belonging to Chlorophyta (26 taxa), Bacillariphyta (18 taxa), Euglenophyta (8 taxa), Charophyta (5 taxa), Cyanophyta (4 taxa), Miozoa (3 taxa) and Ochrophyta (1 taxa) were identified.

Key words: Phytoplankton, Daily vertical composition, Chlorophyll-a, Kadıköy Reservoir.

Özet: Bu çalışma Ağustos 2012 tarihinde Edirne ili Keşan ilçesinde bulunan Kadıköy barajında yapılmıştır. Barajdan su çekiminin yapılmadığı tarihler içerisinde en derin noktası istasyon olarak seçilmiştir. Çalışma süresince 3'er saat ara ile 24 saatlik bir periyotta ve 6 farklı derinlikten örnekler alınmıştır. Çalışma süresince Chlorophyta (26 taxa), Bacillariphyta (18 taxa), Euglenophyta (8 taxa), Charophyta (5 taxa), Cyanophyta (4 taxa), Miozoa (3 taxa) ve Ochrophyta (1 taxa) divizyolarına ait toplam 65 fitoplanktonik alg taksonu belirlenmiştir.

Introduction

Reservoirs are generally formed with barriers in front of rivers and they are described as hybrid systems between the lakes and the rivers (Thornton *et al.* 1990). Being described as artificial lakes for different purposes like flood control, power generation, irrigation and recreation, reservoirs are separated from natural lakes with some of their characteristics like high water flow velocity, solid matter presence in influent suspend and short term water exchange (Harper *et al.* 1999). Naturally, as a result of changing environmental factors and irrigation activities, there might be some changes on the organisms living in an aquatic ecosystem (Wetzel 2001). The responses of the organisms affected by these changes would be either as disappearing or increasing in a population (Wetzel 2001, Harper *et al.* 1999). Therefore, aquatic systems must be under scientific control and physical, chemical and biological characteristics should be recorded.

As algae live in a wide range, they are the most important oxygen source of their environment and they provide oxygen and food requirements for many organisms, ranging from benthic invertebrates to fishes, in aquatic ecosystems (Round 1984). In addition to their surface distributions on the water body, it is also important how algae distribute vertically in the water column in different depths. Turbidity occuring as a result of excess increase on some water layers depending on some environmental factors (food substances, water temperature, climate condition etc.) will also suppress the community structure of the species in the lower layers (Reynolds *et al.* 2002, Akçaalan *et al.* 2006, Akçaalan *et al.* 2014, Becker *et al.* 2009). To understand the factors regulating the key species in aquatic environments, it might be important to determine daily and vertical distributions of phytoplankton communities (Takamura & Yasuno 1984). Phytoplanktonic organisms have remodelled their morphology and physiology for surviving in different environments (Reynolds 1988), including adaptations to particular daily and vertical dynamics.

This study is performed to determine the daily vertical distributions of phytoplankton in Kadıköy Reservoir which provides tap water to Keşan district of Edirne.

Materials and Methods

Study area

Kadıköy Reservoir is located in Keşan city borders in Edirne province and was constructed in 1973 on Derbent

Stream in order to provide water both for agricultural irrigation and industrial and daily use and to prevent flood. The reservoir is located at an altitude of 74m a.s.l and its surface area is 6.20km^2 at normal water-level. The total agricultural area using irrigation water from the reservoir covers about 4,428 hectares and the average amount of daily water use is 2hm^3 (http://www.dsi.gov.tr) (Fig. 1).

The sampling station was selected near the water intake (40°47'40.04"N, 26°46'24.31"E) in the deepest part of the reservoir. The reservoir shows the characteristics of a mesoeutrophic system and its maximum depth is 18.5m (Öterler *et al.* 2015). The annual mean temperature is 13.5°C. The reservoir was thermally stratified, as clear mixed epilimnion and slightly alkaline conditions. Some physico-chemical parameters were formerly measured in the water column (Öterler *et al.* 2015) (Table 1).

Sampling and analyses

Phytoplankton and water samplings were taken in the reservoir on 16 August 2012. Samplings started at mid-day (13:00) and phytoplankton communities were sampled every 3h during a 24-h period in a vertical profile. Samplings were performed from surface water and from 6 depths of 1, 3, 5, 9, 12 and 15 meters. Samplings were made during the period when the dam covers were closed and there was no water intake.

Water samples were taken from under water surface using a Van Dorn type water sampler in order to determine some environmental values of the reservoir such as water temperature, dissolved oxygen (DO), pH, nitrogen in nitrite and nitrate forms and phosphate values. Water temperature, DO, pH and electrical conductivity were measured on sampling station using field type equipments and chlorophyll-*a* was measured spectrophotometrically according to Nusch (1980). Water transparency was measured once using a 20-cm Secchi disk.

For the phytoplankton samples, 1L of the water samples taken from the reservoir using Whatmann GF/A filter papers were filtered, planktonic samples were condensed and identification of the algae other than diatoms in temporary slides were done on Olympus brand CX41 model microscope. For identifications of diatoms, samples brought to the laboratory were purified from organic matter by boiling in 1:1 volume H_2SO_4+HNO_3 and by rinsing in distilled water for a couple of times. Neutral pH was provided and a drop was taken and dried on lamella, then it was covered by Naphrax and permanent slides were prepared and identified under the microscope. From the water samples, 25-50 and 100ml sub-samples were prepared, precipitated according to Utermöhl (1958) and organism counts and calculations were done under an Olympus brand CK2 model inverted microscope. The taxonomic books (Huber-Pestalozzi 1982, John *et al.* 2003, Krammer & Lange-Bertalot 1986-2004, Round *et al.* 1990, Komarek & Anagnostidis 2005, Hindák 2008, Kristiansen & Preisig 2011) were used for the identification of algal species. All species were checked online on algaebase cite (Guiry & Guiry. 2017).

Table 1. Some environmental variables in the water column (Öterler *et al.* 2015).

Parameters	Values
NO_2^-	0.037mg.L^{-1}
NO_3^-	1.778mg.L^{-1}
SRP	0.009mg.L^{-1}
Hardness	22°F
Salinity	0.083‰
Secchi Disc	98 cm (Midday)

Fig. 1. The map showing the locations of Kadıköy Reservoir and the sampling point. The solid circle in the left figure corresponds to the sampling point.

Results

The sky was open during samplings and the mean wind speed varied from 3 to 8ms^{-1} depending on the sampling time. In morning samplings, generally a relatively gentle breeze occurred while evening and night time samplings were generally characterized by a moderate and a light breeze, respectively.

Air temperature was lower than surface water temperature from 22:00 to 07:00h, but higher from 10:00 to 19:00h. The daily water temperature ranged from 18.5°C to 27°C, but the surface water (0-5m) lost heat throughout the night when the air temperature fell below the surface water but gained heat during the day (Fig. 2).

The highest value of average DO was measured in 1m samples of 04.00pm with 11.86mg.L^{-1} and the descending ranking in terms of DO was as 5m>1m>3m>Subsurface>9m>12m>15. The pH was measured between 7.41 and 8.94 and conductivity average was 0.52µS.cm^{-1} (Table 2). The laboratory analysis showed that there was no significant difference between sampling depths and times in the measured parameters.

Phytoplankton species diversity and chlorophyll-a

Identification of the sampled material showed that a total of 65 taxa were present in the reservoir during the sampling period. Chlorophyta was identified as the group having the maximum number of taxa with 26, followed by Diatoms (Bacillariophyta) with 18 taxa, Euglenophyta with 8 taxa, Carophyta with 5 taxa, Cyanophyta with 4 taxa, Miozoa with 3 taxa and Ochrophyta with 1 taxon (Table 3).

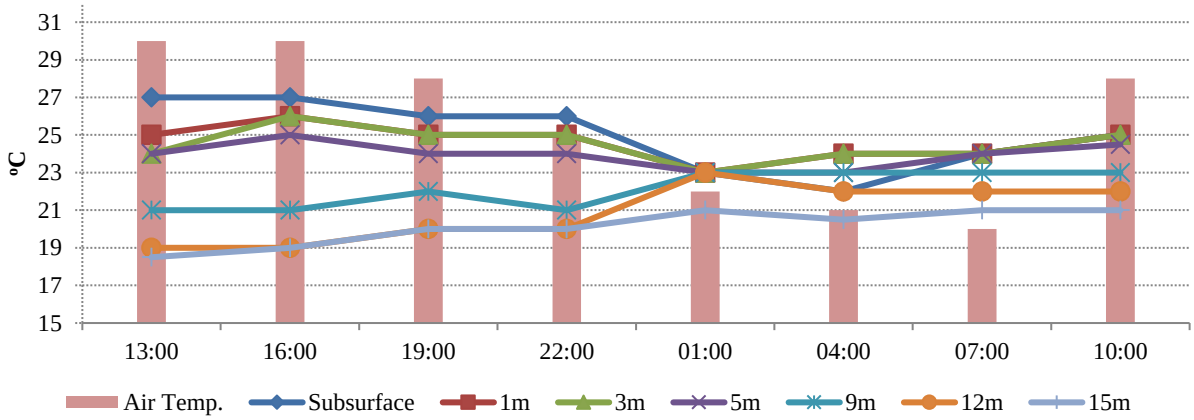

Fig. 2. The relationship between water and air temperature values during a sampling day.

Table 2. Daily variation of Dissolved Oxygen (DO) concentration (mg.L^{-1}), pH and Electric Conductivity (EC) (µS.cm^{-1}) of the water column in the reservoir.

	Depth/Hours	13:00	16:00	19:00	22:00	01:00	04:00	07:00	10:00
DO (mg.L^{-1})	Surface	7.71	7.09	6.14	9.92	9.23	11.71	9.4	8.6
	1m	7.57	7.6	6.15	9.65	9.9	**11.86**	10.41	8.4
	3m	7.54	7.48	6.28	9.58	9.64	11.62	10.58	8.36
	5m	7.47	7.91	6.69	9.55	9.59	11.54	11.61	8.41
	9m	7.28	7.35	6.18	8.84	9.51	10.75	11.34	7.32
	12m	6.99	6.88	6.35	8.33	9.54	10.08	10.82	6.92
	15m	6.5	**4.26**	5.24	7.86	9.63	10.13	9.94	6.82
pH	Surface	8.47	**7.41**	**8.94**	8.6	8.56	8.33	8.25	7.88
	1m	8.24	8.8	8.9	8.29	8.6	8.45	8.25	7.86
	3m	8.18	8.71	8.73	8.3	8.41	8.43	8.32	7.78
	5m	8.17	8.58	8.21	8.33	8.63	8.52	8.53	7.76
	9m	8.15	8.21	8.05	8.33	8.57	8.21	8.23	7.58
	12m	8.12	8.11	7.79	8.24	8.27	8.07	8.14	7.52
	15m	8.07	7.71	7.73	8.05	8.14	7.86	7.88	7.46
EC (µmS.cm^{-1})	Surface	0.58	0.54	0.51	0.51	0.5	0.5	0.49	0.52
	1m	0.57	0.54	0.51	0.5	0.5	0.5	0.49	0.52
	3m	0.57	0.55	0.51	0.51	0.52	0.5	0.5	0.52
	5m	0.59	0.55	0.51	0.5	0.5	**0.49**	**0.49**	0.54
	9m	0.6	0.55	0.52	0.51	0.51	0.51	0.5	0.54
	12m	0.62	0.56	0.52	0.51	0.51	0.51	0.5	0.54
	15m	**0.64**	0.58	0.53	0.5	0.51	0.51	0.5	0.55

Table 3. The list of the planktonic algal species determined in the reservoir with respect tothe sampling depths. Represents presence of a taxon in a particular depth sample.

	Surface	1m	3m	5m	9m	12m	15m
BACILLARIOPHYTA							
Bacillariophyceae							
Cocconeis placentula Ehrenberg		X		X		X	
Cymatopleura elliptica (Brébisson) Smith	X	X	X	X		X	
Cymatopleura solea (Brébisson) Smith	X	X		X		X	X
Cymbella cymbiformis Agardh	X						
Cymbella tumida (Brébisson) Van Heurck		X	X		X		
Gyrosigma attenuatum (Kützing) Rabenhorst		X		X			X
Hippodonta capitata (Ehren.) Metzeltin&Witkowski		X	X	X	X	X	X
Navicula sp.	X	X	X	X	X	X	X
Navicula viridula (Kützing) Ehrenberg	X						
Nitzschia acicularis (Kützing) Smith	X	X	X	X	X	X	X
Nitzschia palea (Kützing) Smith	X	X	X	X	X	X	X
Nitzschia sp.	X	X	X	X	X	X	X
Coscinodiscophyceae							
Aulacoseira italica (Ehrenberg) Simonsen	X	X	X	X			
Melosira varians Agardh	X		X		X	X	X
Fragilariophyceae							
Diatoma vulgaris Bory	X						
Fragilaria crotonensis Kitton	X		X				
Ulnaria ulna (Nitzsch) Compère	X	X	X	X	X	X	X
Mediophyceae							
Cyclotella meneghiniana Kützing	X	X	X	X	X	X	X
CHAROPHYTA							
Conjugatophyceae							
Closterium pronum Brébisson	X	X	X	X	X	X	X
Closterium sp.	X	X					
Cosmarium sp.	X	X	X	X		X	X
Staurastrum paradoxum Meyen	X	X	X	X	X	X	X
Staurastrum punctulatum Bréb.	X	X	X	X	X		
CHLOROPHYTA							
Chlorophyceae							
Coelastrum astroideum De Notaris	X	X	X	X	X	X	X
Coelastrum microporum Nägeli		X	X	X		X	X
Desmodesmus abundans (Kirchner) Hegewald		X	X	X	X	X	X
Hariotina reticulata Dangeard		X	X	X	X		
Kirchneriella sp.	X	X	X	X	X	X	
Monactinus simplex (Meyen) Corda	X	X	X	X	X		X
Monoraphidium contortum (Thuret) Komárková-Leg.	X	X	X	X		X	X
Monoraphidium minutum (Nägeli) Komárková-Leg.	X	X	X	X	X	X	
Pediastrum duplex Meyen	X	X	X	X		X	X
Pseudopediastrum boryanum (Turpin) Hegewald		X	X		X		
Scenedesmus bijuga (Turpin) Lag.	X	X		X		X	X
Scenedesmus quadricauda (Turpin) Brébisson	X	X	X	X		X	X
Schroederia sp.	X	X	X	X	X		X
Tetradesmus dimorphus (Turpin) Wynne	X		X	X	X		
Tetradesmus lagerheimii Wynne & Guiry	X	X	X	X	X	X	
Tetradesmus obliquus (Turpin) Wynne	X	X	X	X	X		X

	Surface	1m	3m	5m	9m	12m	15m
Tetraedron caudatum (Corda) Hans.	X	X	X	X	X	X	X
Tetraedron trigonum (Nägeli) Hans.			X	X			
Tetrastrum staurogeniiforme (Schröder) Lemm.	X	X	X	X	X		X
Eustigmatophyceae							
Tetraëdriella regularis (Kützing) Fott	X	X	X	X	X	X	X
Trebouxiophyceae							
Botryococcus braunii Kützing	X	X	X	X			
Chlorella sp.	X	X	X	X	X	X	X
Crucigenia tetrapedia (Kirchner) Kuntze	X	X	X	X	X	X	X
Dictyosphaerium sp.	X	X	X	X	X		
Oocystis sp.	X	X	X	X	X	X	X
Willea rectangularis (Braun) Wynne & Tsarenko	X	X	X	X	X	X	X
CYANOBACTERIA (Cyanophyta)							
Cyanophyceae							
Microcystis aeruginosa (Kützing) Kützing	X	X	X	X	X	X	X
Chroococcus sp.	X	X	X	X	X	X	X
Merismopedia sp.	X	X	X	X	X		X
Oscillatoria limosa Agardh						X	X
EUGLENOPHYTA							
Euglenophyceae							
Euglena granulata (Klebs) Schmitz	X	X		X	X	X	X
Euglena texta (Duj.) Hüb.	X	X	X	X	X	X	X
Lepocinclis acus (Müller) Marin & Melkonian	X	X	X	X	X	X	X
Phacus acuminatus Stokes	X	X	X	X	X	X	X
Phacus longicauda (Ehrenberg) Dujardin	X	X	X	X		X	
Strombomonas sp.	X	X	X	X	X	X	X
Trachelomonas hispida (Perty) Stein	X	X	X	X	X	X	X
Trachelomonas volvocina (Ehrenberg) Ehrenberg	X	X		X	X	X	
MIOZOA							
Dinophyceae							
Ceratium hirundinella (Müller) Duj.		X	X	X			
Peridiniopsis cunningtonii Lem.	X	X	X	X	X	X	X
Peridinium cinctum (Müller) Ehrenberg	X	X	X	X	X	X	X
OCHROPHYTA							
Chrysophyceae							
Dinobryon divergens Imhof	X	X	X	X	X		

A total of 53 taxa were determined in the samples taken from subsurface while the taxa determined in 1, 3, 5, 9 12 and 15m depths were 56, 53, 55, 44, 43 and 42, respectively (Fig. 3). The hourly samplings showed that the minimum and maximum taxa numbers were obtained at 19:00 (31 taxa) and at 01:00 (19 taxa) for subsurface samplings, at 10:00 (29 taxa) and at 19:00 (22 taxa) for 1m samplings, at 07:00 (31 taxa) and at 22:00 (21 taxa) for 5m samplings and at 01:00 (26 taxa) and at 13:00 (12 taxa) for 15m samplings (Fig. 4).

During the study, phytoplankton numbers in liter were calculated from all depths and for chosen sampling hours. The highest cell number (80790ind.L^{-1}) was calculated in the sampling at 01:00 on the surface and the lowest (19540ind.L^{-1}) was calculated at 07:00 in 15m (Fig. 5). Average cell number and percentage distributions according to the groups in the reservoir are given in Table 4. Spectrophotometric measurements showed that the highest average chlorophyll-*a* was measured at 22:00 for the surface sampling (12.57µg.L^{-1}) and the lowest was measured at 16:00 for 15m sampling (3.1µg.L^{-1}) (Fig. 6).

Crucigenia tetrapedia, Monactinus simplex, Scenedesmus quadricauda and *Coelastrum astroideum* from the green algae, *Peridiniopsis cunningtonii* and *Peridinium cinctum* from Miozoa, *Staurastrum paradoxum* from Charophyta and *Microcystis aeruginosa* from Cyanobacteria were identified as the dominant organisms of Kadıköy Reservoir during the study.

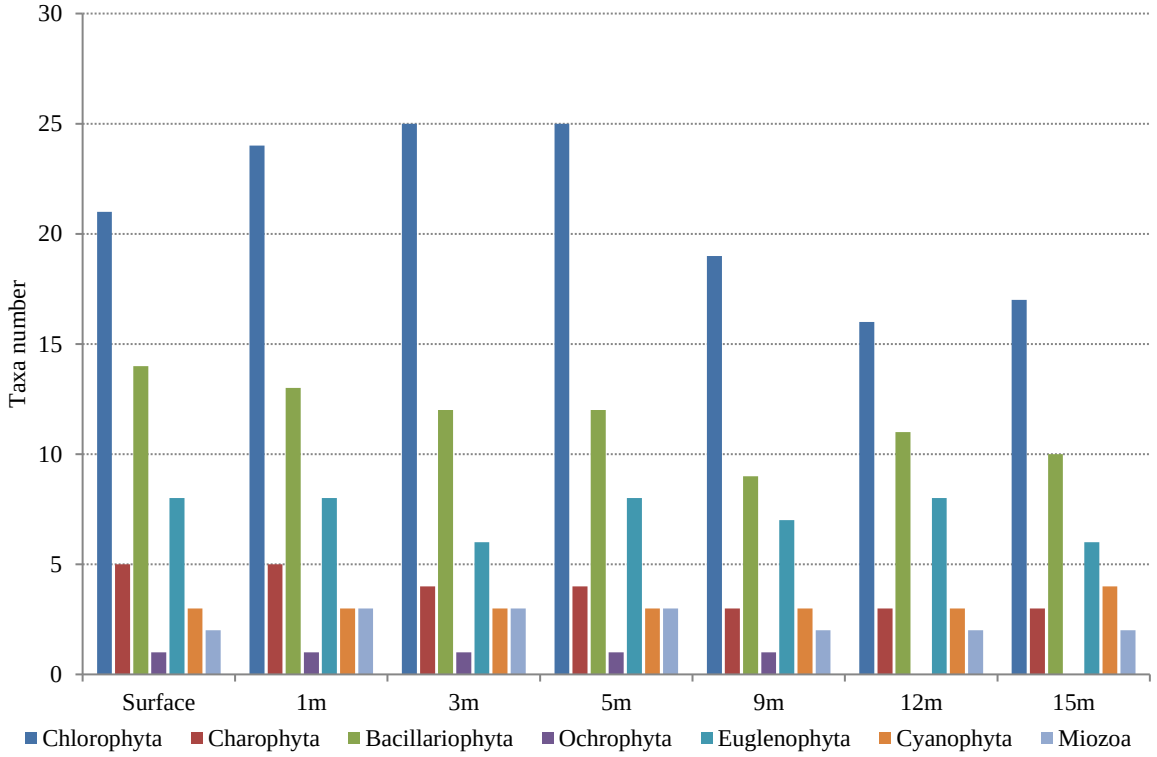

Fig. 3. The spatial distribution of phytoplankton taxa identified in the reservoir with respect to depths.

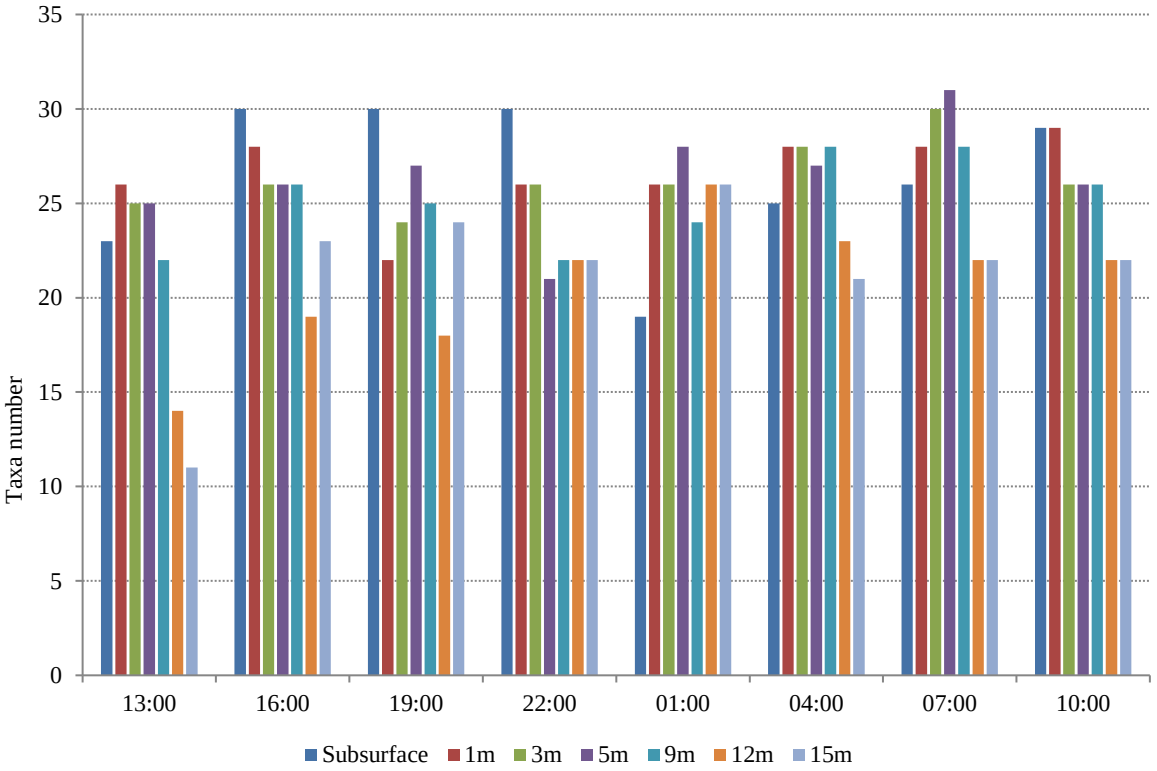

Fig. 4. The spatial distribution of phytoplankton taxa identified in the reservoir with respect to sampling hours.

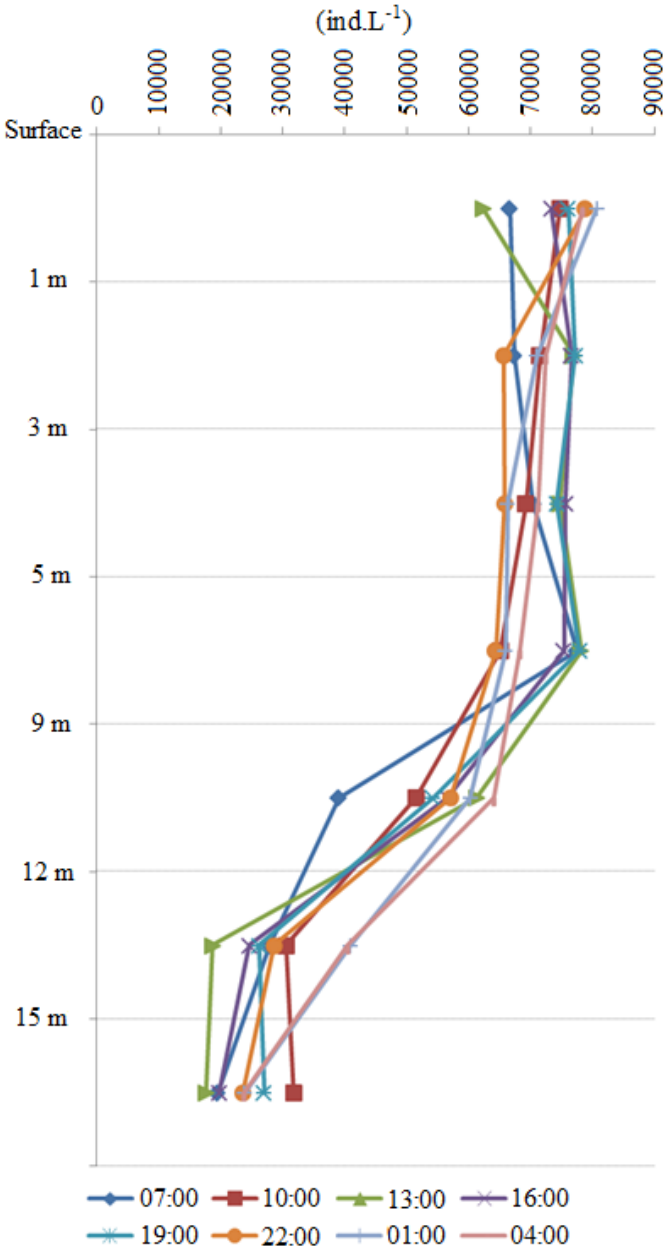

Fig. 5. Organism numbers (in total) determined with respect to depths (ind.L^{-1}).

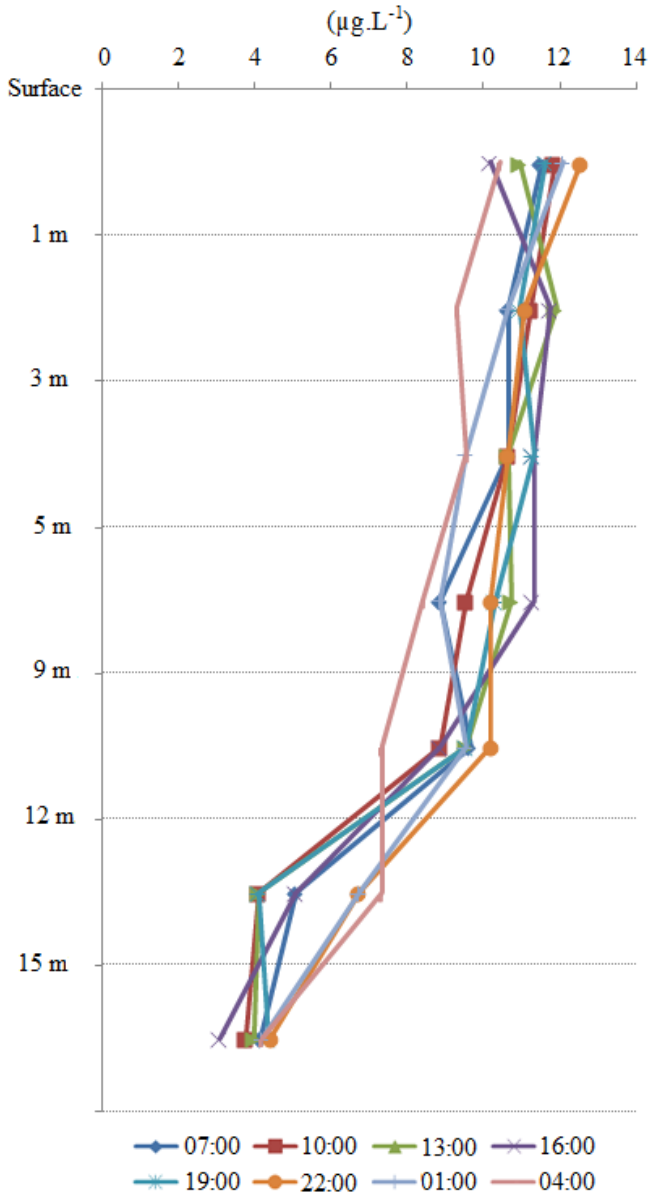

Fig. 6. Chlorophyll-a quantities identified with respect to depths (µg.L^{-1}).

Table 4. Abundance of taxonomic groups of phytoplankton and their contributions to total phytoplankton abundance in the reservoir (ind.L^{-1}).

	Subsurface	**1m**	**3m**	**5m**	**9m**	**12m**	**15m**
Bacillariophyta	2453	2051	2476	2406	2389	1806	1871
Charophyta	5963	6096	7012	6798	7096	2227	1745
Chlorophyta	44121	40662	40195	40368	28287	16912	13020
Cyanophyta	6461	6519	5526	6149	4994	2068	1068
Euglenophyta	3225	3792	4651	5103	2954	3417	3619
Miozoa	11733	13263	11144	10795	9825	3388	2075
Ochrophyta	6157	7214	7200	7886	8243	7909	7460
Total	80113	79597	78203	79505	63789	37726	30857

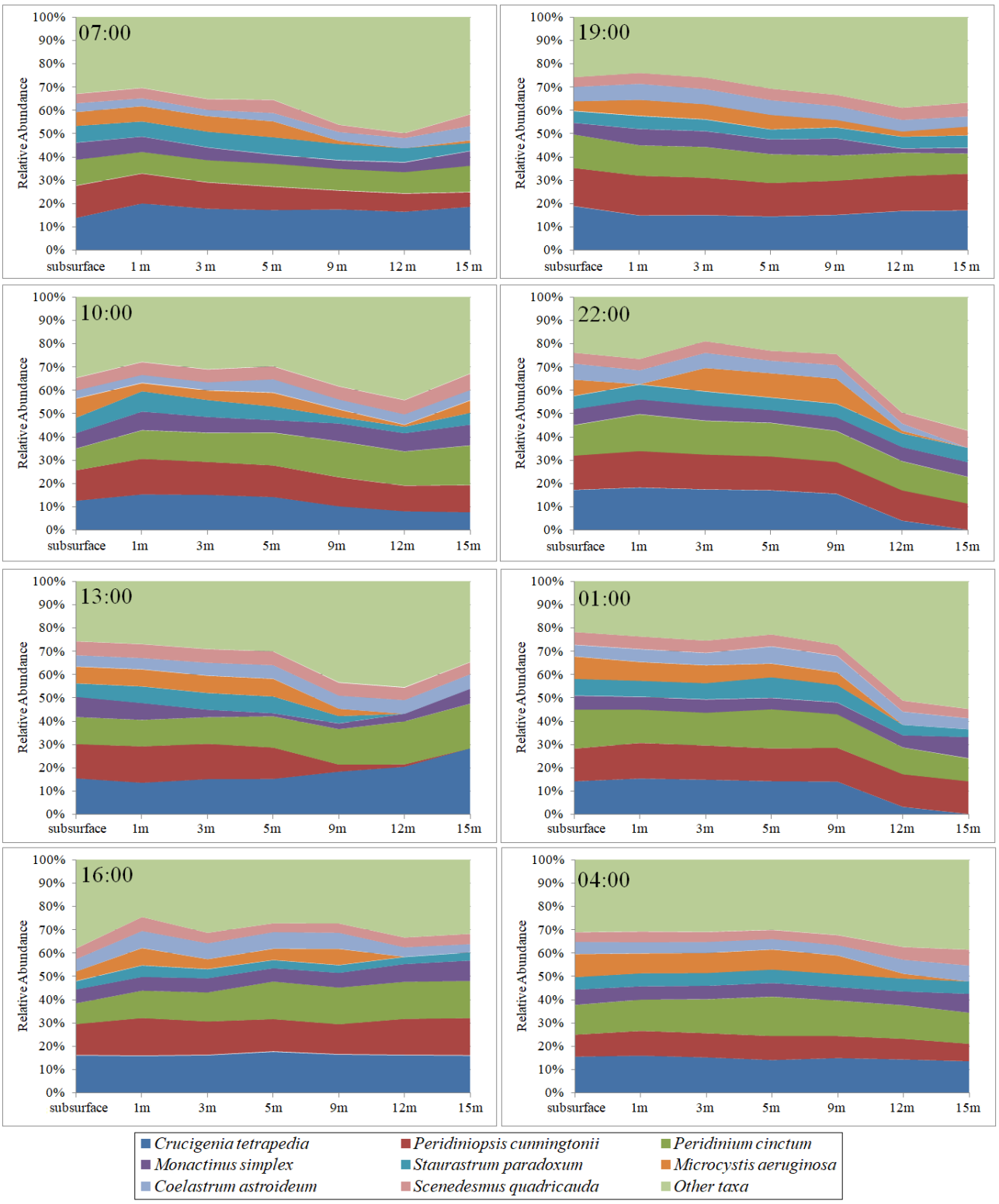

Fig. 7. Relative abundance changes of the dominant species found in Kadıköy reservoir phytoplankton with respect to the months (% abundance).

Especially, *C. tetrapedia* being in the first place, *P. cunningtonii* and *P. cinctum* were identified having the highest relative abundance in every sampling hour and in almost all depths. Although *M. aeruginosa* was identified in the first 5m, it was not found below 5m or identified hardly. In the samplings done at 10:00, 19:00, 22:00 and 04:00, *S. quadricauda* was found in 12 and 15m depths in higher numbers. *C. astroideum* was found in high numbers close to the bottom in early morning. *S. paradoxum* was positioned between 1-5m while *M. simplex* was found to be vertically distributed almost homogeneously. Hourly identified dominant species and their relative abundances in Kadıköy reservoir are given in Fig. 7.

Discussion

Weather and water temperatures measured during the study period were in seasonal normal, but when water temperatures of the surface water and in 1, 3, 5, 9, 12 and 15 m depths were evaluated according to YSKYY (Surface Water Quality Management Framework), it appeared that they do not exceed I. class water quality values (YSKYY 2015). A pH value between 6.5 and 8.5 is needed to provide a suitable environment for aquatic organisms and to not to pose endangering risks on the life of organisms (Küçükyılmaz *et al.* 2010). High pH values in particular lead to increase of detrimental effects of ammoniac and nitrogen compounds and therefore it is stated that pH changes in water is very important in aquatic environments to provide chemical balance thus making sure that aquatic life continues (Küçükyılmaz *et al.* 2010, Boztuğ *et al.* 2012). When data taken from the measurements are considered from the perspective of YSKYY, it is found that it does not significantly exceed the I. class quality values in terms of pH value of the reservoir. Besides being directly related with photosynthesis and respiration and decomposition, dissolved oxygen is also related with light density and temperature (Cunha-Santino *et al.* 2013). In our study, dissolved oxygen levels were found to be I. class water quality according to YSKYY criteria (YSKYY 2015). In fresh water ecosystems, 250-500µS.cm^{-1} conductivity level is suitable for fresh water organisms (Tanyolaç 2011). In Kadıköy reservoir, conductivity levels were measured daily and were found to be suitable for fresh water ecosystems and for the organisms living there. Salinity value is important for drinking water and in our study the values showing the salinity indicate that Kadıköy reservoir is suitable as a freshwater reserve (Tanyolaç 2011).

In this study, 65 taxa have been identified, the highest abundance was determined in the sampling done at 01:00 as 80790ind.L^{-1} on the subsurface. In the lake phytoplankton, Chlorophyta (56.21%) was found to be dominant followed by Miozoa (15.64%), Charophyta (9.29%), Cyanophyta (8.24%), Euglenophyta (6.73%) and Bacillariophyta (3.89%). Diatoms are represented in low numbers in every sampling hour while Euglonids were higher in number in evening hours under 5m depth. *Microcystis aeruginosa*, which is an eutrophic indicator species, is among the dominant organisms at around 5m in the lake phytoplankton. This situation is similar to the results of the study in Ömerli Reservoir performed by Albay and Akçalan (2003). Besides, *M. aeruginosa* is identified in many reservoir studies like Hasan Uğurlu, Kemer, Çamlıdere, Derbent, Hirfanlı, Ömerli and Kadıköy Reservoir (Fakıoğlu *et al.* 2011). This species is also identified in the studies performed in the lakes and rivers of Trakya (Öterler 2013, Öterler *et al.* 2014, Öterler *et al.* 2015). Other Cyanophyta group members were not dominant in the reservoir.

In our study area, Chlorophyta is the phytoplankton group that is represented by the highest taxa and organism number. This situation resembles many mesotrophic and eutrophic lakes' phytoplankton compositions (Gönülol & Obalı 1998b, İşbakan-Taş *et al.* 2002; Ongun-Sevindik 2010). Among the dominant organisms of the reservoir, *C. tetrapedia* is a planktonic, cosmopolitan and eutrophic species that is highly seen in lakes and rivers (John *et al.* 2003). Other Chlorococcales members we came across in our study, *Scenedesmus*, *Monactinus* and *Pediastrum* species, are highly seen in oligomesotrophic reservoirs and eutrophic lakes in our country (Aykulu & Obalı 1981, Aykulu *et al.* 1983, Obalı 1984, Gönülol & Obalı 1998a, 1998b, Atıcı 2001-2002, İşbakan-Taş *et al.* 2002, Albay & Akçaalan 2003, Atıcı 2003, Baykal *et al.* 2004, Kıvrak & Gürbüz 2005, Özyalın & Ustaoğlu 2008, Ongun-Sevindik, 2010, Çelekli & Öztürk 2014).

Among Charophyta, 5 taxa have been identified and most of them are *Staurastrum*, known as the characteristic of oligotrophic lakes (Rawson 1956, Hutchinson 1967, Wetzel 2001). These species can be seen in many of the oligotrophic and mesotrophic lakes in our country (Baykal *et al.* 2004, Karacaoğlu *et al.* 2004, Atıcı *et al.* 2005, Şahin & Akar 2007, Ustaoğlu *et al.* 2010, Ongun-Sevindik 2010, Atıcı & Alaş 2012).

Miozoa, the 2nd dominant organism group of the reservoir after Chlorophyta was identified as determinant of mesotrophic waters by Rawson (1956): *Ceratium hirundinella* is common in oligotrophic and mesotrophic waters and *Peridinium cinctum* and *Peridiniopsis cunningtonii* are found in high numbers (Rawson 1956, Eloranta 1995, Reynolds *et al.* 2002). Being identified in water poor in phosphorus, *Dinobryon divergens* is found in the reservoir in the first 9m depth (Hutchinson 1944, Lee 1980, Sandgren 1988).

There is a strong relation between the physical and chemical features of water bodies and the phytoplankton distribution and structure. The abiotic water conditions show variations naturally throughout the day and over the seasons of the year. These variations which may be related to stratification and mixing of the water column can be either vertical or horizontal. As a result, the availability of light and nutrients for the development of the phytoplankton community result in changes. Stratification, buoyancy, capacity for vertical regulation, light regime and grazing are the factors which have the potential of controlling phytoplankton biomass and species composition (Lopes et al., 2005). It can be said that temperature and especially light are effective in daily vertical changes of phytoplankton in Kadıköy dam. Phytoplanktonic organisms, which are concentrated in 1-3 meters depending on the amount of light during the day, approach the surface water in order to make more efficient use of light in the evening hours. During the study period, the sunset time in Edirne province was around 21:30. This may explain the detection of high chlorophyll_*a* and high cell counts in the sample taken at 22:00, which appears to be a paradox. Phytoplanktonic organisms sink deep into the night with a decrease in the amount of light and temperature.

In conclusion, the present study provided evidence for a relation with the 24-h cycle phytoplankton distribution and environmental conditions. The highest values of phytoplankton densities and biomass were found during the second half of a day and for the first 9m depth. Phytoplankton taxa without self-regulating capacity and those able to regulate their vertical position were differentially distributed in the segregated layers during the 24-h cycle. The results are regarded as evidence to conclude that temperature and light have effect on daily vertical movements of phytoplankton in Kadıköy reservoir.

Editor-in-Chief note: Burak Öterler is a member of Editorial Board of Trakya University Journal of Natural Sciences. However, he was't involved in the decision process during manucript evaluation.

References

1. Akçaalan, R., Young, F., Metcalf, J., Morrison, L., Albay, M. & Codd, G.A. 2006. Microcystin analysis in single filaments of *Planktothrix* spp. in laboratory cultures and environmental blooms. *Water Research*, 40(8): 1583-1590.
2. Akçaalan, R., Köker, L., Gürevin, C. & Albay M. 2014. *Planktothrix rubescens*: a perennial presence and toxicity in Lake Sapanca. *Turkish Journal of Botany*, 38: 782-789.
3. Albay, M. & Akçaalan, R. 2003. Factors influencing the phytoplankton steady-state assemblages in a drinkingwater reservoir (Ömerli Reservoir, İstanbul). *Hydrobiologia*, 502(1): 85-95.
4. Atıcı, T. 2001–2002. Sarıyar Barajı Planktonik Algleri Kısım: II- Chlorophyta, *Süleyman Demiral Üniversitesi Eğirdir Su Ürünleri Fakültesi Dergisi*, 8: 128-151.
5. Atıcı, T. 2003. Sarıyar Barajı Planktonik Algleri Kısım II – Chlorophyta. *Süleyman Demiral Üniversitesi Eğirdir Su Ürünleri Fakültesi Dergisi*, 8: 128-151.
6. Atıcı, T., Obalı, O. & Calışkan, H. 2005. Su kirliliğinin ve fitoplanktonik alg florasının Bayındır Baraj Gölü'nde kontrolü. *Su Ürünleri Dergisi*, 22(1): 79-82.
7. Atıcı, T. & Alas, A. 2012. A study on the trophic status and phytoplanktonic algae of Mamasin Dam Lake (Aksaray-Turkey). *Turkish Journal of Fisheries and Aquatic Sciences*, 12: 595-601.
8. Aykulu, G. & Obalı, O. 1981**.** Phytoplankton biomass in the Kurtboğazı Dam Lake. *Communications de la Facultê des Sciences del"Université de Ankara Serie C 2*, 24: 29-45.
9. Aykulu, G., Obalı, O. & Gönülol, A. 1983. The distribution of the phytoplankton of some lakes of Ankara around (in Turkish with English abstract). *Doğa Bilim Dergisi*, 7: 277-288.
10. Baykal, T., Açıkgöz, İ., Yıldız, K. & Bekleyen, A. 2004. A study on algae in Devegeçidi Dam Lake. *Turkish Journal of Botany*, 28: 457-472.
11. Becker, V., de Souza Cardoso, L. & Huszar, V.L.M., 2009. Diel variation of phytoplankton functional groups in a subtropical reservoir in southern Brazil during an autumnal stratification period. *Aquatic Ecology*, 43: 285.
12. Boztuğ, D., Dere, T., Tayhan, N., Yıldırım, N., Danabaş, D., Yıldırım, N.C., Önal, A.Ö., Danabaş, S., Ergin, C., Uslu, G. & Ünlü, E. 2012. Uzunçayır Baraj Gölü (Tunceli) fiziko-kimyasal özellikleri ve su kalitesinin değerlendirilmesi. *Adıyaman Üniversitesi Fen Bilimleri Dergisi*, 2(2): 93-106.
13. Cunha-Santino, M.B., Bitar, A.L. & Bianchini Jr., I. 2013. Chemical constraints on new man-made lakes. *Environmental Monitoring Assessment*, 185: 10177-10190.
14. Çelekli, A. & Öztürk, B. 2014. Determination of ecological status and ecological preferences of phytoplankton using multivariate approach in a Mediterranean reservoir. *Hydrobiologia*, 740: 115–135.
15. Eloranta, P. 1995. Phytoplankton of the national park lakes in central and southern Finland. *Annales Botanici Fennici*, 32: 193-209.
16. Fakıoğlu, Ö., Atamanalp, M. & Demir, N. 2011. Baraj göllerinde toksik mavi yeşil algler. *Ankara Üniversitesi Çevrebilimleri Dergisi*, 3(2): 65-71.
17. Guiry, M.D. & Guiry, G.M. 2017. *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org (Date accessed: 06 November 2017).
18. Gönülol, A. & Obalı, O. 1998a. A study on the phytoplankton of Hasan Uğurlu Dam Lake (Samsun-Turkey), *Turkish Journal of Biology*, 22: 447-461.
19. Gönülol, A. & Obalı, O. 1998b. Seasonal variations of phytoplankton blooms in Suat Uğurlu (Samsun - Turkey) *Turkish Journal of Botany*, 22: 93-97.
20. Harper, D., Brierley, B., Ferguson, A.J.D. & Phillips G. 1999. The ecological basis of lake and reservoir management. *Hydrobiologia*, 395/396: 469 pp.
21. Hindák, F. 2008. *Colour Atlas of Cyanophytes*. VEDA, Bratislava, 253 pp.
22. http://www2.dsi.gov.tr/bolge/dsi11/edirne.htm (Date accessed: 16 August 2017).
23. Huber-Pestalozzi, G. 1982. *Das Phytoplankton des Susswasser Teil: 8 E. Schweizerbart'sche Verlagsbuchhandlund (Nagele U. Obermiller)*, Stuttgart, 539 pp.
24. Hutchinson, G.E. 1944. Limnological studies in Connecticut. VII. A critical examination of the supposed relationship between phytoplankton periodicity and chemical changes in lake waters. *Ecology*, 25: 3-26.
25. Hutchinson, G.E. 1967. *A Treatise on Limnology. volume 2, ıntroduction to lake biology and the limnoplankton*. New York, John Wiley and Sons, Inc. 1115 pp.
26. İşbakan-Taş, B., Gönülol, A. & Taş, E. 2002. A study on the seasonal variation of the phytoplankton of Lake Cernek (Samsun-Turkey), *Turkish Journal of Fisheries and Aquatic Sciences*, 2: 121-128.
27. John, D.M., Whitton, B.A. & Brook, J.A. 2003. *The freshwater algal flora of British Isles, An identification guide to freshwater and terrestrial algae*. Cambridge University Press, 700 pp.

28. Karacaoğlu, D., Dere Ş. & Dalkıran, N. 2004. A taxonomic study on the phytoplankton of Lake Uluabat (Bursa). *Turkish Journal of Botany*, 28: 473-485.

29. Kıvrak, E. & Gürbüz, H. 2005. Seasonal variations in phytoplankton composition and physical-chemical features of Demirdöven Dam Reservoir, Erzurum, Turkey, *Biologia, Bratislava*, 60(1): 1-8.

30. Komarek, J. & Anagnostidis, K. 2005. *Cyanoprokariota. 2. Teil: Oscillatoriales. In: Büdel B, Gärtner G, Krienitz L, Schagerl M(eds) Süßwasserflora von Mitteleuropa*. Elsevier, Heidelberg, 759 pp.

31. Krammer, K. & Lange-Bertalot, H. 1986-2004. *Bacillariophyceae. 1-4 Teil. Süsswasserflora von Mitteleuropa*. H. Ettl, J. Gerloff, H. Heynig, and D. Mollenhauer (editors). Fischer-Verlag, Stuttgart, Germany.

32. Kristiansen, J. & Preisig, H.R. 2011. *Phylum Chrysophyta (Golden Algae). In: The freshwater algal flora of the British Isles. An identification guide to freshwater and terrestrial algae. Second edition. (John, D.M., Whitton, B.A. & Brook, A.J. Eds)*. Cambridge: Cambridge University Press, 896 pp.

33. Küçükyılmaz, M., Uslu, G., Birici, N., Örnekçi, N.G., Yıldız, N. & Şeker, T. 2010. Karakaya Baraj Gölü su kalitesinin incelenmesi. *International Sustainable Water and Wastewater Management Symposium*, 26-28 October 2010, Konya, Turkey. 445-454.

34. Lee, R.E. 1980. *Phycology*. Cambridge University Press, Cambridge, 561 pp.

35. Lopes, M.R.M., Bicudo, C.E.M. & Ferragut, M.C. 2005. Short term spatial and temporal variation of phytoplankton in a shallow tropical oligotrophic reservoir, southeast Brazil. *Hydrobiologia*, 542(1): 235-247.

36. Nusch, E. 1980. Comparison of different methods for chlorophyll and phaeopigment determination. *Archiv für Hydrobiologie*, 14: 14-36.

37. Obalı, O. 1984. Mogan Gölü fitoplanktonunun mevsimsel değişimi. *Doğa Bilim Dergisi, A2*, 8(1): 91-104.

38. Ongun-Sevindik, T. 2010. Phytoplankton composition of Çaygören Reservoir, Balikesir-Turkey. *Turkish Journal of Fisheries and Aquatic Sciences*, 10: 295-304.

39. Öterler, B. 2013. The phytoplankton composition of Kadiköy Reservoir (Keşan Edirne). *Trakya University Journal of Natural Sciences*, 2(14): 69-76.

40. Öterler, B., Kirgiz, T. & Albay, M. 2014. Seasonal variations of water quality parameters and algal flora of Tundzha (Tunca) River (Edirne, Turkey). *Open Journal of Ecology*, 4: 807-819.

41. Öterler, B., Albay, M, Çamur-Elipek, B., Güher, H. & Kirgiz, T. 2015. Spatial and temporal distribution of phytoplankton in Lake Gala (Edirne/TURKEY). *Trakya University Journal of Natural Sciences*, 16 (2): 71-80.

42. Özyalın, S. & Ustaoğlu, M.R. 2008. Kemer Baraj Gölü (Aydın) net fitoplankton kompozisyonunun incelenmesi. *E.U. Journal of Fisheries & Aquatic Sciences*, 25 (4): 275-282.

43. Rawson, P.S. 1956. Algal indicators of trophic lake types. *Limnology and Oceanography*, 1(1): 18-25.

44. Reynolds, C.S. 1988. *Funcional morphology and the adaptative strategies of freshwater phytoplankton. In Sandgren, C.D. (ed.), Growth and Reproductive Strategies of Freshwater Phytoplankton*. Cambridge University Press, Cambridge: 388-426.

45. Reynolds, C.S., Huszar, V., Kruk, C., Naselli- Flores, L. & Melo, S. 2002. Towards a functional classification of the freshwater phytoplankton, *Journal of Plankton Research*, 24(5): 417-428.

46. Round, F.E. 1984. *The ecology of the algae*. Cambridge Universty Press., Cambridge, 653 pp.

47. Round, F.E., Crawford, R.M. & Mann, D.G. 1990. *The diatoms, biology & morphology of the genera*. Cambridge University Press, 746 pp.

48. Sandgren, C.D. 1988. *The ecology of chrysophyte flagellates: their growth and perennation strategies as freshwater phytoplankton. In Sandgren, C. D. (ed.), Growth and Reproductive Strategies of Freshwater Phytoplankton*. Cambridge University press, Cambridge: 9-104.

49. Şahin, B. & Akar, B. 2007. The desmid flora of some high mountain lakes of the Turkish Eastern Black Sea Region. *Pakistan Journal of Botany*, 39(5): 1817-1832.

50. Takamura, N. & Yasuno, M. 1984. Diurnal changes in the vertical distribution of phytoplankton in hypertrophic Lake Kasumigaura, Japan. *Hydrobiologia*, 112: 53-60.

51. Tanyolaç, J. 2011. *Limnoloji*. Hatiboğlu Yayınları, Ankara, Türkiye, 290 p. (in Turkish).

52. Thornton, K.M., Kimmel, L.B. & Fonest, E.P. 1990. *Reservoir limnology ecological perspectives.* New York: J Wiley & Sons, New York, USA, 246 pp.

53. Ustaoğlu, M.R., Balık, S., Gezerler-Şipal, U., Özdemir, D. & Aygen, C. 2010. Buldan Baraj Gölü (Denizli) planktonu ve mevsimsel değişimi. *E.U. Journal of Fisheries & Aquatic Sciences*, 27(3): 113-120.

54. Utermöhl, H. 1958. Zur Ver vollkommung der quantitativen phytoplankton-methodik. *Mitteilungen der Internationalen Vereinigung für Limnologie*, 9: 39 pp.

55. Wetzel, R.G. 2001. *Limnology, lake and river ecosystems.* Third edition. Academic Press, New York, 1006 pp.

56. YSKYY. 2015. *Surface Water Quality Management Framework* (In Turkish). Official Journal of the publication: Date 15.04.2015, No. 29327.

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Araştırma Makalesi/Research Article

TRAKYA ÜNİVERSİTESİ BALKAN YERLEŞKESİ'NİN FLORASI

Vildan SALIK, Necmettin GÜLER*

Trakya Üniversitesi, Fen Fakültesi, Biyoloji Bölümü, 22030, Edirne, TURKEY
*Corresponding author: e-mail: nguler@trakya.edu.tr

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Özet: Bu çalışma Trakya Üniversitesi Balkan Yerleşkesi florasının belirlenmesi amacıyla yapılmıştır. Araştırma materyalini 2013-2014 yılları arasında Trakya Üniversitesi Balkan Yerleşkesi'nde yapılan arazi çalışması sonucu toplanan ve EDTU Herbaryumu'ndaki 1986-2013 tarihleri arasında toplanmış örnekler oluşturmuştur. Araştırma alanında 77 familya 250 cinse ait 428 takson tespit edilmiştir. 428 taksondan 1 (*Onopordum acanthium* L.)'i Trakya Bölgesi florası, 53'ü ise Edirne florası için yeni kayıt niteliğindedir. Alanda en çok takson içeren familyalar sırasıyla Asteraceae (%15,88), Fabaceae (%10,51) Rosaceae (%7) familyalarıdır. Çalışma alanında belirlenen taksonlardan 298 tanesi fitocoğrafik bölgesi bilinmeyen, 59 tanesi Avrupa-Sibirya elementi, 50 tanesi Akdeniz elementi, 14 tanesi Doğu Akdeniz elementi, 4 tanesi kozmopolit, 2 tanesi İran- Turan elementi ve 1 tanesi ise Akdeniz ve İran-Turan elementi olarak belirlenmiştir. Endemik bitki tür sayısı 4'tür.

Anahtar kelimeler: Flora, Trakya Üniversitesi, Balkan Yerleşkesi, Edirne.

The Flora of Trakya University Balkan Campus

Abstract: This study was performed to determine the flora of Trakya University Balkan Campus. The study material included plant specimens collected during field studies carried out between 2013 and 2014 and herbarium specimens collected from 1986 to 2013 and deposited in EDTU Herbarium. 428 taxa belonging to 77 families and 250 genera were determined in the study area. Amont the 428 taxa, *Onopordum acanthium* L. is a new record for flora of Thrace Region and 53 taxa are new records for flora of Edirne. The families with the highest number of taxa are Asteraceae (15.88%), Fabaceae (10.51%) and Rosaceae (7%). Among the determined taxa, 298 plants were unknown phytogeographical region data, 59 plants as Euro-Siberian elements, 50 as Mediterranean elements, 14 as East Mediterranean elements, 4 as cosmopolitans, 2 as Irano-Turanian elements and 1 plant as element Mediterranean-Irano-Turanian element. The number of endemic plant species is 4.

Key words: Flora, Trakya University, Balkan Campus, Edirne.

Giriş

Edirne İli Merkez İlçesine 6km uzaklıkta yer alan Trakya Üniversitesi Balkan Yerleşkesi, dere kenarında bulunan ağaçlık alan ve çevresinde açık otlu alanlar ve tarım arazileriyle çevrili iki dere yatağı ve arasında kalan bir tepeden oluşan ve çok çeşitli habiatatları içeren bölge olması itibari ile potansiyel olarak zengin bir biyoçeşitliliğe sahip olabileceği için son derece önemlidir.

Çalışma alanında floristik olarak şimdiye kadar 5 çalışma gerçekleştirilmiştir. Bu çalışmalardan ilki, Yerleşke içindeki Balkan Arboretum alanı ile ilgili olup 26 odunsu bitki (çalı, ağaççık ve ağaç) türü tespit edilmiştir (Özyavuz & Korkut 2008). İkinci çalışmada ise Dalgıç (2003) tarafından Edirne'nin Park ve Bahçelerinin florasının belirlenmesi kapsamında, Yerleşke içinde bulunan iki alan incelenmiş, Tıp Fakültesi Bahçesi olarak ele alınmış alanda 40 adet süs bitkisi belirlenmiş, şimdi ki Balkan Arboretumu alanından ise 28 adet doğal odunsu bitki taksonu tespit edilmiş ve Yerleşke alanında toplam tür sayısını (odunsu takson) 61 olarak belirlemiştir. Bunların haricinde, alandan toplanan Asteraceae familyasına ait 6 türün birçok organında kalsiyum oksalat kristalleri incelenmiştir (Meriç 2008, 2009). Bir diğer çalışmada kelebek bitki ilişkileri incelenmiş ve alanda 66 bitki türü tespit edilmiştir (Yurtsever & ark. 2010).

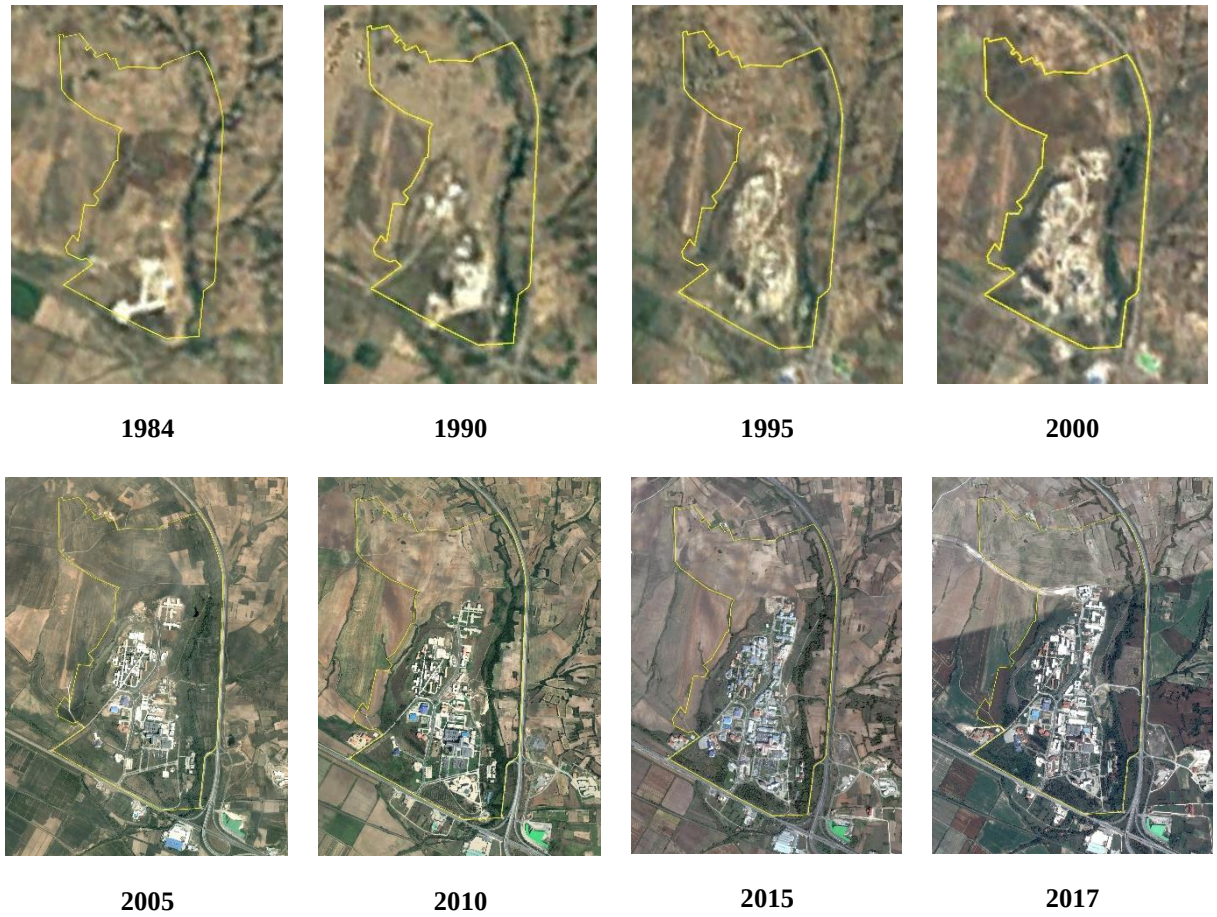
Alanının jeolojik yapısını tersiyer kuvarterner yaşlı birimler oluşturmaktadır. Bu birimler Pliyosene ait Ergene Formasyonu ve Kuvarternere ait Genç Çökeller yani alüvyonlar oluşturmaktadır. Genellikle grumosol toprakların hakim olduğu alanda çok fazla olmamakla birlikte yer yer dere içlerinde ve birikme alanlarında alüvyal topraklar görülmektedir. Çoğunlukla kireçli ve bazik bir yapıya sahiptir (Dönmez 1968, Anonim 2014). Alandaki yoğun yapılaşma ve buna bağlı olarak yapılan çevre düzenlemesi amacıyla dışarıdan getirilen topraklar nedeniyle hem alanın toprak yapısı hem de florası sürekli değişim altındadır.

Marmara Bölgesi'nin Trakya kesiminde bulunan Edirne ve civarı karasal iklim özelliklerine sahiptir. Yazları sıcak ve kurak, kışlar çok soğuk ve sert geçmektedir (Anonim 2014). Marmara ve Meriç Havzalarında yer alan Edirne İli genel nemlilik indislerine göre de yarı nemli veya Step-Nemli arası iklim tiplerine girmektedir (Anonim 2017a).

Trakya vejetasyonu incelendiğinde Balkan Yerleşkesi antropojen step sahası içinde yer almaktadır (Dönmez 1968). Genellikle tarım arazileri arasına sıkışmış olan yerleşke bölgesi, daha önceden tarım arazisi olarak kullanılmış ve yerleşke oluşumundan sonra bu alanların büyük bir kısmı terkedilmiş tarım arazisine dönüşürken bir kısmı hala tarım arazisi olarak kullanılmaya devam edilmektedir. Dere içleri ve yamaçlarda yer alan yoğun çalılık-ağaçlık alanlar doğal yapısını az-çok korumaya devam etmektedir.

Alan, Trakya vejetasyonu incelendiğinde Atropojen Step sahası içinde yer almaktadır (Dönmez 1968). Genellikle tarım arazileri arasına sıkışmış olan yerleşke bölgesi, daha önceden tarım arazisi olarak kullanılmış ve yerleşke oluşumundan sonra bu alanların büyük bir kısmı terkedilmiş tarım arazisine dönüşürken, bir kısmı hala tarım arazisi olarak kullanılmaya devam edilmektedir. Dere içleri ve yamaçlarda yer alan yoğun çalılık-ağaçlık alanlar, doğal yapısını az-çok korumaya devam etmektedir.

Yerleşkelerin oluşturulması sırasında gerek inşaat, gerekse peyzaj çalışmalarının floristik yapıyı geri dönülmez şekilde bozduğu ve değiştirdiği bilinmektedir (Turkmen 1987, İspirgil 1996, Türe & Böcük 2001, Nugan ve ark. 2007, Altay 2012 vb.). Bu durum genellikle alanların floristik kompozisyonunu değiştirmekte ve doğallıktan uzaklaştırmaktadır. Alanda Tıp Fakültesi ile başlayan ve halen devam etmekte olan yapılaşma, alan yapısını sürekli olarak değiştirirken; peyzaj çalışmaları ile de sürekli olarak dışarıdan yeni türler getirilmekte ve floristik yapı da sürekli olarak değişmektedir. Alanın 1984-2017 yılları arasındaki uydu fotoğrafları Şekil 1'de verilmiştir (DigitalGlobe 2017). 2003 öncesi görüntüler çok net olmamakla birlikte alanın büyük bir kısmının tarım arazi şeklinde kullanıldığı ve yapılaşmanın az olduğu görülmektedir. Alanda asıl yapılaşma 1995 sonrası başlamış ve 2000'li yıllarda artarak devam etmiştir. Bu durum uydu fotoğraflarında net olarak görülebilmektedir. Bu görüntülerde yerleşke alanında doğal floranın tespitinin önemini birkez daha ortaya koymaktadır.



Şekil 1. Araştırma alanının 1984-2017 yılları arasındaki arazi kullanımı (DigitalGlobe 2017).

Yapılan bu çalışma ile Balkan Yerleşkesi florasının tespiti ve yapılmakta olan, Balkan Arboretumu için bitki envanterinin çıkarılması ve Biyoloji, Eczacılık ve Peyzaj Mimarlığı öğrencilerinin pratiği için alt yapı oluşturulması amaçlanmıştır.

Materyal ve Metot

Araştırma materyalini 2013-2014 yılları arasında Trakya Üniversitesi Balkan Yerleşkesi'nde yapılan arazi çalışması sonucu toplanan ve 1986-2013 tarihleri arasında farklı araştırıcılar tarafından toplanmış ve T.Ü. Fen Fakültesi Biyoloji Bölümü'nde bulunan EDTU Herbaryumu'ndaki örnekler oluşturmuştur. Arazi çalışmaları 2013-2014 yıllarında Şubat-Eylül ayları arasında aylık periyotlarda yapılmıştır. Toplanan bitkiler preslenip kurutulmuş daha sonra da teşhisi yapılmıştır. Teşhis işleminde "Flora of Turkey and the East Aegean Islands (vol. 1-11)" (Davis 1965-1988, Güner ve ark. 2000) başta olmak üzere "Flora Europaea (vol. 1-5)" (Tutin ve ark. 1965-1980) gibi çeşitli kaynaklardan yararlanılmıştır. Latince ve Türkçe isimlendirmeleri Türkiye Damarlı Bitkiler Listesine göre verilmiştir (Güner ve ark. 2012). Teşhisi yapılan örnekler kartonlara yapıştırılıp Trakya Üniversitesi Fen Fakültesi Biyoloji Bölümü Herbaryumu'na (EDTU) teslim edilmiştir.

Çalışma alanı tamamıyla T.Ü. Balkan Yerleşkesi içinde yer almaktadır. Alan yapılaşma ve floristik özelliklerine göre 7 istasyona ayrılarak örnek toplanmıştır (Şekil 2). Örnek toplanan istasyonlar ve bunların minumum ve maksimum yükseklikleri Tablo 1'de verilmiştir.

Tablo 1. Alan yapılaşma ve floristik özelliklerine göre belirlenen istasyonlar.

İstasyon No	İstasyon adı	Yükseklik
1	Balkan Arboretumu alanı	55-75m
2	Balkan Lojmanları çevresi ve T.Ü. Tıp Fakültesi-Otoban arasında kalan bölge	48-65m
3	Kredi Yutlar arkası	46-70m
4	Yerleşke alanı kuzey bölgesi	70-85m
5	Konservatuvar çevresi	40-60m
6	Rektörlük çevresi	40-55m
7	Fakülteler ve Kredi Yurtlar alanı	45-75m

Şekil 2. Balkan Yerleşkesi Bölünen Alanları

Bitki listesi oluşturulurken familyalar ve taksonlar alfabetik sıraya göre verilmiştir. Tür ve tür altı taksonlarla ilgili bilgi verilirken taksonun latince adı verilip Türkçe adı parantez içinde verilmiş, endemik ise endemik olduğu (END), varsa IUCN tehlike kategorisi, taksonun toplandığı istasyon numarası (koyu olarak), toplanma tarihi, doğal türlerin habitatı veya kültür ve süs bitkisi olma durumu, EDTU numarası ve biliniyorsa fitocoğrafik bölgesi belirtilmiştir. Bitkilerin ait oldukları fitocoğrafik bölgeler ise Takhtajan (1986) göre; Akd. (Akdeniz), Av.-Sib. (Avrupa-Sibirya), D. Akd. (Doğu Akdeniz), İr.-Tur. (İran-Turan) ve Akd.-İr.-Tur. (Akdeniz ve İran-Turan) şeklinde kısaltılarak verilmiştir. Nadir ve endemik (End.) türlerin IUCN kriterlerine göre tehlike kategorileri ise EN (Tehlikede), VU (Hassas), DD (Yetersiz Veri) ve LC (En Az Endişe) şeklinde kısaltılarak verilmiştir. Listede doğal taksonlar olduğu gibi yazılırken, kültür ve peyzaj bitkileri (*) ile işaretlenerek, Trakya ve Edirne için yeni olan kayıtlar koyu yazılarak verilmiştir. Nadir ve endemik bitkilerin tehlike kategorilerinin belirlenmesinde Türkiye Bitkileri Kırmızı Kitabı'ndan (Ekim ve ark. 2000) ve IUCN (IUCN 2001) verilerinden yararlanılmıştır.

Sonuçlar

Balkan Yerleşkesi'nde arazi çalışmaları ve EDTU Herbaryumu'nda bulunan örneklerin incelenmesiyle 77 familya, 250 cinse ait 428 tür ve türaltı takson tespit edilmiştir.

Trakya Ünivesitesi Balkan Yerleşkesi vejetasyon açısından incelendiğinde Antropojen Step Sahası içinde kaldığı gözlemlenmiştir. Yerleşke öncesi büyük bir kısmı tarım arazisi olan ve bu alanlar arasına sıkışıp kalmış çalılık ve ağaçlık yerlerden oluşan vejetasyon yapısı, zamanla yapılaşma nedeniyle, büyük bir kısmının doğal yapısı bozulmuş ve vejetasyonu değişmiştir. 1 nolu istasyon, 1999 yılında çalışmalarına başlanan Balkan Arboretumu içinde yer almaktadır. Bölgenin ortasında Güllapoğlu deresi yer almakta ve derenin büyük bir kısmı doğallığını korumaktadır. Orta Anadolu Galeri Ormanlarına benzeyen vejetasyonda *Salix alba, Populus alba* ve *Ulmus minor* başta olmak üzere *Morus alba, Juglans regia, Pyrus communis, P. elaeagnifolia* subsp. *elaeagnifolia, Fraxinus angustifolia, Quercus robur subsp. robur* gibi ağaçların yanı sıra *Acer tataricum* subsp. *tataricum, Prunus spinosa* subsp. *dasyphylla, Rubus sanctus, Sambucus nigra, Cydonia vulgaris, Crataegus monogyna* var. *monogyna* gibi çalı ve ağaççıklar başta olmak üzere, *Cornus sanguinea* subsp. *sanguinea, Jasminum fruticans, Ligustrum vulgare, Paliurus spina-christii, Rosa canina, R. gallica* ve *Ruscus aculeatus* gibi çalılar görülmektedir. Bölgede görülen sarılıcı bitkiler arasında *Periploca graeca* var. *graeca, Vitis sylvestris* ve *Humulus lupulus* en fazla yaygın olan türlerdir. Bunların dışında *Clematis viticella* ve *Lonicera etrusca* var. *etrusca* diğer sarılıcı türlerdir. Bölgede görülen ve yer yer ağaçlandırma amacıyla kullanılmış türler de yer almaktadır. Bunlar arsında *Robinia pseudoacacia, Populus nigra* subsp. *nigra, Populus x canadensis, Ailanthus altissima, Gleditsia triancanthos, Acer negundo, Cercis siliquastrum, Thuja orientalis, Coryllus avellana* subsp. *avellana* yer almaktadır. Bölgede ayrıca *R. pseudoacacia* ve *P. x canadensis* ağaçlandırma alanları yer almaktadır. Bölgenin orta kısmı terkedilmiş tarım arazisinden oluşmakta ve dışardan gelen ağaç ve çalı türleriyle zamana bağlı olarak örtülmeye devam etmektedir. Yer yer ağaç ve çalı kümeleri alanda görülmektedir. Bölgenin kuzey batısında göletin üst tarafında bulunan dik yamaçlarda dere vejetasyonuna benzer şekilde çok yoğun bir yapı gösterir. Bu alanın baskın türlerini *P. alba, M. alba* ve *C. monogyna* var. *monogyna* oluşturur. 2 nolu istasyon, özellikle dere civarında yer alan vejetasyon açısından, Arboretum alanı ile benzer yapıya sahiptir. Alanın bir kısmı tarım arazisi olarak kullanılmaya devam edilmektedir. Bir kısmında ise yerleşim birimleri, sosyal alanlar ve kısmen peyzaj alanları mevcuttur. 3 nolu istasyon içindeki dere, doğal yapısını korumakla birlikte, derenin doğu tarafı ağaçlandırma alanı haline getirilmiş, batı kısmı ise tarım arazisi olarak kullanılmaktadır. 4 nolu istasyonun büyük bir kısmı tarım arazisinden oluşmakta ve doğusunda bulunan dere, Arboretum alanı ile aynı özellikleri gösterirken, etrafı tarım arazileri ile çevrilidir. 5 ve 6 nolu istasyonlar, Yerleşkenin giriş kısmında yer almakta ve bir kısmında yapılaşma, diğer kısımlarında ise ağaçlandırma alanları yer almaktadır. Kısmen peyzaj alanlarına sahiptir. 7 nolu istasyon, tamamen yapılaşmış ve peyzajı yapılmış bir alandan oluşmaktadır. Alanda tespit edilen türlerin listesi aşağıda verilmiştir.

Floristik Liste

PTERIDOPHYTA (EĞRELTİLER)

EQUISETACEAE (Atkuyruğugiller)

Equisetum arvense L. (Atkuyruğu), **1**, 29.04.2014, sulak alan, EDTU 13730!

GYMNOSPERMAE (AÇIK TOHUMLULAR)

CUPRESSACEAE (Servigiller)

Cupressus arizonica* Greene "Glauca" (Mavi servi), **7, 10.04.2014, kültür, süs bitkisi.

C. sempervirens* L. (Servi), **7, 10.04.2014, kültür, süs bitkisi, Akd.

Juniperus sabina* L. (Sabin ardıcı), **7, 10.04.2014, kültür, süs bitkisi.

Thuja orientalis* L. (Doğu mazısı), **1, **2**, **7**, 10.04.2014, kültür, süs bitkisi.

GINKGOACEAE (Mabetağacıgiller)

Ginkgo biloba* L. (Mabet ağacı), **7, 10.04.2014, kültür, süs bitkisi.

PINACEAE (Çamgiller)

Abies nordmanniana* (Steven) Spach subsp. *equi-trojani* (Asc. & Sint. Ex Boiss.) Coode & Cullen (Kazdağı göknarı), **7, 10.04.2014, kültür, süs bitkisi, Av.-Sib.

Cedrus atlantica* (Endl.) Carr. (Atlas sediri), **7, 10.04.2014, kültür, süs bitkisi, Akd.

******Picea abies* var. *abies* (L.) H.Karst. (Avrupa ladini) **7**, 10.04.2014, kültür, süs bitkisi.

******P. pungens* Engelm. (Mavi ladin), **7**, 10.04.2014, kültür, süs bitkisi. Kozmopolit.

******P. orientalis* (L.) Link (Doğu ladini), **7**, 10.04.2014, kültür, süs bitkisi.

******Pinus nigra* Arn. subsp. *pallasiana* (Lamb.) Holmboe (Kasatura çamı), **2, 3, 5, 6, 7,** 10.04.2014, kültür, süs bitkisi.

******P. brutia* Ten. var. *brutia* (Kızılçam), **7**, 10.04.2014, kültür, süs bitkisi, D. Akd.

******P. pinea* L. (Fıstık Çamı), **7**, 10.04.2014, kültür, süs bitkisi, Akd.

TAXACEAE (Porsukgiller)

******Taxus baccata* L. (Porsuk), **7**, 10.04.2014, kültür, süs bitkisi.

ANGIOSPERMAE (KAPALI TOHUMLULAR)

ADOXACEAE (Mürvergiller)

Sambucus nigra L. (Ağaç mürver), **1**, **2**, 10.04.2014, dere kenarları, ağaçlık alanlarda, Av.-Sib.

******Viburnum opulus* L. (Gilaburu), **7**, 10.04.2014, kültür, süs bitkisi, Av.-Sib.

******V. tinus* L. (Fil burnu), **7**, 10.04.2014, kültür, süs bitkisi, Akd.

ALISMATACEAE (Kurbağakaşığıgiller)

Alisma plantago-aquatica L. (Çobandüdüğü), **1**, 24.05.2013, sulak alan, EDTU 13524! Av.-Sib.

AMARANTHACEAE (Horozibiğigiller)

Amaranthus albus L. (Kömüş mancarı), **7**, 21.09.2013, otlak alan, EDTU 13526!

A. deflexus L. (Sarkık ibik), **7**, 24.05.2013, otlak alan, EDTU 13527!

Chenopodium album L. subsp. *album* var. *album* (Aksirken), **7**, 30.07.2013, otlak alan, EDTU 13525!

C. murale L. (Salmanca), **1**, 25.05.2005, açık arazi, EDTU 9469!

AMARYLLIDACEAE (Nergisgiller)

Allium atroviolaceum Boiss. (Lifli körmen), **1**, 15.05.2013, otlak alan, EDTU 13528!

A. scorodoprasum L. subsp. *rotundum* (L.) Stearn (Deli pırasa), **4**, 11.06.1987, EDTU 1107! 23.05.1985, EDTU 130! 31.05.1988, EDTU 2072!

APIACEAE (Maydanozgiller)

Anthriscus caucalis M. Bieb. (Deligımı), **1**, 19.04.2013, ağaçlık altı, EDTU 13532!

Conium maculatum L. (Baldıran), **1**, 15.05.2013, ağaçlık altı, EDTU 13534!

Daucus carota L. (Havuç), **1**, 31.08.2013, otlak alan, EDTU 13531!

Eryngium campestre L. var. *campestre* (Kırsenet), **3**, 08.07.2013, çamlık altı, EDTU 13529!

Orlaya grandiflora (L.) Hoffm. (Koca dilkanatan), **1**, 15.05.2013, otlak alan, EDTU 13535!

Scandix pecten-veneris L. (Zühre tarağı), **1**, 19.04.2013, yol kenarı, EDTU 13533!

***Tordylium apulum* L. (Kafkalida), 1**, 25.06.2013, otlak alan, EDTU 13530! Akd.

***Torilis leptophylla* (L.) Reichb. (İnce dercikotu), 1**, 15.05.2013, otlak alan, EDTU 13537!

APOCYNACEAE (Zakkumgiller)

******Nerium oleander* L. (Zakkum), **7**, 10.04.2014, kültür, süs bitkisi, Akd.

Periploca graeca L. var. *graeca* (Garipler urganı), **1, 2,** 10.04.2014, dere kenarlarında, sarılıcı, D. Akd.

ARACEAE (Yılanyastığıgiller)

Arum maculatum L. (Yılan ekmeği), **1**, 24.05.2013, ağaçlık altı, EDTU 13537!

ARISTOLOCHIACEAE (Lohusaotugiller)

Aristolochia clematis L. (Lohusa otu), **7**, 06.06.1987, ağaç altı, EDTU 1110! Av.-Sib.

ASPARAGACEAE (Kuşkonmazgiller)

Muscari neglectum Guss. ex Ten. (Arap üzümü), **1**, 01.04.2013, ağaçlık altı, EDTU 13543! **2**, 01.04.2013, otlak alan, EDTU 13542! **4**, 20.04.1988, EDTU 1942! **7**, 21.04.2013, otlak alan, EDTU 13541!

Ornithogalum fimbriatum Willd. (Kirpi sasal) (**DD**), **1**, 02.04.2013, otlak alan, EDTU 13539! **5**, 12.04.2013, ağaçlık altı, EDTU 13538! D. Akd.

O. refractum Kit. ex Schlecht. (Dönek yıldız) (**VU**), **3**, 21.03.1988, EDTU 1863!

O. umbellatum L. (Sunbala), **1**, otlak alan, 19.04.2013, EDTU 13544! **4**, 02.05.1988, EDTU 1980! 2922!

Ruscus aculeatus L. Boiss. (Tavşanmemesi), **1, 2**, ağaçlık altı, 10.04.2014.

******Yucca filamentosa* L. (Gür avize), **7**, 10.04.2014, kültür, süs bitkisi.

ASTERACEAE (Papatyagiller)

Achillea clypeolata Sibth. & Sm. (Yılan çiçeği), **1**, 15.05.2013, otlak alan, EDTU 13602! 30.05.2005, otlak alan, EDTU 9485! 21.07.2005, otlak alan, EDTU 9487! Av.-Sib.

A. coarctata Poir. (Kirpit), **1**, 30.05.1988, EDTU 5011! 18.06.2003, dere kenarı, EDTU 8537!

A. maritima (L.) Ehrend. & Y.P. Guo subsp. *maritima* (Çocuk otu), **1**, 18.06.2013, dere kenarı, EDTU 8539! Akd.

A. setacea Waldst. Et Kit. (Ayvabala), **1**, 30.06.2005, açık arazi, EDTU 9483! Av.-Sib.

Anthemis pseudocotula Boiss. (Acem papatyası), **5**, 08.07.2013, çamlık altı, EDTU 13556!

***A. arvensis* L. (Tarla papatyası), 1**, 19.04.2013, dere kenarı, EDTU 13583! Av.-Sib.

Arctium minus (Hill.) Bernh. (Löşlek), **1**, 03.08.2005, açık arazi, EDTU 9494! Av.-Sib.

Artemisia annua L. (Kabe süpürgesi), **1**, 21.10.2002, dere kenarı, EDTU 8540!

***Bidens tripartita* L. (Üç suketeni), 1**, 13.08.2013, sulak alan, EDTU 13598! 19.09.2004, dere kenarı, EDTU 8575!

Carduus acicularis Bertol. (Sivri kangal), **1**, 15.05.2013, otlak alan, EDTU 13603! Akd.

C. candicans Walds. & Kit. subsp. *candicans* (Toptelli) (**EN)**, **1**, 19.04.2013, EDTU 13599! 09.05.2005, EDTU 8585! Av.-Sib.

C. pycnocephalus L. subsp. *albidus* (Bieb.) Kazmi (Eşek soymacı), **1**, 15.05.2013, otlak alan, EDTU 13604!

C. pycnocephalus L. subsp. *pycnocephalus* (Soymaç), **1**, 18.05.2005, açık arazi, EDTU 8588! 9465!

Carthamus dentatus Vahl. (Kına dikeni), **1**, 30.07.2013, otlak alan, EDTU 13549! 09.07.2003, açık arazi, EDTU 8520!

C. lanatus L. (Sarıdiken), **1**, 30.07.2013, otlak alan, EDTU 13545! 30.06.2003, açık arazi, EDTU 8518!

Centaurea diffusa Lam. (Ak düğme) (**END)**, **1**, 25.06.2013, otlak alan, EDTU 13591! Akd.

C. iberica Trev. ex Sprengel (Deligöz dikeni), **1**, 25.06.2013, otlak alan, EDTU 13547! 02.07.2003, açık arazi, EDTU 8521!

C. salonitana Vis. (Keşan dikeni), **1**, 30.06.2003, açık arazi, EDTU 8522! Av.-Sib.

C. solstitialis L. subsp. *solstitialis* (Çakırdikeni), **1**, 18.06.2003, açık arazi, EDTU 8535! **2**, 28.05.2013, otlak alan, EDTU 13580!

Chondrilla juncea L. var. *juncea* (Karakavuk), **1**, 02.08.2005, açık arazi, EDTU 9491! **7**, 08.07.2013, otlak alan, EDTU 13555!

Cichorium pumilum Jacq. (Dünek), **7**, 10.06.2013, otlak alan, EDTU 13560! D. Akd.

C. intybus L. (Hindiba), **1**, 03.06.2005, açık arazi, EDTU 9481!

Cirsium arvense (L.) Scop. (Köy göçüren), **1**, 25.06.2013, otlak alan, EDTU 13584! 18.08.2004, dere kenarı, EDTU 8533!

C. creticum d'Urv subsp. *creticum* (Eşek çalısı), **1**, 18.08.2004, dere kenarı, EDTU 8532! Akd.

C. vulgare (Savi.) Ten (Yaygın kangal), **1**, 18.08.2004, dere kenarı, EDTU 8531!

Cnicus benedictus L. (Topdiken), **1**, 28.05.2013, otlak alan, EDTU 13552!

Conyza bonariensis (L.) Cronquist (Çakal otu), **1**, 09.08.2004, açık arazi, EDTU 8528! **3**, 08.07.2013, çamlık altı, EDTU 13554!

C. canadensis (L.) Cronquist (Selvi otu), **1**, 09.08.2004, açık arazi, EDTU 8527! **7**, 30.07.2013, otlak alan, EDTU 13553!

***Cota altissima* (L.) J. Gay (Köpek papatyası), 1**, 15.05.2013, otlak alan, EDTU 13588! **6**, 01.04.2013, otlak alan, EDTU 13589!

C. tinctoria (L.) J. Gay ex Guss. var. *tinctoria* (Boyacı papatyası), **1**, 30.07.2013, sulak alan, EDTU 13550! 28.05.2013, ekili alan, EDTU 13581! 15.05.2013, otlak alan, EDTU 13582! 18.06.2004, dere kenarı, EDTU 392! 30.05.2005, açık arazi, EDTU 9466!

C. tinctoria (L.) J. Gay ex Guss. var. *pallida* (DC.) Özbek & Vural (Boyacı papatyası), **1**, 13.05.2005, açık arazi, EDTU 8584!

Crepis sancta (L.) Babcock (Yaban kıskısı), **1**, 07.04.2013, otlak alan, EDTU 13568! 13569! 29.04.2004, açık arazi, EDTU 8512! 11.04.2005, EDTU 8580! 03.06.2005, EDTU 9476! **4**, 13.04.2013, tarla sınırı, EDTU 13567! **5**, 12.04.2013, ağaçlık altı, EDTU 13570! 07.04.2013, otlak alan, EDTU 13571!

C. setosa Hall. (Kılçıklı kıskıs), **1**, 24.05.2013, otlak alan, EDTU 13578! 13.05.2005, açık arazi, EDTU 8583! Av.-Sib.

C. vesicaria L. (Kese kıskısı), **1**, 13.05.2005, dere kenarı, EDTU 8582! Akd.

Cyanus segetum Hill. (Gelin tacı), **1**, 24.05.2013, otlak alan, EDTU 13572! 23.05.1985, EDTU 129! 25.05.1986, EDTU 396! 24.05.1987, EDTU 775! 31.05.1988, EDTU 2083! 18.06.2003, EDTU 8536! **5**, 02.04.2013, yol kenarı, EDTU 13573! 01.04.2013, yol kenarı, EDTU 13574!

Echinops microcephalus Sm. (Papaz kalpağı), **5**, 08.07.2013, çamlık altı, EDTU 13584! Akd.

Filago arvensis L. (Keçe otu) **1**, 30.06.2005; açık arazi, EDTU 9482!

F. eriocephala Guss. (Deli keçe otu), **1**, 18.06.2003, dere kenarı, EDTU 8534! D. Akd.

F. germanica (L.) L. (Alman keçe otu), **1**, 28.05.2013, ekili alan, EDTU 13575!

Galinsoga parviflora Cav. (Beşpat çiçeği) (**DD) 7**, 20.09.2004, yol kenarı, EDTU 8525!

Helminthotheca echioides (L.) Holub. (Billurdüğme), **6**, 10.06.2013, ağaçlık altı, EDTU 13558!

***Hypochaeris radicata* L. (Dağ marulu), 1**, 24.05.2013, otlak alan, EDTU 13576! Av.-Sib.

Inula graveolens (L.) Desf. (Deli sarıot), **1**, 05.10.2004, dere kenarı, EDTU 8576! Akd.

Lactuca saligna L. (Deli marul), **1**, 30.07.2013, otlak alan, EDTU 13579!

L. serriola L. (Eşek helvası), **1**, 30.07.2013, otlak alan, EDTU 13559!

***Matricaria chamomilla* L. var. *recutita* (L.) Grierson (Alman papatyası), 1**, 15.05.2013, otlak alan, EDTU 13593! 19.04.2013, yol kenarı, EDTU 13595! 13596! 09.05.2005, açık arazi, EDTU 8586! **2**, 01.04.2013, yol kenarı, EDTU 13597!

***Onopordum acanthium* L. (Galagan), 1**, 25.06.2013, otlak alan, EDTU 13606!

Pulicaria dysenterica (L.) Bernh. subsp. *dysenterica* (Yaraotu), **1**, 18.08.2004, açık arazi, EDTU 8529!

Scolymus hispanicus L. (Şefketibostan), **6**, 10.06.2013, ağaçlık altı, EDTU 13546! Akd.

Scorzonera cana (C.A. Mey) Griseb. var. *cana* (Tekesakalı), **1**, 01.05.2004, dere kenarı, EDTU 8563!

S. laciniata L. subsp. *laciniata* (Parım), **1**, 15.05.2013, otlak alan, EDTU 13605! 02.06.2004, açık arazi, EDTU 8523!

Senecio aquaticus Hill. subsp. *erraticus* (Bertol.) Matthews (Tarla kanarya otu), **1**, 20.09.2004, dere kenarı, EDTU 8577! Av.-Sib.

S. vernalis Waldst. & Kit. (Kanarya otu), **1**, 19.04.2013, ağaçlandırılan alan, EDTU 13564! **4**, 13.04.2013, tarla sınırı, EDTU 13562! **5**, 12.04.2013, çamlık altı, EDTU 13563! **6**, 02.04.2013, ağaçlık altı, EDTU 13561! 25.05.1986, EDTU 391! 31.04.2004, EDTU 8513!

S. vulgaris L. (Taşakcıl otu), **1**, 07.04.2013, otlak alan, EDTU 13565! 13566! 31.04.2004, açık arazi, EDTU 8514! 21.10.2002, dere kenarı, EDTU 8542!

Silybum marianum (L.) Gaertner (Deve dikeni), **1**, 15.05.2013, otlak alan, EDTU 13607! 15.05.2003, açık arazi, EDTU 9460! Akd.

***Sonchus asper* (L.) Hill subsp. *glaucescens* (Jordan) Ball (Gevirtlek), 1**, 15.05.2005, açık arazi, EDTU 8589! 16.05.2005, açık arazi, EDTU 8596! 13.05.2005, açık arazi, EDTU 9457! 15.05.2005, açık arazi, EDTU 9461! 9462! 9464! **5**, 19.04.2013, çamlık altı, EDTU 13601!

S. tenerrimus L. (Kovuk), **1**, 10.06.2013, otlak alan, EDTU 13600! Akd.

Symphyotrichum squamatum (Spreng.) G.L. Nesom (Arsız simpatı), **1**, 21.10.2002, dere kenarı, EDTU 8541! 01.10.2004, açık arazi, EDTU 8578! 05.10.2004, EDTU 9470!

Taraxacum scaturiginosum G. Hagl. (Kıvır kıvır), **1**, 15.04.2005, dere kenarı, EDTU 8579!

Tragopogon dubius Scop (At yemliği), **1**, 24.05.2013, otlak alan, EDTU 13577!

T. pratensis L. subsp. *orientalis* (L.) Celak (Sarı salsifin), **1**, 17.05.2004, açık arazi, EDTU 8526! Akd.

Tripleurospermum baytopianum E. Hossain (Sultan papatyası) (**END/EN), 1**, 05.04.2013, otlak alan, EDTU 13585! 19.04.2013, ağaçlandırılan alan, EDTU 13594! **7**, 01.04.2013, otlak alan, EDTU 13587! Akd.

***T. parviflorum* (Willd.) Pobed. (Beybunik), 5**, 01.04.2013, çamlık altı, EDTU 13586!

Tussilago farfara L. (Öksürük otu), **1**, 07.03.1993, ağaç altı, EDTU 5744! Av.-Sib.

Tyrimnus leucographus (L.) Cass. (Dulkarı gömleği), **1**, 15.05.2013, otlak alan, EDTU 13590! 30.05.2005, açık arazi, EDTU 9467! Akd.

Xanthium spinosum L. (Pıtrak), **7**, 08.07.2013, otlak alan, EDTU 13551!

X. strumarium L. subsp. *strumarium* (Koca pıtrak), **1**, 30.07.2013, otlak alan, EDTU 13557!

Xeranthemum annuum L. (Kağıt çiçeği), **1**, 10.07.2013, açık arazi, EDTU 8519!

BERBERIDACEAE (Karamukgiller)

Berberis vulgaris* L. (Kızıl karamuk), **7, 10.04.2014, kültür, süs bitkisi

B. thunbergii* DC. (Kadın tuzluğu), **7, 10.04.2014, kültür, süs bitkisi

Mahonia aquifolium* (Pursh) Nutt. (Sarıboya ağacı), **7, 10.04.2014, kültür, süs bitkisi

BETULACEAE (Huşgiller)

Corylus avellana* L. var. *avellana* (Fındık), **1, 2, 10.04.2014, dere kenarı, ağaçlık altı. Av.-Sib.

BORAGINACEAE (Hodangiller)

Anchusa azurea Mill. var. *azurea* (Sığırdili), **5**, 08.07.2013, çamlık altı, EDTU 13614!

A. officinalis L. (Ballağan), **1**, 03.05.2013, otlak alan, EDTU 13620! Av.-Sib.

Buglossoides arvensis (L.) Johnston (Tarla taşkeseni), **1**, 13.04.2013, otlak alan, EDTU 13629! 28.04.2013, tarla sınırı, EDTU 13631! **5**, 12.04.2013, ağaçlık altı, EDTU 13630! 01.04.2013, çamlık altı, 13632!

Echium italicum L. (Kurt kuyruğu), **1**, 28.05.2013, otlak alan, EDTU 13613! Akd.

E. plantagineum L. (Kırk batıran), **1**, 30.04.2013, otlak alan, EDTU 13609! 13624! 10.06.2013, ağaçlık altı, EDTU 13610! 28.05.2013, ekili alan, EDTU 13621! **7**, 03.05.2013, otlak alan, EDTU 13605! Akd.

Heliotropium europaeum L. (Akrep otu), **1**, 25.06.2013, otlak alan, EDTU 13623! 30.04.2013, otlak alan, EDTU 13622! İr.-Tur.

***H. suaveolens* M. Bieb. (Itırlı bambul), 1**, 25.06.2013, otlak alan, EDTU 13611! 10.06.2013, otlak alan, EDTU 13612! D. Akd.

Myosotis arvensis (L.) Hill. subsp. *arvensis* (Kardeşboncuğu), **1**, 15.05.2013, otlak alan, EDTU 13626! 13627! 03.05.2013, otlak alan, EDTU 13628! 29.04.2014, otlak alan, EDTU 13633! Av.-Sib.

M. ramosissima Rochel ex Schultes (Kuşgözü), **1**, 19.04.2013, ağaçlık altı, EDTU 13625! **2**, 01.04.2013, otlak alan, EDTU 13618!

M. stricta Link ex Roemer et Schultes (Yitik unutmabeni), **1**, 29.04.2013, otlak alan, EDTU 13615! 01.04.2013, otlak alan, EDTU 13616! 28.04.2013, otlak alan, EDTU 13617! Av.-Sib.

M. uncata Boiss. & Balansa (Kısa kuşgözü); **(END); 1**, 01.04.2013; tarla sınırı; EDTU 13619! Akd.

BRASSICACEAE (Turpgiller)

Alyssum strigosum Banks et Sol. var. *strigosum* (Dökük kunduzotu), **1**, 02.04.2013, ağaçlık altı, EDTU 13673! **5**, 01.04.2013, ağaçlık altı, EDTU 13674!

Arabidopsis thaliana (L.) Heynbold (Fenotu), **1**, 21.04.2013, tarla sınırı, EDTU 13662! 13653! 13.04.2013, tarla sınırı, EDTU 13672!

Berteroa mutabilis (Vent.) DC. (Deli tere), **6**, 01.04.2013, çamlık altı, EDTU 13634!

***B. incana* (L.) DC. (Boz deli tere), 1**, 30.07.2013, otlak alan, EDTU 13647!

Calepina irregularis (Asso.) Thell. (Top hardal), **1**, 21.04.2013, otlak alan, EDTU 13649! 07.04.2013, otlak alan, EDTU 13669! **5**, 12.04.2013, ağaçlık altı, EDTU 13658! 13670! 13663!

Capsella bursa-pastoris (L.) Medik. (Çobançantası), **1**, 05.04.2013, otlak alan, EDTU 13641! 21.04.2013, otlak alan, EDTU 13650! 13.04.2013, tarla sınırı, EDTU 13654! 13655! 02.04.2013, otlak alan, EDTU 13656! 07.04.2013, otlak alan, 13657! **7**, 24.02.2013, otlak alan, EDTU 13635! 23.05.1985, 125!

***C. rubella* Reuter (Ayşecik), 1**, 02.04.2013, tarla sınırı, EDTU 13668! Akd.

Cardamine hirsuta L. (Kıllı kodim), **1**, 21.04.2013, otlak alan, EDTU 13671! **5**, 12.04.2013, ağaçlık altı, EDTU 13664!

Diplotaxis tenuifolia (L.) DC. (Türpenk), **1**, 15.05.2013, otlak alan, EDTU 13625!

***Draba muralis* L. (Ak dolama), 1,** 21.04.2013, otlak alan, EDTU 13651!

D. verna L. (Çırçır otu), **1**, 24.02.2013, otlak alan, EDTU 13666! 02.04.2013, ağaçlık altı, EDTU 13667!

Erysimum repandum L. (Çatal zarife), **1**, 01.04.2013, otlak alan, EDTU 13637! **3**, 02.04.2013, çamlık altı, 13640! **7**, 19.04.2013, yol kenarı, EDTU 13636!

Lepidium draba L. (Diğnik), **1**, 19.04.2013, tarla sınırı, EDTU 13638!

Microthlaspi perfoliatum (L.) F.K. Mey. (Giyle), **1**, 24.02.2013, otlak alan, EDTU 13659! 07.04.2013, otlak alan, EDTU 13660! 02.04.2013, otlak alan, EDTU 13661! **5**, 12.04.2013, ağaçlık altı, EDTU 13662!

Rorippa sylvestris (L.) Besser subsp. *sylvestris* (Çakandura), **1**, 03.05.2013, otlak alan, EDTU 13645! **2**, 13.08.2013, ağaçlık altı, EDTU 13642!

R. thracica (Gris.) Fritsch (Tüylü düzbağa) (**EN)**, **1**, 30.04.2013, otlak alan, EDTU 13644!

Sisymbrium altissimum L. (Ergelen otu), **1**, 19.04.2013, yol kenarı, EDTU 13639! 24.05.2013, otlak alan, EDTU 13643!

S. officinale (L.) Scop. (Ergelen hardalı), **1**, 25.06.2013, otlak alan, EDTU 13648!

***Teesdalia coronopifolia* (Berg.) Thellung (Çoban çadırı), 5**, 12.04.2013, ağaçlık altı, EDTU 13646! D. Akd.

BUTOMACEAE (Bataklıkgülügiller)

Butomus umbellatus L. (Bataklık gülü), **5**, 30.07.2013, su kanalı, EDTU 13676! Av.-Sib.

BUXACEAE (Şimşirgiller)

Buxus sempervirens* L. subsp. *sempervirens* (Şimbir), **7, 10.04.2014, kültür, süs bitkisi. Av.-Sib.

CAMPANULACEAE (Çançiçeğigiller)

Campanula rapunculus L. var. *lambertiana* (A. DC.) Rech. f. (Sidikli çançiçeği), **1**, 15.05.2013, otlak alan, EDTU 13677! 03.05.2013 ağaçlık altı, EDTU 13678! **2**, 16.05.2002, EDTU 8468!

C. rapunculus L. var. *rapunculus* (Frenk salatası), **2**, 15.05.2002, açık otlu alanlar, EDTU 8469!

***Legousia pentagonia* (L.) Thellung (Kadın aynası), 1**, 30.04.2013, otlak alan, EDTU 13679! D. Akd.

CANNABACEAE (Kenevirgiller)

***Cannabis sativa* L. (Kenevir), 1**, 08.07.2013, otlak alan, EDTU 13680!

Humulus lupulus L. (Şerbetçi otu), **1**, 29.08.1995, nemli yerlerde ve dere kenarlarında ağaçlık alanlarda sarılıcı, EDTU 3251!

CAPRIFOLIACEAE (Hanımeligiller)

Cephalaria transsylvanica (L.) Schrader (Tarla pelemiri), **1**, 30.07.2013, otlak alan, EDTU 13684!

Lonicera etrusca var. *etrusca* Santi. (Dokuzdon), **1, 2,** 10.04.2014, ağaçlık ve çalılık alanlarda sarılıcı.

L. japonica* Thunb. (Sarılıcı hanımeli), **7, 10.04.2014, kültür, süs bitkisi.

L. fragrantissima* Lindl.& Paxton (Hanımparmağı), **7, 10.04.2014, kültür, süs bitkisi.

***Scabiosa columbaria* L. subsp. *columbaria* var. *columbaria* (Uyuzotu), 1**, 25.06.2013, otlak alan, EDTU 13685!

Symphoricarpos albus* (L.) S.F.Blake (Beyaz inciçalısı), **7, 10.04.2014, kültür, süs bitkisi

***Valerianella carinata* Lois. (Sandal kuzu gevreği), 1**, 19.04.2013, yol kenarı, EDTU 13681! 28.04.2013,

otlak alan, EDTU 13683! **5**, 12.04.2013, ağaçlık altı, EDTU 13682!

***V. coronata* (L.) DC. (Taçlı kuzu gevreği), 1**, 19.04.2013, yol kenarı, EDTU 13686!

CARYOPHYLLACEAE (Karanfilgiller)

***Arenaria serpyllifolia* L. subsp. *leptoclados* (Rchb.) Nyman (Tarla kum otu), 1**, 30.04.2013, yol kenarı, EDTU 13714!

Cerastium dubium (Bastard) O. Schwarz (Mızrak boynuz otu), **1**, 19.04.2013, otlak alan, EDTU 13688! 02.04.2014, ağaçlandırılan alan, EDTU 13692! 13693!

C. glomeratum Thuill. (Boynuz otu), **1**, 28.04.2013, tarla sınırı, EDTU 13717! 01.04.2013, otlak alan, EDTU 13718! 05.04.2013, dere kenarı, EDTU 13722!

C. gracile Duf. (Küçük boynuz otu), **1**, 30.04.2013, otlak alan, EDTU 13719!

Dianthus armeria L. subsp. *armeriastrum* (Wolfner) Velen. (Edirne karanfili); (**EN); 1**, 15.05.2013, otlak alan, EDTU 13723!

D. corymbosus Sibth. & Sm. (Dallı karanfil), **1**, 23.05.1985, EDTU 111! 06.07.1986, EDTU 515!

D. leptopetalus Willd. (Gece karanfili), **1**, 25.06.2013, otlak alan, EDTU 13709! 31.08.2013, otlak alan, EDTU 13720!

Gypsophila muralis L. (Kır çöveni), **3**, 08.07.2013, otlak alan, EDTU 13687! Av.-Sib.

Holosteum umbellatum L. var. *umbellatum* (Şeytan küpesi), **1**, 02.04.2013, otlak alan, EDTU 13712! 07.04.2013, otlak alan, EDTU 13713!

Moenchia mantica (L.) Bertl. subsp. *mantica* (Dördüz otu), **5**, 07.04.2013, çamlık altı, EDTU 13691! **6**, 01.04.2013, çamlık altı, EDTU 13690!

Petrorhagia dubia (Raf.) G. Lopez & Romo (Zar karanfil), **6**, 10.06.2013, ağaçlık altı, EDTU 13711! 01.04.2013, ağaçlık altı, EDTU 13721!

P. prolifera (L.) Ball. et Heywood (Çeri karanfili), **6**, 10.06.2013, ağaçlık altı, EDTU 13710!

***Saponaria officinalis* L. (Sabun otu), 2**, 13.08.2013, ağaçlık altı,13716! 12.07.1995, EDTU 5971!

Silene cretica L. (Ada nakılı), **1**, 03.04.2013, otlak alan, EDTU 13724! Akd.

S. dichotoma Ehrh. subsp. *dichotoma* (Çatal nakıl), **1**, 21.05.1988, EDTU 2504! 22.05.1988, EDTU 2063!

S. italica (L.) Pers. subsp. *italica* (Yuğuş yüreği), **1**, 07.07.1988, EDTU 2301! **6**, 10.06.2013, otlak alan, EDTU 13715! Akd.

S. subconica Friv. (Mahruti nakıl), **1**, 30.04.1988, açık otlu alanlar, EDTU 2494!

S. tenuiflora Guss. (İnce nakıl), **1**, 22.05.1988, açık otlu alanlar, EDTU 2064! 21.05.1988, EDTU 2503! Akd.

***Stellaria graminea* L. (Cücü barsağı), 3,** 02.04.2014, ekili alan, EDTU 13689!

***S. media* (L.) Vill. (Kuş otu), 1**, 13.04.2013, otlak alan, EDTU 13695! 07.04.2013, otlak alan, EDTU 13696! 02.04.2013, otlak alan, EDTU 13697! **5**, 07.04.2013, çamlık altı, EDTU 13694!

S. pallida (Dumort.) Piré (Kuşmak), **1**, 01.04.2013, tarla sınırı, EDTU 13699! otlak alan, EDTU 13702! 13703! 05.04.2013, otlak alan, EDTU 13700! 13.04.2013, otlak alan, EDTU 13701! 07.04.2013, otlak alan, EDTU 13704! 02.04.2013, otlak alan, EDTU 13705! 13706! 13708! **5**, 12.04.2013, ağaçlık altı, EDTU 13707!

Vaccaria hispanica (Mill.) Rauschert (Ekin ebesi), **1**, 23.05.1985, açık otlu alanlar, EDTU 131!

CELASTRACEAE (İğağacıgiller)

Euonymus japonicus* Thunb. (Japon papaz külahı, Japon taflanı), **7, 10.04.2014, kültür, süs bitkisi.

CONVOLVULACEAE (Sarmaşıkgiller)

Convolvulus arvensis L. (Tarla sarmaşığı), **1**, 15.05.2013, otlak alan, EDTU 13725! 30.07.2013, otlak alan, EDTU 13726! Kozmopolit.

C. cantabrica L. (Çadır çiçeği), **6**, 10.06.2013, çamlık altı, EDTU 13727! Akd.

C. betonicifolius Miller subsp. *betonicifolius* (Büyük yayılgan), **3**, 23.05.1985, EDTU 122! Akd.-İr.-Tur.

C. elegantissimus Mill. (Mahmude otu), **6**, 25.05.1986, çamlık altı, EDTU 395! Akd.

CORNACEAE (Kızılcıkgiller)

Cornus sanguinea L. subsp. *sanguinea* (Kiren), **1, 2,** 10.04.2014, dere kenarlarında doğal, peyzaj alanlarında süs bitkisi.

CYPERACEAE (Hasırotugiller)

Carex muricata L. (Çengel sazı), **1**, 29.04.2014, ağaçlık altı, EDTU 13728! Av.-Sib.

Cyperus rotundus L. (Topalak), **3**, 08.07.2013, otlak alan, EDTU 13729!

ELEAGNACEAE (İğdegiller)

Elaeagnus angustifolia* L. var. *angustifolia* (İğde), **7, 10.04.2014, kültür, süs ve meyve bitkisi.

EUPHORBIACEAE (Sütleğengiller)

Euphorbia aleppica L. (Haşul), **5**, 08.07.2013, ağaçlık altı, EDTU 13734!

E. helioscopia L. (Feriban otu), **5**, 12.04.2013, ağaçlık altı, EDTU 13731! **7**, 24.02.2013, otlak alan, EDTU 13732!

E. pannonica Host. (Macar sütleğeni), **1**, 15.05.2013, otlak alan, EDTU 13733! Av.-Sib.

E. seguieriana Necker subsp. *seguieriana* (Tasmaotu), **1**, 21.05.1986, açık otlu alanlar, EDTU 393! 25.05.1985, EDTU 118! 06.06.1987, EDTU 1102! Av.-Sib.

FABACEAE (Baklagiller)

Astragalus hamosus L. (Koç boynuzu), **1**, 03.05.2013, otlak alan, EDTU 13745! 15.05.2013, otlak alan, EDTU 13775! 25.06.2013, otlak alan, EDTU 13776! 28.05.1993, EDTU 5806!

Cercis siliquastrum* L. subsp. *siliquastrum* (Erguvan), **1, 2, 10.04.2014, kültür, süs bitkisi.

Galega officinalis L. (Keçi sedefi), **1**, 25.06.2013, otlak alan, EDTU 13777! Av.-Sib.

Gleditsia triacanthos* L. (Gıladiçya), **1, 2, 10.04.2014, kültür, süs bitkisi, yer yer istilacı bitki.

Laburnum anagyroides* Medik. (Sarı salkım), **7, 10.04.2014, kültür, süs bitkisi.

Lathyrus annuus L. (Dağ dırılcası), **1**, 03.05.2013, otlak alan, EDTU 13751! Akd.

L. cicera L. (Colban), **1**, 03.05.2013, otlak alan, EDTU 13747! 02.04.2013, yol kenarı, EDTU 13748! 11.05.1987, EDTU 692! Akd.

L. sphaericus Retz. (Çam burçağı), **7**, 06.06.1987, yol kenarı, EDTU 1111! D. Akd.

Lotus angustissimus L. (Kurtlu ot), **6**, 10.06.2013, çamlık altı, EDTU 13773!

L. corniculatus L. var. *tenuifolius* L. (Gazal boynuzu), **1**, 10.06.2013, ağaçlık altı, EDTU 13772!

Medicago arabica (L.) Huds. (Benli yonca), **1**, 03.05.2013, otlak alan, EDTU 13774!

***M. lupulina* L. (Bitçik otu), 6**, 10.06.2013, çamlık altı, EDTU 13770!

***M. minima* (L.) Bart. var. *minima* (Gurnik), 1**, 03.05.2013, otlak alan, EDTU 13746!

M. orbicularis (L.) Bart. (Paralık), **1**, 01.04.2013, otlak alan, EDTU 13788! **6**, 01.04.2013, otlak alan, EDTU 13737! 07.06.2013, yol kenarı, EDTU 13787!

M. sativa L. subsp. *sativa* (Kara yonca), **6**, 10.06.2013, çamlık altı, EDTU 13771!

Melilotus albus Desr. (Ak taş yoncası), **2**, 03.05.2013, otlak alan, EDTU 13750!

M. officinalis (L.) Desr. (Kokulu yonca), **1**, 23.05.1989, EDTU 3432!

Ononis spinosa L. subsp. *antiquorum* (L.) Briq. (Acram), **1**, 31.08.2013, otlak alan, EDTU 13762! **6**, 10.06.2013, ağaçlık altı, EDTU 13763! Akd.

Robinia pseudoacacia* L. (Yalancı akasya), **1, 2, 10.04.2014, kültür, süs bitkisi, bazı alanlarda istilacı.

Trifolium angustifolium L. subsp. *angustifolium* (Nefel), **2**, 03.05.2013, otlak alan, EDTU 13741! 25.05.1986, EDTU 399! 2074!

T. campestre Schreb. (Üçgül), **1**, 03.05.2013, otlak alan, EDTU 13743! 23.05.1985, EDTU 115! 31.05.1988, EDTU 2077!

T. constantinopolitanum Ser. (Üç kulak otu), **1**, 18.05.2002, EDTU 8333!

T. echinatum Bieb. (Kirpi üç gülü), **1**, 24.05.2013, otlak alan, EDTU 13783! D. Akd.

T. hirtum All. (Deli yonca), **1**, 15.05.2013, otlak alan, EDTU 13786! 31.05.1988, EDTU 2073! Akd.

T. hybridum L. var. *hybridum* (Melez üç gül), **1**, 25.06.2013, otlak alan, EDTU 13780!

***T. leucanthum* Bieb. (Yapışık üç gül), 1**, 15.05.2013, otlak alan, EDTU 13735!

T. michelianum Savi var. *balansae* (Boiss.) Azn. (Uzun diş), **1**, 19.04.2013, otlak alan, EDTU 13778! **VU**

T. nigrescens Viv. subsp. *nigrescens*, **1**, 15.05.2013, otlak alan, EDTU 13785!

T. pratense L. var. *pratense* (Yanık üç gül), **1**, 10.07.2001, EDTU 8027!

T. purpureum Lois. var. *purpureum* (Mor üç gül), **2**, 28.05.2013, otlak alan, EDTU 13764! 06.06.1987, EDTU 1108! D. Akd.

T. repens L. var. *repens* (Aküçgül), **2**, 10.06.2013, otlak alan, EDTU 13781!

T. striatum L. (Çizik yonca), **1**, 15.05.2013, otlak alan, EDTU 13784! 31.05.1988, EDTU 2076!

Vicia cracca L. subsp. *stenophylla* Vel. (Meşefiği), **2**, 03.05.2013, otlak alan, EDTU 13740!

V. hirsuta (L.) F. S. Gray (Bozfiğ), **1**, 19.04.2013, otlak alan, EDTU 13759! 28.05.1993, açık arazi, EDTU 5870! **7**, 19.04.2013, yol kenarı, EDTU 13760!

V. hybrida L. (Melez bakla), **2**, 03.05.2013, tarla kenarı, EDTU 13749! 22.05.1993, EDTU 5873! 5874! 05.05.1993, EDTU 6172!

V. lathyroides L. (Çam fiği), **1**, 05.04.2013, otlak alan, EDTU 13761! **5**, 12.04.2013, ağaçlık altı, EDTU 13738! **7**, 21.04.2013, otlak alan, EDTU 13739!

V. melanops Sibth. & Sm. (Sülük fiği), **1**, 05.05.1994, açık arazi, EDTU 6185!

V. pannonica Crantz var. *pannonica* (Macar fiği), **1**, 15.05.2013, otlak alan, EDTU 13757! 13758!

V. pannonica Crantz var. *purpurascens* (DC.) Ser. (Macar fiği), **1**, 03.05.2013, otlak alan, EDTU 13742! **5**, 19.04.2013, çamlık altı, EDTU 13755! 22.05.1993, EDTU 6141! 6165! 6163! 28.05.1993, tarla kenarı, EDTU 6147! 6151!

V. sativa L. subsp. *incisa* (Bieb.) Arc. var. *incisa (*Ekin fiği); (**DD), 1**, 03.05.2013, otlak alan, EDTU 13765! 22.05.1993, nemli ağaç altı, EDTU 6851! 16.04.1994, dere kenarı, EDTU 6852! 10.05.1997, EDTU 6853! 01.05.1997, EDTU 6854!

V. sativa L. subsp. *nigra* (L.) Ehr. var. *nigra* (Eşek gürülü), **7**, 19.04.2013, otlak alan, EDTU 13736!

01.04.2013, yol kenarı, EDTU 13754! 22.05.1993, EDTU 5881!

V. sativa L. subsp. *nigra* (L.) Ehr. var. *segetalis* (Thuill.) Ser. ex DC., (Eşek gürülü), **7**, 19.04.2013, yol kenarı, EDTU 13752!

V. sativa L. subsp. *sativa* (Fiğ), **1**, 15.05.2013, otlak alan, EDTU 13756! 03.05.2013, otlak alan, EDTU 13766! **5**, 01.04.2013, otlak alan, EDTU 13753!

V. villosa Roth subsp. *dasycarpa* (Ten.) Cav. (Dağ efereği), **1**, 25.06.2013, otlak alan EDTU 13767! **7**, 08.07.2013, çamlık altı, EDTU 13768! 28.05.1993, EDTU 6177!

V. villosa Roth subsp. *villosa* (Tüylü fiğ), **1**, 19.04.2013, otlak alan, EDTU 13769! 23.05.1985, EDTU 116! 25.05.1986, EDTU 397! 30.05.1987, yol kenarı, EDTU 666! 28.05.1993, tarla kenarı, EDTU 6193!

FAGACEAE (Kayıngiller)

Quercus petraea* (Mattuschka) Liebl. subsp. *petraea* (Sapsız meşe), **7, 10.04.2014, kültür, süs bitkisi.

Q. pubescens* Willd. subsp. *pubescens* (Tüylü meşe), **7, 10.04.2014, kültür, süs bitkisi.

Q. robur L. subsp. *robur* (Saplı meşe), **1, 2,** 10.04.2014, dere kenarlarında ağaçlık alanlarda. Av.-Sib.

GERANIACEAE (Turnagagasıgiller)

Erodium cicutarium (L.) L'Herit subsp. *cicutarium* (İğnelik), **1**, 02.04.2013, otlak alan, EDTU 13793! 24.02.2013, otlak alan, EDTU 13794! **4**, 13.04.2013, tarla sınırı, EDTU 13792! **6**, 10.06.2013, çamlık altı, EDTU 13796!

Geranium dissectum L. (Dilimli ıtır), **1**, 19.04.2013, dere kenarı, EDTU 13790! **6**, 01.04.2013, ağaçlık altı, EDTU 13789!

G. molle L. (Yumuşak ıtır), **1**, 19.04.2013, otlak alan, EDTU 19795! dere kenarı, EDTU 13791!

HYDRANGEACEAE

Philadelphus coronarius* L. (Filbahri), **7, 10.04.2014, kültür, süs bitkisi.

HYPERICACEAE (Kantarongiller)

Hypericum perforatum L. (Kantaron), **1**, 24.05.2013, ağaçlık altı, EDTU 13797! 23.05.1985, EDTU 114! 06.06.1987, EDTU 1101! 08.06.1988, EDTU 2090!

IRIDACEAE (Süsengüller)

Crocus pallasii Goldb. subsp. *pallasii* (Güz çimi*)*, **4**, 20.11.1990, çalılık ve açık otlu alanlarda, EDTU 5748!

Iris pseudacorus L. (Batak süseni), **1**, 29.04.2014, sulak alan, EDTU 13798!

JUGLANDACEAE (Cevizgiller)

Juglans regia L. (Ceviz), **1, 2,** 10.04.2014, doğal olarak dere kenarlarında, ayrıca peyzaj alanlarında kültür bitkisi olarak dikilmiş.

LAMIACEAE (Ballıbabagiller)

***Glechoma hederacea* L. (Yer nanesi), 2**, 29.04.2014, otlak alan, EDTU 13810! Av.-Sib.

Lamium amplexicaule L. (Baltutan), **4**, 13.04.2013, tarla sınırı, EDTU 13804! **5**, 12.04.2013, ağaçlık altı, EDTU 13803!

L. purpureum L. var. *purpureum* (Ballıbaba), **1**, 07.04.2013, otlak alan, EDTU 13805! 02.04.2013, tarla sınırı, EDTU 13807! 13809! 24.02.2013, EDTU 13808! **7**, 08.04.2013, otlak alan, EDTU 13806! Av.-Sib.

Lavandula angustifolia* Miller subsp. *angustifolia* (Lavanta), **7, 10.04.2014, kültür, süs bitkisi.

Lycopus europaeus L. (Kurtayağı), **1**, 21.09.2013, sulak alan, EDTU 13799!

Mentha pulegium L. (Yarpuz), **7**, 30.07.2013, otlak alan, EDTU 13800!

Prunella laciniata (L.) L. (Bodur fesleğen), **6**, 10.06.2013, çamlık altı, EDTU 13801! Av.-Sib.

***P. vulgaris* L. (Gelincikleme otu),** **7**, 10.06.2013, otlak alan, EDTU 13802! Av.-Sib.

Rosmarinus officinalis* L. (Biberiye), **7, 10.04.2014, kültür, süs bitkisi.

Stachys cretica L. subsp. *bulgarica* Rech. fil. (Kızıl deliçay), 23.05.1985, EDTU 123! D. Akd.

S. thirkei K. Koch (Kestere), 25.05.1986, EDTU 400!

Thymus longicaulis C.Presl subsp. *longicaulis* (Aş kekiği), **1**, 23.05.1985, dere kenarı, EDTU 124!

LILIACEAE (Zambakgiller)

Gagea peduncularis (J. &C. Presl) Pascher (Karga sarımsağı), **1**, 07.04.2013, otlak alan, EDTU 13811! 13812! 13.04.2013, otlak alan, EDTU 13813! 02.04.2013, otlak alan, EDTU 13814! **5**, 12.04.2013, ağaçlık altı, EDTU 13815! Akd.

G. pratensis (Pers.) Dumort. (Çayır yıldızı), **3**, 21.03.1988, EDTU 1862! Av.-Sib.

LINACEAE (Ketengiller)

Linum bienne Mill. (Deli keten), **1**, 25.06.2013, otlak alan, EDTU 13819! 28.05.2013, otlak alan, EDTU 13820! **6**, 10.06.2013, çamlık altı, EDTU 13818! 06.06.1987, EDTU 1116!

L. trigynum L. (Otlak keteni), **1**, 15.05.2013, otlak alan, EDTU 13816! 25.06.2013, otlak alan, EDTU 13817! Akd.

LYTHRACEAE (Aklarotugiller)

Lythrum salicaria L. (Hevhulma), **3**, 08.07.2013, otlak alan, EDTU 13821! Av.-Sib.

L. virgatum L. (Çamur aklarotu), **3**, 08.07.2013, otlak alan, EDTU 13822! Av.-Sib.

Punica granatum* L. (Nar), **7, 10.04.2014, kültür, süs bitkisi.

MALVACEAE (Ebegümecigiller)

Althaea cannabina L. (Gülhannaz), **1**, 30.07.2013, otlak alan, EDTU 13826!

Hibiscus trionum* L. (Kerkede), **7, 10.04.2014, kültür, süs bitkisi.

Malva sylvestris L. (Ebegümeci), **1**, 15.05.2013, otlak alan, EDTU 13823! 10.06.2013, ağaçlık altı, 13834! 08.07.2013, yol kenarı, EDTU 13825!

Malvella sherardiana (L.) Jaub. & Spach (Pubazi), **2**, 31.08.2013, ağaçlık altı, EDTU 13827!

Tilia tomentosa Moench (Gümüşi ıhlamur), **7**, 10.04.2014, kültür, süs bitkisi.

MELIACEAE

Melia azedarach* L. (Tesbih ağacı), **7, 10.04.2014, kültür, süs bitkisi.

MORACEAE (Dutgiller)

Ficus carica subsp. *carica* L. (İncir), **1, 2,** 10.04.2014, açık otlu alanlarda tek veya küme şeklinde, bazı binaların çevresinde istilacı.

Morus alba L. (Ak dut), **1, 2, 7,** 10.04.2014, ağaçlık alanlarda veya açık otlu alanlarda tek veya küme şeklinde, doğallaşmış, bazı binaların çevresinde istilacı, bazı ağaçlandırma alanlarında kültür bitkisi olarak dikilmiş.

M. rubra L. (Mor dut), **1, 2, 7,** 10.04.2014, ağaçlık alanlarda veya açık otlu alanlarda tek veya küme şeklinde, doğallaşmış.

OLEACEAE (Zeytingiller)

Forsythia x intermedia* Zab. (Altın çanak), **7, 10.04.2014, kültür, süs bitkisi.

Fraxinus angustifolia Vahl. (Sivri meyveli dişbudak), **1, 2, 7,** 10.04.2014, ağaçlık sulak alanlarda, ayrıca süs bitkisi olarak yol kenarlarında.

F. ornus* L. subsp. *ornus* (Çiçekli dişbudak), **7, 10.04.2014, kültür, süs bitkisi. Av.-Sib.

Jasminum fruticans L. (Boruk), **1**, 15.05.2013, ağaçlık altı, EDTU 13828! Akd.

Syringa vulgaris* Mill. (Leylak), **1, 2, 7, 10.04.2014, kültür, süs bitkisi.

Ligustrum vulgare L. (Kurtbağrı), **1, 2,** 10.04.2014, doğal olarak ağaçlı alanlarda, ayrıca süs birkis olarak çit şeklinde. Av.-Sib.

ONAGRACEAE (Yakıotugiller)

Epilobium hirsutum L. (Hasan hüseyin çiçeği), **1**, 08.07.2013, otlak alan, EDTU 13830! **2**, 08.07.2013, otlak alan, EDTU 13831! **3**, 08.07.2013, çamlık altı, EDTU 13829!

***E. tetragonum* L. subsp. *tetragonum* (Ezber yakısı), 1**, 08.07.2013, otlak alan, EDTU 13832!

ORCHIDACEAE (Salepgiller)

Anacamptis morio (L.) R.M.Bateman, Pridgeon & M.W. Chase subsp. *morio* (Gelincik salebi), **2**, 04.05.1995, otlak alan, EDTU 6066!

Anacamptis papilionacea (L.) R.M. Bateman, Pridgeon & M.W. Chase subsp. *papilionacea* (Dil çıkık) **(LC)**, **1**, 15.05.2013, otlak alan, EDTU 13834! **2**, 09.05.1995, EDTU 6123! Akd.

Ophrys mammosa Desf. (Kedi kulağı), **1**, 19.04.2013, ağaçlık altı, EDTU 13833! **2**, 01.05.1995, meralık, EDTU 6048! **4**, 26.04.1996, EDTU 5906! Akd.

OROBANCHACEAE (Canavarotugiller)

Odontites vulgaris Moench (Davun otu), 15.09.1993, EDTU 5868! Av.-Sib.

Parentucellia latifolia (L.) Caruel subsp. *latifolia* (Üç dil otu), **1**, 03.05.2013, otlak alan, EDTU 13835! Akd.

OXALIDACEAE (Ekşiyoncagiller)

Oxalis corniculata L. (Sarı ekşi yonca), **7**, 10.06.2013, otlak alan, EDTU 13836! Kozmopolit.

PAPAVERACEAE (Haşhaşgiller)

Fumaria densiflora DC. (Ergen döşeği), **5**, 12.04.2013, ağaçlık altı, EDTU 13846!

F. officinalis L. (Şahdere), **1**, 30.04.2013, otlak alan, EDTU 13844! 13.04.2013, tarla sınırı, EDTU 13845! 28.04.2013, tarla sınırı, EDTU 13847! 01.04.2013, otlak alan, EDTU 13848!

Hypecoum procumbens L. subsp. *procumbens* (Yavruağzı)(**DD)**, **1**, 13.04.2013, otlak alan, EDTU 13738! 01.04.2013, tarla sınırı, EDTU 13740! otlak alan, EDTU 13741! 23.05.1985, EDTU 117! Akd.

Papaver argemone L. subsp. *argamone* (Kum haşhaşı), 08.05.1989, EDTU 3689!

***P. dubium* L. (Köpek yağı), 1**, 10.06.2013, otlak alan, EDTU 13842! 29.04.2014, otlak alan, EDTU 13843!

P. rhoeas L. (Gelincik), **1**, 15.05.2013, otlak alan, EDTU 13837!

PAULOWNIACEAE

Paulownia tomentosa* (Thunb.) Mill. (Pavlonya), **7, 10.04.2014, kültür, süs bitkisi.

PLANTAGINACEAE (Sinirotugiller)

Cymbalaria muralis G. Gaertn., B. Mey. & Scherb. subsp. *muralis* (Ak Nakkaş otu), **1**, 29.04.2014, sera, EDTU 13869! 23.05.1990, 6305!

Linaria genistifolia (L.) Mill. subsp. *artvinense* Davis (Artvin nevruzotu) (**END?)**, **2**, 10.06.2013, ağaçlık altı, , EDTU 13868! Av.-Sib.

***L. genistifolia* (L.) Mill. subsp. *genistifolia* (Som nevruzotu), 1**, 31.08.2013, otlak alan, EDTU 13870! 25.06.2013, otlak alan, EDTU 13871! Av.-Sib.

L. pelisseriana (L.) Mill. (Mor nevruzotu), **1**, 03.05.2013, otlak alan, EDTU 13851! Akd.

Plantago lanceolata L. (Damarlıca), **1**, 15.05.2013, otlak alan, EDTU 13849! 06.06.1987, EDTU 1112!

P. major L. subsp. *intermedia* (Gilib.) Lange (Yedi damar otu), **1**, 30.07.2013, otlak alan, EDTU 13850!

Veronica arvensis L. (Ekin mavişi), **1**, 19.04.2013, otlak alan, EDTU 13864! 05.04.2013, otlak alan, EDTU 13865! **7**, 19.04.2013, otlak alan, EDTU 13863! Av.-Sib.

V. hederifolia L. (Bahar mavisi), **1**, 02.04.2013, ağaçlık altı, EDTU 13853! 13859! 08.04.2013, tarla sınırı, EDTU 13860! 04.02.2013, tarla sınırı, EDTU 13862!

V. persica Poiret (Cırcamuk), **1**, 02.04.2013, tarla sınırı, EDTU 13856! **3**, 02.04.2013, çamlık altı, EDTU 13867! 7, 07.04.2013, otlak alan, EDTU 13866!

V. polita Fries (Maviş ot), **1**, 02.04.2013, ağaçlık altı, EDTU 13852! 02.04.2013, otlak alan, EDTU 13858! 01.04.2013, otlak alan, EDTU 13861!

V. triloba (Opiz) Kerner (Üç maviş), **1**, 02.04.2013, tarla sınırı, EDTU 13857! **2**, 02.04.2013, tarla sınırı, EDTU 13855!

V. triphyllos L. (Bahçe mavişi), **1**, 02.04.2013, ağaçlık altı, EDTU 13854!

PLATANACEAE (Çınargiller)

Platanus orientalis* L. (Çınar), **7, 10.04.2014, kültür, süs bitkisi.

PLUMBAGINACEAE (Kardikenigiller)

***Plumbago europaea* L. (Karakına), 1**, 13.08.2013, otlak alan, EDTU 13873! **2**, 21.09.2013, otlak alan, EDTU 13872! Av.-Sib.

POACEAE (Buğdaygiller)

***Aegilops triuncialis* L. subsp. *triuncialis* (Üç kılçık), 1**, 15.05.2013, otlak alan, EDTU 13877!

***Aira elegantissima* Schur subsp. *elegantissima* (Tül çiçeği), 2**, 03.05.2013, yol kenarı, EDTU 13881! Akd.

Avena barbata Pott ex Link subsp. *barbata* (Narin yulaf), **1**, 15.05.2013, otlak alan, EDTU 13883! Akd.

***Bothriochloa ischaemum* (L.) Keng. (Sakal otu), 6**, 08.07.2013, otlak alan, EDTU 13893!

Bromus arvensis L. (Tarla bromu), **1**, 15.05.2013, otlak alan, EDTU 13876!

***B. lanceolatus* Roth (Kılıç bromu), 1**, 28.05.2013, ekili alan, EDTU 13888!

***B. rigidus* Roth (Sert brom), 1**, 15.05.2013, otlak alan, EDTU 13882!

Cynodon dactylon (L.) Pers. var. *villosus* Regel (Köpek dişi), **6**, 10.06.2013, otlak alan, EDTU 13892!

Cynosurus echinatus L. (Top tarak otu), **6**, 24.05.2013, otlak alan, EDTU 13897! 15.05.2013, otlak alan, EDTU 13898! Akd.

Dactylis glomerata L. var. *hispanica* (Roth) Nyman (Kıllı domuza yrığı), **1**, 28.05.2013, ekili alan, EDTU 13886! **2**, 03.05.2013, yol kenarı, EDTU 13880!

Eragrostis minor Host (Bodur yulaf), **2**, 13.08.2013, otlak alan, EDTU 13884!

Glyceria notata Chevall. (Kıvrık tatlı çim), **1**, 24.05.2013, otlak alan, EDTU 13887!

***Hordeum murinum* Hudson subsp. *leporinum* (Link) Arc. (Kılçık arpa), 1**, 15.05.2013, otlak alan, EDTU 13878! İr.-Tur.

H. bulbosum L. (Boncuk arpa), **1**, 15.05.2013, otlak alan, EDTU 13894!

***Lolium rigidum* Gaudin var. *rottbollioides* Heldr. Ex Boiss (Sert çim), 1**, 15.05.2013, otlak alan, EDTU 13875! 28.05.2013, ekili alan, EDTU 13885! D. Akd.

Phleum bertolini DC. (Kumul itkuyruğu), **1**, 15.05.2013, otlak alan, EDTU 13874!

***P. phleoides* (L.) Karsten (Bayır itkuyruğu), 1**, 28.05.2013, ekili alan, EDTU 13900! Av.-Sib.

Phragmites australis (Cav.) Trin. ex Steud. (Kamış), **1**, 30.07.2013, sulak alan, EDTU 13890! Av.-Sib.

Poa bulbosa L. (Yumrulu salkım), **1**, 19.04.2013, yol kenarı, EDTU 13879! 13889!

***P. trivialis* L. (Kaba salkım otu), 1**, 19.04.2013, yol kenarı, EDTU 13896! 15.05.2013, otlak alan, EDTU 13895!

Polypogon monspeliensis (L.) Desf. (Hıtır), **1**, 25.06.2013, otlak alan, EDTU 13889!

Setaria glauca (L.) P. Beauv. (Sıçan saçı), **1**, 09.01.2001, otlak alan, EDTU 9617!

Sorghum halepense (L.) Pers. var. *halepense* (Ekin süpürgesi), **2**, 10.06.2013, otlak alan, EDTU 13891!

POLYGONACEAE (Madımakgiller)

Polygonum aviculare L. (Köy otu), **1**, 10.06.2013, otlak alan, EDTU 13907! **7**, 15.05.2013, kaldırım kenarı, EDTU 13903!

P. patulum Bieb. subsp. *pulchellum* (Lois.) Leblebici (Soğan bağı), **1**, 30.07.2013, otlak alan, EDTU 13906!

P. persicaria L. (Söğüt otu), **7**, 25.06.2013, otlak alan, EDTU 13901!

Rumex acetosella L. (Kuzukulağı), **1**, 15.05.2013, otlak alan, EDTU 13902! 08.06.1988, EDTU 2081!

R. cristatus DC. (Lapuşa), **1**, 28.05.2013, ekili alan, EDTU 13908!

R. pulcher L. (Ekşilik), **1**, 24.05.2013, otlak alan, EDTU 13905!

R. tuberosus L. subsp. *tuberosus* (Kuzu kıkırdağı), **1**, 19.04.2013, otlak alan, EDTU 13904!

PORTULACACEAE (Semizotugiller)

Portulaca oleracea L. (Semizotu), **1**, 30.07.2013, otlak alan, EDTU 13909!

PRIMULACEAE (Çuhaçiçeğigiller)

Anagallis arvensis L. var. *arvensis* (Fare kulağı), **7**, 15.05.2013, kaldırım kenarı, EDTU 13910!

***A. foemina* Mill. (Bağırsak otu), 1**, 15.05.2013, otlak alan, EDTU 13911! Akd.

RANUNCULACEAE (Düğünçiçeğigiller)

Clematis vitalba L. (Akasma), **1**, 07.03.1994, ağaçlık alanlarda sarılıcı, EDTU 2154!

C. viticella L. (Yakmuk), **2**, 30.07.2013, yol kenarı, çalılar üzerinde sarılıcı, EDTU 13924!

Consolida regalis S.F. Gray subsp. *paniculata* (Host) Soó var. *paniculata* (Horoz kuyruğu), **1**, 15.05.2013, otlak alan, EDTU 13917! 21.09.2013, otlak alan, EDTU 13918!

C. orientalis (Gay) Schröd. (Mor çiçek), **1**, 15.05.2013, otlak alan, EDTU 13919! 31.05.1988, EDTU 2087!

***Nigella elata* Boiss. (Deli çörekotu), 5**, 08.07.2013, çamlık altı, EDTU 13926!

Ranunculus arvensis L. (Mustafa çiçeği), **1**, 19.04.2013, otlak alan, EDTU 13921! **7**, 19.04.2013, otlak alan, EDTU 13920!

R. ficaria L. subsp. *calthifolius* (Reichb.) Arc. (Çöp salebi), **5**, 12.04.2013, ağaçlık altı, EDTU 13916!

R. ficaria L. subsp. *ficariiformis* Rouy & Fouc. (Arpacık salebi), **1**, 13.04.2013, ağaçlık altı, EDTU 13912, 02.04.2013, otlak alan, EDTU 13913! 13914! 24.02.2013, otlak alan, EDTU 13915!

R. neapolitanus Ten. (Çiçeğezer), **1**, 19.04.2013, otlak alan, EDTU 13922! ağaçlık altı, EDTU 13923!

***R. velutinus* Ten. (Kadife yağ otu), 1**, 19.04.2013, ağaçlık altı, EDTU 13927! Akd.

***Thalictrum lucidum* L. (Çayırsedefi), 1**, 10.06.2013, otlak alan, EDTU 13925!

RHAMNACEAE (Cehrigiller)

Paliurus spina-christii P. Mill. (Karaçalı), **1, 2,** 10.04.2014, ağaçlık alanların kenarında küme şeklinde.

ROSACEAE (Gülgiller)

Amygdalus communis L. (Badem), **1, 2, 7,** 10.04.2014, ağaçlık alanlarda veya tek tek açık otlu alanlarda.

Chaenomeles speciosa* (Sweet) Nak. (Bahar dalı), **7, 10.04.2014, kültür, süs bitkisi.

Cotoneaster franchetii* Bois. (Dağ muşmulası), **7, 10.04.2014, kültür, süs bitkisi.

Crataegus monogyna Jacq. var. *monogyna* (Yemişen), **1, 2,** 10.04.2014, ağaçlık alanlarda büyük kümeler şeklinde veya açık otlu alanlarda tek tek kümeler şeklinde.

C. oxicantha* L. "Flore Coccinea Pleno" (Kırmızı çiçekli geyik dikeni), **1, 10.04.2014, kültür, süs bitkisi.

Cydonia oblonga Mill. (Ayva), **1, 2,** 10.04.2014.

Geum urbanum L. (Meryam otu), **1**, 03.05.2013, otlak alan, EDTU 13930! Av.-Sib.

Kerria japonica* (L.) DC. (Kanarya gülü), **7, 10.04.2014, kültür, süs bitkisi.

Malus sylvestris* Miller (Elma), **1, 2, 7, 10.04.2014, kültür, meyve bitkisi.

M. floribunda* Siebold ex Van Houtte (Süs elması), **7, 10.04.2014, kültür, süs bitkisi.

Mespilus germanica* L. (Muşmula), **1, 2, 10.04.2014, kültür, süs ve meyve bitkisi, Av.-Sib.

Potentilla argentea L. (Gümüş parmak otu), **1**, 03.05.2013, otlak alan, EDTU 13928! **5**, 08.07.2013, çamlık altı, EDTU 13932!

***P. detommasii* Ten. (Şehir parmak otu), 1**, 24.05.2013, otlak alan, EDTU 13931!

***P. inclinata* Vill. (Eğri parmak otu), 1**, 03.05.2013, otlak alan, EDTU 13929!

P. recta L. (Su parmak otu), **1**, 15.05.2013, otlak alan, EDTU 13933!

Prunus spinosa L. subsp. *dasyphylla* (Schur) Domin (Çakal eriği), **1, 2,** 10.04.2014, ağaçlık alanlarda veya açık otlu alanlarda çok yoğun kümeler şeklinde. Av.-Sib.

P. x domestica L. (Erik), **1, 2,** 10.04.2014, ağaçlık alanlarda veya tek tek açık otlu alanlarda, kültür olarak yeşil alanlarda meyve bitkisi.

P. mahaleb L. (Mahleb), **1, 2,** 10.04.2014, ağaçlık alanlarda veya tek tek açık otlu alanlarda.

P. avium* (L.) L. (Kiraz), **7, 10.04.2014, kültür, meyve bitkisi.

P. persica* (L.) Stokes (Şeftali), **7, 10.04.2014, kültür, meyve bitkisi.

P. serrulata* Lindl. "Kanzan" (Süs kirazı), **7, 10.04.2014, kültür, süs bitkisi.

P. cerasus* L. (Vişne), **1, 2, 7, 10.04.2014, kültür, meyve bitkisi.

Pyracantha coccinea* M. Roem. (Ateş dikeni), **7, 10.04.2014, kültür, süs bitkisi

Pyrus communis L. (Armut), **1, 2,** 10.04.2014, ağaçlık alanlarda veya tek tek açık otlu alanlarda, ayrıca kültür bitkisi olarak çim alanlarında.

P. elaeagnifolia Pall. subsp. *elaeagnifolia* (Ahlat), **1, 2,** 10.04.2014, ağaçlık alanlarda veya tek tek açık otlu alanlarda.

R. canina L. (Kuşburnu), **1, 2,** 10.04.2014, ağaçlık alanlarda veya açık otlu alanlarda küme şeklinde.

R. x damascena* Mill. (Isparta gülü), **7, 10.04.2014, kültür, süs bitkisi.

***R. gallica* L. (Hokka gülü),1,** 15.05.2013, ağaçlık altı, EDTU 13935!

R. sempervirens* L. (İtburnu), **7, 10.04.2014, kültür, süs bitkisi. Akd.

Rubus sanctus Schreber (Böğürtlen), **1, 2,** 10.04.2014, ağaçlık alanlarda veya açık otlu alanlarda yoğun kümeler şeklinde.

Sanguisorba minor Scop. subsp. *muricata* (Spach) Briq. (Çayır düğmesi), **1**, 15.05.2013, otlak alan, EDTU 13934!

Spiraea japonica* L. (Pembe ispir), **7, 10.04.2014, kültür, süs bitkisi.

RUBIACEAE (Kökboyagiller)

Cruciata laevipes Opiz. (Sarılık otu), **1**, 05.04.2013, çeşme yanı, EDTU 13938! 02.04.2013, çeşme yanı, EDTU 13939! Av.-Sib.

***C. pedemontana* (Bellard.) Ehrend. (Tüylü sarılık otu), 1**, 24.05.2013, ağaçlık altı, EDTU 13941! 19.04.2013, otlak alan, EDTU 13944!

Galium aparine L. (Çobansüzgeci), **1**, 03.05.2013, otlak alan, EDTU 13939! 13937!

G. verum L. subsp. *verum* (Boyalık), **7**, 10.06.2013, otlak alan, EDTU 13942! Av.-Sib.

***Sherardia arvensis* L. (Gökören otu), 1**, 19.04.2013, otlak alan, EDTU 13943! **2**, 02.04.2013, çamlık altı, EDTU 13940! Akd.

SALICACEAE (Söğütgiller)

Populus alba L. var. *alba* (Akkavak),**1, 2,** 10.04.2014, doğal olarak dere yataklarında, ayrıca bina çevrelerinde, yeşil çim alanlarında. Av.-Sib.

P. x *canadensis* Moench (Boz kavak), **1, 2,** 10.04.2014, doğal olarak dere yataklarında, ayrıca bina çevrelerinde, yeşil çim alanlarında.

P. nigra* L. subsp. *nigra* (Karakavak), **1, 2, 7, 10.04.2014, dere kenarında kültür bitkisi. Av.-Sib.

P. tremula L. subsp. *tremula* (Titrek kavak), **1**, 10.04.2014, dere kenarları, kültür bitkisi.

Salix alba L. var. *alba* (Ak söğüt), **1, 2,** 10.04.2014, doğal olarak dere yataklarında, ayrıca bina çevrelerinde, yeşil çim alanlarında. Av.-Sib.

S. babylonica* L. (Salkım söğüt), **7, 10.04.2014, kültür, süs bitkisi.

S. caprea* L. (Sorgun), **7, 10.04.2014, kültür, süs bitkisi. Av.-Sib.

S. matsudana* Koidz. (Tirbişon söğüdü), **7, 10.04.2014, kültür, süs bitkisi.

SANTALACEAE (Güvelekgiller)

Osyris alba L. (Morcak), **1, 2,** 10.04.2014, doğal olarak çalılık alanlarda. Akd.

SAPINDACEAE (Akçaağaçgiller)

Acer tataricum L. subsp. *tataricum* (Tatar akçaağacı), **1, 2,** 10.04.2014, doğal olarak dere yataklarında ve ağaçlık alanlarda. Av.-Sib.

A. negundo* L. (İsfendan), **1, 2, 10.04.2014, kültür, süs bitkisi, bazı alanlarda istilacı.

A. pseudoplatanus* L. (Dağ akçaağacı), **7, 10.04.2014, kültür, süs bitkisi. Av.-Sib.

A. palmatum* Thunb. (Japon akçaağacı), **7, 10.04.2014, kültür, süs bitkisi.

SCROPHULARIACEAE (Sırıcaotugiller)

**Buddleja davidii* Franch. (Kelebek Çalısı), 10.04.2014, kültür, süs bitkisi.

Verbascum blattaria L. (Tutan sığırkuyruğu), **1**, 01.08.2002, EDTU 10114!

V. lagurus Fisch. et Mey. (Tavşan sığırkuyruğu), 09.07.1990, EDTU 4475! Av.-Sib.

V. phlomoides L. (Yünotu), **2**, 13.06.1990, yol, EDTU 4488! 4489! Av.-Sib.

V. sinuatum L. var. *sinuatum* (Bodan otu), **6**, 10.06.2013, çamlık altı, EDTU 13945! 03.07.2001, çamlık altı, EDTU 8335! Akd.

SIMAROUBACEAE (Kokarağaçgiller)

Ailanthus altissima (*Miller) Swingle (Kokar ağaç), **1, 2, 10.04.2014, kültür, süs bitkisi, bazı alanlarda istilacı.

SMILACEAE (Dikenucugiller)

Smilax excelsa L. (Diken ucu), **1**, 10.04.2014, dere kenarı. Av.-Sib.

SOLANACEAE (Patlıcangiller)

Datura stramonium L. (Boru çiçeği), **7**, 30.07.2013, otlak alan, EDTU 13949! Kozmopolit.

Solanum americanum Mill. (İt üzümü), **1**, 13.08.2013, otlak alan, EDTU 13947!

***S. decipiens* Opiz. (Ecavlusu), 1**, 31.08.2013, ağaçlık altı, EDTU 13946! **3**, 08.07.2013, çamlık altı, EDTU 13948!

S. dulcamara L. (Sofur), **1**, 25.06.2013, otlak alan, EDTU 13950! Av.-Sib.

TAMARICACEAE (Ilgıngiller)

Tamarix parviflora* DC. (Deli ılgın), **1, 2, 10.04.2014, kültür, süs bitkisi.

ULMACEAE (Karaağaçgiller)

Ulmus minor Mill. (Ova karaağacı), **1, 2,** 10.04.2014, dere kenarlarında büyük kümeler şeklinde. D. Akd.

U. laevis Pallas (Hercai karaağaç), **7**, 10.04.2014, doğal bitki, bazı alanlarda süs bitkisi olarak kullanılmış. Av.-Sib.

VERBENACEAE (Mineçiçeğigiller)

Verbana officinalis L. (Mine çiçeği), **6**, 10.06.2013, ağaçlık altı, EDTU 13951!

VIOLACEAE (Menekşegiller)

Viola kitaibeliana Roem. & Schultz. (Yabani menekşe), **1**, 13.04.2013, otlak alan, EDTU 13952! 02.04.2013, tarla sınırı, EDTU 13953! 19.04.2013,

ağaçlık altı EDTU 13955! **5**, 12.04.2013, ağaçlandırılan alan, EDTU 13954!

VITACEAE (Asmagiller)

Vitis vinifera L. (Asma), **1, 2,** 10.04.2014, yabani formları dere kenarlarında sarılıcı, kültür bitkisi olarak da örnekleri bulunmaktadır.

XANTORRHOEACEAE (Çirişgiller)

***Asphodeline lutea* (L.) Reichb. (Sarı çiriş), 2**, 29.04.2013, otlak alan, EDTU 13956! Akd.

ZYGOPYLLACEAE (Çobançökertengiller)

Tribulus terrestris L. (Çoban çökerten), **3**, 08.07.2013, otlak alan, EDTU 13957!

Sonuçlar ve Tartışma

Çalışma alanında tespit edilen 77 familya 250 cinse ait 428 tür ve türaltı takson incelendiğinde, bunların 1'i Pteridophyta ve diğer 427 takson Spermatophyta'ya aittir. Bunlardan da 14 tanesi Gymnospermae, 413 tanesi Angiospermae'lere aittir. Angiospermae'lerin 369'u Dicotyledoneae, 44'ü Monocotyledoneae sınıflarında bulunmaktadır. Tespit edilen 428 taksondan 296'sı (%69,23) otsu, 81'i (%18,88) ağaç, 44'ü (%9,32) çalı ve 7'si (%1,63) ise sarılıcı formlardır. Bu taksonların 358'i alanda yer alan doğal veya doğallaşmış taksonlardan oluşurken, 70 takson peyzaj amacıyla süs bitkisi veya meyve bitkisi olarak dikilmiş kültür bitkileriden oluşan egzotik türlerdir.

Araştırma sonucunda tespit edilen 428 taksondan 54'ü alandan yeni kayıt olarak verilmiştir. Bu kayıtlardan *Onopordum acanthium* Trakya, diğer 53'ü Edirne için yeni kayıttır. Bu türler; *Aegilops triuncialis* subsp. *triuncialis, Aira elegantissima* subsp. *elegantissima, Anagallis foemina, Anthemis arvensis, Arenaria serpyllifolia* subsp. *leptoclados, Asphodeline lutea, Berteroa incana, Bidens tripartita, Bothriochloa ischaemum, Bromus rigidus, B. lanceolatus, Cannabis sativa, Capsella rubella, Cota altissima, Cruciata pedemontana, Draba muralis, Epilobium tetragonum* subsp. *tetragonum, Glechoma hederacea, Heliotropium suaveolens, Hordeum murinum* subsp. *leporinum, Hypochaeris radicata, Saponaria officinalis, Sonchus asper* subsp. *glaucescens, Stellaria graminea, S. media, Legousia pentagonia, Linaria genistifolia* subsp. *genistifolia, Lolium rigidum* var. *rottbollioides, Matricaria chamomilla* var. *recutita, Medicago minima* var. *minima, M. lupulina, Nigella elata, Papaver dubium, Phleum phleoides, Plumbago europaea, Poa trivialis, Potentilla inclinata, P. detommasii, Prunella vulgaris, Ranunculus velutinus, Rosa gallica, Scabiosa columbaria* subsp. *columbaria* var. *columbaria, Sherardia arvensis, Solanum decipiens, Thalictrum lucidum, Teesdalia coronopifolia, Tordylium apulum, Torilis leptophylla, Trifolium leucanthum, Tripleurospermum parviflorum, Valerianella carinata, V. coronata* ve *Veronica triloba*'dır.

Türkiye 3 fitocoğrafik bölgenin kesişme noktasındadır. Trakya Bölgesi'ne bakıldığında Akdeniz ve Avrupa-Sibirya fitocoğrafik bölgelerinin kesişme bölgesi olmasına karşılık, İran-Turan elemanlarını da bünyesinde barındırır (Yaltırık 1963). Araştırma bölgesinde tespit edilen türlerin fitocoğrafik bölgelere göre dağılımlarına bakıldığında bu görülebilir (Tablo 2). Tabloda görüldüğü üzere en fazla görülen 298 (%69,63) bitki ile fitocoğrafik bölgesi bilinmeyen taksonlar olmuştur. Diğerleri sırasıyla Avrupa-Sibirya, Akdeniz, Doğu Akdeniz, kozmopolitler, İran-Turan ve Akdeniz ve İran-Turan Elementleri gelmektedir. Değerlendirme yapılırken kültür bitkileri ayrı tutulmamıştır. Çalışma alanı, Avrupa-Sibirya fitocoğrafik bölgesiyle Akdeniz fitocoğrafik bölgesinin kesişme noktasında olduğu için, Avrupa-Sibirya elementlerinin sayısıyla Akdeniz elementlerinin sayısı yakınlık göstermektedir. Akdeniz ve Doğu Akdeniz elementlerinin toplam olarak fazla olması, bölgenin biraz daha Akdeniz fitocoğrafik bölgesine yakın olduğunu gösterir. Ayrıca bölgenin vadi tabanı olarak kabul edilen dere yataklarını barındırması, alana yakın olan ve genellikle nem isteği fazla olan Avrupa-Sibirya elementlerinin fazla olmasına neden olmuştur. İran-Turan elementlerinin az sayıda bulunması ise araştırma alanının, İran-Turan flora bölgesinden coğrafik ve ekolojik olarak farklılığına bağlanabilir.

Tablo 2. Araştırma Alanında Tespit Edilen Taksonların Fitocoğrafik Bölgelere Göre Dağılımı.

Floristik Bölge	Takson Sayısı	% Oranı
Avrupa-Sibirya	59	13,79
Akdeniz	50	11,68
Doğu Akdeniz	14	3,27
İran-Turan	2	0,47
Akdeniz ve İran-Turan	1	0,23
Kozmopolit	4	0,93
Bilinmeyen	298	69,63
Genel Toplam	**428**	**100**

Tespit edilen taksonlar incelendiğinde en fazla Asteraceae üyeleri görülmektedir (Tablo 3). Bunu sırasıyla Fabaceae, Rosaceae, Poaceae, Caryophyllaceae, Brassicaceae, Ranunculaceae, Lamiaceae, Plantaginaceae, Boraginaceae, Apiaceae, Pinaceae, Caprifoliaceae, Salicaceae, Polygonaceae ve Papaveraceae familyaları izler. Araştırma alanında en çok cins içeren familyaların sıralamasına bakıldığında da benzer sıralama görülebilir (Tablo 3). En fazla takson içeren üç familya, içerdikleri takson sayısı bakımından tespit edilen tüm floranın %28,25'ini oluşturmaktadır. Bunun nedeni Türkiye florasının en fazla takson içeren familyaları arasında olmalarından kaynaklanmaktadır. Asteraceae familyasının en fazla takson içermesinin nedeni familya üyelerinin çoğunun ekolojik toleranslarının geniş olması, meyvalarının pappuslu olması ve rüzgarla kolayca uzaklara taşınabilmesine bağlanmaktadır. Araştırma alanında en fazla takson içeren diğer bir familya ise

Fabaceae'dir. Bunun nedeni ise, bu familyaya ait *Vicia* L. ve *Trifolium* L. cinslerinin çok fazla tür ile temsil edilmesi söylenebilir. Akdeniz iklimini ve nemli bölgeleri seven, tahrip edilen orman vejetasyonunda öncü bitki olarak kolayca yetişebilen, çayır-mera vejetasyonuna daha uyumlu *Vicia* cinsi, araştırma alanında ilk sırada yer almakta olup, ikinci sırada da *Trifolium* gelmektedir. Ayrıca alanın büyük bir kısmının terk edilmiş tarım arazilerinde oluşması bunu kolaylaştırmaktadır.

Tablo 3. Araştırma alanında en çok tür içeren familyaların cins ve tür sayılarının oranları

Floristik Bölge	Cins Sayısı	Toplam Cins Sayısına % Oranı	Tür Sayısı	Toplam Tür Sayısına % Oranı
Asteraceae	39	15,60	68	15,88
Fabaceae	19	7,60	30	7,00
Rosaceae	16	6,40	23	5,37
Poaceae	14	4,60	22	5,14
Caryophyllaceae	12	4,80	19	4,43
Brassicaceae	11	4,40	12	2,80
Ranunculaceae	8	3,20	11	2,57
Lamiaceae	7	2,80	11	2,57
Plantaginaceae	7	2,80	11	2,57
Diğer	126	50,04	221	51,67
Genel Toplam		**100**	**428**	**100**

Çalışma alanında 4 (*Centaurea diffusa, Tripleurospermum baytopianum, Myosotis uncata* ve *Linaria genistifolia* subsp. *artvinense*) endemik takson bulunmaktadır. Endemik türlerin floradaki oranı %0,9'dur. Türkiye genelindeki endemizm oranının %31,4 olduğu düşünüldüğünde, araştırma alanında endemizmin oranı oldukça düşüktür. Bu türler üzerindeki en büyük tehditin alanın yapılaşmaya açık olması, ağaçlandırma alanlarının sürekli olarak tarım arazisi şeklinde işlenmesi ve bölgenin büyük bir kısmının yeşil alanlara çevrilmesi doğal yapıyı bozmakla birlikte, alandaki nadir ve endemik türlerin geleceğini ciddi şekilde tehdit etmektedir. Ancak alanda güçlü bir yapıya sahip olan Balkan Arboretum alanı içindeki populasyonların korunma olasılığı oldukça yüksektir. Endemik türlerden sadece *T. baytopianum* IUCN kriterlerine göre EN kategorisinde yer alırken, diğerleri değerlendirilmemiştir (n/l). Ayrıca alanda 10 nadir takson bulunmaktadır. Bunlar IUCN kriterlerine göre incelendiğinde EN kategorisinde 3 tür (*Carduus candicans* subsp. *candicans, Dianthus armeria* subsp. *armeriastrum* ve *Rorippa thracica*), VU kategorisinde 2 tür (*Ornithogalum refractum* ve *Trifolium michelianum* var. *balansae*), LC kategorisinde 1 tür (*Anacamptis papilionacea*) ve DD kategorisinde ise 4 tür (*Galinsago parviflora, Hypecoum procumbens* subsp. *procumbens, Ornithogalum fimbriatum* ve *Vicia sativa* subsp. *incisa* var. *incisa*) yer almaktadır.

Araştırma alanından elde edilen taksonlar üzerinde yapılan çalışmalar neticesinde 3 taksonda bazı morfolojik farklılıklar tespit edilmiştir. Bunlar: Türkiye Florası kayıtlarına göre, Lamiaceae familyasından bu bölgede yayılış gösteren *Lamium purpureum* var. *purpureum* taksonunun korolla rengi morumsu-pembe olduğu belirtilmiştir. Ancak araştırma bölgesinde bulunan taksonların bir kısmı, diğer bütün morfolojik özellikleri uymasına rağmen, korolla rengi beyazdır. Geraniaceae familyasında *Geranium molle* taksonun da aynı şekilde korolla rengi pembe olduğu belirtilmiş ve araştırma bölgesinde bulunan taksonların bir kısmı, diğer bütün özellikleri morfolojik olarak uymasına rağmen korolla rengi beyazdır. Boraginaceae familyasından *Echium plantagineum* türünün de korolla rengi koyu maviden mora değiştiği belirtilmiş, ancak bu çalışmada tespit edilen türlerin bazılarının diğer morfolojik özellikleri uymasına rağmen korolla rengi beyazdır. Bu türlerde görülen renk farklılığı, şimdilik bir renk formu olarak değerlendirilmiştir.

Araştırma bölgesinin küçük, bir kısmının yapılaşma alanı ve tarım arazisi olmasına rağmen 428 (358 doğal veya doğallaşmış, 70 egzotik tür) takson bulunmuştur. Bu sayıya doğal ve egzotik taksonların hepsi dahildir. Bu kadar çok taksonun dar bir alanda bulunması, alanın iklim ve toprak bakımından çok sayıda bitkinin yetişmesinde uygun bir alan olduğunu göstermektedir. Ayrıca en fazla tür 1 ve 2 nolu alanlardan toplanmıştır. Bunun nedeni, doğal yapısının bozulmamış olması ya da çok az bozulmuş olmasıdır. En az takson sayısı içeren 4 ve 7 nolu alanların çoğunlukla tarım arazisi olarak kullanılması veya inşaat yapımı için kullanılması sebebiyle takson sayıları diğer alanlara göre çok daha azdır. Alanın 1984 yılından günümüze kadar geçen sürede oldukça değişmiş ve bundan sonra da değişmeye devam edecektir. Yapılan peyzaj çalışmaları ve dışarıdan getirilen toprak, hem alanların toprak yapısını, hemde buna bağlı olarak floristik yapıyı kalıcı olarak geri dönüşülmez şekilde değiştirmektedir ve değiştirmeye devam edecektir. Araştırma alanın yakın geçmişe kadar büyük bir kısmının tarım arazisi olması ve bu alanların bazı kısımlarının bina yapımına tahsisisinden sonra, kademeli olarak tarımsal amaçlarla kullanılmaması, alanda doğal türlerin zamanla gelişip yerleşmesini sağlamıştır. İlerleyen zaman içerisinde, bölge florasının kararlı aşamaya gelinceye kadar sürekli olarak değişeceği düşünülmektedir. Bu değişim dere civarında ki doğal ağaçlık alanlarda çok fazla olması beklenemez. Fakat bu bölgelere yapılacak müdahaleler, alanın kararlı bir klimaksa ulaşmasını ve doğal dengeyi kurmasını engelleyecektir. Alanda görülen istilacı türler *Acer negundo, Ailanthus altissima, Robinia pseudoacacia, Gleditsia triacanthos* ve *Morus alba* flora açısından ciddi sorunlara yol açabilecek türlerdir. Örneğin, *A. negundo* Dalgıç (2003) tarafından verilen listede Arboretum sahasında yer almamakla birlikte, yaptığımız çalışmada arboretum alanında varlığı saptanmıştır. *A. altissima* arboretum alanı içinde ciddi kümeler oluşturmakta ve hızlı yayılma başarısı nedeniyle, boş alanlara doğru genişlemektedir. *R. pseudoacacia* dere içinde bir plantasyon sahasına sahip ve dere vejetasyonunun önemli türleri arasına girmeye başlamıştır. *G. triacanthos* yine dere içinde birçok

noktada gözlemlenmiştir. *M. alba* diğer istilacı türlere oranla daha fazla alana yayılmış ve yer yer vejetasyonun dominant bitkisi halini almıştır.

Bu çalışma, Balkan Yerleşkesi'nin bundan sonraki takiplerinde ilk çalışma olması açısından son derece önemlidir. Çalışma sonunda Balkan Yerleşkesi'nin florası tespit edilmiş, Balkan Arboretumu için bitki envanteri çıkarılmış ve Biyoloji, Eczacılık ve Peyzaj Mimarlığı öğrencilerinin pratiği için alt yapı oluşturulmuştur. Ayrıca Trakya ve Edirne florası için yeni türler ile diğer bilim dalları ve Türkiye Florasına katkı sağlamıştır.

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Kaynaklar

1. Altay, V. 2012. Mustafa Kemal Üniversitesi Tayfur Ata Sökmen Kampüsü (Hatay)'nün süs Bitkileri. *Karadeniz Fen Bilimleri Dergisi*, 2(6): 11-26.
2. Anonim, 2014. Edirne İlinin Karasal ve İç Su Ekosistemleri Biyolojik Çeşitlilik Envanter ve İzleme Projesi, Doğa Koruma ve Milli Parklar Genel Müdürlüğü, Edirne.
3. Anonim, 2017a. İklim Sınıflandırmaları, https://www.mgm.gov.tr/FILES/iklim/iklim_siniflandirmalari.pdf (Erişim tarihi: 1.11.2017).
4. Dalgıç, G. 2003. *Edirne'nin Yeşil Alanları I,* Mesut Matbaası, Edirne, s. 25-26.
5. Davis, P.H. (ed.) 1965-1988. *Flora of Turkey and the East Aegean Islands*. Vol. 1-10, University Press, Edinburgh.
6. DigitalGlobe, 2017. Google Earth Pro (V 7.3.0.3832) Edirne uydu fotoğrafları 1984-2017, http://www.earth.google.com (Erişim tarihi: 30.10.2017).
7. Dönmez, Y. 1968. *Trakya'nın Bitki Coğrafyası*, İ. Ü. Yayınları No:1321, Taş Matbaası, İstanbul, 476 s.
8. Ekim, T., Koyuncu, M., Vural, M. Aytaç, Z. & Adıgüzel, N. 2000. *Türkiye Bitkileri Kırmızı Kitabı*, Türkiye Tabiatını Koruma Derneği ve Van 100. Yıl Üniv. Yayını, Ankara, 246 s.
9. Güner, A. Aslan, S., Ekim, T., Vural, M. & Babaç, M.T.(edlr.), 2012. *Türkiye Bitkileri Listesi (Damarlı Bitkiler)*, Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını, İstanbul, 1290 s.
10. Güner, A., Özhatay, N., Ekim, T. & Başer, H.S. (edlr.) 2000. *Flora of Turkey and the East Aegean Islands*, vol. 11, Edinburgh Univ. Press. Edinburgh, 656 s.
11. IUCN (International Union for Conservation of Nature) 2001. IUCN Red List categories and criteria, Version 3.1. IUCNSSC, Gland. www.iucnredlist.org/ static/categories_criteria_3_1.
12. İspirgil, Y. 1996. Çevresel Etki Değerlendirme Çalışmalarına Esas Olmak Üzere Kampüs Alanının Bitkisel Çeşitliliğinin Tespiti. Yüksek Lisans Tezi, Mersin Üniversitesi, Mersin, 51 s.
13. Meriç, Ç. 2008. Calcium oxalate crystals in *Conyza canadensis* (L.) Cronq. and *Conyza bonariensis* (L.) Cronq. (Asteraceae: Astereae). *Acta Biologica Szegediensis*, 52(2): 295-299.
14. Meriç, Ç. 2009. Calcium oxalate crystals in some species of the Tribe Inuleae (Asteraceae). *Acta Biologica Szegediensis*, 51(1): 105-110.
15. Mutlu, B., Erik, S. & Tarikahya, B. 2008. New Contributions to the Flora of Beytepe Campus (Ankara) and Floristic Comparison with Neighboring Floras and Other Campus Floras. *Hacettepe Journal of Biology and Chemistry*, 36(3): 181-195.
16. Nugan, Ö.Z., Duran A. & Doğan, B. 2007. Kırıkkale Üniversitesi Kampüs Florası. Selçuk Üniversitesi Fen Edebiyet Fakültesi Fen Dergis, 30: 79-92.
17. Özyavuz, M. & Korkut, A.B. 2008. Trakya Üniversitesi Güllapoğlu Arboretumu Peyzaj Planlama Çalışmaları. *Tekirdağ Ziraat Fakültesi Dergisi*, 5(3): 297-302.
18. Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.W., Webb, D.A., 1965-1980. *Flora Europaea vol. 1-5*, Cambridge University Press, Cambridge.
19. Takhtajan, A. 1986. *Floristic regions of the world*. University of California Press, California, 544 s.
20. Türe, C. & Böcük, H. 2001. Anadolu Üniversitesi Kampüsü Florası (Eskişehir-Türkiye). *Anadolu Üniversitesi Bilim ve Teknoloji Dergisi*, 2(1): 83-95.
21. Yaltırık, F. 1963. Belgrad Orman Vejetasyonunun Floristik Analizi Ana Meşcere Tiplerinin Komposizyonu Üzerinde Araştırmalar. *İstanbul Üniversitesi Orman Fakültesi Dergisi*, A 13(1): 33-80.
22. Yurtsever, S., Okyar, Z. & Güler, N. 2010. What colour flowers do some Lepidoptera prefer foraging? *Biologia, Section Zoology*, 65(6):1049-1056.

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Research Article

NEW TRAMP ANT SPECIES FOR TURKEY: *Tetramorium lanuginosum* Mayr (HYMENOPTERA: FORMICIDAE)

Celal KARAMAN*, Kadri KIRAN

Trakya University, Faculty of Science, Department of Biology, Balkan Campus, 22030, Edirne, TURKEY
*Corresponding author: e-mail: celalkaraman@trakya.edu.tr

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Abstract: Human activities such as tourism, developed transportation and increased trade lead to the introduction of faunal elements into non-native habitats and consequently affect native fauna. These introduced species are called as non-native, exotic, invasive or tramp species. Here we record the well-known tramp species *Tetramorium lanuginosum* Mayr, for the first time from Turkey (Antalya-Alanya), and present first locality records for *Paratrechina longicornis* (Latreille) from Antalya-Alanya and Adana. Thus, the number of tramp ant species of Turkey is increased to 19.

Key words: Tramp species, new record, Northeastern Mediterranean, Antalya-Alanya.

Özet: Turizm, gelişmiş ulaşım ve artan ticaret gibi insan faaliyetleri faunal elemanların dağılım alanları dışındaki habitatlara taşınmasına neden olmakta ve dolayısıyla yerli faunayı etkilemektedir. Bu faaliyetlerle taşınan organizmalar yerli olmayan, egzotik, istilacı veya tramp türler olarak adlandırılır. Bu çalışmada, çok iyi bilinen tramp karınca türü olan *Tetramorium lanuginosum* Mayr'u Türkiye'den (Antalya-Alanya) ilk defa kayıt edilmekte ve *Paratrechina longicornis* (Latreille)'e ait ilk lokalite kayıtları ise Antalya-Alanya ve Adana'dan verilmektedir. Böylece, Türkiye'de tramp karınca tür sayısı 19'a yükselmiştir.

Introduction

The increasing rate of human activities (e.g. trade, tourism, developing projects, globalization, import and export etc.) cause the spread of faunal elements out of their native ecosystems (Chown *et al.* 1998, IUCN 2000, Clavero & Garcia-Berthou 2005). The transferred species are called "non-native", "alien", "exotic", "invasive", and "tramp" species (McGlynn 1999, Ivanov 2016). Mcglynn (1999) recorded 146 ant species belonging to 48 genera within 7 subfamilies, while AntWeb (2017) listed more than 200 ant species belonging to 65 genera within 18 subfamilies as tramp, but the real number is most probably higher (Miravete *et al.* 2014).

Turkey is an important destination for tourism (Tursab 2016), with more than 35 million tourists who visited the country each year in the last decade. The geographic position of Turkey as a peninsula and the increased internal and external trade activities by shipping resulted in the introduction of non-native ant species to the country. For instance, *Anoplolepis gracilipes* (F. Smith), *Camponotus compressus* (Fabricius), *C. maculatus* (Fabricius), *Hypoponera punctatissima* (Roger), *Linepithema humile* (Mayr), *Solenopsis geminata* (Fabricius), *Paratrechina longicornis* (Latreille), and *Tetramorium bicarinatum* (Nylander) have recently been recorded from Turkey as tramp species (Borowiec 2014), but without their distribution localities. According to the records of Borowiec (2014) and AntWeb (2017) the current number of tramp species known from Turkey is 32.

Here we present a new tramp ant species *T. lanuginosum* Mayr from Antalya-Turkey and give the first locality based records of *P. longicornis* (Latreille) from Antalya and Adana.

Materials and Methods

Tetramorium lanuginosum Mayr

Turkey, Antalya-Alanya (36° 32′ 41″ N, 31° 59′ 59″ E), 13m., 12.ix.2016, 16/0112e, 6 ☿☿; (36° 32′ 15″ N, 31° 59′ 53″ E), 17m., 12.ix.2016, 16/0115c, 3 ☿☿, leg. C. Karaman.

Paratrechina longicornis (Latreille)

Turkey, Antalya-Alanya (36° 32′ 00″ N, 31° 59′ 25″ E), 221m., 12.ix.2016, 16/0113a, 20 ☿☿, leg. C. Karaman;

Adana (36° 58′ N, 35° 20′ E), 30m., 01.vii.2016, 16/0104b, 4 ☿☿, S. Yıldız.

The specimens of both species were collected by an aspirator. Digital images were prepared using Nikon D800E DSLR camera with 3.2x and 8x microscope objectives and Combine-Z (2008) free software. The images were cropped with Adobe Photoshop CS2.

Antalya-Alanya and Adana is characterized by a Mediterranean type climate with hot and dry summers and mild and wet winters. The average annual temperature and precipitation values are 18.7°C and 1087mm for Antalya-Alanya and 19.3°C and 679mm for Adana, respectively. Moreover, the average temperature of winter season of these two provinces never fall below 10°C (Climate-data.org).

Results

Tetramorium lanuginosum Mayr (Fig. 1)

Diagnosis: *Tetramorium lanuginosum* with *T. bicarinatum* are among the few Turkish ants with antennal scrobe. *Tetramorium lanuginosum* can be readily discriminated from *T. bicarinatum* and from other Turkish *Tetramorium* species by the long and profuse bifid pilosity.

Paratrechina longicornis (Latreille) (Fig. 2)

Diagnosis: *Paratrechina longicornis* is differentiated from all other species of the genus by 5 toothed mandibles, the stiff and blunt hairs and the bare antennal scape.

Discussion

The tramp ant species have been known since more than one century. Forel (1911) recorded fifteen ant species spread by human activities in 1911 and Wetterer (2010) mentioned that eight of them [*Anoplolepis gracilipes*, *Linepithema humile*, *Trichomyrmex. destructor*, *Monomorium pharaonis*, *Paratrechina longicornis*, *Pheidole megacephala*, *Solenopsis geminata*, and *Tapinoma melanocephalum* (Fabricius)] became “serious pest species” worldwide.

Fig. 1. *Tetramorium lanuginosum*: A- Head in full-face view, B- Body in profile.

Fig. 2. *Paratrechina longicornis*: A- Head in full-face view, B- Body in profile.

Four of these serious pest ant species (*A. gracilipes*, *L. humile*, *S. geminata* and *P. longicornis*) were reported from Turkey by Borowiec (2014) without exact locality records. *Monomorium pharonis* and *P. megacephala* were recorded by different researchers from different localities in Turkey. The other problematic species, *T. destructor*, is a native ant species for Turkish ant fauna and only one of them (*Tapinoma melanocephalum*) has not been recorded so far from Turkey.

The records available from Borowiec (2014) and AntWeb (2017) point out presence of at least 32 tramp ant species from Turkey (Table 1). However, 14 ant species (Table 1; written as red) from this list are native to Turkey because their native distribution range comprise Turkey. As a result, the exact and real tramp ant species number of Turkey needs to be revised as 18 (Table 1; written as black).

Tetramorium lanuginosum is widely distributed across tropical and subtropical regions with several records in countries in Western Palearctic (Egypt, England, Israel, Lebanon, Libya, Malta, Netherlands, Spain, Tunisia and Saudi Arabia). We recorded *T. lanuginosum* from two different urban habitats, pavements of historical bazaar and a coastal road in Alanya (Figure 3A). These findings let us to conclude without doubt that *T. lanuginosum* is settle to Alanya and became a putative permanent tramp species.

Paratrechina longicornis has been recorded in the Western Palearctic Region from Algeria, Azores, Balearic Islands, Belgium, Canary Islands, Czech Republic, Denmark, Egypt, England, Estonia, France, Netherlands, Germany, Gibraltar, Greece, Iran, Iraq, Israel, Italy, Lebanon, Libya, Malta, Morocco, Saudi Arabia, Spain, Sweden, Switzerland, Syria and United Arab Emirates. We recorded *P. longicornis* in a semi-rural area of Alanya castle (Figure 3B). The workers were collected from *Pinus brutia* Tenore trunk where they were most probably feeding with aphid honeydew. The Adana record of the species was also from semi-rural area but no information on its biology is available.

Boer & Vierbergen (2008) divided the tramp ant species into 4 groups as intercepted tramps, temporary tramps, local tramps and permanent tramps according to their existence in a country. According to Boer and Vierbergen's classification, local tramps are non-native ant species moved away from the entry medium and dispersed to settle a temporary population. Permanent tramps are also non-native ant species who settled permanent populations and spread different locations. We, therefore, can categorize *T. lanuginosum* as a local tramp and *P. longicornis* as a permanent tramp species. Many tropical and subtropical ant species will become tramp ant species in the future due to global warming (Boer & Vierbergen 2008).

Table 1. Tramp ant species of Turkey according to the list in AntWeb (regular: native ant species; **bold**: tramp ant species).

SUBFAMILY DOLICHODERINAE	SUBFAMILY MYRMICIANE
1. *Dolichoderus quadripunctatus* (Linnaeus)	17. *Cardiocondyla emeryi* Forel
2. *Linepithema humile* (Mayr)	**18. *Cardiocondyla mauritanica* Forel**
SUBFAMILY FORMICINAE	19. *Monomorium monomorium* Bolton
3. *Anoplolepis gracilipes* (F. Smith)	**20. *Monomorium pharaonis* (Linnaeus)**
4. *Camponotus compressus* (Fabricius)	**21. *Monomorium subopacum* (F. Smith)**
5. *Camponotus maculatus* (Fabricius)	22. *Myrmica rubra* (Linnaeus)
6. *Camponotus vagus* (Scopoli)	23. *Myrmica specioides* Bondroit
7. *Camponotus variegatus* (F. Smith)	**24. *Pheidole indica* Mayr**
8. *Lasius alienus* (Foerster)	**25. *Pheidole megacephala* (Fabricius)**
9. *Lasius fuliginosus* (Latreille)	**26. *Strumigenys membranifera* Emery**
10. *Lasius neglectus* Van Loon, Boomsma & Andrásfalvy	**27. *Solenopsis geminata* (Fabricius)**
11. *Lepisiota frauenfeldi* (Mayr)	**28. *Tetramorium bicarinatum* (Nylander)**
12. *Nylanderia jaegerskioeldi* Mayr	29. *Tetramorium* cf. *caespitum* (Linnaeus)
13. *Nylanderia vividula* (Nylander)	30. *Trichomyrmex destructor* (Jerdon)
14. *Paratrechina longicornis* (Latreille)	SUBFAMILY PONERINAE
15. *Plagiolepis pygmaea* (Latreille)	**31. *Hypoponera eduardi* (Forel)**
SUBFAMILY MYRMICIANE	**32. *Hypoponera punctatissima* (Roger)**
16. *Crematogaster sordidula* (Nylander)	

Fig. 3. Microhabitat of the species: A- *T. lanuginosum;* B- *P. longicornis.*

In conclusion, the current and exact number of tramp ant species in Turkey, after the exclusion of 14 species, all which were regarded as to have a native distribution in Turkey, from the list one can deduce considering the data in Borowiec (2014) and AntWeb (2017) (see Table 1), is increased to 19 by the present record of *T. lanuginosum*. We are expecting that this number will increase in the future with more collecting efforts in urban and semi-rural areas especially in southern parts of Turkey.

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References

1. AntWeb, 2017. Available from: https://www.antweb.org/taxonomicPage.do?rank=species&project=introducedants&images=true. (Date accessed: September 2017).
2. Boer, P. & Vierbergen, B. 2008. Exotic ants in The Netherlands (Hymenoptera: Formicidae). *Entomologische Berichten*, 68: 121-129.
3. Borowiec, L. 2014. Catalogue of ants of Europe, the Mediterranean Basin and adjacent regions (Hymenoptera: Formicidae). *Genus*, 25(1-2): 1-340.
4. Chown, S.L., Gremmen, N.J.M. & Gaston, K.J. 1998. Ecological biogeography of Southern Ocean islands: species-area relationships, human impacts, and conservation. *The American Naturalist*, 152: 562-575.
5. Clavero, M. & Garcia-Berthou, E. 2005. Invasive species are a leading cause of animal extinctions. *Trends in Ecology & Evolution*, 20: 110.
6. Climate-data.org. Climate data for cities worldwide. http://en.climate-data.org. (Date accessed: September 2017).
7. Forel, A. 1911. Aperçu sur la distribution géographique et la phylogénie des fourmis, 81-100. Memoires Ier Congres International d'Entomologie, Bruxelles, Belgium.
8. IUCN, 2000. *Guidelines for the prevention of biodiversity loss due to biological invasion. Gland:* IUCN –The World Conservation Union; 21 pp.
9. Ivanov, K. 2016. Exotic ants (Hymenoptera, Formicidae) of Ohio. *Journal of Hymenoptera Research*, 51: 203-226.
10. McGlynn, T.P. 1999. The worldwide transfer of ants: geographical distribution and ecological invasions. *Journal of Biogeography*, 26: 535-548.
11. Miravete, V., Roura-Pascal, N., Dunn, R.R. & Gómez, C. 2014. How many and which ant species are being accidentally moved around the world? *Biology Letters*, 10: 20140518.
12. Tursab, 2016. Turizm verileri. http://www.tursab.org.tr/tr/turizm-verileri/istatistikler/turist-sayisi-ve-turizm-geliri/2003-gelirsayi-ve-ortalama-harcama_68.html. (Date accessed: March 2017).
13. Wetterer, J.K. 2010. Worldwide spread of the wooly ant, *Tetramorium lanuginosum* (Hymenoptera: Formicidae). *Myrmecological News*, 13: 81-88.

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Research Article

ASYMMETRIC VARIATIONS IN SOME SPECIES OF THE GENUS *Raphignathus* Dugès (ACARI: RAPHIGNATHIDAE)

Meryem BİNGÜL, Sibel DOĞAN*, Salih DOĞAN

Erzincan University, Faculty of Arts and Sciences, Department of Biology, Erzincan, TURKEY
*Corresponding author: e-mail: sdilkara@erzincan.edu.tr

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Abstract: In this study, asymmetric variations in dorsal and ventral body setae in some adult females in three species of the genus *Raphignathus* Dugès; *R. gracilis* (Rack), *R. hecmatanaensis* Khanjani & Ueckermann and *R. kuznetzovi* Doğan & Ayyıldız were given and photographed. Records on asymmetric variations in *Raphignathus* species found in the literature were also reviewed.

Key words: Anomaly, asymmetric variation, *Raphignathus*, morpholology.

Özet: Bu çalışmada, *Raphignathus* Dugès cinsine ait *R. gracilis* (Rack), *R. hecmatanaensis* Khanjani & Ueckermann ve *R. kuznetzovi* Doğan & Ayyıldız türlerinin bazı dişilerinde görülen sırt ve karın kıllarındaki asimetrik varyasyonlar verilmiş ve fotoğrafları alınmıştır. Ayrıca, literatürde geçen *Raphignathus* türleriyle ilgili asimetrik varyasyon kayıtları gözden geçirilmiştir.

Introduction

The mites show a variety of morphological variations, as in other living organisms, among which variations in the form of unilateral or bilateral absences of exoskeletal structures are very common, resulting in asymmetrical individuals. Asymmetric variations can be considered as the deviations from symmetry in bilaterally paired structures and are called as anomalies. Morphological variations in mites are inevitable results of a series of changes in genetic structures and ecological changes occurring in the environments. Considering that fact that asymmetric anomalies of taxonomically important structures may cause taxonomic errors, taxonomists should have more knowledge about morphological anomalies (Bingül *et al*. 2017a).

The genus *Raphignathus* Dugès has a worldwide distribution with 68 species of which 23 have been recorded from Turkey so far (Koç & Ayyıldız 1996, Doğan 2003, Doğan & Ayyıldız 2003, Koç & Akyol 2004, Koç & Kara 2005, Akyol & Koç 2006, Akyol & Koç 2007, Doğan 2007, Erman *et al*. 2007, Bagheri *et al*. 2013, Dönel & Doğan 2013, Khanjani *et al*. 2013, Bingül *et al*. 2017b). Representatives of the genus are predaceous and can be found underneath tree barks and in litter, moss, lichen, soil, stored products, house dust and bird nests. They are easily recognized by the fused cheliceral bases, forming a stylophore, cervical peritremes not embedded in dorsal surface of stylophore and confluent coxae (Fan & Zhang 2005, Dönel & Doğan 2013).

In spite of many taxonomic and faunal works on mites of the genus *Raphignathus*, data on morphological variations in this group is very limited. Some asymmetric variations in only three species, *Raphignathus gracilis* (Rack), *R. hecmatanaensis* Khanjani & Ueckermann, *R. collegiatus* Atyeo, Baker & Crossley, of the genus were reported by Gerson (1968), Khanjani & Ueckermann (2003), Koç & Akyol (2004), Doğan (2006) and Akyol (2014).

During a faunal study on urban mites of Erzincan province in Turkey (Bingül 2016), asymmetric variations were observed in 13 specimens of *Raphignathus gracilis*, *R. hecmatanaensis* and *R. kuznetzovi* Doğan & Ayyıldız. In this study, we aimed to contribute to the knowledge on anomalies observed in *Raphignathus* mites.

Materials and Methods

Mite specimens were collected from grass, moss, soil and litter under *Pinus nigra* Arnold, *P. sylvestris* L., *Pyrus* sp., *Rosa canina* L. and *Thuja* sp. during a study carried on from 2014 to 2016 on biodiversity of urban mites in Erzincan city center (Turkey). The specimens were extracted by using Berlese funnels, cleared in 60% lactic acid and mounted on microscopic slides in Hoyer's medium. Asymmetric characters of some specimens were

determined and photographed by using Olympus BX63-CBH DIC microscopes.

Results

Our microscopic investigations showed that 13 (1.68%) of the 773 specimens investigated showed asymmetric variations. Most of the variations were determined on *Raphignathus hecmatanaensis* (n=10, 76.92%), followed by *R. gracilis* (n=2, 15.38%) and *R. kuznetzovi* (n=1, 7.69%). The 13 specimens of the three species differed from others by asymmetric variations in the number of their coxal setae (*4c*), aggenital setae (ag_2), genital setae (g_2), internal pair of humeral setae (c_1) and the location of setae f_1 (Table 1).

Table 1. Morphological variations in *Raphignathus* mites.

Species	Asymmetric variations
R. hecmatanaensis	1 ♀, right seta ag_2 is duplex (Fig. 1) 2 ♀♀, right genital valve bears an extra seta (Fig. 2) 3 ♀♀, left genital valve bears an extra seta (Fig. 3) 1 ♀, left seta g_2 is absent (Fig. 4) 3 ♀♀, right seta f_1 located on striated integument (Fig. 5)
R. gracilis	1 ♀, left aggenital seta ag_2 is duplex (Fig. 6) 1 ♀, left seta f_1 located on striated integument (Fig. 7)
R. kuznetzovi	1 ♀, left seta c_1 and left seta *4c* are absent (Figs 8 and 9)

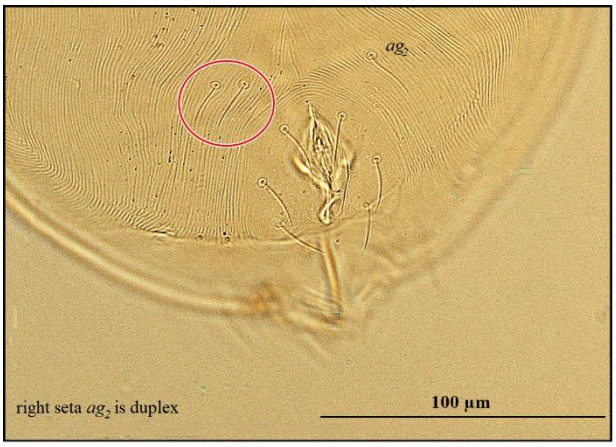

Fig. 1. *Raphignathus hecmatanaensis* (female): variation in the number of aggenital setae.

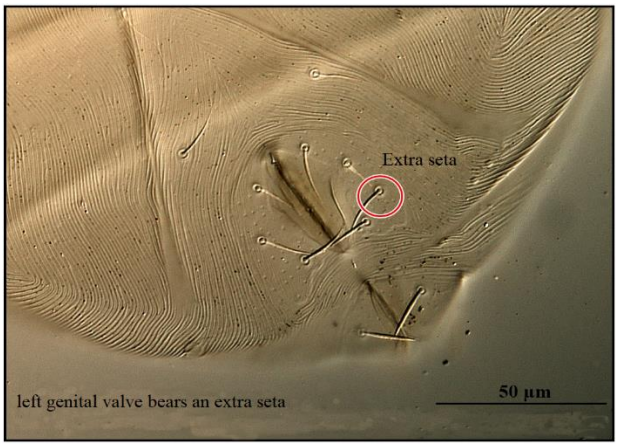

Fig. 3. *Raphignathus hecmatanaensis* (female): variation in the number of genital setae.

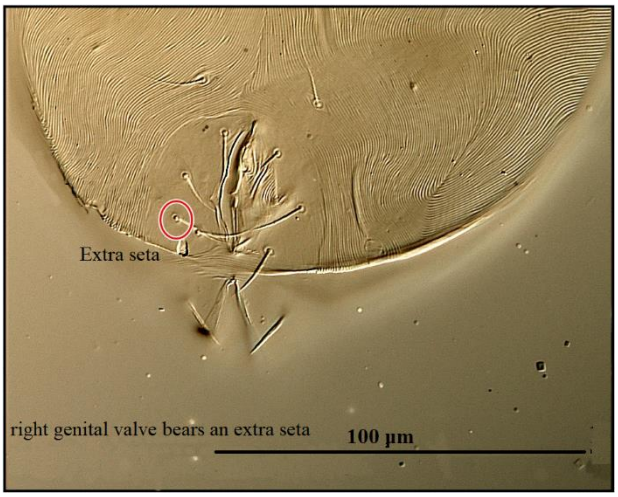

Fig. 2. *Raphignathus hecmatanaensis* (female): variation in the number of genital setae.

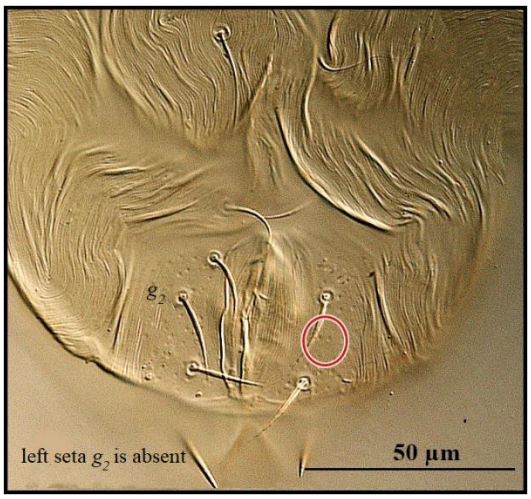

Fig. 4. *Raphignathus hecmatanaensis* (female): variation in the number of genital setae.

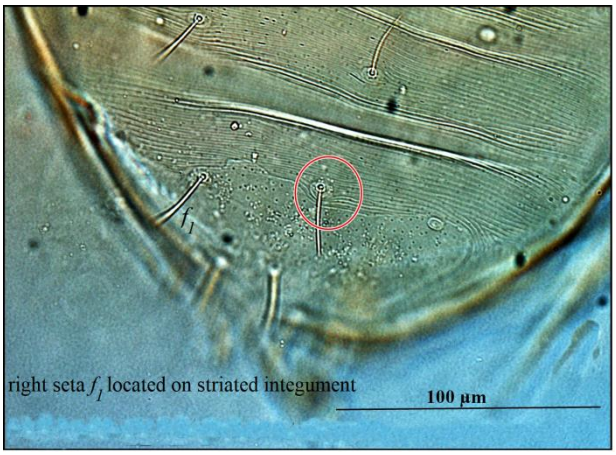

Fig. 5. *Raphignathus hecmatanaensis* (female): variation in the location of setae f_1

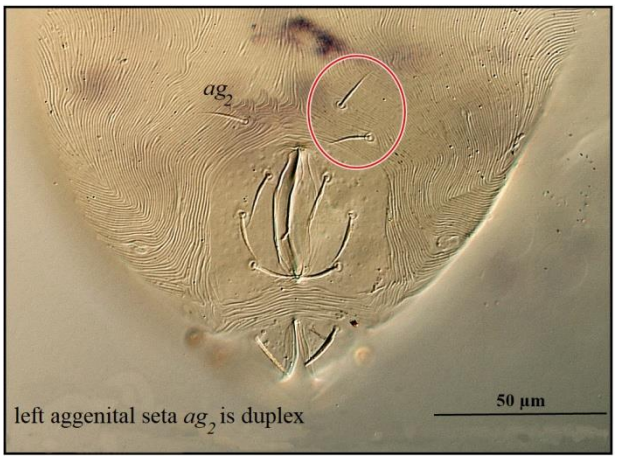

Fig. 6. *Raphignathus gracilis* (female): variation in the number of aggenital setae.

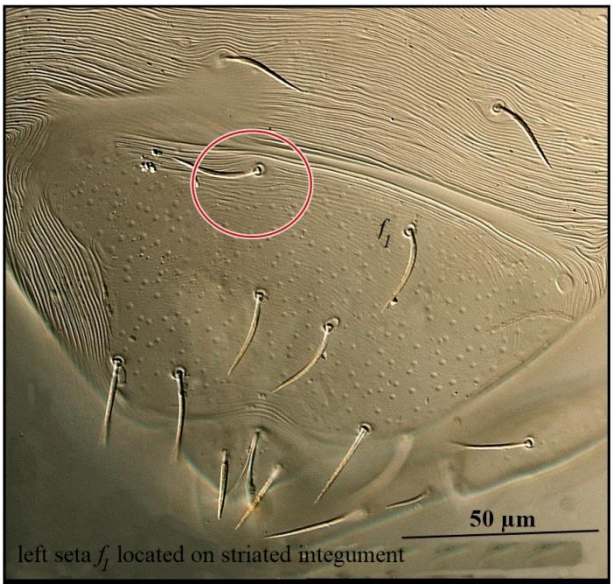

Fig. 7. *Raphignathus gracilis* (female): variation in the location of setae f_1

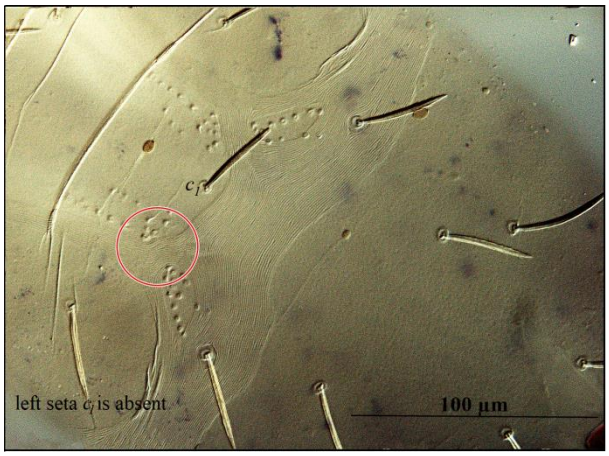

Fig. 8. *Raphignathus kuznetzovi* (female): variation in the number of setae c_1

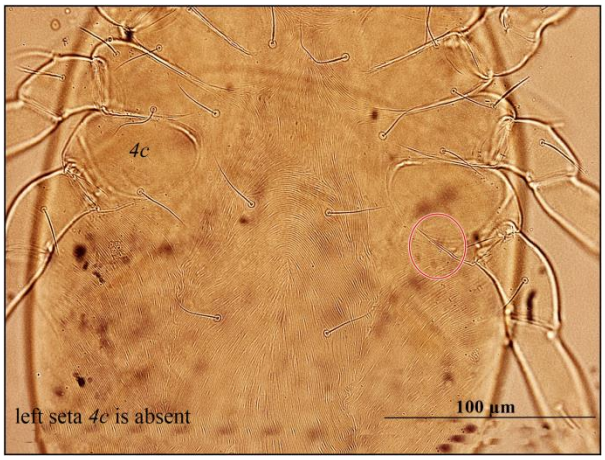

Fig. 9. *Raphignathus kuznetzovi* (female): variation in the number of setae *4c*

Discussion

Some authors reported asymmetric variations in the genus *Raphignathu*s. Gerson (1968) reported that some specimens of *R. gracilis* either had ventral aggenital setae duplicated or had four genital setae on one side. Khanjani & Ueckermann (2003) determined variation in the number of genital setae in *R. hecmatanaensis*. The authors observed in one specimen that there were three setae on one side and four setae on other side on genital valves. Similar variations in the number of genital setae were recently reported for females of *R. hecmatanaensis* by Akyol (2014). Nine of the females Akyol (2014) investigated had 7 genital setae and one female had 5 genital setae. Koç & Akyol (2004) reported that one adult specimen of *R. collegiatus* Atyeo, Baker & Crossley had four genital setae on one of the genital valves while another specimen had two genital setae. Doğan (2006) reported two genital setae, instead of three, were present on the left side of holotype of *R. ozkani* Doğan.

The authors observed asymmetric variations only in the number of aggenital and genital setae in the studied mite group. Variations in the number of coxal setae (*4c*),

humeral setae (c_1) and the location of setae f_1 are reported for the first time with this study. Furthermore, asymmetric variation in *R. kuznetzovi* is reported for the first time.

Within the same faunal study (Bingül 2016) with which the current study material was collected, morphological abnormalities in some stigmaeid species were also reported by Bingül *et al*. (2017a). Bingül *et al*. (2017a) mentioned numerical variations on some body setae and differences in length and shape of some body setae in the stigmaeid species. In our observations on *Raphignathus* mite specimens, numerical variations were also found in some setae as in the case of stigmaeid species.

Data on asymmetric variations in *Raphignathus* is very limited. Observed structural changes in the present work are easily detectable variations distorting bilateral symmetry. Such morphological variations, in other words anomalies, can be considered as minor changes distorting bilateral symmetry. These asymmetric variations may be induced by random environmental stresses, genetic problems during development or epigenetic changes, and are accord with the concept of fluctuating asymmetry (Bingül *et al*. 2017a). More detailed studies should be performed for confirmation of factors causing asymmetric variations.

Acknowledgement

This study is a part of the first author's MSc thesis, and was presented as a short summary with oral presentation at 3rd International Symposium on EuroAsian Biodiversity (SEAB 2017), held from July 5 to 8, 2017 in Minsk, Belarus.

References

1. Akyol, M. 2014. *Raphignathus hecmatananensis* (Acari: Raphignathidae) türünde görülen genital kıl sayısındaki morfolojik varyasyonlar. 22nd National Congress on Biology, 23-27 June, University of Eskişehir Osmangazi, Eskişehir (In Turkish).
2. Akyol, M. & Koç, K. 2006. *Raphignathus* mites from Turkey (Acari: Raphignathidae). *Journal of Natural History*, 40: 1149-1165.
3. Akyol, M. & Koç, K. 2007. Two new records of the genus *Raphignathus* (Acari: Actinedida) for the Turkish Fauna. *Journal of Applied Biological Sciences*, 1: 15-18.
4. Bagheri, M., Jafari, S. & Saeej, S.P. 2013. A new species of the genus *Raphignathus* (Acari: Raphignathidae) from western Iran. *Persian Journal of Acarology*, 2(3): 481-486.
5. Bingül, M. 2016. An evaluation in terms of urban biodiversity: Erzincan urban mites. Erzincan University, Graduate School of Natural and Applied Sciences, MSc thesis, 145 p.
6. Bingül, M., Doğan, S. & Doğan, S. 2017a. Morphological abnormalities in some stigmaeid species of *Eustigmaeus*, *Stigmaeus* and *Storchia* (Acari: Raphignathoidae: Stigmaeidae). *Systematic and Applied Acarology*, 22: 2119-2126.
7. Bingül, M., Doğan, S. & Dilkaraoğlu, S. 2017b. Contributions to the knowledge of the mite genus *Stigmaeus* Koch (Acari: Stigmaeidae) of Turkey. *European Journal of Taxonomy*, 307: 1-16.
8. Doğan, S. 2003. Descriptions of three new species and two new records of *Raphignathus* Dugés (Acari: Raphignathidae) from Turkey. *Archives des Sciences*, 56: 143-153.
9. Doğan, S. 2006. Contributions to the knowledge of the raphignathoid mites of Turkey (Acari: Raphignathoidea) with description of a new species. *International Journal of Acarology*, 32: 371-375.
10. Doğan, S. 2007. Checklist of raphignathoid mites (Acari: Raphignathoidea) of Turkey. *Zootaxa*, 1454: 1-26.
11. Doğan, S. & Ayyıldız, N. 2003. Mites of the genus *Raphignathus* (Acari: Raphignathidae) from Turkey. *New Zealand Journal of Zoology*, 30: 141-148.
12. Dönel, G. & Doğan, S. 2013. Two new mite species of the genus *Raphignathus* Dugés (Acari: Raphignathidae) from Turkey. *Turkish Journal of Zoology*, 37(2): 179-183.
13. Erman, O., Özkan, M., Ayyıldız, N. & Doğan, S. 2007. Checklist of the mites (Arachnida: Acari) of Turkey. Second supplement. *Zootaxa*, 1532: 1-21.
14. Gerson, U. 1968. Some raphignathoid mites from Israel. *Journal of Natural History*, 2: 492-537.
15. Fan, Q.-H. & Zhang, Z.-Q. 2005. *Raphignathoidea (Acari: Prostigmata). Fauna of New Zealand*, Manaaki Whenua Press, Lincoln, Catenbury, New Zealand, 400 pp.
16. Khanjani, M. & Ueckermann, E.A., 2003. Two new species of the genus *Raphignathus* Dugés (Acari: Raphignathidae) from Iran. *Acarologia*, 43: 299-306.
17. Khanjani, M., Pishavar, S. & Mirmoayedi, A. 2013. A new species of *Raphignathus* Dugés (Acari: Raphignathidae) from Iran. *Acarina*, 21(1): 62-68.
18. Koç, K. & Akyol, M., 2004. *Favognathus afyonensis* sp. nov. with notes on *Raphignathus collegiatus* Atyeo, Baker et Crossley, 1961 (Acari: Raphignathoidea) from Turkey. *Annales Zoologici*, 54: 475-479.
19. Koç, K. & Ayyıldız, N. 1996. Two species of *Raphignathus* Dugés (Acari, Prostigmata, Raphignathidae) new to the Turkish fauna. *Turkish Journal of Zoology*, 20: 209-214. (In Turkish).
20. Koç, K. & Kara, M. 2005. Two new species of Raphignathoidea (Acari: Raphignathidae; Camerobiidae) from Turkey. *Acarologia*, 45: 195-202.

http://dergipark.gov.tr/trkjnat
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Research Article

FIRST LARVAL RECORD OF *Lipiniella moderata* Kalugina, 1970 IN TURKISH INLAND WATERS

Nurcan ÖZKAN

Trakya University, Faculty Education, Department of Science Education, 22030, Edirne, TURKEY
e-mail: nurcanozkan@hotmail.com

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Abstract: In this study, *Lipiniella moderata* Kalugina, 1970 (Diptera, Chironomidae) is reported for the first time in larval form from the part of Tunca River in Edirne, Turkey. The larvae were collected from three different sampling stations along the river. One of the stations was selected in the river segment passing next to Edirne city center and the other two stations were selected in Suakacağı and Değirmenyeni villages located to the north of city center. A total of 145 *L. moderata* from the stations were sampled from June to October 2012. The highest number of larvae (n=89) were determined in the station in Edirne city center in August. Some morphological characters of the larvae and the sampling habitat characteristics are given, and they are discussed in terms of the biogeographical distribution of the species.

Key words: Chironomidae, *Lipiniella moderata*, first record, Tunca River, Turkey.

Özet: Bu çalışmada *Lipiniella moderata* Kalugina, 1970 (Diptera, Chironomidae) Tunca Nehri (Edirne, Türkiye)'nden ilk kayıt olarak bildirilmiştir. Larvalar nehir boyunca üç farklı örnekleme istasyonundan toplanmıştır. İstasyonlardan bir tanesi nehrin Edirne şehir merkezi yanından geçen bölümünden, diğer iki istasyon ise şehir merkezinin kuzeyinde yer alan Suakacağı ve Değirmenyeni Köyleri'nden seçilmiştir. İstasyonlardan Haziran – Ekim 2012 döneminde toplam 145 adet *L. moderata* larvası örneklenmiştir. En fazla larva (n=89), Ağustos ayında Edirne il merkezindeki istasyonda tespit edilmiştir. Larvaların bazı morfolojik ve habitat özellikleri verilmiş ve biyocoğrafik dağılım yönünden tartışılmıştır.

Introduction

Chironomidae is a taxonomically diverse group with representatives from nine subfamilies (Telmatogetoninae, Orthocladiinae, Chironominae, Tanypodinae, Diamesinae, Podonominae, Prodiamesinae, Buchonomyiinae and Aphroteniinae) and includes species adapted a life style in intertidal zones of coasts all over the world. *Lipiniella* Shilova, 1961 is a Holarctic genus within the tribe Chironomini from the subfamily Chironominae and is widely distributed in Europe (Ashe *et al.* 1987, Klukowska *et al.* 2011, Orendt & Faasch 2011, Sæther & Spies 2011).

The genus is represented in Europe with three species; *Lipiniella araenicola* Shilova, 1961, *L. moderata* Kalugina, 1970, and *L. prima* Shilova, Kerkis & Kiknadze, 1993, among which *L. araenicola* has the widest distributional range covering 10 countries [Belarus, Czech Republic, Finland, France, Germany, The Netherlands, Norway, East Palaearctic, Russia (Central, East, North, South) and Slovakia] (Hamerlik 2006). *L. araenicola* is associated with stagnant waters with sandy substratum, while the other two species inhabit large rivers (Shilova *et al.* 1992, Moller Pillot 2009, Orendt & Faasch 2011). *L. prima* has been recorded so far only from Finland and Russia while *L. moderata* has been reported from Belarus, France, Germany, Hungary, The Netherlands, Norway, Poland, Serbia and the Far East Russia (Shilova *et al.* 1992, Sæther & Spies 2011).

Lipiniella moderata was recorded from nearly all surrounding countries of Turkey except Kaliningrad Region of Russian Federation (Sæther & Spies 2011, Bitušík *et al.* 2006, Hamerlík 2006). It was first recorded in Russia in riparian sand of a fish pool near the shore of the River Oki near Serpuchova (Kalugina 1970). In central and western parts of Europe, the species was recorded from the rivers Vistula and Bug in Poland (Klukowska *et al.* 2011), from the potamal reaches of rivers in Hungary (Móra 2008), Czech Republic (Biró 2000), France (Laville & Serratosio 1996), the Netherlands and Germany (Orendt & Faasch 2011). The first record of *L. moderata* presented the present study come from habitats typical for this species – mainly sand bottom with silt or gravel and rocks. Sandy substrate is characteristic for the bottom of most of the large Polish lowland rivers (Moller Pillot 2009).

Lipiniella has previously been reported in Turkey from Kiremitlik stream in Çokal village, Gelibolu, Çanakkale (Özkan 2007) but the material was identified at genus level. This present study reports *L. moderata* for the first time in Turkey.

Materials and Methods

Tunca River originates in the Yumurkçal hill in Middle-Balkans in Bulgaria and merges with Meriç River in Edirne-Turkey as its main branch after running for 350km. It enters Turkey in Suakacağı village, which is one of the stations included in the present study (see below), runs for 30km in Turkey and 12km part of this 30km segment forms the border between Turkey and Bulgaria.

The study material was collected from three stations along the river (Fig. 1). Sampling was performed in monthly intervals from June to October 2012. The larvae were collected from benthos with a hand-net. The riverbed width in the sampled parts of the river was approximately 15m for each station and its depth ranged from 25 to 125cm. The river had a continuous water flow during samplings.

The material collected was placed in plastic bottles containing 70% ethyl alcohol, labelled and transferred to the laboratory. Chironomid larvae were cleaned under a binocular microscope (Olympus SZ61) and separated from other groups. Temporary and permanent preparations were done, and the material was identified using the keys of Shilova *et al.* (1992), Makarchenko & Makarchenko (1999), Klink & Moller Pillot (2003), Kobayashi *et al.* (2007), Moller Pillot (2009), Orendt & Spies (2010) and Klukowska *et al.* (2011). The study material is deposited in the personal collections of the author.

Fig. 1. The map showing the sampling stations along Tunca River. **1.** Suakacağı village (46°54′09,00″E; 46°32′519,00″N, 47m a.s.l.), **2.** Değirmenyeni village (46°10′67,00″E; 46°23′425,00″N, 40m a.s.l.), **3.** Edirne city center (46°29′65,63″E; 46°19′414,88″N, 37m a.s.l.).

Results and Discussion

This present study reports *L. moderata* for the first time in Turkey. A total of 145 *L. moderata* larvae were collected from sand, mud and vegetation. In Tunca River sampling zones, 11 larvae were found in the first station, 1 larvae in the second station and 133 larvae in the third station. The evaluation of distributions of the larvae in the stations considering the sampling months showed that August was the richest month for larval presence with 89 larvae, followed by July with 17 larvae, September with 16 larvae, October with 15 larvae and June with 7 larvae. 15 of these larvae were made into a permanent preparation.

Description:

Lipiniella moderata Kalugina, 1970

Head: Head capsule is wider compared to other Chironomini (Fig. 2 a).

Mentum: Mentum with median tooth comprising 4 small, subequal toothless, with 6 pairs of laterals, regularly decreasing in size. Ventromental plates contiguous medially, very narrow and 2x as wide as mentum, gently curved with fine continuous striate and dark longitudinal band becoming slenderer laterally. Setae submental simple (Fig. 2e, h).

Mandible: Mandible with a pale dorsal tooth and three moderately darker inner teeth. The dorsal tooth is shorter than the apical inner teeth. Seta subdentalis short, simple. Seta internal of 4 plumose branches. Pecten mandibularis comprising 10-15 long setae (Fig. 2f).

Labrum: SI seta coarsely plumose on both side, SII simple, SIII fine, simple, SIVa simple sensillum. Labral lamellae normal. Pecten epipharyngis long, apparently simple but actually finely divided into 3 parts, each with distal margin equipped with large and small teeth, without surface teeth (Fig. 2i). Premandible with 5 teeth, strong brush; seta premandibularis simple (Fig. 2d).

Antenna: The antennae are 5 jointed, and a big Ring organ (RO) exists at the bottom of the 1/3 of the 1st joint, seta absent, antennae breech extends to the middle of the 4th joint and has an aiding antennae breech. There exist two opposite Lauterborn organs (LO) in the distal of the 2nd joint as big and wide as the joint of the 3rd antennal segment (Fig. 2c).

Body: *L. moderata* larvae are relatively large and lengths up to 11mm and are light red in colour. At the end of the penultimate body segment there are two short and inflated ventral respiratory tubules (Fig 2b).

Remarks:

The larvae can be identified easily under a microscope. Members of *Lipiniella* have some common characters such as the shape of the mentum and ventromental plates, premandible with numerous teeth and pecten mandibularis with distally divided lamella (Paraskeva *et al.* 1992). *L. moderata* could easily be discriminated from the two-other species of the genus by the presence of ventral tubules on 11th abdominal segment (Shilova *et al.* 1992).

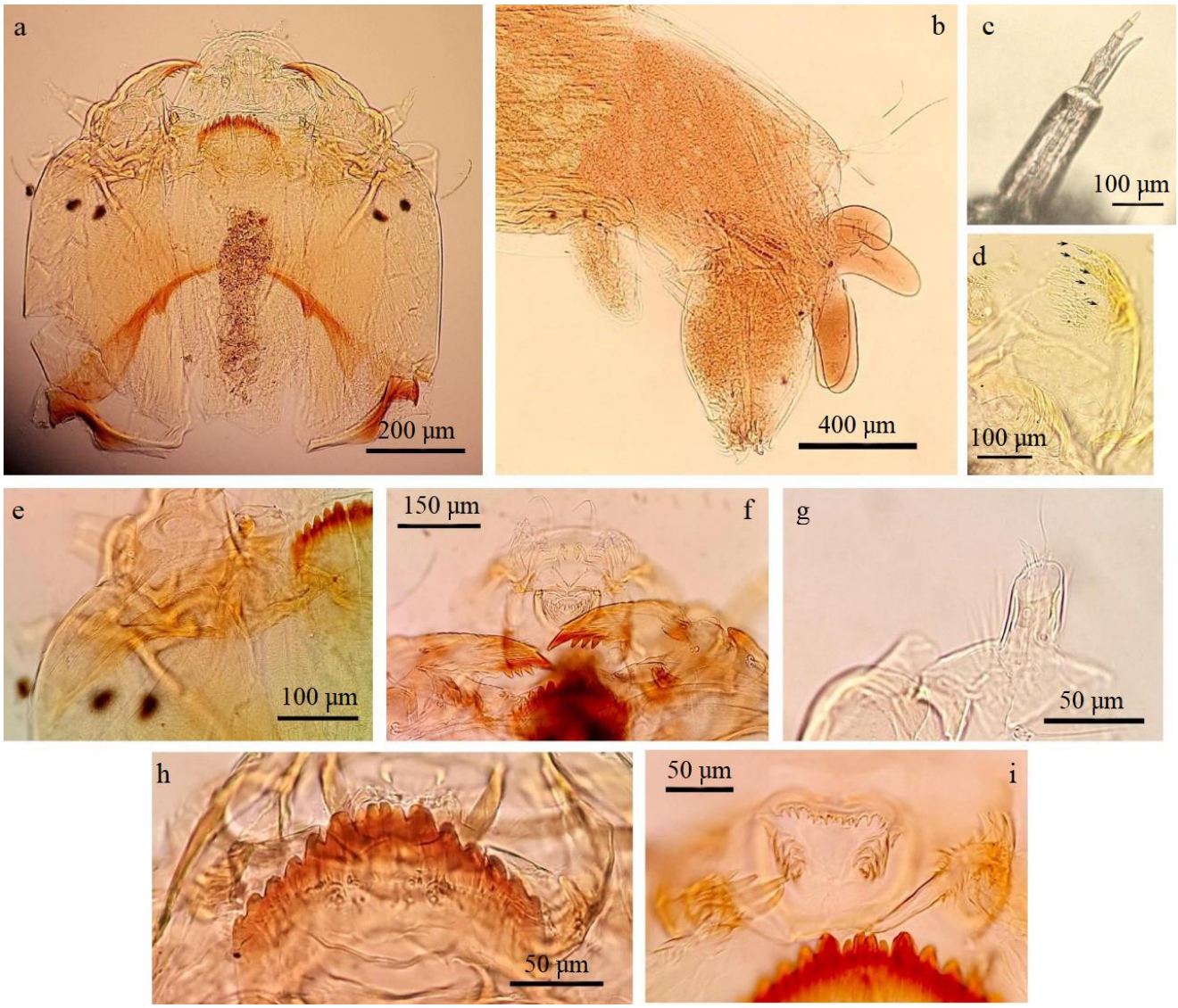

Fig. 2. *Lipiniella moderata* Kalugina, 1970. Ventral views of (except b) a) larval head capsule, b) last abdominal segments, c) antenna, d) premandible, e) mentum and ventromental plates, f) mandible, g) maxillary palp, h) mentum, and i) pecten epipharyngis, premandible.

Community, habitat and ecology

The macroinvertebrate community obtained with *L. moderata* included *Potamothrix hammoniensis* (Michaelsen, 1901), *Limnodrilus hoffmeisteri* Claparede, 1862, *L. claparedeianus* Ratzel, 1868 from Oligochaeta, *Harnischia fuscimana* (Kieffer, 1921), *Polypedilum scalaenum* (Schrank, 1803), *P. nubeculosum* (Meigen, 1804), *P. exsectum* (Kieffer, 1916), *P. bicrenatum* Kieffer, 1921, *Microtendipes chloris* (Meigen, 1818), *Chironomus anthracinus* Zetterstedt 1860, *Procladius* (*Holotanypus*) *sp.* from Chironomidae, *Erpobdella octoculata* (Linnaeus, 1758) from Hirudinea, *Platycnemis pennipes* (Pallas, 1771), *Ischnura elegans* (Van der Linden, 1820), *Gomphus vulgatissimus* (Linnaeus, 1758), *Gomphus flavipes* (Charpentier, 1825), *Orthetrum albistylum* (Selys, 1848) from Odonata, *Potamantus luteus* (Linne, 1767), (Burmeister, 1839), *Caenis luctuosa* Burmeister, 1838 (Ephemeroptera), *Asellus aguaticus* (Linnaeus, 1758) from Isopoda, *Ecnomus tenellus* (Rambur, 1842), *Cyrnus trimaculatus* (Curtis, 1834) from Trichoptera) and *Planorbella trivolvis* (Say, 1817) from Gastropoda.

The 1st and 2nd sampling stations are characterized by mud, sand and vegetation while the 3rd station is lacking the latter. The third station has a simpler appearance than the other stations in terms of trees, grass, and ground. The surrounding area is covered with grass. The population density of *L. moderata* was found to be quite high particularly in the 3rd sampling station in August. The fact that August is one of the hottest months has allowed the growth of plants and animals living in the water over time, and accordingly the breakup of the dead plants and animals. Under these conditions, the larvae may have increased their numbers by finding better nutrition. Population densities of *L. moderata* were reported to be low in Netherlands and Yugoslavia (Klink *et al.* 1995;

Biró 2000), but Kiknadze *et al*. (1989) and Móra (2008) obtained the species in high numbers in Siberian waters (up to 100 larvae in the reservoir).

The features of the habitats from which the study material was sampled resemble to those reported by other researchers. The samples were found in large rivers in sand, vegetation and muddy habitats (Shilova *et al.* 1992, Moller Pillot 2009, Klukowska *et al*. 2011, Orendt & Faasch 2011).

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References

1. Ashe, P., Murray, D.A. & Reiss, F. 1987. The zoogeographical distribution of Chironomidae (Insecta: Diptera). *Annales de Limnologie*, 23: 27-60.
2. Biró, K. 2000. Chironomidae (Insecta, Diptera, Chironomidae) from Hungary 2. new records of *Lipiniella moderata* Kalugina, 1970. *Spixiana,* 23: 175-158.
3. Bitušík, P., Hamerlík, L. & Haviar, M. 2006. First records of chironomids (Diptera, Chironomidae) from Slovakia. *Biologia*, 61: 639-641.
4. Hamerlík, L. 2006. Faunistic records of Chironomidae (Diptera) from the Morava River, new for Czech Republic. *Lauterbomia*, 58: 79-81.
5. Kalugina, N.S. 1970. A new species of the genus *Lipiniella* (Diptera, Chironomidae). *Zoologiceskij zurnal*, 49: 1034-1038.
6. Kiknadze, I.I., Kerkis, I.E. Shilova, A.I. & Filippova, M.A. 1989. A review of the species of the genus *Lipiniella* Shilova (Diptera), *L. arenicola* Shil. and *L. moderata* Kalug. *Acta Biologica Debrecina Supplementum Oecologica Hungarica*, 2: 115-128.
7. Klink, A., Mulder, J. Wilhelm, M. & Jansen, M. 1995. *Ecologische ontwikkelingen in de wateren van Blauwe Kamer 1989- 1995*.- Hydrobiologisch Adviesburo Klink bv Wageningen. Rapporten en Mededelingen 58. In opdracht van Rijkswaterstaat Dir. Oost Nederland en Stichting Het Utrechts Landschap, Wageningen, 107 pp.
8. Klink, A.G. & Moller Pillot, H.K.M. 2003. Chironomidae larvae. Key to the higher taxa and species of the lowlands of Northwestern Europe. World biodiversity database, CD-ROM Series. Expert Center for Taxonomic Identification, University of Amsterdam.
9. Klukowska, M., Płóciennik, M. & Żbikowski. J. 2011. *Lipiniella moderata* (Diptera, Chironomidae) – new species for the Polish fauna. *Lauterbornia*, 72: 1-6.
10. Kobayashi, T., Nakazato, R. & Higo, M. 2007. The identity of Japanese *Lipiniella* Shilova species (Diptera: Chironomidae). In: *Contributions to the systematics and ecology of aquatic Diptera*. Atribute to Ole A. Sæther. In: T. Andersen (ed.), the Caddis Press, Columbus, pp. 155-164.
11. Laville, H. & Serratosio, B. 1996. Addition et correction à l´inventaire des chironomidés (Diptera, France) depuis 1990. *Annales de Limnologie*, 32:115-121.
12. Makarchenko, E.A. & Makarchenko, M.A. 1999. *Chironomidae. Non-biting midges.- In: Tsalolikhin, S. J. (ed.): Key to freshwater invertebrates of Russia and adjacent lands.* Vol. 4. Higher Insects, Diptera, St. Peterburgh, pp. 210-295 and 670-857, (in Russian).
13. Moller Pillot, H.K.M. 2009. *Chironomidae larvae*. Biology and ecology of the Chironomini. (KNNV Publishing), Zeist, the Netherlands, 270 pp.
14. Morá, A. 2008. Faunistic particularities of the chironomid assemplages (Diptera: Chironomidae) of the Upper-Tisza, NE Hungary. *Boletim do Museu Municipal do Funchal Suplemento*, 13: 5-12.
15. Orendt, C. & Spies, M. 2010. *Bestimmungsschlüssel Chironomini: Illustrierter Bestimmungsschlüssel für die mitteleuropäischen Larven der Zuckmücken-Unterfamilien (Diptera: Chironomidae) sowie der Unterfamilie Chironominae, Tribus Chironomini*. Unter Verwendung vorwiegend makroskopischer Merkmale, (Selbstverlag) Leipzig, 59 pp.
16. Orendt, C. & Faasch, H. 2011. First record of *Lipiniella moderata* Kalugina, 1970 (Diptera, Chironomidae) from Germany. *Lauterbornia*, 72: 7-12.
17. Özkan, N. 2007. Larvae of Çanakkale region and their distribution. *Trakya University Journal of Science*, 8(2): 123-132.
18. Paraskeva, V.L., Michailova, L.C. & Ferrington, J.R. 1992. The Karyotype of *Axarus festivus* (Say) and a comparison with species of *Lipiniella* (Chironomidae: Diptera). *Netherlands Journal of Aquatic Ecology*, 26(2-4): 181-185.
19. Sæther, O.A. & Spies, M. 2011. *Fauna Europaea: Chironomidae*. In Beuk, P. & Pape, T. (eds) Fauna Europaea: Diptera "Nematocera". Fauna Europaea version 2.4. http://www.faunaeur.org [accessed 3.2.2011].
20. Shilova, A.I., Kerkis, I.E. & Kiknadze, I.I., 1992. *Lipiniella prima* sp. nov. (Diptera, Chironomidae), larva and karyotype. *Netherlands Journal of Aquatic Ecology*, 26: 197-201.

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Research Article

FcMgv1, *FcStuA* AND *FcVeA* BASED GENETIC CHARACTERIZATION IN *Fusarium culmorum* (W.G. Smith)

Emre YÖRÜK*, Özlem SEFER

İstanbul Yeni Yüzyil University, Faculty of Arts and Sciences, Department of Molecular Biology and Genetics, Istanbul, TURKEY
*Corresponding author: e-mail: emreyoruk@outlook.com

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Abstract: *Fusarium culmorum* (W.G. Smith) leads to economic losses in wheat and barley fields in Turkey as well as in many countries worldwide as a result of head blight and crown rot diseases. In this study, *in vitro* growth capacity of 33 *F. culmorum* isolates originating from Turkey and the relationship between phenotypic and genetic characteristics obtained based on similarities of *FcMgv1*, *FcStuA* and *FcVeA* genes were investigated. Linear growth rate values were recorded at 4th and 7th days of incubation. The mean linear growth rate values ranged from 7.58±1.06 to 14.7±1.26mm/day. The isolates F2 and 18F with relatively high linear growth values and the isolates 12F and F19 with relatively low linear growth values, were selected to be used in multiloci based genotyping analysis. *FcMgv1*, *FcStuA* and *FcVeA* genes were amplified in lengths of 1733, 2001 and 1898bp, respectively. The genes were sequenced, aligned and then subjected to BLASTn and to maximum likelihood topology analysis. Nucleotide sequence of each gene showed maximum hit with associated genes deposited in NCBI with 0.0-0.0 E-values and 1188 to 3256 bit scores. Alignment analysis resulted in at least 89% bootstrap support. Moreover, isolates with similar linear growth rates were co-clustered in phylogenetic analysis. The findings obtained in this study showed that the three genes which are essential for fungal survival could be used in genetic characterization analysis and in revealing the associations between their genetic and phenotypic characteristics.

Key words: *Fusarium culmorum*, PCR, genetic characterization, genetic diversity, linear growth rate.

Özet: Dünya çapında pek çok ülkede olduğu gibi Türkiye'de de *Fusarium culmorum* (W.G. Smith) başak yanıklığı ve kök çürüklüğü hastalıkları ile arpa ve buğday tarım alanlarında ekonomik kayıplara yol açar. Bu çalışmada, Türkiye'den köken alan 33 *F. culmorum* izolatının *in vitro* büyüme kapasitesi ile *FcMgv1*, *FcStuA* ve *FcVeA* genetik benzerliği aracılığı ile elde edilen fenotipik ve genetik karakterlerin ilişkisi incelenmiştir. Doğrusal büyüme oranı değerleri inkübasyonun 4. ve 7. günlerinde kaydedilmiştir. Ortalama doğrusal büyüme oranlarının 7,58±1.06 ve 14,7±1.26mm/gün arasında olduğu görülmüştür. Göreceli olarak yüksek LGR değerlerine sahip olduğu belirlenen F2 ile 18F izolatları ile göreceli olarak düşük LGR değerlerine sahip olduğu belirlenen 12F ve F19 izolatları multilokus temelli genotiplendirme analizlerinde kullanılmak üzere seçilmiştir. *FcMgv1*, *FcStuA* ve *FcVeA* genlerine ait sırasıyla 1733, 2001 ve 1898bç ürünler elde edilmiştir. Genler dizilenmiş, bir araya getirilmiş ve BLASTn ile maksimum olasılık topoloji analizi yapılmıştır. Her bir genin nükleotid dizisi NCBI'da 0,0-0,0 E-değeri ve 1188-3256 arası bit skoru vermiştir. Hizalama analizi en az %89 ön yükleme değeri ile sonuçlanmıştır. Ayrıca, benzer büyüme oranına sahip izolatlar filogenetik analizlerde aynı alt kümede yer almıştır. Bu çalışmada elde edilen bulgular, fungal yaşam için gerekli olan bu üç genin, genetik karakterizasyonda ve fenotipik ve genotipik özellikleri arasında ilişki kurulmasında kullanılabileceğini ortaya konmuştur.

Introduction

Fusarium culmorum (W.G. Smith) has been reported to be associated with head blight, ear blight and crown rot, diseases of cereals including wheat, barley, and maize (Miedaner *et al.* 2008). This fungus is the second major causal agent of head blight, one of the most destructive diseases affecting cereals worldwide and it is a predominating agent particularly in cooler regions of the world (Yli-Mattila *et al.* 2013, Pasquali & Migheli 2014, Yörük *et al.* 2016). *F. culmorum* is known as a quarantined phytopathogen and it is capable of producing some major and minor mycotoxins such as trichothecenes, zearalenon, fusarin C, and butanolide. In addition to its hazardous effects on cereals, *F. culmorum* also have adverse effects on human and animal health (e.g. carcinogenic and estrogenic effects) especially through class B trichothecenes and zearalenone (Desjardins & Proctor 2007, Niessen 2007). Therefore, detailed characterization of *F. culmorum* isolates is a requisite in studies considering fight against diseases associated with *F. culmorum*.

The necrotrophic *F. culmorum* is an asexually reproducing fungal plant pathogen, lacking a sexual stage.

However, potential parasexual reproduction is also assumed for *F. culmorum* since two mating types, *MAT1-1* and *MAT1-2*, were reported to be present approximately in equal proportions (Obanor *et al.* 2010, Albayrak *et al.* 2016). The number of chromosomal nucleotide sequence data of *F. culmorum* has recently increased by registrations of growing sequence data in GenBank and some other databases. However, the number of annotated and well characterized *F. culmorum* genes is still limited. Since the species has a high level of genetic variation worldwide (Miedaner *et al.* 2008), detailed and comprehensive genetic and genomic characterization of isolates are crucial in terms of effective control of the fungus. Several different polymerase chain reaction (PCR) based strategies including single primer based genotyping, sequencing based locus screening, and restriction endonuclease digestion based genotyping have been used in *F. culmorum* genotyping. SCAR-RAPD-PCR (Sequence characterized amplified region-random amplified polymorphic DNA-polymerase chain reaction), inter simple sequence repeat (ISSR), microsatellite PCR, restriction fragment length polymorphisms (RFLP), and single locus genotyping are the most common methods used in *F. culmorum* identification studies. SCAR and AFLP (Amplified fragment length polymorphism) are less frequently used in genotyping studies (Nicholson *et al.* 1998, Miedaner *et al.* 2001, Mishra *et al.* 2003, Chung *et al.* 2008, Albayrak *et al.* 2016). These techniques are generally cost effective, non-laborious, and not time-consuming and they do not need high amount of DNA but minimum prior nucleotide sequence knowledge. On the other hand, non-reproducible bands and inconsistent findings are the two major disadvantages of these techniques. In addition, a majority of these marker techniques do not provide co-dominant markers meaning that there is no possibility to distinguish the heterozygote individuals from the homozygote one (Williams *et al.* 1990, Bornet & Branchard 2001, Llorens *et al.* 2006).

Multilocus genotyping methods are promising in terms of providing a fast, cost effective, reliable, and reproducible strategy for *F. culmorum* genotyping. The most commonly used strategy in *Fusarium* spp. studies is the multiloci genotyping applied for distinguishing of *F. graminearum* (Schwabe) species complex members via 12 different genes of approximately 16kb (O'Donnell *et al.* 2000, Yli-Mattila *et al.* 2009, Sarver *et al.* 2011). In contrast to its efficient use in species complex differentiation of *F. graminearum*, this strategy is not useful for *F. culmorum* which was reported as a monophyletic species (Obanor *et al.* 2010, Przemieniecki *et al.* 2014). The use of multiloci genotyping analysis led so far to precise investigation of even if not all phenotypic characteristics and genotypic similarities and to well recognition of geographic region and genotypic diversity associations. Multiloci genotyping analysis showed that *F. graminearum* could be defined as a species complex with 15 different members (van der Lee *et al.* 2015, Pasquali *et al.* 2016). Despite the relatively long nucleotide sequence regions that have been used in multiloci genotyping, a low level of genetic diversity was detected. However, because presence of high level of intra-specific variation could not be detected by multiloci genotyping via 12 genes, novel strategies can be adapted to *Fusarium* spp. characterization. These novel strategies could be useful in making associations with genetic similarities and some other phenotypic characteristics such as host species, chemotype etc. in *Fusarium* isolates. Yörük & Sefer (2017) used a genotyping strategy based on sequencing of *FgMgv1, FgStuA,* and *FgVeA* genes in *F. graminearum* to make association between some characteristics such as 15-acetyldeoxynivalenol producing capacity, radial growth capacity, and genetic similarity. The findings obtained from *FgMgv1, FgStuA* and *FgVeA* sequencing and alignment assays showed that genotyping of these genes could be useful in detailed differentiation of fungal species complex members. Since *FgMgv1*, *FgStuA* and *FgVeA* genes whose expressions are essential in sexual/asexual development, cell wall formation and survival of fungal cells possess high level of genetic similarity in terms of nucleotide sequence data, variation in these genes can lead to significant differences in phenotypic characteristics (Hou *et al.* 2002, Jiang *et al.* 2011, Pasquali *et al.* 2013). Macroconidia and/or microconidia production, radial growth rate, host type, and chemotype characteristics can be potential phenotypic traits that can be associated with nucleotide sequence variation. In this study, it was aimed to develop novel multiloci genotyping by amplification and sequencing of *FcMgv1, FcVeA,* and *FcStuA* genes and to make association between genetic diversity and phenotypic characteristics.

Materials and Methods

Fungal materials and linear growth rate (LGR) assays

Fusarium culmorum isolates were provided by Dr. Berna Tunali from Ondokuz Mayis University, Agricultural Faculty, Department of Plant Protection. Each of the 33 isolates (see Table 1) included in the study was given a specific code, purified and identified according to standard morphological and genetic methods (Yörük *et al.* 2016).

Czapek dox agar (CDA) medium was used for *in vitro* growth of the isolates. Fresh *F. culmorum* cultures were initiated by placing the mycelia plug in the middle of the CDA medium. The cultures were incubated at 25°C for 7 days. Linear growth rates (LGR) were calculated as mm/day at 4th and 7th days of incubation (Irzykowska *et al.* 2013). Mean and standard derivation values for LGR data were calculated using column statistics (Graphpad Prism 5.0 software, USA).

Genomic DNA extraction

Genomic DNA (gDNA) of the isolates was isolated from 7-day-old cultures using the CTAB-based DNA isolation kit (BioBasic, Canada). Approximately 100mg of mycelium on the culture surface was collected and after washing with 96% ethanol for 3 minutes, mycelium was homogenized in liquid nitrogen with sterile mortar and

pestle. The homogenate was transferred to a microtube and the binding, washing and elution steps were carried out according to manufacturer's recommendations. The purities and quantities of gDNA was analysed via spectrophotometer (Thermo, USA) and 1% agarose gel electrophoresis. gDNA was visualized under UV light of gel documentation system (Maestrogen, Taiwan) by staining with 0.2µg/mL ethidium bromide. Electrophoresis was carried out under 60V constant power for 60 minutes.

Table 1. Codes, host plants, the region of isolations, the mating types and the isolation years of the *F. culmorum* isolates.

Code	Host	Region/Province	Mating	Year
F1	Wheat	Marmara	*MAT1-1*	2006
F2	Wheat	Marmara	*MAT1-2*	2006
F3	Wheat	Konya	*MAT1-2*	2006
F4	Wheat	Marmara	*MAT1-2*	2006
F10	Wheat	Bilecik	*MAT1-1*	2006
F12	Wheat	Balıkesir	*MAT1-2*	2006
F14	Wheat	Bilecik	*MAT1-2*	2006
F15	Wheat	Sinop	*MAT1-1*	2006
F16	Wheat	Konya	*MAT1-1*	2006
F17	Wheat	Konya	*MAT1-1*	2006
F19	Wheat	Konya	*MAT1-1*	2006
F20	Wheat	Bilecik	*MAT1-2*	2006
F21	Wheat	Uşak	*MAT1-1*	2006
F24	Wheat	Konya	*MAT1-2*	2006
8F	Wheat	Ankara	*MAT1-2*	2009
9F	Wheat	Isparta	*MAT1-1*	2008
10F	Wheat	Samsun	*MAT1-2*	2007
11F	Wheat	Corum	*MAT1-2*	2009
12F	Wheat	Amasya	*MAT1-2*	2009
13F	Wheat	Konya	*MAT1-1*	2008
17F	Wheat	Ankara	*MAT1-1/2*	2009
18F	Wheat	Eskişehir	*MAT1-1/2*	2010
19F	Wheat	Eskişehir	*MAT1-1/2*	2010
20F	Barley	Afyon	*MAT1-1*	2011
14-1TR	Barley	Sivas	*MAT1-2*	2014
14-2TR	Wheat	Samsun	*MAT1-1*	2014
15-1TR	Wheat	Tekirdağ	*MAT1-1/2*	2015
14-3TR	Wheat	Yozgat	*MAT1-1/2*	2014
09-1TR	Wheat	Kastamonu	*MAT1-1/2*	2009
15-2TR	Wheat	Edirne	*MAT1-2*	2015
15-3TR	Barley	Tekirdağ	*MAT1-2*	2015
15-4TR	Wheat	Edirne	*MAT1-1/2*	2015
14-8TR	Wheat	Amasya	*MAT1-2*	2014

FcMgv1, FcStuA and FcVeA amplification and cloning

Allele specific or chromosomal nucleotide sequence data of *FcMgv1*, *FcStuA* and *FcVeA* genes were obtained from the GenBank with accession numbers AF492766.1, HG970332.2 and HQ436464.1, respectively. The primers of 20 nucleotides in length were designed using the "Primer 3" software (Rozen & Skaletsky 2000) for the polymerase chain reaction (PCR). PCRs were carried out in a reaction volume of 50µL including 1X PCR mix (Takara, Japan), 10pmol of each primer (Table 2), and 50ng of DNA. PCR amplification was performed by pre-denaturation at 98°C for 2min, followed by 34 cycles of amplification at 98°C for 30s, 55°C for 30s, 72°C for 3min and final extension at 72°C for 5min. PCR bands were separated on 1.5% agarose gels. Qualitative analysis of bands was carried out via gel documentation system.

Table 2. Primers used in this study.

Primer name	Sequence 5'-3'	Amplicon Size
MGVSPANF	ATGGGCGACCT ACAAGGACG	1733bp
MGVSPANR	TTATCTTCGAG AAGCATCCA	
STUASPANF	ATGAACCAAA GTCATCACCA	2001bp
STUASPANR	CTATCCAAAGG ACTGTTGCC	
VELVETSPANF	CTGGGTTCCTC TCTGCCTTA	1898bp
VELVETSPANR	TGTCGCTCATG TATCTTCCAT	

PCR bands were purified from agarose gels by using a gel extraction kit (BioBasic, Canada). Pieces (100-200mg) including amplicons were cut form the gels and the purification was carried out according to the protocol provided by the manufacturer.

Bioinformatics assays

The sequencing process was maintained based on Sanger dideoxy termination method using "DYEnamic ET Terminator Cycle Sequencing" kit (Amersham, USA) on ABI PRISM 310 system. The nucleotide sequence was displayed in chromatograms using the "Chromas Pro" software. The sequences translated to FASTA format were subjected to BLASTN analysis. The nucleotide sequences generated via forward and reverse primers were aligned via DNA Dragon software. Assembled DNA sequences were used in multiple alignment analysis via ClustalW 1.8. software and in neighbor joining (NJ) topology analysis via Mega 6.0 software (Tamura *et al.* 2013). Pairwise distance and bootstrap support values were recorded for each assay. Accession numbers for *FgMgv1*, *FgStuA* and *FcVeA* genes (see above) were used as reference control in alignment analysis. The potential amino acids changes were characterized in "HOPE amino acid mutation" analysis.

Results

LGR analysis and fungal sample selection

Single spore *F. culmorum* isolates were grown successfully on CDA media with a main pigmentation of red to tan colour (data not shown). Each isolate was used in LGR assays. The minimum and maximum mean LGR values of the isolates were 7.58±1.06 and 14.7±1.26mm/day, respectively (Fig. 1). Isolates with relatively high (F2 and 18F) and low (12F and F19) LGR values were used in multilocus sequencing based genotyping analysis.

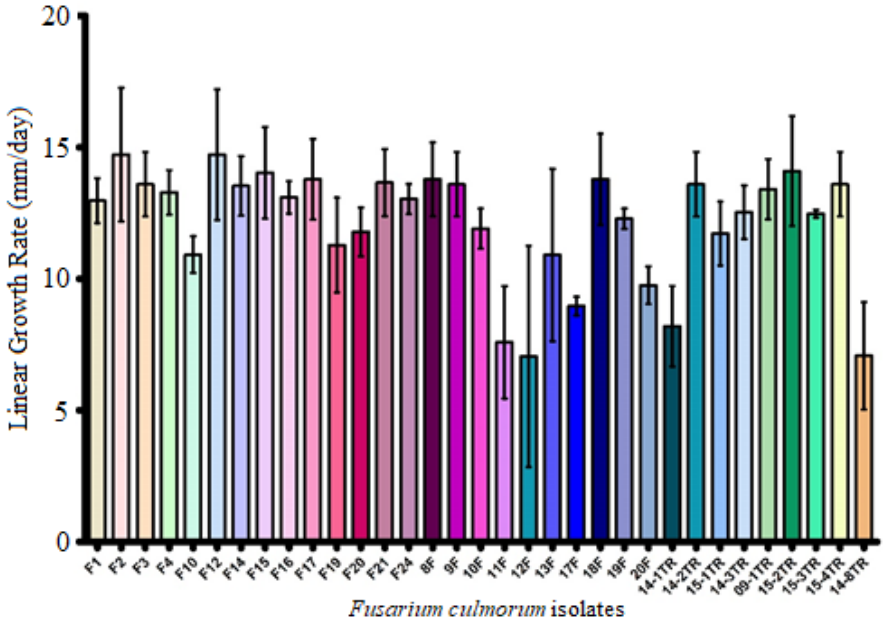

Fig. 1. LGR data of the *F. culmorum* isolates.

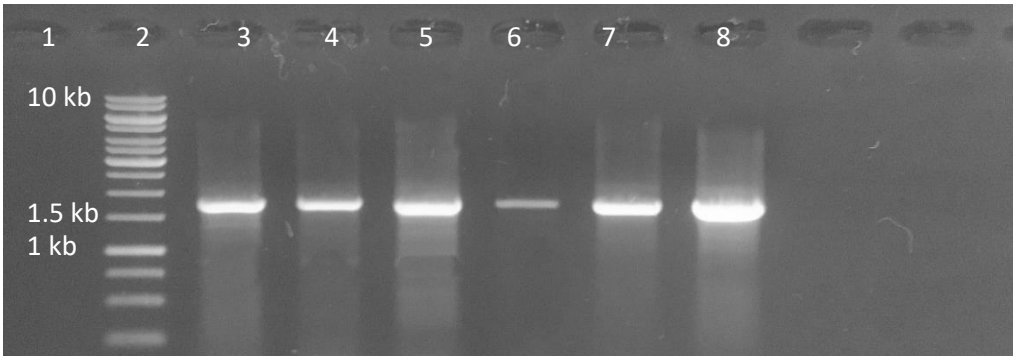

Fig. 2. *FcMgv1*, *FcStuA*, and *FcVeA* genes amplified from F2, F19 isolates.1: negative control, 2: 1kb size marker (Thermo, ABD), 3, 5, 7: from F2 isolate, 4, 6 and 8: from F19 isolate, 3, 4; 5, 6 and 7, 8 *FcMgv1*, *FcStuA* and *FcVeA* PCR product, respectively.

gDNA Isolation and PCR analysis

gDNAs were extracted from 7-day-old cultures of the isolates F2, 18F, 12F and F19. High quality ($A_{260/280}$=1.7-1.9) and quantity (1-2.5µg/µL) of gDNAs were obtained from 100mg fresh mycelia.

FcMgv1, *FcStuA* and *FcVeA* genes were used in genetic characterizations. 1733, 2001 and 1898bp amplicons were obtained from each isolate (Fig. 2). PCR amplicons purified from agarose gels were checked on agarose gel again. The PCR bands visible on gels (approximately 20-100ng/µL) were subjected to sequencing analysis.

Alignment analysis

Nucleotide sequence data of *FcMgv1*, *FcStuA* and *FcVeA* genes were screened and subjected to BLASTn analysis. The similarity of the sequence data with target associated genes recorded on GenBank (Table 3) was found to be significant. The E-values and maximum bit scores ranged from 0.0 to 0.0 and from 1188 to 3256, respectively.

The assembled *FcMgv1*, *FcStuA* and *FcVeA* sequences were subjected to ClustalW and NJ topology analysis. The nucleotide sequence data obtained from the three genes were merged and used as a single locus in homology analysis. According to ClustalW analysis minimum bootstrap support value was 89% (Fig. 3). The minimum and maximum genetic similarity values were ranged from 89 to 99%. According to topology analysis, isolates with the similar LGR values were included in the same sub-cluster and they were genetically closer to each other (Fig. 3).

Alignment analysis showed that isolates with similar LGR values had the same type of amino acid changes in 430 and 693 points (Fig. 4). HOPE amino acid mutation analysis showed that these mutations occurred as conversion of proline to leucin and glycin to serine could directly affect ligand and substrate binding interactions with proteins coded by the genes used.

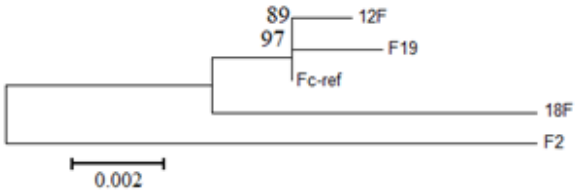

Fig. 3. Maximum Likelihood phylogram of the isolates. Bootstrap support values above 70% are shown above the branches.

Table 3. BLASTn analysis of three genes aligned in this study.

Sample	Gene	Corresponding organism	Accession no	E value	Bit score
F2	*FcMgv1*	*F. culmorum*	LT598659.1	0.0	1653
F2	*FcStuA*	*F. culmorum*	LT598659.1	0.0	3256
F2	*FcVeA*	*F. culmorum*	LT598659.1	0.0	2761
18F	*FcMgv1*	*F. culmorum*	LT598659.1	0.0	1687
18F	*FcStuA*	*F. culmorum*	LT598659.1	0.0	3201
18F	*FcVeA*	*F. culmorum*	LT598659.1	0.0	2854
12F	*FcMgv1*	*F. culmorum*	LT598659.1	0.0	2843
12F	*FcStuA*	*F. culmorum*	LT598659.1	0.0	3201
12F	*FcVeA*	*F. culmorum*	LT598659.1	0.0	1188
F19	*FcMgv1*	*F. culmorum*	LT598659.1	0.0	1698
F19	*FcStuA*	*F. culmorum*	LT598659.1	0.0	3253
F19	*FcVeA*	*F. culmorum*	LT598659.1	0.0	1995

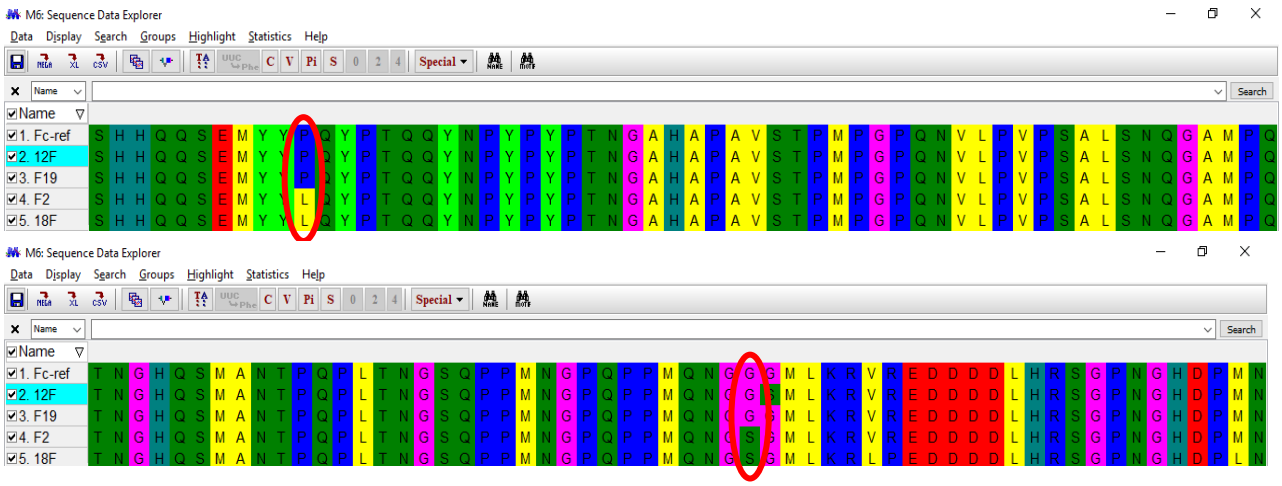

Fig. 4. Phylogeny analysis obtained with MEGA 6.0 software. The boxes within the red circle show the mutations. Fc-ref means the merged nucleotide sequences of *FcMgv1*, *FcStuA* and *FcVeA*, respectively.

Discussion

Fusarium head blight and crown rot of cereals are among the fungi-based destructive diseases worldwide (Miedaner *et al.* 2008, Yli-Mattila *et al.* 2013, Pasquali & Migheli 2014). Diagnosis and genetic characterization of causal agents of these diseases form the crucial step in disease management. The comprehensive genotyping strategies would provide useful data and knowledge in order to fight with *F. culmorum* which is major causal agent of head blight and crown rot in many countries. The majority of studies on population structure of *F. culmorum* mainly include the use of molecular marker techniques. These techniques are generally associated with targeting and amplifying unknown or random sites on genomes. These marker strategies have some disadvantageous in usage because they do not allow obtainment of co-dominant markers, the data obtained through these strategies are non-reproducible and they are likely to yield false positive results (Miedaner *et al.* 2008, Ward *et al.* 2008). However, multilocus genealogical concordance analysis provides more allele specific and reliable findings about genetic characteristics of fungal

samples. Moreover, co-dominant markers could be provided via multiloci genotyping. *F. graminearum* species complex members have been successfully identified via genealogical concordance analysis of genes with highly conserved nucleotide sequence data (O'Donnell *et al.* 2000, Yli-Mattila *et al.* 2009, Sarver *et al.* 2011). However, there is no additional usage of these conserved markers for making correlation between genetic diversity and some phenotypic characteristics including radial growth capacity, host type, macroconidium reproduction, chemotype differences or mating type. Yörük & Sefer (2017) used *FgMgv1*, *FgStuA* and *FgVeA* genes in order to make association with genetic similarity and chemotype and radial growth rate in *F. graminearum*. The results obtained from *FgMgv1*, *FgStuA* and *FgVeA* sequencing in *F. graminearum* showed that nucleotide sequence of these genes could be used as a supportive tool for detailed characterization of hemi-biotrophic species. However, there exist no data about detailed analysis of these genes for monophyletic species such as *F. culmorum, F. semitectum*, *F. poae* etc. and about amino acids alignments. Also, genotypic diversity and bootstrap support values obtained from this study were relatively close to the results obtained from individuals of *F. graminearum* species complex (O'Donnell *et al.* 2000, Yli-Mattila *et al.* 2009, Sarver *et al.* 2011). It was shown that *Mgv1*, *StuA* and *VeA* genotyping could be useful in differentiation of highly divergent individuals of fungal populations. In this study, we used *FcMgv1, FcStuA* and *FcVeA* genes for the first time as a simple gene set for making association between genetic and phenotypic characteristics in. LGR and associated amino acid sequence alterations of *FcMgv1*, *FcStuA* and *FcVeA* were evaluated for necrotrophic fungus *F. culmorum* for the first time. These three genes could also be used in detailed genetic characterization of phytopathogenic fungi which could lead to devastating diseases worldwide such as *F. fujikori* (Niren-berg) species complex and *F. oxysporum* (Sacc.) species complex. Aggressiveness, host range and genotypic diversity in members of these species complexes are issues which are not well known. Thus, in addition to genotyping of *F. culmorum* which is monophyletic phytopathogen, these three genes could be used in genotyping of fungal species complexes. Moreover, these genes could provide additional data for some important characteristics such as chemotype profiles which is still problematic worldwide (Pasquali & Migheli 2014, Pasquali *et al.* 2016). These kinds of novel approaches could contribute to disease management of monophyletic and polyphyletic fungi with different chemotypes.

We used, considering the data of LGR analysis, the isolates with relatively low and high *in vitro* growth capacity in genotyping analysis. Almost all Turkish *F. culmorum* isolates showed a high level of *in vitro* growth capacity compared to *Fusarium* spp. isolates subjected to LGR analysis in previous studies. (Chung *et al.* 2008, Irzykowska *et al.* 2013). Nucleotide sequence data of *FcMgv1, FcStuA* and *FcVeA* genes were firstly subjected to detailed BLASTn and multiple nucleotide alignment analysis. Predictably, each nucleotide sequence data showed maximum E-value with corresponding gene located at *F. culmorum* chromosomes deposited on GenBank. Assembled sequences were used in multiple alignment and phylogeny assays. Multiple alignment analysis provided effective results with bootstrap support more than 89%. Maximum likelihood topology analysis showed that isolates with the similar LGR values were co-clustered in the same sub-clusters. Moreover, isolates with the similar characteristics showed the same amino acid changes in comparison to isolates with distinct characteristics. Protein modelling analysis showed that the mutations detected in the genes subjected to the multilocus genotyping analysis could lead to alterations in structure and affinity and changes in 3D configurations in the proteins coded by these genes. Our present findings showed that the genes used in this study could be adapted to novel genotyping studies which could be useful in making association of genetic and phenotypic characteristics in *Fusarium* spp. Different isolates originating from different regions should be used in further studies in order to support the techniques used in the present study.

References

1. Albayrak, G., Yörük, E., Gazdagli, A. & Sharifnabi, B. 2016. Genetic diversity among *Fusarium graminearum* and *F. culmorum* isolates based on ISSR markers. *Archieves of Biological Sciences,* 68(2): 333-343.
2. Bornet, B. & Branchard, M. 2001. Nonanchored inter simple sequence repeat (ISSR) Markers: reproducible and specific tools for genome fingerprinting. *Plant Molecular Biology Reporter,* 19: 209-215.
3. Chung, W.H., Ishii, H., Nishimura, K., Ohshima, M., Iwama, T. & Yoshimatsu H. 2008. Genetic analysis and PCR-based identification of major *Fusarium* species causing head blight on wheat in Japan. *Journal of General Plant Pathology*, 10: 110-118.
4. Desjardins, A.E. & Proctor, R.H. 2007. Molecular biology of *Fusarium* mycotoxins, *International Journal of Food Microbiology*, 119: 47-50.
5. Hou, Z., Xue, C., Peng, Y., Katan, T., Kistler, H.C. & Xu, J. 2002. A mitogen-activated protein kinase gene (MGV1) in *Fusarium graminearum* is required for female fertility heterokaryon formation, and plant infection. *International Society for Molecular Plant-Microbe Interactions*, 11: 1119-1127.
6. Irzykowska, L., Bocianowski, J. & Baturo-Cieśniewska, A. 2013. Association of mating-type with mycelium growth rate and genetic variability of *Fusarium culmorum*. *Central European Journal of Biology*, 8(7): 701-711.
7. Jiang, J., Liu, X., Yin, Y. & Ma, Z. 2011. Involment of a Velvet protein FgVeA in the regulation of asexual development lipid and seconder metabolisms in virulance in *Fusarium graminearum*. *PLoS ONE*, 6(11): e28291.
8. Llorens, A., Hinojo, M.J., Mateo, R., Medina, A., Valle-Algarre, F.M., Gonzalez-Jaen, M.T. & Jimenez, M. 2006.

Variability and characterization of mycotoxin producing *Fusarium spp.* isolates by PCR-RFLP analysis of the IGS-rDNA region. *Antonie van Leeuwenhoek*, 89: 465-478.

9. Miedaner, T., Schilling, A.G. & Geiger, H.H. 2001. Molecular genetic diversity and variation for aggressiveness in populations of *Fusarium graminearum* and *Fusarium culmorum* sampled from wheat fields in different countries. *Journal of Phytopathology*, 149: 641-648.

10. Miedaner, T., Cumagun, C.J.R. & Chakraborty, S. 2008. Population genetics of three important head blight pathogens *Fusarium graminearum, F. pseudograminearum* and *F. culmorum. Journal of Phytopathology*, 156: 129-139.

11. Mishra, P.K., Fox, R.T.V. & Culham, A. 2003. Inter simple sequence repeat and aggressiveness analysis revealed high genetic diversity, recombination and long-range dispersal in *Fusarium culmorum. Annals of Applied Biology,* 143: 291-301.

12. Nicholson, P., Simpson, D.R., Weston, G., Rezanoor, H.N. & Lees, A.K. 1998. Detection and quantification of *Fusarium culmorum* and *Fusarium graminearum* in cereals using PCR assays. *Physiological and Molecular Plant Pathology*, 53: 17-37.

13. Niessen, L. 2007. PCR-based diagnosis and quantification of mycotoxin producing fungi. *International Journal of Food Microbiology*, 119: 38-46.

14. Obanor, F., Erginbas-Orakci, G., Tunali, B., Nicol, J.M. & Chakraborty, S. 2010. *Fusarium culmorum* is a single phylogenetic species based on multilocus sequence analysis. *Fungal Biology*, 114: 753-765.

15. O'Donnell, K., Kistler, H.C., Tacke, B.K. & Casper, H.H. 2000. Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. *Proceedings of the National Academy of Sciences*, 97(14): 7905-7910.

16. Pasquali, M., Spanu, F., Scherm, B., Balmas, V., Hoffmann, L., Beyer, M. & Migheli, Q. 2013. *FcStuA* from *Fusarium culmorum* controls wheat foot and root rot in a toxin dispensable manner. *PLoS ONE*, 8(2): e57429.

17. Pasquali, M. & Migheli, Q. 2014. Genetic approaches to chemotype determination in type B-trichothecene producing *Fusaria*. *International Journal of Food Microbiology*, 189: 164-182.

18. Pasquali, M., Pasquali, M., Beyer, M., Logrieco, A., Audenaert, K., Balmas, V., Basler, R., Boutigny, A.L., Chrpová, J., Czembor, E., Gagkaeva, T., González-Jaén, M.T., Hofgaard, I.S., Köycü, N.D., Hoffmann, L., Lević, J., Marín, P., Miedaner, T., Migheli, Q., Moretti, A., Müller, M.E., Munaut, F., Parikka, P., Pallez-Barthel, M., Piec, J., Scauflaire, J., Scherm, B., Stanković, S., Thrane, U., Uhlig, S., Vanheule, A., Yli-Mattila, T. & Vogelgsang, S. 2016. A European Database of *Fusarium graminearum* and *F. culmorum* Trichothecene Genotypes. *Frontiers in Microbiology*, 7: e406.

19. Przemieniecki, S.W., Kurowski, T.P. & Korzekwa, K. 2014. Chemotypes and geographic distribution of the *Fusarium graminearum* species complex. *Environmental Biotechnology*, 10(2): 45-54.

20. Rozen, S. & Skaletsky, H. 2000. Primer3 on the WWW for general users and for biologist programmers. *Methods in Molecular Biology*, 132: 365-386.

21. Sarver, B.A.J., Ward, T.J., Gale, L.R., Broz, K., Kistler, H.C., Aoki, T., Nicholson, P., Carter, J. & O'Donnell, K. 2011. Novel fusarium head blight pathogens from Nepal and Louisiana revealed by multilocus genealogical concordance. *Fungal Genetics and Biology*, 48:1096-1107.

22. Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution*, 30(12): 2725-2529.

23. van der Lee, T., Zhang, H., van Diepeningen, A. & Waalwijk, C. 2015. Biogeography of *Fusarium graminearum* species complex and chemotypes: a review. *Food Additives and Contaminants*: Part A, 32(4): 453-460.

24. Ward, T.J., Clear, R.M., Rooney, A.P., O'Donnell, K., Gaba, D., Patrick, S., Starkey, D.E., Gilbert, J., Geiser, D.M. & Nowicki, T.W. 2008. An adaptive evolutionary shift in Fusarium head blight pathogen populations is driving the rapid spread of more toxigenic *Fusarium graminearum* in North America. *Fungal Genetics and Biology*, 45: 473-484.

25. Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. & Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Asid Research*, 18(22): 6531-6535.

26. Yli-Mattila, T., Gagkaeva, T., Ward, T.J., Aoki, T., Kıstler, H.C. & O'Donnell, K. 2009. A Novel Asian clade within the *Fusarium graminearum* species complex includes a newly discovered cereal head blight pathogen from the Russian Far East. *Mycologia*, 101: 841-852.

27. Yli-Mattila, T., Hietaniemiv, R., Hussientcarlobos-Lopez, A. & Cumagun, C.Jr. 2013. Molecular quantification and genetic diversity of toxigenic *Fusarium* species in Northern Europe as compared to those in Southern Europe. *Microorganisms*, 1: 162-174.

28. Yörük, E., Tunali, B., Kansu, B., Ölmez, F., Uz, G., Zümrüt, I.M., Sarıkaya, A. & Meyva, G. 2016. Characterization of high-level deoxynivalenol producer *Fusarium graminearum* and *F. culmorum* isolates caused head blight and crown rot diseases in Turkey. *Journal of Plant Diseases and Protection*, 123: 177-186.

29. Yörük, E. & Sefer, Ö. 2017. Polymorphisms in *FgMgv1*, *FgStuA* and *FgVeA* genes associated with growth of *Fusarium graminearum*. Pp. 358-366. In: Arapgirlioğlu, H., Atık, A., Elliott, R.L. & Turgeon E. (eds), *Researches on science and art in 21st century Turkey*, Gece Kitaplığı, Ankara Volume 1, 364 pp.

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Research Article

EPILITHIC DIATOM-BASED ECOLOGICAL ASSESSMENT IN TAŞMANLI POND (SINOP, TURKEY)

Fatih GÜMÜŞ[1*], Arif GÖNÜLOL[2]

[1] Sinop University, Faculty of Art and Science, Department of Biology, Sinop, TURKEY
[2]Ondokuz Mayıs University, Faculty of Art and Science, Department of Biology, Samsun, TURKEY
*Corresponding author: e-mail: lakustrin@gmail.com

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Abstract: The common goal of the Water Framework Directive (WFD) published by The European Parliament and European Council and the Regulation on the Management of Surface Water Quality Directive (RMSWQ) published by the Republic of Turkey, Ministry of Forestry and Water Affairs, is to provide all water bodies to achieve a fine ecological status. These directives require the use of relevant biological quality elements and diatoms constitute an important group within these elements. The use of diatom based indices for assessing ecological conditions in inland water systems is increasing day by day. The current study was performed with the aim of determination of the ecological status of the Taşmanlı Pond based on epilithic diatoms, physico-chemical parameters and to estimate pond water quality using diatom indices. For this purpose, epilithic diatom and water samples were collected from 3 stations in the pond in monthly intervals from March 2008 to March 2009. The identifications of the sampled revealed presence of a total of 46 taxa in the sampled stations. *Achnanthidium minutissimum* (Kützing) Czarnecki, *Nitzschia acicularis* (Kützing) W. Smith, *Navicula cincta* (Ehrenberg) Ralfs in Pritchard and *Navicula cryptocephala* Kützing were the dominant taxa in all stations. According to Regulation on the Management of Surface Water Quality directive, the pond water was classified as III-IV quality class and trophic status changed from mesotrophic to eutrophic. Significant correlation was determined between LTDI (Lake Trophic Diatom Index) and EQR (Ecological Quality Ratio). The values of LTDI and EQR indicate that, pond water has class III (poor, moderately polluted site) water quality.

Key words: Epilithic, Diatom, LTDI, EQR, Pond, Taşmanlı, Sinop, Turkey.

Özet: Avrupa Parlementosu ve Avrupa Birliği tarafından yayınlanan Su Çerçeve Direktifi (WFD) ve Türkiye'de "Orman ve Su İşleri Bakanlığı, Su Yönetimi Genel Müdürlüğü" tarafından yayınlanan "Yüzey Suları Su Kalite Yönetmeliği"nin ortak hedefi, tüm su kütlelerinin iyi bir ekolojik statüye ulaşmasıdır. Bu yönetmelikler, amaca uygun biyolojik kalite öğelerini kullanmayı gerektirmektedir ve diyatomeler bu öğeler içerisinde oldukça önemli bir grubu oluşturmaktadır. Diyatome temelli indislerin içsu kütlelerinde ekolojik parametrelerin belirlenmesi amacı ile kullanımı günden güne artış göstermektedir. Bu çalışmada, Taşmanlı Göleti'nin ekolojik durumunun değerlendirilmesinde epilitik diyatomelerin, fizikokimyasal parametrelerin ve diyatome indislerinin ekolojik kalitenin belirlenmesinde, bir araç olarak test edilmesi amaçlanmıştır. Bu nedenle, Mart 2008 - Mart 2009 tarihleri arasında göletten alınan diyatome ve su örnekleri analizleri sonucunda, toplam 46 takson gözlenmiştir. Bunlardan *Achnanthidium minutissimum* (Kützing) Czarnecki, *Nitzschia acicularis* (Kützing) W.Smith., *Navicula cincta* (Ehrenberg.) Ralfs in Pritchard, *Navicula cryptocephala* Kützing tüm istasyonlardaki baskın türlerdir. Yapılan fizikokimyasal testler sonucu gölet suyu "Yüzeysel Su Kalitesi Yönetmeliği" ne göre III-IV kalite sınıfı olarak sınıflandırılmış ve trofik durumu mezotrofiden ötrofiye doğru değişmiştir. LTDI (Göl Trofik Diatom İndeksi) ve EQR (Ekolojik Kalite Oranı) değerleri gölet suyunun sınıf III (tamamen kirli alan) su kalitesine sahip olduğunu göstermektedir.

Introduction

Water is vital for any type of life on earth. The world contains aquatic and terrestrial ecosystems that are in very close contact with each other resulting in a delicate balance. The continuity of life is directly related to the continuity of this dynamic ecosystem interaction. Moreover, the continuity of socioeconomic inputs for humans is realized by the continuity of this system. Algae are primary producers of aquatic ecosystems and ensure the continuity of the systems they are involved in. This essential component creates the natural energy inputs of aquatic ecosystems and forms the most fragile link.

Aquatic systems, freshwater ecosystems in particular, are under a constant and strong pressure of human related activities (Gümüş 2010). Therefore, protection of natural water resources and the dominance of aquatic systems is one of the most important elements to be used in determination of future standard of life quality. Small physico-chemical changes in water initiate a chain

reaction that affects both algae and our life quality. Phytobenthos is one of the biological quality elements that must be contained in The Water Framework Directive (WFD, "Directive 2000/60/EC" The European Parliament and European Council 2000) assessments of ecological status of freshwaters and diatoms are good environmental indicators and are often the main component of phytobenthos (Della Bella *et al.* 2007). Although diatoms are one of the important components of phytobenthos in aquatic systems, only some countries have yielded WFD-compliant phytobenthos tools specifically for lakes (Bennion *et al.* 2014). Diatoms are also taken into account as key organisms used as biological indicators in ecological quality analysis. Diatom-based water quality evaluation is a new process for Turkey and the first studies have begun after 2000s. Some of these studies were performed in Antalya River basin (Kalyoncu 2002, Solak *et al.* 2007a, Kalyoncu *et al.* 2009), Büyük Menderes River basin (Barlas *et al.* 2001, 2002, Solak *et al.* 2007b), Fırat River basin (Gürbüz & Kıvrak 2002), Kızılırmak River basin (Akbulut *et al.* 2010), Sakarya River basin (Solak *et al.* 2009, Solak 2011) and Susurluk River basin (Dalkıran *et al.* 2008, Karacaoğlu *et al.* 2008, Solak *et al.* 2011, Solak & Acs 2011), showing that no study has been performed so far in Taşmanlı Pond on the subject of diatom-based water quality evaluation.

The present study was performed in order to determine the ecological status of Taşmanlı Pond based on epilithic diatoms and to estimate pond water quality using diatom indices.

Materials and Methods

Study Area

Taşmanlı Pond (41°54′N, 35°02′E) is located in the southern part of Sinop (Fig. 1) and covers an area of 222.000 square meters. The maximum depth of the pond is 15m and the minimum is 3-4m. The pond was constructed in 1977 by the General Directorate of State Hydraulic Works (DSI) for irrigation and it does not freeze in winter. The pond is free of industrial waste water discharge and juvenile common carp (*Cyprinus carpio* Linnaeus) were released a few times to the pond with the aim of breeding. Forest and scrub vegetation is observed in the drainage basin of the pond.

Sampling and Chemical Analysis

The samples were collected monthly from March 2008 to March 2009. Epilithic samples were collected from the stones by brushing, brought to the laboratory in plastic cups then preserved in 4% formalin solution. Diatoms were cleaned using hydrogen peroxide (H_2O_2) and diluted hydrochloric acid (3%) was added to purify calcium carbonate (Renberg 1990). After cleaning, the diatom frustules were washed with distilled water and rinsed. Clean diatom frustules were mounted in a synthetic resin (Naphrax). Diatom species were identified according to Krammer & Lange-Bertalot (1991a, 1991b, 1999a, 1999b), Round *et al.* (1990) and Sims (1996). Relative abundances of diatom species were evaluated by counting 300 valves per sample (Round 1953). Identified species were checked in algae databases and necessary taxonomic corrections were made (Guiry & Guiry 2017, Gönülol 2017). The author names were abbreviated according to Brummit & Powell (1992).

The surface water samples for physico-chemical analysis in laboratory were taken from all stations using plastic bottles. Environmental parameters such as dissolved oxygen of the water, temperature were measured in field with portable instruments.

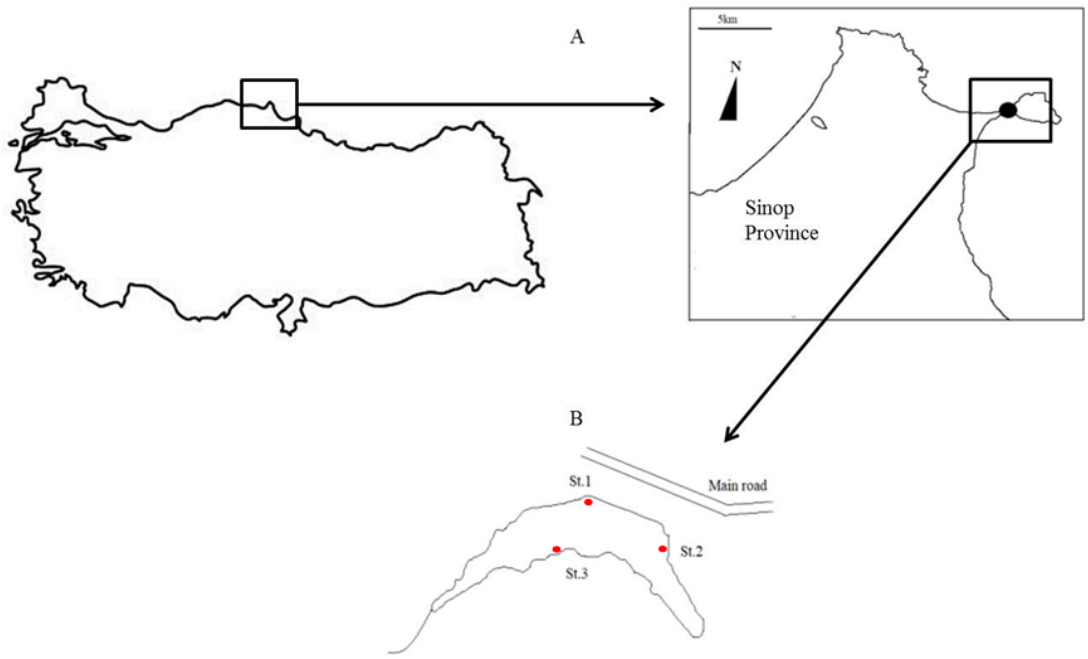

Fig. 1. A) The maps showing locations of Sinop in Turkey (on the top left) and Taşmanlı Pond in Sinop (on the top right) and B) the schematic representations of the locations of the stations in the pond.

Chemical analysis were carried out according to APHA protocols (APHA 1998) and contained nutrients and ions such as chloride, nitrates, alkalinity, silica and phosphates. The water conductivity and pH were measured using a portable digital pH meter (Hach®HQ40d). The temperature and the dissolved oxygen values –as environmental parameters- of the water samples were measured in the filed using a portable handheld dissolved oxygen and temperature system (YSI®55).

Data Analysis

The average values and standard errors of all physico-chemical analysis were computed using "Microsoft® Office Excel 2003". Diatom diversity "H´" (Shannon & Weaver 1949) and evenness "J´" (Pielou 1975) indexes were computed with Primer5 software (Clarke 1993). Trophic diatom indices were computed by two different softwares; i) Trophic Diatom Index for Lakes (TDIL) was computed with the DILSTORE software (Hajnal *et al.* 2009) and ii) Lake Trophic Diatom indices (LTDI) developed based on modification of the trophic diatom index (TDI) (Kelly & Whitton 1995) and Ecological Quality Ratio (EQR) were computed with the DARLEQII Software (WFD-UKTAG 2014).

Results

The identifications of the collected material showed that a total 46 taxa were present in the sampled stations of the pond (Table 1). The numerical distributions of the taxa with respect to the sampling months were given in Figure 2. *Achnanthidium minutissimum* (Kützing) Czarnecki, *Nitzschia acicularis* (Kützing) W.Smith, *Navicula cincta* (Ehrenberg) Ralfs in Pritchard and *Navicula cryptocephala* Kützing were the dominant taxa in all stations. The lowest nitrate value was measured in March 2008 with 1.17mgL^{-1} in St1 and the highest value was measured in August 2008 with 1.46mgL^{-1} in St2. The lowest phosphate value was measured in March 2009 in St2 with 0.14mgL^{-1} and the highest value was measured in August 2008 in St 1 with 2.97mgL^{-1}. The lowest value of silica was recorded in May 2008 in St3 with 0.09mgL^{-1} and the highest value was measured in March 2009 in St1 with 3.10mgL^{-1}. Total alkalinity ranged from 12.20 to 109mgL^{-1} $CaCO_3$. Maximum, minimum and average values and the standard deviations of measured physico-chemical variables were given in (Table 2). The lowest values of Shannon-Weaver and Evenness indices for all three stations were calculated in May. Although the numbers of sampled individuals for the identified taxa were higher than other months, all stations were represented with a less diverse species moiety in May. In particular, *Pantocsekiella ocellata* and *Achnanthidium minutissimum* reached the dominant position by reaching their highest numbers in this month. This is also consistent with the low index values observed in May. Diversity (H') and evenness (J') indices and species richness were shown graphically in Fig. 2.

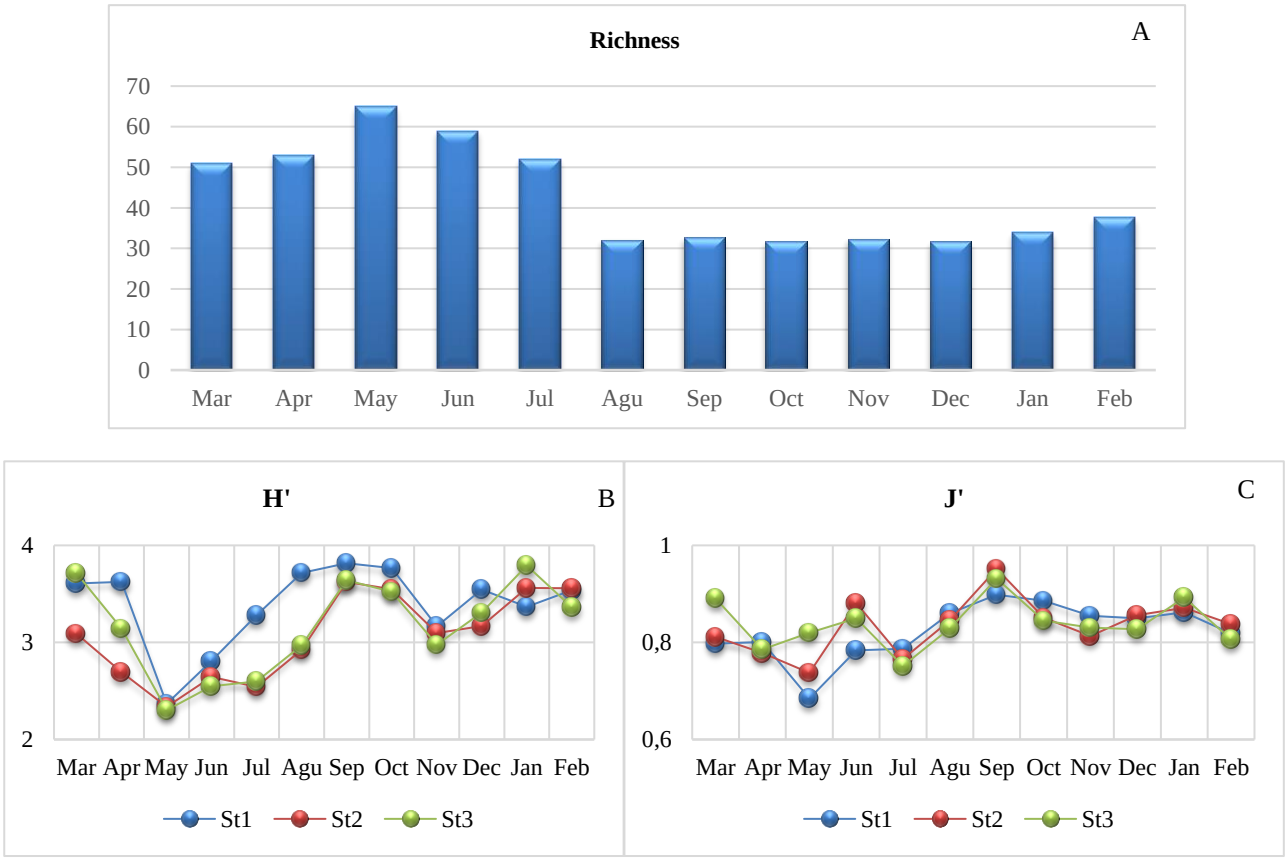

Fig. 2. Graphical representation of A) species richness and B) diversity and C) evenness indexes. The sampling months were given as abbreviations.

Table 1. Epilithic diatom list of Taşmalı Pond.

Phylum: Bacillariophyta
Class: Bacillariophyceae
Order: Mastogloiales
Achnanthes brevipes C.Agardh
Achnanthes coarctata (Bréb. ex W.Sm.) Grunow
Mastogloia baltica Grunow

Order: Cocconeidales
Achnanthidium exiguum (Grunow) Czarnecki
Achnanthidium minutissimum (Kütz.) Czarnecki

Order: Bacillariales
Hantzschia amphioxy (Ehrenb.) Grunow
Nitzschia acicularis (Kütz.) W.Sm.
Nitzschia dissipata (Kütz.) Rabenh.
Nitzschia gracilis Hantzsch
Nitzschia palea (Kütz.) W.Sm.
Nitzschia recta Hantzsch ex Rabenh.
Nitzschia sigmoidea (Nitzsch) W.Sm.

Order: Cymbellales
Cymbella affinis Kütz.
Cymbella sp.
Encyonema silesiacum (Bleisch) D.G.Mann
Gomphonema affine Kütz.
Gomphonema gracile Ehrenb.
Gomphonema parvulum (Kütz.) Kütz.
Placoneis clementis (Grunow) E.J.Cox

Order: Fragilariales
Fragilaria capucina Desmaz.
Fragilaria capucina var. *gracilis* (Oestrup) Hustedt

Order: Licmophorales
Ulnaria ulna (Nitzsch) Compère

Order: Naviculales
Craticula halophila (Grunow) D.G.Mann
Diploneis parma Cleve
Gyrosigma acuminatum (Kütz.) Rabenh.
Halamphora veneta (Kütz.) Levkov
Luticola mutica (Kützing) D.G.Mann
Luticola nivalis (Ehrenb.) D.G.Mann
Luticola ventricosa (Kütz.) D.G.Mann
Navicula cari Ehrenb.
Navicula cincta (Ehrenb.) Ralfs
Navicula cryptocephala Kütz.
Navicula rhynchocephala Kütz.
Navicula trivialis Lange-Bert.
Navicula veneta Kütz.
Neidium dubium (Ehenb.) Cleve
Sellaphora pupula (Kütz.) Mereschk.

Order: Surirellales
Surirella amphioxys W.Sm.
Surirella angusta Kütz.
Surirella librile (Ehrenb.) Ehrenb.
Surirella minuta Bréb. ex Kütz.
Surirella ovalis Bréb.

Order: Thalassiophysales
Amphora ovalis (Kütz.) Kütz.

Class: Mediophyceae
Order: Stephanodiscales
Cyclotella meneghiniana Kütz.
Cyclotella sp.
Pantocsekiella ocellata (Pant.) Kiss & Acs

According to the Regulation on the Management of Surface Water Quality Directives "Republic of Turkey, Ministry of Forestry and Water Affairs, 2015", the water bodies of all stations in Spring/Winter and Summer/Fall seasons have been identified as Class III and Class IV, polluted areas respectively. According to Trophic Classification System Limit Values in the directive, the water body of Taşmanlı Pond is in the limit values from mesotrophic to eutrophic waters. Index values of measured based on the sampled epilithic diatoms were given Table 3.

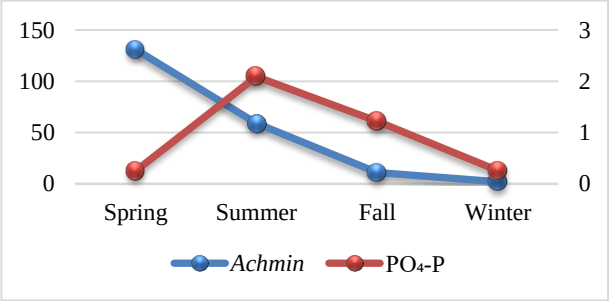

Fig. 3. The relationship between phosphate (PO_4-P) values determined in the pond with respect to seasons and the abundance of *Achnanthidium minutissimum* (Achmin).

Table 2. The minimum, maximum and average values of the measured physic-chemical parameters of surface water in all sampling stations.

Parameter	Min.	Max.	Average
Dissolved O_2 (mgL^{-1})	5.40	15.62	9.88 ± 3.32
pH	7.28	9.06	8.14 ± 0.56
Alkalinity (mgL^{-1})	12.20	109.80	49.26 ± 0.62
Chloride (mgL^{-1})	46.40	72.70	59.24 ± 7.18
$Temperature_W$ (°C)	6.00	26.00	15.00 ± 7.37
Nitrate (mgL^{-1})	0.17	1.46	0.52 ± 0.44
Phosphate (mgL^{-1})	0.14	2.97	0.98 ± 0.12
Silica (mgL^{-1})	0.09	3.10	0.85 ± 0.47

Table 3. Index values for the sampling stations.

	St.1	St.2	St.3
Trophic Diatom Index for Lakes (TDIL)	1.38	1.42	1.48
Lake Trophic Diatom Index (LTDI)	76.57	80.17	75.21
Ecological Quality Ratio (EQR)	0.36	0.31	0.38

Table 4. Ecological Quality Ratio (EQR) class boundaries for the 3 alkalinity types (Bennion *et al.* 2014). LA: low alkalinity, MA: medium alkalinity, HA: high alkalinity. H: high, G: good, M: moderate, P: poor, B: bad ecological status.

Alkalinity type (Annual mean $CaCO_3$ mgL^{-1})	H/G	G/M	M/P	P/B
LA (<10)	0.92	0.70	0.46	0.23
MA (10-50)	0.95	0.70	0.46	0.23
HA (>50)	0.92	0.70	0.46	0.23

Table 5. Class boundaries and trophic status according to TDIL.

Class boundary	Ecological status
4 – 5	Excellent
3 < 4	Good
2 < 3	Medium
1 < 2	Tolerable
0 < 1	Bad

Discussion

Achnanthidium minutissimum (Kütz.) Czarnecki, was described as the most common and abundant diatom in well-oxygenated, neutral or alkaline lakes and streams with low or moderate concentrations of nutrients and organic pollution. It can also be present in high numbers in streams exposed to heavy metal pollution and in eutrophic inland waters (Bennion *et al.* 2014, Stenger-Kovács *et al.* 2007, Kelly *et al.* 2005). Some former studies suggested that taxa in the *A. minutissimum* complex can be assigned to morphological groups, each with differing ecological preferences though these morphological groups are not discontinuous (Potapova and Hamilton 2007). However, palaeoecological works indicates that a slightly change in planktonic diatoms suggest the early stages of nutrient enrichment rather than changes in non-planktonic diatoms (Bennion *et al.* 2004). Indeed, as revealed in the present study, *A. minutissimum* became a dominant species in spring months when the pond water began to be enriched with nutrients (Fig. 3). *Cyclotella meneghiniana* Kütz., *Gyrosigma acuminatum* (Kütz.) Rabenh., *Luticola mutica* (Kütz.) D.G.Mann, *Sellaphora pupula* (Kütz.) Mereschk., *Navicula rhynchocephala* Kütz., *N. cryptocephala* Kütz., *N. veneta* Kütz., *N. trivialis* Lange-Bert., *N. cincta* (Ehrenb.) Ralfs, *Gomphonema parvulum* (Kütz.) Kütz., *Nitzschia palea* (Kütz.) W.Smith, *N. gracilis* Hantzsch, and *N. acicularis* (Kütz.) W.Smith were detected as constant species of Taşmanlı pond. According to the literature the species listed above were pollution-tolerant and they were found in eutrophic and mesotrophic waters (Taylor *et al.* 2007, Kelly *et al.* 2005, Van Dam *et al.* 1994, Hall *et al.* 1999).

The common goal of both WFD and RMSWQT is to obtain water bodies with better ecological status. According to RMSWQT directives, the pond water were classified as III-IV quality class and the trophic status change from mesotrophic to eutrophic. Significant correlation was determined between LTDI (Lake Trophic Diatom Index) and EQR (Ecological Quality Ratio). The values of TDIL, LTDI and EQR indicate that the pond water have class III (poor, moderately polluted site) water quality. Consequently, it can be said that The Taşmanlı Pond is under pollution effect and diatom community content is closely related with water quality. Therefore, the epilithic diatom metrics can be used in monitoring in Taşmanlı Pond.

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References

1. Akbulut, N. Akbulut, A. Günsel, S. and Solak, C.N. 2010. *Use of epilithic diatoms to evaluate water quality of Kızılırmak River.* Hacettepe University Fund, No: 0701601006, final report [in Turkish].
2. Anonymous. 2000. Water Framework Directive (WFD). Directive 2000/60/EC, The European Parliament and European Council.
3. Anonymous. 2015. Regulation on the Management of Surface Water Quality in Turkey (RMSWQT). Republıc of Turkey, Ministry of Forestry and Water Affairs.
4. APHA.1998. *Standard Methods for the Examination of Water and Wastewater*. 20th ed., Washington.
5. Barlas, M. Mumcu, M.F. Dirican, S. and Solak, C.N. 2001. Sarıçay (Milas)'da yaşayan epilitik diyatomelerin su kalitesine bağlı olarak incelenmesi, 5-8 Ekim 2001 Bodrum.IV. Ulusal Ekoloji ve Çevre Kongresi.
6. Barlas, M. Mumcu, M.F. Solak, C.N. and Çoban, O. 2002. Akçapınar Deresi ve Kadın Azmağı Deresi (Muğla) epilitik alg florasının su kalitesine bağlı olarak incelenmesi. 4-7 Eylül 2002 Malatya VI. Ulusal Biyoloji Kongresi.
7. Bennion, H., Fluin, J. & Simpson, G. L. 2004. Assessing Eutrophication and Reference Conditions for Scottish Freshwater Lochs Using Subfossil Diatoms. *Journal of Applied Ecology*, 41: 124-138.
8. Bennion, H., Kelly, M. G., Juggins, S., Yallop, M. L., Burgess, A., Jamieson, J. & Krokowski, J. 2014. Assessment of Ecological Status in UK Lakes Using Benthic Diatoms. *Freshwater Science*, 33(2): 639-654.
9. Brummitt, R.K. & Powell, C.E. 1992. *Authors of Plant Names. A List of Authors of Scientific Names of Plants, With Recommended Standard Forms of Their Names, İncluding Abbreviations*. Royal Botanic Gardens, Kew: Pp [4],732 p. ISBN: 0-947643-44-23.
10. Clarke, K.R. 1993. Non-Parametric Multivariate Analyses of Changes in Community Structure. *Australian Journal of Ecology*, 18: 117-143.
11. Dalkıran, N. Karacaoğlu, N. Dere, Ş. and Şentürk, E. 2008. Orhaneli Çayı'nın Kirlilik Düzeyinin Diyatomelere Dayandırılarak Saptanması. 23-27 Haziran, 19. Ulusal Biyoloji Kongresi Özet Kitapçığı, 228. sayfa, Trabzon.
12. Della Bella, V., Puccinelli, C., Marcheggiani, S. & Mancini, L. 2007. Benthic diatom communities and their relationship to water chemistry in wetlands of central Italy. In *Annales de Limnologie-International Journal of Limnology* (Vol. 43, No. 2, pp. 89-99). EDP Sciences.
13. Hall, R. I., Smol, J. P., & Smol, J. P. 1999. Diatoms as indicators of lake eutrophication. *The diatoms: applications for the environmental and earth sciences*, 128-168.
14. Gönülol, A. 2017. Turkishalgae Electronic Publication, Samsun, Turkey. http://turkiyealgleri.omu.edu.tr Date accessed: Jun 2017

15. Guiry, M.D. & Guiry, G.M. 2017. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. www.algaebase.org Date accessed: 15 Jun 2017, 16 p.

16. Gümüş, F. 2010. *Taşmanlı Göleti (Sinop) Kıyı Bölgesi Algleri Üzerine Nitel ve Nicel Araştırmalar.* Ondokuz Mayıs Üniversitesi, Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi, 48 p. Samsun.

17. Gürbüz, H. and Kıvrak, E. 2002. Use of epilithic diatoms to evaluate water quality in the Karasu River of Turkey. *J. Environmental Biology*, 23(3): 239-246.

18. Hajnal, É., Stenger-Kovács, C. & Padisák, J. 2009. DILSTORE Software for Ecological Status Assessment of Lakes Based on Benthic Diatoms. *Fottea*, 9: 351-354.

19. Kalyoncu, H. 2002. *Aksu Çayı'nın Fiziksel, Kimyasal ve Biyolojik Olarak İncelenmesi*. Doktora Tezi. S.D.Ü. Fen Bilimler Enstitüsü, Isparta, 155 s.

20. Kalyoncu, H. Çiçek, N.L. Akköz, C. and Yorulmaz, B. 2009. Comparative performance of diatom indices in aquatic pollution assessment. *African Journal of Agricultural Research*, 4(10): 1032-1040.

21. Karacaoğlu, N. Dalkıran, N. Dere, Ş. and Şentürk, E. 2008. Emet Çayı'nın Kirlilik Düzeyinin Diyatomelere Dayandırılarak Saptanması. 23-27 Haziran, 19. Ulusal Biyoloji Kongresi Özet Kitapçığı, 228. sayfa, Trabzon.

22. Kelly, M. G., Bennion, H., Cox, E. J., Goldsmith, B., Jamieson, J., Juggins, S., Mann, D.G. & Telford, R. J. 2005. *Common Freshwater Diatoms of Britain and Ireland: An İnteractive Key.* Environment Agency, Bristol.

23. Krammer, K. & Lange-Bertalot, H. 1991a. *Bacillariophyceae, Band, 2/3 3,. Teil :Centrales, Fragillariaceae, Eunotiaceae*. Gustav Fischer-Verlag, Stuttgart, 576 pp.

24. Krammer, K. & Lange-Bertalot, H. 1991b. *Süßwasserflora von Mitteluropa Bacillariophyceae, Band, 2/4 4,. Teil:Acnanthaceae, Kritische Erganzungen zu Navicula (Lineolatae) und Gomphonema Gesamtliteraturverzeichnis*. Gustav Ficher- Verlag, Stuttgart, 437 pp.

25. Krammer, K. & Lange-Bertalot, H. 1999a. *Süßwasserflora von Mitteluropa Bacillariophyceae, Band 2/1, 1.Teil :Naviculaceae*. Spectrum Academischer-Verlag, Heidelberg, Berlin.

26. Krammer, K. & Lange-Bertalot, H. 1999b. *Süßwasserflora von Mitteluropa Bacillariophyceae, Band 2/2, 2.Teil: Bacillariaceae, Epithemiaceae, Surirellaceae*. Spectrum Academischer -Verlag, Heidelberg, Berlin.

27. Kelly, M.G. & Whitton, B.A. 1995. The Trophic Diatom Index: A New İndex for Monitoring Eutrophication in Rivers. *Journal of Applied Phycology*, 7: 433-444.

28. Pielou, E.C. 1975. *Ecological Diversity*. John Wiley and Sons, New York, 165 p.

29. Potapova, M., and P. B. Hamilton. 2007. Morphological and ecological variation within the Achnanthidium minutissimum (Bacillariophyceae) species complex. Journal of Phycology 45:561-575.

30. Renberg, I. 1990. A Procedure for Preparing Large Sets of Diatom Slides from Sediment Cores. *Journal of Paleolimnol*ogy, 4: 87-90.

31. Round, F.E. 1953. An Investigation of Two Benthic Algal Communites in Malharm Tarn, Yorkshire. *Journal of Ecology*, 41:174-197.

32. Round, F.E., Crawford, R.M. & Mann, D.G. 1990. *The Diatoms: Biology and Morphology of the Genera*. Cambridge University Press, Cambridge, 746 p.

33. Shannon, C.E. & Weaver W. 1949. *The Mathematical Theory of Communication*. Univ. Of Illionis Press, Urbana, 144 p.

34. Sims P.A. (1996). *An Atlas of British Diatoms*. Biopress Ltd., England, 601 s.

35. Solak, C.N. 2009. *The Determination of Pollution in Felent Stream by using Some Aquatic Organisms.* Ph.D. Thesis, Dumlupınar University, 143pp [in Turkish].

36. Solak, C.N. 2011. The application of diatom indices in the Upper Porsuk River, Kütahya-Turkey. *Turkish Journal of Fisheries and Aquatic Sciences*, 11(1): 31-36.

37. Solak, C.N. Fehér, G. Barlas, M. and Pabuçcu, K. 2007a. Use of epilithic diatoms to evaluate water quality of Akçay Stream (Büyük Menderes River) in Mugla/Turkey. Archiv Für Hydrobiologie Suppl., 161 (3-4), Large Rivers, 17 (3-4): 327-338.

38. Solak, C.N. Ács, É. and Pabuçcu, K. 2007b. BDI (Biyolojik Diyatome İndeksi) İndeksine Göre Düden Şelalesi (Antalya) Su Kalitesinin Durumu. 10-13 Eylül 2007 Malatya 7. Ulusal Ekoloji ve Çevre Sempozyumu Özet Kitapçığı 184 s.

39. Solak, C.N. Àcs, É. and Dayioglu, H. 2009. The application of diatom indices in Felent Creek (Porsuk-Kütahya). *Diatomededlingen*, 33: 107-109.

40. Solak, C.N. & Ács, É. 2011. Water Qualıty Monıtorıng ın European and Turkısh Rıvers Usıng Diatoms. *Turkish Journal of Fisheries and Aquatic Sciences*, 11(2): 329-337.

41. Stenger-Kovács, C., Buczko, K., Hajnal, E. & Padisák J. 2007. Epiphytic, Littoral Diatoms as Bioindicators of Shallow Lake Trophic Status: Trophic Diatom Index for Lakes (TDIL) Developed in Hungary. *Hydrobiologia*, 589(1): 141-154.

42. Taylor, J.C., Harding, W.R. & Archibald, C.G.M. 2007. *An Illustrated Guide to Some Common Diatom Species from South Africa*. Pretoria: Water Research Commission, 225 p.

43. Van Dam, H., Mertens, A. & Sinkeldam, J. 1994. A coded checklist and ecological indicator values of freshwater diatoms from the Netherlands. *Aquatic Ecology*, 28(1): 117-133.

44. WFD-UKTAG, 2014. Phytobenthos – Diatoms for Assessing River and Lake Ecological Quality (DARLEQ2). Water Framework Directive – United Kingdom Technical Advisory Group (WFD-UKTAG), Scotland.

http://dergipark.gov.tr/trkjnat
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Research Article

MICROMORPHOLOGICAL AND ANATOMICAL CHARACTERS OF THE TURKISH ENDEMIC *Marrubium trachyticum* Boiss. (LAMIACEAE)

Tülay AYTAŞ AKÇİN*, Burcu CAMİLİ

Ondokuz Mayıs University, Faculty of Arts and Science, Department of Biology, 55139 Samsun, TURKEY
*Corresponding author: e-mail: aytasakcin@hotmail.com

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Abstract: In the present study, micromorphological characters of stem, leaf, calyx and mericarp of the Turkish endemic *Marrubium trachyticum* Boiss were investigated using scanning electron microscopy (SEM) and the anatomy of root, stem and leaf using light microscopy (LM). Stellate trichomes with unequal rays and branched hairs were observed on the stem, leaf and calyx. The distributions and densities of glandular trichomes on these vegetative organs were less than the eglandular trichomes. The mature mericarps of the species were ovate in shape and sculpturing pattern was penta-hexagonal colliculate. According to the anatomical results, *M. trachyticum* has secondary root structure. The stem has a distinct collenchyma layer. The stem is surrounded by oval or rectangular epidermal cells with a thick cuticle and is quadrangular in shape. The leaves are bifacial (dorsiventral) with anomocytic stomata in both the upper and the lower surfaces.

Key words: Anatomy, Lamiaceae, Scanning electron microscopy (SEM), Turkey.

Özet: Bu çalışmada, Türkiye için endemik bir tür olan *Marrubium trachyticum* Boiss.'un taramalı elektron mikroskobu (SEM) kullanılarak gövde, yaprak, kaliks ve fındıkçığının mikromorfolojik özellikleri ve ışık mikroskobu kullanılarak kök, gövde ve yaprak anatomisi araştırıldı. *M. trachyticum*'un gövde, yaprak ve kaliksi üzerinde eşit olmayan ışınlı stellat tüyler ve dallanmış tüyler gözlendi. Bununla beraber, *M. trachyticum*'un bu vejetatif organları üzerindeki salgı tüylerinin dağılışı ve yoğunluğu, örtü tüylerinden daha azdır. Türün olgun fındıkçıkları, ovat şekilliydi ve yüzey süslemesi penta-hexagonal colliculate (beşgen-altıgen şekilli kabartılı)'dir. Anatomik sonuçlara göre, araştırılan tür, sekonder kök yapısına sahiptir. Gövdenin belirgin bir kollenkima tabakasına sahip olduğu gözlendi. Gövde enine kesiti dört köşelidir ve ince bir kutikulaya sahip oval veya dikdörtgen epidermis hücreleri ile çevrilidir. *M. trachyticum*'un yaprakları bifasiyaldir (dorsiventral). Bu tür, yaprağın hem alt hem de üst yüzeyinde anomositik tip stomalara sahiptir.

Introduction

The genus *Marrubium* L. belongs to the Lamiaceae family and includes annual and perennial herbs. The species of the genus have an important distribution in Irano-Turanian and Mediterranean phytogeographic regions. The genus comprises about 40 taxa throughout the world (Hedge 1992) and is represented in Turkey by 21 taxa of which 12 are endemic (Cullen 1982, Davis *et al*. 1988, Ekim *et al.* 2000, Aytaç *et al*. 2012). It is thought that Turkey is the main centre of diversity for the genus *Marrubium* (Akgül & Ketenoğlu 2014). *M. trachyticum* Boiss., growing in steppe, slopes and fields inside the altitude range from 900 to 2500 m a.s.l. (Cullen 1982), is one of the endemic species of the genus in Turkey and has been included in the Red Data Book of Turkish Plants in near threatened (LR-nt) status (Ekim *et al.* 2000).

Marrubium has been used as a traditional medicine for asthma, pulmonary infections, inflammation, and hypotension and also as pain reliever (Meyre-Silva & Cechinel-Filho 2010). Furthermore, there are some reports on *Marrubium* species about their effects on reducing oxidative stress and inflammatory reactions due to their high amount of polyphenol and flavonoid contents (Yousefi *et al.* 2013, 2014). However, studies on *Marrubium* focused mainly on palynological, micromorphological and chemical features of the taxa studied (Akgül *et al.* 2008, Ahvazi *et al.* 2016, Kharazian & Hashemi 2017). Pollen and seed micromorphology generally support identifications of members of some genera such as *Marrubium* and *Stachys* L. (Abu-Assab & Cantino 1994).

Trichomes are the most useful taxonomic characters in the Lamiaceae family and taxonomical significance of trichome structure in this family is well known (Abu-Assab & Cantino 1987, Marin *et al.* 1994, Navarro & El Qualidi 2000, Kahraman *et al*. 2011, Celep *et al.* 2014, Atalay *et al.* 2016, Haratym & Werszko-Chmielewska

2017). Trichomes are widely distributed in the vegetative and reproductive parts of plants of Lamiaceae and distinguished as glandular and non-glandular trichomes (Cantino 1990, Navarro & El- Qualidi 2000). Glandular hairs are widely distributed over the aerial reproductive and vegetative organs of members of the family and their structures have been investigated in a number of studies (Bosabilidis 1990, Ascensao *et al.* 1999, Kaya *et al.* 2003). However, non-glandular trichomes are more common than glandular trichomes within Lamiaceae (Cantino 1990).

Studies on mericarp micromorphology in Lamiaceae have been a useful tool for classification within the family (Navarro & El Qualidi 2000, Dinç & Doğan 2006, Kaya & Dirmenci 2008). The usefulness of nutlet micromorphological characters in Lamiaceae taxonomy were also proved (Jamzad *et al.* 2000, Guerin 2005, Moon & Hong 2006, Kahraman *et al*. 2011). Furthermore, the importance of analysis based on Scanning Electron Microscopy (SEM) has been reported in many genera of the family (Budantsev & Lobova 1997, Jamzad *et al*. 2000, Kahraman *et al.* 2011, Satıl *et al.* 2012).

There exist many anatomical studies concerning Lamiaceae in Turkey (Kaya *et al.* 2000, Uysal 2003, Erken 2005, Celep *et al*. 2011). Although some of these studies are related with *Marrubium* species, detailed investigations and publications on anatomical and micromorphological characters of Turkish endemic *M. trachyticum* are very limited (Akgül 2004, Büyükkartal *et al.* 2016). The present study is the first comprehensive study of anatomy and micromorphology of this endemic species.

The aim of this study is to investigate the detailed characteristics of micromorphological structures of stem, leaf, calyx and mericarp of *M. trachyticum* using SEM and its anatomy of root, stem and leaf using light microscopy.

Materials and Methods

Plant samples were collected from May to July 2014 from natural populations in the vicinity of Bağcılı village in Çorum, Turkey. The specimens were dried according to standard herbarium techniques and stored in the Ondokuz Mayıs University, Faculty of Art and Science Herbarium (OMUB). The taxonomical descriptions of the plants were made according to Flora of Turkey (Cullen 1982).

Dried stem, leaf, calyx and mericarp samples were mounted directly on stubs using double-sided adhesive tape for SEM investigations. The stubs were coated with gold for 5 minutes and then observed and photographed in JEOL-JSM 7001 Scanning Electron Microscope. The terminology of Cantino (1990) and Koul *et al*. (2000) was followed for micromorphological investigations.

Anatomical investigations were performed using an average of 30 fresh specimens which were kept in 70% alcohol. Transverse sections of roots, stems and leaves and surface sections of leaves were used to make permenant/temporary slides and the slides were viewed and photographed using a Nikon Coolpix 5200 digital camera. All anatomical measurements were done in computer media based on the photographs with the help of the Image J program. Stomatal index was calculated according to the method described by Meidner & Mansfield (1968) for both surfaces of the epidermis.

Results

Micromorphological characteristics

Observation of stem using SEM showed that eglandular and glandular trichomes are present on stems of *M. trachyticum* (Fig. 1A-B). Lanate eglandular trichomes, in particular, are more abundant at the base of the stem. The head of glandular trichomes with short stalk are composed of a spheric cell (Fig. 1B).

Leaf surface is more or less densely covered with eglandular stellate trichomes and eglandular trichomes on the abaxial surface are longer than the adaxial surface (Fig. 1C-D). Glandular trichomes are rare on both side of leaf surfaces. The leaves of the investigated species are amphistomatous and with anomocytic stomata (Fig. 1C). The mean number of stomata per mm^2 of leaf surface is 15.01±3.01 on adaxial epidermis and 25.64±4.89 on the abaxial epidermis (Table 1). Stomata are more abundant on the abaxial side of the leaf. The upper and lower epidermis consist of cells with strongly sinous walls (Fig. 1C-D).

SEM observation showed that the throat of calyx has more greyish stellate trichomes (Fig. 1E). However, these trichomes are more sparsely on the calyx teeth (Fig. 1F). The distribution and density of the glandular trichomes on calyx are less than eglandular ones (Fig. 1E-F).

The mature mericarps are ovate in outline (1.01×2.05mm) and dark-brown in color (Fig. 1G). The sculpturing pattern of mature mericarps are penta-hexagonal colliculate (Fig. 1H).

Anatomical characteristics

Root anatomy

The root structure in *M. trachyticum* is more or less uniform. In cross-sections, the periderm layer on the outermost surface is multilayered (Fig. 2A). Cortex consists of 11-12 layers of oval or rectangular parenchymatous cells. Cambium and phloem cells are distinguishable. The thickness of phloem layer is 59.99±8.27µm (Table 2). The xylem consists of vessel members and tracheids. The xylem rays are composed of 7-8 rowed rectangular cells. The pith is completely filled with xylem elements.

Table 1. Stomata index of leaf of *M. trachyticum*.

Stomata index	Mean±SD
Upper leaf surface	15.01±3.01
Lower leaf surface	25.64±4.89

SD: Standard deviation

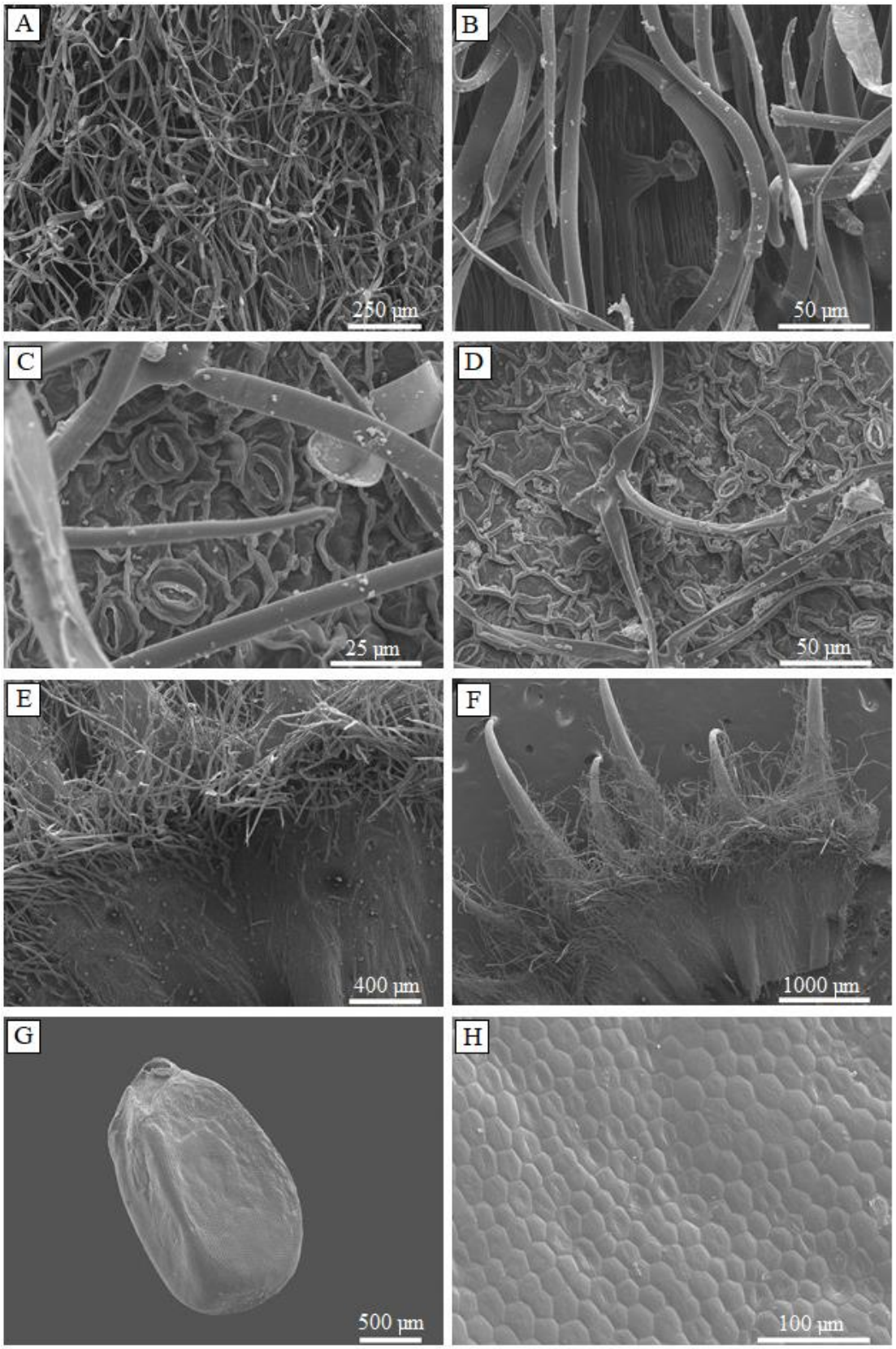

Fig. 1. Scanning electron micrographs (SEM) of *M. trachyticum*. A-Eglandular trichomes on stem. B-Glandular trichomes on stem. C-Upper surface of leaf. D-Lower surface of leaf. E-Trichomes on the calyx throat. F-Trichomes on the calyx teeth. G-General appearance of mericarps. H-Surface details of mericarps.

Stem anatomy

Cross-section of the stem is clearly quadrangular in shape. The epidermis consists of oval or rectangular cells and is covered by a thick cuticle. There are one-celled or multicellular non-glandular or glandular hairs on the epidermis (Fig. 2B). The collenchyma tissue consisting of 10-12 layers of ovoidal cells is located underneath the epidermis. The cortex tissue is composed of ovoidal and quadrangular cells with thin walls and the thickness of this layer is 130.70±23.31µm (Fig. 2B, Table 2). Beneath the cortex parenchyma, small groups of phloem sclerenchyma cells are located above the phloem and vascular cambium is indistinguishable. Xylem elements are thick-walled. The mean diameter of vessel elements is 20.20±4.35µm (Table 2). The pith consists of large and cylindrical parenchymatic cells and pith cells become smaller towards the central part of the stem (Fig. 2B).

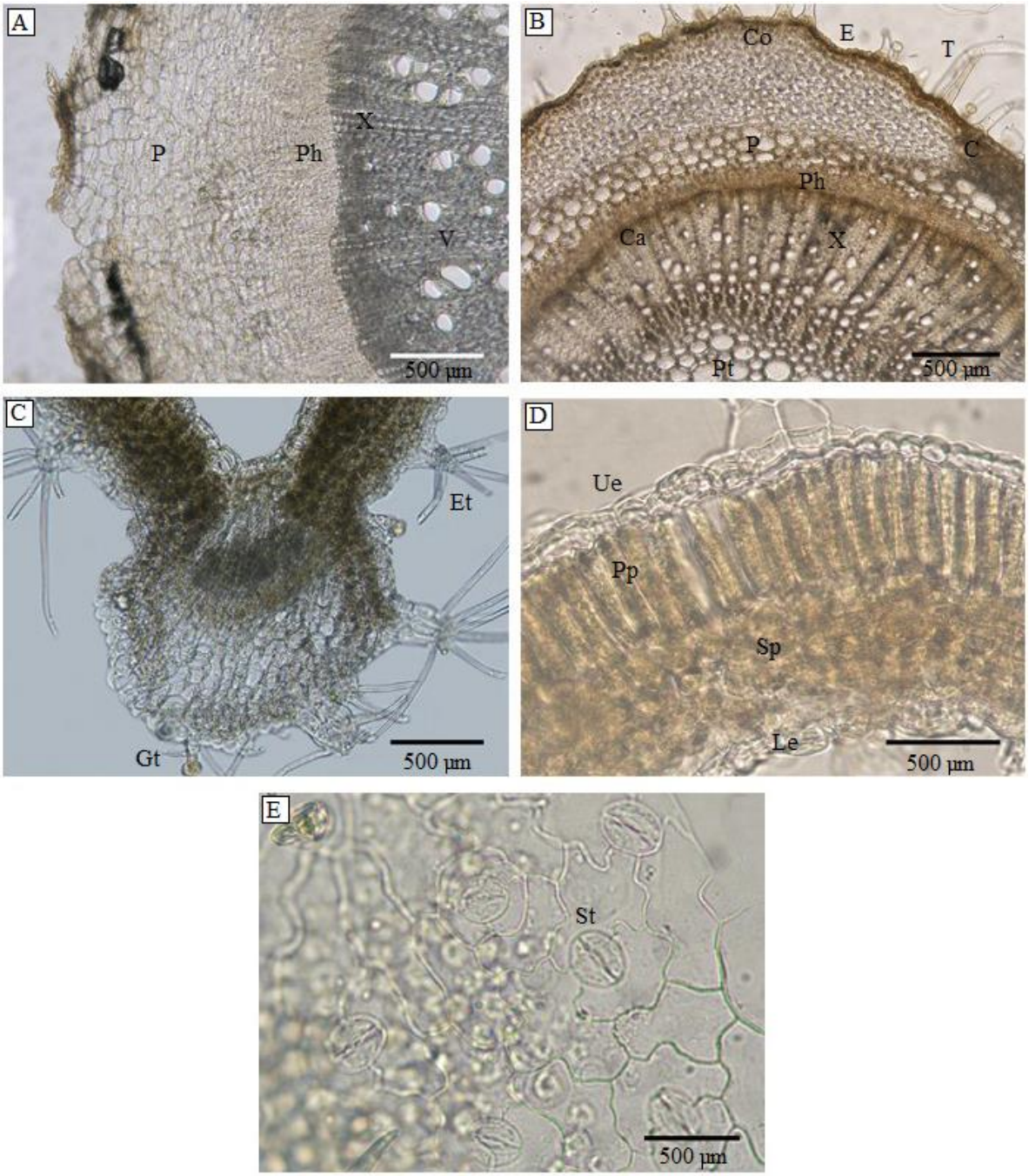

Fig. 2. Root, stem and leaf anatomical structures of *M. trachyticum*. A-Cross-sections of the root (A), the stem (B) and the leaf (C, D). E- Lower surface of the leaf. (C-cortex, Ca-cambium, Co-collenchyma, E-epidermis, Et-eglandular trichome, Gt-glandular trichome, Le-lower epidermis, P-parenchyma, Ph-phloem, Pp-palisade parenchyma, Pt-pith, Sp-spongy parenchyma, St-stomata, T-trichome, Ue-upper epidermis, V-vessel elements).

Table 2. Anatomical characteristics of *M. trachyticum*.

		Width (µm) (mean±SD)	Length (µm) (mean±SD)
Root	Thickness of periderm	36.46±6.56	-
	Parenchyma cells	23.65±5.97	15.77±3.94
	Thickness of phloem	59.99±8.27	-
	Thickness of xylem	443.34±27.42	-
	Diameter of vessels	24.68±7.76	-
Stem	Thickness of epidermis	71.79±2.91	-
	Parenchyma cells	20.22±3.99	12.60±2.87
	Thickness of cortex	130.70±23.31	-
	Thickness of collenchyma	163.17±33.31	-
	Thickness of phloem	49.11±12.77	-
	Thickness of xylem	114.42±40.50	-
	Diameter of vessels	20.20±4.35	-
	Pith cells	26.66±8.75	20.65±7.80
Leaf	Upper epidermis cells	9.81±2.28	6.93±0.97
	Thickness of palisade parenchyma	41.29±2.47	-
	Thickness of spongy parenchyma	39.71±2.56	-
	Lower epidermis cells	10.38±3.00	10.52±2.89
	Adaxial stomata	22.22±1.22	26.72±0.62
	Abaxial stomata	18.73±1.39	19.46±2.11

SD: Standard deviation

Leaf anatomy

In transverse sections, the upper and the lower epidermises comprise of uniseriate, quadrangular and oval cells. However, lower epidermal cells are larger than upper epidermis cells (Table 2). Both epidermises are covered with non-glandular and glandular hairs (Fig. 2C). 2-3 layers of collenchyma cells are present in the midrib located between the upper and the lower epidermis. The leaves are bifacial (dorsiventral) (Fig. 2D). Palisade tissue is composed of 1-2 layered cylindrical cells with e plenty of chloroplasts. Spongy parenchyma with large intercellular spaces are round or irregular in shape and the thickness of this layer is 39.71±2.56µm (Fig. 2D, Table 2). Vascular bundles are collateral type. In their surface views, the leaves are amphistomatic and epidermal cells have wavy walls (Fig. 2E). Adaxial stomata length and width is higher than the abaxial stomata dimensions (Table 2). The number of stomata per mm^2 in lower surface is higher than in upper surface (Table 1).

Discussion

In this study, micromorphological and anatomical properties of *M. trachyticum* studied by light and scanning electron microscopies were determined to be useful characters.

The taxonomic significance of trichome micromorphology in some members of Lamiaceae has already been evident (Dinç *et al*. 2009, Xiang *et al*. 2010, Kahraman *et al.* 2010, Osman 2012, Celep *et al*. 2014). This study shows that the main hair type in *M. trachyticum* is stellate with unequal rays and branched hairs. Stellate and branched hairs have been previously reported in some other genera of Lamiaceae such as *Stachys* L. (Salmaki *et al*. 2009), *Nepeta* L. (Jamzad 2001), *Lavandula* L. (Upson & Andrews 2004) and *Phlomis* L. (Azizian & Cutler 2008). Our findings are consistent with the results of Seyedi & Salmaki (2015) and Ahvazi *et al*. (2016) indicating that the stellate and branched hairs were found to be in Lamiaceae family members. However, in the present study, the distributions and densities of glandular trichomes on stem, leaf and calyx of *M. trachyticum* are less than the non-glandular trichomes. It was previously reported that non-glandular trichomes are more common than glandular trichomes in Lamiaceae (Cantino 1990, Osman 2012, Ahvazi *et al.* 2016).

Mericarp surface sculpturing patterns have diagnostic value for species recognition in some members of Lamiaceae (Jamzad *et al.* 2003, Kaya & Dirmenci 2008, Kahraman *et al*. 2010). Furthermore, the importance of the micromorphology of mericarp surfaces has been determined for *Marrubium* (Akgül 2004). Akgül *et al.* (2008) reported that the ornamentation surface in *Marrubium* species is verrucate and the seed shape in the Turkish *Marrubium* species can be related to their habitat. Similar results were also reported by Aytaç *et al*. (2012). According to the findings of our investigations, the mericarp surface in *M. trachyticum* is penta-hexagonal colliculate. Our results confirmed previous studies investigating the mericarp surface in *Marrubium* genus (Akgül *et al*. 2008, Aytaç *et al*. 2012).

Marrubium trachyticum was found to have the same general root anatomy characteristics as in other members of the Lamiaceae family. In root cross sections, the protective tissue was comprised of periderm and the center of the root was composed of tracheary elements. It was previously determined that the pith rays of some species within Lamiaceae, e.g. *Lamium lycium* Boiss. are 1-4 rowed while *Salvia chrysophylla* Stapf. has 1-24 rowed and *S. quezelii* Hedge & Afzal-Rafii has 1-3 (-4) rowed pith rays (Baran & Özdemir 2009, Kahraman *et al.* 2010, Celep *et al.* 2014). Root transverse sections showed that *M. trachyticum* has 7-8 rows of ray cells.

Watson & Dalwitz (1978) stated that stems of many members of the Lamiaceae family are quadrangular with well-defined collenchyma on each corners. This character was previously reported in some other *Marrubium* species (Akgül *et al*. 2008, Büyükkartal *et al*. 2016, Tüylü *et al.* 2017). A well developed multilayered collenchyma was distinguishable at the corners of the stem of *M. trachyticum*. Vessel elements were more distinct in stem. The pith cells were parenchymatic becoming smaller towards the central part of the stem.

Anatomical studies showed that the leaves of the *M. trachyticum* was bifacial (dorsiventral). Palisade parenchyma cells were presented in upper surface of the leaf and spongy parenchyma cells had large intercellular spaces in the lower surface. These leaf anatomy characteristics coincide with those previously reported by Akgül *et al.* (2008) and Büyükkartal *et al.* (2016) for some other *Marrubium* species. In leaf cross-sections, vascular bundles were collateral type in *M. trachyticum* and the phloem surrounded xylem. Our observation showed that *M. trachyticum* has amphistomatic leaves. Akgül (2004) and Tüylü *et al.* (2017) also reported that the leaves of the some other *Marrubium* species are amphistomatic. In surface sections of leaf, anomocytic type stomata were observed with higher numbers in abaxial surface of the leaf.

In conclusion, some characteristic micromorphological and anatomical features of *M. trachyticum* were reported in detail. The results presented here have the potential to contribute to further taxonomic studies of the genus.

References

1. Abu-Asab, M.S. & Cantino, P.D. 1994. Systematic implications of pollen morphology in subfamily Lamioideae and Pogostemonoideae (Labiatae). *Annals of the Missouri Botanical Garden*, 81(4): 653-686.
2. Abu-Asab, M.S. & Cantino, P.D. 1987. Phylogenetic implications of leaf anatomy in subtribe Melittidinae (Labiatae) and related taxa. *Journal of the Arnold Arboretum*, 68: 1-34.
3. Ahvazi, M., Jamzad, Z., Balali, G.R. & Saeidi, H. 2016. Trichome micro-morphology in *Marrubium* L. (Lamiaceae) in Iran and the role of environmental factors on their variation. *Iranian Journal of Botany*, 22(1): 39-58.
4. Akgül, G. 2004. The revision of the genus *Marrubium* L. (Lamiaceae) of Turkey PhD. thesis, Institute of Science and Technology, Ankara University, Ankara-Turkey.
5. Akgül, G. & Ketenoğlu, O. 2014. A new subpecies for Flora of Turkey, *Marrubium cephalanthum* Boiss. & Noe subsp. *montanum* Akgul & Ketenoğlu (Lamiaceae). *Ot Sistematik Botanik Dergisi*, 21(1): 21-28.
6. Akgül, G., Ketenoğlu, O., Pınar, N.M. & Kurt, L. 2008. Pollen and seed morphology of the genus *Marrubium* L. (Labiatae) in Turkey. *Annales Botanici Fennici*, 45: 1-10.
7. Ascensao, L., Mota, L. & Castro, M.D.M. 1999. Glandular trichomes on the leaves and flowers of *Plectranthus ornatus*: Morphology, distribution and histochemistry. *Annals of Botany*, 84(4): 437-447.
8. Atalay, Z., Celep, F., Bilgili, B. & Doğan, M. 2016. Pollen morphology of the genus *Lamium* (Lamiaceae) and its systematic implications. *Flora*, 219: 68-84.
9. Aytaç, Z., Akgül, G. & Ekici, M. 2012. A new species of *Marrubium* (Lamiaceae) from Central Anatolia, Turkey. *Turkish Journal of Botany*, 36: 443-449.
10. Azizian, D. & Cutler, D.F.F. 2008. Anatomical, cytological and phytochemical studies on *Phlomis* L. and *Eremostachys* Bunge (Labiatae). *Botanical Journal of the Linnean Society*, 85(4): 249-281.
11. Baran, P. & Özdemir, C. 2009. The morphological and anatomical properties of *Lamium lycium* (Lamiaceae) endemic to Turkey. *Nordic Journal of Botany*, 27: 388-396.
12. Bosabalidis, A.M. 1990. Glandular trichomes in *Satureja thymbra* leaves. *Annals of Botany*, 65: 71-78.
13. Budantsev, A.L. & Lobova, T.A. 1997. Fruit morphology, anatomy and taxonomy of Tribe Nepeteae (Labiatae). *Edinburgh Journal of Botany*, 54(2): 183-216.
14. Büyükkartal, H.N., Çölgeçen, H. & Akgül, G. 2016. Comparative leaf, stem and root anatomies of taxa *Marrubium bourgaei* and *Marrubium heterodon* (Lamiaceae). *Australian Journal of Crop Science*, 10(11): 1516-1522.
15. Cantino, P.D. 1990. The phylogenetic significance of stomata and trichomes in the Labiatae and Verbenaceae. *Journal of the Arnold Arboretum*, 71(3): 323-370.
16. Celep, F., Kahraman, A., Atalay, Z. & Doğan, M. 2011. Morphology, anatomy and trichome properties of *Lamium truncatum* Boiss. (Lamiaceae) and their systematic implications. *Australian Journal of Crop Science*, 5(2): 147-153.
17. Celep, F., Kahraman, A., Atalay, Z. & Doğan, M. 2014. Morphology, anatomy, palynology, mericarp and trichome micromorphology of the rediscovered Turkish endemic *Salvia quezelii* (Lamiaceae) and their taxonomic implications. *Plant Systematic and Evolution*, 300: 1945-1958.
18. Cullen, J. 1982. *Marrubium* L. In: Davis, P.H. (eds), *Flora of Turkey and the Aegean Islands*. Vol. 7. Edinburgh University Press, Edinburgh, 165-178.
19. Davis, P.H., Mill, R.R. & Kit, T. 1988. *Flora of Turkey and the East Aegean Islands*. Suppl. 1. Edinburgh University Press, Edinburgh, 202-203.
20. Dinç, M. & Doğan, H.H. 2006. *Stachys yildirimlii* Dinc (Lamiaceae), a new species from South Anatolia, Turkey. *Annales Botanici Fennici*, 43: 143-147.
21. Dinç, M., Pınar, N.M., Doğu, S. & Yıldırımlı, Ş. 2009. Micromorphological studies of *Lallemantia* L. (Lamiaceae) species growing in Turkey. *Acta Biologica Cracoviensia Series Botanica*, 51(1): 45-54.
22. Ekim, T., Koyuncu, M., Vural, M., Duman, H., Aytaç, Z. & Adıgüzel, N. 2000. *Türkiye Bitkileri Kırmızı Kitabı,* Van Y.Y. Üniversitesi Doğa Derneği, Barışcan Ofset, Ankara, 246 pp.

23. Erken, S. 2005. Morphological and Anatomical Studies on *Thymbra sintenisii* Bornm. & Aznav. (Labiatae). *Turkish Journal of Botany*, 29: 389-397.

24. Guerin, G.R. 2005. Nutlet morphology in *Hemigenia* R.Br. and *Microcorys* R.Br. (Lamiaceae). *Plant Systematic and Evolution*, 254(1/2): 49-68.

25. Haratym, W. & Weryszko-Chmielewska E. 2017. Ultrastructural and histochemical analysis of glandular trichomes of *Marrubium vulgare* L. (Lamiaceae). *Flora*, 231: 11-20.

26. Hedge, I.C. 1992. *A global survey the biogeography of the Labiatae*, Royal Botanic Gardens, Kew. Science, 7-17.

27. Jamzad, Z. 2001. *A phylogenetic study of Nepeta L. (Lamiaceae)*. Ph. D. thesis, Birkbeck College, University of London.

28. Jamzad, Z., Harley, M.M., Ingrouille, M., Simmonds, M.S.J. & Jalili, A. 2000. Pollen exine and nutlet surface morphology of the annual species of *Nepeta* L. (Lamiaceae) in Iran. Pp. 385-397. In: Harley M.M., Morton, G.M. & Blackmore, S. (eds). *Pollen and Spores: Morphology and Biology*. Royal Botanic Gardens, Edinburgh, 567 pp.

29. Jamzad, Z., Ingrouille, M. & Simmonds M.S.J. 2003. Three new species of *Nepeta* L. (Lamiaceae) from Iran. *Taxon*, 52(1): 93-98.

30. Kahraman, A., Celep, F. & Doğan, M. 2010. Anatomy, trichome morphology and palynology of *Salvia chrysophylla* Stapf (Lamiaceae). *South African Journal of Botany*, 76: 187-205.

31. Kahraman, A., Celep, F., Doğan, M., Guerin, G.R. & Bagherpour, S. 2011. Mericarp morphology and its systematic implications for the genus *Salvia* L. section Hymenosphace Benth. (Lamiaceae) in Turkey. *Plant Systematic and Evolution*, 292(1/2): 33-39.

32. Kaya, A. & Dirmenci, T. 2008. Nutlet surface micromorphology and taxonomy of species of the genus *Nepeta* L. (Lamiaceae) in Turkey. *Turkish Journal of Botany*, 32: 103-112.

33. Kaya, A., Başer, K.H.C., Satıl, F. & Tümen, G. 2000. Morphological and anatomical studies on *Cyclotrichium origanifolium* (Labill.) Manden and Scheng. (Labiatae). *Turkish Journal of Botany*, 24: 273-278.

34. Kaya, A., Demirci, B. & Başer, K.H.C. 2003. Glandular trichomes and essential oils of *Salvia glutinosa* L. *South African Journal of Botany*, 69: 422-427.

35. Kharazian, N. & Hashemi, M. 2017. Chemotaxonomy and morphological studies in five *Marrubium* L. species in Iran. *Iran Journal of Science Technology Transactions A: Science*, 41: 17-31.

36. Koul, K.K., Ranjna, N. & Raina, S.N. 2000. Seed coat microsculturing in *Brassica* and allied genera (subtribes Brassicinae, Raphaninae, Moricandiinae). *Annals of Botany*, 86: 385-397.

37. Marin, D.P., Petkovic, B. & Duletic, S. 1994. Nutlet sculpturing of selected *Teucrium* species (Lamiaceae): a character of taxonomic significance. *Plant Systematic and Evolution*, 192: 199-214.

38. Meidner, H. & Mansfield, T.A. 1968. *Physiology of Stomata*, McGraw-Hill, London, 178 pp.

39. Meyre-Silva, C.H. & Cechinel-Filho, V. 2010. A review of the chemical and pharmacological aspects of the genus *Marrubium*. *Current Pharmaceutical Design*, 16: 3503-3518.

40. Moon, H.K. & Hong, S.P. 2006. Nutlet morphology and anatomy of the genus *Lycopus* (Lamiaceae: Menthae). *Journal of Plant Research*, 119(6): 63-64.

41. Navarro, T. & El Qualidi, J. 2000. Trichome morphology in *Teucrium* L. (Labiatae). A taxonomic review. *Annales de Jardin Botanico de Madrid*, 57(2): 277-297.

42. Osman, A.K. 2012. Trichome micromorphology of Egyptian *Ballota* (Lamiaceae) with emphasis on its systematic implication. *Pakistan Journal of Botany*, 44(1): 33-46.

43. Salmaki, Y., Zarre, S.H., Jamzad, Z. & Brauchler, C. 2009. Trichome micromorphology of Iranian *Stachys* (Lamiaceae) with emphasis on its systematic implication. *Flora*, 204: 371-381.

44. Satıl, F., Kaya, A., Akcicek, E. & Dirmenci, T. 2012. Nutlet micromorphology of Turkish *Stachys* sect. Eriostomum (Lamiaceae) and its systematic implications. *Nordic Journal of Botany*, 30: 352-364.

45. Seyedi, Z. & Salmaki, Y. 2015. Trichome morphology and its significance in the systematic of *Phlomoides* (Lamiaceae; Lamioideae; Phlomideae). *Flora*, 213: 40-48.

46. Tüylü, M., Büyükkartal, H.N., Akgül, G. & Kalyoncu, H. 2017. *Marrubium lutescens* Boiss. ve *M. cephalanthum* Boiss. & Noe subsp. *akdaghicum* (Lamiaceae)'un gövde ve yaprak özelliklerinin anatomik olarak karşılaştırılması. *Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, 21(1): 113-117.

47. Upson, T.M. & Andrews, S. 2004. *The Genus Lavandula*. *A Botanical Magazine Monograph.* Royal Botanical Gardens, Kew, London, 442 pp.

48. Uysal, İ. 2003. *Stachys thirkei* Koch (Kekikgiller) türünün morfolojisi, anatomisi ve ekolojisi üzerinde araştırmalar. *Ot Sistematik Botanik Dergisi*, 10: 129-141.

49. Xiang, C.L., Dong, Z.H., Peng, H. & Liu, Z.W. 2010. Trichome micromorphology of the East Asiatic genus *Chelonopsis* (Lamiaceae) and its systematic implications. *Flora*, 205: 434-441.

50. Watson, L. & Dallwitz, M.T. 1978. *The families of flowering plants*. Oxford University Press, London.

51. Yousefi, K., Soraya, H., Fathiazad, F., Khorrami, A., Hamedeyazdan, S., Maleki-Dizaji, N. & Garjani, A. 2013. Cardioprotective effect of methanolic extract of *Marrubium vulgare* L. on isoproterenol-induced acute myocardial infarction in rats. *Indian Journal of Experimental Biology*, 51(8): 653-660.

52. Yousefi, K., Fathiazad, F., Soraya, H., Rameshrad, M., Maleki-Dizaji, N. & Garjani, A. 2014. *Marrubium vulgare* L. methanolic extract inhibits inflammatory response and prevents cardiomyocyte fibrosis in isoproterenol-induced acute myocardial infarction in rats. *Bioimpacts*, 4(1): 21-27.

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Research Article

Poecilimon zonatus Bolívar (ORTHOPTERA, TETTIGONIIDAE) REVISITED: GENETIC DATA REVEALED TWO NEW SPECIES AND ONE NEW SUBSPECIES

Sarp KAYA

Vocational School of Health Services, Mehmet Akif Ersoy University, Burdur, TURKEY
Corresponding author: e-mail: sarpkaya@mehmetakif.edu.tr

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Abstract: *Poecilimon* Fischer (Orthoptera, Tettigoniidae) is the most species-rich genus of the respective order and family in Anatolia. However, intrageneric diversity still needs to be documented. *Poecilimon zonatus* Bolívar is a species with a relatively wide range of distribution in Anatolia compared to other congeners. The present study aims to examine the diversity of *P. zonatus* using an integrated approach. The diversity of the species was documented using data based on DNA sequences, male calling song and morphology. Automatic species delimitation tests applied to genetic data revealed a cryptic diversity in *P. zonatus* complex and suggested two new species and one new subspecies. The song data supported the new species, but morphology remained less productive in this respect. The new taxa defined in the light of the obtained data are; *Poecilimon isozonatus* sp. n., *Poecilimon ciplaki* sp. n. as *Poecilimon ciplaki ciplaki* subsp. n. and *Poecilimon ciplaki denizliensis* subsp. n. These taxa together with *P. zonatus* were considered a species complex within *P. zonatus* group. The phylogenetic tree produced from *nad2* gene sequences supported the following relationships of *P. tauricola* + (*P. ciplaki* + (*P. zonatus* +*P. isozonatus*)) relationship.

Key words: Anatolia, *Poecilimon isozonatus* sp. n., *Poecilimon ciplaki* sp. n., *Poecilimon ciplaki ciplaki* subsp. n., *Poecilimon ciplaki denizliensis* subsp. n., phylogeny.

Özet: *Poecilimon* Fischer (Orthoptera, Tettigoniidae), dahil olduğu takım ve familyanın Anadolu'da tür sayısı en fazla olan cinstir. Ancak cins içi çeşitlilik halen ortaya konmayı beklemektedir. Cinsin diğer türleri ile karşılaştırıldığında *Poecilimon zonatus* Bolívar Anadolu'da nispeten geniş yayılışa sahip bir türdür. Bu çalışma entegre bir yaklaşımla türün çeşitliliğini saptamayı amaçlamaktadır. DNA dizileri, erkek çağrı sesi ve morfolojiden üretilen verilerle *P. zonatus* türünün çeşitliliği tanımlanmıştır. Genetik verilere uygulanan otomatik tür sınırları testleri *P. zonatus*'un kriptik bir çeşitlilik içerdiğini ve iki yeni tür ile bir yeni alttürün varlığına işaret etmiştir. Erkek çağrı sesi verileri yeni türleri desteklemiştir. Ancak, bu açıdan morfoloji daha az kullanışlı olmuştur. Elde edilen bu veriler ışığında tanımlanan yeni taksonlar şunlardır; *Poecilimon isozonatus* sp. n. *Poecilimon ciplaki* sp. n. ve *Poecilimon ciplaki ciplaki* subsp. n. ve *Poecilimon ciplaki denizliensis* subsp. n. Bu yeni taksonlar *P. zonatus* ile birlikte *P. zonatus* kompleksi olarak tanımlanmış ve bu kompleksin *P. zonatus* tür grubu içinde yer aldığı sonucuna varılmıştır. *nad2* gen dizilerinden üretilen filogenetik ağaç *P. tauricola* + (*P. ciplaki* + (*P. zonatus* +*P. isozonatus*)) akrabalık ilişkisini desteklemiştir.

Introduction

Poecilimon Fischer is one of the largest genera of Phaneropterinae by its species number running into 130 (Cigliano *et al.* 2018). Most of these species were arranged into 19 species groups, but 18 species still have not been assigned to any of the known groups (Cigliano *et al.* 2018). Ramme (1933), the first who arranged *Poecilimon* in groups, listed eight species in Group III and proposed *P. zonatus* Bolivar, 1899 and *P. varicornis* (Haan, 1843) to be closely related among others. Another related species is *P. variicercis* Miram. Ramme (1951) in the original description of *P. tauricola* compared it with *P. zonatus* and *P. varicornis*, but he did not mention *P. variicercis*. Cigliano *et al.* (2018) reported that *Poecilimon zonatus* species group included *P. zonatus*, *P. variicercis*, and *P. tauricola*, but not *P. varicornis*. Ullrich *et al.* (2010) presented a phylogeny of Barbitistini based on two genetic markers and both of the trees supported monophyly of the group, but *P. variicercis* was not among the species studied. Among the taxa listed above, *P. zonatus* has by far the widest range, but animals from different parts within the range of the species exhibit differences supporting the idea that a complex of taxa are included under this name (Çıplak *et al.* 1996, Ünal 2010, Sevgili *et al.* 2012) and thus, need to be examined more in detail.

Although the species number of the genus *Poecilimon* is high, its range size is comparatively narrow. Vast majority of the species are known from Anatolia and Greece/Balkans and only a few species occur outside this core area (La Greca 1999). Based Çıplak (2004) suggested, considering such distributional pattern of members of the genus, that the genus originated from an ancestral stock within the historical Aegeid Plate. Diversity in ecological preferences of generic members seems to be another factor promoting intra-generic diversity (Kaya *et al*. 2015). Some intra generic lineages are confined to lowlands while some others to mountain chains. Anatolia harbours a pronounced diversity of the genus (Çıplak 2008). *Poecilimon zonatus* is a species endemic to Anatolia and its distribution is mostly associated with highlands. The distribution pattern indicates that both tectonic evolution of the area and the ecological diversity provided by highlands may have played as evolutionary drivers leading to radiation of the *P. zonatus* species complex (Çıplak 2004, 2008, Kaya *et al*. 2015). Inferring from this pattern, we concluded that further localities in Anatolia should be studied, which in turn allowed us to detect an extra diversity of the species group, especially throughout Taurus Mountain range along Mediterranean Anatolia.

The results of the studies on the species group was presented and new taxa belonging to *P. zonatus* group were described by providing necessary illustrations, presenting male calling songs for a better description of the new taxa and diagnosing them. The study, on the other hand, does not aim a detailed phylogeny or phylogeography of the species group. During our ongoing studies, DNA sequence data were obtained indicating a cryptic diversity in *P. zonatus* s. str. (*P. zonatus* complex here after). Thus, a sufficient number of sequences per population was analysed by applying basic phylogenetic analyses and automatic species delimitation tests applied to detect independent evolutionary or reproductive units.

Materials and Methods

Sampling, molecular studies, phylogenetic analyses and automatic species delimitation tests

Samples of *P. zonatus* group were collected from different location throughout Anatolia during 1992–2015. Twenty different populations were sampled and of these the Muğla-Fethiye population is from lowland (285m) while remaining 19 are from Anatolian higlands. Samples collected prior to 2000 were prepared as dry material while those after this date were preserved in 96% ethanol for molecular studies.

Studies on members of Phaneropterinae showed that mitochondrial NADH dehydrogenase 2 (*nad2*) gene is highly variable and contains a significant amount of phylogenetic information (Chobanov *et al.* 2017). We obtained and used a few sequences per population to test distinctness of the taxa in the group. For amplification of the *nad2* region the forward primer TM-J210 and reverse primers TW-N1284 or TY-N1433 (Simon *et al*. 2006) were used. The protocols given in Chobanov *et al.* (2017) were followed for DNA isolation and PCR.

The sequences were aligned manually in Sequencher v. 4.1 (Gene Codes Corporation, Ann Arbor, MI, USA) and checked manually by eye. Each sequence of *nad2* was checked using DnaSP v.5 (Librado & Rozas 2009) to detect NUMTs (nuclear mitochondrial genes) and to determine unique haplotypes. Nucleotide sequences of each unique haplotype identified in this study are deposited in the Genbank database (see Table 1). The parameters and best fit evolutionary model for data matrix were estimated by jModelTest v.0.1.1 (Darriba *et al*. 2012). The matrix was analysed with maximum likelihood (ML) and ML-bootstrap using RaXML v.1.3.1 (Silvestro & Michalak 2012) with ML-rapid 1000 bootstrap option. The selected model was implemented in ML analysis. One sequence per *Poecilimon cervus* Karabag (GenBank acc: MH168578) and *P. inflatus* Brunner von Wattenwyl (GenBank acc: MH168579) were chosen as outgroups. Four different DNA sequence-based species delimitation tests were applied to detect independently evolved lineages in the dataset and to make a taxonomic decision more objectively: (i) statistical parsimony (SP), (ii) distance-based test SpeciesID (SpeID), (iii) the Automatic Barcode Gap Discovery (ABGD), and (iv) the Bayesian implementation of the Bayesian Poisson Tree Process model (bPTP). See Kaya & Çıplak (2017) for the application procedures and the software used.

Morphology and bioacoustics

Specimens collected during field studies were prepared as museum material by standard methods or preserved in 96% alcohol. The material examined in this study is preserved in Akdeniz University, Department of Biology, Zoological Museum, Antalya, Turkey (AUZM). We also benefitted from the images of type specimens given in Orthoptera Species File2 (Cigliano *et al*. 2018) and SysTax-DORSA. Morphological structures of the investigated specimens were photographed, qualitatively examined and measured using a digital camera attached to a Leica MZ6/DC200 stereomicroscope and Image J v.1.36 (http://rsb.info.nih.gov/ij/). We paid particular attention for qualitative examinations of the cerci in males, the pronotum, tegmina and subgenital plate in males and females, and ovipositor in females since former descriptions and diagnoses of *Poecilimon* species were based mainly on these characters (Ramme 1933, Bey-Bienko 1954, Harz 1969, Ünal 2010, Kaya *et al.* 2012).

For sound recordings, a FOSTEX FR-2 (frequency response 22.05-192kHZ, all records have been made in the range of 46-98 kHz frequency responses) digital recorder was used with a G.R.A.S. Type 40BF microphone (frequency response 10Hz - 40kHz ± 1.0dB, 4Hz - 100kHz ± 1.0dB). Oscillograms and sound analyses were made using Turbolab (Stemmer AG) and CoolEdit Pro. V. 2.0 (Syntrillium Software Corporation). The figures of waveform (oscillogram) and power of frequency spectra (spectrogram) were produced using

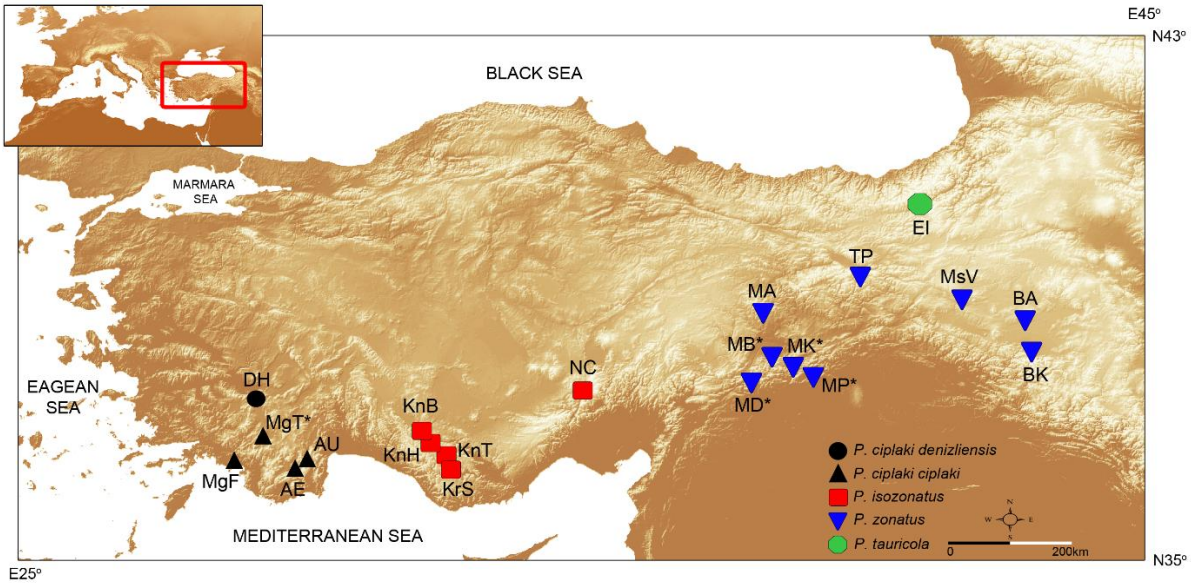

Fig. 1. Distribution map of sampling localities of *P. zonatus* complex and *P. tauricola*. (AE: Antalya-Elmalı, AU: Antalya Uzunkarış Mt., BA: Bitlis-Adilcevaz, BK: Bitlis-Kuskunkıran pass, DH: Denizli-Honaz, MgF: Muğla-Fethiye, MgT: Muğla-Tuzla pass, KnB: Konya-Bozkır, KnH: Konya-Hadim, KnT:Konya-Taşkent, KrS: Karaman-Sarıveliler, MA: Malatya-Arguvan, MB: Malatya-Beydağı Mt., MD: Malatya-Doğanşehir, MK: Malatya-Kubbe Mt., MP: Malatya-Pütürge, MsV: Muş-Varto, NC: Niğde-Çamardı, EI: Erzurum-İspir) * samples not used for molecular studies.

Seewave package version 2.1.0 (Sueur *et al.* 2008) for the R v. 3.3.4 (R Development Core Team 2018) with the following settings: proper (46-98kHz) sample ratio, 16-bit amplitude resolution, mono channel, 128 points FFT length, Hanning window function with 80% overlap.

In song descriptions, the terminology given in Heller (1988) and Kaya *et al.* (2012) was used as considering *Calling song*- song produced by an isolated male, s*yllable*- the song produced by one opening-closing movement cycle of the tegmina, *impulse*- a simple, undivided transient train of sound waves, and *ms*- millisecond/s.

Results

Taxonomy on the basis of phylogeny

After alignment and trimming of the sequences, the final sequence length in the *nad2* matrix was 992bp along which 597 sites were constant, 395 were variable, and 239 were parsimony informative. We identified 16+2 unique haplotypes of ingroup + outgroup from a total of 58 sequences (Table 1). The jModeltest calculated TrN+I+Γ model with gamma correction (Γ) of 0.8380 and the frequency of invariable sites (I) of 0.39 for data sets including outgroups.

ML analysis produced a tree with significantly high bootstrap values for all nodes (Fig. 2). Next to the outgroups on the tree, *P. tauricola* branches off basally and the other haplotypes constitute a monophyletic group. There are three phylogroups in the crown monophyletic group (*P. zonatus* complex) subsequent to *P. tauricola*,. The haplotypes from westernmost populations (Antalya, Denizli, and Muğla) constitute a sister haplogroup to the remaining haplotypes. The last phylogroup consists of two compact phylogroups, one including haplotypes from the populations sampled from the eastern Mediterranean Part of Anatolia (Konya and Niğde) and the other including the population from the highlands of East Anatolia (Bitlis, Erzincan, Malatya, and Muş).

The automatic species delimitation tests suggested consistent results. The following geographically outlined populations were suggested as distinct species/taxa (Fig. 2); (i) the Erzurum population, (ii) the populations from East Anatolia (Bitlis, Erzincan, Malatya, and Muş) (iii) the populations from the eastern part of Mediterranean Anatolia (Konya and Niğde), and (iv) the populations from the westernmost Mediterranean part of Anatolia (Antalya, Denizli, and Muğla) (see Fig. 1). The first population corresponds to *Poecilimon tauricola* and the second to *P. zonatus* s. str. as the type locality Binboğa Mts. in association with mountain chains of East Anatolia (see Çıplak 2008). The third and fourth populations represent the new species named below as *Poecilimon isozonatus* sp. n. and *Poecilimon ciplaki* sp. n. Denizli population is suggested as a different species by the statistical parsimony, the species ID and Bayesian poison tree process (bPTP) except for automatic barcode gap discovery (it was included in a species together with Antalya and Muğla populations) (Fig. 2). Considering the fact that there are also phenotypic differences distinguishing this population, we considered *P. ciplaki* sp. n. as polytypic with two subspecies: *P. ciplaki ciplaki* subsp. n. and *P. ciplaki denizliensis* subsp. n.

Table 1. The unique haplotypes of *nad2* gene for each population of *P. zonatus* complex and *P. tauricola* (there are no populations sharing haplotypes among taxa) Numbers next to the locality names correspond to the number of haplotypes of *nad2* in each locality.

GenBank Acc. no	Species/ Subspecies	*P. tauricola*	*P. zonatus*				*P. isozonatus sp. n.*			*P. ciplaki ciplaki subsp. n.*		*P. ciplaki denizliensis subsp. n.*	Total
	Haplotype/ Locality	Erzurum	Bitlis	Erzincan	Muş	Malatya	Niğde	Karaman	Konya	Antalya	Muğla	Denizli	
MH168590	Erzurum-1	2											2
MH168582	Bitlis-1		6										6
MH168595	Erzincan-1			3									3
MH168589	Muş-1				1								1
MH168586	Malatya-1					3							3
MH168587	Malatya-2		2			1							3
MH168588	Malatya-3					1							1
MH168583	Niğde-1						11						11
MH168593	Niğde-2						2						2
MH168594	Karaman-1							1					1
MH168584	Konya-1								2				2
MH168585	Konya-2							4	8				12
MH168591	Antalya-1									3			3
MH168580	Muğla-1										1		1
MH168581	Muğla-2										1		1
MH168592	Denizli-1											4	4
Total		2	8	3	1	5	13	5	10	3	2	4	56

Table 2. Some song parameters for the species in *P. zonatus* complex. Mean values of each parameter were given with minimum-maximum values in parenthesis (N: number of measured syllables).

Species (population recorded)	N	Syllable duration	Impulse number per syllable	Recording Temperature (°C)
P. zonatus (Bitlis)	49	8.90 (7-11)	11.35 (7-16)	25.8, 25.9, 26.0
P. isozonatus sp. n. (Konya-Taşkent)	52	18.36 (14-25)	28.02 (16-36)	25.2
P. ciplaki sp. n. (Muğla-Fethiye)	20	49.00 (42-72)	19.30 (16-24)	23.5

The pattern of male calling songs is similar in all examined populations. The song consisted of single isolated syllables repeated in irregular periods (see Figs. 3, 4). The main differences between the species were observed in syllable duration and impulse number per syllable (Table 2). The species ordered by syllable duration from highest to the lowest are as follows: *P. ciplaki* sp. n. - 49.0ms (42-72ms), *P. isozonatus* sp. n. - 18.4ms (14-25ms), and (iii) *P. zonatus* - 8.9ms (7-11ms). *P. isozonatus* sp. n. was represented with 28.03 (16-36), *P. ciplaki* sp. n. with 19.30 (16-24) and *P. zonatus* with 11.35 (7-16) impulses per syllable. It is clear from these results that there are clear gaps between the species in both song parameters allowing us to diagnose them from each other.

Species and subspecies diagnoses

The data presented here mainly concern *P. zonatus*. We also added a sample population of *P. tauricola*, which is suggested to be a member of the *P. zonatus* group. *P. zonatus* can be easily distinguished from other members of the group by its greenish coloration (black is dominant in others), species specific shape of male cerci and the higher number of stridulatory teeth (64-73 in *P. tauricola* while less than 56 in others). It is also genetically very distant to the remaining three taxa (Table 3).

P. zonatus and *P. isozonatus* sp. n. are very similar and cannot be distinguished from each other by the traditionally used structures such as pronotum, cerci, tegmina, and ovipositor, except minor differences exhibited in more slender and apically more tapered male cerci.

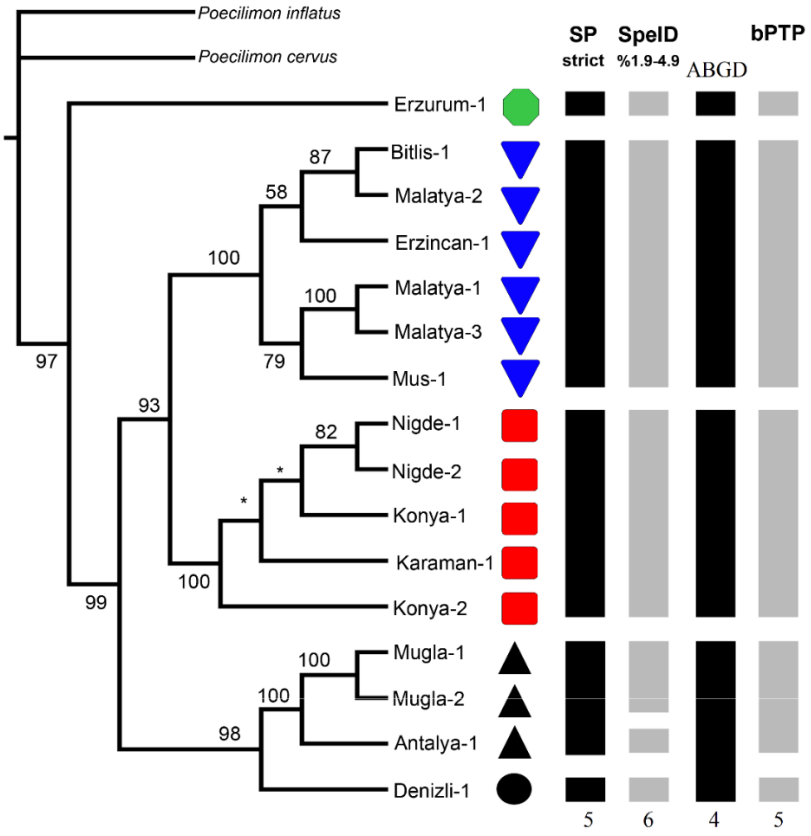

Fig. 2. Phylogenetic tree produced from *nad2* matrix and results of automatic species delimitation tests mapped on the tree (SP- statistical parsimony, SpeID- SpeciesID, the ABGD- Automatic Barcode Gap Discovery, bPTP Bayesian implementation of the Poisson Tree Process model).

Table 3. Some measurements for the species in P. zonatus complex and P. tauricola (lengths of pronotum (Pr), hind femur (Hf), ovipositor (Ov), number for cercal denticles (Cd), and stridulatory teeth (Sp)). All measurement values were given in mm and presented as means and their maximum-minimum. NA: not applicable.

Species	Gender (N)	Pr	Hf	Cd	Sp	Ov
P. zonatus	♂ (8)	5.54 5.68-5.42	17.03 17.90-16.20	9.67 13-8	48.70 53-43	NA
	♀ (6-8)	6.07 6.59-5.57	18.41 19.50-17.32	NA	NA	12.25 12.88-11.67
P. isozonatus sp. n.	♂ (10-15)	5.16 5.82-4.83	16.85 17.72-15.50	10.00 14-7	48.60 56-45	NA
	♀ (11)	5.09 6.42-3.06	18.27 19.15-17.05	NA	NA	11.50 12.70-9.24
P. ciplaki sp. n.	♂ (3-4)	5.69 6.10-5.38	16.82 17.65-16.00	7.66 8-5	53.33 48-57	NA
	♂ (7-10)	6.08 6.10-6.07	17.66 18.82-16.50	NA	NA	11.58 12.17-10.99
P. tauricola	♂ (4-5)	5.95 6.35-5.46	16.66 17.30-16.00	8.00 9-7	67.25 73-64	NA
	♀ (4)	6.65 7.06-6.22	18.03 18.80-17.30	NA	NA	9.50 9.72-9.37

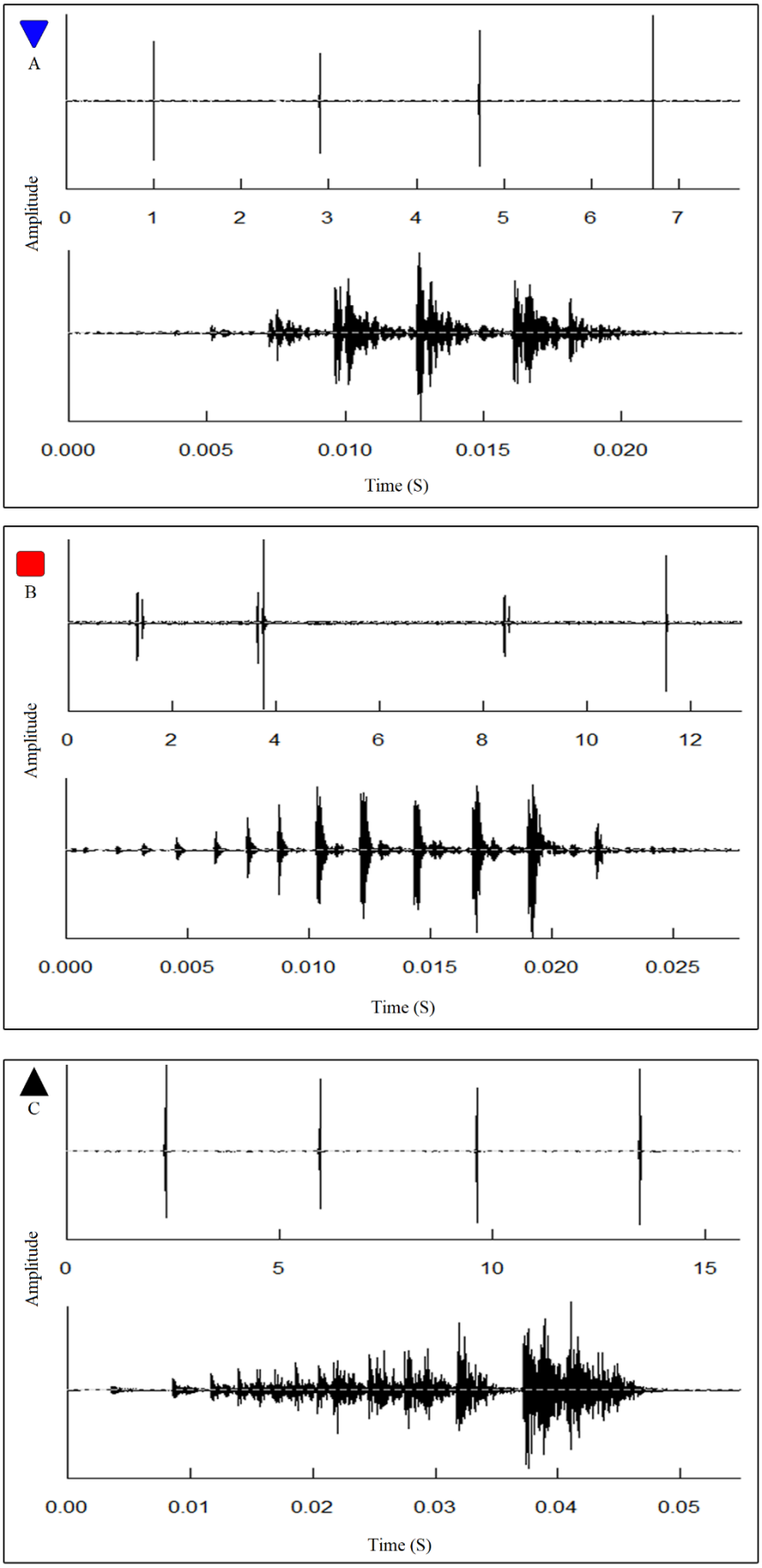

Fig. 3. Oscillograms of male calling songs (syllables): A- *P. zonatus*, B- *P. isozonatus* sp. n., C- *P. ciplaki ciplaki* subsp. n.

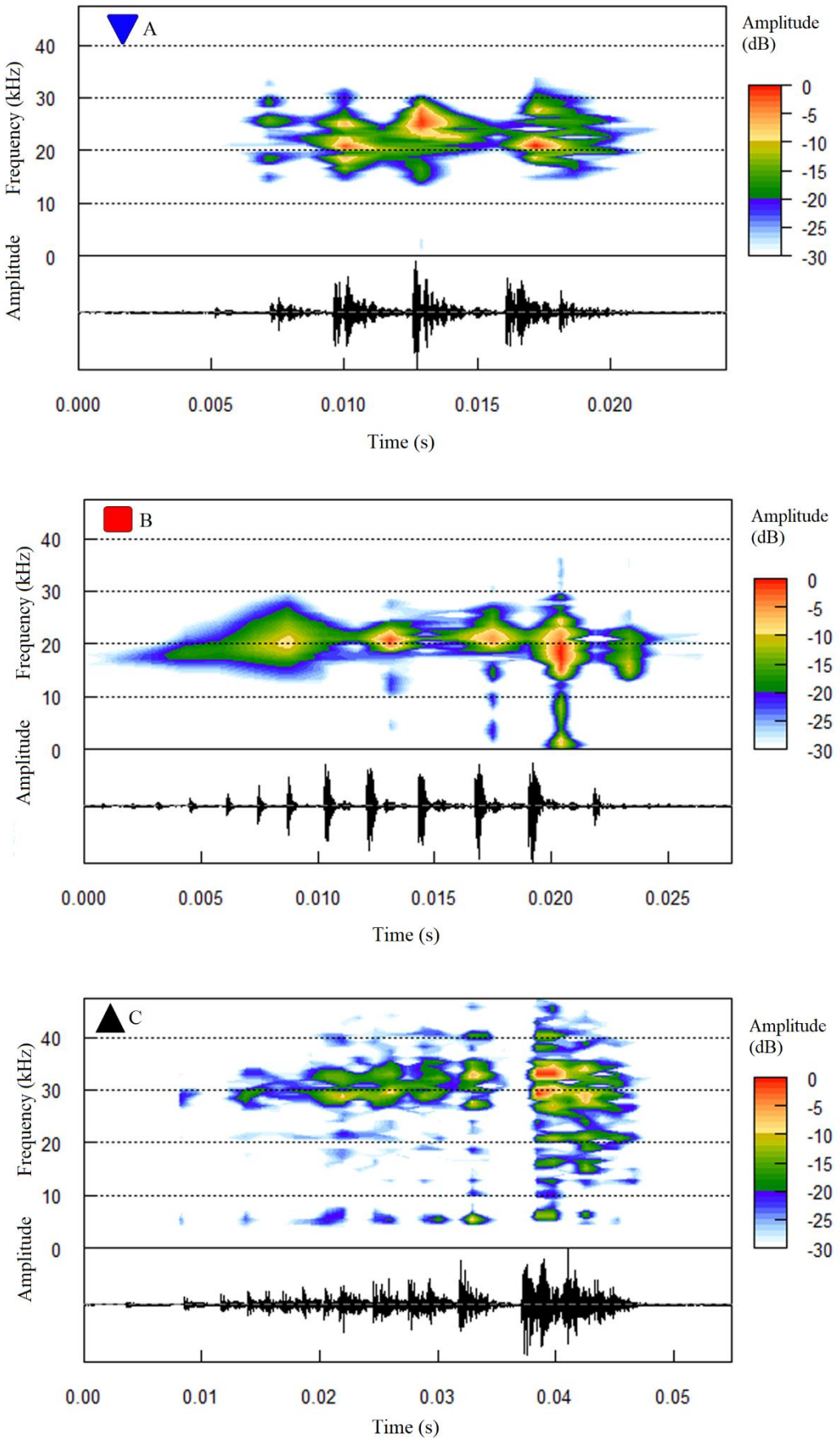

Fig. 4. Power spectra of male calling songs: A- *P. zonatus*, B- *P. isozonatus sp. n.*, C- *P. ciplaki ciplaki* subsp. n.

However, genetic data supported a clear distinction as eachshowed unique haplotypes. The diagnostic characters distinguishing the two taxa are based on male calling songs. The syllable duration is 8.9ms (7-11ms) and impulse number per syllable is 11.35 (7-16) in *P. zonatus*, while they are 18.6 (14-25) and 28 (16-36), respectively, in *P. isozonatus* sp. n. (Table 2, Figs. 3, 4).

Poecilimon zonatus Bolívar, 1899
Poecilimon zonatus Bolívar, 1899: 597

Type information. Lectotype male; Marach (Kahramanmaraş), Bimbogha-Dagh (Binboğa Mt.) in Museo Nacional de Ciencias Naturales, Madrid.

Material examined. 2♂♂, 3♀♀, TURKEY: Van, road to Bitlis-Kuskunkıran Pass, N: 38.37700, E: 42.78647, 2234m, 18.VII.2011, leg. S. Kaya, Z. Boztepe, Ö. Pekter; 8♂♂, 9♀♀, Bitlis-Adilcevaz-Harmantepe Vill., N: 38.86667, E: 42.73333, 2215m, 4.VII.2015, leg. S. Kaya and B. Çıplak; 1♀, Muş-Varto, road to Hınıs, N: 39.19940, E: 41.55625, 2000m, 6.VIII.2012, leg. S. Kaya, Z. Boztepe, Ö. Pekter; 1♂, 2♀♀, Road between Erzincan-Tunceli,-Pülümür, N: 36.65000, E: 32.63333, 1697m, 8.VII.2009, leg. M. Korkmaz; 5♂♂, 5♀♀, Malatya-Arguvan-Çobandere-Eşkınlı, N: 38.98532, E: 38.18311, 1600m, 22.VI.2013, leg. B. Çıplak; 7♂♂, 10♀♀, Malatya-Doğanşehir-Erkenek-Deveyatağı, N: 37.92338, E: 37.94709, 1300m, 27.V.1990, leg. B. Çıplak; 4♂♂, 7♀♀, Malatya-Pütürge-Esencik Village, N: 38.09296, E: 39.00990, 1600 m, 2.VI.1989, Leg. B. Çıplak; 1♂, 3♀♀, Malatya-Beydağı-Rafa, N: 38.329632, E: 38.324065, 2000m, 23.VII.1990, leg. B. Çıplak; 1♂, 1♀, Malatya-Pütürge, Kubbe Mt., N:38.247580, E:38.705044, 1600m, 20.VI.1990, leg. B. Çıplak (all in AUZM).

Description. The description by Bolivar is functional. Also see Figs. 1, 2; A in Figs. 3, 4; B in Figs. 5-14.

Poecilimon isozonatus sp. n.
http://zoobank.org/urn:lsid:zoobank.org:act:FD646C32-75BB-4BD4-BC00-BE27211321B7, Figs. 1, 2; B in Figs. 3, 4; C in Figs. 5-14.

Material examined. Holotype, male: TURKEY: Konya-Taşkent-Afşar, N:36.90000, E:32.50000, 1682m, 16.VI.2014, leg. S. Kaya and D. Chobanov; *Paratypes*, 20♂♂ (2 nymph), 12♀♀ (2 nymph), same data as holotype; 5♂♂, 10♀♀ (3 nymph); Niğde-Çamardı Vill., road fromYahyalı to Demirkazık Mt., N:37.83333, E:35.01667, 1539m, 17.VI.2014, leg. S. Kaya and D. Chobanov; 5♂♂, 2♀♀, Konya-Hadim, road to Gündoğmuş, N:36.88333, E:32.11667, 1887m, 15.VI.2014, leg. S. Kaya and D. Chobanov; 2♂♂, Konya, road between Bozkır-Hadim, 16.VI.2014, leg. S. Kaya and D. Chobanov; 13♂♂, 8♀♀, Karaman-Sarıveliler Village, 1610m 16.VI.2014, leg. S. Kaya and P.D. Chobanov (all in AUZM).

Diagnosis. P. zonatus and *P. isozonatus* sp. n. are very similar in traditionally used structures and metric characters (see Table 3), but genetic data clearly suggest each as a separate species (Table 1 and Fig. 2). The phenotypical characters distinguishing both are based on from male calling songs. Although these two species are morphologically similar, there is minor differences between both taxa such as the male cerci more slender, and more tapered distalward along incurved part in *P. isozonatus* sp. n. The coloration of abdominal terga is distinct and mostly population specific and may differ in young and elder individuals, but do not correlate with species. Apart from these differences, a single description can be done for both species.

Description (holotype, male). Fastigium of vertex slightly narrower than scapus. Pronotum short, slightly constricted in the middle, median sulcus located after the middle, cylindrical in prozona and weakly raised in metazona, caudal margin of the disc slightly concave, medial carina occurs as a white line or totally absent, disk bordered by large light lines constituting roughly as “)(“ shape; paranotal margin almost straight along prozona and oblique along metazona. Tegmina short, extends beyond the posterior margin of pronotum, stridulatory vein covered by pronotum; stridulatory file with 43-53 teeth. Cerci cylindrical, roundly incurved as a bow; curvature is more prominent apicalward, with a robust but slightly tapered apex and 3-5 small denticles on its external margin and a more prominent one at the tip internally. Subgenital plate slightly longer than wide and oval in its distal half, distal margin is slightly concave.

Female. Similar to male in general. Pronotum just slightly raised in metazona, tegmina slightly extended beyond hind margin of pronotum. Subgenital plate triangular, ovipositor typical of the group.

Coloration. General coloration black with a light pattern; vertex black or with black spots on a greenish brown background, antennae black with regular white rings. Disc of pronotum with black dots or spots on a greenish brown background at the beginning of prozona, black in the middle and reddish brown in metazona; paranota with black spots on a greenish brown background; tegmina with typical black/light (marble or brown) pattern; all legs are black dorsally. Abdominal terga black in front 2/3 and light in the remaining part, the black and light bands extend into each other showing a population-specific pattern.

Etymology. Although genetic and song data suggest discrimination of the new species, it is morphologically very similar to *P. zonatus*. The name *isozonatus* expresses this similarity.

Poecilimon ciplaki sp. n.
http://zoobank.org/urn:lsid:zoobank.org:act:6F157257-B942-4981-A806-B0BBFDC47C72, Figs. 1, 2; C in Figs. 3, 4; D and E in Figs. 5-14.

Diagnosis. All four species delimitation tests suggested *P. ciplaki* sp. n. as a distinct species, sister to *P. zonatus* + *P. isozonatus* sp. n. It can be easily diagnosed from other three species by the species-specific cerci (almost “L” shaped in *P. ciplaki* sp. n. while roundly curved in others with a more robust apex) and male calling

song with longer syllable duration [48ms (42-72ms) in *P. ciplaki* sp. n., while <25ms in *P. zonatus* and *P. isozonatus* sp. n.]. Additionally, *P. ciplaki* sp. n. can be distinguished by a more slender general appearance.

Three of four automatic species delimitation tests suggest with high supporting values that the Denizli population is different from Antalya and Muğla populations. We therefore split the species into two subspecies and established *P. ciplaki ciplaki* subsp. n. and *P. ciplaki denizliensis* subsp. n. The male cerci with a short inward curved part, more robust apex and extending beyond tip of subgenital plate distinguish *P. ciplaki denizliensis* subsp. n. from *P. ciplaki ciplaki* subsp. n. Below given description is valid for both subspecies.

Description (holotype, male). Fastigium of vertex narrower than scapus. Pronotum short, slightly constricted in the middle and widened front and backward, typical sulcus located in the middle or slightly behind middle; in profile pronotal disc concave being more raised in metazona, caudal margin of the disc slightly concave, medial carina absent, disc by irregular light lines; paranotal margin almost straight along prozona and oblique along metazona. Tegmina short, hardly extends to end of first abdominal tergum, its proximal one-third covered by pronotum, cubital vein covered by pronotum; stridulatory file with 43-53 teeth. Cerci cylindrical, incurved almost with a right angle, with a robust but slightly tapered apex and 5-8 small denticles along the tip most of which on external margin. Subgenital plate slightly longer than wide and oval in its distal half, distal margin slightly concave.

Female. Similar to male in general. Pronotum less raised in metazona, tegmina slightly extended beyond the hind margin of pronotum. Subgenital plate triangular, ovipositor typical of the group.

Coloration. Typical of *P. zonatus* complex. General coloration black with a light pattern. The vertex of fastigium black or with black spots on a brown background, antennae black with regular white rings. Disc of pronotum with black spots on a greenish brown background at the beginning of prozona, black in the middle and reddish brown in metazona; paranota with black spots on a greenish brown background; tegmina with typical black/light (marble or brown) pattern; all legs are black dorsally. Abdominal terga black in the proximal 2/3 and light in the remaining part, the black and light bands extend into each other with a population-specific pattern.

Etymology. The name of the new species is dedicated to Prof. Dr. Battal ÇIPLAK who made a great contribution to our knowledge on Orthoptera.

Poecilimon ciplaki ciplaki subsp. n.
http://zoobank.org/urn:lsid:zoobank.org:act:6F157257-B942-4981-A806-B0BBFDC47C72, Figs. 1, 2; C in Figs. 3, 4; D Figs. 5-14.

Material examined. *Holotype*, male; TURKEY: Muğla-Fethiye, road to Dalaman, N:36.75000, E:28.90000, 258m, 14.V.2011, leg. S. Kaya, Z. Boztepe, Ö. Pekter; *paratypes*: 6♂♂, 7♀♀, same data as holotype; 1♂, 4♀♀, Muğla-Fethiye-Tuzlabeli, 1650m, 29.VII.1997, leg. B. Çıplak; 11♂♂, 5♀♀, Antalya-Elmalı-Bozöyük, Uzunkarış Hill, N:36.71667, E:30.11667, 1691m, 15.V.2011, leg. S. Kaya, Z. Boztepe, Ö. Pekter; 3♂♂, 3♀♀; Antalya-Elmalı-Çamkuyusu, N:36.59112, E:30.00234, 1600m, 27.VI.1997, leg. B. Çıplak (all in AUZM).

Poecilimon ciplaki denizliensis subsp. n.
http://zoobank.org/urn:lsid:zoobank.org:act:6CB12F26-DD4E-4947-AEEF-82E9C1B49391, Figs. 1, 2; E in Figs. 5-14.

Material examined. *Holotype*, male; TURKEY: Denizli, Honaz Mt., N:37.65027, E:29.25021, 1530m, 15.V.2011, leg. S. Kaya, Z. Boztepe, Ö. Pekter; *Paratypes*, 3♀♀, same data as holotype (all in AUZM).

Etymology. The type locality of the new subspecies is in Denizli Province and the name of the new subspecies is based on Denizli province.

Poecilimon tauricola Ramme, 1951
Poecilimon tauricola Ramme, 195:331

Type information. Holotype male; Turkey, Niğde, Ulukışla (Museum für Naturkunde, Berlin).

Material examined. 10♂♂, 6♀♀, TURKEY: Erzurum-İspir, İkizdere, Ovit Mt., N:40.55097, E:40.91595, 1928m, 29.VII.2008, leg. S. Kaya, E. M. Korkmaz, M.S. Taylan (AUZM).

Description. See Ramme (1951) and also Figs. 1, 2; A in Figs. 5-14.

Remarks. The type locality of this species is located in the southern part of the Anatolian Diagonal (see Çıplak *et al*. 1993). The locality reported here is inside the Black Sea region, but still in association with the Diagonal. Comparison of the material genetically may provide interesting and more illuminative results. However, such a distribution pattern is not unexpected as there are further taxa ranging along the Diagonal (see Kaya & Çıplak 2017).

Discussion

The present taxonomic study is based on three kinds of data; DNA sequences, male calling songs and traditionally used qualitative morphology. In our case, the power of mtDNA sequences to document biodiversity of a lineage seems to be the highest. Automatic species delimitation tests based on bioinformatic applications suggested a cryptic diversity within *P. zonatus*, and two new species and a new subspecies (Fig. 2). The high value of statistical support to each of the new species and absence of shared haplotypes provide significant support to this suggestion. Song characteristics are regarded as supportive to genetic data in exploring the biodiversity as documented in other species within Orthoptera (Heller 1988, Ragge & Reynolds 1998). The syllable duration and the impulse number per syllable in particular allowed us

to diagnose the genetically distinct units (Table 2). However, song data of some populations is still unavailable and obtaining song from these populations may provide a better description of biodiversity of this group. Contrary to DNA sequence and bioacoustics data, morphology was not so productive and remained insufficient in biodiversity documentation (compare the images given in Figs. 5-14). Only some taxa in the *P. zonatus* complex can be distinguished by morphology and cerci seem to be the most valuable diagnostic structure (Fig. 11). We can propose two reasons for the low use of morphology for taxa discriminations. First, the rate of morphological evolution may be lower than the DNA and bioacoustic data. Second, if the morphological divergence between these taxa cannot be explored by traditional examination of morphology, then application of geometric morphometry may provide a better resolution. It is worth noting that the linear metric morphology has not provided any diagnostic characters (see Table 3). In conclusion, the use of the combination of characters from different sources allowed a better documentation of biodiversity, and helped to discover two new species and one new subspecies. Thus, we consider *P. zonatus*, *P. isozonatus* sp. n., and *P. ciplaki* sp. n. as a complex within *P. zonatus* species group which also includes *P. tauricola*, *P. variicercis* and *P. varicornis*. Among the latter three species, the features of *P. varicornis* from Lebanon seem to fit the *P. zonatus* complex, while *P. tauricola* and *P. variicercis* are more divergent [as much as inferred from the original description and the data presented in Orthoptera Species File2 (Cigliano *et al.* 2018)].

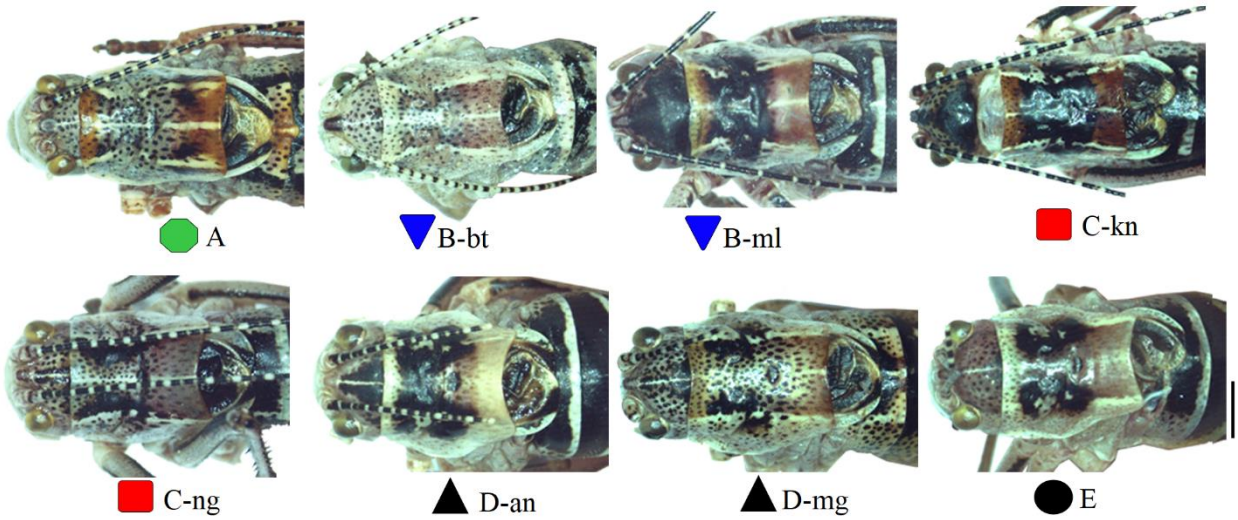

Fig. 5. Male pronotum from above (scale: 2mm): A- *P. tauricola*, B- *P. zonatus* (bt- Bitlis, ml- Malatya), C- *P. isozonatus* sp. n. (kn- Konya, ng- Niğde), D- *P. ciplaki ciplaki* subsp. n. (an- Antalya, mg- Muğla), E- *P. ciplaki denizliensis* subsp. n.

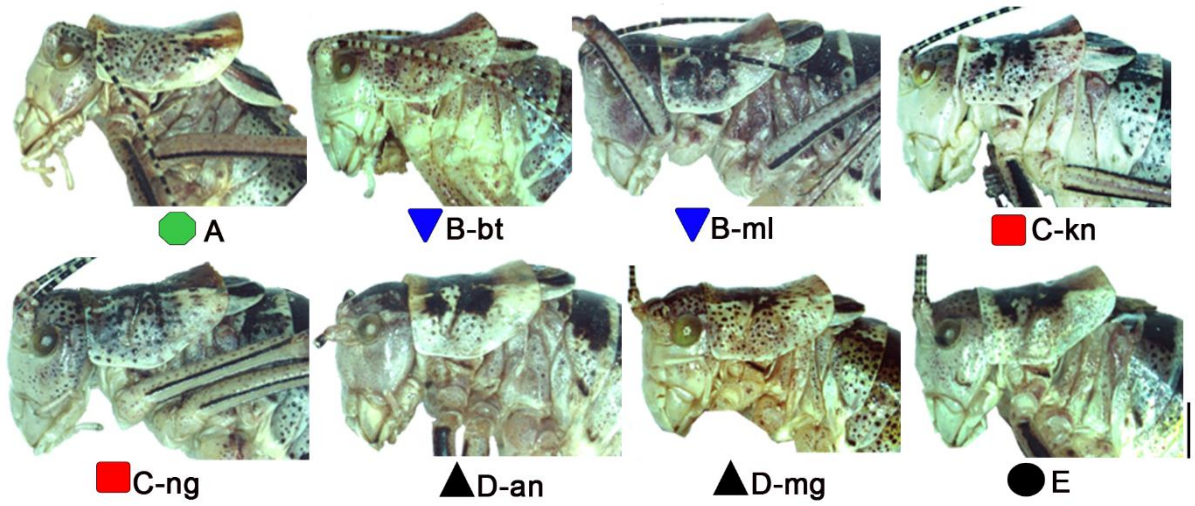

Fig. 6. Male pronotum in profile (scale: 2mm): A- *P. tauricola*, B- *P. zonatus* (bt-Bitlis, ml-Malatya), C- *P. isozonatus* sp. n (kn-Konya, ng- Niğde), D- *P. ciplaki ciplaki* subsp. n. (an-Antalya, mg-Muğla), E- *P. ciplaki denizliensis* subsp. n.

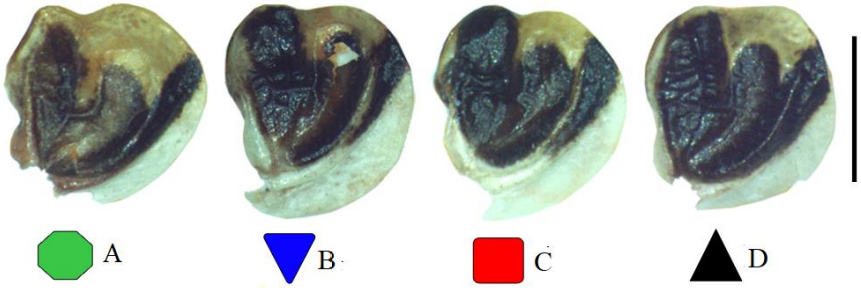

Fig. 7. Male tegmina (scale: 2 mm): A- *P. tauricola*, B- *P. zonatus*, C- *P. isozonatus* sp. n., D- *P. ciplaki ciplaki* subsp. n.

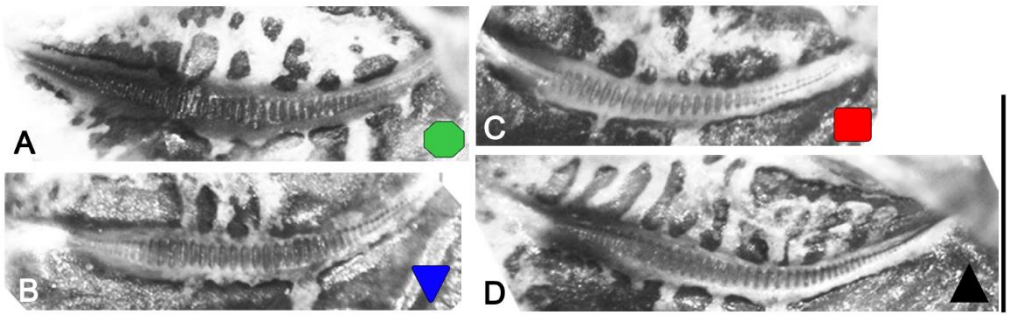

Fig. 8. Stridulatory file (scale: 1mm): A- *P. tauricola*, B- *P. zonatus*, C- *P. isozonatus* sp. n., D- *P. ciplaki ciplaki* subsp. n.

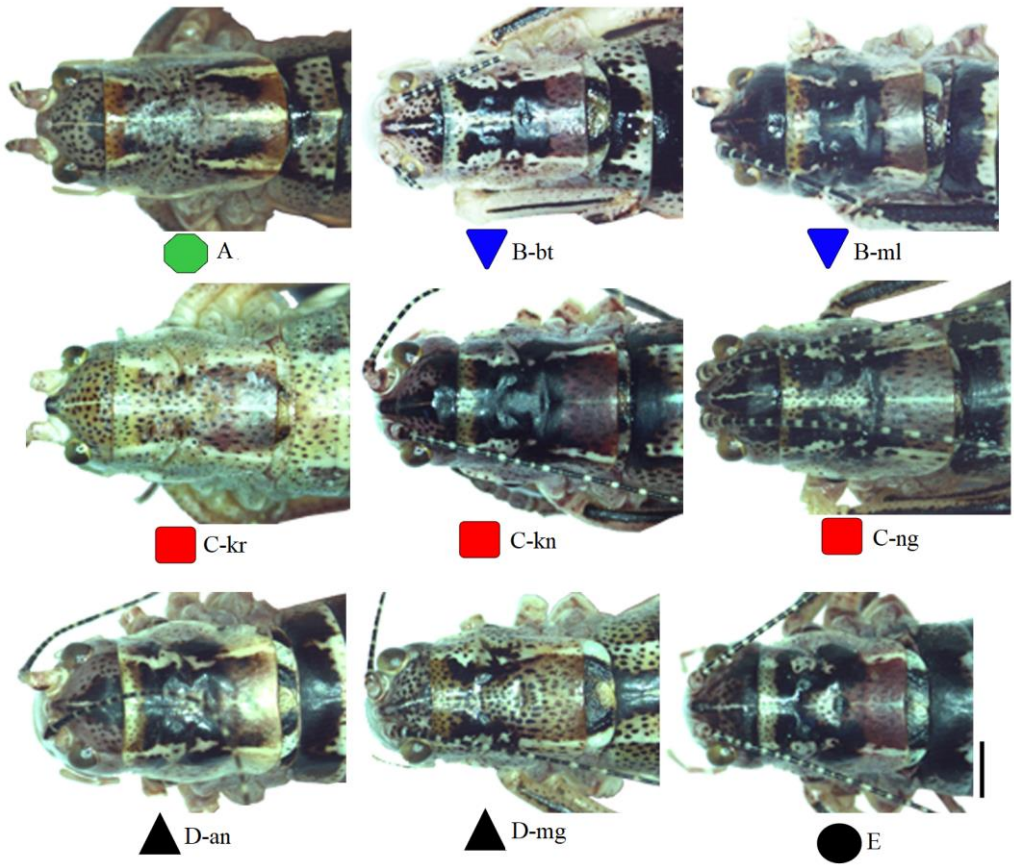

Fig. 9. Female pronotum from above (scale: 2mm): A- *P. tauricola*, B- *P. zonatus* (bt-Bitlis, ml-Malatya), C- *P. isozonatus* sp. n. (kr-Karaman, kn-Konya, ng- Niğde,), D- *P. ciplaki ciplaki* subsp. n. (an- Antalya, mg- Muğla), E- *P. ciplaki denizliensis* subsp. n.

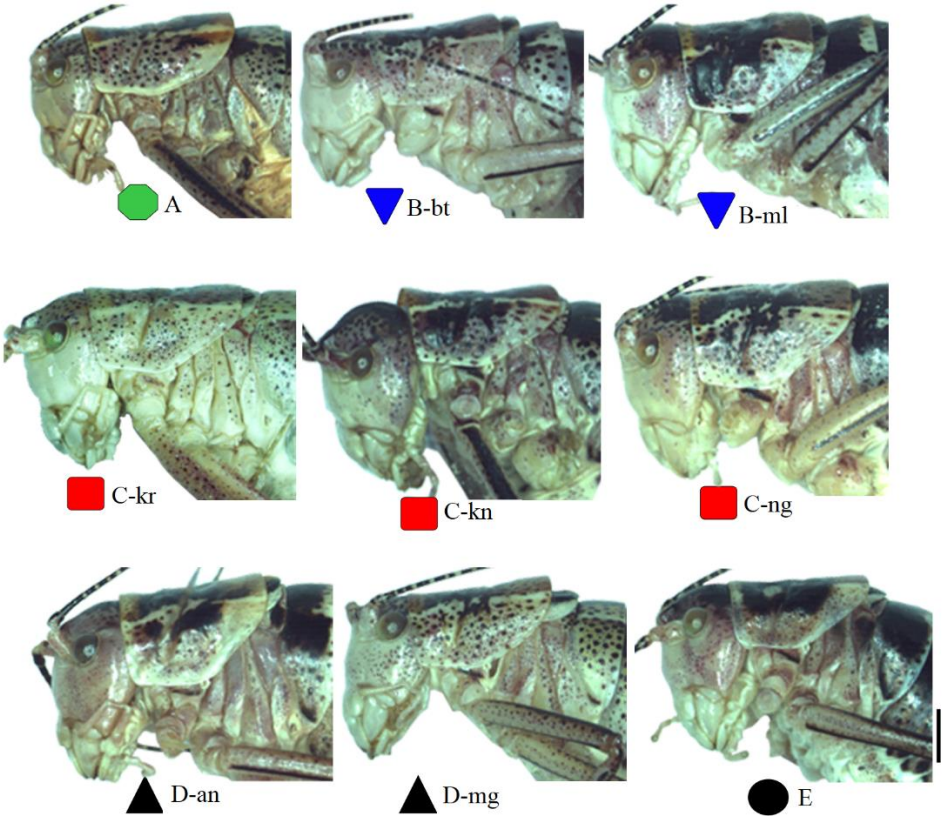

Fig. 10. Female pronotum in profile (scale: 2mm): A- *P. tauricola*, B- *P. zonatus* sp. n. (bt-Bitlis, ml-Malatya), C- *P. isozonatus* (kr-Karaman, kn- Konya, ng- Niğde,), D- *P. ciplaki ciplaki* subsp. n. (an-Antalya, mg- Muğla), E- *P. ciplaki denizliensis* subsp. n.

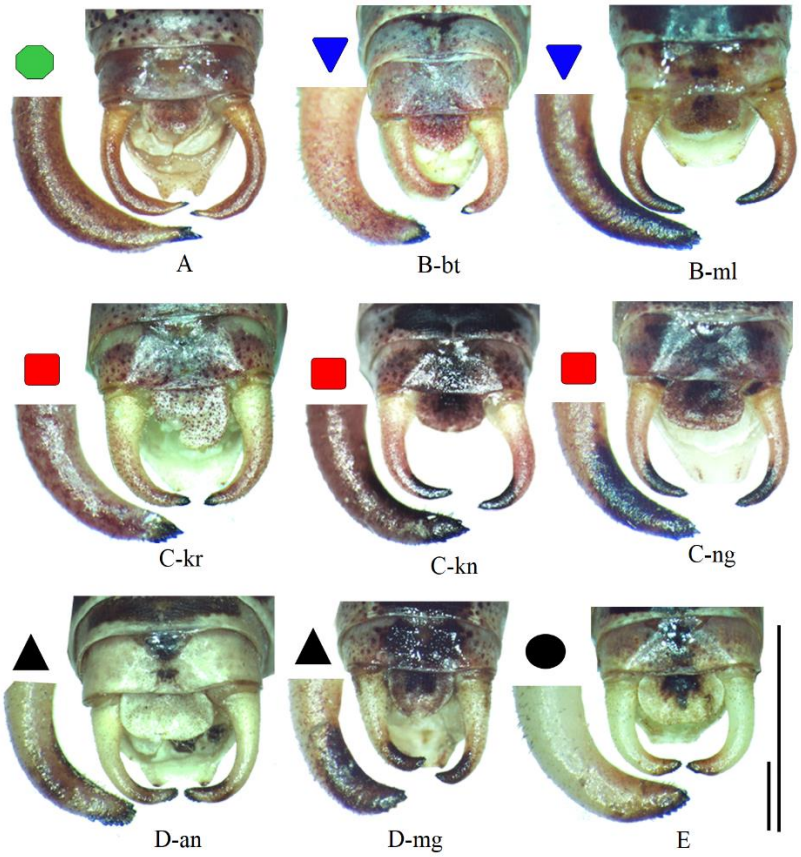

Fig. 11. Male cerci (scale: 2mm): A- *P. tauricola*, B- *P. zonatus* sp. n. (bt- Bitlis, ml- Malatya), C- *P. isozonatus* (kr- Karaman, kn- Konya, ng- Niğde,), D- *P. ciplaki ciplaki* subsp. n. (an- Antalya, mg- Muğla), E- *P. ciplaki denizliensis* subsp. n.

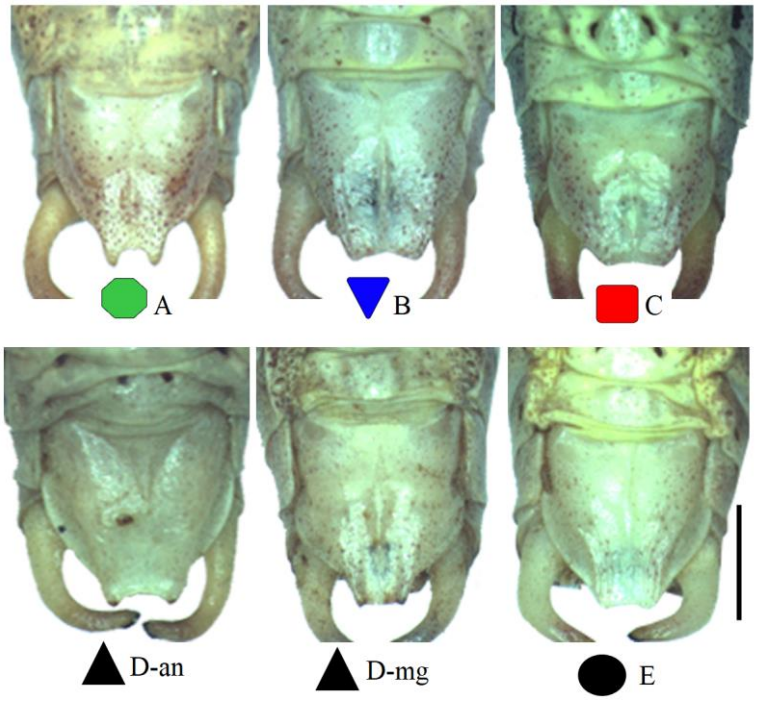

Fig. 12. Male subgenital plate (scale: 2mm): A- *P. tauricola*, B- *P. zonatus*, C- *P. isozonatus* sp. n., D- *P. ciplaki ciplaki* subsp. n. (an- Antalya, mg- Muğla), E- *P. ciplaki denizliensis* subsp. n.

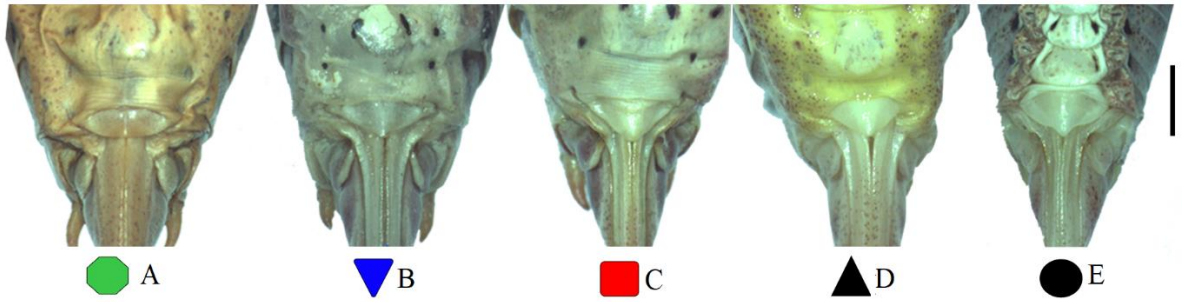

Fig. 13. Female subgenital plate (scale: 2mm): A- *P. tauricola*, B- *P. zonatus*, C- *P. isozonatus* sp. n., D- *P. ciplaki ciplaki* subsp. n., E- *P. ciplaki denizliensis* subsp. n.

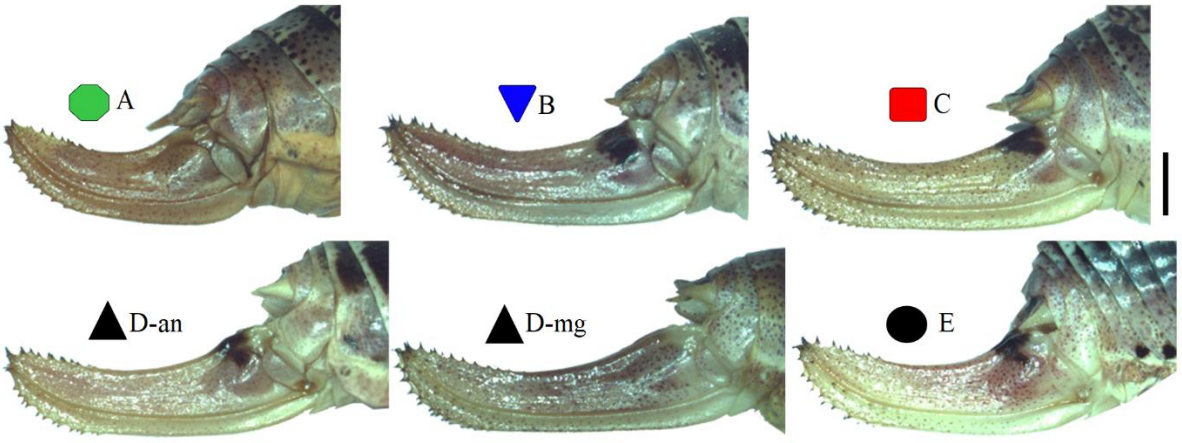

Fig. 14. Female ovipositor (scale: 2mm): A- *P. tauricola*, B- *P. zonatus*, C- *P. isozonatus* sp. n., D- *P. ciplaki ciplaki* subsp. n. (an- Antalya, mg- Muğla), E- *P.ciplaki denizliensis* subsp. n.

The second main implication of the present data is related to the specialty of Anatolian biodiversity, especially the one associated with mountain belts. Most of the populations reported here are restricted to highlands (Fig. 1). The range of *P. tauricola* correlates with the Anatolian Diagonal and that of *P. zonatus* with the East Anatolian highlands. *P. isozonatus* sp. n. ranged along the eastern part of Anatolia and *P. ciplaki* sp. n. in the western part of Mediterranean Taurus. More strikingly the total range of these species well fit the "Taurus Way" – an altitudinal entity providing a faunal connection between northeast and southwest Turkey (Çıplak 2008) and even between the Caucasus and the Balkans through southern Anatolia (Kaya & Çıplak 2017). Thus, *P. zonatus* complex may constitute a model group to test the Taurus Way assumption by applying detailed phylogeographic analyses.

In conclusion, as suggested by present data and reported by other studies (see Çıplak 2003), Anatolian Mountains are the main areas harbouring Anatolian biodiversity. As documented for *P. zonatus* complex, the specialty of the mountainous diversity is the existence of endemic forms. Thus, mountainous diversity requires special attention for conservation.

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References

1. Bey-Bienko, G.Y. 1954. *Orthoptera. Leaf bush-crickets (Phaneropterinae).* Fauna of the USSR, Vol. II, Sect. 2. Zoological Institute of the Academy of Sciences of the USSR, New Series, 59, 385 pp. (in Russian)
2. Cigliano, M.M., Braun, H., Eades, D.C. & Otte, D. 2018. *Orthoptera Species File*. Version 5.0/5.0. http://Orthoptera.SpeciesFile.org (Date accessed: March 2018).
3. Chobanov, D.P., Kaya, S., Grzywacz, B., Warchałowska-Śliwa, E. & Çıplak, B. 2017. The Anatolio-Balkan phylogeographic fault: a snapshot from the genus *Isophya* (Orthoptera, Tettigoniidae). *Zoologica Scripta*, 46(2): 165-179 (doi:10.1111/zsc.12194).
4. Çıplak, B. 2003. Distribution of Tettigoniinae (Orthoptera, Tettigoniidae) bush-crickets in Turkey: the importance of the Anatolian Taurus Mountains in biodiversity and implications for conservation. *Biodiversity and Conservation*, 12: 47-64.
5. Çıplak, B. 2004. Biogeography of Anatolia: the marker group Orthoptera. *Memorie della Società Entomologica Italiana,* 82: 357-372.
6. Çıplak, B. 2008. The analogy between glacial cycles and global warming for the glacial relicts in a refugium: a biogeographic perspective for conservation of Anatolian Orthoptera, pp. 135-163, in: Fattorini, S. (ed) *Insect Ecology and Conservation*. Research Signpost, Kerela, India.
7. Çıplak, B., Demirsoy, A. & Bozcuk, A.N. 1993. Distribution of Orthoptera in relation to the Anatolian Diagonal in Turkey. *Articuluta,* 8: 1-20.
8. Çıplak, B., Demirsoy, A. & Bozcuk, A.N. 1996. Malatya Ensifera (Orthoptera, Insecta) faunası. *Turkish Journal of Zoology*, 20: 247-254.
9. Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, 9: 772.
10. Harz, K. 1969. *Die Orthopteren Europas 1*. Series Entomologica 5, The Hague (Dr. W. Junk N.V.), 749 pp.
11. Heller, K.-G. 1988. *Bioakustik der europäischen Laubheuschrecken*. Verlag Josef Margraf, Weikersheim, 358 pp.
12. Kaya, S., Çıplak, B., Chobanov, D. & Heller, K-G. 2012. *Poecilimon bosphoricus* group (Orthoptera, Phaneropterinae): iteration of morpho-taxonomy by song characteristics. *Zootaxa*, 3225, 1-71.
13. Kaya, S. & Çıplak, B. 2017**.** Phylogeography and taxonomy of the *Psorodonotus caucasicus* (Orthoptera, Tettigoniidae) group:independent double invasion of the Balkans from the Caucasus. *Systematic Entomology*, 42: 118-133.
14. Kaya, S., Boztepe, Z. & Çıplak, B. 2015. Phylogeography of the *Poecilimon luschani* species group (Orthoptera, Tettigoniidae): a radiation strictly correlated with climatic transitions in the Pleistocene. *Zoological Journal of the Linnean Society*, 173(1): 1-21.
15. La Greca M. 1999. Il contributo degli Ortotteri (Insecta) alla conoscenza della biogeografia dell'Anatolia: la componente gondwaniana. *Biogeographia*, 20: 179-200.
16. Librado, P. & Rozas, J. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, 25: 1451-1452.
17. Miram, E.F. 1938. New species of the genera *Poecilimon* Fisch. Fr. and *Isophya* Br. W (Subf. Phaneropterinae Fam. Tettigonoidea - long-horned grasshoppers) of the fauna of the USSR. *Zoologicheskii zhurnal*, 17: 348-372.
18. R Development Core Team 2018. R: A language and environment for statistical computing, Version 3.3.4. - Available at http://www.R-project.org
19. Ragge, D.R. & Reynolds, W.J. 1998. *The Songs of the Grasshoppers and Crickets of Western Europe*. Harley Books, London, 612 pp.

20. Ramme, W. 1933. Revision der Phaneropterinen-Gattung *Poecilimon* Fisch. (Orth. Tettigon). *Mitteilungen aus dem Zoologischen Museum in Berlin*, 19: 497-575.

21. Ramme, W. 1951. Zur Systematik, Faunistik und Biologie der Orthopteren von Südost-Europa und Vorderasien. *Mitteilungen aus dem Zoologischen Museum in Berlin*, 27: 1-421.

22. Silvestro, D. & Michalak, I. 2012. raxmlGUI: a graphical front-end for RAxML. *Organisms Diversity & Evolution* 12: 335-337.

23. Simon, C., Buckley, T.R., Frati, F., Stewart, J.B. & Beckenbach, A.T. 2006. Incorporating Molecular Evolution into Phylogenetic Analysis, and a New Compilation of Conserved Polymerase Chain Reaction Primers for Animal Mitochondrial DNA. *Annual Review of Ecology, Evolution and Systematics*, 37: 545-579.

24. Sevgili, H., Demirsoy, A. & Durmuş, Y. 2012. Orthoptera fauna of Kemaliye (Erzincan). *Hacettepe Journal of Biology and Chemistry*, 40(4): 317-335.

25. Sueur J., Aubin T. & Simonis C. 2008. Seewave: a free modular tool for sound analysis and synthesis. *Bioacoustics*, 18: 213-226.

26. Ullrich, B., Reinhold, K., Niehuis, O. & Misof, B. 2010. Secondary structure and phylogenetic analysis of the internal transcribed spacers 1 and 2 of bush crickets (Orthoptera: Tettigoniidae: Barbitistini). *Journal of Zoological Systematics and Evolutionary Research*, 48: 219-228.

27. Ünal, M. 2010. Phaneropterinae (Orthoptera: Tettigoniidae) from Turkey and the Middle East II. *Transactions of the American Entomological Society*, 136: 125-183.

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Research Article

THE GROWTH OF *Tanypus punctipennis* Meigen (DIPTERA, CHIRONOMIDAE) LARVAE IN LABORATORY CONDITIONS AND THE EFFECTS OF WATER TEMPERATURE AND pH

Firuze GÜLBUNAR, Gazel Burcu AYDIN*, Belgin ÇAMUR-ELİPEK

Trakya University, Faculty of Science, Department of Biology, Edirne, TURKEY
*Corresponding author: e-mail: gburcuaydin@trakya.edu.tr

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Abstract: Recent taxonomic studies suggest that findings of larval chironomids should be supported also by adult findings in order to obtain more robust and reliable results on the studied group. Moreover identifications of larvae of some species can be made to genus level only due to similarities of some larval characteristics in different species. In such cases, species level identifications can be achieved by the growth of larvae in laboratory conditions. Also, larval culturing under optimum growth conditions will not only make it easy to provide materials for experimental studies and but also achievement of larvae with a higher biomass value to be used as food in the sector. In this study, *Tanypus punctipennis* Meigen (Diptera, Chironomidae), a very common species in Turkish Thrace, was used as the model organism for culture studies. Individual larvae were cultured from third instar stage to adult form under laboratory conditions. A simple and cheap method is offered for experimental studies on larval growths of chironomids and the effects of water temperature and pH, both with very important roles in larval culturing, were determined. The overall results of laboratory tests showed that the temperature value of 25°C and 7-8 pH interval were the optimal laboratory conditions for culture of *T. punctipennis* larvae.

Key words: Freshwater, midge, environmental conditions, rearing, identification.

Özet: Son zamanlarda yapılan taksonomik çalışmalarda larval chironomidlerin erginleri ile birlikte değerlendirilmesinin daha güvenilir sonuçlar verdiği belirtilmektedir. Ayrıca, bazı türlerin teşhisleri, larval safhadaki bazı karakterlerin türler arasındaki benzerlikleri nedeniyle ancak cins düzeyine kadar yapılabilmektedir. Bu durumda larvaların laboratuvar koşullarında yetiştirilerek erginleştirilmesi sayesinde tür düzeyinde teşhisleri mümkün olabilmektedir. Ayrıca, uygun yetiştirme koşulları belirlenerek yapılan larva yetiştiriciliği, bir taraftan deneysel çalışmalarda gerekli materyalin eldesi için kolaylık sağlarken, diğer taraftan balık yemi sektöründe daha ileri instar evrelerine ve dolayısıyla yüksek biyomass değerine hızla erişecek larva temini için de yararlı olacaktır. Bu araştırmada, yetiştirme çalışmaları için model tür olarak Trakya'da oldukça yaygın olan *Tanypus punctipennis* Meigen (Diptera, Chironomidae) larvaları kullanıldı. Bireyler, üçüncü instar safhasından ergin forma erişinceye dek laboratuvar koşullarında yetiştirildi. Böylelikle, laboratuvar koşullarında larval chironomid yetiştirme amaçlı çalışmalar için basit ve ucuz bir yöntem deneyimlenerek sunulurken, larvaların yetişmesinde oldukça önemli rolü olan su sıcaklığı ve pH gibi çevresel faktörlerin etkileri de araştırıldı. Çalışmanın sonucunda *T. punctipennis* larvalarının gelişiminde 25°C ve 7-8 pH aralığının en uygun koşullar olduğu belirlendi.

Introduction

Chironomidae commonly called as midges or non-biting midges, is a dipteran family in which adult forms live in terrestrial environments while the larval forms adapted to an aquatic lifestyle. The larval period represented with four stages is the longest period of the whole life cycles of these insects (Armitage *et al.* 1995, Specziar 2008). Individual chironomids in metamorphosis leave their exuviae on water surface after a long fourth stage and stay in a relatively short pupal stage to emerge as adults.

Larval chironomids are known as one of the most abundant macroinvertebrate groups in freshwater environments and they often account for the majority of aquatic insects (Epler 2001). Although they have high adaptation abilities to aquatic ecological conditions, species can show different reactions to changing microhabitat conditions (Armitage *et al.* 1995, Maasri *et al.* 2008). Their growth and development can also be influenced by a number of conditions some of which are temperature, food, and photoperiodicity (Maier *et al.* 1990, Vos *et al.* 2000). Furthermore, morphological, physiological or behavioural adaptation abilities of larval chironomids differ from one to another species (Ferrington 2008). Therefore, some environmental variables affect larval dynamics in an aquatic environment in different rates depending on the species

that the larvae belong to, which in turn makes larval chironomids as potential organisms to be considered as indicator organisms for aquatic environments (Kenney *et al.* 2009).

Larval chironomids are one of the most important groups in benthic fauna in terms of their roles in food web since they are live food sources for higher aquatic organism, mainly for fish and macroinvertebrates (Habibi *et al.* 1992, Nath *et al.* 2017). Chironomid larvae are also of paramount importance in aquaculture and they are widely used as live food for fish larvae (Sahandi 2011). They can also be used as food material for commercial fish because of their high protein contents. Therefore, larval chironomids can and are readily reproduced in *in vitro* conditions to be used for this particular aim.

Larval stages of chironomids have been widely used for species identifications in taxonomical studies performed in aquatic ecosystems. However, identifications based on immature specimens sometimes can allow researchers for identification of a specimen up to genus level because of the high taxonomic complexity of the family (for example *Chironomus (Holotanypus)* sp. larvae). One common technique used by researchers from various countries to overcome this problem is larval rearing to obtain adults, but this technique has not been applied in Turkey so far (Namayandeh & Beresford 2012). Recent studies suggest that larval identifications should be supported by adult findings to provide the most reliable results.

Tanypus punctipennis Meigen, is a chironomids species with a wide Palaearctic distribution and is found in freshwater ecosystems. The immature stages of the species are abundant in a wide range of lentic and lotic habitats in Turkey, making the species a suitable material to be used as commercial fish food. *T. punctipennis* chosen as the experimental model organism for this study, is very common in aquatic environments in Turkish Thrace (Özkan & Çamur-Elipek 2006, 2007, Özkan 2006, 2007, Özkan et al. 2010, Çamur-Elipek et al. 2006, 2010, 2012, Aydın & Güher 2017). Although some studies have been performed on growth of *T. punctipennis* larvae in laboratory conditions, effects of environmental conditions on larval growth were not addressed in these studies (Vallenduuk & Lipinski 2009). Moreover, no similar study on growth of larval chironomids has been performed in Turkey so far. We aimed in the present study to provide an inexpensive and rapid method for growth of larval chironomids in laboratory conditions with an experimental study on *T. punctipennis*. We also tested the most suitable water temperature and pH levels for larval growth of the species in laboratory conditions. The effectiveness of the culture apparatus used was tested for possible use of it for chironomids with identification problems. The apparatus allowed us to define optimal culture conditions for experimental studies and fishing worms.

Materials and Methods

Larval chironomids were obtained from surface-sediment samples of an artificial shallow pond (depth less than 2m) in Balkan Campus of Trakya University in Edirne. Samples were collected in the field in May 2017 and were immediately transported to laboratory in their natural sediment and water. A total of 15 *T. punctipennis* larvae in 3rd instar stage were selected under a stereobinoculer microscope to establish the experimental groups. Saether (1980), Fittkau & Roback (1983), Epler (2001) were used in identifications of the larvae. A total of five experimental groups [1 control group and 4 test groups (2 groups for temperature and 2 groups for pH effect tests)] with three larvae in each were obtained and each group was transferred to different aquariums providing conditions similar to their natural conditions. The control group was same for both experimental paradigms. The aquariums were placed in laboratory with an ambient air temperature of +18°C during day and they were covered with a mesh net to retain emerging adults (Fig. 1). An oxygeniser was used to provide two oxygen bubbles per second to each aquarium. All larvae were fed everyday by putting a drop mixture of milk powder and water (1:1) into the aquariums (Namayandeh & Beresford 2012). The experiments were ended when all specimens in the groups reached to adult stage or when all larvae/pupae died. Wiederholm (1989), Armitage *et al.* (1995) and Langton & Pinder (2007) were used in identifications of adults and pupae.

Testing Effects of Temperature: In order to test the effects of ambient temperature on larval growth, three experimental groups were placed in three different temperature conditions. The control group was placed under controlled room temperature (18±2°C) while the two test groups were placed at two climate room temperatures, 25±2°C and 10±2°C, respectively. Temperature levels were measured by an ordinary thermometer at 6 hours intervals during the experiment. The pH levels of water in all groups were measured as 7-8 and were kept stable during the experiments. By doing so, we could determine the effects of temperature on larval growth for each group, considering that all other parameters were adjusted to be same for all groups.

Testing Effects of pH: The pH levels of water in all experimental groups were measured as 8 at the beginning of the experiment. All three groups were placed under controlled room temperature (18±2°C) to provide an environment similar to the natural environments of the larvae. To determine the effects of pH variations on larval growth, the temperature value of 18±2°C was kept the same for each experimental group. The control group of the first experimental condition for which pH was measured as 7-8 was also the control group of this experiment. The pH level of one of the two test groups was decreased to 5-6 with the help of addition of HCl solution and pH level of the second group was increased to 9-10 adding NaOH solution into the environment. The pH levels were measured by a pHmeter at 6 hours intervals during the experiment. Other conditions that have the potential to effect larval growth, i.e. light, pH, food, oxygen, sediment type and water level, were standardized for each group in both testing conditions.

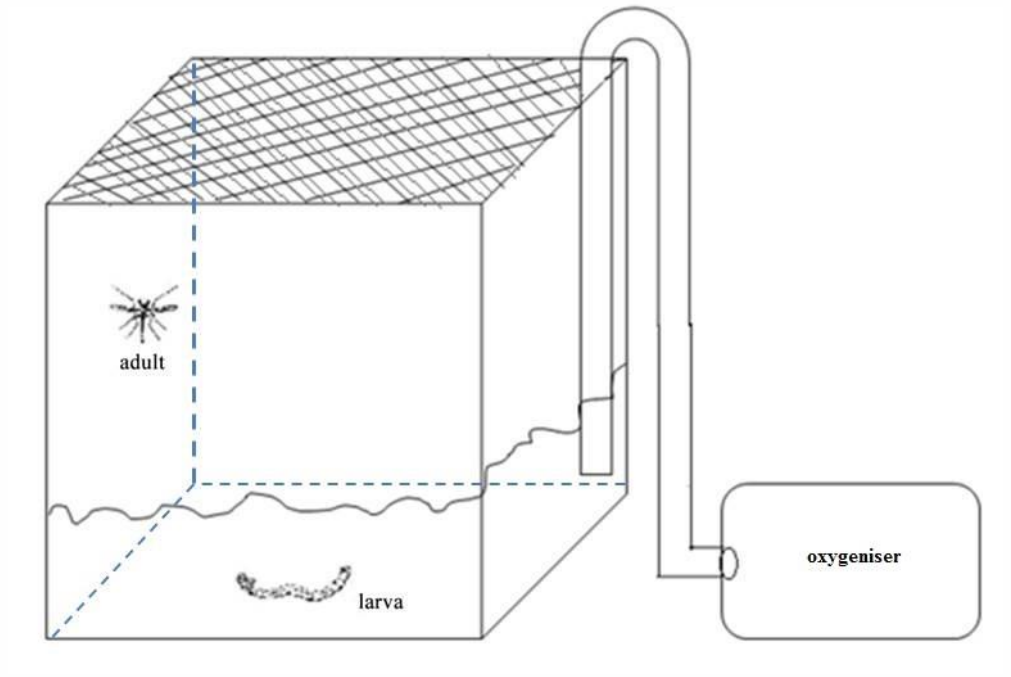

Fig.1. A schematic representation of the experimental setup in the laboratory. The aquariums were covered with a mesh to prevent escape of emerging adults.

Results and Discussion

In this study, a simple and cheap apparatus was tested for the culture of chironomid larvae using *Tanypus punctipennis* as the experimental organism. This apparatus have the potential to be used in culturing of chironomid larvae having identification problems and supplying fishing worm for other studies. The effects of water temperature and pH on larval growth of *T. punctipennis* which is very common chironomid species in Turkish Thrace were also investigated. The results of the experiments testing effects of temperature showed that adult emergence in the control group (+18°C) occurred within 4-5 days. The increasing and decreasing temperature conditions in the test groups revealed different adult emergence periods. All larvae in high temperature (+25°C) group emerged as adults within 2 days, while it took 8-10 days for larvae in low temperature (+10°C) group to emerge as adults (Table 1). In the beginning of the experiments testing effects of pH on larval growth, the natural pH values of the water body containing experimental larvae were measured as 7-8. All larvae of the pH=9-10 group died after changing the pH level in the setup, the larvae placed in the pH=5-6 group survived for only 3 days but no adult emergence occurred after the pupal stage (Table 2).

It is reported that Chironomidae members have relatively a short time period from egg to adult ranging from a few days to one month (Tokeshi 1995). *T. punctipennis* larvae in our study was found to emerge as adults in relatively shorter time period at 25°C compared to control and low temperature conditions. The results in this study showed that this species has high growth ratio at +25°C level.

It is reported that pH levels are also very important in aquatic ecosystems (Makela & Oikari 1992, Courtney & Clements 1998, Weisse & Stadler 2006). The adaptation abilities of benthic macroinvertebrates to the environment can change by pH fluctuations. Increasing alkalinity and pH were reported to have significant effects on the enhancement of protein amounts and body lengths of larval chironomids (Nath *et al*. 2017). Rapid pH changes in an aquatic ecosystem can lead to changes in dynamics of larval chironomid populations, which in turn affect the food chain in a negative manner.

The present results also revealed evidence for that *T. punctipennis* is a species with fast larval growth and high mortality rate in different conditions, and easily maintained in laboratory for experimental studies. More studies on larval chironomids including their growth, feeding, and behaviour are needed for a better understanding of their biology.

This study identified an inexpensive and rapid method that could be used for growth of *T. punctipennis* larvae in laboratory conditions and showed the optimal cultivation conditions of the species. In addition, to provide the natural environment to the larvae, uncontrolled discharges to an aquatic ecosystem must be prevented and monitoring of the water qualities must be made periodically.

Acknowledgement

The preliminary results of the experiments in this study were presented in XIII. Congress of Ecology and Environment with International Participation, held from 12-15 September 2017 in Edirne, Turkey.

Table 1. The results of the experiments on effects of temperature on larval growth.

Embodiments → Days↓	(Control group) (+18°C)	(+25°C)	(+10°C)
1st day	3 alive larvae	3 alive larvae	3 alive larvae
2nd day	3 alive larvae	3 pupae	3 alive larvae
3rd day	1 alive larvae, 2 pupae	3 alive adults	3 alive larvae
4th day	2 pupae, 1 alive adult		3 alive larvae
5th day	3 alive adults		3 alive larvae
6th day			2 alive larvae, 1 pupae
7th day			2 alive larvae, 1 pupae
8th day			2 pupae, 1 alive adult
9th day			2 pupae, 1 alive adult
10th day			3 alive adults

Table 2. The results of the experiments on effects of pH on larval growth.

Embodiments → Days↓	(Control group) (pH=7-8)	(pH=5-6)	(pH=9-10)
1st day	3 alive larvae	3 alive larvae	3 alive larvae
2nd day	3 alive larvae	2 alive larvae, 1 pupae	3 dead larvae
3rd day	1 alive larvae, 2 pupae	3 pupae	
4th day	2 pupae, 1 alive adult	3 dead pupae	
5th day	3 alive adults		

References

1. Armitage, P., Cranston, P.S.V. & Pinder, L.C.V. 1995. *The Chironomidae - The biology and ecology of non-biting midges*, Chapman and Hall, London, 572 pp.
2. Aydın, G.B. & Güher, H. 2017. The Chironomidae (Diptera) fauna of Kırklareli province. *Turkish Journal of Zoology*, 41: 335-341.
3. Courtney, L.A. & Clements, W.H. 1998. Effects of acidic pH on benthic macroinvertebrate communities in stream microcosms. *Hydrobiologia*, 379: 135-145.
4. Çamur-Elipek, B., Arslan, N., Kırgız, T., & Öterler, B. 2006. Benthic macrofauna in Tunca River (Turkey) and their relationship with environmental variables. *Acta Hydrochimica et Hydrobiologica*, 34: 360-366.
5. Çamur-Elipek, B., Arslan, N., Kırgız, T., Öterler, B., Güher, H., & Özkan, N. 2010. Analysis of benthic macroinvertebrates in relation to environmental variables of Lake Gala, a national park of Turkey. *Turkish Journal of Fisheries and Aquatic Sciences*, 10: 235-243.
6. Çamur-Elipek, B., Güher, H., Kırgız, T. & Özkan, N. 2012. A comparative study on larval chironomid limnofauna (Insecta, Diptera) of some lakes in İğneada (Kırklareli, Turkey). *Review of Hydrobiology*, 5(1): 57-70.
7. Epler, J. 2001. *Identification manual for the larval Chironomidae (Diptera) of North and South Carolina.* Orlando, Department of Environmental and Natural Resources, 526 pp.
8. Ferrington, L.C. 2008. Global diversity of non-biting midges (Chironomidae; Insecta-Diptera) in freshwater. *Hydrobiologia*, 595: 447-455.
9. Fittkau, E.J. & Roback, S.S. 1983. *The Larvae of Tanypodinae (Diptera: Chironomidae) of the Holarctic Region –Keys and Diagnoses*, Entomologica Scandinavica Supplement, 19, 109 pp.
10. Habibi, M.A.B., Ali, M.M. & Dey, N. 1992. Culture of chironomids larvae in artificial medium. *Bangladesh Journal of Fisheries*, 20: 63-70.
11. Kenney, M.A., Sutton-Grier, A.E., Smith, R.F. & Gresens, S.E. 2009. Benthic macroinvertebrates as indicators of water quality: The intersection of science and policy. *Terrestrial Arthropod Reviews*, 2: 99-128.
12. Langton, P.H. & Pinder, L.C.V. 2007. *Keys to the Adult Male Chironomidae of Britain and Ireland*, Freshwater Biological Association, 1, 64 pp.
13. Maasri, A., Fayolle, S., Gandouin, E., Garnier, R. & Franquet, E. 2008. Epilithic chironomid larvae and water enrichment: Is larval distribution explained by epilithon quantity or quality? *Journal of the North American Benthological Society*, 27(1): 38-51.

14. Maier, K.J., Kosalwat, P. & Knight, A.W. 1990. Culture of *Chironomus decorus* (Diptera: Chironomidae) and the effect of temperature on its life history. *Environmental Entomology*, 19: 1681-1688.

15. Makela, T.P. & Oikari, A.O.J. 1992. The effects of low water pH on the ionic balance in freshwater mussel *Anodonta anatine* L. *Annales Botanici Fennici*, 29: 169-175.

16. Namayandeh, A. & Beresford, D.V. 2012. An easy to make and simple designed rearing apparatus for Chironomidae. *Chironomus Newsletter for Chironomidae Research*, 25: 33-34.

17. Nath, S., Podder, R. & Kumar, B. 2017. Seasonal trend of body protein and growth of Chironomid larvae. *Proceedings of the Zoological Society*, 70(1): 88-91.

18. Özkan, N. 2006. Fauna of chironomid (Chironomidae; Diptera) in Turkish Thrace Region (Kırklareli, Tekirdağ, İstanbul and Çanakkale). *Ege University Journal of Fisheries and Aquatic Sciences*, 23(1-2): 125-132.

19. Özkan, N. 2007. Chironomidae (Diptera) larvae of Çanakkale Region and their distribution. *Trakya University Journal of Natural Sciences*, 8(2): 123-132.

20. Özkan, N. & Çamur-Elipek, B. 2006. The dynamics of Chironomidae larvae (Diptera) and the water quality in Meriç River (Edirne/Turkey). *TISCIA*, 35: 49-54.

21. Özkan, N. & Çamur-Elipek, B. 2007. Relationships between chironomid communities (Diptera: Chironomidae) and environmental parameters in Sazlıdere Stream (Turkish Thrace). *TISCIA*, 36: 29-34.

22. Özkan, N., Moubayed-Breil, J. & Çamur-Elipek, B. 2010. Ecological analysis of chironomid larvae (Diptera, Chironomidae) in Ergene River Basin (Turkish Thrace). *Turkish Journal of Fisheries and Aquatic Sciences*, 10: 93-99.

23. Sæther, O.A. 1980. *Glossary of Chironomid Morphology Terminology (Diptera, Chironomidae)*, Scandinavian Entomology, Sandby, Sweden, 51 pp.

24. Sahandi, J. 2011. Natural food production for aquaculture: Cultivation and nutrition of chironomid larvae (Insecta, Diptera). *Advances in Environmental Sciences- Bioflux*, 3(3): 268-271.

25. Specziar, A. 2008. Life history patterns of *Procladius choreus*, *Tanypus punctipennis* and *Chironomus balatonicus* in Lake Balaton. *Annales de Limnologie - International Journal of Limnology*, 44(3): 181-188.

26. Tokeshi, M. 1995. Life cycles and population dynamics, Pp 225-250 In: P.D. Armitage, P.S. Cranston and L.C.V. Pinder (Editors) *The Chironomidae: biology and ecology of non-biting midges.* Chapman and Hall. London UK, 572 pp.

27. Vallenduuk, H. & Lipinski, A. 2009. Neglected and new characters in Chironomidae: Tanypodinae (Larvae). *Lauterbornia*, 68: 83-93.

28. Vos, J.H., Ooijevaar, M.A.G., Postma, J.E. & Admiraal, W. 2000. Interaction between food availability and food quality during growth of early instar chironomid larvae. *Journal of North American Benthological Society*, 19(1): 158-168.

29. Weisse, T. & Stadler, P. 2006. Effect of pH on growth, cell volume, and production of freshwater ciliates, and implications for their distribution. *Limnology and Oceanography*, 51(4): 1708-1715.

30. Wiederholm, T. 1989. *The Adult Males of Chironomidae (Diptera) of the Holarctic Region-Keys and Diagnoses*, Entomologica Scandinavica Supplement, 34, 532 pp.

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Örnek:

Tek yazarlı Makale için

Soyadı, A. Yıl. Makalenin adı. (Sözcüklerin ilk harfi küçük). *Yayınlandığı derginin açık ve tam adı*, Cilt(Sayı)**:** Sayfa aralığı.

Kıvan, M. 1998. *Eurygaster integriceps* Put. (Heteroptera: Scuteleridae)'nin yumurta parazitoiti *Trissolcus semistriatus* Nees (Hymenoptera: Scelionidae)'un biyolojisi üzerinde araştırmalar*. Türkiye Entomoloji Dergisi*, 22(4): 243-257.

İki ya da daha çok yazarlı makale için

Soyadı1, A1. & Soyadı2**,** A2. Yıl. Makalenin adı**.** (Sözcüklerin ilk harfi küçük). *Yayınlandığı derginin tam adı*, Cilt(Sayı): Sayfa aralığı.

Lodos, N. & Önder, F. 1979. Controbution to the study on the Turkish Pentatomoidea (Heteroptera) IV. Family: Acanthasomatidae Stal 1864. *Türkiye Bitki Koruma Dergisi*, 3(3): 139-160.

Soyadı1, A1., Soyadı2, A2. & Soyadı3, A3**.** Yıl. Makalenin adı**.** (Sözcüklerin ilk harfi küçük). *Yayınlandığı derginin tam adı*, Cilt (Sayı): Sayfa aralığı.

Önder, F., Ünal, A. & Ünal, E. 1981. Heteroptera fauna collected by light traps in some districts of Northwestern part of Anatolia. *Türkiye Bitki Koruma Dergisi***,** 5(3): 151-169.

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Önder F., Karsavuran, Y., Tezcan, S. & Fent, M. 2006. *Türkiye Heteroptera (Insecta) Kataloğu*. Meta Basım Matbaacılık, İzmir, 164 s.

Lodos, N., Önder, F., Pehlivan, E., Atalay, R., Erkin, E., Karsavuran, Y., Tezcan, S. & Aksoy, S. 1999. Faunistic *Studies on Lygaeidae (Heteroptera) of Western Black Sea, Central Anatolia and Mediterranean Regions of Turkey.* Ege University, İzmir, ix + 58 pp.

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Jansson, A. 1995. Family Corixidae Leach, 1815—The water boatmen. Pp. 26–56. In: Aukema, B. & Rieger, Ch. (eds) Catalogue of the Heteroptera of the Palaearctic Region. Vol. 1. Enicocephalomorpha, Dipsocoromorpha, Nepomorpha, Gerromorpha and Leptopodomorpha. The Netherlands Entomological Society, Amsterdam, xxvi + 222 pp.

Kongre, Sempozyum: Yazarlar, Yıl. "Bildirinin adı (Sözcüklerin ilk harfi küçük), sayfa aralığı". Kongre/Sempozyum Adı (Tarihi (gün aralığı ve ay), Toplantı Yeri) Bildirileri, (varsa) Yayınlayan Kurum, toplam sayfa sayısı s./pp.

Örnek:

Önder, F., Karsavuran, Y., Pehlivan, E. & Turanlı, F. 1995. Güneydoğu Anadolu Projesi (GAP) uygulama alanında saptanan Pentatomoidea (Heteroptera) türleriyle ilgili bir değerlendirme. GAP Bölgesi Bitki Koruma Sorunları ve Çözüm Önerileri Sempozyumu, 27-29 Nisan, Şanlıurfa, 120-130.

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Hatch, S., 2001. Studentsperception of online education. Multimedia CBT Systems. http://www.scu.edu.au/schools/sawd/moconf/papers2001/hatch.pdf (Erişim: May 2009).

Kaynaklara metin içinde numara verilmemeli ve aşağıdaki örneklerde olduğu gibi belirtilmelidir.

Örnekler:
... x maddesi atmosferde kirliliğe neden olmaktadır (Landen 2002). Landen (2002) x maddesinin atmosferde kirliliğe neden olduğunu belirtmiştir. İki yazarlı bir çalışma kaynak olarak verilecekse, (Landen & Bruce 2002) veya Landen & Bruce (2002)'ye göre. ... şeklinde olmuştur; diye verilmelidir. Üç veya daha fazla yazar söz konusu ise, (Landen *et al.* 2002) veya Landen *et al.* (2002)'ye göre .... olduğu gösterilmiştir; diye yazılmalıdır.

Şekil ve Tablolar: Tablo dışında kalan fotoğraf, resim, çizim ve grafik gibi göstermeler "Şekil" olarak verilmelidir. Resim, şekil ve grafikler, net ve ofset baskı tekniğine uygun olmalıdır. Her tablo ve şeklin metin içindeki yerlerine konmalıdır. Tüm tablo ve şekiller yazı boyunca sırayla numaralandırılmalı (Tablo 1., Şekil. 1), başlık ve açıklamalar içermelidir. Şekillerin sıra numaraları ve başlıkları, alta, tabloların ki ise üstlerine yazılır.

Şekiller (tablo dışında kalan fotoğraf, resim, çizim ve grafik gibi) tek tek dosyalar halinde en az **300 dpi** çözünürlükte ve **tif** dosyası olarak şekil numaraları dosya isminde belirtilmiş şekilde ayrıca sisteme ek dosya olarak yüklenmelidir.

Sunulan yazılar, öncelikle Dergi Yayın Kurulu tarafından ön incelemeye tabii tutulur. **Dergi Yayın Kurulu, yayınlanabilecek nitelikte bulmadığı veya yazım kurallarına uygun hazırlanmayan yazıları hakemlere göndermeden red kararı verme hakkına sahiptir.** Değerlendirmeye alınabilecek olan yazılar, incelenmek üzere iki ayrı hakeme gönderilir. Dergi Yayın Kurulu, hakem raporlarını dikkate alarak yazıların yayınlanmak üzere kabul edilip edilmemesine karar verir.

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Balkan Yerleşkesi
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Tel : 0284 235 82 30
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Phone : +90 284 235 82 30
Fax : +90 284 235 82 37
e-mail : tujns@trakya.edu.tr