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İçindekiler (Contents)

Orijinal araştırmalar (Original articles)

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Original article (Orijinal araştırma)

Parasitoids of the apple ermine moth, *Yponomeuta malinellus* Zeller, 1838 (Lepidoptera: Yponomeutidae), in the Çoruh Valley, Erzurum Province, Turkey

Çoruh Vadisi'nde (Erzurum, Türkiye) elma ağ kurdu [*Yponomeuta malinellus* Zeller, 1838 (Lepidoptera: Yponomeutidae)]'nun parazitoidleri

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Summary

Parasitoids of *Yponomeuta malinellus* Zeller, 1838 (Lepidoptera: Yponomeutidae), in various host plants (especially apple) were investigated in the Coruh Valley, Erzurum Province, Turkey, during 2015 and 2016. The parasitoids associated with *Y. malinellus* were reared in a laboratory, with a total of 255 individual parasitoids emerging from three families, Braconidae, Ichneumonidae (Hymenoptera), and Tachinidae (Diptera). Six parasitoid species, *Habrobracon concolorans* (Marshall, 1900) (Hymenoptera: Braconidae), *Diadegma armillatum* (Gravenhorst, 1829), *Trieces tricarinatus* (Holmgren, 1858), *Itoplectis tunetana* (Schmiedeckneckt, 1914), *Itoplectis maculator* (Fabricius, 1775) (Hymenoptera: Ichneumonidae) and *Bessa parallela* (Meigen, 1824) (Diptera: Tachinidae), were determined. Of these, *H. concolorans* was reared from *Y. malinellus* for the first time. Apple ermine moth is therefore a new host for this parasitoid. The combined contribution of the parasitoids in parasitizing apple ermine was 25.5%, with *D. armillatum* being the most numerous accounting for 5.5% of all parasitoids reared.

Keywords: Coruh Valley, *Habrobracon concolorans*, parasitoid, Turkey, *Yponomeuta malinellus*

Özet

Çoruh Vadisi'nde 2015-2016 yıllarında yürütülen bu çalışma, özellikle elma ağaçlarında konukçu olan, elma ağ kurdu [*Yponomeuta malinellus* Zeller, 1838 (Lepidoptera: Yponomeutidae)]'nun parazitoidlerini belirlemek amacıyla yapılmıştır. Braconidae, Ichneumonidae ve Tachinidae familyalarına bağlı toplam 255 parazitoid örneğinin laboratuvarda çıkışı sağlanmıştır. *Habrobracon concolorans* (Marshall, 1900) (Hymenoptera: Braconidae); *Diadegma armillatum* (Gravenhorst, 1829), *Trieces tricarinatus* (Holmgren, 1858), *Itoplectis tunetana* (Schmiedeckneckt, 1914), *Itoplectis maculator* (Fabricius, 1775) (Hymenoptera: Ichneumonidae) ve *Bessa parallela* (Meigen, 1824) (Diptera: Tachinidae) olmak üzere belirlenen 6 parazitoid tür içerisinde, *H. concolorans* için elma ağ kurdu yeni bir konukçudur. %25.5 oranında parazitlenmenin görüldüğü çalışmada, en fazla çıkış %5.5 ile *D. armillatum*'da görülmüştür.

Anahtar sözcükler: Çoruh Vadisi, *Habrobracon concolorans*, parazitoid, Türkiye, *Yponomeuta malinellus*

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Introduction

Yponomeuta malinellus Zeller, 1838 (Lepidoptera: Yponomeutidae), is widespread in the Palearctic region (Kuhlmann et al., 1988) and is known as an important pest of some mahlep cherry, cultivars of apples and twigs of large poplar trees. This pest is a univoltine defoliator of *Malus* spp. in Europe and Asia. It is a member of a complex of host-differentiated defoliators known as the small ermine moths (Menken et al., 1992).

The main hosts of *Y. malinellus* are *Malus* spp. (apple). Some sources state that this pest exclusively feeds on *Malus spp.* (Carter, 1984; Philip and Edwards, 1991; CFIA, 2006), while others include a broader host range (Menken et al., 1992). The most commonly reported hosts are *Malus* spp. and *Pyrus communis* (pear) (Philip & Edwards, 1991; Menken et al., 1992).

This species is found throughout most of Europe and parts of Asia. Some countries where this pest is found are Asia (China, Japan, Kazakhstan and Korea), Europe (Czech Republic, Finland, France, Georgia, Germany, Italy, Lithuania, the Netherlands, Sweden, Turkey, Ukraine and the United Kingdom), Middle East (Armenia, Azerbaijan, Iran, Pakistan and Uzbekistan), North America (Canada) (Gershenzon, 1970; Pustovarov, 1980; Mamedov & Makhmudova-Kurbanova, 1982; Arduino et al., 1983; Kuhlmann et al., 1988; Orr, 1991; Unruh et al., 1993; Jonaitis, 2001; Gençer, 2003; Hrudová, 2003; Lee & Pemberton, 2005; CFIA, 2006; Kimber, 2011).

The parasitoids of the small ermine moths of Europe and the former Soviet Union have been extensively studied (Beirne, 1943; Junnikkala, 1960; Friese, 1963; Affolter & Carl, 1986; Dijkerman et al., 1986; Kuhlmann, 1996), while those in Korea, Japan and China are less well known (Friese, 1963). More than 50 species of parasitoids or hyperparasitoids have been associated with the small ermine moths in Europe, but only a few of these are common (Affolter & Carl, 1986). Several authors attribute regulation of ermine moths in Eurasia to parasitoids (Vaclav, 1958; Pyornila & Pyornila, 1979; Affolter & Carl, 1986; Kuhlmann et al., 1988).

In Turkey, *Y. malinellus* has not been studied in detail, although it is an important defoliator of a range of plants particularly in eastern and central Turkey. This species has been reported by Koçak (1989), and several other authors (Iren, 1960; Bulut & Kılınçer, 1989; Erol & Yaşar, 1996; Tozlu et al., 2000; Gençer, 2003, Çoruh, 2005; Çoruh & Özbek, 2008; Çoruh, 2010) have reported finding this pest in Amasya, Ankara, Erzurum, Manisa and Van.

A total of 97% of the fruit produced in Erzurum Province is produced in the Coruh Valley, so a range of pests and diseases are common in this area and cause considerable damage and economic loss (Güçlü et al., 1998). *Yponomeuta malinellus* is a very important pest, especially on *Malus* spp., in this region.

Also, parasitoids of this species have not been a subject of detailed study in Turkey (Iren, 1960; Gençer & Doğanlar, 1996; Gençer, 2003). In this study, our aims were to (1) determine the species parasitoids associated with *Y. malinellus* in Erzurum Province of Turkey, (2) determine natural parasitism rates, (3) consider the potential of parasitoids for classical biological control of this species.

Material and Methods

Study area

This study was conducted during 2015 and 2016. *Yponomeuta malinellus* feeding as caterpillars on the leaves of apple were collected in the Coruh Valley (Erzurum Province) (Figure 1).

The Coruh Valley, with its geological and geomorphological diversity, and unique of vegetation, has extraordinary importance for nature conservation. Its rich biological diversity is the basis for its recognition as one of the most important 25 ecoregions under threat by International Environmental Protection Agency, the World Bank and the Global Environment Fund (Aslantaş et al., 2011).

The climate of the Coruh Valley is particularly suitable for fruit production. Consequently, fruit production is a long-established tradition in many districts within the valley and many localities are known by names of fruit. There are many villages named after the fruit such as almond, walnut, cherry and apple (Karlıdağ & Eşitken, 2006).

Sampling and collection method

A total of 1000 *Y. malinellus* larvae were collected by hand from trees in study area (Figure 1) and each sample was placed in a box with apple leaves and covered with cheesecloth (Figure 2).

Samples were collected from different apple orchards (Figure 3) at about 1200 m altitude. The common apple trees were *Malus pumila* Mill. cultivar Golden Delicious, one of the most important apple cultivars of the 20th century. *Malus pumila* is a highly important commercial crop in the valley.

Figure 1. Map of the study area.

Larvae were reared in a laboratory at ambient temperature to obtain parasitoids and were placed in groups of 10 in boxes (10 by 20 cm) for moth or parasitoid emergence.

Periodically, withered leaves were replaced with fresh ones and checked every 1 or 2 days for 4 to 5 weeks. Emerging adults of parasitoids in the boxes were transferred to a killing jar.

Parasitoids identifications was verified by comparison with the preserved specimens in the Entomology Museum, Erzurum, Turkey (EMET). The unidentified specimens were determined by specialists (Dr. Janko Kolarov, Dr. Miktat Doğanlar, Dr. Kenan Kara and Dr. Saliha Çoruh).

Figure 2. Rearing boxes.

Results

All the parasitoids that emerged in the laboratory were members of the orders Diptera and Hymenoptera. From a total of 255 samples, six parasitoid species were reared from *Y. malinellus* during 2015 and 2016 (Table 1). Among these parasitoids, four species, *Diadegma armillatum* (Gravenhorst, 1829), *Trieces tricarinatus* (Holmgren, 1858)*, Itoplectis tunetana* (Schmiedeckneckt, 1914) and *Itoplectis maculator* (Fabricius, 1775), belong to the family Ichneumonidae (Hymenoptera); one species, *Bessa parallela (*Meigen, 1824) belongs to the family Tachinidae (Diptera) and one species, *Habrobracon concolorans* (Marshall, 1900) belongs to the family Braconidae (Hymenoptera). The adults of parasitoids and moths were deposited in the EMET (as detailed in Table 2).

Figure 3. Infestation of *Yponomeuta malinellus* larvae on *Malus pumila*.

Table 1. List of the parasitoids obtained from the *Yponomeuta malinellus* (2015-2016)

Parasitoid species	Order	Family	Number of individual parasitoids	Number of females	Number of males
Diadegma armillatum (%22)	Hymenoptera	Ichneumonidae	55	28	27
Itoplectis maculator (%16)			42	23	19
Trieces tricarinatus (%15)			39	18	21
Itoplectis tunetana (%11)			27	11	16
Habrobracon concolorans (%19)	Hymenoptera	Braconidae	48	27	21
Bessa parallela (%17)	Diptera	Tachinidae	44	23	21
Total	3	6	255	130	125

Table 2. List of records of parasitoids on *Yponomeuta malinellus* (Yu et al., 2012)

Accepted scientific name	Original name	Synonyms	Parasitism	Geographic area*	Associated plant
Diadegma armillatum	*Campoplex armillatus*	*Angitia monospila* *Angitia pseudocombinata* *Campoplex tibialis*	Endoparasitoid	AUS, EP, E, WP	*Alnus glutinosa* *Medicago sativa* *Peucedanum oreoselinum* *Picea* sp.
Itoplectis maculator	*Ichneumon maculator*	*Ichneumon arlequinatus* *Ichneumon graminellae* *Ichneumon lateratoriu* *Ichneumon plaesseus* *Ichneumon scanicus* *Itoplectis rufiventris* *Pimpla castaniventris* *Pimpla cruentata* *Pimpla maculatrix* *Pimpla sexpunctata* *Pimpla tricolor* *Pimpla vincta*	Endoparasitoid	EP, E, WP	*Adonis vernalis* *Alnus glutinosa* *Chaerophyllum bulbosum* *Cnicus paluster* *Daucus carota* *Epilobium angustifolium* *Euphorbia nicaeensis* *Fraxinus excelsior* *Heracleum sphondylium* *Peucedanum oreoselinum* *Picea abies* *Picea excelsa* *Pinus sylvestris* *Quercus ilex* *Quercus sessiliflora* *Rubus* sp. *Taxus baccata*
Trieces tricarinatus	*Chorinaeus tricarinatus*	*Chorinaeus facialis*	Endoparasitoid	E, WP	
Itoplectis tunetana	*Pimpla tunetana*	*Itoplectis alternoides* *Itoplectis europeator* *Itoplectis haemorrhoidalis* *Itoplectis mediorufa*	Endoparasitoid	EP, E, WP	
Habrobracon concolorans	*Bracon concolorans*	*Bracon opacus* *Habrobracon mongolicus* *Habrobracon nigricans*	Endoparasitoid	EP, E, ORR, WP	
Bessa parallela		*Tachina parallela*	Endoparasitoid	PR	

* Geographic area: AUS: Australian region, E: Europe, EP: Eastern Palearctic, NEAR: Nearctic region, NTR: Neotropical, ORR: Oriental, P: Palearctic, WP: Western Palearctic.

Discussion

The valley that takes its name from the Coruh River, which flows for 442 km through Turkey, possesses a landscape as spectacular as it is vast. The Coruh River, which carved out this valley and which, owing to its topographical structure, ranks among the world's fastest flowing rivers, begins on the western slopes of Mt Mescit between the cities of Ispir and Erzurum.

Parasitoids of *Y. malinellus* have been reported in previous studies in Turkey (Gençer, 2003).

Lill et al., (2002) reported that host plant species had a large influence on infestation rates herbivores. There are significantly different infestation rates of apple ermine moth between geographical locations sampled, which is likely due to the habitat type and host plants (Lee & Pemberton, 2005). In this study of the Coruh Valley, the mean parasitism rate was 25.5%. In other studies, in Turkey and internationally, rates between 30 and 90% have been reported (İren, 1960; Junnikkala, 1960; Dijkerman et al., 1986; Kuhlmann, et al., 1988; Gençer & Doğanlar, 1996; Gençer, 2003).

Diadegma armillatum is known to be an important parasitoid in Europe and Eurasia. This species has been obtained from 64 different hosts worldwide. It is a major parasite of ermine moths in Europe (Junnikkala, 1960), causing relatively high percentage of parasitism, ranging from 10 to 40% (Balachowsky, 1966; Zayanchkauskas et al., 1979). In contrast, in Northeast Asia the mean parasitism rate of the moth was 0.3% in Korea and below 0.05% in the other regions. We found that, *D. armillatum* had highest abundance of the parasitoids obtained from *Y. malinellus* in apple in the Coruh Valley. It caused 5.5% mortality which was the highest of the the six parasitoids found in this study. It was obtained from about 22% of the parasitized larvae (55 of 255) in this study. This parasitoid species is considered to provide potentially useful biological control of *Plutella (Plutella) xylostella* (Linnaeus, 1758) (Lepidoptera: Yponomeutidae) and *Y. malinellus* elsewhere in the world (Yu et al., 2012).

Itoplectis maculator has a large range of host species. Yu et al., (2012) listed about 158 host species in lepidopteran families including Lasiocampidae, Noctuidae, Nolidae, Notodontidae, Nymphalidae, Pterophoridae and Pyralidae. This parasitoid has been reared from *Archips* sp. (Lepidoptera: Tortricidae) (İren, 1952, 1960, 1977; Doğanlar, 1982, 1987; Ulu, 1983; Kansu et al., 1986; Özdemir & Kılınçer, 1990), *Archips rosana* (Linnaeus, 1758) (Lepidoptera: Tortricidae) (Ulu, 1983; Doğanlar, 1987, 2003; Öncüer, 1991; Özdemir & Özdemir, 2002; Çoruh & Özbek, 2008), *Tortrix viridana* Linnaeus, 1758 (Lepidoptera: Tortricidae) (Özdemir & Kılınçer, 1990; Öncüer, 1991); *Acleris rhombana* (Denis & Schiffermüller, 1775) (Lepidoptera: Tortricidae) (Çoruh & Özbek, 2008), *Yponomeuta* sp. (Lepidoptera: Yponomeutidae) (İren, 1977; Ulu, 1983; Kansu et al., 1986; Doğanlar, 1987), *Yponomeuta evonymella* (Linnaeus, 1758) (Lepidoptera: Yponomeutidae) (Çoruh & Özbek, 2008), *Y. malinellus* (İren, 1952, 1960; Soydanbay, 1978; Ulu, 1983; Özdemir & Kılınçer, 1990; Öncüer, 1991; Erol & Yaşar, 1996), *Yponomeuta padella* (Linnaeus, 1758) and *Yponomeuta rorrella* (Hübner, 1796) (Lepidoptera: Yponomeutidae) (İren, 1952, 1960; Soydanbay, 1978; Ulu, 1983; Özdemir & Kılınçer, 1990; Öncüer, 1991), *Malacosoma (Clisiocampa) neustria* (Linnaeus, 1758) (Lasiocampidae: Lepidoptera) (Özder, 1999), *Lamprosticta culta* (Denis & Schiffermüller, 1775) (Lepidoptera: Noctuidae) (Okyar & Yurtcan, 2007); *Autographa gamma* (Linnaeus, 1758) (Lepidoptera: Noctuidae) (Okyar & Yurtcan, 2007), *Rhagoletis cerasi* (Linnaeus, 1758) (Diptera: Tephritidae) (Özder, 1999), *Myzus (Myzus) cerasi* (Fabricius, 1775) (Homoptera: Aphididae) (Özder, 1999), and *Hypera variabilis* (Herbst, 1795) (Coleoptera: Curculionidae) (İren, 1952, 1960; Özdemir & Kılınçer, 1990; Öncüer, 1991) in Turkey. It caused 4.2% mortality of the *Y. malinellus* specimens collected in this study, being the second highest of the ichneumonid parasitoids, and was obtained from 16% of the parasitized larvae (42 of 255).

Trieces tricarinatus has been obtained from *Y. malinellus*, *Y. padella*, *Y. rorrella* and *Yponomeuta sedella* (Treitschke, 1832) (Lepidoptera: Yponomeutidae) (Dijkerman et al., 1986), *Yponomeuta cagnagella* (Hübner, 1813) (Lepidoptera: Yponomeutidae) (Aliev, 1983) and *Y. evonymella* (Haeselbarth, 1989). Also, this species is used as a biological control agent of *Y. malinellus* in Canada and the USA (Dijkerman et al., 1986). Nevertheless, studies on this parasitoid are limited in Turkey. Gençer (2003), obtained it from the larvae apple ermine moth in Sivas at a rate of 0.6%. It caused 3.9% mortality of the specimens collected in this study and was obtained from 15% of the parasitized larvae (39 of 255).

Itoplectis tunetana is parasitoid with of some biocontrol importance. In Turkey, this parasitoid has been obtained from *Y. evonymella* (Çoruh & Özbek, 2008), *Y. malinellus* (Özdemir & Kılınçer, 1990; Erol & Yaşar, 1996; Gençer, 2003), *Y. padella* (Özdemir & Kılınçer, 1990) and *Y. rorrella* (Özdemir & Kılınçer, 1990). *Itoplectis tunetana* has 15 different known hosts worldwide (Talebi et al., 2005; Yu et al., 2012). It caused 1.1% mortality of the specimens collected in this study, was obtained from 11% of the parasitized larvae (27 of 255). *Habrobracon concolorans* is a Trans-Eurasian species (Samartsev & Belokobylskij, 2013), being widely distributed in the Palearctic and Oriental regions (Yu et al., 2012). It has seven known hosts worldwide. Beyarslan et al. (2005), listed 62 species of Braconidae from the western Black Sea region in Turkey and reported that *H. concolorans* obtained from *Etiella zinckenella* (Treitschke, 1832) (Lepidoptera: Pyralidae), *Pexicopia malvella* (Hübner, 1805) (Lepidoptera: Gelechiidae), *Cnephasia (Cnephasia) sedana* (Constant, 1884) (Lepidoptera: Tortricidae), all of which are microlepidoptera. It caused 4.8% mortality of the specimens collected in this study and was obtained from 19% of parasitized larvae (48 of 255). Notably, *Y malinellus* is considered to a new host record for *H. concolorans*.

Bessa parallela was the only tachinid parasitoid obtained from the *Y. malinellus*. It is broadly distributed in the Palearctic region and has more than 20 recorded lepidopterous hosts from many families (Herting, 1960). *Bessa parallela* is a gregarious larval parasitoid of some important lepidopteran pests, such as *Pieris rapae* (Linnaeus, 1758) (Lepidoptera: Pieridae) and *Pryeria sinica* Moore, 1877 (Lepidoptera: Zygaenidae) (Shima, 1999). The catalog of Kara & Tschorsnig (2003) lists tachinid parasitoids obtained from different hosts in Turkey. *Bessa parallela* has been obtained from *Yponomeuta* sp., *Y. malinellus*, *Y. padella* and *Nycteola* sp. (Kansu et al., 1986; Kara, 1998; Kara & Özdemir, 2000; Kara & Tschorsnig, 2003). It caused 4.4% mortality of the specimens collected in this study and was obtained from 17% of the parasitized larvae (44 of 255).

The study has provided useful new information on the parasitoids of *Y. malinellus* the Coruh Valley, which will underpin future laboratory and field studies.

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Original article (Orijinal araştırma)

Morphometric analysis of wild-caught flies of *Drosophila* (Diptera: Drosophilidae) species: Altitudinal pattern of body size traits, wing morphology and sexual dimorphism

Doğadan yakalanan *Drosophila* (Diptera: Drosophilidae) türlerinin morfometrik analizi: Vücut büyüklüğü özelliklerinin yüksekliğe bağlı değişimi, kanat morfolojisi ve eşeysel dimorfizmi

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Summary

Literature concerning phenotypic variation among wild-caught drosophilids inhabiting varied ecological habitats is relatively rare. The present study explores pattern of body size traits along altitudinal gradients, and compensation to colder environments and reduced air pressure via adjustment of wing morphology at higher altitudes. Wild adult flies were collected in two extensive surveys during September-October 2014 and April-May 2015. All traits were measured for both the sexes to obtain data on sexual dimorphism. It was found that though these populations differed significantly in their size, as already known, they deviated from the expected reaction norms of size increase along altitudinal gradients as observed in several previous studies. This deviation from normal clinal trend can be attributed to variation in growth rates and development times at different altitudes which has important implications in overall reproductive success. Also, a significant increase in wing area of flies at higher altitude was recorded with dramatically lower wing loadings than flies that developed in comparatively warmer habitats, giving them an aerodynamic advantage at cold temperatures. Thorax width was also analyzed, possibly for the first time in wild-caught flies of Indian populations, revealing sexual dimorphism. The ratio of thorax length to width was greater than one for all species indicating that the thorax is more elongated in females, which may also influence the flight capacity of the sexes.

Keywords: Bergmann rule, Diptera, Drosophilidae, morphometric traits, plasticity

Özet

Çeşitli ekolojik habitatlarda yaşayan doğadan toplanmış Drosophila türleri arasındaki fenotipik çeşitlilik ile ilgili literatür sayısı nispeten azdır. Bu çalışmada vücut boyutu özelliklerinin yükseklik eğrileri boyunca olan uyumu ve daha yüksek yerlerde kanat morfolojisinin değişimiyle daha soğuk ortamlara ve daha düşük hava basıncına uyum sağlanması incelenmiştir. Doğadan ergin sinekler, Eylül-Ekim 2014 ve Nisan-Mayıs 2015 tarihlerinde iki kapsamlı sürvey ile toplanmıştır. Eşeysel dimorfizmi hakkında bilgi edinmek için her iki cinste de tüm özellikler ölçülmüştür. Bilindiği gibi, bu popülasyonların boyutlarında önemli farklılıklar olmasına rağmen, daha önceki birçok çalışmada gözlemlendiği gibi, yükseklik eğrileri boyunca boyut artışının beklenen reaksiyon normlarından sapmış oldukları bulunmuştur. Normal klinal eğimindeki bu sapma, genel üreme başarısında önemli etkileri olan farklı yüksekliklerde büyüme hızlarındaki ve gelişim zamanlarındaki farklılıklara bağlanabilir. Ayrıca, yüksek irtifadaki sineklerin kanat alanlarındaki önemli bir artış, karşılaştırmalı olarak daha sıcak habitatlarda gelişen sineklerden dramatik olarak çok daha düşük kanat yükleri ile rekor kırmış olmaları sayesinde onlara soğuk hava koşullarında aerodinamik bir avantaj sağlamıştır. Bu arada, muhtemelen Hint popülasyonlarının doğadan yakalanmış sineklerinde ilk kez, Thoraks genişliği, cinsel dimorfizmi açığa çıkararak analiz edilmiştir. Thoraks uzunluğunun genişliğe oranı tüm türler için birden fazla olup; bu da eşeylerin uçuş kapasitesini etkileyebilen thoraksın dişilerde daha uzun olduğunu göstermektedir.

Anahtar sözcükler: Bergmann kuralı, Diptera, Drosophilidae, morfometrik özellikler, plastisite

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Introduction

Analyzing variability of biological characteristics of organisms in response to some geographical gradient has been the prime approach to interpret macro-ecological patterns. The most widely cited studies are those of Bergmann, Allen, Gloger and Jordan (Huxley, 1942). Bergmann (1847) illustrated an eco-geographical pattern where organisms show increased body size or mass in colder climates, reflecting an altitudinal or latitudinal cline, with larger organisms at higher altitudes or latitudes. Ray (1960) first proposed the explanation that ectotherms might also follow Bergmann's rule as the average temperature decreases with increasing altitude or latitude and ectotherms reared at lower temperatures typically matured at larger sizes as compared to their conspecifics reared at higher temperatures. The related Allen's rule explained the significance of shorter limbs and less surface area in colder regions and vice versa (Daly, 1985). This has received little attention in insects (Ray, 1960; Peat et al., 2005) possibly because the results are complicated and conflict with Bergmann's rule. Some studies have argued Allen's rule as an exception rather than a rule, since protruding parts may be under strong selective pressures rather than other body parts related to thermoregulation (Stevenson, 1986).

The inverse Bergmann's rule has also been documented for insects (Van Voorhies, 1997; García-Barros, 2000) hinting that for diverse taxa body size even decreases from the tropics towards the poles, i.e., from warmer to colder climates (Mousseau, 1997). A species inhabiting different climatic conditions, adapts to the local climate often resulting in progressive genetic variations among populations. Also, the phenotypic plasticity, which is a general property of living beings, can contribute to geographical adaptation, if there is genetic variation for such plasticity (DeWitt & Scheiner, 2004). Phenotype along an environmental gradient is determined by its genotype. Though rigorous genetic studies should be conducted, a thorough understanding of geographic variation in morphology of ectotherms is prerequisite to compare the response curves to an environmental gradient of different populations, or the shape of the reaction norms. Understanding such adaptive capacity of natural populations and species has remained a central problem for evolutionary biologists, and comparative methods have long been powerful tools for exploring such capacities.

With thousands of described species, drosophilid flies appear as an irreplaceable model for investigating both phenotypic and genotypic adaptations. Biogeographically these species are usually classified either as tropical (cold sensitive) or temperate (cold tolerant). Only a few drosophilids can proliferate in both tropical and temperate environments and are termed widespread or often cosmopolitan (David & Tsacas, 1981; Powell, 1997). Geographical gradients as a proxy for climatic adaptations in such cosmopolitan flies have remained a fascinating arena for drosophilid researchers. Most studies have focused on latitudinal body size variations, in various species including *Drosophila robusta* Sturtevant, 1916 (Diptera: Drosophilidae) (Stalker & Carson, 1947), *Drosophila subobscura* Collin, 1936 (Prevosti, 1955), *Drosophila melanogaster* Meigen, 1830 and *Drosophila simulans* Sturtevant, 1919 (Capy et al., 1993; Gibert et al., 2004), *Drosophila kikkawai* Burla, 1954 (Karan et al., 1998) and *Zaprionus indianus* Gupta, 1970 (Karan et al., 2000; David et al., 2006a). Body size traits have been observed as highly-plastic showing increasing trend towards higher latitudes and colder places, and vice versa (Angilletta et al., 2004) often referred to as the temperature-size rule. Like the small size of wild flies (the expected result of natural selection) in warm tropical conditions, can be attributed to small genetic size due to the cline and smaller phenotypic size due to plasticity, favoring better fitness of small individuals in warm environments (Atkinson & Sibly, 1997; James et al., 1997).

However, no clinal pattern has been observed in some species (Loeschke et al., 2000), the temperature-size rule is not always convincing for traits such as thorax size (David et al., 2006a) and distinct phenotypes have been observed for distant geographic populations inhabiting the same thermal climatic conditions (Capy et al., 1993). Pitchers et al. (2013) studied variation in wing shape and size in *D. melanogaster* derived from populations at varying altitudes and latitudes across sub-Saharan Africa suggesting that selection responsible for these phenotypic clines may be more complex than just thermal adaptation. Klepsatel et al. (2014) also suggested that clinal patterns in morphology are not a simple function of changes in body size; instead, each trait might be subject to different selection pressures while Carreira et al. (2016) revealed weak clinal signals and a strong population effect on morphological variation and within-population genetic variation associated to the second chromosome. Singh (2015) has reviewed the work conducted on *Drosophila ananassae* Doleschall, 1858, *D. melanogaster*, *Drosophila nasuta* Lamb, 1914, *Drosophila bipectinata* Duda, 1923 and other species in India highlighting that these species vary in degree and pattern of genetic diversity and have evolved different mechanisms for adjusting to their environments. Evidently, such range of quantitative variation observed among geographic populations, call for deeper and more accurate investigations on these paradigmatic drosophilid species.

With most investigations along latitudes emphasizing the role of temperature in shaping different morphological traits, altitudinal gradient provides more rapid change in environmental conditions occurring over relatively small distance compared to equivalent distances over latitude. The Himalayan range is among the most intricate and diverse mountain systems in the world. It forms distinct geological and ecological entity, influencing climate and biotic aspects of the region. The varying topography promotes environmental heterogeneity at both temporal and spatial scales affecting diversity and distribution patterns of biodiversity elements. Uttarakhand State located in Central Himalayan region of India encompasses highly varied tropical to temperate like regimes in span of just few hundred kilometers due to its variable altitudinal terrain. Extensive explorations over the past decade identified more than 90 species from this region (Sarswat et al., 2015), with a significant number of novel species. Prior to this around 300 drosophilid species had been recorded throughout varied eco-geographical zones in India (Gupta, 2005; Kumar & Ajai, 2009). The change in environmental conditions occurring over short geographic distance in this Himalayan range profoundly effect the morphology, physiology and evolution of these flies.

The present study attempts to explore phenotypic variation among wild-caught drosophilids inhabiting varied ecological habitats, i.e., patterns of body size traits along altitudinal gradients (several traits were investigated along with different body shape indices) and compensation to colder environment and reduced air pressure via adjustment of wing morphology at higher altitudes (flight related traits such as wing length, wing width and wing area, along with wing aspect ratio and wing loading). All traits were measured for both the sexes to obtain data on sexual dimorphism. Thorax width was also analyzed, possibly for the first time in wild-caught flies of Indian populations, revealing difference between the sexes, with more elongated female thorax than male. In this study, it was found that though these populations differed significantly, they deviated from the expected, i.e., increasing size and shape related traits observed along altitudinal gradients in several previous studies.

Material and Methods

Sampling locations

Wild adult flies were collected in two surveys during September-October 2014 and April-May 2015, the most favorable months with optimum climatic conditions for proliferation of drosophilid population. Flies were collected by a range of sampling techniques along altitudinal transects starting from Srinagar-Garhwal (District-Pauri), Augustyamuni (District-Rudraprayag), Upper Chamoli (District-Chamoli), Mandal (District-Chamoli), Kanchula-Kharak (District-Chamoli) and Chopta (District-Rudraprayag). Data on weather conditions were obtained from local weather stations as well as the published climatological literature of the Indian Meteorological Department, Government of India (Table 1).

Table 1. Geographical locations and climatic conditions for different drosophilid populations analyzed in this study

	Geographical location			Climatic conditions			
Sampling station	Altitude (m)	Latitude (N)	Longitude (E)	T_{max} (°C)	T_{min} (°C)	T_{avg} (°C)	Annual precipitation (mm)
Srinagar-Garhwal	550	30° 22′	78° 78′	36.1	6.8	21.7	1371
Augustyamuni	800	30° 39′	79° 02′	34.7	6.5	20.7	1553
Upper Chamoli	1150	30° 24′	79° 21′	29.3	3.8	16.7	1305
Mandal	1600	30° 46′	79° 26′	28.8	3.6	16.4	1292
Kanchula-Kharak	2100	30° 49′	79° 22′	23.4	1.9	11.6	1445
Chopta	2700	30° 34′	79° 05′	20.8	-2.7	9.7	1626

The cosmopolitan or wide ranging species of fruit fly were collected from natural habitats employing range of techniques; the trap-bait method (small containers baited with yeasted banana or some other fermenting fruits, such as oranges, tomato, guava and apples, suspended on strings from the branches of bushes and trees), net sweeping (over natural feeding sites, such as decaying fruits and leaves, wild grasses and cultivated vegetation) and direct collection with aspirator (to trap flies directly while they were either courting or resting over the leaves, petals and fungi).

Identification and morphological study

Collected flies were etherized, categorized and subsequently identified through species specific morphological patterns common to both males and females according to Gupta (2005) and Markow & O'Grady, (2006), and online identification tools like BioCIS, JDD and FlyBase. For confirmation the detailed structures of male and female terminalia were observed under stereo microscope (Magnus MLX-DX model, at 10X magnification). The respective genital organs were detached from the adult body and cleared by warming in 10% KOH to around 100°C for 20-30 minute and observed in a droplet of glycerol. The morphological terminology, and the definitions of measurements and indices mostly followed McAlpine (1981), Zhang & Toda (1992) and Hu & Toda (2001). The examined specimens of all species were deposited in the Cytogenetics and Molecular Systematics Laboratory, Department of Zoology, HNB Garhwal University, Chauras Campus, Srinagar-Garhwal, Uttarakhand, India.

Measurement of morphometric traits

Twenty-five wild-caught flies of each sex per sampling location of five species viz., *Drosophila immigrans* Sturtevant, 1921, *Drosophila nepalensis* Okada, 1955, *Drosophila repleta* Wollaston, 1858, *Scaptomyza himalayana* Takada, 1970 and *Zaprionus grandis* Kikkawa & Peng, 1938 were measured for various morphometric traits related to head, thorax and wings, along with several body indices and flight traits. Major metric traits (related to size) analyzed were wing length (W) measured from the thoracic articulation to the tip of post-scutellum laterally, wing width (w) along the mid vertical line of the wing and thorax length (T) laterally from the neck to the tip of scutellum. Thorax width (t) was measured probably for the first time in wild-caught flies of Indian population, from a ventral view as the distance between the bases of the two major, posterior sternopleural bristles. Though much literature is available on wing and thorax length of several drosophilid species, thorax width has only been rarely reported.

An ocular micrometer was used for all measurements, and micrometer observations were transformed according to the magnifications and expressed in mm. Apart from these size related traits different ratios were also calculated. The W/T ratio, which describes the relative proportion of wing with respect to thorax, has been shown to have strong negative correlation with wing loading and provides information on flight capacity (David et al., 2006b). The elongation index, the ratio of thorax length to thorax width, increase with elongation of the thorax. The ratio of wing length to thorax width was also calculated. These ratios provide useful indices of the shape of drosophilid flies and have been considered as shape indices. All the morphometric studies were done in a temperature-controlled room set to 25ºC.

The standard methods widely reported in literature to calculate wing area, wing aspect ratio and wing-load index were followed to estimate flight related traits in this study (Stalker, 1980; Azevedo et al., 1998; Van't Land et al., 1999). Wing area (mm^2) was estimated as the product of wing length and wing width. Wing aspect ratio was measured as the ratio of wing length^2 to wing area. It is an important metric index which provides information about wing shape. Wing-load index was also calculated for the populations along altitudinal gradient, as the ratio of thorax volume to wing area. Two methods were followed in the cited studies for estimating wing loading, i.e., wing loading = body weight / wing area or thoracic volume / wing area. According to previous studies in wild-caught flies, variations in body weight due to age are difficult to control in females, however, such variations have been shown not to be significant in males. Accordingly, the age-related effects were nullified using thorax volume instead of body weight for wild-caught flies, as suggested by Stalker (1980). Thorax volume and body weight show positive linear correlation and thus it can be used to reduce uncontrolled variations in body weight due to age as well as nutrition. The thorax volume was calculated as the product of thorax length, thorax width and thorax depth.

All statistical analysis of the various traits was performed in IBM SPSS Statistics 20.0 software. Mean±SD values of 25 male and 25 female individuals per population were calculated for wild-caught drosophilid flies. ANOVAs were performed to examine the effects of the location altitude on the phenotypic traits. For almost all the quantitative traits, data on male and female individuals were treated separately. An attempt was also made to obtain data on sexual dimorphism for homologous traits that can be measured on both the sexes. Comparisons were made using the mean values of the females and males of wild-caught flies. Two methods have been published for estimating the extent of sexual dimorphism; difference between female and male trait values (F-M) and ratio of female to male trait values (F/M). Both measures were considered in previous studies (David et al., 2003; Huey et al., 2006) and the ratio method was considered to be preferable as it has no dimensionality and allows comparisons between different characters.

Results

Body size related traits

Body size related traits, in particular, are known to increase considerably with altitude and latitude as both genetics and temperature strongly mediate plasticity effects influencing these traits. In the present study, fly collection was done during the most favorable months of September-October and April-May (in 2014 and 2015, respectively), when the climatic conditions are optimum for proliferation of drosophilid population. Consistent with several earlier studies, an increasing trend for these traits in all species analyzed was also observed. There was a sharp increase in mean values up to Mandal (1600 m asl) and a significant decrease in values from Mandal to Chopta (2700 m asl). Further, the effect of altitude was also similar between the sexes, i.e., a similar trend of size variation with altitude was observed for both the sexes. Size variation was considerably marked across species. *Drosophila immigrans* had the maximum values for male body length and thorax length, while *Z. grandis* had the maximum mean value for female body length and thorax length, and maximum wing length for both males and females. The lowest values were observed for *S. himalayana* for body length and thorax length in both the sexes (Figure 1).

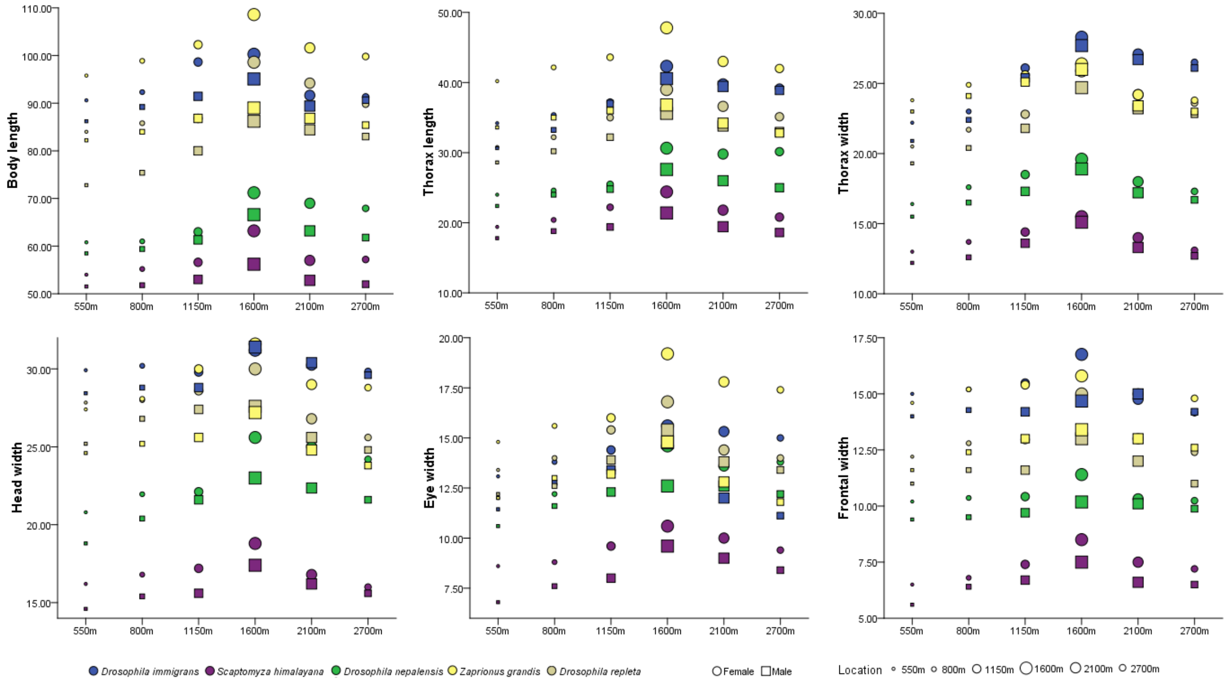

Figure 1. Variation in body size related traits along six sampling locations (symbol size is only indicative, representing trait measurements).

The variation in the traits with altitude was highly significant and there was also highly significant variation within and between groups for each trait sampled (Table 2). The shorter traits exhibited more variation because of a higher relative magnitude of measurement errors (Imasheva et al., 2000). Therefore, on average, males were more variable than females, and the thorax more variable than the wing length. Body length, and wing and thorax lengths varied significantly not only within species but also between species and also between sexes. The distributions for the sexes, however, overlapped considerably, such that males of some big species are much bigger than females of some small species.

Though these populations differed significantly along with altitude, they deviated from the expected increasing norm observed in other studies. The body size traits after quadratic transformation are presented in Figure 2. The analysis of the derivative curves reveals a fairly complex and sometimes biphasic shape, thus polynomial models are convenient for adjusting the response curve (David et al., 1997, 2004). A higher degree provides a better fit between the observations and the model; however, these are difficult to interpret biologically. There is, thus, a practical tradeoff between the need to increase the polynomial degree for a better fit and the use of a simple polynomial for an easier biological interpretation. The quadratic has obvious biological significance and may be called the characteristic values of the reaction norm (David et al., 1997).

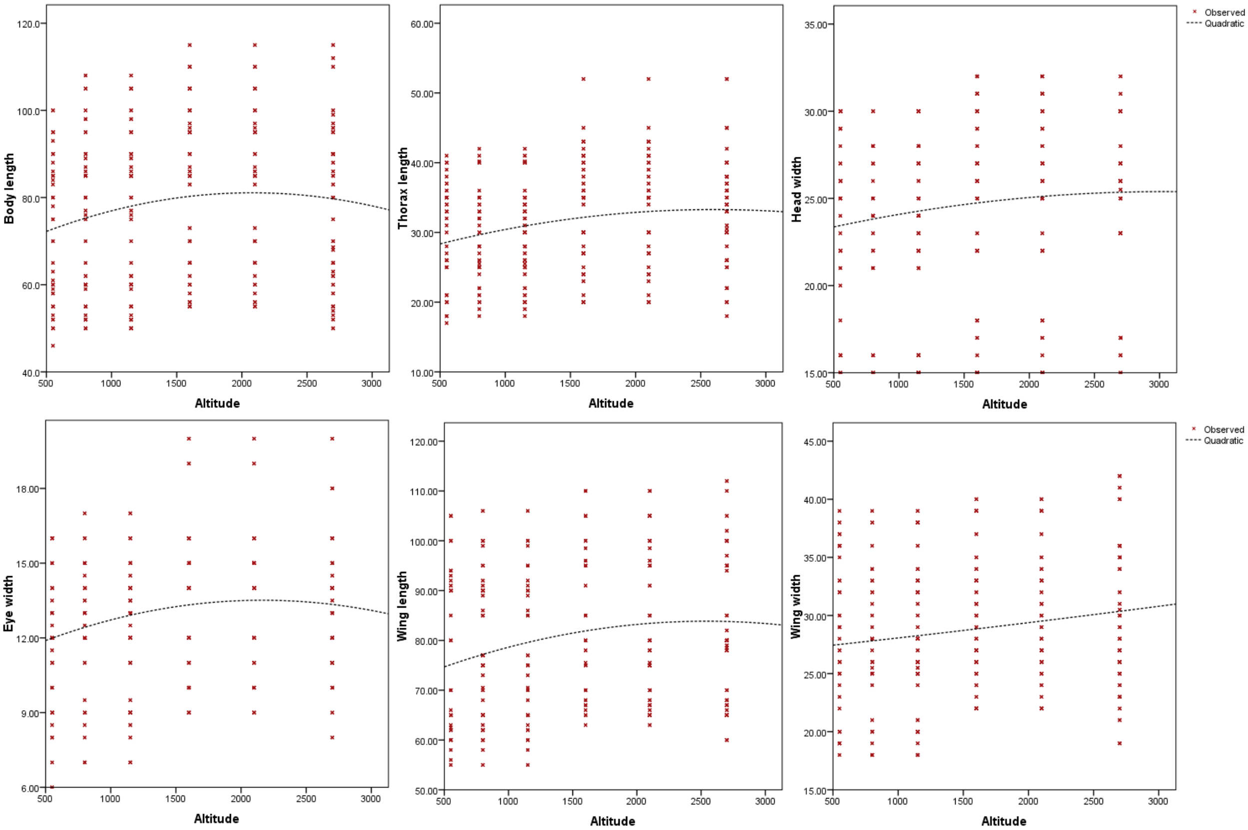

Figure 2. Results obtained after quadratic transformation of all trait reaction norms at the locations sampled.

Table 2. Variance of different traits explained by altitude as a factor (ANOVA) and post hoc analysis (Tukey HSD)

	Mean±SD						P value	Multiple comparisons P value						
	550m	800m	1150m	1600m	2100m	2700m								
									1	2	3	4	5	6
Body length	73.48±16.70	75.52±17.21	75.52±17.21	81.70±16.98	81.70±16.98	79.25±17.10	0.0087	1		0.076	0.076	0.00001	0.00001	0.002
								2	0.076		1	0.001	0.001	0.014
								3	0.076	1		0.001	0.001	0.014
								4	0.0001	0.001	0.001		1	0.059
								5	0.0001	0.001	0.001	1		0.059
								6	0.002	0.014	0.014	0.059	0.059	
Thorax length	29.60±7.23	29.09±6.28	29.09±6.29	33.48±7.73	33.48±7.73	32.85±7.97	0.0009	1		0.0971	0.0971	0.00001	0.00001	0.00009
								2	0.0971		1	0.00001	0.00001	0.00001
								3	0.0971	1		0.00001	0.00001	0.00001
								4	0.00001	0.00001	0.00001		1	0.0927
								5	0.00001	0.00001	0.00001	1		0.0927
								6	0.00009	0.00001	0.00001	0.0927	0.0927	
Head width	23.92±5.08	23.50±4.73	23.50±4.73	25.32±5.26	25.32±5.26	25.17±4.94	0.0025	1		0.0937	0.0937	0.022	0.022	0.060
								2	0.0937		1	0.001	0.001	0.003
								3	0.0937	1		0.001	0.001	0.003
								4	0.022	0.001	0.001		1	0.0999
								5	0.022	0.001	0.001	1		0.0999
								6	0.060	0.003	0.003	0.0999	0.0999	

Table 2. (Continued)

	Mean±SD						P value	Multiple comparisons P value Tukey HSD[#]						
	550m	800m	1150m	1600m	2100m	2700m								
Eye width	12.18±2.63	12.40±2.33	12.40±2.33	13.64±2.35	13.64±2.35	13.24±2.44	0.0019	1		0.0911	0.0911	0.00001	0.00001	0.00002
								2	0.0911		1	0.00001	0.00001	0.001
								3	0.0911	1		0.00001	0.00001	0.001
								4	0.00001	0.00001	0.00001		1	0.0430
								5	0.00001	0.00001	0.00001	1		0.0430
								6	0.00002	0.001	0.001	0.0430	0.0430	
Frontal width	11.35±3.19	10.30±2.58	10.30±2.58	11.24±3.18	11.24±3.18	11.36±2.89	0.0005	1		0.001	0.001	0.0998	0.0998	1
								2	0.001		1	0.005	0.005	0.001
								3	0.001	1		0.005	0.005	0.001
								4	0.0998	0.005	0.005		1	0.0998
								5	0.0998	0.005	0.005	1		0.0998
								6	1	0.001	0.001	0.0998	0.0998	
Wing length	75.95±16.18	77.15±13.73	77.15±13.73	83.68±14.78	83.68±14.78	83.43±14.20	0.00001	1		0.942	0.942	0.00001	0.00001	0.00001
								2	0.942		1	0.00001	0.00001	0.00002
								3	0.942	1		0.00001	0.00001	0.00002
								4	0.00001	0.00001	0.00001		1	1
								5	0.00001	0.00001	0.00001	1		1
								6	0.00001	0.00002	0.00002	1	1	
Wing width	28.38±6.37	27.11±5.70	27.11±5.70	29.82±5.40	29.82±5.40	30.07±6.10	0.0003	1		0.140	0.140	0.061	0.061	0.014
									0.140		1	0.00003	0.00003	0.00001
								3	0.140	1		0.00003	0.00003	0.00001
								4	0.061	0.00003		0.00003	1	0.997
								5	0.061	0.00003	0.00003		1	0.997
								6	0.014	0.00001	0.00001	0.997	0.997	

[#] In multiple comparisons Tukey HSD, numbers 1-6 represent the six sampling locations from lowest to highest.

Wing size and shape

Mean values of the wing size estimates showed an increasing trend, reaching the maximum at the highest altitude. Wing area was larger at higher altitudes and compensated for variation in body size (Figure 3). The relationship between wing and thorax lengths a useful parameter because it provides critical information on wing loading, wing beat frequency and presumably flight capacity. Maximum values for the ratio of wing length to thorax length were 3.77±0.05 in males and 3.55±0.07 in females, and for the ratio of wing length to thorax width were 5.28±0.08 in males and 5.63±0.09 in females observed at highest altitude, i.e., Chopta (2700 m asl) for *S. himalayana*. Owing to different shape and these variations in individuals their respective wing loads will not be exactly the same. Thorax width variability, a measure of elongation index (the ratio of thorax length to thorax width) was significantly higher in females indicating thorax is more elongated in female flies (Figure 4).

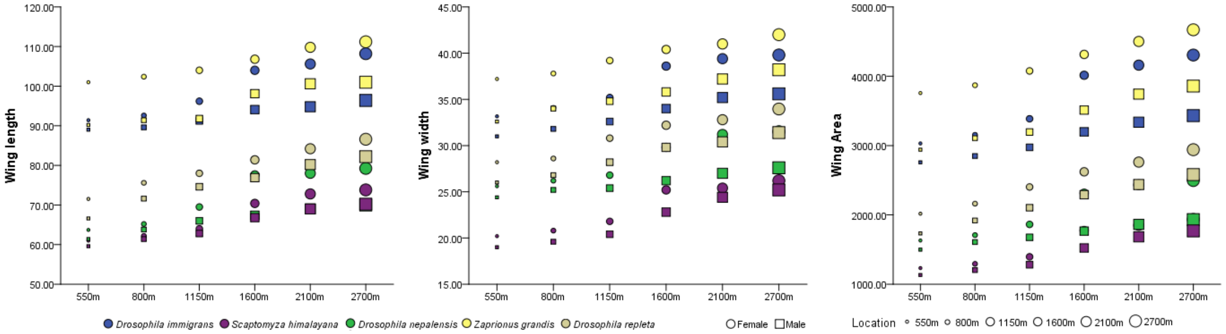

Figure 3. Wing length, wing width and wing area analyzed at six sampling locations (symbol size is only indicative, representing trait measurements).

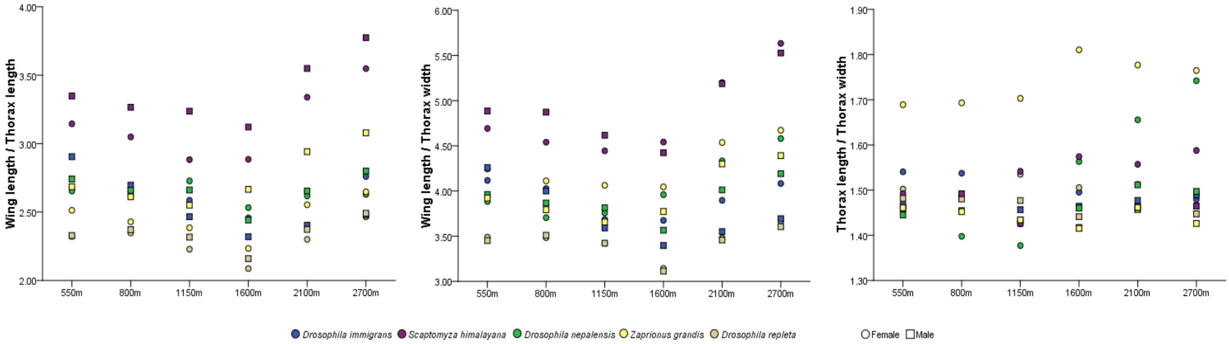

Figure 4. Variation in body size indices observed along the altitude gradient sampled.

Wing aspect ratio, an interpretation of the wing shape, also increased significantly from Mandal to Chopta, indicating elongation of wings at high altitude. The flies developing in cold habitats also had decreased wing loading compared to those developing at mid elevations with optimum temperature and longer growing season (Figure 5). Overall all traits, the mean square between subgroups was always superior to the mean square within subgroups and F was greater than unity. Such variations between populations are mainly due to long-range geographic variations. These were higher and statistically significant for almost all traits. This indicates that different traits do not always follow the same rules of variation exactly. Such a result gives evidence of parallel variation and suggests an adaptive significance.

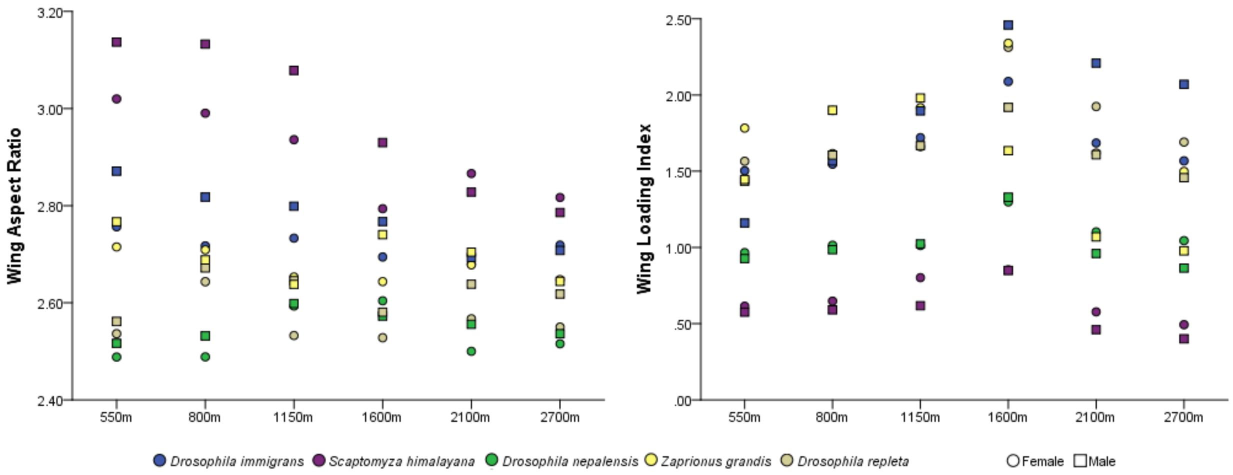

Figure 5. Flight performance indices: wing aspect ratio and wing loading for populations studied along an altitudinal transect.

Sexual dimorphism

The size related traits (e.g., body length, thorax length, thorax width, wing length and wing width) were much greater in females; however, a significant trend of increasing size with altitude was observed for both sexes. Two possible indices, the F to M ratio and the difference between F and M, provided the same information, however, the ratio was preferred as it is a non-dimensional measure not influenced by variations of the mean, thus allowing comparison between different traits. Values obtained at different altitudes were grouped to obtain a mean value of F to M ratio for a species (Table 3). For body length, and thorax and wing lengths, the F to M ratios were the highest, ranging up to 1.18, 1.24 and 1.11, respectively, all for *Z*. *grandis*. The ratio for thorax width, however, was much less with maximum value of 1.05 for *D*. *nepalensis, D*. *repleta* and *S*. *himalayana* indicating that males are more similar to females for this trait. The ratio of thorax length to thorax width, i.e., the elongation index, was greater than 1 obtained for all species, which indicates that the male thorax is more rounded than their female counterparts. A similar trend was observed for the wing length to thorax width ratio. The wing length to thorax length ratio was, however, very close to one in all five species, which can be attributed to the fact that these two traits have minimal dimorphism.

Discussion

The body size related traits vary both across and within species in response to the environment (e.g., nutrition, developmental temperature and stressors) as well as genetic factors. Our findings however, deviated from general temperature-size rules as the highest value for these traits were observed at mid elevation, hinting an adaptive interpretation that size is maximum under optimal physiological conditions. A similar trend was observed in both male and female flies. This deviation from normal clinal trend can be attributed to variation in growth rates and development times at different altitudes, which has important implications for overall reproductive success. It has been observed that some species show increasing size with altitude, although the reverse has also been seen (Chown & Klok, 2003), due to shortening of developmental time, in order to facilitate successful reproduction before the end of the season.

Table 3. Analysis of sexual dimorphism considering the F to M ratio with the percentage of variance obtained through ANOVA

	F/M Ratio, % Variance, P value														
	Drosophila immigrans			*Drosophila nepalensis*			*Drosophila repleta*			*Scaptomyza himalayana*			*Zaprionus grandis*		
Body length	1.042	17.66	*0.00091*	1.059	21.87	*0.00062*	1.120	33.21	*0.00098*	1.081	27.23	*0.00083*	1.180	37.19	*0.00099*
Thorax length	1.041	16.5	*0.06729*	1.098	26.72	*0.00091*	1.079	22.8	*0.00092*	1.117	29.12	*0.00075*	1.241	31.89	*0.00097*
Thorax width	1.028	26.8	*0.00072*	1.052	45.23	*0.00082*	1.050	39.17	*0.00075*	1.054	46.77	*0.00094*	1.029	27.56	*0.00091*
Wing length	1.076	19.1	*0.00088*	1.088	19.23	*0.00092*	1.056	16.82	*0.0008*	1.036	15.69	*0.00092*	1.109	21.87	*0.00083*
Head width	1.022	11.28	*0.05916*	1.093	22.13	*0.00083*	1.060	18.5	*0.00099*	1.074	19.21	*0.00082*	1.157	26.88	*0.00072*
Eye width	1.164	26.56	*0.00081*	1.107	22.13	*0.00099*	1.083	18.75	*0.00096*	1.159	24.6	*0.00082*	1.301	37.92	*0.00096*
Frontal width	1.058	13.78	*0.00081*	1.071	15.5	*0.00072*	1.116	18.7	*0.00084*	1.118	18.9	*0.00081*	1.196	24.51	*0.00084*
W/T[#]	1.036	11.23	*0.00082*	0.992	9.6	*0.07121*	0.979	8.77	*0.06111*	0.928	8.12	*0.05102*	0.895	6.63	*0.08121*
W/t[#]	1.047	21.32	*0.00092*	1.034	20.26	*0.00064*	1.006	18.45	*0.00093*	0.984	18.2	*0.00099*	1.078	24.56	*0.00062*
T/t[#]	1.012	19.56	*0.00073*	1.044	24.3	*0.00066*	1.028	22.95	*0.00091*	1.060	25.69	*0.00096*	1.207	37.98	*0.00077*

[#] W, wing length; T, thorax length; t, thorax width.

Initial increases in these traits observed in our data can generally be explained by a negative relationship between developmental temperature and size in a non-resource-limited environment up to mid altitude (T_{avg} = 16.5°C and ~1300 mm of annual precipitation at mid elevation, i.e., Mandal), having optimum habitat conditions, such as a warmer, but still cool, non-seasonal environment. Support for these observations has been given by several workers studying different insect genera (Smith et al., 2000). . Mid elevation peaks observed for most of the size related traits in our study also support the hypothesis that insect body size clines are influenced by the length of the insect's generation time relative to local season length (Mousseau, 1997; Dillon et al., 2006; Chown & Gaston, 2010). The insect populations living in the colder regions of the range of their species are more likely to experience significant resource limitations (T_{avg} = 11.6°C and ~1450 mm of annual precipitation at Kanchula-Kharak; T_{avg} 9.7°C and ~1600 mm of annual precipitation at Chopta) leading to size decrease as they must have more rapid development to complete a generation during the available growing season, or they are under risk of running out of optimum time or resources prior to completing development. The generation time can be shortened by either maturing to adult at a smaller body size or by increasing the growth rate. However, insects can increase their growth rate only to certain extent due to thermodynamic constraints (Gillooly et al., 2002).

These patterns in body size traits, although being supported by the Bergmann’s, Allen’s or temperature–size rules, include several instances showing a reverse or even the absence of pattern in body size clines along altitudinal gradients. The optimal size of a trait is thus a reflection of a trade-off between the costs and benefits of body size in a particular environment. As studied by Ray (1960), Bergmann’s and Allen’s rules apply to both endotherms and ectotherms but only at the intraspecific level (*sensu* Mayr 1942). Ray considered temperature as the only factor related to Allen’s rule and his results for *Drosophila* species followed both Bergmann’s and Allen’s rules as leg proportions with respect to body size decreased with decreasing temperature. In species showing inverse Bergmann’s pattern, the geographical variation in body size proportions of protruding parts may show differential allometric growth in different areas due to time constraints on development and growth imposed by abiotic factors that regulate season and time available for reproduction. Alternatively, it also reflects true Allenian variation related to thermoregulation (Bidau et al., 2012).

Other environmental factors challenging to insects are reduced mean temperatures and low barometric pressure at higher altitude. Wing morphology was also studied, as it has been shown that decreased temperature and air pressure severely compromises walking speed and flight performance. A significant increase in wing length and width was observed with altitude. Another factor for improved flight performance of flies at cold temperatures was a dramatic increase in wing area relative to body mass. Flies from cold environments have been shown to have improved flight performance from increased wing area relative to their body mass, which reduces induced power requirements and increases lift production (Dudley, 2000). Wing aspect ratio, an interpretation of the wing shape, also increased significantly from Mandal to Chopta, indicating elongation of wings at high altitude. Changes in wing shape may also improve flight performance. Elongation of wings, while maintaining the wing area improves some aspects of flight performance as higher, translational velocity of wing tips at same angular velocity produces greater aerodynamic forces (Frazier et al., 2008). With a significant increase in wing area, flies at higher altitude had dramatically lower wing loadings than flies that developed in comparatively warmer habitats, giving them an aerodynamic advantage at cold temperatures, as has been observed in several other studies (Gilchrist & Huey, 2004; Frazier et al., 2008). Theoretically reduced wing loading is advantageous for generating lift during flight via increasing mechanical power output, thus a compensatory mechanism for improved flight performance (Norry et al., 2001).

Though several studies focus on altitudinal variation in overall body size, most of them ignore sexual size dimorphism. Here, we assessed female to male ratio depicting that the size variations between the sexes were significant for different traits. It indicates that some or the other environmental/ecological factor varying with altitude is differentially affecting selection on the sexes. Also, though a similar trend in size traits with altitude was observed for both the sexes, females were larger than males, a pattern that is consistent with the general female-biased dimorphism observed in most species of insects (Fairbairn, 1997). It has also been suggested that variation in sexual size dimorphism among populations is due to canalization of traits closely associated with fitness, thus the F to M ratios for body length, and thorax and wing lengths, were much higher indicating these traits are more important to fitness in females thus were developmentally canalized in response to environmental perturbations (Fairbairn, 2005) as compared to thorax width for which the ratio was smaller indicating little influence of this trait in female or male fitness. The different optima of these traits for each sex might also be a consequence of different ecological or social roles as suggested under dimorphic niche hypothesis (Slatkin, 1984), i.e., if there are intrinsic differences between sexes for their energetic needs to ensure successful reproduction, then it is likely that different optima exist for each sex. This could be reasonable a explanation, as reproductive capacity of most species is limited by the size of females, and adult female size is probably dependent, to an extent, on limited ecological resources, as along an altitudinal gradient.

Further, three ratios were calculated from three size-related traits. The higher value obtained for thorax length to thorax width ratio, i.e., the elongation index, indicate that the thorax is more elongated in the females, which may significantly influence the flight capacity between sexes. The second ratio for the wing length to thorax width showed similar trend, however its biological meaning is less obvious. The wing length to thorax length ratio characterizes the relative proportion of the wing with respect to the thorax. As established in previous studies (Petavy et al., 1997; David et al., 2006b), this ratio is strongly negatively correlated with wing loading, and provides information on flight capacity thus a value closer to unity in all five species hints that flight compensatory adjustments via wing loading are similar among species as well as sexes.

It is concluded that the general pattern of body size related traits along altitudinal gradient cannot be well predicted by any single eco geographic rule. The correlation between any environmental gradient to body size is much more complex and depends upon its life history and several environmental aspects. As the populations facing greater time constraint for development and reproduction are likely to mature at smaller body size at high altitudes (contrary to Bergmann). Notably, some initial reports of this “inverted” empirical body size pattern seem to support Allen’s rule (Ruibal, 1955; Martof & Humphries, 1959; Ray 1960). Also, some studies suggest that beneficial plasticity or acclimation may contribute to the ability of *Drosophila* species to occupy varied habitats while others have shown it may not be an evolutionarily important mechanism. As for wing-loading, phenotypic plasticity may be more important than population level genetic differences at higher altitude. It can be assumed that there is strong developmental plasticity of flight performance due to decreased wing loading in response to cold developmental temperatures at higher elevations. Developmental plasticity and/or acclimatization thus may be an imperative phenomenon, especially when local genetic adaptation may be hindered by high gene flow in mobile insects occupying large geographic ranges as drosophilids.

The future studies in this region could explore, the relative contributions of size variation and growth rate to early development, to determine the evolutionary constraints on these important fitness related variables. Also, the significant values obtained for sexual dimorphism of almost all body size related traits suggests that these traits share a common genetic basis. Investigating how the sex-determining genetic cascade interferes with various quantitative traits would also be worthwhile.

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Original article (Orijinal araştırma)

Incidence and economic impact of the mint aphid, *Eucarazzia elegans* (Ferrari) (Hemiptera: Aphididae) on common sage[1]

Adaçayında nane yaprakbiti *Eucarazzia elegans* (Ferrari) (Hemiptera: Aphididae)'ın zararı ve ekonomik etkisi

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Summary

A survey and laboratory experiments were conducted on incidence rates and economic impact of *Eucarazzia elegans* (Ferrari) (Hemiptera: Aphididae) in *Salvia officinalis* L. (Lamiaceae). The investigations were purposively performed on conventionally grown common sage in Bornova and Menemen, Izmir-Turkey. Experiments were set up with zero, normal and double population densities of the aphid. The corresponding treatments 0 (no exposure), 2, 4, 6, 8, 10 and 12 weeks (complete exposure) were used for evaluating economic impact. *Eucarazzia elegans* infested old leaves with an incidence rate of 9.5% in the early spring and then migrated to young leaves and blossom at the beginning of the summer with the incidence rate of 1.1%. The seasonal distribution of the aphid was affected more by temperature than humidity and rainfall. Although the aphid infestation had no differences on the quantity of the essential oil, some of the essential oil components were markedly different from the control. The total plant weights (fresh and dry) with the normal and double infestation rate were decreased about a third and more than a half compared to the control, respectively. Aphids feeding on common sage beyond 8-10 weeks caused more than 50% yield loss. The highest cost-benefit ratio was achieved in plots where six insecticide applications were made.

Keywords: Economic impact, *Eucarazzia elegans*, incidence rate, *Salvia officinalis*

Özet

Eucarazzia elegans (Ferrari) (Hemiptera: Aphididae)'ın *Salvia officinalis L.* (Lamiaceae) üzerindeki zarar oranını ve ekonomik etkisini belirlemek üzere sürvey ve laboratuvar çalışmaları gerçekleştirilmiştir. Çalışmalar Bornova ve Menemen'de bilimsel amaçlı kurulmuş parsellerde gerçekleştirilmiştir. Denemeler yaprakbitinin sıfır, normal ve iki katı olmak üzere üç farklı popülasyon yoğunluğunda kurulmuştur. Çalışmada zararlının ekonomik etkisini belirlemek için karşılaştırma yapmak üzere 0 (salım öncesi), 2, 4, 6, 8, 10 ve 12 haftalar boyunca yapılan sayımlardan elde edilen veriler kullanılmıştır. Çalışma sonuçlarına göre, *E. elegans*'ın, erken ilkbahar döneminde %9.5 zarar oranı ile bir önceki yıldan kalan kışlamış yapraklar üzerinde beslendiği belirlenmiş ve daha sonra yaz başında %1.1 zarar oranı ile genç yapraklara ve çiçeklere göç ettiği saptanmıştır. Yaprakbiti mevsimsel dağılımının nem ve yağışa göre sıcaklıktan daha fazla etkilendiği görülmüştür. Yaprakbiti zararı uçucu yağ miktarı üzerinde bir farklılık oluşturmamasına rağmen, uçucu yağ bileşenlerinden bazıları kontrol grubuna göre büyük ölçüde değişime uğramıştır. Toplam yaş ve kuru yaprak ağırlık artışı iki kat popülasyon seviyesinde sırasıyla kontrol ve normal yaprakbiti popülasyon seviyesinde 1/3 ve 1/2 oranında azalmıştır. Yaprakbitlerinin, adaçayında 8-10 haftadan fazla beslenmesi sonrasında %50'den fazla ürün kaybına neden olduğu saptanmıştır. En yüksek maliyet yarar oranı altı kez insektisit uygulanmış parsellerde gerçekleşmiştir.

Anahtar sözcükler: Ekonomik etki, *Eucarazzia elegans*, zarar oranı, *Salvia officinalis*

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Introduction

The mint aphid, *Eucarazzia elegans* (Ferrari, 1872) (Hemiptera: Aphididae) is one of the important aphid species feeding on aromatic and herbal plants in the family Asteraceae, Solanaceae and Lamiaceae, such as *Mentha, Salvia, Lavandula, Coleus, Melissa, Nepeta* and *Origanum* (Blackman & Eastop, 2006). It is a small green aphid which lives generally on the undersides of leaves, shoots and flowers (Stoetzel, 1985; Hales et al., 2009). The alatae of *E. elegans* is easily identifiable by observing the siphunculi which are dark, narrow at the base and swollen towards the tip. The swollen part is more than two times the diameter of the stem of the pale cylindrical base. The wings have dark triangular spots at the ends of all the veins (Stoetzel, 1985; Blackman & Eastop, 2006).

Eucarazzia elegans has been recorded indigenously in the Mediterranean region (Stoetzel, 1985) and has spread to the Madeira (Aguiar & Ilharco, 1997), Middle East (Hussain et al., 2015), Central Asia (Moktari et al., 2012), Pakistan, India, Australia (Hales et al., 2009), Africa (Muller & Scholl, 1958), western USA (Stoetzel, 1985) and South America (Bolivia, Argentina and Brazil) (Avila et al., 2014). The genus of *Eucarazzia* has only two or three species (Blackman & Eastop, 1984), but only *E. elegans* has spread (Blackman & Eastop, 2006). This aphid species has demonstrated the capacity to spread readily via modern transport pathways (Hales et al., 2009).

Research on *E. elegans* infestations, especially on common sage, is limited. In Turkey, the first *E. elegans* record was by Bodenheimer & Swirski (1957), however, there are no reports of economic impacts and losses for most host plants. This aphid species is a well-known pest of *Salvia splendens* F. Sellow ex Roem. & Schult. (Özdemir & Toros, 1997) and *Mentha* sp. (Özdemir, 2004). Ülgentürk et al. (2013) reported *E. elegans* as a honeydew producer on shrubs and herbaceous plants in Aegean, Marmara and Mediterranean Regions, but did not report on damage and yield loss.

In this study, we evaluated *E. elegans* populations to predict incidence rates on *S. officinalis* by survey and its economic impact by laboratory experiments. These data were also used to determine control required for optimal common sage production.

Material and Methods

Field experiment

Seasonal distribution of the mint aphid, *E. elegans* was determined for January to December 2016. The common sage fields used were 0.5 and 1 ha plantings of the experimental farms of the Field Crops Department, Faculty of Agriculture, Ege University Bornova and Aegean Agricultural Research Institute, Menemen, respectively, in Izmir, Turkey. A total 50 blocks from the two plantings were monitored using the incident random sampling. Aphid populations were recorded every 2 weeks for three selected clumps of plant per block. The aphids were counted each infested leaf in each clump. Weather data was obtained for the Second Region of Izmir from the Turkish State Meteorological Service. The relationships between temperature, humidity and rainfall, and seasonal changes in aphid population were determined by multiple regression analysis. Principal component analysis was used to explore the relationship between variables observed.

Laboratory experiment

The experiment had a randomized complete design with 21 replications of three treatments *i.e* zero, normal and double aphid population densities on 2-month old *S. officinalis.* The plants were grown in 15-cm diameter plastic pots under laboratory condition. The zero-population density had no aphids with aphids controlled by azadirachtin insecticide 0.3 g/L EC at the recommended field dose of 5 ml/L applied as a spray every 2 week. The normal population density was 100-200 aphids/plant and the double population density was 300-400 aphids/plant. Final instar or adult aptera aphids reared on *S. officinalis* in the laboratory were used and the aphid population densities were predicted by pre-experimental observations. The aphid populations were maintained every week by reinfesting or removal by hand using a brush. The experiment was conducted for 90 d under the laboratory conditions of 25±2ºC, 65±5% RH and 16:8 h L:D photoperiod. The plant parameters measured were fresh and dry weight, and essential oil concentration. Data were analyzed by repeated measures mixed model analyses of variance.

Water distillation and gas chromatography were used to determine the essential oil concentration and quality, respectively. The essential oil was separated by hydrodistillation for 3 h using a Clevenger-type apparatus, according to the procedure described in the German Pharmacopoeia (Wichtl, 1971). The components of essential oil were determined using an Agilent 6890 N gas chromatograph equipped with a flame ionization detector (GC-FID) at the Central Laboratory, Ege University. Compounds were separated on a high-polarity capillary column (DB-Wax, 30 m x 0.25 mm, 0.25 µm film thickness), with helium as the carrier gas, at a constant flow rate of 1 ml/min. The oven temperature program was as follows: hold for 2 min at 45ºC, the ramp from 45 to 250ºC at 3ºC/min, and hold at 250ºC for 34 min. The injector and detector temperatures were 250ºC. The GC-FID was calibrated using authentic standards (Sigma-Aldrich).

Economic impact of *Eucarazzia elegans*

A randomized complete block experiment was conducted under laboratory conditions with 21 replications of 2-month-old *S. officinalis* plants grown in 15-cm diameter plastic pot. The plants were infested at the normal aphid population density (as above) and were sprayed with azadirachtin applied at 2-weekly intervals either 0, 1, 2, 3, 4, 5 or 6 times to give aphid exposure periods from 12 to 0 weeks. Aphid populations were counted a day before application of the insecticide. The percentages of aphid infestation were measured by determining the ratio of fallen and remaining leaves per plant. At harvest, yields were recorded for each replicate. The plant parameter measured were fresh and dry weight, and plant height. The yield data were subjected to analysis of variance to determine the minimum number of sprays required to produce the maximum yield. Yield data and number of aphids per plant within each treatment were used to calculate the linear regression: $y = a - bx$, where y is the potential yield, a is the expected yield loss at zero infestation level, b is the regression coefficient or yield loss in g/plant caused by one aphid per plant, and x is the percentage infestation.

Results and Discussion

Seasonal distribution and incidence of *Eucarazzia elegans*

The mint aphid appeared in both Bornova and Menemen in the first week of February (the end of winter, Figure 1). The aphids had started to establish in the fields by early spring and then migrated to young leaves and blossom by early summer. The more aphids were captured at Bornova than Menemen ($T = 0.935$; $df = 41.5$; $P < 0.05$), with two distinct peaks of aphid flights observed at Bornova. These were between late February to March and late October to mid-November. The peak observed in late winter to early summer was the highest.

The population dynamics and incidence of *E. elegans* at Bornova were different from Menemen but similar in pattern (Figure 1). At Bornova, the population of total aphids was relatively high from February until the last week of March compared to other periods. However, a sharp decline in the density of aphid population was observed until the beginning of September with the number of aphids captured at this period was lower compared to Menemen. The minimum and maximum incident rate of the aphid was from 1.1 to 9.5% and 0.9 to 8.2% for Bornova and Menemen, respectively. The aphid incidence patterns in both areas were similar with the highest density in March and the lowest in August. This was probably due to the presence of the large populations of natural enemies of aphids, such as Coccinellidae (Coleoptera), Chrysopidae (Neuroptera), Cecidomyiidae and Syrphidae (Diptera) species found feeding on the aphids in spring. Also, considerable variation in weather occurred in the following season.

The aphid population dynamics were strongly affected by weather variations (Figures 2 to 4). The analysis of variance of multiple regression revealed that temperature and humidity had a strong effect on the changes in populations of aphids for both locations (Bornova, R = 0.682, F = 3.136, df = 5, P < 0.033 and Menemen, R = 0.628, F = 3.094, df = 5, P < 0.040). Moreover, it was also clear that at both locations the changes in aphid population was affected more by maximum temperature than humidity (Bornova, P < 0.007 and Menemen, P < 0.038) and minimum temperature (Bornova, P < 0.010 and Menemen, P < 0.039). In contrast, the variation in the aphid populations at both locations was not significantly correlated with rainfall (Bornova, P > 0.088 and Menemen, P > 0.154).

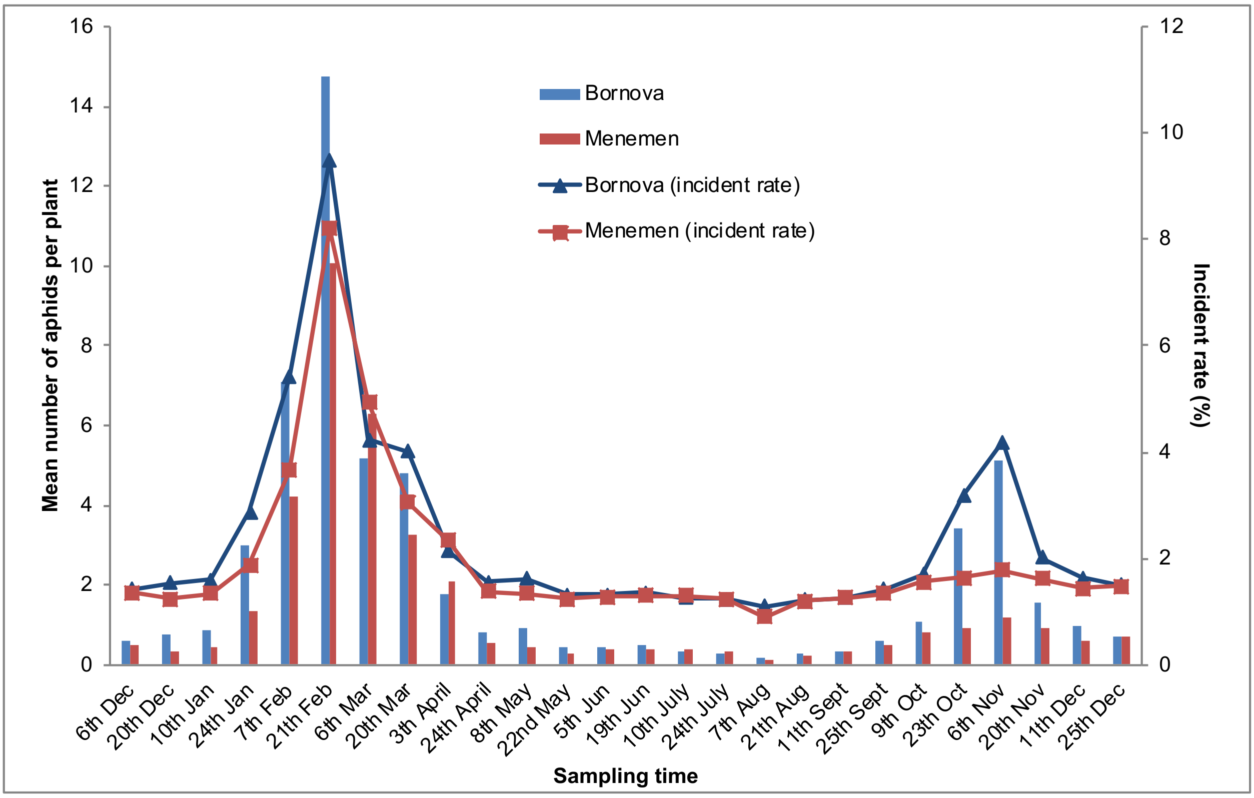

Figure 1. Seasonal distribution and incident rates of *Eucarazzia elegans* on *Salvia officinalis* at Bornova and Menemen in 2016.

The analysis of seasonal changes of aphid population density at Bornova and Menemen showed that peak and low aphid densities were significantly different, however, this was not due variation in rainfall. Whereas, minimum and maximum temperatures appeared to be drivers of aphid population change (Figures 2 to 4). When the two locations were compared, rainfall had a less effect on weekly mean aphid numbers at Menemen than Bornova, but this was not sufficient to reduce the population density. In this study, there was no clear effect of rainfall, which contrasts with several studies that found a negative relationship between rainfall and aphid population density (Mann et al., 1995). Rainfall mainly washes aphids off plants and effects to flight activity, restricting their ability to move within and between plants (Wains et al., 2010; Alyokhin et al., 2011). *Salvia officinalis* has a large, dense canopy which prevents the penetration of raindrops. In fact, mint aphids are able to easily crawl across and between the plants. *Eucarazzia elegans* is also temperate species (Stoetzel, 1985), which is active and develops faster at low temperatures than tropical species. Also, its rate of development at high temperatures allows its population to increase and range to expand when the low-temperature limitation abates (Parry et al., 2006; Hazell et al., 2010; Brabec et al., 2014). Therefore, early emergence in the late winter can lead to an outbreak, if the population of natural enemies in early spring is low.

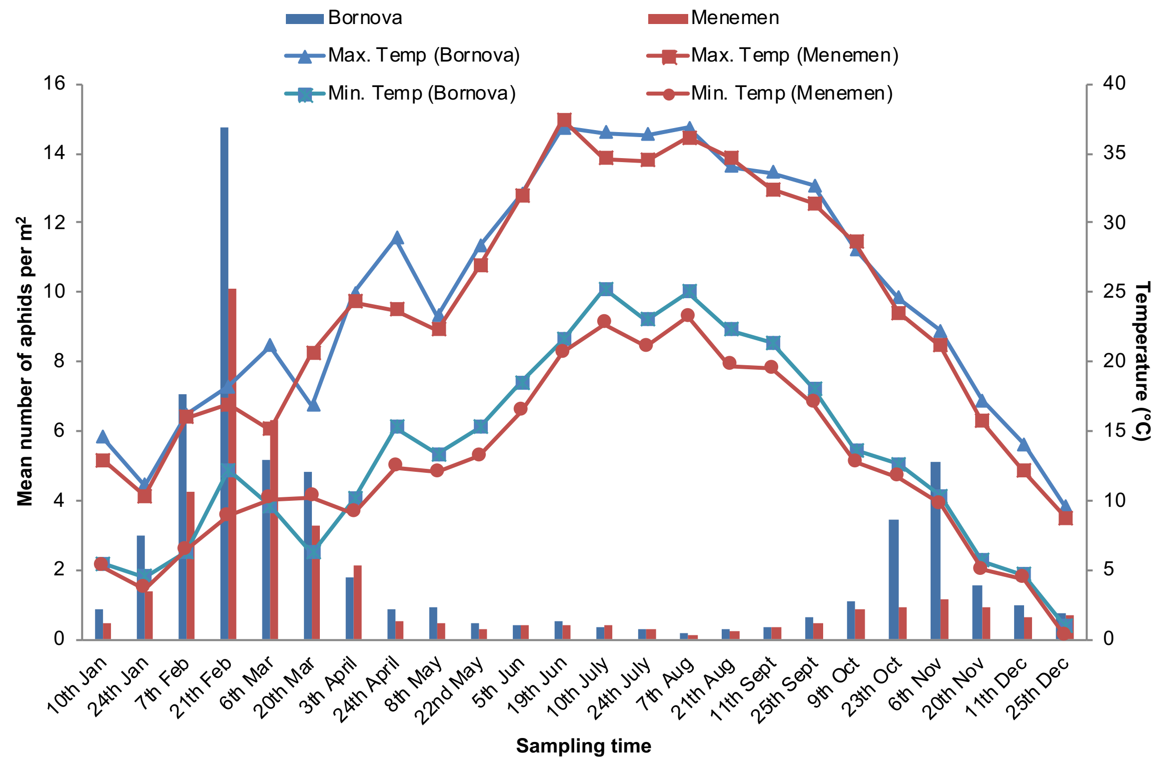

Figure 2. Weekly mean population density of *Eucarazzia elegans,* and maximum and minimum temperatures at Bornova and Menemen in 2016.

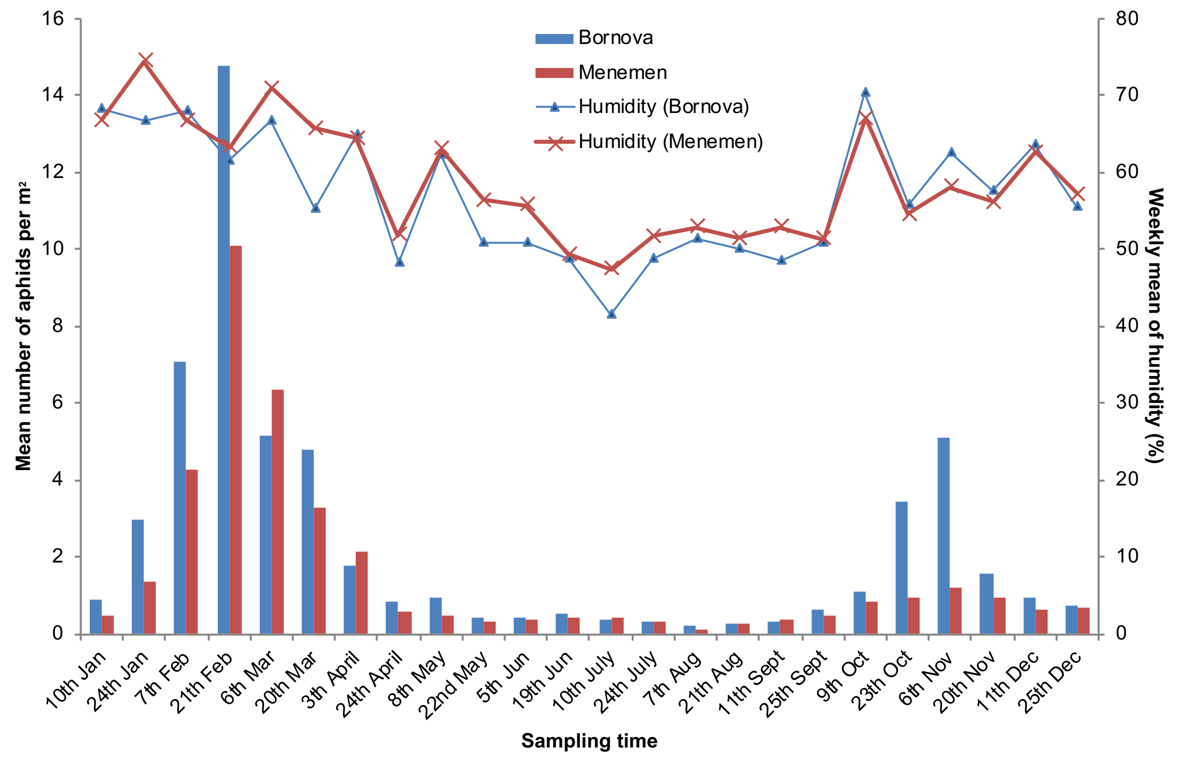

Figure 3. Weekly mean population density of *Eucarazzia elegans* and humidity at Bornova and Menemen in 2016.

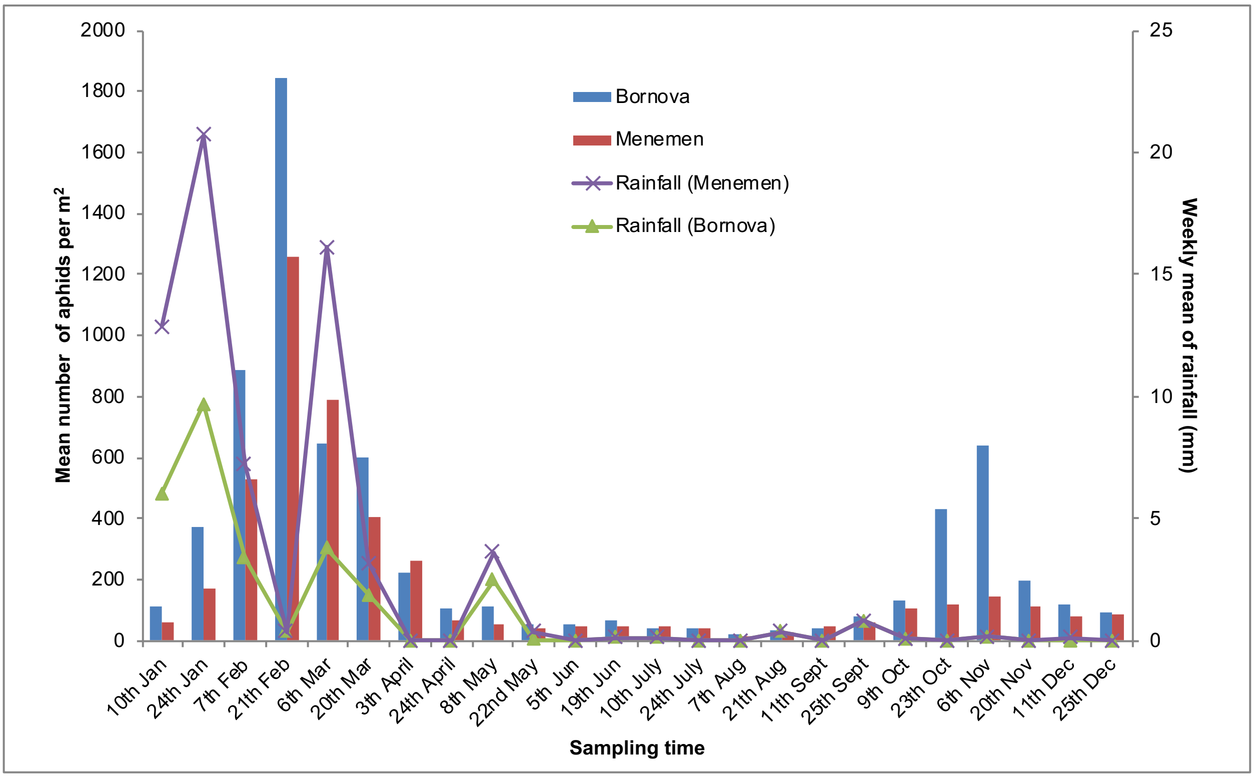

Figure 4. Weekly mean population density of *Eucarazzia elegans* and rainfall at Bornova and Menemen in 2016.

Impact of normal and double *Eucarazzia elegans* population densities on *Salvia officinalis*

There was significant difference between the effect of normal and double density aphid infestations on fresh and dry weight, and essential oil concentrations of *S. officinalis* compared to the control (Table 1). The fresh and dry weight loss caused by aphid populations was about 30 and 60% for normal and double densities, respectively, compared to the control. However, there was no significant difference in essential oil production between normal and double density aphid infestations, they were both about 20% less than the control.

Table 1. The impact of *Eucarazzia elegans* infestation on the fresh and dry weight, and essential oil plant production

Treatments	Fresh weight±SEM* (g)	Dry weight±SEM (g)	Essential oil concentration±SEM (%)
Double Population	0.79±0.04 a**	0.56±0.02 a	1.25±0.90 a
Normal Population	1.53±0.08 b	1.07±0.06 b	1.30±0.41 a
Zero Population (Positive Control)	2.17±0.10 c	1.52±0.07 c	1.59±0.97 b

* SEM: Standard error of the mean;
** Means in a column followed by the same letter are not statistical significantly different (ANOVA P < 0.05, Tukey's test).

The aphids tend to infest old leaves and cause serious defoliated. The resultant leaf fall, as a plant defense mechanism, contributes to the magnitude of the plant weight losses (Matsuki, 2004; Ballhorn et al., 2008; Gong & Zhang, 2014). In the laboratory experiment, infestation of about 50-80 aphids/leaf was enough to cause leaf fall within 5-7 days. Moreover, over two months a normal population density increased to a double density and caused leaf fall over the next 1-2 months. This clearly demonstrates that *E. elegans* has the potential to be a serious pest of common sage if there are no factors limiting population growth, such as environmental conditions, availability of resources and impact of natural enemies, to keep the population below an economic threshold.

The quality of the sage essential oil analyzed by GC-FID showed that normal and double aphid infestation levels influenced the concentrations the oil's components (Table 2). Some essential oil components, such as camphor and camphene, were greatly reduced, while other components, such as borneol, thymol, β-caryophyllen and limonene, were only slightly decreased. In contrast, α,β-thujone, β-pinene and bornyl acetate were increased, in case some components such as linalool and 1,8-cineol were either stable or showed no consistent response. The differences in percentage of essential oil components may reflect resistance and tolerance traits in plant defense mechanisms (Gong & Zhang, 2014), or impact of degraded development resulting from plant cell disruption (Steinbauer et al., 2014). Changes in chemical components, such as terpenoids; phenolic compounds; nitrogen compounds; tannins, lignin and cellulose; plant hormones and lectin; protease inhibitors; and volatile compounds, are made to defend plants against herbivores, and can be used as indicators of chemical defense capacity (Fürstenberg-Hägg et al., 2013; Schiestl et al., 2014).

Table 2. The impact of *Eucarazzia elegans* infestation on the essential oil components

Essential Oil Components	Zero Population (Positive Control) (%)	Normal Population (%)	Double Population (%)
α,β-Thujone	51.47	58.03	61.55
Camphor	16.95	12.04	11.70
Camphene	9.24	6.75	6.36
1,8-Cineole	5.62	8.32	4.34
Borneol	2.58	1.86	1.34
Limonene	2.14	1.49	1.50
β-Pinene	1.45	1.82	1.50
Thymol	0.94	0.75	0.68
β-Caryophyllene	0.49	0.35	0.30
Linalool	0.06	0.11	0.06
Bornyl acetate	0.08	0.18	1.44

The effect of the series insecticide applications against *E. elegans* is shown in Table 3. The aphid population increased significantly when period of exposure (i.e. the period without insecticide application) exceeded 4 weeks. A single insecticide application had no significant effect on plant weight. Also, 3-5 insecticide applications showed no significant differences for all parameters. Six insecticide applications caused a reduction in plant dry weight. However, the plant height was almost the same with three to five insecticide applications. The greatest plant height occurred with complete exposure (no insecticide application) due to the positive response to the substantial leaf fall caused by aphid infestation.

The benefit-per-unit cost of insecticide varied with exposure period, which influenced the yield and determined the number of sprays. The highest yield was obtained when plants were sprayed every two weeks. Allowing aphids to feed on common sage beyond 4 weeks resulted yield loss from 25 to over 64% (Table 3). The highest benefit-to-cost ratio was obtained maximum with a 4-week aphid exposure. Then, the highest gross profit was evident when common sage was kept free of aphids and decreased with an increase in aphid exposure period in both seasons.

Table 3. Effects of insecticide application on development of *Eucarazzia elegans*

Aphid exposure period (weeks)	Number of insecticide sprays	Fresh weight±SEM* (g)		Dry weight±SEM (g)		Plant height±SEM (cm)		Aphid infestation±SEM (%)	
0	6	2.78±0.15	a**	1.60±0.09	a	19.02±0.81	bc	0.79±0.27	a
2	5	2.16±0.15	b	1.24±0.09	b	19.35±0.80	b	4.89±0.37	b
4	4	2.07±0.12	b	1.19±0.07	b	18.21±0.84	bc	32.94±0.66	b
6	3	1.97±0.11	bc	1.13±0.06	bc	17.50±0.73	bc	46.56±0.62	bc
8	2	1.65±0.13	c	0.95±0.07	c	16.83±0.40	c	66.14±1.42	c
10	1	1.13±0.07	d	0.65±0.04	d	19.10±0.71	bc	84.39±0.71	d
Complete	0	1.00±0.05	d	0.57±0.03	d	21.91±0.71	a	86.90±0.46	d

* SEM: Standard error of the mean;
** Means in a column followed by the same letter are not statistical significantly different (ANOVA $P < 0.05$, Tukey's test).

The relationship between aphids' infestation and dry weight production is described by a regression equation: $y = 1.488 - 0.010x$ (Figure 5). This formula revealed that the aphid infestation inflicted significant reductions on sage yield as the number of aphids per plant increased. The reduction in yield and yield components are attributed to the feeding activities of aphids. This activity increased with duration of aphid exposure, with complete aphid exposure still permitting 30% plant development, though in some cases it caused plant mortality. The conversion of aphid population per plant to percentage aphid infestation follow the formula $y = 19.8x$ (Figure 6), which every percentage of aphid infestation being about 19-20 aphid per plant. These equations are useful for pest control policy given that this aphid species continues to be accidentally spread and introduced to other countries (Hales et al., 2009).

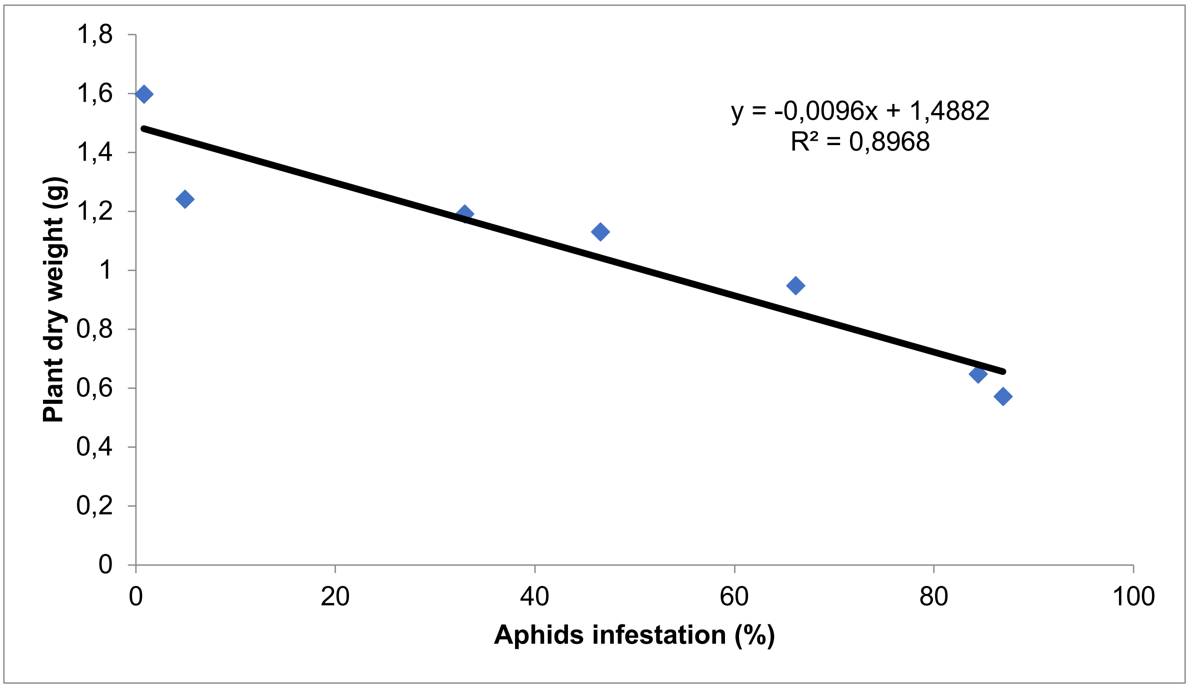

Figure 5. Regression line shows the relationship between plant dry weight and percentage of *Eucarazzia elegans* infestation.

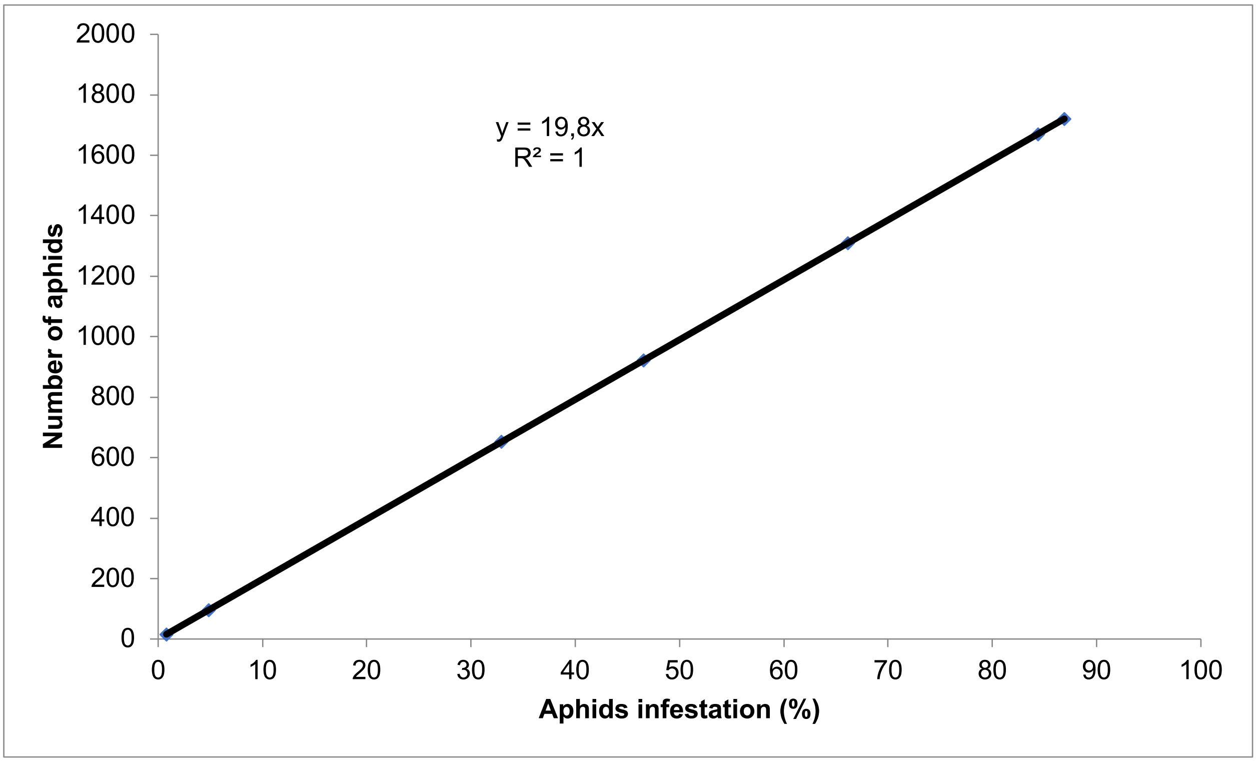

Figure 6. Regression line shows the relationship between number of aphids/plant and percentage of *Eucarazzia elegans* infestation.

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Original article (Orijinal araştırma)

Numerical taxonomy of *Ormyrus* Westwood, 1832 (Ormyridae: Hymenoptera) species based on general morphology in Sivas[1]

Sivas İli *Ormyrus* Westwood, 1832 (Ormyridae: Hymenoptera) türleri üzerinde nümerik taksonomik çalışmalar

Funda ARAS[2] **Lütfiye GENÇER**[3*]

Summary

The main objectives of the study were to determine the adequacy and reliability of characters used for identification of *Ormyrus* (Ormyridae: Hymenoptera) species, and the relationships between species within the genus by numerical taxonomic analysis. The specimens studied were collected in Sivas Province in 2010. Individual females of five species in to *Ormyrus* were examined. One hundred morphological characters were measured for 249 specimens. Three different numeric taxonomic analyses were performed, principal component, principal coordinate, and cluster analyses, using computer package software PAST. Principal components and coordinate analyses revealed the with characters of sufficient value to reliability distinguish five species of *Ormyrus*. Examination of the relationship between the five species by clustering analysis showed that the species belonged to two different main groups. The robustness of the currently available identification keys and new characters that could be used for of species identification was also determined.

Keywords: Chalcidoidea, numerical taxonomy, Ormyridae, *Ormyrus*

Özet

Çalışmanın asıl amacı nümerik taksonomik analiz ile Ormyrus cinsine ait türlerin teşhisinde kullanılan karakterlerin kalite ve güvenilirliğini ve türler arasındaki akrabalık ilişkilerini belirlemektir. Çalışılan örnekler 2010 yılında, Sivas ilinden toplanmıştır. *Ormyrus* Westwood (Ormyridae: Hymenoptera) cinsine ait 5 türün dişi bireyleri incelenmiştir. 249 örnek için 100 morfolojik karakter ölçülmüştür. PAST bilgisayar paket programı kullanılarak Temel Bileşenler Analizi, Temel Koordinat Analizi ve Kümeleme Analizi olmak üzere üç farklı nümerik taksonomik analiz yapılmıştır. Yapılan Temel Bileşen ve Koordinat analizleri sonucunda, belirlenen karakterlerin, *Orymrus* cinsine ait 5 türü tamamen birbirinden ayıracak yeterlilikte ve güvenilirlikte olduğu gösterilmiştir. Yapılan kümeleme analizi ile *Orymrus* cinsine ait 5 türün birbirleriyle olan akrabalık dereceleri belirlenerek, bu türlerin iki ayrı ana grup oluşturduğu gösterilmiştir. Mevcut teşhis anahtarlarının güvenilirliği ve teşhislerde kullanılabilecek yeni karakterler belirlenmiştir.

Anahtar sözcükler: Chalcidoidea, numerik taksonomi, Ormyridae, *Ormyrus*

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[1] This study was presented as an oral presentation at the 22nd National Biology Congress (23-27 June 2014, Eskişehir, Turkey).

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Introduction

Numeric taxonomy is a means of classification based on numerical principals. Briefly, it is the use numerical data within the field of systematics. Basing classification upon many characters instead of single character (not monothetic but polythetic) requires a simultaneously evaluating a range of characters. According to the Gilmour (1940) principle, the more characters are used in classification the greater the likelihood of achieving reliable results, both for the status of characters in different groups and for accurate determination of taxonomic relationships.

Taxon that are classified by numeric taxonomy are known as operational taxonomic units (OTU). All of the measurements taken on OTUs, which are to be classified in numeric taxonomy, constitute a data matrix. The most suitable geometric pattern for the data matrix is based on a spatial representation of the OTUs. Numeric taxonomy tries to reveal the hierarchical structure between the species by using clustering and permutation procedures from numerical methods (Sneath & Sokal, 1973; Podani, 1994).

The Ormyridae family, which belongs to the Chalcidoidea superfamily with the order Hymenoptera, is distinguished from related families by non-elongated cercus, metasomal tergum and the structure of ovipositor sheath on females, and consists of 153 species within three genera (Narendran, 1999; Noyes, 2017). Most species of Ormyridae are inhabitants of plants gall. Some species may be partially phytophagous, while others are either entomophagous or phytophagous (Zerova & Seryogina, 2006). *Ormyrus* is the largest and the most widespread genus, and consists of 151 species.

In the past, four different genera, *Ormyrus* Westwood, 1832, *Cyrtosoma* Perris, 1840, *Avrasyamyrus* Doğanlar, 1991 and *Ormyrulus* Boucek, 1986, were established using characters found on gaster (Doğanlar, 1991a). Doğanlar (1991b) reestablished the genus *Ormyrus* and divided the genus into three subgenera (*Monobaeus* Forster, *Tribaeus* Forster and *Crytosoma* Perris). Some authors consider that the family contains of only a single genus, *Ormyrus*. However, family has recently been seen as having three genera (Narendran, 1999; Noyes, 2017). Only the genus *Ormyrus* is recorded in Palearctic region. Several generic names have been proposed in this group, all based on the number of anelli and on the shape of gaster. Despite this proposal, and because they do not form any evident natural groups, only the genus *Ormyrus* is generally recognized (Zerova & Seryogina, 2006). Therefore, it is important to be able to identify species within the genus *Ormyrus*.

In all studies, some characters have been given more weight. Also, keys used for identification of species and characters used in these keys are limited. For this reason, it is desirable to use the numerical taxonomy as it gives equal value to all characters. The main objective of this study was to determine the adequacy and reliability of selected characters for identification of species and determination of relationships between species, and also to contributed to the development of an identification method based on numerical taxonomic analysis.

Material and Methods

Supply of material

The material of this study constituted of preserved specimens of *Ormyrus* from the collection of Entomology Museum of the Biology Department, Science Faculty, Cumhuriyet University. These specimens had been collected from Sivas Province in 2010. Five *Ormyrus* species were used and were identified using identification keys applicable to the Palearctic region (Doğanlar, 1991a, b; Hanson, 1992; Narendran, 1999; Zerova & Seryogina, 2006). The species, localities and collection dates of the specimens are given in Table 1.

Table 1. Localities and collection dates of female specimens of five *Ormyrus* species used from numerical taxonomic analysis

Taxon name	Number of specimens	Locality	Collection date
Ormyrus bingoeliensis	13	Kayapınar/Ulaş-Sivas	18/08/2010
Ormyrus cupreus	1	Sorguncuk/Merkez-Sivas	25/06/2010
O. cupreus	2	Tatlıca/Gemerek-Sivas	25/06/2010
O. cupreus	1	Karacaören/Yıldızeli-Sivas	11/07/2010
O. cupreus	1	Karaman/Divriği-Sivas	04/08/2010
O. cupreus	1	İğdeli/Kangal-Sivas	04/08/2010
O. cupreus	1	Gürpınar/Ulaş-Sivas	05/08/2010
O. cupreus	1	Elmalı/Şarkışla-Sivas	06/08/2010
O. cupreus	3	Kuluncak/Divriği-Sivas	18/08/2010
O. cupreus	4	Taşgeçit/Kangal-Sivas	18/08/2010
O. cupreus	4	Karasarbeli/Divriği-Sivas	18/08/2010
O. cupreus	1	Şenyurt/Ulaş-Sivas	19/08/2010
O. cupreus	7	Çetinkaya/Kangal-Sivas	20/08/2010
O. cupreus	6	Koşutdere/Hafik-Sivas	20/08/2010
O. cupreus	1	Eskibağ/Akıncılar-Sivas	28/08/2010
O. cupreus	35	Yellice/ Kangal-Sivas	17/09/2010
O. cupreus	8	Çetinkaya/Kangal-Sivas	17/09/2010
O. cupreus	10	Kuluncak/Divriği-Sivas	17/09/2010
O. cupreus	6	Ödek/Divriği-Sivas	17/09/2010
O. cupreus	15	Tödürge/Zara-Sivas	19/09/2010
Ormyrus orientalis	1	Kömürkaya/Şarkışla-Sivas	25/06/2010
O. orientalis	2	Taşgeçit/Kangal-Sivas	18/08/2010
O. orientalis	5	Çetinkaya/Kangal-Sivas	20/08/2010
O. orientalis	1	Yellice/Kangal-Sivas	17/09/2010
O. orientalis	1	Çetinkaya/Kangal-Sivas	17/09/2010
O. orientalis	4	Kuluncak/Divriği-Sivas	17/09/2010
Ormyrus salmanticus	1	Güllüce/Divriği-Sivas	18/07/2010
O. salmanticus	1	Koşutdere/Hafik-Sivas	20/08/2010
Ormyrus tschami	1	Karaman/Divriği-Sivas	18/07/2010
O. tschami	1	Yusufşeyh/Divriği-Sivas	04/08/2010
O. tschami	11	Ödek/Divriği-Sivas	04/08/2010
O. tschami	30	Kuluncak/Divriği-Sivas	18/08/2010

Table 1. (Continued)

Taxon name	Number of specimens	Locality	Collection date
O. tschami	19	Kasarbeli/Divriği-Sivas	18/08/2010
O. tschami	40	Taşgeçit/Kangal-Sivas	18/08/2010
O. tschami	40	Koşutdere/Hafik-Sivas	20/08/2010
O. tschami	3	Çetinkaya/Divriği-Sivas	20/08/2010
O. tschami	10	Yellice/Kangal-Sivas	17/09/2010
O. tschami	3	Kuluncak/Divriği-Sivas	17/09/2010

Specimen selection for numerical taxonomy

When selecting the specimen, the aim was to select specimens that represented the whole of the sampled locality and that exhibited intraspecific variation. By examining the female individuals of the five species, 100 morphological characters (Table 2) were measured for each of 249 specimens.

Table 2. Morphological characters used in numerical taxonomic analysis

ANTENNA	
Scape width/length	Club segment number
Pedisel width/length	Club segment width/length
Anelli 2 width/length*	Longitudinal sensilla number*
Anellus number	Longitudinal sensilla row *
Funicular segment number	Flagellum shape
Funicular segment width/length (mm)	Scape*, Pedicel*, Anelli*, Funicular segment color
FOREWING	
Submarginal/ Marginal vein	Wing width/length
Submarginal/ Postmarginal vein	Costal cell width/length
Submarginal/ Stigmal vein *	Admarginal setae number
Submarginal setae number	Basal setal line
Speculum open/ closed	Cubital setal line*
HEAD	
Head ventral width/length	Distance between toruli/distance between torulus -clypeus
Eye width/length	Distance between toruli/ distance between toruli-median ocellus
Clypeus entire	POL / OOL
Malar space length/mouth width	Head dorsal width/length *
Distance between toruli -clypeus and toruli-median ocellus	Head width / plagellum+Pedicel length

Table 2. (Continued)

THORAX	
Thorax width/length	Dorsellum width/length
Pronotum width/length	Propodeum width/length
Mesoscutum width/length	Spiracle diameter / distance to metanotum
Scutellum width/length	Thorax (Lateral) width/length
Metanotum width/length	Notauli / Axilla length
GASTER	
Carina	T6 width/length
T1 width/length	T6 color*
T1 color*	T6 sculpture
T3 width/length*	Epipygium width/length
T3 color*	Epipygium color*
T3 sculpture	Ovipositor width/length
T4 width/length	Ovipositor color*
T4 color*	Gaster (dorsal) width/length
T4 sculpture	Gaster (lateral) width/length
T5 width/length	Sercal setae 1/3
T5 color*	Sercal setae 2/4
T5 sculpture	
LEG	
Fore Coxa width/length	Tibia 1 width/length
Hind Coxa width/length	Tibia 2 width/length
Femur color*	Tibia 3 width/length
Femur 1 width/length	Spur 1 (mm) / Tibia 1 length
Femur 2 width/length	Spur 2 (mm) / Tibia 2 length
Femur 3 width/length	Spur 3 (mm) / Tibia 3 length
Tibia color*	

Results and Discussion

Five species of *Ormyrus* were determined, with *Ormyrus salmanticus* Nieves-Aldrey, 1984 being placed in two groups according to the number of anelli (one or two). Therefore 6 OTUs were recognized (Table 3).

Table 3. List of OTUs for numerical taxonomic analysis

OTU1	*Ormyrus bingoeliensis*
OTU2	*Ormyrus cupreus*
OTU3	*Ormyrus orientelis*
OTU4	*Ormyrus tschami*
OTU5	*Ormyrus salmanticus*, 1 anellus
OTU6	*Ormyrus salmanticus*, 2 anelli

Morphological characters not included in the current identification keys where chosen to improve the reliability the numeric taxonomic analysis. The identifications made from numeric taxonomical analysis were consistent with identification by existing keys. To maximize the reliability of the data care was taken to always use the same angle when taking morphological measurements. In this manner, the variations that could be result from different angles were minimized.

The 100 morphological characters used for separating OTUs from each other by principal component analysis were described from the first two components that were verifiably the highest. As shown in Table 4, variation described by components seems to reduce gradually from the first component. The first two components accounted for 95.5% of the variation. This revealed more than 90% differences between OTU based on principal component analysis (Figure 1). These results confirm the reliability of the characters chosen for numerical taxonomic analysis. Also, the effectiveness of selecting a large number of characters was confirmed. The grouping of OTUs can be seen in the graphical comparison of the first three components. In particular, it is evident that OTUs 4, 5 and 6 represent a separate group and are close to each other. The reason for this observed closeness is considered to be related to the similarity of the habitat of these species and the similarity of their hosts.

Table 4. Eigen values of the first two components measured for six operational taxonomic units and Eigen value percentages

Measured character	Eigen value	% Variance
1	1389.190	91.733
2	57.772	3.815
3	7.748	3.153

The characters listed in Table 5 are the important characters that best delineate the six OTUs (Figures 2 & 3). The characters indicated by an asterisk in Table 2 are the characters with the highest ratio for separating OTUs. These characters, especially 30, 31, 32, 54, 68, 70, 73, 76, 79, 82 and 91, when used to evaluate *O. salmanticus* with 1 or 2 anelli, indicated the possibility that these two OTUs represent two different species. It is recommended that these two OTUs be evaluated in molecular studies to assess the possibility are two different species. It is also considered that these characters are important for identification of species, that they are adequate and reliable enough to be used in identification keys. The characters listed in Table 2 are not used in existing identification keys.

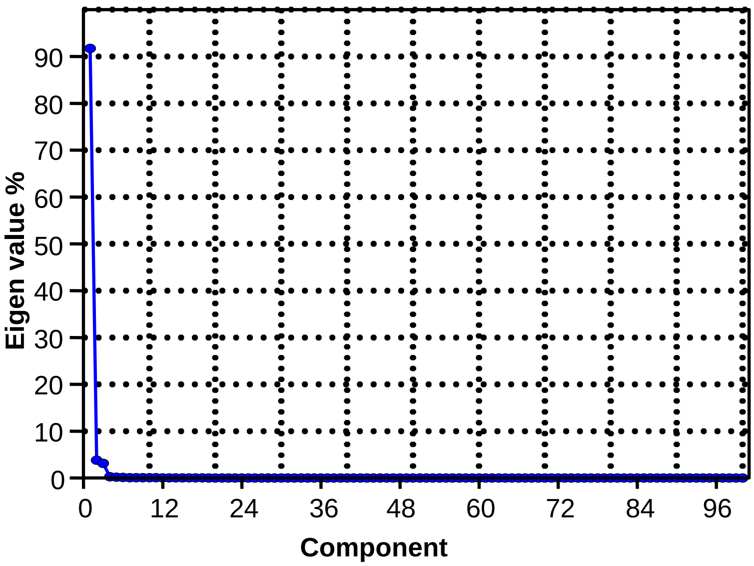

Figure 1. Percentage of Eigen values of the first two components measured for six operational taxonomic units.

Table 5. Characters values for principal components 1 and 2

Character Symbol/Number	Character name	Character values Component 1	Character values Component 2
F	Anelli (2) width/length	-0.02726	0.05653
Z	Longitudinal sensilla number	0.01476	-0.05669
27	Longitudinal sensilla row	0.05391	-0.06080
30	Scape color	0.31550	0.17640
31	Pedicel color	0.29420	-0.32350
32	Anelli color	0.29420	-0.32350
43	Head dorsal width/length	-0.02310	0.07293
47	Submarginal /stigmal vein	0.02720	0.09104
54	Cubital setal line	-0.12900	0.54860
68	T1 color	0.31550	0.17640
69	T3 width/length	-0.02922	0.07240
70	T3 color	0.31550	0.17640
73	T4 color	0.31550	0.17640
76	T5 color	0.31550	0.17640
79	T6 color	0.31550	0.17640
82	Epipygium color	-0.17330	0.45650
84	Ovipositor color	0.25190	-0.13290
91	Femur color	0.31550	0.17640
92	Tibia color	-0.09597	0.05062

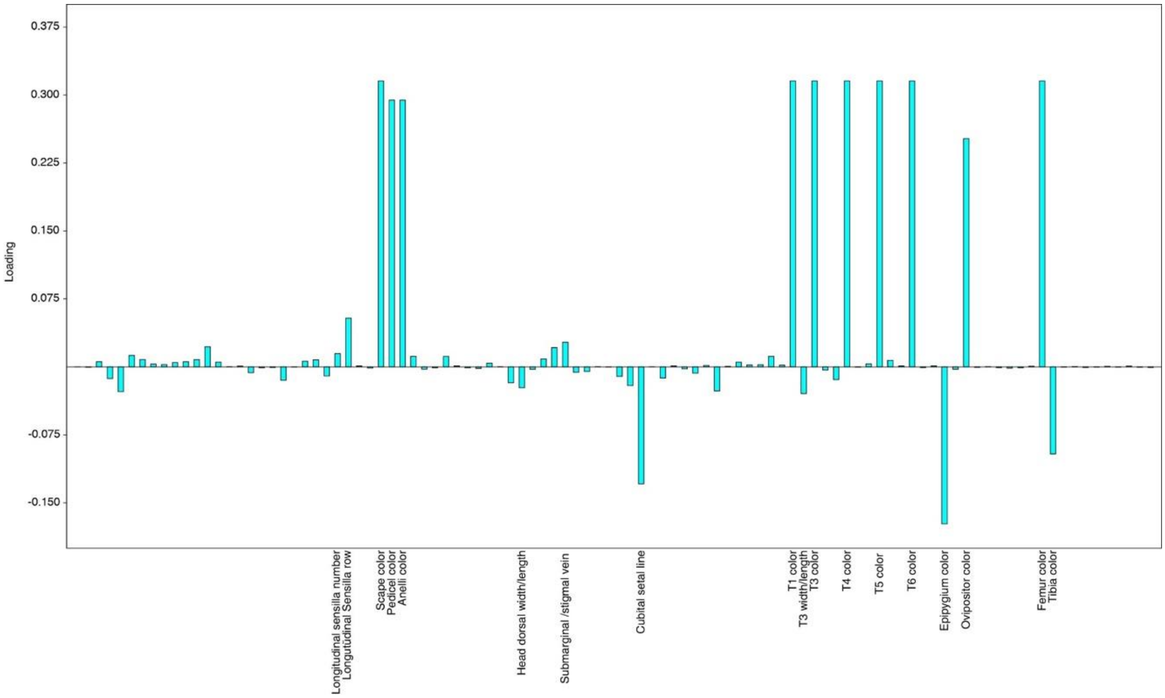

Figure 2. The first principal component graphic of measured characters.

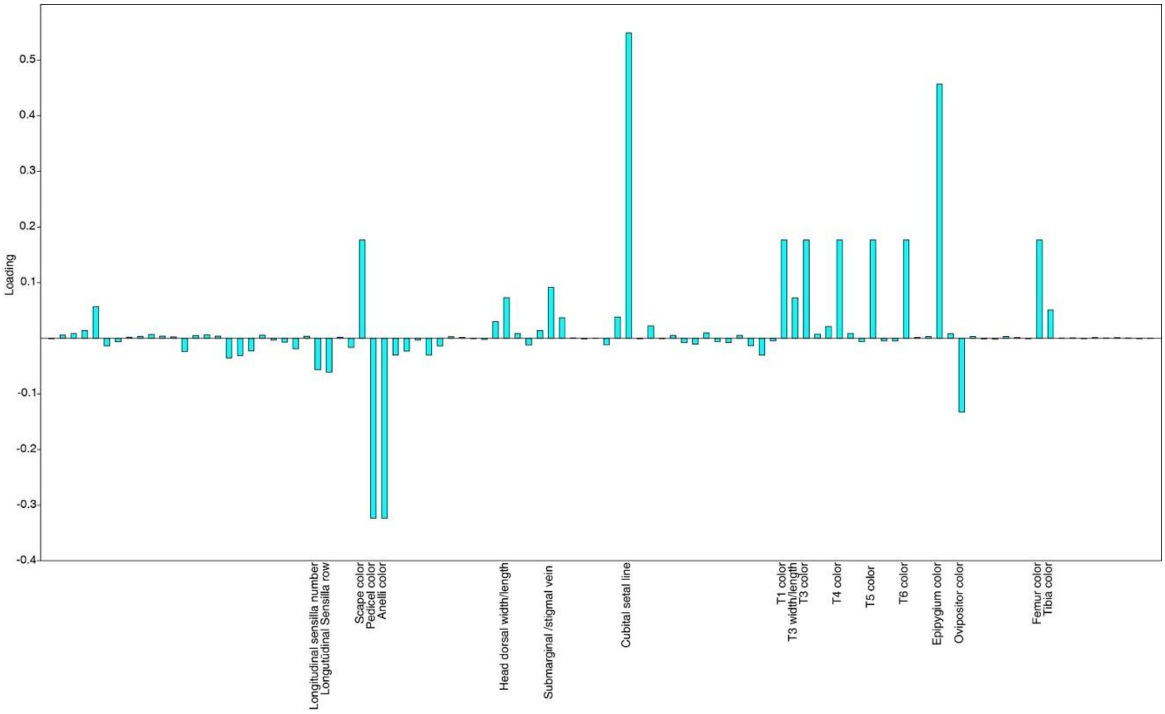

Figure 3. The second principal component graphic of measured characters.

The Eigen values of the first two components from the principal coordinate analysis explain more than 90% difference between the OTUs. In Table 6 it is evident that the amount of variation explained by the coordinates from the principal component analysis, reduce gradually from the first coordinate and describe 95.54% of the variation of the first two coordinates. The characters which revealed 95.54% of the variation at OTUs seem to be highly effective in separating of OTUs (Figure 4).

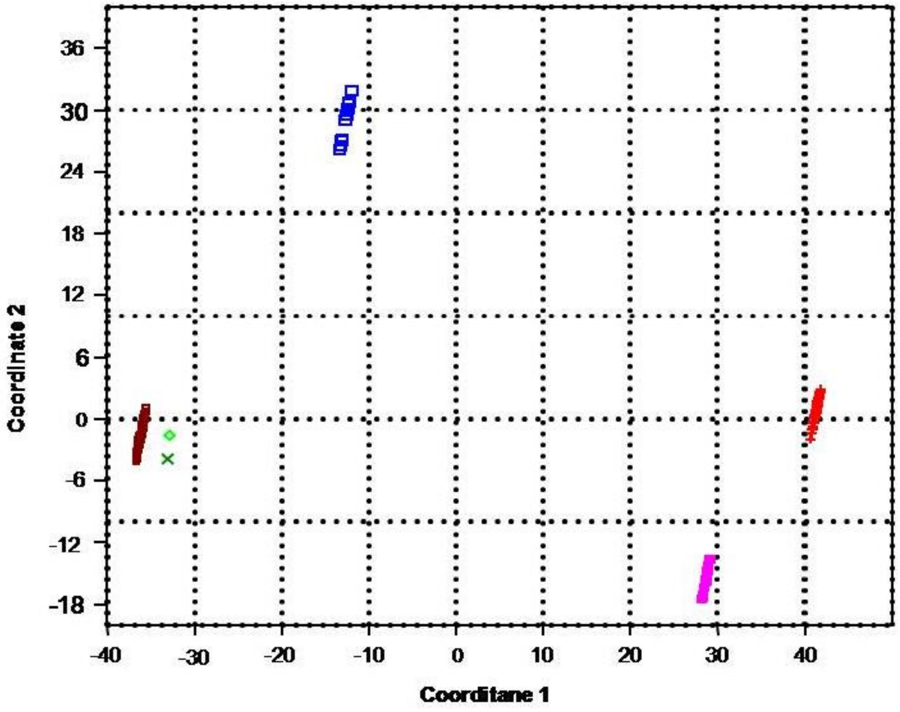

Figure 4. Distance and locations of six operational taxonomic units the first two coordinates.

In the unweighted pair group method dendrogram (Figure 5) from the cluster analysis the OTUs were put in two major groups. *Ormyrus bingoeliensis* Doğanlar, 1991 and *Ormyrus cupreus* Askew, 1998 are close to each other and in a separate group. Also, *Ormyrus orientalis* Walker, 1871 and *Ormyrus tschami* Doğanlar, 1991 are close to each other and in a separate group. *Ormyrus salmanticus* (1 anellus), *O. salmanticus* (2 anelli) and *O. orientalis* are relatively close to each other and in a separate group.

Ormyrus salmanticus (1 anellus) and *O. salmanticus* (2 anelli) are the closest taxons to each other and in a separate group (Figure 5). According to principal components, principal coordinate and cluster analyses, *O. salmanticus* (1 anellus) and *O. salmanticus* (2 anelli) could potentially be regarded as two distinct species. However, given that only two specimens of *O. salmanticus* were available for the present study, it is recommended that molecular analysis be used to determine the status of these two OTUs.

The results of all these analyses support the adequacy and reliability of the morphological characters selected for distinguishing the five species. The classification obtained by numerical taxonomy seem to be compatible with identification made using existing identification keys (Doğanlar, 1991a,b; Hanson, 1992; Zerova & Seryogina, 2006; Narendran, 1999; Gençer, 2014). The analyses performed were quite efficient for distinguishing the species. The closeness of species to each other was explained and it is suggested that the selected characters should be considered for inclusion in any future identification keys for this genus.

Table 6. Eigen values of the first two coordinates measured for six operational taxonomic units and Eigen value percentages

Measured character	Eigen value	% Variance
1	34452	91.733
2	14327	3.815
3	11842	3.153

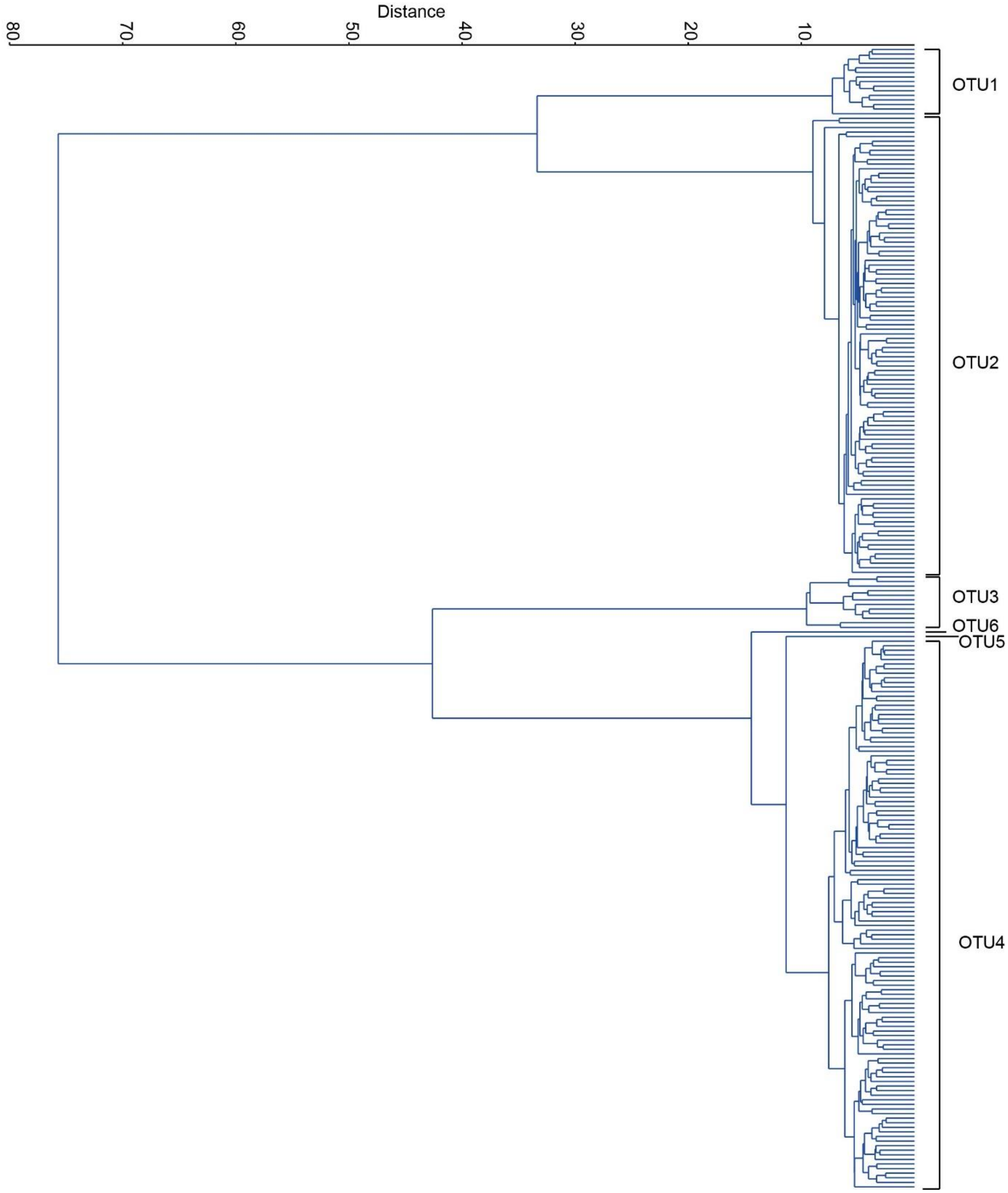

Figure 5. Distance tree created by clustering method for six operational taxonomic units.

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Original article (Orijinal araştırma)

Notes on the genus *Astenus* Dejean, 1833 from the Palearctic Region (Coleoptera: Staphylinidae: Paederinae)[1]

Palaearktik Bölgedeki *Astenus* Dejean, 1833 cinsi üzerine notlar (Coleoptera: Staphylinidae: Paederinae)

Sinan ANLAŞ[2*]

Summary

The genus *Astenus* Dejean, 1833 (Coleoptera: Staphylinidae: Paederinae) contains more than 200 species in the Palearctic Region. In this paper, new and additional distributional data concerning 20 species of the genus *Astenus* in the Palearctic Region are presented. The studied material has been collected between 1928-2016 and also contained the types and additional specimens from some European museums. Among them nine species are first country records: Armenia (1), Azerbaijan (1), Bulgaria (2), Bosnia Herzegovina (1), France (1), Iraq (2), Israel (1), Syria (2), Kazakhstan (2), Turkey (1) and Turkmenistan (4). Besides, the original specimens of *Astenus rufopacus* Reitter, 1909 have been studied, the lectotype designated, the species redescribed and illustrated.

Keywords: *Astenus*, fauna, lectotype, Paederinae, Palearctic Region

Özet

Astenus Dejean, 1833 (Coleoptera: Staphylinidae: Paederinae) cinsi Palearktik Bölgede 200'den fazla tür içermektedir. Bu çalışmada, Palearktik Bölgede bulunan *Astenus* Dejean cinsine bağlı 20 türe ait yeni ve ek yayılışsal kayıtlar sunulmuştur. İncelenen materyal 1928-2016 yılları arasında toplanmış olup, bazı Avrupa müzelerindeki tip ve diğer örnekleri de içermektedir. Bu 20 türden dokuz tür ilk ülke kaydı niteliğindedir: Ermenistan (1), Azerbaycan (1), Bulgaristan (2), Bosna Hersek (1), Fransa (1), Irak (2), İsrail (1), Suriye (2), Kazakistan (2), Türkiye (1) ve Türkmenistan (4). Ayrıca, *Astenus rufopacus* Reitter, 1909 türünün tip örnekleri incelenmiş, lektotipi seçilmiş ve bu tür yeniden tanımlanarak şekillendirilmiştir.

Anahtar sözcükler: *Astenus*, fauna, lektotip, Paederinae, Palaearktik Bölge

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Introduction

The genus *Astenus* (Coleoptera: Staphylinidae: Paederinae) was described by Dejean (1833) and is attributed to the subtribe Astenina Hatch, 1957 of the tribe Paederini Fleming, 1821 (Schülke & Smetana, 2015). Members of this genus occur in many places, mainly in dry and semi-dry biotopes, most often under stones and in the nests of ants at a wide range of elevations, from near sea-level to almost 3000 m. They also found sometimes in wet areas and on the banks of rivers and lakes.

Reliable identification of *Astenus* species is possible mostly on the basis of the male primary and sexual secondary characters, particularly the shape of the ventral process of aedeagus. Many external characters, such as body size, coloration, relative density of puncturation and microsculpture, are highly variable even in specimens from the same locality, so that an identification of females is usually very difficult. Especially, the widespread species were found to be extremely variable. According to recent catalog by Schülke & Smetana (2015; updated), the genus *Astenus* contains more than 200 species in the Palearctic Region. The genus divided into four subgenera: the nominate subgenus (92 species); *Astenopleuritus* Coiffait (11 species); *Eurysunus* Reitter (60 species); *Mecognathus* Wollaston (three species). In addition, 35 species as incertae sedis and one species as nomen dubium are known. However, in the opinion of the author the subgeneric concept currently in use is not phylogenetic. For this reason, the application of DNA data should be made to reveal the subgeneric status of the genus. Molecular approach to systematic and taxonomic studies is a helpful way to complement traditional morphological methods of species identification.

In the present study, 20 species of the nominate subgenus and the subgenus *Astenopleuritus* are reported from different countries of the Palearctic Region, including some records of zoogeographic interest. Additionally, the original specimens of *Astenus rufopacus* Reitter, 1909 have been studied, the lectotype designated, the species redescribed and the habitus and genitalic structures illustrated. The main aim of the study was to contribute to the knowledge of *Astenus* fauna of the Palearctic Region.

Material and Methods

Primary and secondary sexual characters of the species redescribed herein are termed following Coiffait (1984). The morphological studies were conducted using a Stemi 2000-C microscope (Zeiss, Germany). For the photographs a digital camera (Zeiss Axiocam ERC5s) was used. The following abbreviations are used for the measurements, which are given in mm:

AL, length of antenna; AW, maximal width of abdomen; EL, length of elytra from apex of scutellum to posterior margin; EW, combined width of elytra; HL, head length from anterior margin of clypeus to posterior margin of head; HW, head width (including eyes); ML, length of aedeagus from apex of ventral process to base; PL, length of pronotum along median line; PW, maximal width of pronotum; TaL, length of metatarsus; TiL, length of metatibia; and TL, total body length.

The studied material has been collected between 1928-2016 and contained types and additional male specimens from the museums. The material referred to in this study stored in the following collections:

AZMM – Alaşehir Zoological Museum, Manisa, Turkey (S. Anlaş);

HNHM – Hungarian Natural History Museum, Budapest, Hungary (G. Makranczy and O. Merkl);

IRSNB – Inst. Royal des Sciences Naturelles de Belgique, Bruxelles, Belgium (W. Dekoninck);

MHNG – Muséum d'Histoire Naturelle, Genève, Switzerland (G. Cuccodoro);

NMNHS – National Museum of Natural History, Sofia, Bulgaria (R. Bekchiev); and

NMPC – National Museum, Praha, Czech Republic (M. Fikáček).

Results

Genus *Astenus* Dejean, 1833

Subgenus *Astenopleuritus* Coiffait, 1982

Astenus melanurus (Küster, 1853)

Material examined: Bulgaria: 2 exs, 19.IV.2010, Strandzha Mts., Bliznak vill., 42°04'41" N, 27°42'59" E, leg. Bekchiev (NMNHS). Cyprus: 3 exs, XII.1965, Cypre, Yermasoyia (MHNG). Egypt: 1 ex., 12.VI.1995, Beni Suef, El Shanawaya (MHNG); 10 exs, 29.V.1996, Cairo, Dahshur, ca. 23 km S Cairo, 29°48'00" N, 31°14'30" E, light trap collection, leg. Ullrich (MHNG). Iran: 2 exs, 25.V.1974, Kohkiluyeh Basht, 30°20' N, 51°15' E, leg. Senglet (MHNG). Morocco: 1 ex., 08.IV.1974, Morocco, Sud Oued Massa, leg. Besuchet (MHNG); 3 exs, Morocco (NMPC). Turkey: 5 exs, Adana, Asia Min., leg. Boyadjani (NMPC); 1 ex., 04.V.1998, Antakya 7 km E, Yeşilkent 350-400 m, leg. Besuchet & Löbl (MHNG); 1 ex., 18.IV.1978, Konya (=Karaman), Sertavul Pass, 1500-1600 m, leg. Besuchet & Löbl (MHNG); 1 ex., 29.IV.1978, Mersin, Yeniköy, 1000-1200 m, leg. Besuchet & Löbl (MHNG); 1 ex., 15.V.1976, Zonguldak, Ereğli-Beliköy, leg. Besuchet & Löbl (MHNG); 1 ex., 06.V.1978, Kayseri, Sultan Sazlığı, 1000 m, leg. Besuchet & Löbl (MHNG); 3 exs, 15.IV.2013, Afyonkarahisar, İhsaniye, Döğer 2 km E, 39°09'13" N, 30°25'52" E, 1286 m, leg. Anlaş, Yağmur & Örgel (AZMM); 1 ex., 22.III.2015, Aydın, Dilek peninsula national park, 37°39'49" N, 27°12'57" E, 969 m, leg. Yağmur & Örgel (AZMM); 2 exs, 12.VI.2013, Denizli, Acıpayam, Alaattin, Bozdağ, 37°24'55" N, 29°13'11" E, 1470 m, leg. Yağmur & Örgel (AZMM); 2 exs, 12.IV.2015, Denizli, Güney, 38°04'26" N, 28°59'02" E, 210 m, leg. Anlaş, Yağmur & Örgel (AZMM); 5 exs, 14.XI.2014, İzmir, Karaburun 5 km SW, 38°37'39 N, 26°29'26 E, leg. Anlaş, Yağmur & Örgel (AZMM); 5 exs, 13.IV.2015, Kütahya, Simav, Akdağ, 39°14'58" N, 28°49'41" E, 1670 m, leg. Anlaş & Örgel (AZMM); 1 ex., 21.V.2015, Muğla, Dalaman, 36°53'37" N, 28°53'37" E, 127 m, leg. Yağmur & Örgel (AZMM); 1 ex., 30.IV.1975, Muğla, Gökova, Akyaka, leg. Besuchet & Löbl (MHNG); 2 exs, 19. IX.2013, Kütahya, Gediz, Sandıklı 2 km S, 38°55’03" N, 29°525’44" E, 854 m, leg. Özgen & Örgel (AZMM).

Distribution: This species is known from southern Europe, North Africa, Cyprus, Iran, Syria, Turkey in Palearctic Region, and also in Oriental and Afrotropical Region (Schülke & Smetana, 2015). The records from Bulgaria are reported for the first time.

Subgenus *Astenus* Dejean, 1833

Astenus adonis Coiffait, 1960

Material examined: Turkey: 1 ex., 19.V.1962, Samsun, Bafra, leg. Besuchet (MHNG); 1 ex., 26.VII.1969, Istanbul, Halkalı, leg. Besuchet (MHNG); 1 ex., 17.VII.1969, Bergama, leg. Besuchet (MHNG). Greece: 1 ex., Attica (MHNG). Syria: 1 ex., Syrie (=Syria) (MHNG).

Distribution: *Astenus adonis* was known from Greece, Lebanon, Saudi Arabia and Turkey (Schülke & Smetana, 2015). The above specimen in Syria represents the first records for this country.

Astenus assingi Bordoni, 1994

Material examined: Turkey: 2 exs, 03.I.2010, Antalya, Gazipaşa, Beyrebucağı, 36°13'10" N, 32°24'17" E leg. Kunt (AZMM); 2 exs, 17.IV.1978, Antalya, 18 km SE of Gazipaşa, leg. Besuchet & Löbl (MHNG).

Distribution: *Astenus assingi* is endemic to southwestern Anatolia (Bordoni, 1994; Schülke & Smetana, 2015).

Astenus bimaculatus bimaculatus (Erichson, 1840)

Material examined: Algeria: 1 ex., 13.V.1988, Algérie, Gyde Kabylie, 9 km E of Yakouren, 930 m, leg. Besuchet, Löbl & Buchardt (MHNG). Bosnia Herzegovina: 1 ex., V.1928, Bosnia, Hrid, Goražde, leg. Fodor (HNHM). Cyprus: 2 exs, 23.VII.1977, Chypren, Polis, leg. Besuchet (MHNG). Iran: 1 ex., VII.2006, Mazandaran, Savadkooh, leg. Ghahari (AZMM); 3 exs, 14.VI.1975, Esfahan, Falâvariân, 32°34' N, 51°31' E, leg. Senglet (MHNG); 1 ex., 20.VI.1974, Bakhtiyari, N-E du Zardeh-Küh, 2700 m, 32°23' N, 50°07' E, leg. Senglet (MHNG); 1 ex., 05.VII.1973, Guilan, Lahidjan, 37°11' N, 49°54' E, leg. Senglet (MHNG). Italy: 5 exs, 22.IX.1996, Basilicata, str. Irsina-Grassanosu F. Bradano Mts., 150 m, leg. Angelini (AZMM). Kazakhstan: 2 exs, 05.IV.2010, Yuzhno-Kazakhistan Region, Boralday Range, Satur Mts, hole of the Kulan nv., high Krasnye Vorota pass, 1000 m, 42°35'13" N, 70°26'53" E, leg. Matalin (AZMM); 2 exs, Turkestan, Reitter, Leder (NMPC). Spain: 1 ex., 25.V.1991, Espana, Bilboa, Marquina, leg. Podlussány (HNHM). Tunusie: 1 ex., Lekef, coll. J. Clermont (MHNG). Turkey: 3 exs, 15.V.2011, Gümüşhane, Gümüşhane-Bayburt road 5 km, leg. Anlaş (AZMM). Turkmenistan: 1 ex., 8-10.V.1952, Repetek (IRSNB); 2 exs, 25.VI.1992 Turkmenisian, Kopet-Dagh, 400-600 m, Fkyuza, 37°50' N, 58°05' E, leg. Fábián, Herczig, Podiussány & Varga (HNHM).

Distribution: This species is widespread in Europe, North Africa, Afghanistan, Cyprus, Iran, Israel, Pakistan, Syria, Turkey, Tajikistan and Uzbekistan (Schülke & Smetana, 2015). Thus, this species is reported for the first time from Bosnia Herzegovina, Kazakhstan and Turkmenistan.

***Astenus bucharensis* Bernhauer, 1902**

Material examined: Kazakhstan: 1 ex., "Aulia Atia" (=Almaty), "Syr Dari" (=Syr Darya) (NMPC).

Distribution: This species is known from Kazakhstan and Turkey (Coiffait, 1984; Schülke & Smetana, 2015). Coiffait (1984) reported this species from eastern Turkey without exact locality. In my opinion, occurrence of this species in Turkey is highly doubtful. This record should be confirmed by future studies.

***Astenus bulgaricus* Coiffait, 1971**

Material examined: Bulgaria: 1 ex., 19.IV.2010, Strandzha Mts., Bliznak vill., 42°04'41" N, 27°42'59" E, leg. Bekchiev (AZMM); 2 exs, 18.IV.2010, Strandzha Mts., Malko Tarnova env., 41°58'52" N, 27°52'46" E, leg. Bekchiev (NMNHS). Russia: 1 ex., 22.VII.1999, Rostov Region, Veshenskaya vill., leg. Khachikov (AZMM). Turkey: 4 exs, 25.V.2016, Karaman, Sarıveliler, Göktepe, Saçaktepe, 36°38'22" N, 32°32'09" E, 1847 m, leg. Anlaş, Örgel & Yaman (AZMM); 1 ex., 21.V.2015, Muğla, Dalaman, 36°53'37" N, 28°53'37" E, 127 m, leg. Yağmur & Örgel (AZMM).

Distribution: This species is known from Bulgaria, southern European territory of Russia, Turkey and Ukrania (Schülke & Smetana, 2015).

***Astenus cribrellus* (Baudi di Selve, 1870)**

Material examined: Turkey: 1 ex., 28.VI.2016, Yozgat, Aydıncık, Kuşsaray, 40°05'07" N, 35°11'59" E, 1341 m, leg. Örgel & Yaman (AZMM); 3 exs, 22.III.2015, Aydın, Dilek peninula national park, 37°39'49" N, 27°12'57" E, 969 m, leg. Yağmur & Örgel (AZMM); 3 exs, 17.IV.2013, Eskişehir, İncesu, Porsuk Dam, 39°37'09" N, 30°14'07" E, 917 m, leg. Yağmur & Örgel (AZMM).

Distribution: According to Schülke & Smetana (2015), this species occurs in Belgium, Croatia, France, Georgia, southern European territory of Russia and Turkey.

***Astenus fauveli* (Eppelsheim, 1888)**

Material examined: Turkmenistan: 3 exs, 25.VI.1992 Turkmenisian, Kopet-Dagh, 400-600 m, Fkyuza, 37°50' N, 58°05' E, leg. Fábián, Herczig, Podiussány & Varga (HNHM).

Distribution: This species was reported from Kyrgyzstan, Tajikistan and Uzbekistan (Schülke & Smetana, 2015). It is recorded from Turkmenistan for the first time.

***Astenus gracilis* (Paykull, 1789)**

Material examined: Azerbaijan: 2 exs, Caucasus, Leder, Reitter (NMPC); 1 ex., 9-10.V.1959, Caucase oriental, Talysh: Lerik (IRSNB). Czech Republic: 1 ex., 04.VI.1947, Hradec Králové (NMPC). Greece: 1 ex., 14.III.1976, Krete, umg. Anogia (MHNG). Iraq: 3 exs, 17-20.V.2008, northern Iraq, ca 10 km NW Suleimaniyah province, leg. Sevinç (AZMM). Morocco: 1 ex., 08.IV.1974, Morocco, s-Oued, Massa, leg. Besuchet (MHNG).

Distribution: *Astenus gracilis* is known from Europe, North Africa, west and east Siberia, Iran, Uzbekistan and Turkey (Schülke & Smetana, 2015). The records from Iraq are reported for the first time.

***Astenus immaculatus* Stephens, 1833**

Material examined: Azerbaijan: 1 ex., Caucasus, Leder, Reitter (NMPC). Italy: 1 ex., 22.VII.1993, Basilicata, F. Sinni a Episcopia (PZ), leg. Angelini (AZMM). Iran: 2 exs, 18.VI.1974, Bakhtiyari, barrage de Kührang, 32°26' N, 50°06' E, leg. Senglet (MHNG). Russia: 1 ex., 05.V. 1991, Rostov Region, Rostov-on-Don city, leg. Khachikov (AZMM). Turkey: 15 exs, 22.VI.2016, Ankara, Beypazarı, Üreğil, 40°17'07" N, 32°04'11" E, 1375 m, leg. Örgel & Yaman (AZMM); 1 ex., 24.V.2016, Konya, Sarıveliler 25 km W, 36°52'08" N, 32°25'19" E, 1976 m, leg. Anlaş, Örgel & Yaman (AZMM); 5 exs, 14.VI.2013, Denizli, Acıpayam, Yassıca Tepe, 37°16'52" N, 29°13'06" E, 1700 m, leg. Yağmur & Örgel (AZMM); 3 exs, 14.XI.2014, İzmir, Karaburun 5 km SW 38°37'39 N, 26°29'26E, leg. Anlaş, Yağmur & Örgel (AZMM); 2 exs, 13.IV.2015, Kütahya, Simav, Akdağ, 39°14'58" N, 28°49'41" E, 1670 m, leg. Anlaş & Örgel (AZMM); 1 ex., 30.XI.2014, Manisa, Spil Mts., 38°33'44" N, 27°23'10" E, 1100 m, leg. Yağmur & Örgel (AZMM); 4 exs, 17.XI.2014, Muğla, Datça, 36°47'27" N, 28°03'05" E, 100 m, leg. Yağmur & Örgel (AZMM); 4 exs, Adana, Asia min. (NMPC).

Distribution: This species is known from Europe, Algeria, Iran and Turkey (Schülke & Smetana, 2015).

***Astenus lithocharoides* (Solsky, 1874)**

Material examined: Turkmenistan: 2 exs, 09.V.1991, USSR, Turkmenia, Karakum desert, 200 m, 20 km SW Repetek, 38°25' N, 63°09' E, leg. Gsoroba, Fabian, Herczig, Hrebiay & Ronkay (HNHM).

Distribution: This species was known from Georgia, Afghanistan, Iran, Kyrgyzstan, Tajikistan and Uzbekistan (Schülke & Smetana, 2015). It is recorded from Turkmenistan for the first time.

***Astenus lyonessius* (Joy, 1908)**

Material examined: Morocco: 1 ex., 21.III.1982, Morokko, Hoher Atlas, umg. Tahanaoute (as *A. brevelytratus* Lohse, 1987 det. V. Assing) (MHNG). Turkey: 2 exs, 28.V.2016, Karaman, Ayrancı, Yüğlük Mts., 37°00'57" N, 33°46'48" E, 1942 m, leg. Anlaş, Örgel & Yaman (AZMM); 1 ex., 29.V.2016, Ayrancı, Berendi 5 km E, 37°15'59" N, 34°05'39" E, 1986 m, leg. Anlaş, Örgel & Yaman (AZMM); 1 ex., 27.V.2016, Konya, Ereğli, Kartal Hill, 37°23'34" N, 34°02'06" E, 1900 m, leg. Anlaş, Örgel & Yaman (AZMM); 3 exs, 14.IV.2016, Konya, Beyşehir, Dumanlı, 37°27'51" N, 31°19'44" E, 1828 m, leg. Örgel & Yaman (AZMM); 2 exs, 21.V.2016, Beyşehir, Geyik Mts., 37°25'36" N, 31°43'00" E, 2000 m, leg. Örgel & Yaman (AZMM); 1 ex., 12.IV.2013, Afyonkarahisar, Düzağaç 2 km N, 38°48'01" N, 30°09'03" E, 1172 m, leg. Anlaş, Yağmur & Örgel (AZMM); 1 ex., 24.III.2014, Aydın, İmambaba Hill, 37°56'06" N, 27°53'56" E, 1644 m, leg. Anlaş & Örgel (AZMM); 2 exs, 13.04.2014, Balıkesir, Sındırgı, Ulus Mts., leg. Özgen & Örgel (AZMM); 2 exs, 15.IV.2014, Denizli, Çal, Küçük Çökelez Mts., 38°02'48" N, 29°22'11" E, 1575 m, leg. Anlaş & Örgel (AZMM); 6 exs, 06.IV.2015, İzmir, Bergama, Kozak, Güneşli, 39°22'20" N, 27°06'40" E, 1002 m, leg. Yağmur & Örgel (AZMM); 1 ex., 23.VI.2013, Kütahya, Simav, Kabaarmut, 39°10'24" N, 29°01'02" E, 1244 m, leg. Yağmur & Örgel (AZMM); 1 ex., 01.XI.2013, Manisa, Demirci, Bardakçı, 39°07'06" N, 28°33'02" E, 1386 m, leg. Anlaş & Örgel (AZMM); 2 exs, 04.IV.2013, Muğla, Datça, Emecik 2 km SW, 36°46'01" N, 27°48'39" E, 110 m, leg. Yağmur & Örgel (AZMM).

Distribution: This species is known from Europe, North Africa, Israel and Turkey (Schülke & Smetana, 2015).

***Astenus misellus* (Mulsant & Rey, 1880)**

Material examined: Algeria: 4 exs, 13.V.1988, Algérie, Gyde Kabylie, 9 km E of Yakouren, 930 m, leg. Besuchet, Löbl & Buchardt (MHNG).

Distribution: This species is known from France, Italy, Spain, Switzerland, Algeria and Morocco (Schülke & Smetana, 2015).

***Astenus pictipennis* Fauvel, 1900**

Material examined: Turkmenistan: 2 exs, 30.VI-04.VII.1992 Turkmenisian, Kopet-Dagh, 800-1500 m, valley of the river Ipey-kala and Polnt-kala, 38°13'15" N, 59°54'57" E, leg. Fábián, Herczig, Podiussány & Varga (HNHM).

Distribution: This species was reported from Afghanistan and Iran (Schülke & Smetana, 2015). It is recorded from Turkmenistan for the first time.

Astenus procerus (Gravenhorst, 1806)

Material examined: Cyprus: 1 ex., XII.1965, Cypre, Yermasoyia (MHNG). Greece: 1 ex., 14.III.1976, Krete, umg. Anogia (MHNG); 1 ex., 30.III.1973, Krete, Ide-Geb. b. Anogia, leg. Fülscher & Meybohm (MHNG). Iraq: 3 exs, 19.V.2008, ca 6 km NW Suleimaniyah (AZMM). Kazakhstan: 3 exs, 05.IV.2010, Yuzhno-Kazakhistan Region, Boralday Range, Satur Mts, hole of the Kulan nv., high Krasnye Vorota pass, 1000 m, 42°35'13" N, 70°26'53" E, leg. Matalin (AZMM). Serbia: 2 exs, 15.IV.1934, Arilje, Vrané (NMPC). Turkey: 1 ex., 08.V.1978, Antalya, Bakaran-Cevizli, 1400 m, leg. Besuchet & Löbl (MHNG); 2 exs, 02.IV.2010, Manisa, Spil Mts, 1200 m, 38°33'20" N, 27°23'17" E, leg. Anlaş (AZMM); 1 ex., 27.IX.2010, Eskişehir, Alpu, Mihalıççık 25 km N, leg. Kunt (AZMM); 1 ex., 11.VII.2014, Konya, Karapınar, Meke Tuzlası, 37°41'13" N, 33°38'03" E, 995 m, leg. Anlaş (AZMM); 2 exs, 11.V.2015, Kütahya, Emet, Eğrigöz Mts., 39°22'57" N, 29°06'45" E, 1900 m, leg. Yağmur & Örgel (AZMM). 1 ex., 16.V.2013, Simav, Kabaarmut, 39°10'24" N, 29°01'02" E, 1224 m, leg. Yağmur & Örgel (AZMM); 1 ex., 27.VI.2016, Çankırı, Ilgaz Mts., Serçeler Köyü, 40°58'32" N, 33°34'44" E, 1320 m, leg. Örgel & Yaman (AZMM); 1 ex., 25.V.2016, Karaman, Sarıveliler, Göktepe, 36°37'11" N, 32°35'54" E, 1702 m, leg. Örgel & Yaman (AZMM); 1 ex., 28.V.2016, Ayrancı, Yüğlük Hill, 37°00'57" N, 33°46'48" E, 1942 m, leg. Anlaş, Örgel & Yaman (AZMM); 1 ex., 26.V.2016, Ermenek, Oyukdağ, 36°51'24" N, 32°48'45" E, 2020 m, leg. Örgel & Yaman (AZMM); 1 ex., 24.V.2013, Kayseri, Lifos Mts., 38°36'29" N, 35°29'35" E, 1690 m, leg. Erdek (AZMM); 1 ex., 08.VI.2016, Sarız, Binboğa Mts., 38°19'30" N, 36°32'05" E, 2654 m, leg. Yağmur, Örgel & Yaman (AZMM); 2 exs, 09.VI.2016, Pınarbaşı, Eskiyassıpınar, Gövdeli Mts., 38°44'03" N, 36°38'21" E, 1921 m, leg. Yağmur, Örgel & Yaman (AZMM); 3 exs, 14.IV.2016, Konya, Beyşehir, Dumanlı, 37°27' N, 31°19' E, 1800 m, leg. Örgel & Yaman (AZMM); 4 exs, 19.IV.2016, Beyşehir, Erenler Mts., 37°45'54" N, 31°58'53" E, 2200 m, leg. Örgel & Yaman (AZMM); 4 exs, 30.V.2016, Meke, leg. Anlaş, Örgel & Yaman (AZMM); 2 exs, 01.VI.2016, Niğde, Madenköy, 37°24'19" N, 34°33'31" E, 2641 m, leg. Örgel & Yaman (AZMM); 4 exs, 03.VI.2016, Niğde, Çamardı, Aladağlar, Demirkazık Mts., 2277 m, leg. Örgel & Yaman (AZMM); 2 exs, 15.IV.2013, Afyonkarahisar, İhsaniye, Döğer 2 km E 39°09'13" N, 30°25'52" E, 1286 m, leg. Anlaş, Yağmur & Örgel (AZMM); 2 exs, 11.IV.2015, Denizli, Kale 5 km E, 37°25'37" N, 28°53'30" E, 1335 m, leg. Anlaş, Yağmur & Örgel (AZMM). Locality not found: 1 ex., 01.VII.1928, Karpaty, Kuzy (NMPC).

Distribution: *Astenus procerus* widespread in Europe, North Africa and Cyprus, Iran, Lebanon, Tajikistan, Turkey, Uzbekistan and West Siberia (Schülke & Smetana, 2015). This species is recorded for the first time from Iraq and Kazakhstan.

Astenus pulchellus (Heer, 1839)

Material examined: Azerbaijan: 1 ex., 2-6. VI. 2006, Astara, Motlayatag vill., leg. Snegovaya (AZMM). Turkey: 1 ex., 25.V.2016, Karaman, Sarıveliler, Göktepe, 36°37'16" N, 32°34'31" E, 1717m, leg. Örgel & Yaman.

Distribution: According to Schülke & Smetana (2015), *A. pulchellus* is known from Europe, Kyrgyzstan and Uzbekistan. Thus, this species is reported for the first time from Azerbaijan and Turkey.

Astenus rufopacus Reitter, 1909 (Figures 1A-H)

Astenus rufopacus Reitter, 1909: 150

Type examined: Azerbaijan: Lectotype (here designated) 1 ♂: Lenkoran (=Lankaran), coll. Reitter (HNHM); with red printed label reading “Lectotype”, *Astenus rufopacus* Reitter, 1909; des. S. Anlaş 2017. Paralectotypes: 1 ♂, same data as lectotype (HNHM), with red printed label reading “Paralectotype” *Astenus rufopacus* Reitter, 1909; des. S. Anlaş 2017. Azerbaijan or Armenia 2 ♀♀ Caucasus, "Araxesthal" (=Aras Valley), Leder, Reitter (HNHM); with red printed label reading “Paralectotype” *Astenus rufopacus* Reitter, 1909; des. S. Anlaş 2017.

Additional material examined: Azerbaijan or Armenia: 1 ♀, Kaukas (=Caucasus), Leder, coll. Reitter (HNHM). Armenia: 1 ♂, 1 ♀, 15.VII.1977, Armenia, Djruezh, leg. Zombori (HNHM). Syria: 1 ♂, Syria, coll. Reitter (HNHM).

Redescription: Measurements (in mm) and ratios (range, n = 8): AL 1.18-1.23; HL 0.67-0.74; HW 0.61-0.66; PW 0.56-0.60; PL 0.62-0.69; EL 0.70-0.74; EW 0.66-0.70; AW 0.57-0.63; ML 0.67-0.70 (n = 4); TL 5.0-5.5 HL/HW 1.10-1.12; PW/HW 0.91-0.92; PW/PL 0.87-0.90; EL/PL 1.07-1.13; EW/PW 1.17-1.18; EL/EW 1.06; AW/EW 0.86-0.90.

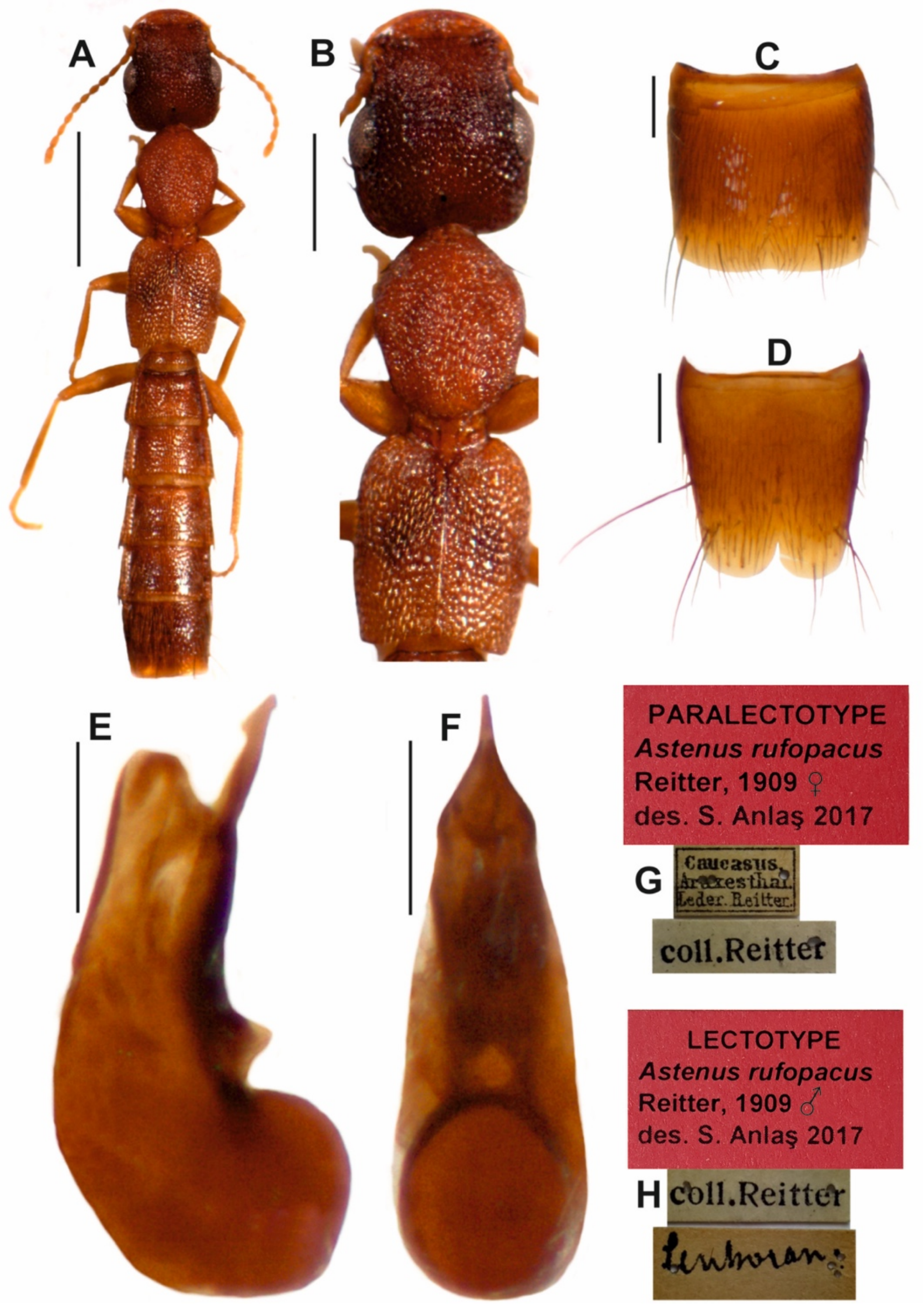

Figure 1. Detail of *Astenus rufopacus* Reitter, 1909: A) Habitus; B) forebody; C) male sternite VII; D) male sternite VIII; E) aedeagus, lateral view; F) aedeagus, ventral view; G) Paralectotype, labels; and H) Lectotype labels. Scale bars, A) 1.0 mm, B) 0.5 mm and C-F) 0.2 mm.

Habitus as in Figure 1A. Coloration: head reddish brown, pronotum reddish, elytra reddish yellow, each with one slightly black spot, abdomen reddish yellow, antennae rufous, legs pale yellow.

Head oblong (see ratio HL/HW and Figures 1A-B); closely punctured with intervals reduced to narrow ridges; eyes convex, large and prominent; pubescence short and reddish brown. Antennae long and slender, antennomere III about three times as long as wide; all antennomeres oblong, about twice as long as wide (Figure 1A). Pronotum convex and oval; oblong and narrower than head (see ratios PW/PL and PW/HW, and Figures 1A-B); distinctly narrowed anteriorly and posteriorly; two long setae on each side of pronotum and smaller setae present on front margin near neck; punctation similar to that of head, but denser, slightly granulose, surface somewhat glossier than that of head; closely punctured with intervals reduced to narrow ridges; pubescence yellowish, of similar length as that of head, but coarser and more conspicuous. Elytra oblong and wider than pronotum (see ratios EL/EW and EW/PW, and Figures 1A-B); microsculpture absent; punctation moderately dense and granulose, interstices narrower than diameter of punctures; pubescence yellowish, more distinct than that of head and pronotum. Hind wings present. Abdomen elongate and narrower than elytra at base (see ratio AW/EW and Figure 1A), distinctly widening to posterior; punctation very dense and very fine; interstices with distinct microsculpture; pubescence fine and yellowish.

Male: sternite VII in posterior median area slightly depressed and with some modified dark stout setae, posterior margin weakly concave (Figure 1C). Sternite VIII deeply and acutely incised at posterior margin, pubescence unmodified (Figure 1D). Ventral process of aedeagus expanded at apex and projected part of the apical area in lateral view (Figure 1E). Median lobe of aedeagus strongly narrowed to apical region, apex pointed in ventral view (Figure 1F).

Comparative notes: The species is distinguished from all its consubgeners by the male sexual characters, especially by the morphology of the aedeagus, in both ventral and lateral view. *A. rufopacus* distinguished from its consubgeners particularly by the presence strongly acute pointed apex of ventral process of aedeagus in ventral view and by the presence of the projected part of the apical area in lateral view.

Distribution: Reitter (1909) described *A. rufopacus* from Caucasus Region of Azerbaijan. Recently, Anlaş & Rose (2009) recorded this species from Turkey. Thus, it is here recorded from Armenia and Syria for the first time.

Comments: Although the types have labels “Holotypus” and “Paratypus”, the author did not select any holotype while describing the new species. The curators in HNHM most probably labeled the types as holotypus and paratypes. The types of *A. rufopacus* actually were syntypes. Reitter (1909) mentioned specimens from two localities, “Lenkoron” (i.e., Lankaran) and “Araxesthal” (i.e., Aras Valley), without specifying the numbers of specimens. The specimens from Lankaran are the only males, and one of them, a dissected male, is designated here as a lectotype, in order to fix the identity of the species for future studies. Also, Coiffait (1984) illustrated the aedeagus of *A. rufopacus,* but this illustration is imprecise. For these reasons, the species is redescribed above and illustrated in Figures 1A-F.

***Astenus thaboris* (Saulcy, 1865)**

Material examined: Syria: 2 exs, Syria, coll. Reitter (HNHM).

Distribution: This species is known from Cyprus, Middle East, Central Asia and Turkey (Schülke & Smetana, 2015).

***Astenus thoracicus* (Baudi di Selve, 1857)**

Material examined: Bulgaria: 2 exs, 09.VIII.2010, Belasitsa Mts., Kongura hut, leg. Bekchiev (AZMM, NMNHS). Cyprus: 1 ex., XII.1965, Cyprus, Yermasoyia (MHNG). Israel: 1 ex., 14.V.1973, Galilée, audessous Safad, 500 m, leg. Löbl (MHNG); 1 ex., 27.V.1973, Galilee, Mt. Meron, 900 m, leg. Löbl (MHNG). Turkey: 1 ex., 13.V.1978, Bursa, Uludağ, 800 m, leg. Besuchet & Löbl (MHNG); 1 ex., 20.V.1976, Sinop, Lala, leg. Besuchet & Löbl (MHNG); 1 ex., 19.V.1976, Kastamonu, Ilgazdağ, 15 km N of Tosya, 1600-1700 m, leg. Besuchet & Löbl (MHNG); 1 ex., 18.V.1976, Kastamonu, Küre 5 km S, 1200 m, leg. Besuchet & Löbl (MHNG); 1 ex., 06.VI.1986, Erczincan (=Erzincan), Tercan, Euphrate, 1400 m, leg. Besuchet, Löbl & Burckhardt (MHNG); 1 ex., 16-20.IV.2004, Antalya, Göynük, 40 km SW from Antalya, leg. Hula (NMPC); 4 exs, 29.V.2016, Karaman, Ayrancı, Berendi 5 km E, 37°16' N, 34°04' E, 1980 m, leg. Anlaş, Örgel & Yaman (AZMM); 1 ex., 05.VI.2016, Kayseri, Yahyalı, 38°03'01" N, 35°24'01" E, 1427 m, leg. Yağmur, Örgel & Yaman (AZMM); 2 exs, 14.IV.2016, Konya, Beyşehir, Dumanlı, 37°27'51" N, 31°19'44" E, 1828 m, leg. Örgel & Yaman (AZMM); 2 exs, 19.IV.2016, Beyşehir, Erenler Mts., 37°45'54" N, 31°58'53" E, 2200 m, leg. Örgel & Yaman (AZMM); 1 ex., 03.VI.2016, Niğde, Çamardı, Pınarbaşı, 37°54'39" N, 35°07'09" E, 1652 m, leg. Örgel & Yaman (AZMM); 1 ex., 16.IX.2011, Afyonkarahisar, Sandıklı Mts., 38°27'45" N, 30°21'23" E, 1548 m, leg. Anlaş (AZMM); 3 exs, 02.V.2015, Ahır Mts., Büyükkavşak Hill, 38°43'08" N, 30°03'48" E, 1810 m, leg. Yağmur & Örgel (AZMM); 2 exs, 24.III.2014, Aydın, İmambaba Hill, 37°56'41" N, 27°53'46" E, 1430 m, leg. Anlaş & Örgel; (AZMM); 2 exs, 25.IV.2014, Denizli, Beyağaç, Yelken Mts., 37°16'22" N, 28°56'03" E, 1211 m, leg. Yağmur & Örgel (AZMM); 2 exs, 27.VI.2014, Eskişehir, Musaözü Dam, 39°41'41" N, 30°19'21" E, 950 m, leg. Yağmur & Örgel (AZMM); 3 exs, 06.IV.2014, Kütahya, Simav, Kabaarmut, 39°10'12" N, 29°00'34" E, 1271 m, leg. Yağmur & Örgel (AZMM); 3 ex., 14.IV.2015, Türkmen Mts., 39°23'31" N, 30°16'33" E, 1686 m, leg. leg. Anlaş, Yağmur & Örgel (AZMM); 3 exs, 04.IV.2013, Muğla, Datça, Emecik 2 km SW, 36°46'01" N, 27°48'39" E, 107 m, leg. Yağmur & Örgel (AZMM).

Distribution: This species is known from Azerbaijan, Georgia, Greece, Italy, Canary Islands, Cyprus, Iran, Syria, Turkey and Uzbekistan (Schülke & Smetana, 2015). The records from Israel and Bulgaria are reported for the first time.

***Astenus tristis* (Erichson, 1840)**

Material examined: France: 1 ex., Corsica, coll. Lokay (NMPC).

Distribution: This species was known only from Sardegna and Sicilia Islands in Italy (Schülke & Smetana, 2015). Thus, it is recorded here for the first time from Corsica Island, France.

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