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New and Additional Records for the Ant Fauna (Hymenoptera, Formicidae) of Morocco

Ahmed TAHERI[1*] Joaquín L. REYES-LÓPEZ[2]

[1*]Laboratory of Plant Biotechnology, Ecology and Ecosystem Valorization / URL-CNRST n°10. Faculty of Sciences of El Jadida, Chouaïb Doukkali University, P.O. Box 20, El Jadida 24000, MOROCCO
[2]Area of Ecology, University of Cordoba, Building C-4 “Celestino Mutis,” Rabanales Campus, 14071-Cordoba, SPAIN
e-mails : [1*]taheri.ahmed@ucd.ac.ma, [2]cc0reloj@uco.es
ORCID IDs: [1]0000-0001-7402-525X, [2]0000-0002-0702-4588
*Corresponding Author

ABSTRACT

Morocco hosts a high diversity of animal and plant species, including a diverse ant fauna. Here, we report collection records of four ant species not previously known from Morocco: *Goniomma kugleri* Espadaler, 1986, *Strumigenys rogeri* Emery, 1890, *Temnothorax longipilosus* (Santschi, 1912) and *Proceratium numidicum* Santschi, 1912. *G. kugleri* is mentioned for the first time for continental Africa. This raises the number of ant species known from Morocco to 242. In addition, we report new data on six ant species in Morocco for which there is little information: *Technomyrmex vexatus* (Santschi, 1919), *Temnothorax convexus* (Forel, 1894), *Messor brevispinosus* Santschi, 1923, *Strumigenys baudueri* (Emery, 1875), *Strumigenys membranifera* Emery, 1869 and *Monomorium andrei* Saunders, 1890. This number of ant species known from Morocco greatly exceeds from neighboring countries in North Africa. In addition, vast regions of Morocco have never been surveyed and probably host many undiscovered species.

Keywords: Ants, Biodiversity, *Goniomma kugleri,* Morocco, *Proceratium numidicum, Strumigenys rogeri, Temnothorax longipilosus*.

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INTRODUCTION

Morocco possesses a diverse geography, from the highest mountains in Africa north of the Sahara to long coastlines on both the Atlantic Ocean and the Mediterranean Sea. Morocco's wide variety of climates have given rise to diverse ecosystems that are home to a high diversity of animal and plant species (ONEM, 2001).

Arthropods are the most diverse animal phylum in Morocco, with 17893 known species, constituting 73% of listed land animal species (SNCUDB, 2004). These include 13461 insect species, nearly 75% of the known arthropods (SNCUDB, 2004). The most recent published list of the ants of Morocco (Cagniant, 2006) includes 214 species, and subsequent studies have increased this number to 238 (Taheri & Reyes-López, 2015; 2018; Ajerrar, Gomez, Bouharroud, Zaafrani, & Cagniant, 2018; Seifert, 2020). Here, we report collection records of four ant species not previously known from Morocco, and six ant species for which there is interesting additional information about their natural history and distribution.

MATERIAL AND METHODS

Between 2014 and 2021, we sampled ants in three regions of Morocco : the northern Tangiers-Tetouan region (Talassemtane National Park, Bouhachem Natural Park, Atlantic coast of Tangier, Dardara, Jebha, Ksar El Majaz and Ksar El Kebir), central Morocco (Casablanca, Rabat and Maamora), and Marrakech city. The specimens were collected using an aspirator, through sifting leaf litter, and by soil washing.

Specimens were studied under a Leica S4D stereomicroscope and identified using available keys (Cagniant & Espadaler, 1997; Bolton, 2000; Barech, Khaldi, Espadaler, & Cagniant, 2017; 2020, Galkowski & Cagniant, 2017, Sharaf, Al Dhafer, & Aldawood, 2018), following the taxonomic nomenclature of Bolton (2023). The examined specimens were deposited in the insect collections of Chouaïb Doukkali University (AT collection, Morocco) and Cordoba University (JRL collection, Spain).

We presented the results as follows: AT-XXXX: # ☿, where AT-XXXX refers to the collection code of Ahmed TAHERI, and where # ☿, refers to # workers. This information is followed by the date, locality, GPS coordinates, altitude in meters above sea level and habitat.

RESULTS

We collected ten notable ant species in Morocco. Four are new records for the country (Figs. 1-5): *Goniomma kugleri* Espadaler, 1986, *Strumigenys rogeri* Emery, 1890, *Temnothorax longipilosus* (Santschi, 1912) and *Proceratium numidicum* Santschi, 1912, and six were rarely collected species with new data on the distribution and natural history: *Technomyrmex vexatus* (Santschi, 1919), *Temnothorax convexus* (Forel, 1894), *Messor brevispinosus* Santschi, 1923, *Strumigenys baudueri* (Emery, 1875), *Strumigenys membranifera* Emery, 1869, and *Monomorium andrei* Saunders, 1890.

New records

Goniomma kugleri Espadaler, 1986

Material examined: AT-2341: 32 ☿, 01/11/2019, Bouhachem Natural Park, Tétouan. 35.2603, -5.4333, 1081 m., open area surrounding a peat bog, dominated by the *Cistus* spp. border of a *Q. suber* forest. AT-1031: 6 ☿, 15/01/2014, Atlantic coast of Tangier. 35.6988, -5.9096, 93 m, very degraded forest of *Quercus suber* L., 1753. AT-1034: 4 ☿, 15/01/2014, Atlantic coast of Tangier. 35.6988, -5.9096, 93 m, very degraded forest of *Q. suber*.

This Iberian endemic ant species was previously known solely from a few southern Spanish and Portuguese localities (Espadaler, 1985; Boieiro, Espadaler, Azedo & Serrano, 2002); its presence in the African continent was unknown. Based on our capture, the species' range is extended to the southern Mediterranean shore (Figs. 1, 5a).

Figure 1. Distribution map of *Goniomma kugleri* Espadaler, 1986. Green area = countries where the species is cited as native, blue circle = new records.

Strumigenys rogeri Emery, 1890

Material examined: AT-2700: 1♀, 30/06/2021, Forest of Maamora, Khémisset. 34.0193, -6.5815, 175 m, cork oak forest.

S. rogeri is native to tropical Africa, but has spread through with human commerce to many parts of the globe (Wetterer, 2012). It occurs in Africa, Americas, Asia, Europe, and Oceania (Wetterer, 2012). There are no reports from the North African countries nor in the Mediterranean Basin (Borowiec, 2014; AntWeb.org) (Figs. 2, 5b). It constitutes the sixteenth known invasive ant species in Morocco, and the seventeenth in all the Maghrebian countries (Taheri & Reyes-López, 2018, Oussalah, Marniche, Espadaler, & Biche, 2019). The species was captured in a natural habitat, 100 m from the road, in the largest cork oak forest in the world.

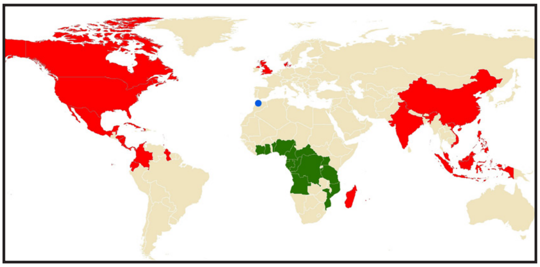

Figure 2. Distribution map of *Strumigenys rogeri* Emeryi, 1890. Green area = countries where the species is cited as native, red area = countries where the species is cited as non-native, blue circle = new record.

Proceratium numidicum Santschi, 1912

Material examined: AT-2701: 1 ☿, 04/04/2021, Bouhachem Natural Park, Beni Layeth, Tétouan. 35.258470, -5.418806, 992 m, cork oak forest with a dense undergrowth of *Arbutus unedo* L., 1753, *Erica arborea* L., 1753, *Pistacia lentiscus* L., 1753, *Cistus crispus* L., 1753 and *Cistus monspeliensis* L., 1753.

Very little is known about the biology of this species. Its known distribution is very scattered across several countries of the southern and eastern Mediterranean (Antmaps.org) (Figs. 3, 5c). But given the general rarity of all species in this genus, much remains to be discovered.

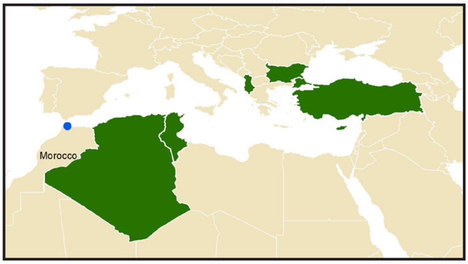

Figure 3. Distribution map of *Proceratium numidicum* Santschi, 1912. Green area = countries where the species is cited as native, blue circle = new record.

Temnothorax longipilosus (Santschi, 1912)

Material examined: AT-0974: 4 ☿, 13/06/2013, Bouhachem Natural Park, Dardara, Chefchaouen. 35.1059, -5.2995, 484 m, *Q. suber* forest with thick, tall under storey of *Erica arborea* L., 1753, *Cistus crispus* L., 1753, *Cistus monspeliensis* L., 1753 and *Arbutus unedo* L., 1753, adjacent to an open area with low scrub of *C. crispus* and some grassy clearings. AT-0976: 1 ☿ and AT-0977: 3 ☿ (same data as AT-0974).

Santschi (1912) described this species from two workers from Le Kef, Tunisia. Our record from Morocco is the only additional record of this species (Figs. 4, 5d). This rediscovery was presented in a poster at the 11th Iberian Congress of Myrmecology (Reyes-López & Taheri, 2016), but without including any data about the material collected nor the exact coordinates.

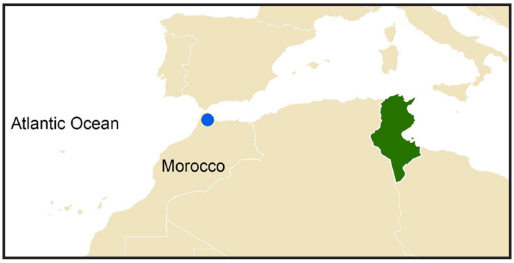

Figure 4. Distribution map of *Temnothorax longipilosus* (Santschi, 1912). Green area = countries where the species is cited as native, blue circle = new record.

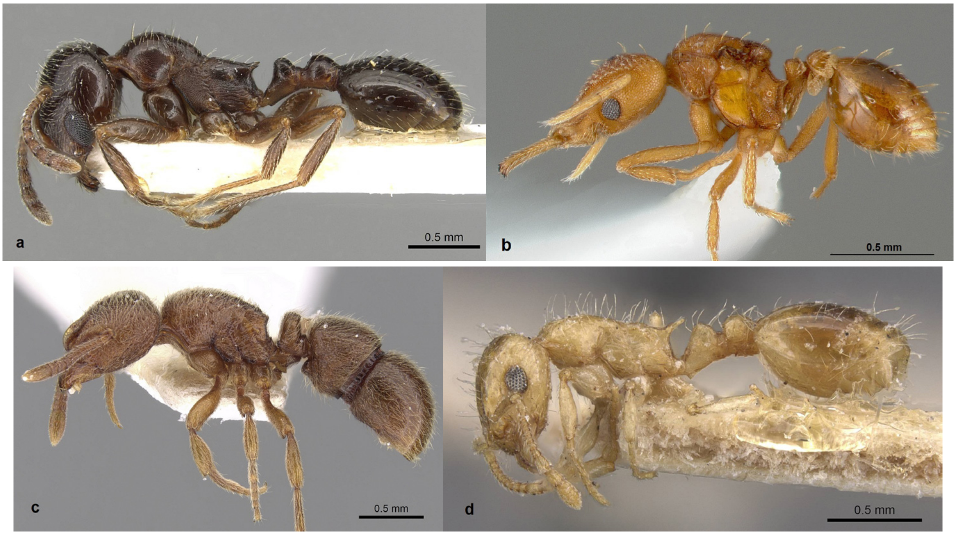

Figure 5. Profile view of a) *Goniomma kugleri*, photo by Zach Lieberman, AntWeb.org (CASENT0915447); b) *Strumigenys rogeri*, photo by April Nobile, AntWeb.org (CASENT0006035); c) *Proceratium numidicum*, photo by Estella Ortega, AntWeb.org (CASENT0281855) and d) *Temnothorax longipilosus*, photo by Will Ericson, AntWeb.org (CASENT0912959).

New Data on Distribution and Natural History

Technomyrmex vexatus **(Santschi, 1919)**

Material examined: AT-0709, 5 ☿, 31/08/2012, Zaouiyat sidi Kassem, Tamrabat, Tetouan, 35.5322, -5.1916, 88 m, reforested pine forest. AT-2293, 20 ☿, 23/10/2019, Jebha, Chefchaouen, 35.1656, -4.6315, 757 m, reforested pine forest on a scrub of *Q. ilex* and *P. lentiscus*.

Technomyrmex vexatus was originally described from Tangier in north Morocco, based on a male (Santschi, 1919). It was subsequently reported from Ceuta by Cagniant & Espadaler (1993) as *Technomyrmex* sp., but later confirmed as *T. vexatus* (Guillem & Bensusan, 2008). The species was omitted in the latest list of Moroccan ants (Cagniant, 2006), which had only mentioned *Technomyrmex* sp 1 (not determined with certainty) located in Tetouan (north Morocco) and Ceuta (Spain). In Europe, it was recorded twice; in Gibraltar (Guillem & Bensusan, 2008) and in Spain (Guillem & Bensusan, 2019). It is also newly recorded from Yemen and the Arabian Peninsula (Sharaf et al., 2018). It seems that the species exploits thick maquis as a habitat on both sides of the Strait of Gibraltar. In Morocco, these maquis have recently been reforested with *Pinus halepensis* Mill., 1768. Nests have been found in dead branches of *P. lentiscus*, in the ground, and on trees in Europe (Guillem & Bensusan, 2008; Guillem & Bensusan, 2019). In Morocco, all the colonies were found under stones. It should be noted that there are two other Tertiary relict ant species that have a similar ecology and natural history as *T. vexatus*: *Anochetus ghilianii* (Spinola, 1951) and *Stigmatomma emeryi* (Saunders, 1890). The status of *A. ghilianii* was clarified by Jowers, Taheri, & Reyes-López (2015) following a genetic approach; it is not a native species in Europe, but a recent introduction from North Africa, possibly via maritime traffic between ports on either side of the Strait of Gibraltar. In the absence of a genetic study, the hypothesis of an accidental introduction of *T. vexatus* from northern Morocco into Spain still remains possible.

Temnothorax convexus (Forel, 1894)

Material examined: AT-1454, 7 ☿, 27/04/2017, Ryade El Ouchak, Tetouan, 35.5673, -5.3687, 63 m, public garden.

This species was originally described as *Leptothorax convexus* from workers captured in northern Algeria in M’Sila cork oak *Quercus suber* forest. Few published records exist. In Morocco, it is only known from Tangier (= *L. submuticus* Emery, 1915 and *L. convexus* var. *timida* Santschi, 1912). In their revision of the *Temnothorax angustulus* group, to which this species belongs, Galkowski & Cagniant (2017) state that *T. convexus* has not been collected again either in Morocco or in Algeria since the description of it and of its junior synonym. Recently, the species has been reported in southern Iberia in Gibraltar, and in the Spanish province of Cadiz (Guillem & Bensusan, 2019). Our findings confirm that it also inhabits the Rif area in Morocco.

Messor brevispinosus Santschi, 1923

Material examined: AT-2343, 19 ☿, 19/07/2019, Talassemtane National Park, Chefchaouen. 35,1738, -5,1390, 1520 m, riverside, degraded area.

The workers we examined correspond to *Messor foreli brevispinosus* Santschi, 1923 described from the Atlantic coast of the Sahara (*sensu* Cagniant & Espadaler, 1997) with a shorter and straight propodeal spine. Currently, this species is only known from Atlas Saharien Oranais in Algeria and from the south of Agadir to Tan-Tan along the Atlantic coast. Our record from Talassemtane National Park (Chefchaouen) indicates that this species also exists in the Rif area in the north of the country.

Strumigenys baudueri (Emery, 1875)

Material examined: AT-1041, 28 ☿, 13/08/2014, Ain Chouka, Ksar El Majaz, Fahs-Anjra, 35.8289, -5.5481, 162 m, open scrub dominated by *P. lentiscus*. AT-0810, 1 ☿, 15/01/2013, Akerrat forest, Dardara, Chefchaouen. 35.1173, -5.2907, 490 m, dense cork oak forest.

The species is recorded in all Mediterranean countries except those in the southeast (Borowiec, 2014; Antmaps.org). In Morocco, it has been reported once in Agadir, fallen in a pool (Bolton, 2000; Cagniant, 2006), and once in the Rif, extracted from a soil sample (Taheri & Reyes-López, 2015). Its cryptic lifestyle (Braschler, 2002; Marko, 2008) makes it difficult to be detected. The capture of these kinds of subterranean ants requires specific methods (Wong & Guénard, 2017). Here, we have used the "washing soil" technique developed by Normad (1911) for the capture of endogenous beetles. Thanks to this method, several species of ants, qualified as rare (Cagniant, 2006), have been recently collected in Morocco. In particular the genera of *Hypoponera*, *Leptanilla*, *Proceratium*, *Ponera*, *Solenopsis*, *Stenamma* and *Strumigenys* (unpublished data).

Strumigenys membranifera **Emery, 1869**

Material examined: AT-1946, 2 ☿, 16/07/2018, Forest of Maamora, Salé. 34.0193, -6.7183, 124 m, cork oak forest.

This species of Afrotropical origin was distributed worldwide through commerce and human activities (Wetterer, 2011). It has successfully established in a wide range of habitats including forests, cultivated fields, pastures, and even manicured gardens and lawns (Deyrup, 1997). Its first discovery in Morocco beginning in 2011 (Taheri & Reyes-López, 2011; Taheri & Reyes-López, 2018). In all cases, the records were from urban areas. The importance of our discovery is that this species has been recorded for the first time in a natural habitat which is the largest cork oak forest in the world. It should also be noted that the forest area where the species has been found is heavily frequented by families for entertainment. Moreover, a continuous urbanization expansion is also remarkable such as: the construction of sports complexes, roads and gardens, which can probably explain the introduction of this tramp species.

Monomorium andrei **Saunders, 1890**

Material examined: AT-1514: 1 ☿, 17/04/2017, Ksar El Kebir, 35.0095, -5.9068, 11 m, AT-1527: 3 ☿, 17/04/2017, Ksar El Kebir, 34.9972, -5.9117, 16 m, public garden. AT-1311: 1 ☿, 13/04/2017, Mnar, Marrakech, 31.6183, -8.0084, 466 m, public garden. AT-1520: 8 ☿, 17/04/2017, Ksar El Kebir, 34.9964, -5,9135, 12 m, urban area.

The species is known in the western Mediterranean region: Algeria, Gibraltar, Spain (including Balearic Islands), and Lebanon (Borowiec, 2014; Tohmé & Tohmé, 2014). In Algeria, two subspecies can be distinguished: *M. a. bernardi* Ettershank, 1966 in Tassili and *M. a. fur* Forel, 1894 in Oran (Barech et al, 2017). In Morocco, the species was recently captured for the first time, in an oasis in the south of the country (Taheri, El Mahroussi, Reyes-López, Bennas & Brito, 2021). So far, it was only detected in human-disturbed areas and could be considered as an introduced species. In Marrakech, it was collected using the sifting leaf litter method and in Ksar El Kbeir by pitfall trap.

CONCLUSION

The number of ant species known from Morocco has increased from 214 species in 2006 (Cagniant, 2006) to 242 with the data presented here. This number greatly exceeds that of ants in neighboring countries, estimated at 180 species in Algeria and Tunisia combined (Cagninat, 2006). The number of exotic ant species known has been steadily increasing (Taheri and Reyes-López, 2018). In addition, vast regions of Moroccan Sahara and in the Atlas Mountains have never been surveyed and probably host many undiscovered species.

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Taxonomic Revision of Genus *Pareronia* Bingham, 1907 (Papilionoidea : Pieridae) from India with New Status for Two Taxa

Manpreet KAUR[1*] Jagbir Singh KIRTI[2] Avtar Kaur SIDHU[3]

[1,2]Department of Zoology and Environmental Sciences, Punjabi University, Patiala-147 001, INDIA
[3]High Altitude Regional Centre, Zoological Survey of India, Saproon, Solan-173 211, INDIA
[1*]preetpup@gmail.com, [2]prjagbir2005@gmail.com, [3]avtarkaur667@gmail.com
ORCID ID's: [1]0009-0007-8695-0101, [2]0000-0002-9670-5931
[3]0000-0003-0302-4158
*Corresponding author

ABSTRACT

This study concentrates on the taxonomic situation of the genus *Pareronia* (Bingham, 1907) in India and recommends revisions. There are three species of genus *Pareronia* in India and all these three species are evaluated under this study using the specimens from the collections located at the Zoological Survey of India (ZSI), Kolkata. Based on the critical examination of morphological characters and well pronounced differences in the male genitalia, the status of *Pareronia valareia hippia* (Fabricius) is raised to species level and the dry season form of *Pareronia avatar* (Moore) form tarina, Fruhstorfer (1903), is upgraded to species level, *Pareronia tarina*.

Keywords: Pareronia avatar, Pareronia ceylanica, Pareronia valareia hippia, dry season form, genitalia

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INTRODUCTION

Bingham (1907) erected the genus *Pareronia* Bingham with type species *P. valeria* Cramer from Java with range Indo-Malayan region. This genus is represented by 15 species which are confined to south-east Asia (Nishimura, 1996; Schroder & Treadaway, 2004). From India, three taxa have been recorded so far i.e., *Pareronia valareia hippia* (Fabricius), *P. ceylanica* (Felder), *P. avatar* (Moore) (Bingham, 1907; Evans, 1932; Talbot, 1939; Varshney and Smetacek, 2015). Varshney and Smetacek (2015) states that the genus is distributed allover India except the northern parts. However when the collections from National Zoological Collections (NZC) of ZSI, Kolkata are studied it can be seen that *Pareronia valareia hippia* (Fabricius) is also distributed in the northern regions also (Evans, 1932). In this study all the species of genus *Pareronia* were re-evaluated and their male genitalia studied in detail. Based on the distinct differences in male genitalia, the status of *Pareronia valareia hippia* (Fabricius) is raised to species level i.e. *Pareronia hippia* (Fabricius) and the dry season form of *Pareronia avatar* (Moore) form tarina given by Fruhstorfer (1903) is upgraded to species level, *Pareronia tarina* (Fruhstorfer).

MATERIAL AND METHODS

To study taxonomically, the species belonging to genus *Pareronia* preserved in the National Museum of Lepidoptera, Zoological Survey of India, Kolkata, West Bengal were examined and identified by consulting the fauna, keys and catalogues (Evans, 1932; Talbot, 1939; Kehimkar, 2008; Varshney and Smetacek, 2015). The adult images both from the dorsal and ventral sides were taken with a digital camera (Nikon digital camera 18-105 mm). The genitalia dissections were performed by adopting the method proposed by Robinson (1976). The terminology for the male genitalia was adopted from Klot (1970). The photography of the male external genitalia were taken by using Leica microscope equipped with a photographic unit.

Abbreviations: Sc = Subcosta vein; R1 = Radius vein 1; R2 = Radius vein 2; R3 = Radius vein 3; R4+5 = Radius vein 4+5; Rs = Radial sector; M1 = Median vein 1; M2 = Median vein 2; M3 = Median vein 3; Cu1 = Cubitus vein1; Cu2 = Cubitus vein 2; 1A+2A = Anal vein 1+2; 3A = Anal vein 3; AED = Aedeagus; TG = Tegumen; UN = Uncus; VLV = Valva; VIN = Vinculum; SA = Saccus; DU.EJ = Ejaculatory duct; HRP = Harpe; VES = Vesica.

RESULTS AND DISCUSSION

Genus *Pareronia* Bingham, 1907

Common name: Wanderers

Bingham, 1907; Fauna Brit. Ind., Butts 2:276.

Valeria, Winhard, 2000, Butterflies of the World 10:6.

Pareronia, Vane-Wright & de Jong, 2003; Zool. Verh. Leiden 343: 102.

Type species: *Papilio valeria* Cramer, 1776

Cramer, 1776; Uitl. Kapellen 1(8): 133.

Diagnosis: Forewing veins M1 and R5 originate from a point; discocellular veins between M1-M2 angled, discocelluar veins between M2-M3 straight; vein R3 present; in male genitalia uncus undivided, down-curved, valve triangular with apex narrow, aedeagus slender, vesica antriodorsad.

Distribution: Borneo, China, Hainan, India, Java, Lombok, Moluccas, Myanmar, New Guinea, Peninsular Malaya, Philippines, Singapore, Sri Lanka, Sumatra.

Key to species of the genus Genus *Pareronia* Bingham

1) Upperside with narrow black marginal borders. Male genitalia with costa convex, apex of valva finger like……………………………………………………………..…..........…2

- Upperside with broad black marginal borders. Male genitalia with costa straighter, bears pilose ridge near middle, apex of valve narrow but without finger like process…………………………………………………………………….………..........……..3

2) Apex widely black, termen marginal black boarder broader, broadly bordered the veins. Male genitalia with uncus in dorsal view Y-shaped, median arm longer than lateral arms.....………………………………………………………………..……..*P. avatar* (Moore)

- Apex narrowly black, termen marginal black border narrow, narrowly bordered the veins. Male genitalia with uncus in dorsal view Y-shaped, median arm almost as long as lateral arms………………………………………..............…*P. tarina* (Fruhstorfer)

3) Forewing black border dorsally without marginal spots. Male genitalia with costa raised to a broad ridge with sparse setae……….........…………*P. ceylanica* (Felder)

- Forewing black border dorsally with marginal spots. Male genitalia with costa straight, in middle raised to form a dome-shaped ridge hirsute with dense setae………………………………………………………............…..…*P. hippia* (Fabricius)

Pareronia avatar (Moore, 1858) (Plate 1, 2)

Common name: Pale Wanderer

Eronia avatar Moore, 1858; Cat. Lep. Ins. Mus. East India Coy 1: 161.

Pareronia avatar, Bingham, 1907; Fauna Brit. Ind. Butts. 2: 277.

Pareronia avatar avatar, Evans, 1932 ; Ident. Indian Butts: 84.

Adult (Male): Wings dorsally pale bluish in colour; forewing costa dorsally black, apex widely black; termen blackish inwardly dentate, broadly bordered the veins near margin; the black margin thin on the tornus; veins light black; ventral surface silver-blue; veins well marked, brownish black. Hindwing dorsal surface with thin black margin, light black veins; ventral side silver blue, veins conspicuous, brownish black.

Wingspan: 60-95 mm

Male Genitalia: Uncus narrow, long, with sharply down-curved tip, in dorsal view Y-shaped, mediam (median) arm longer than lateral arms, inner margins of the lateral arms more sclerotized, arc-shaped; tegumen broad at base, narrow posteriorly, in dorsal view two narrow, pointed projections widely apart, arising from the posterior part of tegumen, pressed against the lateral sides, reaches up to middle of the tegumen; vinculum almost straight, slightly curved, broad towards tegumen; saccus spatula-shaped, smaller than tegumen, appendices angulares small, slightly broad towards valva, narrow towards tegumen; juxta u-shaped; valva oblique in orientation, protrudes beyond uncus, costa arch shaped, apex of the valve narrow, finger like, moderate in length, curved upwards to form acute pointed tip, sides and apex of valve setosed,; aedeagus short, robust, slightly curved, vesica anteriodorsad, subzonal area short, slightly broader than suprazone, coecum absent, ductus ejaculatorious enters porteriodorsal.

Female Genitalia: Not studied.

Material examined: ZSI, Kolkata, National Zoological Collections: 1♂, 1860, Darjeeling; 1♂, 15.10.1880, Sikkim; 3♂♂, 22.88.1883, 5♂♂, 3♀♀ (without abdomen) , Sikkim; 5♂♂, Sikkim.

Distribution India: Arunachal Pradesh, Mizoram, Nagaland, Sikkim, North East India.

Elsewhere: Nepal, Thailand, Laos, Myanmar

Remarks: Bingham (1907) described the species *avatar* Moore under the genus *Pareronia* wherein he considered species *paravatar* as a variety of *P. avatar.* Fruhstorfer (1910) and Evans (1932) considered *paravatar* Bingham as the subspecies of *P. avatar* and Talbot (1939) also considered *paravatar* as the subspecies of *avatar* but under the genus *Valeria* Horsfield. DÁbrera (1982) and Pinratana (1983) illustrated paravatar and avatar as an independent species. Nishimura (1996) also considered P. *avatar* and *P. paravatar* as two independent species on the basis of the male genital organ. The same nomenclature *Pareronia avatar* is followed here.

***Pareronia tarina* (Fruhstorfer, 1903) st. nov.** (Plate 3)

Nepheronia avatar f. *tarina* Fruhstorfer, 1903, Berl. ent. Zs. 48 (1/2):101.

Adult (Male): Wings dorsal side pale bluish in colour; forewing dorsal surface costa narrowly black, apex black; marginal black border narrowly bordered the veins; black margin narrow on the tornus; veins light black; ventral surface silver-blue, veins brownish- green. Hindwing dorsal surface with thin black marginal border; ventral side bluish-white with yellow tinge, veins brownish yellow.

Wingspan: 60-70mm

Male Genitalia: Uncus narrow, thicker, moderate in length, downcurved, in dorsal view Y-shaped, median arm almost as long as lateral arms, proximal inner margins of the lateral arms more sclerotized; tegumen broad at base, narrow posteriorly, dorsally two narrow, widely separated pointed processes, arising from the posterior part of tegumen, reaches up to middle of the tegumen; vinculum almost straight, slightly curved, broad towards tegumen; saccus spatula-shaped, narrow, appendices angulares short, broad near valva, narrow towards tegumen; juxta u-shaped; valva oblique in orientation, protrudes beyond uncus, costa strongly convex, apex of the valve narrow, long, finger like, curved upwardly to form pointed tip, margins of valve setosed; aedeagus small, robust, curved, vesica anteriodorsad, subzonal area short, slightly broader than suprazone, coecum absent, ductus ejaculatorious enters posteriodorsad.

Female Genitalia: Not studied.

Material Examined: ZSI, Kolkata, National Zoological Collections: Sikkim; 1♂, Naga hills; 2♂♂, 1♂, 10.03.1969, Teju, (150m), Lohit (Coll. S. K. Tandon).

Distribution India: Sikkim, Nagaland, Arunachal Pradesh

Elsewhere: North Myanmar

Remarks: Fruhstorfer (1903) proposed a new name tarina for dry season form from Sikkim of *Pareronia avatar*, stating that “this dry season differs so much from avatar typical, that I want to give her a name tarina, as which suggests and almost gives the impression of a special kind”. He differentiates the form tarina from typical avatar by small size, only very narrow black border on forewing and the absence of the black marginal margin of the hindwing. Evans (1932) synonymized *tarina* under *avatar* though he considered dry and wet season forms of *avatar*. Talbot (1939) again gave the nomenclature tarina for dry season form. In this study, two males from Sikkim and Darjeeling of typical avatar and two males from Lohit, Teju (Arunachal pradeh) and Sikkim of Dry Season form of *avatar* were dissected from the collections housed in National Zoological Collections (NZC) from Kolkata. Their male genital organs are studied in detail. In *P. avatar* the uncus from dorsal view is Y-shaped with median arm longer than lateral arms whereas in case of *P. tarina* the uncus from dorsal view is Y-shaped but the median arm is almost as long as lateral arms. The apical process of valve is longer and stout in *P. tarina* whereas it is shorter and weakly sclerotised in *P. avatar*. The saccus is slightly small and broad in case of *P. avatar*. In addition to these characters, the adult males also differ in wing maculation. The black markings on the dorsal side of *P. avatar* are broader than in *P. tarina*. In latter, the ventral side is bluish-white with yellow tinge and veins brownish yellow whereas in case of *P. avatar* the ventral side is silver blue and veins are brownish black.

In view of above strong differences, the form tarina is well separated from species avatar and hence raised to species level i.e. *Pareronia tarina.*

***Pareronia ceylanica* (Felder, 1865)**

Common name: Dark Wanderer

Eronia ceylanica, Felder, 1865; Reise Fregatte Novara, Bd 2 (Abth. 2) (2): 191.

Nepheronia fraterna, Moore, (1881); Lepid. Ceylon 1 (4):139.

Nepheronia spiculifera, Moore, (1881); Lepid. Ceylon 1 (4):139.

Pareronia ceylanica ceylanica, Evans, 1932; Ident. Indian Butts: 84.

***Pareronia ceylanica ceylanica* (Felder, 1865)** (Plate 4)

Eronia ceylanica, Felder, 1865; Reise Fregatte Novara, Bd 2 (Abth. 2) (2): 191.

Adult (Male): Forewing dorsally dark blue with broad black boarders, the latter generally unspotted, costa thick black, apex and termen thick, broadly black, all veins prominently black; ventral surface bluish-silver with dark brownish veins. Hindwing dorsal surface dark blue with thick broad black marginal border; ventral surface bluish-silver with thick brownish veins.

Wingspan: 65-80 mm

Male Genitalia: Uncus Y-shaped dorsally with median arm as much long as the two lateral arms of Y, in lateral view median arm narrow, arched, blunt distal tip; tegumen broad, somewhat triangular, sclerotized, longer than uncus, somewhat rectangular from dorsal view, in dorsal view two narrow, pointed projections arising from the posterior part more inclined towards lateral sides, reaches up to middle of the tegumen; vinculum broad, straight; saccus rounded distally, smaller than tegumen; juxta u-shaped; valva triangular in shape, broad proximally, narrow distally with short, upturned blunt apical process, costa convex in the middle, bears broad ridge with sparse setae, valve pilose; aedeagus small, robust, slightly curved, subzone smaller than suprazone, ductus ejaculatorious entering dorso- caudally; apex of aedeagus with broad anterio-dorsal vesica.

Material examined: ZSI, Kolkata, National Zoological Collections: 1♂, 18.09.1880, 2♂♂, 1♀, 27.09.1880, Calicut; 2♂♂, 10.1885, 2♂♂, 09.1886, 1♂, 10.1886, Kanara; 2♂♂, 05.09.1915, Coorg; 1♂, 1♀, Travancore; 2♂♂, Malabar.

Distribution India: South India (Kerala, Maharashtra, Goa, Karnataka, Tamil Nadu).

Elsewhere: Sri Lanka.

Remarks: The species *Pareronia ceylanica* (Felder) is endemic to South India and Sri Lanka and is represented by two subspecies i.e. *P. c. ceylanica* and *P. c. naraka* (Moore). The former subspecies is distributed in South India and Sri Lanka where as *P. c. naraka* is confined to Andaman Islands. The material has been examined from NZC, Kolkata and its male genitalia is described and illustrated for the first time.

***Pareronia hippia* (Fabricius, 1787) n. stat.** (Plate 5, 6)

Common name: Common Wanderer

Papilio hippia Fabricius, 1787; Mantissa Insectorum 2: 55.

Danais anais Lesson, 1837; in Bougainville, Voyage Thetis, 2: 343.

Eronia gaea Felder, 1865; Reise Fregatte Novara, Bd 2 (Abth. 2) (2): 190.

Nepheronia valeria persides Fruhstorfer, 1903; Berl. ent. Zs. 48 (1/2): 98.

Pareronia valeria persides Fruhstorfer, 1910; in Seitz, Gross-Schmett. Erde 9: 178.

Adult (Male): Forewing dorsal side dark blue, veins well marked with black, costa thick dark black, apex and margin thick black, marginal spots present, increase in size towards apex; ventral surface bluish-silver, apex and termen grayish-silver, dark black veins. Hindwing dorsal surface dark blue with broad black marginal border; veins Sc+R1, RS, and M1 thick black; ventral surface bluish-silver, vein M1 thick black.

Adult (female): Wings dorsally black with blue streaks, cilia white, marginal spots prominent, forewing cell bears two bluish-white stripes, anterior stripe longer than posterior stripe, post-discal stripes of unequal lengths, two parallel stripes in area CuA2 separated by thin black line, streak in area 3 smaller, series of bluish white submarginal spots; ventral side dull blackish with dusky bluish markings, cell possess two bluish-white stripes, post-discal stripes of unequal lengths, two parallel stripes in area CuA2 separated by thin black line, streak in area 3 smaller, series of dusky bluish-white submarginal spots. Hindwing cell possess two bluish-white stripes, bar of post-discal stripes, series of bluish-white submarginal spots; ventral surface dull black, veins dark blackish-grey, cell possess two bluish-white stripes, post discal stripes of unequal lengths, series of dusky bluish rounded submarginal spots.

Wingspan: 65-80mm

Male genitalia: Uncus Y-shaped dorsally with median arm much longer than the two lateral arms of Y, in lateral view mediam arm is down curved, tube-like with blunt apex; tegumen broad anteriorly, gradually narrow towards posterior, longer than uncus, in dorsal view two narrow, pointed, spine-like projections arising from the posterior part, more inclined towards centre, reaches up to the middle of the tegumen; vinculum broad, sclerotized, slightly curved towards saccus; the latter flattened, broadly rounded distally; juxta u-shaped with curved arms; valva triangular in shape, broad proximally, narrow distally, with apical process, the latter very short with blunt tip, costa straight, in middle raised to form a dome-shaped ridge hirsute with dense setae, valve pilose sparsely; aedeagus rounded, broad, curved, subzone smaller than suprazone, suprazone curved upwards, coecum absent, ductus ejaculatorious enters caudally, apex of aedeagus of same width with anterio-dorsal wide vesica.

Female genitalia: Corpus bursae large, somewhat oval in shape, wall of corpus bursae minutely setose; signum v-shapes, arms narrow, strongly divergent, studded with spines, middle constriction protrudes upwardly with pointed tip, well sclerotized; appendix bursae rounded, smaller than corpus bursae, well separated from corpus bursae; ductus bursae slender, moderately long, well sclerotised at base; papilla analis sclerotized and setosed; posterior apophysis thick, well sclerotized, blunt tips, almost as long as the length of anterior apophysis; anterior apophysis slightly curved upwardly, blunt tips.

Material examined: ZSI, Kolkata, National Zoological Collections: 2♂♂, 2♀♀, 07.11.1867, Lucknow (Coll. Jerdon); 3♂♂, 3♀♀; 1♂ 07.08.1879, Calcutta, (Coll. de Niceville); 1♀, 27.07.1880, Barrackpore; 1♂, 10.1882, Poona; 1♀, 06.12.1882, Khurda; 2♂♂, Malabar; Central India; 1♀, 04.11.1884, Orissa; 07.11.1884, 1♂, 1♀, N.W Himalaya; 1♂, 23.08.1885, Bangalore; 1♂, 26.03.1886, 1♂, 1♂,1♀, 12.1896, Calcutta; 1♀, 08.1886, Bombay; 1♂, 08.04, Khandalla; Masuri, (Coll. Jerdon); 1♂, Rurki, (Coll. Jerdon); 1♀, North India; 1♀, 03.1906, Jabalpur; 1♂, 1♂ 19.09.1964, Calcutta, (H. Ghosh); 2♂♂,18.11.1965, Chandanagar, Hooghly, West Bengal, (P.K Maiti); 1♂ 20.10.1998, East Godavari, Andhra Pradesh,(Coll. Chakraborty); 1♂, 12.12.1999 Guntimadugo black forest, Andhra Pradesh (Coll. Roy); 1♀, 30.10.2000, Dehradun, Uttarakhand,(Mukhopadhaya); 1♂, 20.09.2001, 1♂, 1♀, 21.09.2001, Barivali, Mumbai, Maharashtra,(P.H.Roy); 1♀, 24.02.2006, Saharanpur, Uttar Pradesh, (B. Biswas); 1♂ 18.09.2006, Prayagpur, Sultanpur, Uttar Pradesh, (Coll. P.C. Gura); 1♂, 13.12.2010, Dhalkidanga, (B. Mitra); 1♂, 03.10.2016, Madanpur, West Champaran, Bihar, (Coll. Mondal); 1♀, 06.10.2016, Hazra Tolla, West Champaran, (T.K. Mondal); 6♂♂, 5♀♀, 19.10.2017, Biahnupur , Behraich, Uttar Pradesh; 1♀, 21.11.2017, Lion Safari, Haveli, Dadra Nagar, (Coll.G.P. Mandal); 1♂, 1♀, Ganjam; 1♂, Bombay.

Distribution India: All over India except Union territories of J&K and Ladakh, Punjab and Rajasthan.

Elsewhere : South Myanmar, Sumatra, Philippines, Peninsular Malaya, Singapore, Borneo, Nias , Lombok, Bawean, Kangean, Sumbawa, Sumba, Flores, Hainan, Jawa.

Remarks: Evans, 1932 considered hippia Fabricius as a subspecies of *Pareronia valeria* (Cramer). Talbot, 1939 also kept the subspecies status of *hippia* under *Valeria valeria*. However while discussing the species status of *Valeria valeria*, he divided it into two groups hippia and valeria on the basis of small ventral process which he called the harpe. This is his statement: “In hippia group, this process (harpe) is well rounded, broad ventrally and projects beyond edge of the valve; aedeagus is strongly curved posteriorly, whereas in valeria group this harpe is long, narrow, lying entirely within the valve; aedeagus straight or slightly curved and anteriorly broader than hippia forms”. He also stated only the hippia group is known to occur in Indian area. Varshney, 2015 also considered *hippia* as subspecies of *valeria*, which is known to occur throughout India except Jammu & Kashmir, Punjab and Rajasthan. In National Zoological Collections present at ZSI, Kolkata, the *hippia* is reported as subspecies under species *P. valeria* i.e. *Pareronia valeria hippia*.

In the present work, the male genitalia of *Pareronia hippia* has been studied and compared with the structure of male genitalia of *Pareronia valeria* as given by Klots (1931). In the male genitalia of *Pareronia hippia* valve process rounded, costa straight, whereas in figure of male genitalia given by Klots (1931), the valve process is small, within valve, costa highly arched, crescent shaped. From the strong differences in male genital characters, it is hereby inferred that the *Pareronia hippia* should be raised to species level and is distinct from *Pareronia valeria*.

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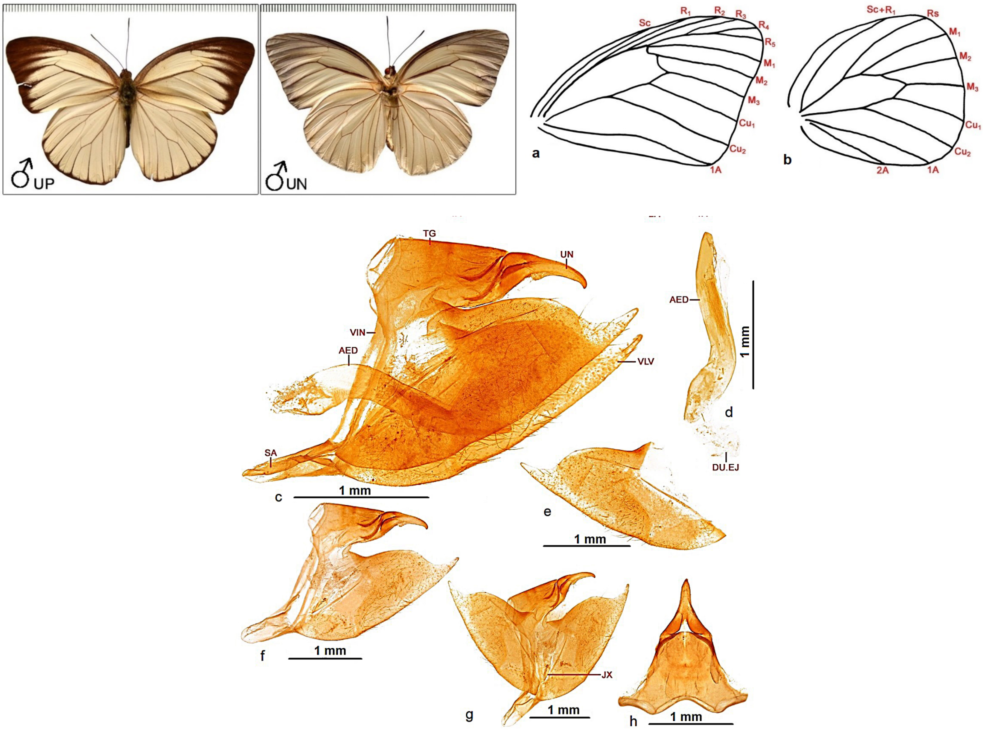

Plate 1. *Pareronia avatar* (male): a) forewing, b) hindwing, c) male genitalia, d) aedeagus, e) valva, f) male genitalia (lateral view), g) male genitalia (lateral view), h) uncus.

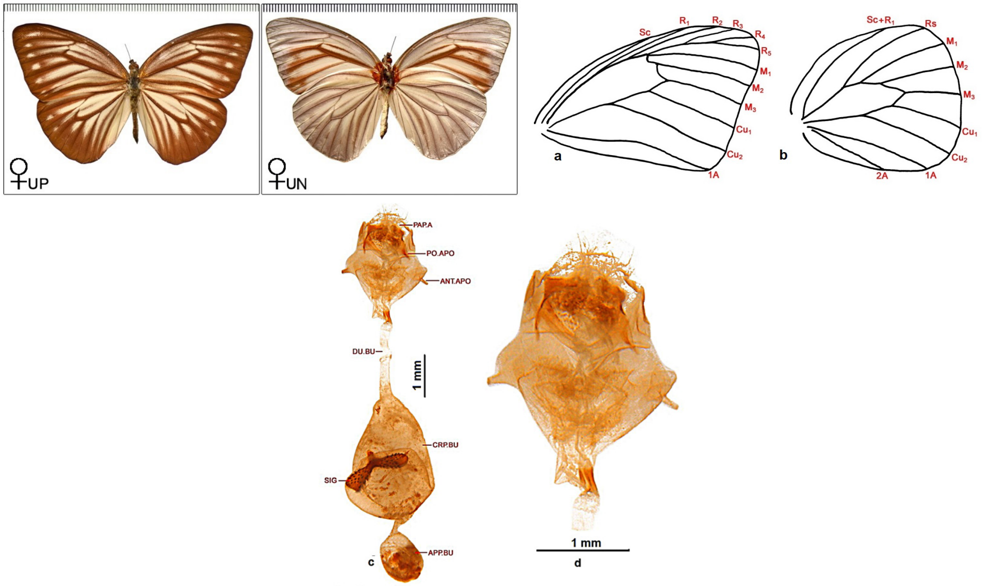

Plate 2. *Pareronia avatar (*female): a) forewing, b) hindwing, c) female genitalia, d) papilla analis.

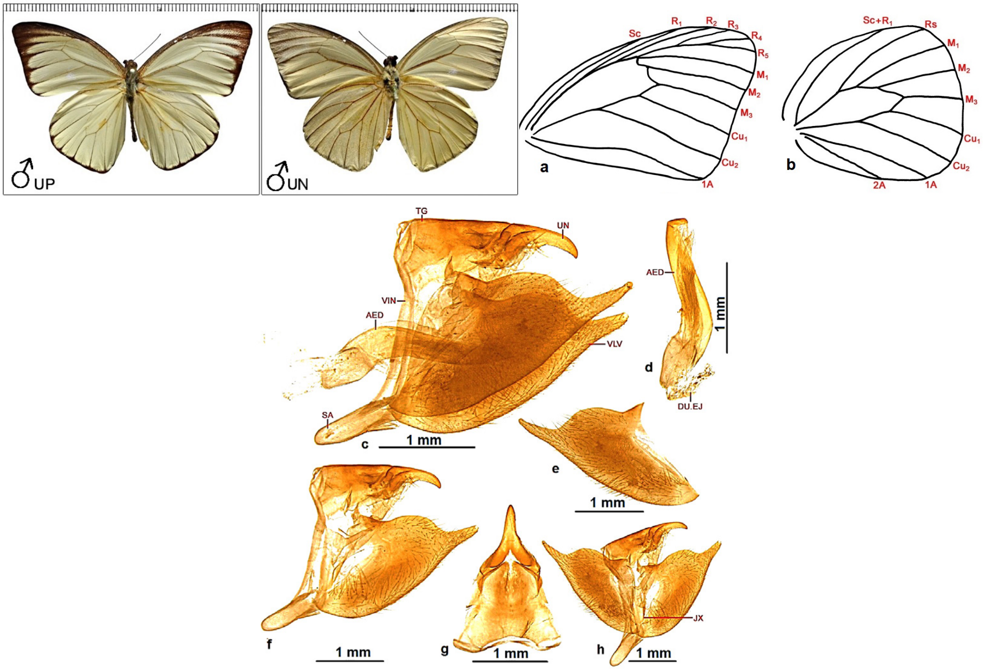

Plate 3. *Pareronia tarina* (male): a) forewing, b) hindwing, c) male genitalia, d) aedeagus, e) valva, f) male genitalia (lateral view), g) uncus, h) male genitalia (lateral view).

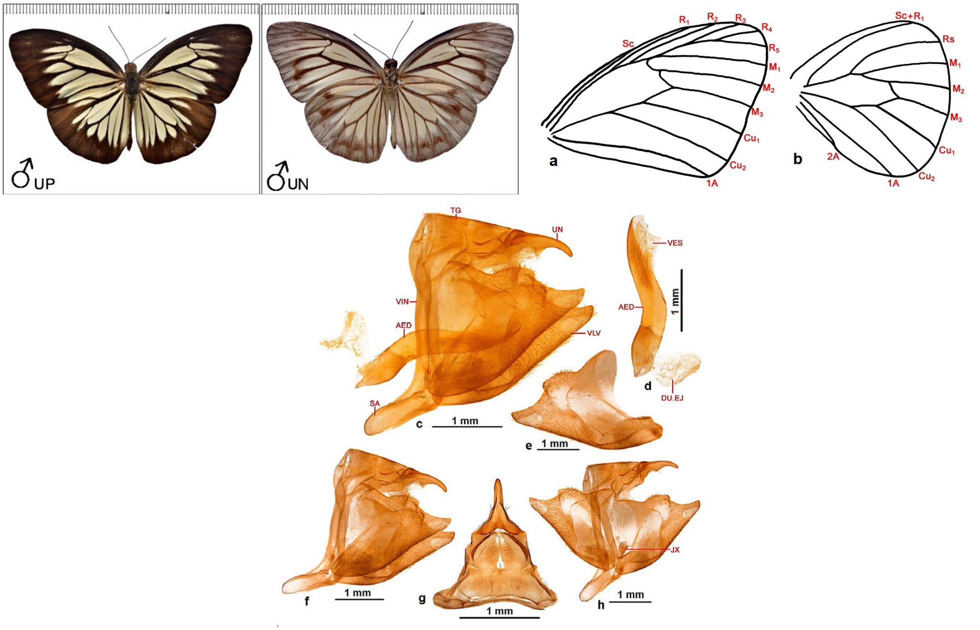

Plate 4. *Pareronia ceylanica* (male): a) forewing, b) hindwing, c) male genitalia, d) aedeagus, e) valva, f) male genitalia (lateral view), g) uncus, h) male genitalia (lateral view).

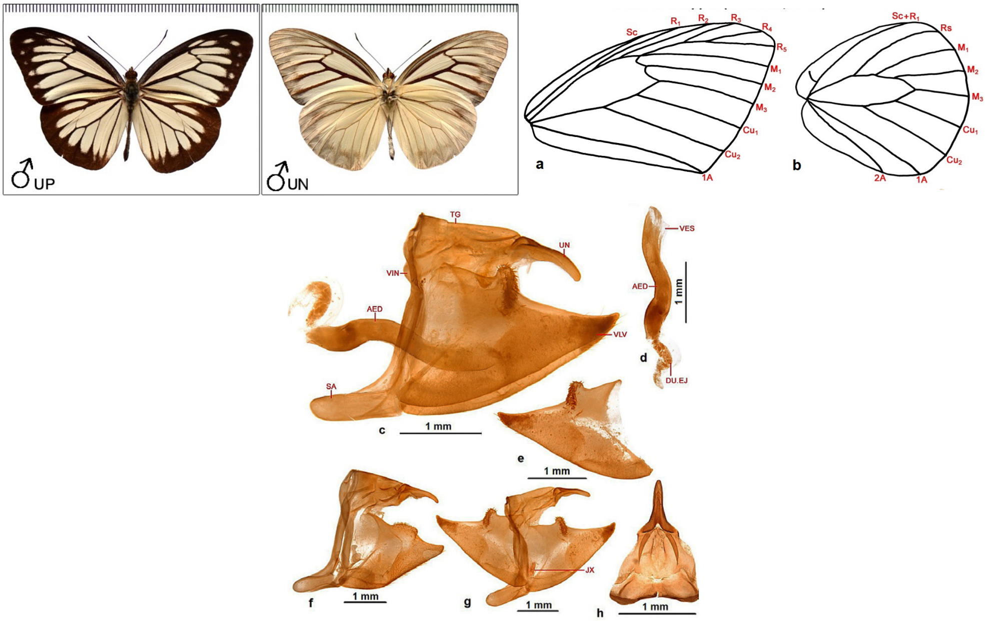

Plate 5. *Pareronia hippia* (male): a) forewing, b) hindwing, c) male genitalia, d) aedeagus, e) valva, f) male genitalia (lateral view), g) male genitalia (lateral view), h) uncus.

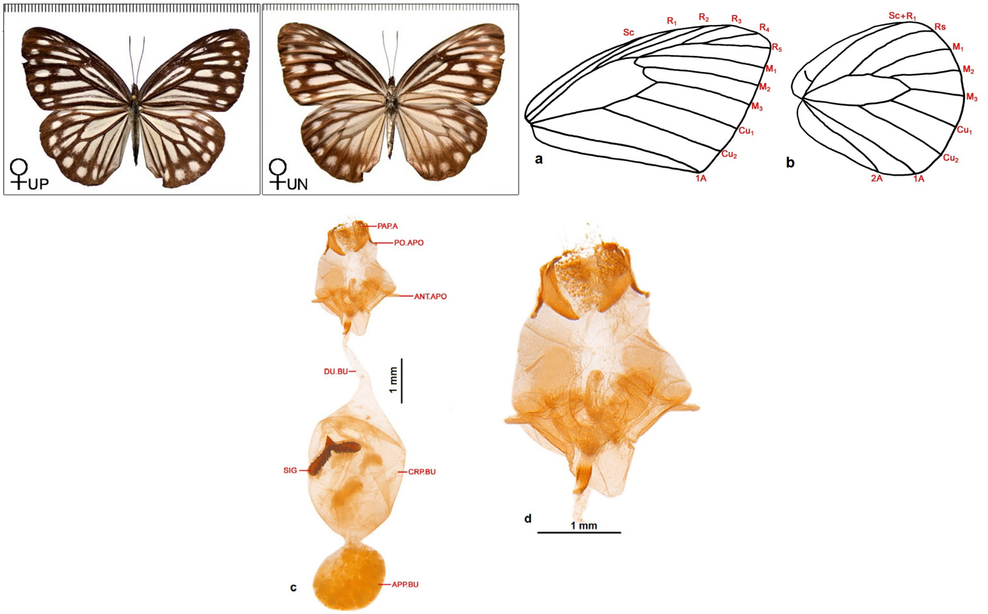

Plate 6. *Pareronia hippia* (female): a) forewing, b) hindwing, c) female genitalia, d) papilla analis.

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New Delphacidae Species (Hemiptera) for the Turkish Fauna

Murat KARAVİN[1,*] Ünal ZEYBEKOĞLU[2] Başak AKYÜREK[3]

[1,*]Amasya University, Suluova Vocational School, Department of Plant and Animal Production, 05500, Suluova, Amasya, TURKEY
[2]Ondokuz Mayıs University, Faculty of Arts and Science, Biology Department, 55200, Kurupelit, Atakum, Samsun, TURKEY
[3]Amasya University, Vocational School of Technical Sciences, 05100, Amasya, TURKEY
e-mails: [1*]murat.karavin@amasya.edu.tr, [2]unalz@omu.edu.tr, [3]basak.akyurek@amasya.edu.tr
ORCID IDs: [1*]0000-0003-0957-859X, [2]0000-0001-7595-9572, [3]0000-0001-7317-9193
*Corresponding author

ABSTRACT

Delphacids, are easily distinguished from the other families of Hemiptera by post tibial spurs, are harmful to many agricultural and cultural plants with their piercing-sucking mouthparts. Some of the species have economical importance for agricultural crops. Fifty seven species were reported from Turkey according to the literature. This study was carried out in the Central Black Sea Region of Turkey. Four delphacid species, *Kelisia guttula*, *K. confusa*, *K. sabulicola* and *Ribautodelphax imitans* were new records for the Delphacidae fauna of Turkey. Figures of genitalia, data on examined specimens and general distribution of species have been given.

Keywords: Central Black Sea Region, Delphacidae, Fauna, Hemiptera, Turkey.

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INTRODUCTION

The family Delphacidae belongs to the order Hemiptera. It is a very large family with 2227 species placed in six subfamilies and 427 genera (Asche 1985, 1990; Bourgoin, 2019). The post-tibial spur on the hind legs of the Delphacidae individuals is the most distinctive feature that distinguishes this family from other families of the Hemiptera order (Wilson & Turner, 2010). The delphacid specimens feed by sucking phloem sap from plant species almost exclusively belonging to the Cyperaceae and Poaceae families with their piercing-sucking mouthparts. Most of the species are found at the root and stem parts of the host plant, which are usually close to the ground. Therefore, they do not attract much attention and can be easily overlooked. Delphacidae species damage plants directly by feeding and laying eggs, and indirectly by carrying pathogens such as viruses, ricketsia, bacteria and mycoplasmas from diseased plants to healthy plants (Ossiannilson, 1978; O'Brein & Wilson,1985).

The first studies about the Delphacidae fauna of Turkey were carried out by Fahringer (1922) and Bodenheimer (1958). Then Dlabola (1957, 1971a, 1971b) listed 22 species and described a new genus and a new species. Linnavuori (1965) reported another two species. Asche (1982a) reported 37 species and stated that nine of them were new records. Lodos & Kalkandelen (1980, 1988) listed 52 species and nine of them were new record for Turkish fauna. Finally, Güçlü (1996) reported the new record of the two species. Fifty seven species were distributed in turkey according to the literature (Dlabola, 1957; Lodos & Kalkandelen, 1980, 1988; Asche, 1982a; Güçlü, 1996).

This study aims to contribute to the Turkish Delphacidae fauna by determining species distribution in the Central Black Sea region.

MATERIALS AND METHODS

Specimens were collected from different localities in the Central Black Sea Region in 2009-2010 by sweep net in the day-time. Specimens were prepared according to the standard methods. Species were identified by comparing with the descriptions and figures given in Perris (1857); Fieber (1866); Ribaut (1934); Linnavuori (1957); Le Quesne (1960); Ossiannilsson (1978); Asche (1985); Anufriev & Emeljanov (1988) and Holzinger, Kammerlander, & Nickel (2003). Specimen were deposited in the collection of Suluova Vocational School, Amasya University, Turkey.

RESULTS

Subfamily: Kelisiinae Wagner, 1963

Genus: *Kelisia* Fieber, 1866

Kelisia guttula (Germar, 1818) (Fig. 1,2)

Material examined: Amasya, Gümüşhacıköy, 850 m, 15.06.2010,1 ♂, 1 ♀; Tokat, Tahtoba, 1055 m, 17.10.2009, 2 ♀♀, leg. M. Karavin.

General Distribution: Albania, Algeria, Austria, Azerbaijan, Belarus, Belgium, Bulgaria, Crimea, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lebanon, Lithuania, Luxembourg, Macedonia, M. Siberia, Norway, Netherlands, Poland, Portugal, Romania, Slovenia, Spain, Switzerland, Tadzhikistan, Tunisia, Ukraine, United Kingdom (Dlabola, 1954, 1958, 1964; Holzinger & Seljak ,2001; Holzinger et al, 2003; Borodin, 2004; Maczey et al, 2005; Söderman, 2007; Aguin-Pombo et al, 2007; Niedringhaus et al, 2010; Gębicki et al, 2013; Mühlethaler et al, 2016; Della Guistina, 2019; Borodin & Borodina, 2021).

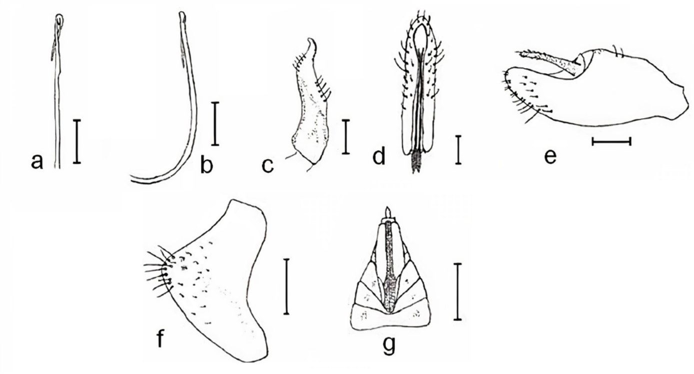

Figure 1. Genital structures, *Kelisia guttula*, a) aedeagus, from ventral, b) aedeagus, from left, c) stylus, d,e) anal tube, from below, right, f) pygopher, from right, g) tip of female abdomen, from ventral (scale a, b, f: 0.2 mm; c, d, e: 0.1 mm; g: 0.5 mm).

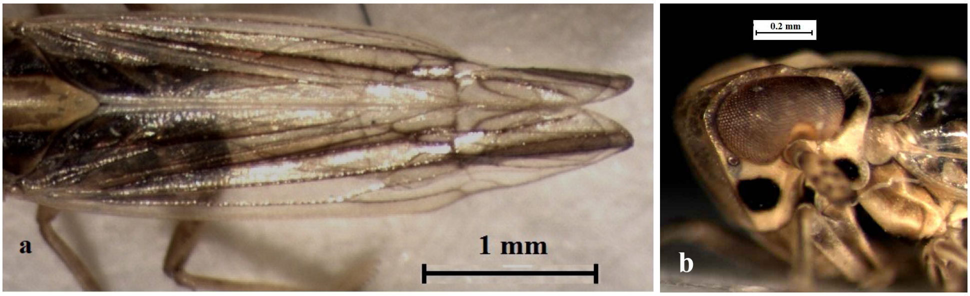

Figure 2. *Kelisia guttula,* the apex of the fore wing (a), and lateral aspect of the head and prothorax (b).

Kelisia confusa Linnavuori, 1957 (Fig. 3, 4)

Material examined: Samsun, Altınkum, 20 m, 14.07.2009, 1 ♂, 1 ♀, leg. M. Karavin.

General Distribution: Austria, Bosnia-Herzegovina, Bulgaria, Croatia, Czech Republic, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Macedonia, Poland, Serbia, Slovenia, Sweden, Switzerland (Nast, 1972; Asche, 1982b; Asche & Hoch, 1982; Holzinger et al, 2003, Holzinger& Kunz, 2006; Söderman et al, 2009; Malenovský, 2013; Mühlethaler et al, 2016; Della Guistina, 2019; Borodin & Borodina, 2021).

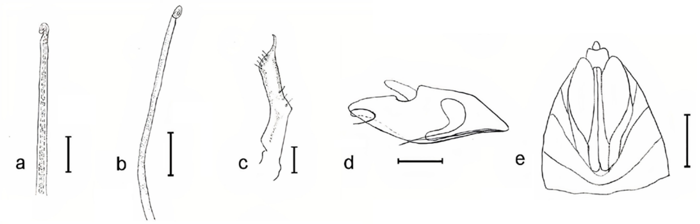

Figure 3. Genital structures, *Kelisia confusa*, a) aedeagus, from ventral, b) aedeagus, from left, c) stylus, d) anal tube, from right, e) tip of female abdomen, from ventral (scale a, b, d: 0.2 mm; c: 0.1 mm; e: 0.5 mm).

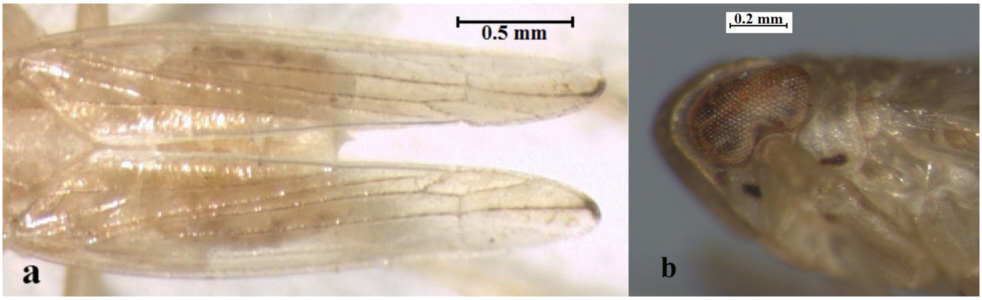

Figure 4. *Kelisia confusa*, a) the apex of the fore wing, b) lateral aspect of the head and prothorax.

Kelisia sabulicola Wagner, 1952 (Fig. 5, 6)

Material examined: Samsun, Kurupelit, OMÜ Campus, 200 m, 20.08.2009, 1 ♂, 28.09.2009, 1 ♀; Çorum, Beydili, 875 m, 10.09.2009, 6 ♂♂, 3 ♀♀; Tokat, Dökmetepe, 550 m, 12.09.2009, 1 ♂, 1 ♀; Amasya, Taşova, 250 m, 16.10.2009,1 ♂; Tokat, Niksar, Çamiçi, 1225 m, 16.10.2009, 2 ♀♀, Tokat, Turhal, 550 m, 18.10.2009, ♂; Sinop, Dikmen, Aşağıakgüney, 160 m, 03.10.2009, 1 ♂; Samsun, Hacıllı, 730 m, 11.06.2010, 1 ♂; Çorum, Ortaköy, Esentepe, 890 m, 14.06.2010, 4 ♂♂, 1 ♀; Amasya, Gümüşhacıköy, 850 m, 15.06.2010, 2 ♂♂, leg. M. Karavin.

General Distribution: Austria, Belgium, England, Estonia, Finland, France, Germany, Ireland, Italy, Jersey, Latvia, Lithuania, Netherlands, Poland, Scotland, Sweden, Wales (Nast, 1972; Baugnée, 1995; Holzinger, 1996; Guglielmino & Bückle, 2007; Söderman et al., 2009; Della Giustina & Remane, 2001; Della Guistina, 2019).

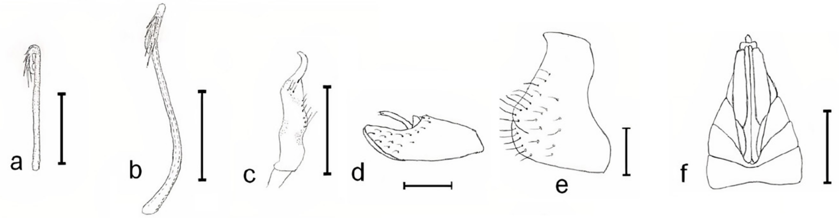

Figure 5. Genital structures, *Kelisia sabulicola*, a) aedeagus, from ventral, b) aedeagus, from left, c) stylus, d) anal tube, from right, e) pygopher, from right, f) tip of female abdomen, from ventral (scale a, b, c, d, e: 0.2 mm; f: 0.5 mm).

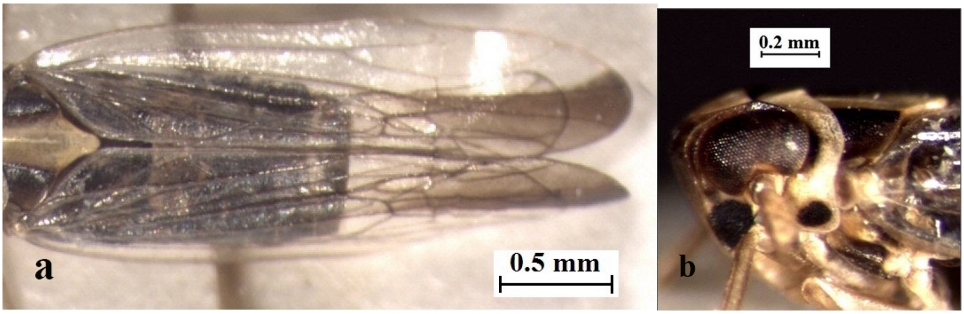

Figure 6. *Kelisia sabulicola*, a) the apex of the fore wing, b) lateral aspect of the head and prothorax.

Subfamily: Delphacinae Wagner, 1963

Genus: *Ribautodelphax* Wagner, 1963

Ribautodelphax imitans (Ribaut, 1953) (Fig. 7)

Material examined: Tokat, Turhal, Şenyurt, 570 m, 13.06.2010, 7 ♂♂, 8 ♀♀, leg. M. Karavin.

General Distribution: Austria, Belgium, Bulgaria, Croatia, Czech Republic, England, France, Germany, Greece; Italy, Kazakhstan, Luxembourg, Netherlands, Poland, Romania, Slovakia, Spain, Switzerland (Nast, 1972, 1987; den Bieman, 1987; Holzinger, 1996; Holzinger & Seljak, 2001; Nickel & Remane 2002; Guglielmino et al, 2005; Aguin-Pombo et al, 2007; den Bieman & Mol, 2010; Niedringhaus et al, 2010; Malenovský & Lauterer, 2012; Gębicki et al, 2013; Mitjaev, 2015; Orosz & Tóth, 2016; Mühlethaler et al, 2018; Della Giustina, 2019; Holzinger et al, 2020; Gjonov, 2022).

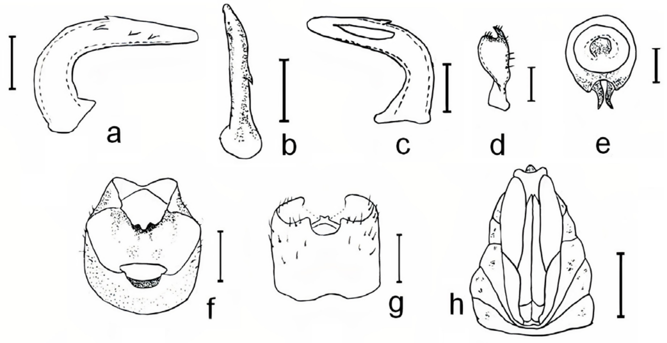

Figure 7. Genital structures, *Ribautodelphax imitans*, a, b, c) aedeagus, left, dorsal, right, d) stylus, e) anal tube f, g) pygopher, anterior, right, h) tip of female abdomen from ventral (scale a, b, c, d, e: 0.1 mm; f, g: 0.2 mm; h: 0.5 mm).

CONCLUSIONS AND DISCUSSION

In this study, it was determined that four species, *Kelisia guttula*, *K. confusa*, *K. sabulicola* and *Ribautodelphax imitans* are new records for the Delphacidae fauna of Turkey. The taxonomic characters of the species examined in this study are in harmony with the drawings and descriptions given in the literature. No significant variation was observed in the taxonomic characters of the species. Considering the general distribution of the identified species, it seems that, except *K. sabulicola*, the others were also reported in other countries close to Turkey. While *K. sabulicola* was known mostly from Northern Europe, it was recorded from several places in Turkey with this study. Male genital characters of *K. sabulicola* were similar to the definitions and drawings given by Ossiannilsson (1978) and Holzinger et al. (2003). It is hoped that these results will contribute to future studies on this subject and provide useful information for plant protection in agricultural fields.

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Research Article
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Temperature-Dependent Demographic Parameters of *Diaeretiella rapae* (M'Intosh, 1855) (Hymenoptera: Braconidae) on *Schizaphis graminum* (Rondani, 1852) (Hemiptera: Aphididae)

Ali Asghar TALEBI[1*] Mahdieh KAZEMI[2] Mehran REZAEI[3]
Mohammad Ali MIRHOSSEINI[4] Saeid MOHARRAMIPOUR[5]

[1,2,3,5] Department of Entomology, Faculty of Agriculture, Tarbiat Modares University, P.O. Box 14115-336 Tehran, IRAN
[4] Department of Plant Protection, Faculty of Agriculture,Persian Gulf University, P.O. Box 75169-13817, Bushehr, IRAN
e-mails: [1]*talebia@modares.ac.ir, [2]mah.k.110@gmail.com, [3]mehran.rezaei@modares.ac.ir, [4]m.mirhosseini@pgu.ac.ir, [5]moharami@modares.ac.ir
ORCID IDs: [1]0000-0001-5749-6391, [2]0000-0002-5490-9055, [3]0000-0002-5994-6939, [4]0000-0002-9236-9678, [5]0000-0001-8389-8861
*Corresponding author

ABSTRACT

Diaeretiella rapae (M'Intosh) (Hymenoptera: Braconidae) is one of the most important biological control agents of aphids on crucifer and cereal plants. The reproduction and demographic parameters of *D. rapae* were investigated at six constant temperatures (10, 15, 20, 25, 27.5, and 30 °C) on the greenbug, *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae) reared on wheat (var. Pishtaz). No development was observed at 30 °C. The highest value of mummified aphids per parasitoid female was obtained at temperature of 20 °C (120.08). However, the highest (0.374 day^{-1}) and lowest (0.019 day^{-1}) values of the intrinsic rate of increase (*r*) were observed at 25 and 27.5 °C, respectively. The shortest times for doubling and mean generation of parasitoid were resulted at 25 °C (1.85 and 7.73 days, respectively). Based on the results, a range of 20 to 25 °C is the most suitable temperature regime for *D. rapae* population growth on *S. graminum*. These findings have potential implications for integrated *S. graminum* management in wheat fields. However, semi-field and field studies are needed to obtain more environmentally relevant results.

Keywords: biological control, parasitoid wasp, environmental condition, life table, intrinsic rate of increase.

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INTRODUCTION

The greenbug, *Schizaphis graminum* Rondani (Hemiptera: Aphididae), is a cosmopolitan and polyphagous aphid which is widely distributed in temperate regions. It is a major pest of cereal crops, particularly wheat (Blackman & Eastop, 2006; van Emden & Harrington, 2007; Jokar, Zarabi, Shahrokhi, & Rezapanah, 2012; Zogli et al, 2020). Damage caused by this species not only weakens the plant, but also transmits viral and phytoplasma diseases ultimately ending up with plant mortality, if the aphid population is not sufficiently controlled (Tofangsazi, Kheradmand, Shahrokhi, & Talebi, 2011; Rezaei et al, 2020b; Zogli et al, 2020). To control aphid infestations, chemical pesticides are commonly used in cereal cultivation. The indiscriminate use of pesticides has led to development of aphid's resistance and decrement of the populations of natural enemies (Jokar et al, 2012; Tazerouni, Talebi, & Rakhshani, 2012; Rezaei & Moharramipour, 2019; Kazemi et al, 2020). Biological control, in particular the augmentative release of biocontrol agents, is an appropriate alternative method to control the aphid infestations (Boivin, Hance, & Brodeur, 2012; Rezaei et al, 2020a).

Among biological control agents, *Diaeretiella rapae* (M'Intosh) (Hymenoptera: Braconidae) has been reported to be an important parasitoid of aphids. It is a solitary and polyphagous endoparasitoid of aphids on crucifer and cereal plants (Silva, Cividanes, Pedroso, & Sala, 2011; Kazemi et al, 2020; Nisar & Rizvi, 2021). The origin of *D. rapae* is considered to be of Western Palearctic and it is currently spread in various parts of the world (Rakhshani et al, 2008; Singh & Singh, 2015). This biocontrol agent is known to parasitize over 98 species of aphids infesting more than 180 plant species belonging to 43 plant families distributed in 87 countries in the world (Bodlah, Naeem, & Mohsin, 2012; Singh & Singh, 2015; Farahani, Talebi, & Rakhshani, 2016). The most economically important hosts for *D. rapae* are *Brevicoryne brassicae* (L.) (Basheer, Aslan, & Asaad, 2014; Karami, Fathipour, Talebi, & Reddy, 2018), *Myzus persicae* (Sulzur) (Bodlah et al, 2012; Nisar & Rizvi, 2021), *Diuraphis noxia* (Mordvilko) (Tazerouni et al, 2012), *Lipaphis erysimi* (Kaltenbach) (Silva et al, 2011; Soni & Kumar, 2021), and *S. graminum* (Kazemi et al, 2020).

Knowledge of the adaptation of natural enemies to climatic conditions is required for predicting the survival and development time (Haghani, Fathipour, Talebi, & Baniameri, 2007; Rezaei et al, 2020b). Temperature is one of the most critical abiotic factors affecting biological traits of many arthropods including, developmental rate, survivorship, adult longevity, sex ratio, fecundity, and fertility (Hayakawa, Grafius, & Stehr, 1990; Haghani et al, 2007; Karimi-Malati, Fathipour, Talebi, & Bazoubandi, 2014; Mirhosseini, Fathipour, & Reddy, 2017; Rezaei et al, 2020a). In addition, some physiological attributes of insects, including extra molting and larval stadia, are influenced by temperature (Ismail et al, 2014; Karimi-Malati et al, 2014; Souza, Veloso, Sampaio, & Davis, 2017).

The biology of *D. rapae* has been studied on different host aphids including, *D. noxia* (Tazerouni et al, 2012), *M. persicae* (Bodlah et al, 2012; Ghorbanian, Fathipour, Talebi, & Reddy, 2019), *B. brassicae* (Karami et al, 2018), and *L. erysimi* (Silva et al, 2011; Nisar & Rizvi, 2021). However, some researchers have focused on development and fecundity of this parasitoid at a single constant temperature or a narrow range of different constant temperatures (Hayakawa et al, 1990; Bernal & Gonzalez, 1997; Tazerouni et al, 2012; Basheer et al, 2014; Souza et al, 2017). For instance, Tazerouni, Talebi, Rakhshani, & Zamani (2013) reported that the highest value of net reproductive rate (R_0) for *D. rapae* is obtained at 10 °C and the parasitoid is potentially able to control *D. noxia* at temperatures of 10 to 15 °C. In addition, Basheer et al (2014) indicated that the optimum temperature for *D. rapae* ranges from 20 to 25 °C based on the developmental rate and mortality. Talebi et al (2021) investigated the functional response of *D. rapae* reared on *S. graminum* at five constant temperatures (10, 15, 20, 25, and 30 °C); their results showed that the optimum temperatures range from 20 to 25 °C. Moreover, the demographic parameters of *D. rapae* at a wide range of temperatures especially on the greenbug, *S. graminum*, have not yet been reported.

The comprehensive knowledge of different biological parameters of *D. rapae* under variable environmental conditions is required to optimize aphid's biological control programs. Demographic parameters are important measurement of population growth potential of a species under specific conditions (Carey, 2001; Amiri, Talebi, Zamani, & Kamali, 2010; Rezaei et al, 2020a). Therefore, the main objective of this study was to elucidate the relationship between the demographic parameters and temperature for *D. rapae* on *S. graminum*. To do so, we compared temperature effects (10, 15, 20, 25, 27.5, and 30 °C) on the reproduction and life table parameters of *D. rapae*. Our study provides additional information on the biology of *D. rapae*, which may be helpful to improve biological control programs of this economically important aphid pest on cereals.

MATERIAL AND METHODS

Plant, host aphid, and parasitoid cultures

Original populations of both *S. graminum* and *D. rapae* were collected from wheat fields of Tarbiat Modares University, Tehran (35°44′N, 51°09′E, 1273 m above sea level) in April 2011. The colony of *S. graminum* was maintained on potted wheat seedlings (about 15 cm height). Seeds of wheat, *Triticum aestivum* L. var. Pishtaz, were obtained from the Seed and Plant Improvement Research Institute in Karaj, Iran. The seeds were planted in plastic pots (20 cm diameter; 13 cm high) without application of any fertilizers or pesticides under glasshouse conditions (25 ± 5ºC, 60 ± 5% RH and 16:8 h L:D photoperiod) until they reached three-week-old. A ventilated cylindrical acrylic plastic (20 cm diameter, 30 cm high) covered the wheat plants. In order to maintain the parasitoid colony, about 10 pairs of one-day-old *D. rapae* were released into the cylindrical plastic cage with wheat plants infested by *S. graminum*. After a 24 h exposure, the parasitoids were removed from the cylindrical plastic cage. Then, the aphids were fed on the wheat plants until mummies appeared. Both the aphid and parasitoid colonies were maintained at suitable environmental conditions (25 ± 1ºC, 70 ± 5% RH and 16:8 h L:D photoperiod). In addition, a cotton ball soaked with 25% honey solution was provided in the cylindrical plastic cage as food for adult parasitoids (Wäckers, 2003).

Demographic parameters

To investigate the demographic parameters of *D. rapae*, the wheat seedlings were initially infested with 50 second instar nymphs of *S. graminum*, the preferred host stage for the parasitoid (Jokar et al, 2012) placed into 1.5 ml micro tube inside a BD Falcon^{tm} 50 ml conical centrifuge tube, and exposed to a pair of *D. rapae*. After a 24 h exposure, the parasitoids were removed, and the exposed aphids were maintained under constant environmental conditions until mummies appeared. Each mummified aphid was separately placed into a 5 ml sample tube. The lids of sample tubes were covered with fine nylon mesh to allow adequate ventilation. The mummies were checked daily until adult parasitoids emerged. The number and sex of emerged adult wasps were recorded. Upon the emergence, naïve (without prior mating or oviposition experience) and one-day-old adult parasitoids were collected and used for the test of life-time fecundity. Also, a pair of one-day-old *D. rapae* was exposed daily to wheat leaves infested with 50 second instar nymphs of the host aphid as described above. The parasitoids were transferred to a new Falcon with a fresh group of 50 second instar nymphs of the host aphids after each day exposure until the female died. If the male died before the female, an alternative male that had been maintained in the same stock culture was introduced. The exposed aphids were maintained under the same experimental conditions until mummies appeared. The numbers of mummies were recorded and was used to approximately estimate the initial number of laid eggs by each female parasitoid. In all experiments, adults of *D. rapae* were fed on a 25% honey solution streaked on the inside wall of each container. These experiments were similarly performed at five constant temperatures of 10, 15, 20, 25 and 27.5 °C, relative humidity of 70 ± 5% and a photoperiod of 16L: 8D h. In addition, the temperature of 30 °C was examined but no adult wasps emerged at this temperature; hence, the demographic parameters could not be estimated for 30 °C.

Statistical analysis

Data from all individuals of parasitoids from different temperatures were subjected to a female age-specific life table procedure. By using the fertility and survivorship schedules, the life table and reproduction parameters were determined according to the formula suggested by Carey (1993, 2001). The jackknife procedure was used to calculate the pseudo-values of life history parameters in order to compare them statistically (Maia, Luiz, & Campanhola, 2000). Data were tested for normality with Kolmogorov-Smirnov test before they were subjected to ANOVA. Data concerning immature development, adult longevity, life span, reproduction, and life table parameters were compared using one-way ANOVA. If significant differences were determined, multiple comparisons were made using Tukey's honestly significant difference test ($P < 0.05$). All statistical analyses were carried out using SAS software (SAS Institute, 2003).

RESULTS

Development time and fecundity

The mean immature development, adult longevity, and life span of *D. rapae* at different temperatures are summarized in Table 1. The parasitoid completed its development at all five evaluated temperatures. The immature development period (egg to adult) significantly decreased with increasing temperatures from 10 to 25 °C and increased at 27.5 °C (df = 4, 496; $F = 65.89$; $P < 0.001$). The immature development periods of *D. rapae* were significantly longer at 10 °C (25.49 days) and shorter at 25 °C (9.24 days), respectively. Both adult longevity (df = 4, 160; $F = 36.67$; $P < 0.001$) and life span (df = 4, 160; $F = 12.64$; $P < 0.001$) were significantly affected by the temperatures. The adult longevity (20.44 days) and life span (45.93 days) were significantly longer at 10 °C. Moreover, the temperatures of 27.5 and 25 °C showed the shortest adult longevity (2.87 days) and life span (12.80 days), respectively.

The effect of temperature was highly significant for the different reproductive parameters including mean number of mummified aphids per female (df = 4, 153; $F = 25.69$; $P < 0.0001$), mean number of adults emerged per female (df = 4, 140; $F = 15.20$; $P < 0.01$), and mean number of adults emerged per female per day (df = 4, 153; $F = 235.12$; $P < 0.0001$). The parasitoid showed the highest value of mummified aphids per female at 20 °C (120.08) and the lowest value was found at 27.5 °C (12.24). There were no significant differences between the numbers of mummified aphids per female and adults emerged per female at 10 °C, 15 °C and 20 °C. Similarly, the maximum and minimum values of adults emerged per female were recorded at 20 °C (105.29) and 27.7 °C (9.46), respectively. Also, the maximum and minimum values of mean fertile eggs per day were estimated to be 17.10 and 3.25 at 25 and 10 °C, respectively (Table 2).

Table 1. Immature development, adult longevity, and life span (days ± SE) of *Diaeretiella rapae* reared on *Schizaphis graminum* at five constant temperatures.

Parameters	**Temperature (°C)**				
	10	**15**	**20**	**25**	**27.5**
Immature development	25.49 ± 0.09a*	22.09 ± 0.10b	17.64 ± 0.05c	9.24 ± 0.05e	11.95 ± 0.21d
Adult longevity	20.44 ± 0.38a	17.41 ± 0.34b	10.11 ± 0.30c	4.05 ± 0.16d	2.87 ± 0.30e
Life span	45.93 ± 0.36a	39.17 ± 0.46b	27.53 ± 0.36c	12.80 ± 0.26e	14.91 ± 0.25d

*****Values followed by the same letter within each row are not significantly different at $P < 0.05$ (Tukey's HSD multiple range test).

Table 2. The reproduction parameters (mean ± SE) of *Diaeretiella rapae* reared on *Schizaphis graminum* at five constant temperatures.

Parameters	**Temperature (°C)**				
	10	**15**	**20**	**25**	**27.5**
Mummified aphids per female	69.10 ± 1.59^{c*}	101.97 ± 2.63^{b}	120.08 ± 1.97^{a}	67.24 ± 2.37^{c}	12.24 ± 0.52^{d}
Adults emerged per female	62.11 ± 1.05^{c}	96.43 ± 2.80^{b}	105.29 ± 1.39^{a}	54.79 ± 2.10^{d}	9.46 ± 0.31^{e}
Adults emerged per female per day	3.25 ± 0.07^{d}	5.66 ± 0.15^{c}	11.34 ± 0.19^{b}	17.10 ± 0.56^{a}	3.91 ± 0.16^{e}

*****Values followed by the same letter within each row are not significantly different at $P < 0.05$ (Tukey's HSD multiple range test).

Survival rate and life expectancy

The age-specific survival rate pattern of *D. rapae* at different temperatures is shown in Fig. 1. *Diaeretiella rapae* completed its developmental from 10 to 27.5°C. The curves of age-specific survival rate (l_x) show the probability that a newly laid egg will survive to age *x*. The survival rate of *D. rapae* at various temperatures was different. The survival rate decreased when temperatures increased from 10 to 25 °C. The nearly similar pattern was observed for survival rate at 25 and 27.5 °C. The longest survival rate (52 days) occurred at 10°C and its shortest value (17 days) was observed at 25 and 27.5 °C (Fig. 1). The life expectancy (e_x) of newly laid eggs of *D. rapae* was 44.07, 38.28, 26.14, 12.59, and 13.82 days at 10, 15, 20, 25 and, 27.5 °C, respectively, which is decreased with increasing age (Fig. 2). The oviposition started after 25, 21, 16, 8, and 10 days at the mentioned temperatures, respectively (Fig. 3). According to our results, the highest value of age-specific fecundity at 10, 15, 20, 25 and, 27.5 °C was 6.61, 11.27, 20.33, 18.04, and 3.00 at the age of 30, 26, 19, 10, and 11 days, respectively.

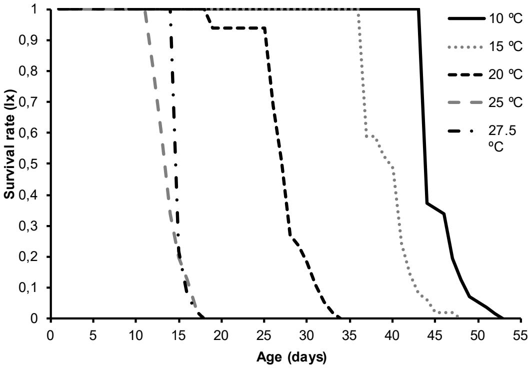

Figure 1. Age-specific survival rate (l_x) of *Diaeretiella rapae* reared on *Schizaphis graminum* at five constant temperatures.

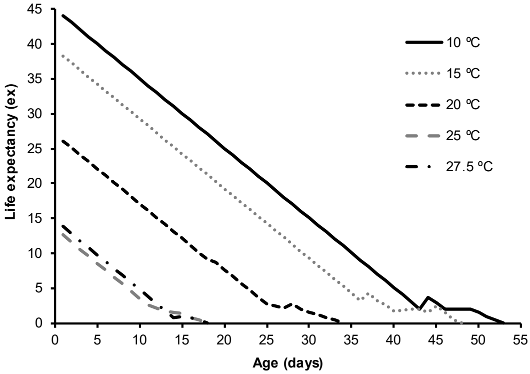

Figure 2. Life expectancy (e_x) of *Diaeretiella rapae* reared on *Schizaphis graminum* at five constant temperatures.

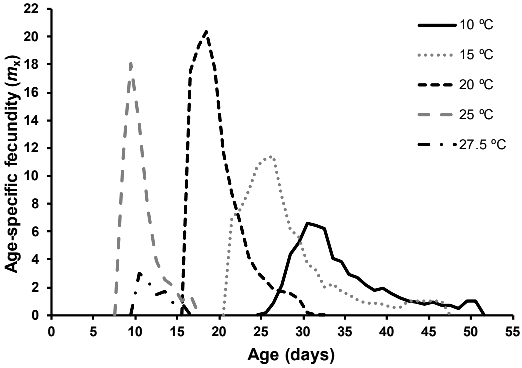

Figure 3. Age-specific fecundity (m_x) of *Diaeretiella rapae* reared on *Schizaphis graminum* at five constant temperatures.

Population growth parameters

The population growth parameters of *D. rapae* at five constant temperatures are summarized in Table 3. The net reproductive rate (R_0) is the average number of female offspring produced in a lifetime by a female and was significantly different at various temperatures (df = 4, 155; F = 20.13; P < 0.01) according to the pattern 20> 15> 25> 10> 27.5 °C. The mean values of R_0 ranged from 52.39 female offspring/female at 25 °C to 0.77 female offspring/female at 27.5 °C. The intrinsic rate of increase (r) was also significantly different at various constant temperatures (df = 4, 155; F = 25.97; P < 0.01), with the highest value (0.374 day^{-1}) at 25 °C and the lowest (0.019 day^{-1}) at 27.5 °C. Similarly, the finite rate of increase (λ) was significantly different among treatments (df = 4, 155; F = 44.54; P < 0.01). The mean values of finite rate of increase ranged from 1.453 to 0.981 day^{-1} at 25 and 27.5 °C, respectively. Also, the temperature showed significantly effects on the doubling time (DT) (df = 4, 155; F = 27.79; P < 0.01), and mean generation time (T) (df = 4, 155; F = 16.14; P < 0.01). The doubling time and mean generation time were significantly lower at 25 ° C (1.85 and 7.73 days, respectively).

Table 3. Population growth parameters (mean ± SE) of *Diaeretiella rapae* reared on *Schizaphis graminum* at five constant temperatures.

Parameters	**Temperature (°C)**				
	10	**15**	**20**	**25**	**27.5**
Gross reproduction rate (GRR)	38.68 ± 0.89^{d*}	50.08 ± 1.35^{c}	66.46 ± 1.24^{a}	57.69 ± 1.27^{b}	9.48 ± 0.42^{e}
Net reproduction rate (R_0; ♀♀/♀)	14.16 ± 0.39^{d}	24.75 ± 0.96^{b}	52.39 ± 0.85^{a}	16.92 ± 0.80^{c}	0.77 ± 0.05^{e}
Intrinsic rate of increase (r, day^{-1})	0.096 ± 0.001^{d}	0.150 ± 0.001^{c}	0.247 ± 0.001^{b}	0.374 ± 0.004^{a}	0.019 ± 0.009^{e}
Finite rate of increase (λ; day^{-1})	1.101 ± 0.001^{d}	1.160 ± 0.001^{c}	1.281 ± 0.001^{b}	1.453 ± 0.006^{a}	0.981 ± 0.005^{e}
Doubling time (DT; days)	7.21 ± 0.08^{a}	4.66 ± 0.04^{b}	2.81 ± 0.01^{c}	1.85 ± 0.22^{d}	7.37 ± 0.52^{a}
Mean generation time (T; days)	27.58 ± 0.16^{a}	21.74 ± 0.10^{b}	15.95 ± 0.62^{c}	7.73 ± 0.06^{e}	13.86 ± 0.11^{d}

* Values followed by the same letter within each row are not significantly different at P < 0.05 (Tukey's HSD multiple range test).

DISCUSSION

Although insects do not live in a constant environmental condition without temperature fluctuation, the results of studies under stable temperatures can provide a valuable insight into the understanding the population dynamics of various insects (Haghani et al, 2007; Amiri et al, 2010; Zamani, Haghani, & Kheradmand, 2012; Rezaei et al, 2020b). This study provides the biological response of *D. rapae* on *S. graminum* cultured on wheat plant to a broad range of constant temperatures, which has not been previously reported. Our findings revealed that temperature had a strong effect on the reproductive and demographic parameters of *D. rapae*. The parasitoid was able to develop across a wide range of temperatures from 10 to 27.5 °C, while the reared parasitoids did not survive and develop at 30 °C. This is in agreement with other studies in which the lethal maximum temperature (T_{max}) of *D. rapae* was estimated from Briere-1 and Lactin-1 Models to be 31.5 and 31.94 °C, respectively (Bernel & Gonzalez, 1993; Tazerouni et al, 2012; Kazemi et al, 2020).

Demographic parameters are important in measurement of population growth capacity of an insect species and they may depend on several factors, in particular temperature (Ismail et al, 2014; Tazerouni et al, 2012; Rezaei et al, 2020a). Many studies have assessed the demographic parameters of parasitoids at various constant temperatures (e.g., Bernal & Gonzalez, 1997; Amiri et al, 2010; Tazerouni et al, 2013; Rezaei et al, 2020a). In the current study, temperature had a significant influence on all measured reproductive and demographic parameters. The intrinsic rate of increase (*r*) is the most important parameter indicating the most favorable temperature for population growth and reflects overall effects of temperature on development, reproduction, and survival (Carey, 1993, 2001; Amiri et al, 2010). The estimated values of *r* in all examined temperatures were positive, indicating that the parasitoid can fully development and reproduction in these temperatures. This is consistent with studies in which *D. rapae* was reared on *D. noxia* (Tazerouni et al, 2012), *L. erysimi*, *B. brassicae*, and *M. persicae* (Karami et al, 2018; Nisar & Rizvi, 2020). The greatest value of *r* was obtained at 25 °C (0.374 day^{-1}), indicating the optimum temperature for this parasitoid reproduction. Moreover, the temperature of 20 °C showed the highest values of mummified aphids and adults emerged per female (120.08 and 105.29, respectively). To date, some studies have reported the intrinsic rate of increase of *D. rapae* at different conditions (Bernal & González, 1997; Tazerouni et al, 2012; Karami et al, 2018; Nisar & Rizvi, 2021). For instance, Ghorbanian et al (2019) reported that the intrinsic rate of increase of *D. rapae* on *M. persicae* established on different cultivars of pepper at 25 °C ranged from 0.279 to 0.389 day^{-1}. In contrast to the current results, Tazerouni et al (2013) showed that *D. rapae* could potentially control *D. noxia* at a temperature range of 10 to 15 °C. They obtained the highest R_0 and *r* for *D. rapae* at 10 °C (22.99) and 20 °C (0.189 day^{-1}), respectively. Also, the *r* value of *D. rapae* on *D. noxia* at 26.7 °C was calculated as 0.202 day^{-1} (Bernal & Gonzalez, 1997). A possible explanation for these differences may be related to host aphid species, as previously reported for the performance of *D. rapae* on different host species (Silva et al, 2011; Souza et al, 2017; Nisar & Rizvi, 2021). The higher value of *r* in the current study indicated that *S. graminum* is a more suitable host than *D. noxia* for the parasitoid. However, the *r* value could be affected by various factors such as host plant (Karami et al, 2018; Ghorbanian et al, 2019), host and parasitoid size (Jervis & Kidd, 1996), and geographic populations of parasitoid (Hayakawa et al, 1990), among other experimental conditions. Moreover, it is mentioned that the intrinsic rate of increase is indicative of population growth only if the population has reached a stable age structure (Carey, 1993). Hence, it is suggested to use the emergence rate, net reproduction rate, and cumulative fecundity curves of the first generation, which are desirable for practical use in biological control program (Ismail et al, 2014; Rezaei et al, 2020a).

The highest net reproductive rate (R_0) occurred at 20 °C (52.39 female offspring/female), which is different from the findings reported by Tazerouni et al (2012) and Bernal & Gonzalez (1997) for *D. rapae* on *D. noxia*. These differences can be explained by the variation in the aphid and host plant species. The lowest R_0 at 27.5 °C (0.77 female offspring/female) resulted in high mortality of the immature life stages or adults from emergence to oviposition peak (Fig. 1). The shortest mean generation time was obtained at 25 °C (7.73 days) indicating that *D. rapae* developed faster at this temperature than the others.

The fecundity rate of a natural enemy is also the important factors to determine its effectiveness (Tazerouni et al, 2012; Zamani et al, 2012). In the present study, the highest value of mummified aphids per female was observed at 20 °C (120.08). In contrast to earlier findings, the reported fecundity rate of *D. rapae* on *D. noxia* at 21.1 °C was 50.20 (Bernal & Gonzalez, 1997). In addition, Nisar & Rizvi (2021) reported that the potential fecundity of *D. rapae* on *L. erysimi*, *M. persicae*, and *B. brassicae* at 26 °C were 101.52, 105.46, and 92.36, respectively. This inconsistency may be due to differences in host aphid species, geographic populations of the parasitoid, and genetic difference as a result of laboratory rearing (Silva et al, 2011; Nisar & Rizvi, 2020).

Several models are used to predict the population dynamics of pest and natural enemies (e.g., Lotka-Volterra model, Nicholson-Baily model, etc), and the intrinsic rate of increase is a key parameter in nearly all of these models (Mirhosseini, Fathipour, & Reddy, 2017). In many cases, the intrinsic rate of increase has been evaluated at a constant environmental condition while the natural enemies are faced with temperature fluctuations in the field and their population growth rate is different at the various temperatures (Amiri et al, 2010; Kant, Minor, & Trewick, 2012). In the present study, the intrinsic rate of increase of *D. rapae* was calculated at different constant temperatures. Hence, it is more helpful to precisely predict population dynamics of *D. rapae* in the field under variable conditions. However, an understanding of thermal requirements of a particular natural enemy (e.g. *D. rapae*) is important for predicting of its potential geographic distribution in different regions. Using the current results, we will able to generate isothermal lines to predict the probable distribution regions for *D. rapae* (Hayakawa et al, 1990; Haghani et al, 2007; Amiri et al, 2010).

The current study shows that the temperature range of 20 to 25 °C may be the best choice for maintenance of a laboratory colony of *D. rapae* on *S. graminum*. In accordance, Basheer et al (2014) revealed that the optimum temperature for *D. rapae* on *B. brassicae* is 20 to 25 °C. Based on the temperature-dependent functional response, the mentioned temperature range was optimized for rearing of *D. rapae* on *S. graminum* (Talebi et al, 2022). Similarly, this temperature range is also optimum for fecundity and fertility rates of *Aphidius matricariae* Haliday and *Aphidius colemani* Viereck (both Hymenoptera: Braconidae) (Zamani et al, 2012). It is important to mention that 26 °C is optimal temperature for *S. graminum* development and growth (Tofangsazi, Kheradmand, Shahrokhi, & Talebi, 2010). Therefore, *D. rapae* could be considered as a good candidate for biological control of *S. graminum* in wheat field at the beginning of spring and autumn before the population of the aphid is built (Tazerouni et al, 2013). Moreover, Kant et al (2012) stated that pre-release of *D. rapae* for suppression of early population of *M. persicae* is required for effective biological control.

CONCLUSIONS

In conclusion, this research confirmed that the *D. rapae* could develop from egg to adult and reproduce successfully within a temperature range of 10 - 27.5 °C. Our laboratory results suggest that the range of 20 to 25 °C is the most suitable temperature regimes for *D. rapae* population growth on *S. graminum*. However, the intrinsic rate of increase (r) was significantly greater at 25 °C compared to other temperatures, which suggest that this is the most suitable temperature for the control of *S. graminum.* Further consideration in semi-field and field applications would help to obtain more reliable results and understanding. In addition, using a temperature-controlled environment is a vital component of mass rearing facilities for consistent rearing of *D. rapae* for biological control of aphids. Thus, the results can be used in mass rearing and mass release programs of *D. rapae*.

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Research Article
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Arthropod Assemblages from La Chimba National Reserve (Antofagasta Region, Chile): Biodiversity, Threats and Conservation

Jaime PIZARRO-ARAYA[1,2,3*] Fermín M. ALFARO[1,2,4,5]
Andrés A. OJANGUREN-AFFILASTRO[6] Mauricio MORA-CARREÑO[7]

[1]Laboratorio de Entomología Ecológica (LEULS), Departamento de Biología, Facultad de Ciencias, Universidad de La Serena, Casilla 554, La Serena, CHILE.
[2]Grupo de Artrópodos, Sistema Integrado de Monitoreo y Evaluación de Ecosistemas Forestales Nativos (SIMEF), CHILE
[3]Instituto de Ecología y Biodiversidad (IEB), Santiago, CHILE
[4]Instituto de Investigación Multidisciplinar en Ciencia y Tecnología, Universidad de La Serena, Casilla 554, La Serena, CHILE
[5]Programa de Doctorado en Biología y Ecología Aplicada, Universidad Católica del Norte, Universidad de La Serena, La Serena, CHILE
[6]División de Aracnología, Museo Argentino de Ciencias Naturales Bernardino Rivadavia, Buenos Aires, ARGENTINA
[7]Proyecto FIC-R Plan de Recuperación Reserva Nacional La Chimba, Vicerrectoría de Investigación y Desarrollo Tecnológico, Universidad Católica del Norte, Antofagasta, CHILE
e-mails: [1,2,3,*]japizarro@userena.cl, [1,2,4,5]fmalfaro@userena.cl, [6]ojanguren@macn.gov.ar
[7]ma.moracarreno@gmail.com
ORCID IDs: [1,2,3*]0000-0002-1595-6924, [1,2,4,5]0000-0001-8790-6638, [6]0000-0003-1093-5822
[7]0000-0001-9730-3726a

ABSTRACT

We provide the first inventory of terrestrial arthropods from La Chimba National Reserve (Antofagasta Region, Chile), a protected area whose purpose is to preserve the biota of the Coastal desert of Tocopilla embedded in the larger transitional coastal desert. The study was conducted during spring of 2019. A total of 2,852 specimens were recorded, distributed across 188 species. Among the collected specimens, 5 new species were identified: two species of scorpions, *Brachistosternus chimba* Ojanguren-Affilastro, Alfaro & Pizarro-Araya, 2021 and *Rumikiru* sp. nov. (Scorpiones: Bothriuridae); a solpugid (Solifugae: Ammotrechidae); a spider (Araneae: Filistatidae), and a centipede (Scutigeromorpha: Scutigeridae). The richness estimations showed large values for most arthropod groups, which is evidence of an incomplete inventory of species richness. The article also discusses the current threats faced by these communities of endemic arthropods and suggests immediate actions to preserve these groups in extremely fragile semiarid ecosystems.

Keywords: Atacama Desert, conservation, coastal desert, endemism, richness estimation.

Pizarro-Araya, J., Alfaro, F. M., Ojanguren-Affilastro, A. A., & Mora-Carreño, M. (2023). Arthropod assemblages from La Chimba National Reserve (Antofagasta Region, Chile): Biodiversity, threats and conservation. *Journal of the Entomological Research Society*, 25(1), 45-63.

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INTRODUCTION

The longitudinal coastal strips of northern Chile are composed of a wide variety of geomorphological, ecological, and climatic zones (Hartley, Chong, Houston, & Mather, 2005; Rundel, Villagra, Dillon, Roig-Juñent, & Debandi, 2007). This wide variety of habitats has favored the evolution of a biota specifically adapted to dry conditions, changes in sea level, as well as humidity and dryness oscillations that are characteristic of the coastal desert (Ceccarelli, Pizarro-Araya, & Ojanguren-Affilastro, 2017), with biodiversity hotspots, endemic species, and relictual distributions in different sections of its geography (Roig-Juñent & Flores, 2001; Cepeda-Pizarro, Pizarro-Araya, & Vásquez, 2005a; Rundel et al, 2007).

The transitional coastal desert of Chile (25° to 32° S) is an important strip in terms of flora diversity, species endemism, desert bloom, and biological conservation (Muñoz, 1991; Vidiella, Armesto, & Gutiérrez, 1999). This region constitutes the northernmost limit of the vegetation biodiversity hotspot recognized for central Chile (Gaston, 2000). Additionally, due to its latitudinal location, the area is subject to the effects of ENOS events (Jaksic, 1998; Cepeda-Pizarro et al, 2005a, Cepeda-Pizarro, Pizarro-Araya, & Vásquez, 2005b) as well as the potential effects of global climate change (Mooney et al, 2001). An important landscape element in the geomorphological diversity of the transitional coastal desert of Chile are interior ravines (Paskoff & Manríquez, 1999). These ravines are attractive for tourism or real estate development, and once they become accessible, they are subject to strong anthropic pressures (Paskoff & Manríquez, 1999).

Terrestrial arthropods are one of the most diverse and abundant biological components of the transitional coastal desert (Cepeda-Pizarro et al, 2005a, 2005b; Pizarro-Araya, Pizarro-Araya, Alfaro, Ojanguren-Affilastro, Perez-Schultheiss, & Thiel, 2023). They play different ecological roles, such as increasing the primary and secondary production of their ecosystems (Ayal, 2007) either due to their ability to pollinate or their ability to dynamize the flow of energy or the nutrient cycle, while at the same time being abundant, high-quality trophic resources (Vidal, Pizarro-Araya, Jerez, & Ortiz, 2011). During the dry season, they function as important macrodecomposers and are significant trophic resources both for invertebrates (Pizarro-Araya, 2010; Cruz-Jofré & Vilina, 2014) and vertebrates (Vidal et al, 2011; Tirado, Trujillo, Pizarro-Araya, Alfaro, González, & Carretero, 2018).

Recent expeditions conducted by an Argentinean-Chilean team have provided relevant biological information regarding the ecology (Ceccarelli, Ojanguren-Affilastro, Mattoni, Ochoa, Ramírez, & Prendini, 2016; Ceccarelli et al, 2017; Alfaro, Pizarro-Araya, & Flores, 2016; Alfaro, Pizarro-Araya & Flores, 2009; Pizarro-Araya & Alfaro, 2018), conservation (Pizarro-Araya, Vergara, & Flores, 2012; Pizarro-Araya, Alfaro, Flores, & Letelier, 2017; Pizarro-Araya & Ojanguren-Affilastro, 2018), biology (Pizarro-Araya, 2010; Ojanguren-Affilastro, Botero-Trujillo, Castex, & Pizarro-Araya, 2016), and taxonomy and systematics of the Arthropoda found in this coastal desert. As a result, several taxonomic groups have been described, e.g., Scorpiones (Ojanguren-Affilastro, Mattoni, & Prendini, 2007a, Ojanguren-Affilastro, Agusto, Pizarro-Araya, & Mattoni, 2007b, Ojanguren-Affilastro, Pizarro-Araya, & Ochoa-Cámara, 2018, Ojanguren-Affilastro, Alfaro, & Pizarro-Araya, 2021; Ojanguren-Affilastro & Pizarro-Araya, 2014), Araneae (Laborda, Ramírez, & Pizarro-Araya, 2013; Grismado & Pizarro-Araya, 2016; Izquierdo & Ramírez, 2017), and Coleoptera (Flores & Pizarro-Araya, 2012, 2014, 2022; Moore, 2017; Mondaca, Pizarro-Araya, & Alfaro, 2019). However, knowledge of the arthropods of the northern part of the Chilean coastal desert, represented by the plant formation known as Coastal Desert of Tocopilla (Gajardo, 1993), is virtually nonexistent. This formation is represented by protected areas such as the Morro Moreno National Park (23° S) and La Chimba National Reserve (23° S). Since these units are isolated from the coastal desert, they are expected to contain a unique endemic arthropod fauna, as documented for the Paposo priority area (25° S) (Pizarro-Araya & Jerez, 2004; Ojanguren-Affilastro & Pizarro-Araya, 2014; Ojanguren-Affilastro et al, 2018; Mondaca et al, 2019; Pizarro-Araya, Alfaro, Ojanguren-Affilastro, & Moreira-Muñoz, 2021). Due to the limited knowledge of the biodiversity from La Chimba National Reserve (Antofagasta Region, Chile), a FIC-R (Innovation Fund for Regional Competitiveness) project was developed during 2019 and 2020 to help recover and preserve its biota. The objectives of this research study were (a) to characterize the richness, abundance, and similarity of the terrestrial arthropod fauna from La Chimba National Reserve, (b) evaluate the level of endemism of the arthropod fauna and the significance of newly discovered species in this natural area, and (c) to identify existing threats to the local fauna and the challenges for its conservation.

MATERIAL AND METHODS

Study sites

La Chimba National Reserve is located in the coastal range of Antofagasta, 15 km north of the city of Antofagasta (Chile), and has a surface area of 2,583 ha (Fig. 1). The purpose of the reserve is to preserve the biota of the Coastal Desert of Tocopilla (Gajardo, 1993; CONAF, 1995). The reserve derives its name from La Chimba ravine (Fig. 2a), an arheic microbasin with a surface area of 1,455.2 ha and a drainage system composed of a third-order main ravine (La Chimba ravine) and the secondary ravines Guanaco (Fig. 2b) and Los Cactus (Fig. 2c). These ravines are important biological relicts of the coastal desert of northern Chile (CONAF, 1995). The vegetation in the area consists of an extremely xeromorphic, open coastal desert shrubland dominated by cacti and succulents, e.g., *Eulychnia iquiquensis* (K. Schum.) Britton & Rose 1920, *Atriplex clivicola* I.M. Johnst 1929, *Frankenia chilensis* C. Presl ex Schult. & Schult.f. 1830, *Nolana lachimbensis* Dillon, Arancio & Luebert 2007, *Ophryosporus triangularis* Meyen 1834, and *Tetragonia angustifolia* Barnéoud 1847 (Luebert & Pliscoff, 2006; Faúndez, 2022).

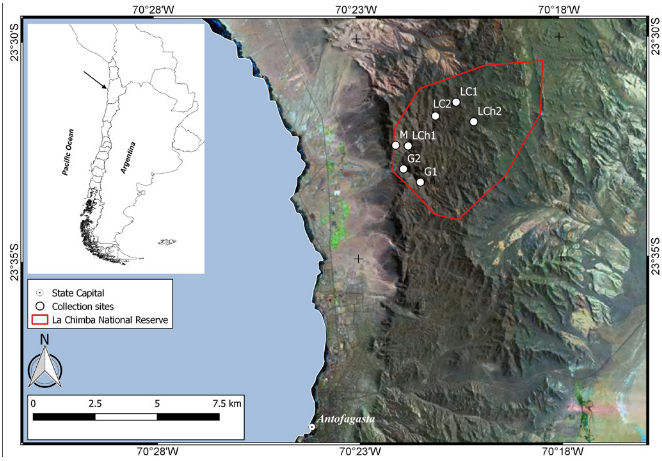

Figure 1. Geographic location of La Chimba National Reserve (Antofagasta Region, Chile) and study sites. Codes as in Table 1.

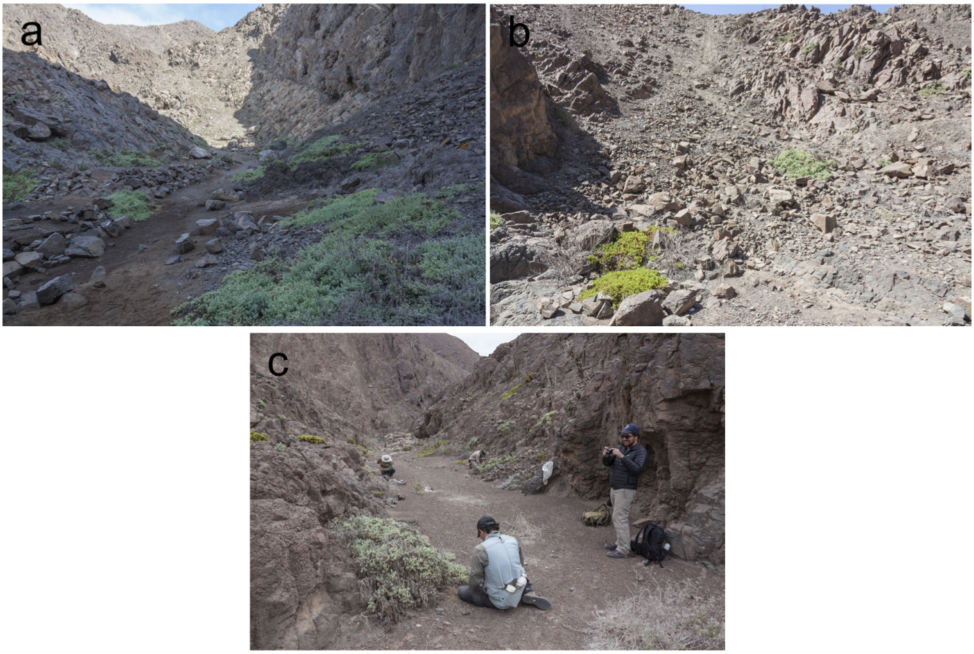

Figure 2. Study sites (habitats) within La Chimba National Reserve. a) La Chimba (LCh1) ravine, b) Guanaco (G1) ravine, and c) Los Cactus (LC2) ravine.

The reserve's geomorphology is complex and dominated by a highly degraded landscape with a broken topography, formed by the pluvial-fluvial action resulting from a wetter past climate (CONAF, 1995). These extreme weather conditions cause cycles of erosion mostly by water, wind, and thermal stress. These elements act selectively and superficially upon large volumes of rock. This superficial action generates shallow saline soil profiles dominated by calcium and sodium sulfides (CONAF, 1995). In a normal year, the most important hydrometereological phenomenon are fog banks with grazing clouds known as “camanchacas”, which cause horizontal rains in the altitudinal strip ranging from 300 to 900 m a.s.l. Although these precipitations occur during the entire year, they are more common in the spring and winter (CONAF, 1995; Carvajal, Mora-Carreño, Sandoval, & Espinoza, 2022).

Sampling methods

The taxonomic richness and relative abundance of epigean arthropods were estimated based on the number of species and the number of collected individuals per species, respectively. For capture, pitfall traps were chosen, a widely used method to assess assemblages of terrestrial arthropods (Cepeda-Pizarro et al, 2005a, 2005b). For sampling, 7 sites representative of the different environments and ravines present in the reserve were selected (Table 1). Following SIMEF procedures (2017), 10 traps were installed in each site, in vegetation representative of that environment. Each trap consisted of two plastic cups one inside the other, with the interior cup easily removable (*sensu* Cepeda-Pizarro et al, 2005a, 2005b). The cups were 7.4 and 7.6 cm in diameter by 10.2 and 12.0 cm in height, respectively. The interior cup was two-thirds filled with a solution of water, domestic washing liquid, and 70% alcohol. The traps remained active between December 18 and December 22, 2019.

Table 1. Geographical location of study sites in La Chimba National Reserve (Antofagasta Region, Chile).

N° site	Site	Site code	Latitude	Longitude	Altitude (m a.s.l.)
1	La Chimba 1 ravine (wetland)	LCh1	23°32'20.76"S	70°21'35.82"W	402
2	Mirador ravine	M	23°32'19.66"S	70°21'53.54"W	568
3	La Chimba 2 ravine	LCh2	23°31'49.71"S	70°20'2.30"W	749
4	Los Cactus 1 ravine	LC1	23°31'23.87"S	70°20'27.11"W	712
5	Los Cactus 2 ravine	LC2	23°31'41.82"S	70°20'56.63"W	712
6	Guanaco 1 ravine	G1	23°33'8.24"S	70°21'18.95"W	434
7	Guanaco 2 ravine	G2	23°32'50.84"S	70°21'42.79"W	308

Scorpions were collected by hand at night using portable UV lamps (395 nm), since they fluoresce under this light (*sensu* Ojanguren-Affilastro & Pizarro-Araya, 2014; Ojanguren-Affilastro et al, 2018). Apart of those specimens collected by traps, the arthropods of the remaining groups were also collected by hand at night using portable lights, or during the day searching under stones, around plants and in rock crevices at each of the sites described above.

In Arachnida (Arthropoda: Chelicerata), the taxonomic identification for Araneae followed Platnick & Shadab (1982), Coyle (1986), Goloboff (1995), and Ramírez (2003); for Solifugae we used Kraus (1966), Muma (1971), and Maury (1987); and for Scorpiones, we followed Ojanguren-Affilastro (2002, 2005), Ojanguren-Affilastro & Ramírez (2009), and Ojanguren-Affilastro et al, (2007a, 2007b). In Insecta (Arthropoda: Mandibulata), the taxonomic identification followed Peña (1966, 1971, 1973, 1974, 1980, 1995); Snelling & Hunt (1975), Cigliano (1989), Cigliano, Ronderos, & Kemp (1989), Chiappa, Rojas, & Toro (1990), Artigas (1994), Estrada & Solervicens (1999), Elgueta, Camousseight, & Carbonell (1999), Roig-Juñent & Domínguez (2001), and Pizarro-Araya & Jerez (2004). It should be noted that some taxa (e.g., Diptera and Hymenoptera) were taxonomically identified only at the level of morphotype/family due to the poor taxonomic knowledge of these groups in the area. All the captured specimens were removed, cleaned, and preserved in alcohol (e.g., insects and myriapods in 70% alcohol; arachnids in 80% alcohol) until their processing and taxonomic identification. The captured specimens were deposited in the entomological and arachnological collection of the Ecological Entomology Laboratory of Universidad de La Serena, Chile (LEULS, Jaime Pizarro-Araya) and the Arachnology Division of the Bernardino Rivadavia Museum of Natural Science, Buenos Aires, Argentina (MACN-Ar, Martín J. Ramírez).

Data analysis

To determine the richness patterns between the study sites, we used species rarefaction curves based on individuals to remove the effects introduced by the sites' difference in area (Gotelli & Colwell, 2001). The analysis was based on the species abundance data obtained from the sample set and performed using PAST 2.16 (Hammer, Harper, & Ryan, 2001). The richness was estimated using the non-parametric estimators ICE, Chao 2, and Jack 2 (second-order jackknife) (Chazdon, Colwell, Denslow, & Guariguata, 1998; Colwell, 2013). These estimators are universally valid for any species abundance distribution and more robust than estimators based on parametric models of species abundance (Chao & Chiu, 2016). For that reason, they provide the most precise estimations of species abundance (Hortal, Borges, & Gaspar, 2006). The incidence-based coverage estimator (ICE) is a robust and precise estimator of species richness (Chazdon et al, 1998), whereas Chao 2 and Jack 2—both based on rare species—provide less biased estimates for small samples (Colwell & Coddington, 1994). All the estimators were computed using EstimateS version 9.1.0 (Colwell, 2013).

The abundance structure of the terrestrial arthropod fauna was analyzed by means of non-metric multidimensional scaling (NMDS) based on Bray-Curtis and Jaccard similarity matrices. Finally, we performed a one-way analysis of similarities (ANOSIM) based on the distance between the Bray-Curtis and Jaccard indices to determine the differences between the main ravines (study sites) in the national reserve. The significance level was calculated using 50,000 permutations. All the analyses were performed in PAST 2.16 (Hammer et al, 2001).

RESULTS

Species richness, abundance and similarity of the terrestrial arthropod fauna

A total of 2,852 arthropod specimens representing 188 species were collected across all the study sites in La Chimba National Reserve. Among these, four classes were identified: Arachnida, Chilopoda, Crustacea, and Insecta. Insecta was the most represented taxon, with 123 species, 56 families, and 11 orders, whereas Arachnida was represented by 62 species, 27 families, and 5 orders. Crustacea, in turn, was represented only by 2 species, *Transorchestia chiliensis* Milne-Edwards, 1840 (Talitridae) and *Scyphoniscus* sp. (Scyphacidae), whereas Scutigeromorpha was represented by a single species (Table 2). Diptera, Hymenoptera, and Lepidoptera were the orders with the largest number of species within Insecta, whereas Acari and Araneae were the more diverse orders within Arachnida (Table 2).

Table 2. Taxonomic composition of terrestrial arthropods registered in La Chimba National Reserve (Antofagasta Region, Chile).

Class	Order	Species	Individuals	Singletons-species
Arachnida	Acari	25	143	8
	Araneae	30	94	10
	Pseudoscorpiones	2	8	1
	Scorpiones	2	66	0
	Solifugae	3	10	1
Chilopoda	Scutigeromorpha	1	1	1
Malacostraca	Amphipoda	1	166	0
	Isopoda	1	261	0
Insecta	Coleoptera	18	120	8
	Collembola	2	371	0
	Diptera	33	312	11
	Hemiptera	9	228	1
	Hymenoptera	32	288	17
	Lepidoptera	18	81	10
	Orthoptera	3	17	1
	Psocoptera	4	161	0
	Siphonaptera	1	1	1
	Thysanoptera	1	519	0
	Thysanura	2	5	1
Totals		188	2,852	71

The accumulation rates for the study sites did not stabilize. Due to the overlapping confidence intervals, no significant differences were observed in sampling levels between study sites (Fig. 3). Although 188 species were identified, the richness values for the different arthropod groups under analysis were large. In this respect, the groups with the largest species values were Diptera and Hymenoptera (Table 3).

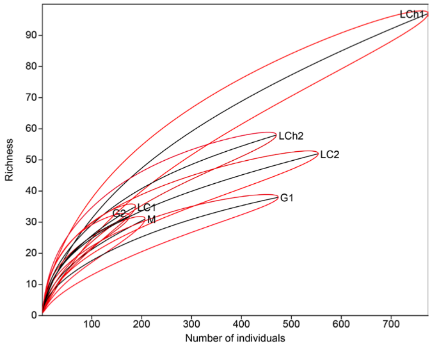

Fig. 3. Individual-based rarefaction curves for the study sites in La Chimba National Reserve. Lines in red indicate the 95% confidence interval.

Table 3. Richness estimated for terrestrial arthropods and principal groups registered.

Group	Richness observed	ICE	Chao 2	Jack 2
All species	188	365.55	305.92	333.31
Arachnida	62	103.15	92.17	105.52
Insecta	123	258.86	206.43	222.24
Acari	25	47.43	43	46.19
Araneae	30	51.14	40.29	49.79
Coleoptera	18	54.21	34.71	36.64
Diptera	33	77.76	57.75	63.4
Hymenoptera	32	58.67	50.1	58.67

The ordination analysis suggests a potential difference in the abundance structure of the arthropod community between the sites located in the ravines under study. For instance, we observed a group composed of the La Chimba, Los Cactus, and Mirador ravines, and another group composed of the sites in the Guanaco ravine (Fig. 4). However, the ANOSIM based on the Bray-Curtis (Global $R = -0.2$, $P = 0.855$) and Jaccard (Global $R = 0.2$, $P = 0.172$) matrices indicated that these differences may not be significant.

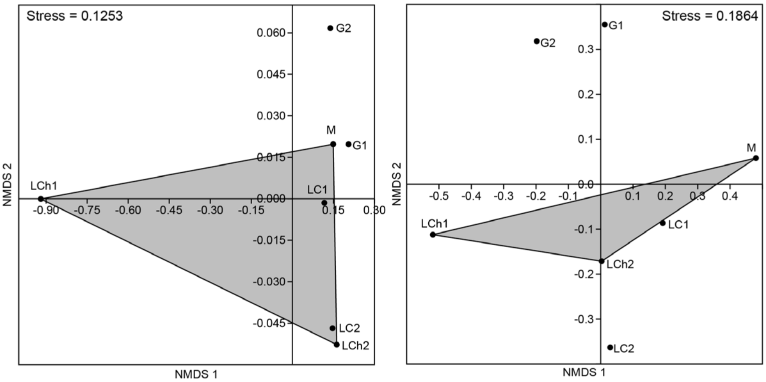

Figure 4. Non-metric multidimensional scaling ordination of study sites analyzed according to terrestrial arthropods from La Chimba National Reserve. Plot based on the Bray-Curtis (left) and Jaccard (right) similarity indices.

New species and endemisms

Even though some groups (e.g., Acari, Diptera, and Hymenoptera) were represented by a large number of morphospecies, it was impossible to identify them at species level -not even preliminary- due to the limited or non-existent knowledge of these groups in the area.

Some of the positively identified species included the locust *Uretacris lilai* Liebermann, 1943 (Orthoptera: Tristiridae), a restricted-range species found only in rocky habitats of the La Chimba 2 and Guanaco 2 ravines. Other endemic species recorded in the reserve were the darkling beetles *Nycterinus* (*Paranycterinus*) *penai* Kulzer, 1961 and *Entomochilus wilsoni* Peña, 1980 (Coleoptera: Tenebrionidae), both recorded exclusively in La Chimba ravine, and the spiders *Anisaedus pellucidas*

Platnick, 1975 (Araneae: Palpimanidae) and *Sicarius thomisoides* Walckenaer, 1847 (Araneae: Sicariidae), recorded in the La Chimba and Guanaco ravines, respectively (Fig. 5). All the individuals from these species were collected from the sparse vegetation of the reserve's desert environments, associated with coastal shrubs such as *Frankenia chilensis*, *Nolana lachimbensis*, *Ophryosporus triangularis,* and *Tetragonia angustifolia* (see Fig. 2).

As a result of this work, we identified *Brachistosternus chimba* Ojanguren-Affilastro, Alfaro & Pizarro-Araya, 2021 a species of scorpion recorded exclusively in La Chimba National Reserve and 4 new species undescribed to date: *Rumikiru* sp. nov. (Scorpiones: Bothriuridae), recorded exclusively in the La Chimba and Los Cactus ravines (Fig. 5); a solpugid from the family Ammotrechidae (Solifugae), recorded only in La Chimba 2 ravine; a spider from the family Filistatidae (Araneae), captured in the La Chimba 1 (wetland) and Guanaco 2 ravines, and a centipede from the family Scutigeridae (Scutigeromorpha), recorded in La Chimba 1 ravine.

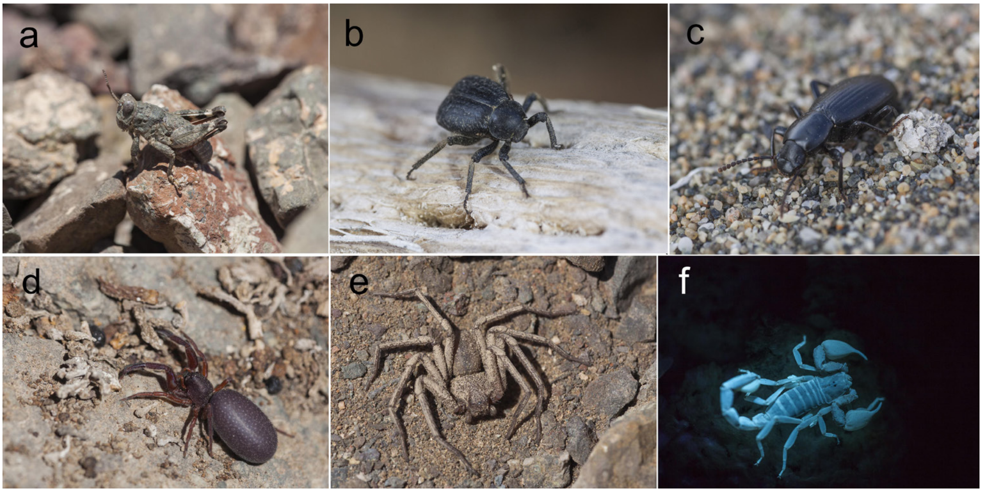

Figure 5. Different species of arthropods recorded in La Chimba National Reserve. a) Lateral view of *Uretacris lilai* Liebermann, 1943 (Orthoptera: Tristiridae); b) lateral view of *Entomochilus wilsoni* Peña, 1980 (Coleoptera: Tenebrionidae); c) lateral view of *Nycterinus* (*Paranycterinus*) *penai* Kulzer, 1961 (Coleoptera: Tenebrionidae); d) lateral view of *Anisaedus pellucidas* Platnick, 1975 (Araneae: Palpimanidae); e) lateral view of *Sicarius thomisoides* Walckenaer, 1847 (Araneae: Sicariidae), and f) lateral view of *Rumikiru* sp. nov. (Scorpiones: Bothriuridae) taken with UV light.

DISCUSSION

Species richness, similarity and singleton-species

Although this study includes only a single seasonal sampling in a representative area of the coastal desert of Chile, it provides the only updated records to date of a poorly studied taxonomic group.

The richness estimations showed high values for most arthropod groups (orders) and the steep slope of the species accumulation curves for all the study sites may be evidence of an incomplete inventory of species richness. This is a recurrent theme in studies of large taxonomic groups using different sampling scales (Legros, Rochat, Reynaud, & Strasberg, 2009; Pryke & Samways, 2009; Andersen, Lanoue, & Radford, 2010). Overall, the similarity analyses revealed comparable communities between the study sites, most of which are coastal desert shrublands (Luebert & Pliscoff, 2006).

The large number of species with only one individual (singletons) in some arthropod groups (see Table 2) suggests the existence of communities with rare species restricted to environments with unique environmental and vegetation conditions embedded in a vast arid matrix. Another possible explanation for this may be the lack of balance in the dynamics of these communities (Coddington, Gnarsson, Miller, Kuntner, & Hormiga, 2009; Richardson & Arias-Bohart, 2011) due to the pressure of predators and parasitoids on common species, the species' grouped distributions (due to the reproductive structure of arthropod species and the uneven distribution of resources) (Longino, Coddington, & Colwell, 2002), or the changing interannual weather conditions, e.g., ENSO years (EL Niño-Southern Oscillation), non-ENSO years, or La Niña years (Azarbayjani, Burgin & Richardson, 1999; Cepeda-Pizarro et al, 2005a, 2005b). Under these conditions, species richness estimates play an increasingly important role in biological and conservation inventories (Cardoso, Gaspar, Pereira, Silva, Henriques, da Silva, & Sousa, 2008; Shen & He, 2008; Schoeman, Nel, & Soares, 2008; Legros et al, 2009; Alfaro & Pizarro-Araya, 2017).

New species and endemisms

One of the most striking findings regarding the arthropod assemblage of La Chimba National Reserve made during the recent survey was the discovery of a new species of scorpion from the genus *Rumikiru* (see Fig. 5). This genus is endemic to northern Chile. It currently includes only two species from a narrow area 200 km in length, extending from central Atacama to the south of the Antofagasta Region. All known species of this genus are remarkable among the family Bothriuridae, because they are lithophilous, inhabiting exclusively in rocky environments of the western slopes of the Chilean "Cordillera de la Costa" (Ojanguren-Affilastro, Ochoa, Mattoni, & Prendini, 2012). This genus is closely related to the genus *Mauryius* Ojanguren-Affilastro & Mattoni 2017 from central Argentina and *Pachakutej* Ochoa, 2004 from southern Peru, and seems to be a relic from ancient fauna that occupied most of the southern South American Andes, and its area of influence, before its last uplift and the consequent extreme aridification of the area (Ojanguren-Affilastro et al, 2012). The presence of this species in this area extends the distribution of the genus about 300 km northward, duplicating its previous known distribution, and projecting it towards a much more arid area. Additionally, due to the high degree of endemicity and unique distribution of the species of the group, this species is likely endemic to the area of La Chimba National Reserve or from an area slightly larger than the reserve itself, since the conditions in which it has been collected are unique to this area and until now it has not been collected in the neighboring protected area of Morro Moreno National Park (Pizarro-Araya *pers. obs.*).

The presence of other arthropod species, such as the scorpion species recently described (*Brachistosternus chimba*), a new solifuge from the family Ammotrechidae, and a new Filistatid spider, were expected since these are common groups in the deserts of northern Chile, and because there were no previous surveys of these groups in the area. Most probably, these findings also represent species with very narrow distributions, limited to the area of the reserve and neighboring areas with similar environments. In the case of the new scorpion from the genus *Brachistosternus*, it has not been collected in the Morro Moreno National Park, *ca* 20 km west from La Chimba National Reserve (Pizarro-Araya *pers. obs.*), nor in the Paposo Norte reserve, located some 100 km to the south, so we can assume that its presence may be, at most, restricted to this area. More field research would be necessary to clearly establish the distribution of all these species. The case of the Scutigeromorpha is also interesting since its most closely related species were collected in central Chile (Andrés Porta *pers. com.*).

All the above findings show that La Chimba National Reserve has a fauna that is unique to this area and very geographically restricted. Besides, this is the only protected area where this particular arthropod community is under some kind of protection since the nearby Morro Moreno National Park and Paposo Norte Natural Monument host communities that are clearly different.

Current threats

Although Chile has recently made some positive advances in terms of the allocation of new protected areas through private donations, and nowadays more than 20% of the country is protected (Terram, 2018), there is still a big differentiation between the designation of protected areas in the north and south of the country. Even though regions in Patagonia, like Magallanes, have more than 50% of their territory under conservation, in the north, places like the Antofagasta Region only have around 3% of their land designated as protected areas (MMA, 2015). Moreover, governmental funding for protected areas is scarce nationwide, with less than USD$2 per hectare every year (FAO, 2010), placing Chile in 9th place among the most under-financed countries in terms of biodiversity conservation worldwide (Waldron et al, 2013). This situation directly affects the capacity of protected areas to achieve the conservation objectives for which they were conceived, and in the case of La Chimba National Reserve, it has implied the absence of park rangers for almost three decades, leading to anthropic damage due to garbage accumulation, gravel extraction, illegal burnings, among others. Moreover, La Chimba National Reserve is located only 700 meters from Antofagasta’s former city dump, one of the biggest of South America, which operated without major sanitary protocols for five decades, until December 2019, generating a complex environmental and social context for conservation to occur. Despite this complex environmental context, recent studies conducted by the FIC-R project have identified the presence of numerous native and endemic species of vascular flora, lichens and vertebrates, confirming the existence of 352 native species (Mora-Carreño, Faúndez, Vargas, Pizarro-Araya, Alfaro, Ojanguren-Affilastro, Mella, Tejeda, & Villalobos, 2022). However, considering recent evidence showing the decrease of rainfall frequency and cloud cover in the coastal region of northern Chile (Schulz, Aceituno, & Richter, 2011), both vital for the maintenance of fog oases ecosystems (Larraín, 2007), it might be expected that the survival of species occurring in La Chimba National Reserve might be highly compromised if these signs of climate change continue to increase (Pinto, 2007a), jeopardizing the survival of more native and endemic species. An example of this worrying signs of climate change is the high mortality rate of the columnar cacti *Eulychnia iquiquensis*, recorded with 70% of dead individuals during botanist surveys (Pinto, 2007b; Faúndez, 2022). These cacti are considered a keystone species for fog oases in the north of Chile due to its capacity to accumulate water from the fog, contributing with water supply for other native species occurring nearby (Pinto, 2014). The habitat perturbation and degradation (conditioned by climate and anthropic factors) observed in La Chimba National Reserve may pose a hidden threat to the arthropod communities that depend upon the availability and seasonality of primary resources.

Threatened species and local experiences

Since 2005 the Chilean Ministry of Environment has been initiating species listing processes based on a review of the species status through the "Reglamento para la clasificación de especies silvestres" (Regulations for the classification of wildlife species). These listing processes are intended to assess the level of threat to biological diversity with the goal of focusing resources on threatened species, developing conservation programs, promoting research on these species, and considering these species in land management efforts (MMA, 2021).

In recent years, several arthropod species have been categorized at a national level based on the restricted geographic distribution criterion proposed by the IUCN (International Union for Conservation of Nature). As part of the classification processes, the level of endemism and the species' habitat have also been of great importance (Jerez, Zuñiga-Reinoso, Muñoz-Escobar, & Pizarro-Araya, 2015; Le Breton et al, 2019).

Considering the current threats faced by this national reserve, we proposed three insect species for the 17th Process for the Classification of Species, namely, *Uretacris lilai*, *Entomochilus wilsoni,* and *Nycterinus* (*Paranycterinus*) *penai* (see Fig. 5), all of them restricted-range and endemic species of the Coastal desert of Tocopilla, a vegetation unit that is only represented in La Chimba National Reserve among all the currently protected areas of Chile (Gajardo, 1993; CONAF, 1995). As a result, the three species proposed were considered as threatened with extinction by the Classification Committee: *Uretacris lilai* was classified as Endangered, while *Entomochilus wilsoni* and *Nycterinus* (*Paranycterinus*) *penai* were both classified as Critically Endangered. Overall, these and other recent species classifications highlight the presence of 13 species in risk of extinction occurring in La Chimba National Reserve (Mora-Carreño et al, 2022).

Conclusion and management recommendations

Complementary to the findings on the arthropod assemblage registered in La Chimba National Reserve, results for other surveys of different taxa conducted by the FIC-R project have confirmed remarkable richness of vascular flora, lichens and vertebrates (Mora-Carreño et al, 2022), some of them at risk of extinction, highlighting the importance to establish effective management and conservation measures in the short term. On the one hand, allocating more financial resources for the management of protected areas in the Antofagasta Region might help enforce the regulations established for protected areas, preventing numerous anthropic actions that nowadays threaten La Chimba National Reserve (Pinto, 2007a; Dillon, Leiva-González, & Quipuscoa-Silvestre, 2007; MMA, 2015). Moreover, establishing monitoring programs for the diverse taxa occurring in the protected area might be crucial to have a better understanding of the ecological processes related with climate change (Schulz et al, 2011) that seem to be silently affecting La Chimba National Reserve (Pinto, 2007a; Mora-Carreño et al, 2022). In this regard, establishing restoration/reforestation programs like those successfully established by Pinto (2014) for keystone species (*Eulychnia iquiquensis*) in other fog oases located in the north of Chile, might have positive cascade effects and be of great value to contribute to the preservation of the unique biodiversity of La Chimba National Reserve.

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Phylogenetic and Haplotype Analyses Offer Insights Towards Conservation of *Pteroptyx tener* Olivier, 1907 (Coleoptera: Lampyridae) Populations from Malaysia

Nur-Athirah ABDULLAH[1a] Madihah HALIM[1b] Mahadimenakbar DAWOOD[2]
Izfa Riza HAZMI[1c] Shahril Mohd. HUSIN[3] Norela SULAIMAN[1d]
Salmah YAAKOP[1*]

[1]Centre of Insect Systematics, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, MALAYSIA
[2]Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, MALAYSIA
[3]TNB Research Sdn. Bhd., No. 1, Lorong Ayer Hitam, Kawasan Institut Penyelidikan, 43000, Kajang, Selangor, MALAYSIA
e-mails: [1a]atyrahabdullah@yahoo.com, [1b]wanmadywanhalim@gmail.com
[2]menakbar@ums.edu.my, [1c]izfahazmi@ukm.edu.my, [3]shahril.husin@tnb.com.my
[1d]vozela@ukm.edu.my, [1*]salmah78@ukm.edu.my
ORCIS IDs: [1a]0000-0002-2239-6152, [1b]0000-0002-5983-1621, [2]0000-0002-7438-5906, [1c]0000-0003-0933-7083, [3]0000-0003-3593-5642, [1d]0000-0002-1317-9957, [1*]0000-0002-2998-8716
*Corresponding author

ABSTRACT

The synchronous-flashing firefly, *Pteroptyx tener* Olivier, 1907 is a congregating firefly with high economic value for the ecotourism industry. However, the populations of *P. tener* are increasingly being threatened due to habitat destruction. The assessment of genetic variation within and among populations of the species from Malaysia might play a major role in conservation and management programs. Objectives of this study were to investigate the relationships among *P. tener* from five populations in Malaysia (Peninsular Malaysia and Borneo (Sabah) using cytochrome oxidase subunit I (*COI*), and to obtain its haplotype information. A total of 50 sequences of *P. tener* were used in the Neighbour-Joining (NJ), Maximum Parsimony (MP), and Bayesian Inference (BI) phylogenetic analyses and successfully formed several distinct clades of West and East Malaysia (East Coast and West Coast of Peninsular Malaysia) with moderately to highly supported bootstrap values and posterior probabilities. Several haplotype analysis parameters are presented; low nucleotide diversity (PiT, 0.01893), haplotype number (Hn, 19), and high haplotype diversity (Hd, 0.89469). Our findings assumed that the geographical isolation due to the inability for flying long distances without help by the wind and flooding in tidal rivers. The data

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presented here are important genetic information on the *P. tener* populations and are important for the conservation programme in Malaysia by conducting the breeding program among populations that have similar genetic information.

Keywords: COI, beetle, conservation, mangrove, mtDNA, Luciolinae, Malaysia

INTRODUCTION

Pteroptyx tener is a species of congregating fireflies that flash synchronously with the purpose of finding mating partners. This species is commonly found in Malaysia (Jusoh, Ballantyne, & Chan, 2013) as well as the southern part of Thailand (Sriboonlert, Swatdipong, Wonnapinij, Teerasak, & Thancharoen, 2015). *Pteroptyx tener* shows high affinity towards *Sonneratia caseolaris*, a tree commonly found in mangrove area of tropical regions such as Peninsular Malaysia (Shahara, Nura Adila, Maimon, & Norela, 2017). The flashing of *P. tener* is generated through a chemical reaction catalyzed by luciferase (Nur Khairunnisa, Nurul Wahida, & Norela, 2016). The flashing of fireflies as they congregate on trees at the riverbank creates a spectacular view at night. This unique beetle is an important ecotourism asset for Malaysia as it captivates many foreign and local tourists alike. The Kampung Kuantan Firefly Park in Kuala Selangor has received a total of 57,368 visitors in 2018 (Kuala Selangor District Council 2019). *Pteroptyx tener* has become a huge commodity for Malaysia, generating income for the local communities through their ecotourism programs.

Anthropogenic activity near the firefly habitat has a huge impact on these populations. Through study and population monitoring, *P. tener* has been observed to have declined during the previous few decades (Seri, Abd Rahman, & Kassim, 2021). This happened as the mangrove areas were extensively converted for other land uses such as agriculture, aquaculture and urbanization (Jusoh & Hashim, 2012; Prasertkul, 2018). The effect is especially inevitable when it concerns land clearing through the logging of mangrove trees, pollution of river by petrol and diesel by-product as well as agricultural chemicals and pesticides. There is also an obvious effect of artificial light on firefly populations as the communication between individuals is being disturbed. Treatment of artificial light on *Photinus* firefly by Owens & Lewis (2021) for example, has led to change in its flashing pattern leading to suppressed courtship activity. Artificial lighting near *P. tener* habitat may have threatened the population to local extinction. This shows the urgent need for conservation of these firefly population.

Study of fireflies in Malaysia involves a lot of firefly monitoring in various localities. Molecular studies have also been done on Malaysian mangrove fireflies to delineate different firefly species in an effort to assist in firefly species identification (Jusoh, Hashim, Saaksjarvi, Adam, & Wahlberg, 2014; Jusoh, Ballantyne, & Chan, 2020). It is also important to note that molecular genetics may help in conservation efforts (Frankham, 2010). With the rate of urbanisation occurring around the natural habitat of *P. tener*, it is very important to have a better understanding of the genetic information of each population through *COI* phylogeny and the haplotype analyses. It is because the conservation measure by implementing the genetic of the *P. tener* may be proposed which is important for conservation and species exploitation by presenting the information on phylogeography and evolution of the species (Wang, 2020). Intraspecific genetic variation depicted by genetic diversity value is a fundamental parameter in conservation practice. A unit within populations with higher genetic diversity will have greater tolerance against human perturbations (Dincă et al, 2018). The molecular genetic approach allows for the determination of significant units within species that are genetically strong to adapt in a changing environment. This may facilitate the conservation effort of *P. tener* where a specific unit within the populations can be selected for the reintroduction of this species to areas from which it has been lost. Moreover, the study on the population genomics of fireflies able to estimate the capacity of populations to evolve and adapt in response to environmental change and manage adaptive variation which was important in a long run conservation efforts (Hohenlohe, Funk, & Rajora, 2021). Hence, the aim of this study is to investigate the phylogeny of the *P. tener* populations from different regions in Malaysia, and to obtain haplotype data and a haplotype network to visualize and support the population relationships. Therefore, the breeding program among populations that having similar genetic information can be conducted effectively.

MATERIAL AND METHODS

Sampling sites

Fresh samples of *P. tener* individuals were collected from East Malaysia (Sungai Klias, Beufort, Sabah; 5°27’07.02”N 115°38’25.10”E, West Coast of Peninsular Malaysia (Sungai Sepetang, Taiping, Perak; 4°54’6.56”N 100°39’35.38”E and Sungai Rembau, Rembau, Negeri Sembilan; 2°26’31.31”N 102° 3’43.60”E) and East Coast of Peninsular Malaysia (Sungai Chukai, Kemaman, Terengganu; 4°18’3.93”N 103°22’58.51”E and Sungai Johor, Kota Tinggi, Johor; 1°39’18.35”N 103°55’37.90”E (Fig. 1). Samples were collected from November 2018 through May 2019. Each set of ten consisted of five males and females were sampled from each location which totalled 50 *P. tener* individuals altogether. The list of samples and localities is in Table 1. The samples were preserved in 70% alcohol to maintain its DNA and to avoid contamination.

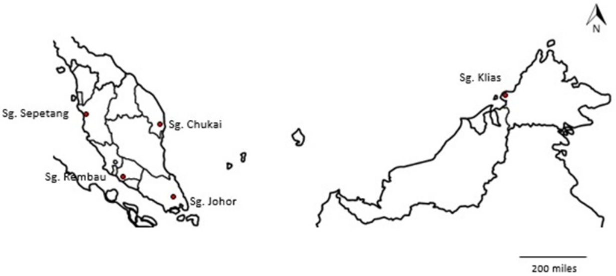

Figure 1. Localities of *Pteroptyx tener* collected in Peninsular Malaysia and Borneo (Sabah).

Table 1. Details of *Pteroptyx tener* used in the study, voucher code, GenBank accession numbers and

sampling locality.

No	Code	GenBank Accession No.	Locality
1	TF1	MN199184	East PM: Terengganu: Kemaman, Sungai Chukai
2	TF2	MN199185	East PM: Terengganu: Kemaman, Sungai Chukai
3	TF3	MN199186	East PM: Terengganu: Kemaman, Sungai Chukai
4	TF4	MN199187	East PM: Terengganu: Kemaman, Sungai Chukai
5	TF5	MN199188	East PM: Terengganu: Kemaman, Sungai Chukai
6	TM1	MN199189	East PM: Terengganu: Kemaman, Sungai Chukai
7	TM2	MN199190	East PM: Terengganu: Kemaman, Sungai Chukai
8	TM3	MN199191	East PM: Terengganu: Kemaman, Sungai Chukai
9	TM4	MN199192	East PM: Terengganu: Kemaman, Sungai Chukai
10	TM5	MN199193	East PM: Terengganu: Kemaman, Sungai Chukai
11	JF1	MN199194	East PM: Johor: Kota Tinggi, Sungai Johor
12	JF2	MN199195	East PM: Johor: Kota Tinggi, Sungai Johor
13	JF3	MN199196	East PM: Johor: Kota Tinggi, Sungai Johor
14	JF4	MN199197	East PM: Johor: Kota Tinggi, Sungai Johor
15	JF5	MN199198	East PM: Johor: Kota Tinggi, Sungai Johor
16	JM1	MN199199	East PM: Johor: Kota Tinggi, Sungai Johor
17	JM2	MN199200	East PM: Johor: Kota Tinggi, Sungai Johor
18	JM3	MN199201	East PM: Johor: Kota Tinggi, Sungai Johor
19	JM4	MN199202	East PM: Johor: Kota Tinggi, Sungai Johor
20	JM5	MN199203	East PM: Johor: Kota Tinggi, Sungai Johor
21	SF1	MN199204	East Malaysia: Sabah, Beufort, Sungai Klias
22	SF2	MN199205	East Malaysia: Sabah, Beufort, Sungai Klias
23	SF3	MN199206	East Malaysia: Sabah, Beufort, Sungai Klias
24	SF4	MN199207	East Malaysia: Sabah, Beufort, Sungai Klias
25	SF5	MN199208	East Malaysia: Sabah, Beufort, Sungai Klias
26	SM1	MN199209	East Malaysia: Sabah, Beufort, Sungai Klias
27	SM2	MN199210	East Malaysia: Sabah, Beufort, Sungai Klias
28	SM3	MN199211	East Malaysia: Sabah, Beufort, Sungai Klias
29	SM4	MN199212	East Malaysia: Sabah, Beufort, Sungai Klias
30	SM5	MN199213	East Malaysia: Sabah, Beufort, Sungai Klias
31	NF1	MN199214	West PM: Negeri Sembilan, Rembau, Sungai Rembau
32	NF2	MN199215	West PM: Negeri Sembilan, Rembau, Sungai Rembau
33	NF3	MN199216	West PM: Negeri Sembilan, Rembau, Sungai Rembau
34	NF4	MN199217	West PM: Negeri Sembilan, Rembau, Sungai Rembau
35	NF5	MN199218	West PM: Negeri Sembilan, Rembau, Sungai Rembau
36	NM1	MN199219	West PM: Negeri Sembilan, Rembau, Sungai Rembau
37	NM2	MN199220	West PM: Negeri Sembilan, Rembau, Sungai Rembau
38	NM3	MN199221	West PM: Negeri Sembilan, Rembau, Sungai Rembau
39	NM4	MN199222	West PM: Negeri Sembilan, Rembau, Sungai Rembau
40	NM5	MN199223	West PM: Negeri Sembilan, Rembau, Sungai Rembau
41	PF1	MN199224	West PM: Perak, Taiping, Sungai Sepetang
42	PF2	MN199225	West PM: Perak, Taiping, Sungai Sepetang
43	PF3	MN199226	West PM: Perak, Taiping, Sungai Sepetang
44	PF4	MN199227	West PM: Perak, Taiping, Sungai Sepetang
45	PF5	MN199228	West PM: Perak, Taiping, Sungai Sepetang
46	PM1	MN199229	West PM: Perak, Taiping, Sungai Sepetang
47	PM2	MN199230	West PM: Perak, Taiping, Sungai Sepetang
48	PM3	MN199231	West PM: Perak, Taiping, Sungai Sepetang
49	PM4	MN199232	West PM: Perak, Taiping, Sungai Sepetang
50	PM5	MN199232	West PM: Perak, Taiping, Sungai Sepetang

Species identification

The samples were identified to the species level by examining morphological characters under a microscope (Stereomicroscope Stemi 4). Identification was done by referring to Ballantyne (1987) and Jusoh, Ballantyne, Lambkin, Hashim, & Wahlberg (2018).

DNA extraction and PCR amplification

DNA was extracted from adult firefly samples collected from the field, using Nucleospin DNA Insect Kit (Düren, Germany) according to the manufacturer's protocol. Firefly specimens had a small incision done in their abdomen in order to expose the tissue for DNA extraction. Standard DNA extraction protocols and the methods of Syarifah-Zulaikha, Halim, Aman, & Yaakop (2021) were followed. A 750 bp sequence of *COI* was amplified via a polymerase chain reaction (PCR) using Mastercycler Nexus (Eppendord North America, Inc). Primer regions used for *COI* were HCO2198 5'- TAA ACT TCA GGG TGA CCA AAA AAT CA-3 and LCO1490 5'- GGT CAA CAA ATC ATA AAG ATA TTG G-3 (Folmer et al. 1994). The PCR was carried out by using a profile with 12.5 µl of Green Mastermix, 7.5 µl of DDH_2O, 3µl of template DNA, and 1.5µl of primer region each from forward and reverse site with the total quantity of 25 µl. The parameters for the PCR process were as follows: 3 minutes of 95 ^{o}C pre-denaturation, 30 cycle of denaturation for 30 seconds at 95 ^{o}C, 1 minute of annealing at 47 ^{o}C, 30 seconds of elongation at 72 ^{o}C and 10 min of final elongation at 72 ^{o}C (Halim, Aman-Zuki, & Yaakop, 2017). PCR products were visualized via electrophoresis on a 1.5% agarose gel.

Sequencing, BLAST and BOLD analyses.

All PCR products of the fireflies were then sent to Apical Sdn. Bhd., Petaling Jaya, Selangor, Malaysia for sequencing. The sequences were manually edited using BioEdit version 7.0.4 (Hall, 1999). The Barcode of Life Data System (BOLD) and Basic Local Alignment Search Tool (BLAST) were applied for comparisons to database sequences (Altschul, Gish, Miller, Myers, & Lipman, 1990) based on several criteria namely expected value, maximum identical, total score, query coverage and maximum score.

Phylogenetic analysis (NJ, MP and BI)

Fifty sequences were analyzed using three phylogenetic analyses which are the Neighbour-Joining (distance criterion), the Maximum Parsimony (parsimony criterion) and the Bayesian Inference (likelihood criterion). For the NJ tree, the Kimura-2- Parameter model was selected for phylogenetic reconstructions. The analysis was constructed by undergoing 1000 bootstrap replications to obtain the bootstrap confidence level. Bayesian tree was generated using MrBayes 3.1.2 software (Ronquist et al. 2012). Prior to tree generating with Bayesian inference, the best nucleotide substitution model was chosen using jModeltest 2.1.4 (Posada, 2008). The robustness of the tree was estimated using bootstrap analysis of 1000 replications; all codons containing gaps or missing data were eliminated. The NJ, MP and BI trees were constructed with the out group of *Luciola cruciata* as the closest related genus to *Pteroptyx* species based on Cheng et al (2019).

Genetic distance and haplotype analyses

Genetic distance between firefly populations from different locations was analyzed using MEGA 7.0 through Kimura-2-Parameter (K2P). Haplotype analysis was conducted on each *P. tener* sequence using DNA Sequence Polymorphism (DnaSP) version 5.10.01 (Librado & Rozas, 2009). The distribution of haplotypes was mapped through Maximum Spanning Network (MNS) in Network 5.0

RESULTS

Sequence variation and genetic distance

A total of 50 samples of *P. tener* were successfully extracted in this study and a 750 bp sequence of *COI* was successfully amplified from each individual. Haplotype diversity differed amongst locality where the lowest was in the east coast region of Penisular Malaysia which is in Sungai Chukai, Terengganu while the highest was in west coast region of Peninsular Malaysia which is Sungai Sepetang, Perak. Correspondingly, the average pairwise distance within population and variation in nucleotide diversity reflect the same trend as haplotype diversity (Table 2). Meanwhile, average pairwise distance among population showed the lowest genetic distance (0.003) between Sungai Chukai-Sungai Johor and Sungai Rembau-Sungai Sepetang while the highest genetic distance was shown between Sungai Sepetang-Sungai Chukai (0.038) (Table 3).

Table 2. Estimates of genetic diversity amongst locality based on different genetic parameter.

Locality	**N**	**h**	**Hd**	**π**	**i**
Sungai Chukai, Tererengganu	10	2	0.20000	0.00031	0.0003
Sungai Johor, Johor	10	3	0.37778	0.00061	0.0006
Sungai Klias, Sabah	10	3	0.37778	0.00061	0.0006
Sungai Rembau, Negeri Sembilan	10	3	0.37778	0.00085	0.0009
Sungai Sepetang, Perak	10	9	0.97778	0.00385	0.0039

Note: N= number of individuals, h= number of haplotypes, Hd- haplotype diversity, π = nucleotide diversity, i = average pairwise distance within population

Table 3. Average pairwise distance among populations of *Pteroptyx tener* in Peninsular Malaysia and Borneo (Sabah).

Locality	**1**	**2**	**3**	**4**	**5**
Sungai Chukai, Tererengganu					
Sungai Johor, Johor	0.003				
Sungai Klias, Sabah	0.011	0.011			
Sungai Rembau, Negeri Sembilan	0.037	0.034	0.032		
Sungai Sepetang, Perak	0.038	0.034	0.033	0.003	

Phylogenetic analysis

For the NJ tree (Fig. 2), the bootstrap values ranged from 63 to 100. The NJ tree was separated into three clades (A, B, C) which consisted of *P. tener* individuals from different population. The three clades were supported by moderate to high bootstrap values of 79, 95 and 98% respectively. Clade A includes the populations of *P. tener* from Sungai Chukai (Terengganu) and Sungai Johor (Johor). *P. tener* populations were separated in Clade B while *P*. *tener* from Sungai Rembau (Negeri Sembilan) and Sungai Sepetang (Perak) were grouped in Clade C (Fig. 2). The topologies of the MP (Fig. 3) and BI (Fig. 4) trees were similar where the trees were separated into three large clades. Clade A includes *P. tener* population from Sungai Rembau (Negeri Sembilan) and Sungai Sepetang (Perak) while Clade B includes population of *P. tener* from Sungai Klias (Sabah) while Clade C grouped population of *P. tener* from Sungai Johor (Johor) and Sungai Chukai (Terengganu).

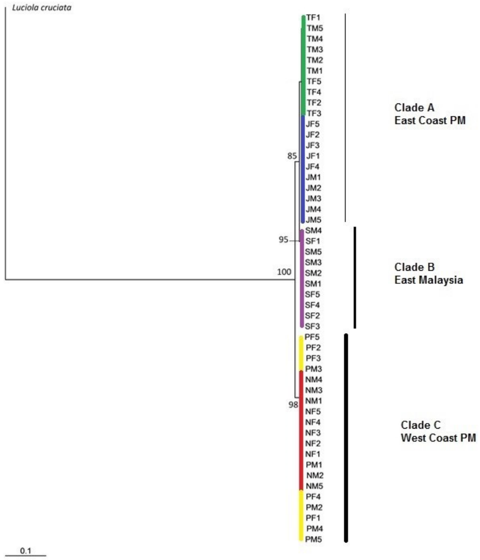

Figure 2. Phylogenetic tree of Neighbour Joining (NJ) analysis of *Pteroptyx tener* using *COI* marker. Bootstrap value was shown on branch of the tree (1000 replications).

Haplotype analyses

A total of 19 haplotypes consisted of 34 nucleotides have been discovered from this study. Populations of *P. tener* in Sungai Johor (Johor), Sungai Rembau (Negeri Sembilan) and Sungai Klias (Sabah) recorded three haplotypes. There were two haplotypes in Sungai Chukai (Terengganu) while Sungai Sepetang (Perak) recorded nine haplotypes (Table 4). The haplotype analysis presented; low nucleotide diversity (PiT, 0.01893), and haplotype number (Hn, 19) with high haplotype diversity (Hd, 0.89469).

Table 4. Haplotype distribution of *Pteroptyx tener* populations using *COI* marker in Peninsular and Malaysia and Borneo (Sabah).

Locality	**Haplotype**																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Sungai Sepetang									1			1	1	1	2	1	1	1	1
Sungai Johor			1	8	1														
Sungai Chukai	1	9																	
Sungai Rembau									8	1	1								
Sungai Klias						1	8	1											

Based on the minimum spanning network (MNS) analysis, there were 18 unique haplotypes comprised of Hap1 and Hap2 (Chukai), Hap3, Hap4 and Hap5 (Johor), Hap6, Hap7 and Hap8 (Klias), Hap10 and Hap11 (Rembau), Hap12, Hap13, Hap14, Hap15, Hap16, Hap17, Hap18 and Hap19 (Sepetang). Only Hap9 was being shared between Sepetang and Rembau showing mixture of *P. tener* population from Sungai Sepetang (Perak) and Sungai Rembau (Negeri Sembilan) (Fig. 5).

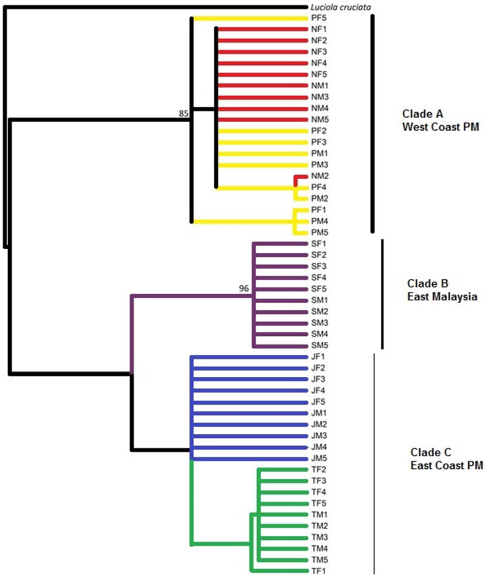

Figure 3. Phylogenetic tree of Maximum Parsimony (MP) analysis of *Pteroptyx tener* using *COI* marker. Bootstrap value was shown on branch of the tree (1000 replications).

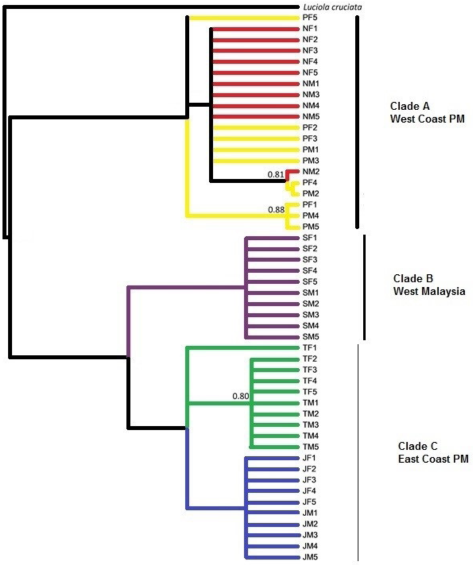

Figure 4. Phylogenetic tree of Bayesian Inference (BI) analysis of *Pteroptyx tener* using *COI* marker. Posterior probabilities value was shown on branch of the tree.

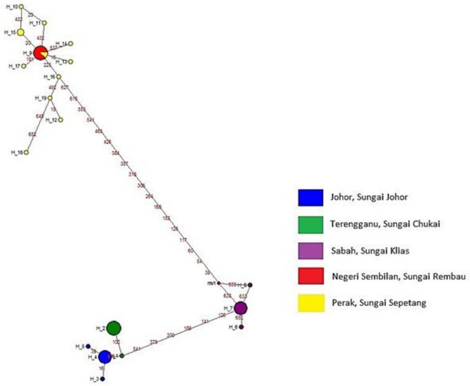

Figure 5. Minimum Spanning Network (MSN) based on sequence analysis of *Pteroptyx tener* using *COI* marker. The size of each circle corresponds to its relative frequency in the total sample.

DISCUSSION

Phylogenetic and haplotype analyses have been conducted on *P. tener* populations from Malaysia using *COI* marker. Lee et al, (2003) also used *COI* to successfully separate populations of *Pyrocoelia rufa* on Jeju do Island, Korea and Tsushima Island, Japan. The use of *COI* data also helped in the identification of various firefly species of the genus *Pteroptyx,* which revealed the existence of cryptic species (Jusoh et al, 2020). This study presents the NJ, MP and BI trees of *COI* sequences of *P. tener* from Malaysia, while *Luciola cruciata* (Luciolinae) was chosen as an outgroup in order to construct comprehensive phylogenetic trees by referring to the previous phylogenetic studies of fireflies (Cheng, Munian, Tan, Mohd Azahari, & Shah-Fadir, 2019).

All trees showed a distinct separation of *P. tener* populations from the West Coast of Peninsular Malaysia in Sungai Sepetang (Perak) and Sungai Rembau (Negeri Sembilan, and the East Coast populations from Sungai Chukai (Terengganu) and Sungai Johor (Johor). The high average pairwise distance between Sungai Sepetang (Perak) and Sungai Chukai (Terengganu) was also manifested in coherent with geographical distance between east coast and west coast. The population of *P. tener* from Sungai Klias (Sabah) was also clearly separated from the Peninsular area. This study is in coherent with Cheng et al (2019) which detected three distinct populations of *P. tener* in Malaysia, one each from east and west coast of Peninsular Malaysia as well as Borneo. There were also numerous other studies showing similar geographic separations in other taxa (Yusof et al, 2016; Ith et al, 2016; Chan, Abraham, Grismer, & Grismer, 2018).

According to Thancharoen (2021), the genetic differentiation of fireflies can be caused by the limitation of dispersal activity, which has a higher probability of reproductive isolation. For example, the discontinuous habitats of desert firefly, *Microphotus octarthrus* that has winged males and apterous larviform females have resulted in genetic isolation (Usener & Cognato, 2005). The isolation of populations in Peninsular was often hypothesized due to the Titiwangsa mountain range acting as a physical barrier that limits the movement of organisms. This isolation was observed in insect species such as *Anopheles* mosquitoes (Sum et al, 2014), and *Coptotermes* termites (Cheng et al, 2014) Nevertheless, since *P. tener* is highly associated with mangrove areas, it was also suggested that the distribution of this species was due to the ancient river network during Pleistocene era (Cheng et al, 2019). However, isolation over distance was not applied to the populations of *P. tener* in Sungai Rembau and Sungai Sepetang. The distance shown to be high at 0.034 suggesting other factors affecting its population structure. As such, it is important to note that the dispersal of *P. tener* may occur through the semi-aquatic larval movement. The female *P. tener* lay their eggs on the wet soil of the riparian area. The hatching larvae of this firefly will easily be dispersed through irrigations of river channels especially during high tide. It has been reported that the eggs of aquatic firefly *Sclerotia aquatilis* in Thailand were found on aquatic vegetation which can be transported with the flow of water (Lewis et al, 2020). Thus, there is a higher possibility for the *P. tener* to establish in a new area if a huge number of larvae survives in the flow of the river.

Apart from the geographical factor, the mtDNA gene flow of *P. tener* may be limited by its flight ability. Considering the small body size of *P. tener*, the dispersal through flight may only occur over short distances explaining the lower average pairwise distance between population in Sungai Sepetang and Sungai Rembau. This mtDNA gene flow is hampered due to *P. tener*'s flight ability as most small insects are easily affected by wind speed (Gatehouse, 1997). As Peninsular Malaysia is subjected to monsoons during long periods of the year (Southwest Monsoon from April to September and Northeast monsoon from October to March), *P. tener* will not be able to overcome the strong moving air to move a larger distance thus limiting its movement.

The haplotypes distribution also corresponds to the others results of this study. Haplotypes from different geographic regions occupied different and non-overlapping parts of the minimum-spanning network suggesting strong phylogeographic structuring. Every sampling site revealed locality-specific haplotype reflecting high diversity even at a small scale. There were 18 unique haplotypes while only one haplotype was being shared between population in Sg. Sepetang and Sg. Rembau. Spatially close populations have a higher tendency sharing haplotypes. For instance, all individuals of *Exocelina manokwariensis* (Dytiscidae) from two different populations shared the same haplotype as the populations located near each other (Lam et al, 2018).

On the other hand, unique haplotypes reflect variation in populations where they do not overlap with other individuals from different populations. *Pteroptyx tener* can be considered as a specialist by referring to its strong association to mangrove habitat, a resource that is distributed rather patchily across Peninsular Malaysia and Sabah. Thus, as weak dispersers, the isolation of extant populations was further emphasized. A similar situation occurred in a deciduous tree specialized beetle, *Osmoderma* (Coleoptera: Scarabaeidae) for which its distribution is highly affected by distribution of its resource (Landvik et al, 2017). All of the information is important in managing populations of congregating fireflies in this region.

From the results, it shows that in conservation and management efforts for *P. tener*, it is important to consider each population in Malaysia as a single genetical unit since there is only scarce overlap between populations of different localities. The populations of *P. tener* are considered unique as they become regionally adapted. As such, the unique populations tend to be very sensitive towards environmental changes (Allendorf, Luikart, & Aitken, 2013). Nevertheless, variations shown in the results were based on a single mitochondrial marker. It is suggested for additional studies to be done using different markers with the possibility of discovering other variations.

The population of *P. tener* in Sungai Chukai requires more attention in respect to the low haplotype diversity in the area. As the population becomes isolated with low gene flow, the rate of inbreeding will increase. Thus, at any influence of genetic drift with detrimental effect, population may experience regional extinction (Allendorf, Luikart, & Aitken, 2013; Allendorf et al, 2013). However, looking at the current status of land use around the area, Sungai Chukai is still largely surrounded by natural mangrove forest habitat which indirectly safeguard the firefly population for the time being. It is suggested for mangrove area around Sungai Chukai to be protected by law in prevention of land use changes which can be detrimental in effect.

In relation to the future prospect of introducing this species to a new area, the population of *P. tener* in Sungai Sepetang will have a higher chance to sustain in reference to its high genetic diversity. It is hypothesized that *P. tener* in Sungai Sepetang will be more resilient towards change in the environment, making it possible to introduced it to a new mangrove habitat. Yet, it is more crucial to focus on protecting the local habitat of this firefly species while considering it an individual genetic pool. Given the specialization of *P. tener* towards mangrove habitats, the species is facing multiple challenges. The most serious threats to mangroves are directly and indirectly a result of increasing human population density (Romañach et al, 2018).

CONCLUSION

Based on our results, we conclude that *P. tener* may only be protected by conservation of the mangrove habitat at the local level. Thus, management and conservation practices should focus on breeding of the species. Genetic diversity and population structure studies are important to be conducted in the near future to measure precisely the genetic movement between populations and the supporting reasons.

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The Community Structure of Ants in *Hordeum Vulgare* and Grass Mixture Conditions in the Southwestern Part of Slovakia

Vladimír LANGRAF[1*] Adrián PURKART[2] Kornélia PETROVIČOVÁ[3]
Janka SCHLARMANNOVÁ[1**]

[1] Department of Zoology and Anthropology, Faculty of Natural Sciences, Constantine the Philosopher University in Nitra, Tr. A. Hlinku 1, Nitra, SLOVAK REPUBLIC
[2] Department of Zoology, Faculty of Natural Sciences, Comenius University, Ilkovičova 6, 842 15 Bratislava, SLOVAK REPUBLIC
[3] Institute of Plant and Environmental Sciences, Faculty of Agrobiology and Food Resources Slovak, University of Agriculture in Nitra, Tr. A. Hlinku 2, 94901 Nitra, SLOVAK REPUBLIC
e-mails: [1*]langrafvladimir@gmail.com, [2]purkart.adrian@gmail.com,
[3]kornelia.petrovicova@gmail.com [1**]jschlarmannova@ukf.sk
ORCID IDs:[1*]0000-0002-3839-3036, [2]0000-0002-8471-5083
[3]0000-0002-1581-2517, [1**]0000-0003-0730-6056
[1]*Corresponding Authors

ABSTRACT

Ants are an important bioindicative group that plays a significant role in agroecosystems. As a result of interspecific competition for food, they can displace native species. The aim of the research was to assess the influence of environmental variables (soil pH, soil moisture, potassium, phosphorus and nitrogen) and the influence of seasons on the dispersion of ants. Between 2018 and 2020, while investigating different types of crops, we recorded 864 individuals belonging to 9 species and 2 unspecified species (sp.). The dispersion of ants was affected by moisture, soil pH, phosphorus, potassium and nitrogen. In addition, an increase in value of the average number of individuals during spring and summer months was confirmed. We confirmed an increasing number of ant individuals with increasing values of potassium, phosphorus, nitrogen and soil moisture. A neutral pH of soil is optimal for ants. Our results yielded new information indicating that agricultural intensification negatively affects ants which are important for the production of biomass and reduces the number of pests which also affect crop yields.

Keywords: ants, abundance, agrosystems, diversity, field margins.

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INTRODUCTION

In a world with a rapidly expanding population of people, there is a growing demand for food and a simultaneous need for higher environmental sustainability. The area available for agricultural production is limited, and methods which do not compromise yields are needed. Insects are one of the largest groups of animals which play a vital part in the conservation of ecosystems, improve the health of an ecosystem, and are the critical component in the food web in both terrestrial and aquatic ecosystems (Courtney, 1994; Brygadyrenko, 2015; Faly, Kolombar, Prokopenko, Pakhomov, & Brygadyrenko, 2017; Avtaeva, Sukhodolskaya, & Brygadyrenko, 2021). Ants are one of the most ecologically dominant groups of insects in terrestrial habitats. Their ecological success can be attributed to the variety and efficiency of their foraging habits, eusocial mode of life, local abundance and the ability to adjust their activity to environmental changes (Ronque, Fourcassié, & Oliveira, 2018). They are a social insect group that carry out various roles such as predator, prey, detritivore and herbivore (Diamé, Rey, Vayssières, Grechi, Chailleux, & Diarra 2018). They vary significantly, there are almost 14,000 species found widely distributed across the earth (Bolton, 2021). They are cosmopolitan and exist across several different ecosystems, including forests, damp places, water sources and drylands. Given this presence, it is no wonder that ant diversity is used as a bioindicator to determine ecosystem and environmental changes (Gibb, et al, 2020; Oberprieler & Andersen, 2020).

Ants contribute to various ecosystem services including soil dynamics and nutrient cycling, they directly affect species composition in animal and plant communities (Toro, Ribbons, & Pelini, 2012), they represent an important component of agricultural ecosystems (Offenberg, 2015) and especially semi-natural habitats within agricultural landscapes (Marshall & Moonen, 2002). They are skilful tillers of soil, dispensers of seeds and microbial propagules, transmitters of N2-fixing bacteria, ecosystem engineers, fungi growers, waste managers, biotechnologists, pest controllers, soldiers and reproducers (Benckiser, 2007). Their densities and compositions in agricultural sites depend on human activities and are predictable in a typical agricultural land-use mosaic such as arable, fallow, grassland fields and forest sites (Dauber, 2001; Braschler, 2005; Purkart, Kollár, & Goffová, 2019). Therefore, many ant species hold desirable characteristics unshared by most other beneficials. They comprise at least one-third of all insect biomass (Hölldobler & Wilson, 2009). With such abundance, any interaction derived from this taxon holds a high potential. Most ant species are polyphagous, cooperative often with polymorphic worker forces, enabling them to deploy a wide range of prey types. They may exert pressure on several pest species and their life stages. Their territorial behaviour makes them attack and deter pests that are far beyond the size of potential prey (Manak, Nordenhem, Bjorklund, Lenoir, & Nordlander, 2013). Weaver ants (*Oecophylla smaragdina* and *O. longinoda*) control more than 50 different pests in 12 different crops. They are able to increase farmers' net income by more than 70% when substituting conventional pesticide regimes (Peng, Christian, & Gibb, 2004; Peng & Christian, 2005). Ants' predation makes them prospects for future integrated pest management strategies in agriculture (Offenberg, 2014).

The objective of this study was to analyse the community structure of ants in the conditions of *Hordeum vulg*are and *Grass mixture*. In the work, we also analysed the effects of environmental variables (soil humidity, soil pH, potassium, phosphorus and nitrogen), which might influence their abundance and population structure. The results of our work might influence for the correct setting of crop management, so that there is no disturbance to the population of ants due to their importance in agroecosystems, where they are part of the biomass and also participate in the reduction of crop pests.

MATERIALS AND METHODS

The research took place in the year 2018 to 2020 and we collected ants in two types of agricultural crops. In the winter crop of *Hordeum vulgare*, ants were collected from November to July. In the *Grass mixture*, ants were collected year-round. These types of agricultural crops were examined throughout each year, the position of crops in the fields changed every year (Klimánek, 2008). Crops were grown in a conventional way. The soil was ploughed three times and turned. Pre-sowing preparation and sowing were combined. Machines with driven working tools were used in conjunction with a seed drill. When sowing, it was possible to use seed coulters with an obtuse angle of penetration into the soil.

Study area

The study area of agricultural crops is located in the geomorphological unit of the Podunajská pahorkatina - the Danubian upland (the south-western part of Slovakia) in the cadastral territory of Nitra Fig. 1. The altitude of the monitored area was approximately 130m above sea level with a brown type of soil. The study area belongs to a warm arid climate area with mild winters (Table 1).

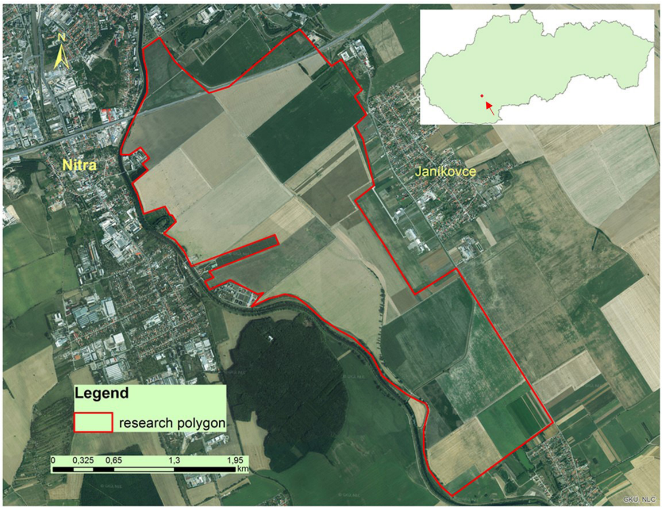

Figure 1. Map of the study area.

Table 1. Average values of temperature and rainfall.

Month	Average temperature (°C)	Average rainfall (mm)
January	−5–5	30
February	−3–6	26
March	0–12	35
April	10–20	12
May	15–22	65
June	18–27	77
July	22–29	41
August	20–29	57
September	15–23	64
October	8–15	54
November	−3–7	40
December	−5–5	55

Collection of samples and application of sprays

We used five pitfall traps for each site, which were placed in a line at a distance of 10 metres. A 4% formaldehyde solution to fix the material was used. Pitfall traps were always in the fields and were collected at two-week intervals. The nomenclature and determination of ants was established according to the work of Seifert (2018).

The insecticide FORCE (Syngenta, Basel, Switzerland), a granular insecticide in-tended for soil application to control soil pests, was applied to the crops. Insects were killed through respiratory and tactile poison ingestion. The preparation had a fast effect and a strong residual (repellent) action against a wide range of soil pests from the orders of Coleoptera, Aranea and Hymenoptera. The applied dose was administered uniformly at a concentration of 12–15 kg per ha each year for the duration of the research. Solinure FX fertiliser (Medilco Hellas S.A., Athens, Greece) containing chlorides and urea, was applied to the crops and was intended for field fertility. Due to its acidifying effect, it contributed to lowering the soil pH.

Measurement of environmental variables

At each pitfall trap location we removed stones and fallen leaves from crops and sampled the soil to a depth of 15 cm for analysis. Five samples (one from each of five sites) were taken from each field every two weeks over the three years of the study period. Subsequently, environmental variables (N, P, K, pH, moisture) were analysed using a soil moisture meter (Rapitest 3 1835, Luster Leaf, Illinois, IL, USA) and a pH meter (Dexxer PH-03, Luboň, Poland). We thoroughly wetted the broken up soil with water (ideally distilled or deionised water) to a muddy consistency. We wiped the meter probe clean with a tissue or paper towel and inserted it into the soil up to the probe base (7-10 cm). We waited one minute and wrote down the value. We converted the measured values into units of mg.

Database quality

The data obtained by research has been saved in the Microsoft SQL Server 2017 database program (Express Edition), consisting of frequency tables for collections and measured environmental variables, (pH, soil moisture, potassium, phosphorus and nitrogen). The database also consisted of code tables for study sites and their variables (crops, habitat, locality name, cadastral area, altitude and coordinates of localities). Matrices for statistical calculations using the Microsoft SQL Server 2017 were programmed.

Statistical analyses

The multivariate analysis (redundancy analysis - RDA) to determine the dependencies between objects (ants, agricultural crops and soil characteristics) was used. We tested the statistical significance of pH, soil moisture, potassium, phosphorus and nitrogen with the Monte Carlo permutation test in the CANOCO5 program (Ter Braak & Šmilauer, 2012).

Analysis in the statistical program Statistica Cz. (StatSoft Inc., 2004) focused on polynomial regression, expressing the relationship between the number of ants and the values of potassium, phosphorus, nitrogen, pH and soil moisture. The Shapiro-Wilk W-test determined the normality of data distribution. Based on the violation of the normality data distribution (p-value = 0.00), we used the nonparametric Friedman test (ANOVA). It was used to test the differences in the number of individuals between the months.

RESULTS

Over a period of three years of research, we found a total of 864 individuals belonging to 9 species and 2 unspecified species (sp.) in the studied area. Species of *Lasius niger* (83.80%) and *Tetramorium caespitum* (11.11%) had a eudominant representation of individuals, other species had subdominant to subrecendent representation (Table 2).

Table 2. Distribution of the ants in the agricultural crops during the years 2018 - 2020.

Species	**Grass mixture**	***Hordeum vulgare***	**∑ ind.**
Formica cunicularia Latreille, 1798	4	11	15
Formica rufibarbis Fabricius, 1793	0	2	2
Formica sp.	0	1	1
Lasius alienus Förster, 1850	0	1	1
Lasius niger (Linné, 1758)	305	419	724
Lasius sp.	3	3	6
Lasius umbratus (Nylander, 1846)	2	11	13
Myrmica sabuleti Meinert, 1861	0	4	4
Polyergus rufescens (Latreille, 1798)	1	0	1
Solenopsis fugax Latreille, 1798	1	0	1
Tetramorium caespitum Santschi, 1927	1	95	96
∑ individuals	317	547	864

Multivariate analysis of the ants between the years 2018 and 2020 was determined using the redundancy analysis (RDA, SD = 1.40 on the first ordination axis). We observed the relationship between ants and environmental variables (pH of the soil, soil moisture, potassium, phosphorus and nitrogen). The values of the explained variability of taxonomic data were 50.9% on the first ordination axis and 54.8% on the second ordination axis. The cumulative variability of the species set explained by environment variables was represented in the first ordination axis 88.9% and in the 2nd axis 95.7%. Using the Monte Carlo permutation test, we identified a statistically significant effect of soil moisture (p = 0.0088, F(1.0276) = 2.0021, df = 5), soil pH (p = 0.0508, F(1.1183) = 1.9297,df = 5), phosphorus (p = 0.0466, F(1.1952) = 2.0805, df = 5), potassium (p = 0.0328, F(1.7145) = 1.9620, df = 5) and nitrogen (p = 0.0490, F(1.7006) = 2.1005, df = 5) on the structure of arthropods. The selected variables were not mutually correlated with the maximum value of the inflation factor = 4.3243. The ordination graph (triplot) contained ants ordered into one cluster (Fig. 2). The first cluster (I) consisted of ants correlated with phosphorus (mg) and moisture. The *Formica rufibarbis* species has links to potassium (mg) and soil pH. *Polyergus rufescens* correlated with nitrogen (mg). *Solenopsis fugax* was not affected by environmental variables.

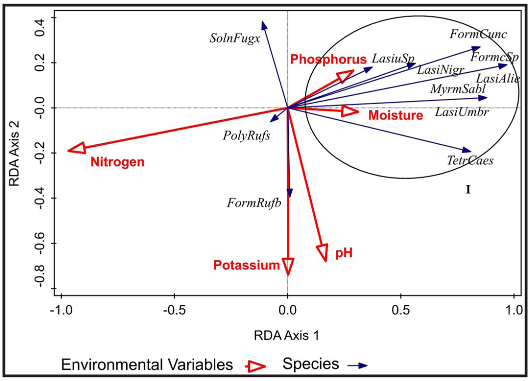

Figure 2. RDA analysis of ants with environmental variables.

The normality data distribution (number of individuals) was violated (p-value = 0.00). Based on that, a nonparametric Friedman test (ANOVA) was used to confirm the statistically significant difference (p-value = 0.04670, F (2.27) = 2.83040, df = 3) (Fig. 3) of individuals between months and crops of the *Hordeum vulgare* and *Grass mixture*. The results showed an increase in the average value of individuals in June - August in the crops *Hordeum vulgare*. Under *Grass mixture* conditions, the number of individuals increased from March to June and decreased in the following months.

The number of individual ants was processed using polynomial regression. Using the regression model, we expressed the relationship (correlation) between the number of individuals of ants and potassium (mg), phosphorus (mg), nitrogen (mg), pH and humidity (%). The correlation coefficient value was high for the number of individuals and pH (r = 0.8140) (Fig. 4, A), potassium (r = 0.9012) (Fig. 4, B), phosphorus (r = 0.8905) (Fig. 4, C), nitrogen (r = 0.7981) (Fig. 4, D) and moisture (r = 0.881) (Fig. 4, E), which indicated a strong relationship. The reliability coefficient for the pH r2 = 0.6899 indicated the capture of 68% variability, potassium r2 = 0.6908 (69% variability), phosphorus r2 = 0.7504 (75% variability), nitrogen r2 = 0.7145 (71% variability) and moisture r2 = 0.7384 (73% variability). The overall suitability of the regression model is statistically significant in all cases: pH (p-value = 0.0015), potassium (p-value = 0.0428), phosphorus (p-value = 0.0298), nitrogen (p-value = 0.0248) and moisture (p-value = 0.0118). The results showed that increasing values of potassium, phosphorus, nitrogen and soil humidity also increased the number of ant individuals. The ideal value for ants was 16 - 22 mg/kg potassium, 1.3 - 1.8 mg/kg phosphorus, 16 - 22 mg/kg nitrogen, 7 pH and 14 – 22 % for moisture.

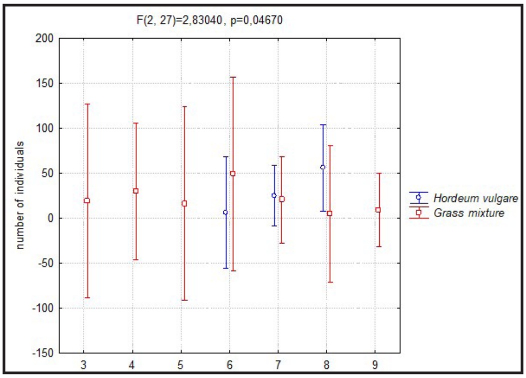

Figure 3. Friedman test (ANOVA) difference in the number of individuals between month and crops.

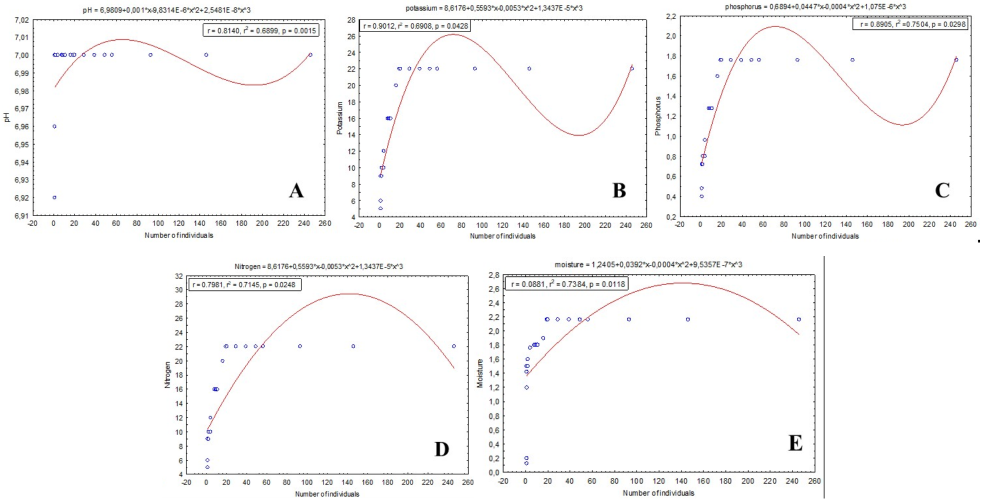

Figure 4. Polynomial regression model potassium, phosphorus, nitrogen, pH and moisture on the number of individuals of the ants.

DISCUSSION

Ants living in agricultural landscapes have a wider tolerance than ants from natural habitats. They can achieve high local density due to the influence of agriculture and field margins support the most diverse community of ants (Bote & Romero, 2012; Oliveira et al, 2012; Magura, Ferrante, & Lövei 2020). We recorded that the ant community was dominated by species *Lasius niger* and *Tetramorium caespitum*. The great abundance of ants affects the maintenance of the natural balance and substance cycle of the biogenic elements in ecosystems such as carbon, nitrogen, sulphur and phosphorus. The dominance of Hymenoptera (Formicidae) and Coleoptera has been indicated as a general trait of ground-dwelling assemblages (Miranda, Piñero, & Megías, 2007; Lenoir & Lennartsson, 2010; Pardee & Philpott, 2011). Their activities accelerated the decomposition of plant residues, aerated the soil and improved soil structure and quality (Holecová, Lukáš, & Harakaľová, 2003; Dieng, Ndiaye, & Taylor, 2016). The dominant representation of the ants (Formicidae) and Coleoptera taxon among epigeic arthropods in the conditions of integrated farming and ecological farming was also recorded by Porhajašová Noskovič, Rakovská, Babošová, & Čeryová (2015); Porhajašová, Babošová, Noskovič, & Ondrišík (2018).

Exploiting biodiversity on ecosystem service provision is a goal of contemporary agriculture, although relationships between diversity and ecosystem services remain largely unexplored for innovative practices (Kalivoda, Petrovič,& Kürthy, 2010; Finney & Kaye, 2016; Griffiths et al, 2000; Špulerová, Petrovič, Mederly, Mojses, & Izakovičová 2018; Dobrovodska, Kanka, & David, 2019). Ants play an irreplaceable role in the decomposition of organic matter, in the cycle of biogenic elements of carbon, nitrogen, sulphur, phosphorus, in transformation and degradation of waste and toxic substances, and their presence is irreplaceable (Fazekašová & Bobuľovská, 2012). Using the multivariate model, we demonstrated the influence of environmental variables (pH of the soil, soil moisture, potassium, phosphorus, nitrogen) on the abundance of ants. Thus, our results agreed with the results of (Attwood, Maron, House, & Zammit (2008)), who observed a change of abundance of ants with increasing land use. Biodiversity loss as a consequence of agricultural intensification can lead to reductions in agroecosystem functions and services. Increasing crop diversity through rotation may alleviate these negative consequences by restoring positive interactions. The impact of ants is an important component of the strategy leading to the sustainability of the soil ecosystem. The diversity of ants, including its abundance in soil, depends on the abiotic and biotic factors that are typical of the biotope (Zak, Holmes, White, Peacock, & Tilman, 2003; Tiemann, Grandy, Atkinson, Spiotta, & McDaniel, 2015).

Arthropod abundance from month to month is usually interpreted as being related to fluctuations in climatic factors (such as temperature, precipitation and day length) (Lionello, Rizzoli, & Boscolo, 2006). The number of ants in March and June was higher from the number of ants captured in July and September in *Grass mixture* conditions. In the *Hordeum vulgare* crop we have seen a steady increase. Simão, Carretero, Amaral, Soares, & Mateos (2015) also confirmed differences in the number of ants affected by different weather during the seasons. Andrew, Roberts, Hill (2012) have suggested that precipitation is more influential on ant diversity at high temperatures than at low temperatures. Greenberg & McGrane (1996); Majeed, Rana, Azevedo, Elmo, & Nargis (2020) confirmed a seasonal trend for the abundance of arthropod groups. It is established that environmental variables and the influence of biogeographic factors account for fluctuations in species abundance. Climatic conditions during the months impact the biodiversity of ant species (Garcia, Cabeza, Rahbek, & Araújo, 2014; Williams & Newbold, 2020). In our study, we confirmed with the help of regression models a strong relationship between the environmental variables potassium (mg), phosphorus (mg), nitrogen (mg), pH, humidity (%) and the abundance of ants. For agricultural management, understanding how species' behaviour varies with environmental variables is imperative in ensuring food security in the future. In addition, ants' predation makes them prospects for future integrated pest management strategies in agriculture (Offenberg, 2014). Ants mineralize nutrients, form soil aggregates, and disperse seeds, are significant and necessary for decorous ecosystem functioning and sustainability (Del Toro, Ribbons, & Pelini, 2012; Pfeiffer, Mezger, & Dyckmans 2013).

CONCLUSION

Our results have provided new knowledge about the preference of ants in the conditions of *Hordeum vulgare* and *Grass mixture* in central Europe. The dispersion of ants was influenced by soil moisture, soil pH, phosphorus, potassium and nitrogen. We confirmed an increase in the average number of individuals during spring and summer months. The ants had a strong correlation with soil moisture (%), soil pH, phosphorus (mg/kg), potassium (mg/kg) and nitrogen (mg/kg). With increasing values of potassium, phosphorus, nitrogen and moisture, the number of individuals also increased. We confirmed that the optimal soil pH value was neutral. A practical, workable approach should be used to preserve the current ant population, achieve sustainable levels of biodiversity, key species to develop conservation and agricultural management strategies. This is of particular importance to those who may face pressure from pest species threatening crop yields. This study can be helpful in the planning of conservation programs as well as provide information to farmers to initiate integrated pest management strategies.

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Two New species of *Dicopomorpha* Ogloblin (Hymenoptera: Mymaridae) from Himalayan belts of Himachal Pradesh, India with the first record of male of *D. albithorax* Rameshkumar & Manickavasagam

Prince Tarique ANWAR[1*] Shahid Bin ZEYA[2] Syeda Uzma USMAN[3]
Syed Kamran AHMAD[4] Farmanur Rahman KHAN[5]

[1,2]Department of Zoology, Aligarh Muslim University, Aligarh – 202002, Uttar Pradesh, INDIA
[3]Department of Zoology, Mohammad Ali Jauhar University, Rampur, 244901, Uttar Pradesh, INDIA
[4]Department of Plant Protection, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, 202 002, INDIA
[5]Department of Biology, Deanship of Educational Services, Qassim University, Buraidah, 51452, Al-Qassim, KINGDOM OF SAUDI ARABIA
e-mails: [1*]ta.friday@gmail.com, [2]drsbz1966@gmail.com, [3]insect.11lab@gmail.com, [4]entosaif@rediffmail.com, [5]insectqh11@gmail.com
ORCID IDs: [1*]0000-0002-1682-0033, [2]0000-0003-1594-0372, [3]0000-0003-2578-2515, [4]0000-0002-3281-6632, [5]0000-0001-8906-709X
[*]Corresponding author

ABSTRACT

Two new mymarid species *Dicopomorpha heratyi* Anwar & Zeya, sp. nov. and *Dicopomorpha mirzai* Anwar & Zeya, sp. nov., are described from Himalayan belts of Himachal Pradesh, India and also male of *D. albithorax* Rameshkumar & Manickavasagam is recorded and described for the first time. The type specimens are deposited in the Insect Collections, Department of Zoology, Aligarh Muslim University, Aligarh, Uttar Pradesh, India.

Keywords: Chalcidoidea, fairy fly, description, record, new species.

Anwar, P. T., Zeya, S. B., Usman, S. U., Ahmad, S. K., & Khan F. R. (2023).Two new species of *Dicopomorpha* Ogloblin (Hymenoptera: Mymaridae) from Himalayan belts of Himachal Pradesh, India with the first record of male of *D. albithorax* Rameshkumar & Manickavasagam. *Journal of the Entomological Research Society*, 25(1), 91-99.

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INTRODUCTION

Himachal Pradesh a state located in the North of India is covered by Himalayan Mountains.The state is a junction of three biogeographic zones of Indian Himalayan regions i.e. West Himalaya, North-West Himalaya and Trans Himalaya. It is a home to many endemic species. Rajmohana, Saini, Girish Kumar, & Patra (2018) documented nearly 475 species of Chalcidoidea in 17 families from the Indian Himalaya which contains 30 species of Mymaridae in 14 genera. The authors have number of unidentified mymarid specimens from Himalayan regions of India which they plan to identify and publish in due course of time.

This paper treats the genus *Dicopomorpha* Ogloblin (1955) which belongs to *Alaptus*-group of genera (Huber & Lin, 1999). With the addition of these two new species (08 from India), the global number of *Dicopomorpha* accounts for a total of 15 species. The genus though small, but widespread and reported from all the regions of the world. Subba Rao (1989) described *Dicopulus indicus* from India which was later transferred to the genus *Dicopomorpha* by Hayat (1992), and this was the first record of the genus from India. Rameshkumar & Manickavasagam (2016) described four species of the genus from India. Rameshkumar et al, (2017) recorded *D. zebra* Huber (2009) from Andaman and Nicobar Islands, India. Here, we describe two new species of *Dicopomorpha* from Himachal Pradesh and additionally, describe the male of *D. albithorax* Rameshkumar & Manickavasagam for the first time.

MATERIAL AND METHODS

The materials for this study were collected using various trapping techniques as were summarized by Noyes (1982). The specimens were killed in ethyl acetate fumes and were initially stored in 80% ethanol. The specimens were mounted on cards for description of coloration and taking measurements of body lengths and were subsequently mounted on slides for detailed morphometric and descriptive studies. Slides were prepared following Noyes (1982) with modifications as mentioned in Anwar, Zeya, & Veenakumari (2020). Length of antennal scape excludes the radicle. Nikon eclipse DM 2500 stereo zoom microscope was used to study the specimens as well as photographs and make measurements. The photographs were retouched using Adobe Photoshop®. The types are deposited in the Insect Collections, Department of Zoology, Aligarh Muslim University, Aligarh, Uttar Pradesh, India. A distributional map (Fig. A) is provided for the Indian species of *Dicopomorpha* from the different Indian states/Union Territory.

Terminology used was follow Zeya & Hayat (1995) and Gibson (1997).

The following abbreviations are used:

ANI = Andaman and Nicobar Islands; F = Funicle segment; mps = multiporous plate sensillum or sensilla (= longitudinal sensilla); HP = Himachal Pradesh; MT = Malaise trap; SN = Sweep net; YPT = Yellow pan trap.

Acronyms used for depositories:

EDAU = Department of Entomology, Annamalai University, Chidambaram, Tamil Nadu, India.

ZDAMU = Insect Collections, Department of Zoology, Aligarh Muslim University, Aligarh, Uttar Pradesh, India.

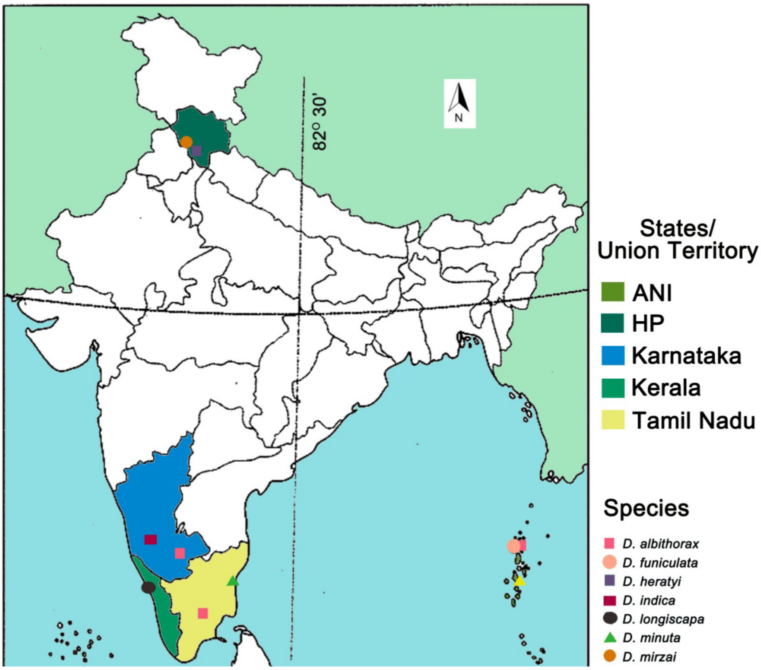

Figure A. Distributional map of *Dicopomorpha* species in India.

RESULTS

Taxonomy

Key to Indian species of *Dicopomorpha* Ogloblin, females

1. Antenna with funicle 6-segmented (Fig. 1c) 2

- Antenna with funicle 7-segmented (Fig. 3b) 3

2. Mesosoma pale yellow; mesoscutum with rugose sculpture .... *D. albithorax*

- Mesosoma yellow medially, sides dark brown; mesoscutum with reticulate sculpture (Fig. 2b) ....*D. heratyi* sp. nov.

3. F2 normal, as long as other funicular segments ....*D. funiculata*

- F2 reduced, subquadrate (Fig. 3b) 4

4. Mesosoma pale yellow with rugose sulptures .... *D. indica*

- Body dark brown; mesosoma with reticulate sculptures 5

5. Mesosoma strongly reticulate (Fig. 3d); ovipositor as long as gaster (Fig. 3e) ....*D. mirzai* sp. nov.

- Mesosoma faintly reticulate; ovipositor at most two-third length of gaster 6

6. Scape with two distinct, transverse, white bands, pedicel white .... *D. zebra*

- Scape and pedicel brown ……...….. 7

7. Scape at most 8× as long as broad; ovipositor longer than mesotibia ..*D. minuta*

- Scape at least 8.5× as long as broad; ovipositor shorter than the length of mesotibia ....*D. longiscapa*

Dicopomorpha heratyi **Anwar & Zeya sp. nov.**

Material examined: Holotype: ♀ (on slide under 4 coverslips, slide No. MYM.220), INDIA: HIMACHAL PRADESH, Shimla, 2.08.2014 (YPT), Coll. K. Veenakumari. (ZDAMU).

Diagnosis

This is the largest *Dicopomorpha* species recorded so far from India with body length 420 µm. Body colour dark brown except mesoscutum and scutellum yellowish brown medially. Antenna with funicle 6-segmented; funicular segments increase in length distally; clava longer than F4-F6 combined. Head with vertex, mesoscutum and scutellum with polygonal reticulation. *Dicopomorpha heratyi* sp. nov. is similar to *D. albithorax* Rameshkumar & Manickavasagam, the only other Indian species with 6-segmented funicle, but otherwise differs in the following characters: mesosoma brown except mesoscutum and scutellum yellowish brown medially (*D. albithorax:* mesosoma largely pale yellow); head triangular; vertex relatively broad with polygonal reticulation (*D. albithorax:* head rounded; vertex relatively narrow with rugose sculpture); fore wing with distinctly curved apex (*D. albithorax:* fore wing with apex relatively less curved).

Description

Female (holotype): Body length, 420 µm. Body brown to dark brown, except mesoscutum and scutellum yellowish brown medially. Antenna brown. Wings subhyaline with brownish tinge. Legs, including coxae, brown.

Head (Fig. 1b). Head, in frontal view, triangular, 1.2× as broad as high; vertex, with polygonal reticulation. Antenna (Fig. 1c) with scape, 5.7× as long as broad; pedicel 2× as long as broad, longer than all funicular segments individually; funicle 6-segmented, all segment longer than broad; F1 smallest and F6 the longest; clava 2.7× as long as broad, longer than F4-F6 combined, with 3 mps.

Mesosoma (Fig. 2b). Mesosoma as long as metasoma; mesoscutum and scutellum with polygonal reticulation; anterior scutellum shorter than frenum; propodeum smooth, subequal to anterior scutellum. Fore wing (Fig. 2a) 9× as long as broad, distinctly curved at apex; disc with a few setae in a single row medially; longest marginal seta 3× as long as maximum wing width. Hind wing (Fig. 2a) 22×as long as broad; longest marginal seta 7× as long as maximum wing width.

Metasoma (Fig. 2c). Ovipositor slightly exserted beyond apex of gaster, 0.7× mesotibia.

Measurements (holotype slide, µm): head width: height, 160: 135; antennal segments length: width — radicle, 15: 13; scape, 100: 18; pedicel, 45:23; F1, 23: 10; F2, 24: 10; F3, 28: 13; F4, 30: 14; F5, 33: 18; F6, 35: 20; clava, 115:43; mesosoma length, 170; mesoscutum, 48; anterior scutellum, 25; frenum, 50; metanotum, 13; propodeum, 28; fore wing length: width, 525:60; longest marginal seta, 188; hind wing length: width, 500: 23; longest marginal seta, 150; protibia, 95; mesotibia, 155; meso basitarsus, 30; metatibia, 153; metasoma, 205; ovipositor, 105.

Male: Unknown.

Hosts: Unknown.

Distribution: India: Himachal Pradesh.

Etymology: The species is named after Dr. John Heraty, Professor in Entomology, University of California, Riverside, California, USA.

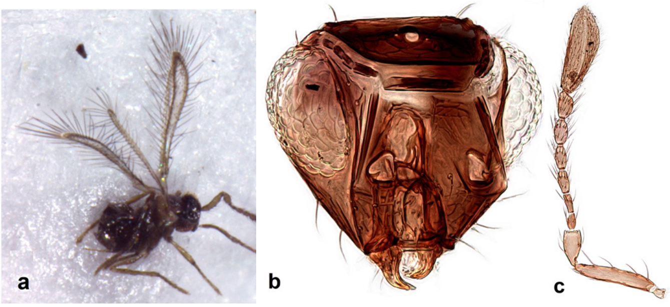

Figure 1. *Dicopomorpha heratyi* sp. nov. (female, holotype). a) habitus, b) head, frontal, c) antenna.

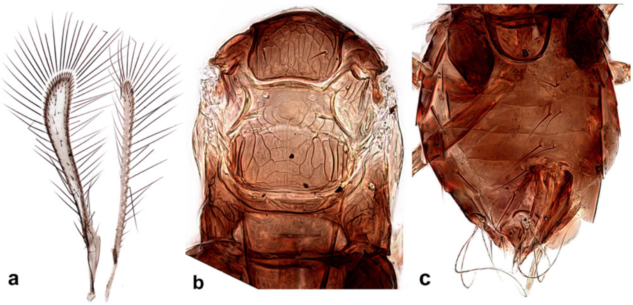

Figure 2. *Dicopomorpha heratyi* sp. nov. (female, holotype). a) wings, b) mesosoma, c) metasoma.

Dicopomorpha mirzai **Anwar & Zeya sp. nov.**

Material examined: Holotype: ♀ (on slide under 4 coverslips, slide No. MYM.101), INDIA: HIMACHAL PRADESH: Hamirpur, Barsar, 1.x.2013 (SN), Coll. P.T. Anwar & F.S.K. Amer. (ZDAMU).

Diagnosis

Dicopomorpha mirzai Anwar & Zeya, sp. nov. is a distinctive species with funicle 7-segmented, and appears close to *D. indica* (Subba Rao, 1989) in having similar antennal configuration and shape of the fore wing. However, both differ from each other by the colour of the body and sculpture on mesosoma. In *D. mirzai* body is dark brown; mesoscutum and scutellum with reticulate sculpture (in *D. indica* mesosoma is largely pale yellow and the mesoscutum and scutellum with rugose sculpture).

Description

Female (holotype): Body length, 260µm. Body dark brown. Antenna with scape, pedicel and funicle yellowish brown, clava dark brown. Legs, including coxae, pale brown. Wings subhyaline with brown suffusion.

Head (Fig. 3a). Head, in frontal view, 1.4× as broad as high; vertex with polygonal reticulations, area below transverse trabecula and facial region with faint reticulations. Antenna (Fig. 3b) with scape 4.4× as long as broad; pedicel 1.7× as long as broad, longer than all funicular segments individually; funicle 7-segmented, all longer than broad except F2, ring-like; F7 longest; clava 3× as long as broad, longer than F5-F7 combined, with 3mps.

Mesosoma (Fig. 3d). Mesosoma subequal to metasoma length; mesoscutum with longitudinal polygonal sculpture; scutellum with polygonal reticulation; anterior scutellum subequal to frenum length; propodeum less than 0.5× length of scutellum. Fore wing (Fig. 3c) more or less straight, slightly curved towards apex, posterior margin wavy; 12× as long as broad, with a few setae scattered irregularly behind venation and on wing disc; longest marginal seta 5× as long as maximum wing width. Hind wing (Fig. 3c) 21×as long as broad; longest marginal seta 6× as long as maximum wing width.

Metasoma (Fig. 3e). Ovipositor, 1.2× as long as mesotibia.

Measurements (holotype slide, µm): head width: height, 170: 120; antennal segments length: width - radicle, 15: 8; scape, 88: 20; pedicel, 38: 23; F1, 15: 10; F2, 8: 8; F3, 23: 9; F4, 28: 13; F5, 30: 10; F6, 28: 13; F7, 35: 18; clava, 103: 33; mesosoma length, 158; mesoscutum, 53; anterior scutellum, 25; frenum, 33; metanotum, 8; propodeum, 28; fore wing length: width, 455: 45; longest marginal seta, 175; hind wing length: width, 425: 20; longest marginal seta, 125; protibia, 88; mesotibia, 123; mesobasitarsus, 30; metatibia, 120; metasoma, 138; ovipositor, 145.

Male: Unknown.

Hosts: Unknown.

Distribution: India: Himachal Pradesh.

Etymology: The species is named in honour of Professor (Late) Babar Mirza, founder of the Department of Zoology, Aligarh Muslim University, Aligarh.

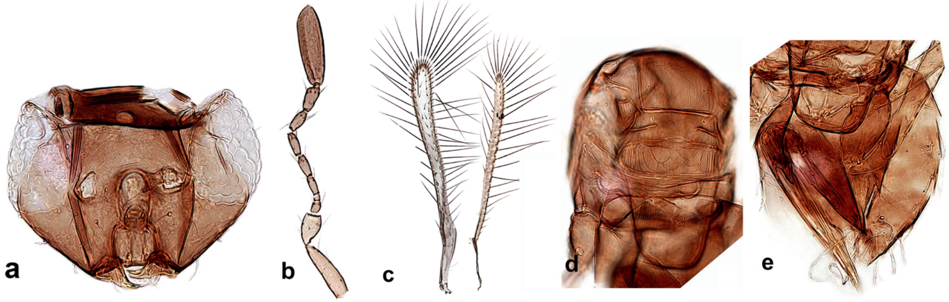

Figure 3. *Dicopomorpha mirzai* sp. nov. (female, holotype). a) head, frontal, b) Antenna, c) wings, d) mesosoma, e) metasoma.

Dicopomorpha albithorax **Rameshkumar & Manickavasagam, 2016**

Dicopomorpha albithorax Rameshkumar & Manickavasagam, 2016: 8384, female. Holotype, female, India, Andaman & Nicobar Islands, Diglipur, Ramnagar, (EDAU), examined.

Material examined: ♂ (on slide under 4 coverslips, slide No. MYM.107) INDIA: KARNATAKA, Bengaluru, Jarakabande Kaval, 14.xi.2013 (MT), Coll. K. Veenakumari. (ZDAMU).

Diagnosis

Male: Body length 270*µ*m. Body mostly dark brown except mesosoma pale yellow. Antenna and legs pale yellow. Head, with rugose sculpture. Antennal with flagellum 9-segmented, longitudinal sensilla on all flagellar segments (Fig. 4a). Mesosoma (Fig. 4c) as long as metasoma; setation and sculpture similar to females. Fore wing (Fig. 4b) 8× as long as broad, disc bare except a few setae in the middle; longest marginal seta 3× as long as maximum wing width. Hind wing (Fig. 4b) 22× as long as broad; longest marginal seta 6× as long as maximum wing width. Genitalia (Fig. 4d) 0.6× mesotibia.

Hosts: Unknown.

Distribution: India: Andaman & Nicobar Islands, Karnataka, Tamil Nadu.

Comments: *Dicopomorpha albithorax* Rameshkumar & Manickavasagam is similar to *D. indica* (Subba Rao, 1989), as both share same body coloration but the later has one extra funicle segment. It may fall under a potential synonymy of *D. koreana* Triapitsyn & Berezovskiy (2003) from South Korea which has similar body colouration and 6-segmented funicle. Both the species hardly differs from each other except for the presence a few setae on the fore wing.

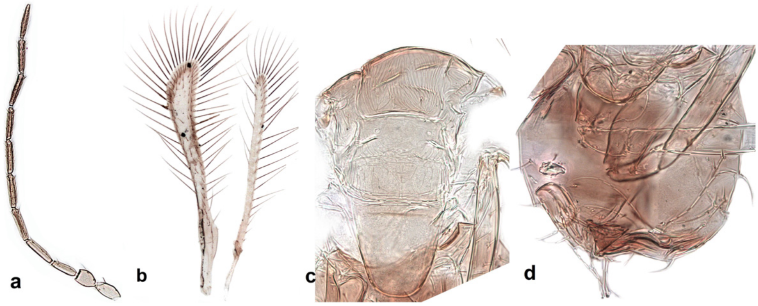

Figure 4. *Dicopomorpha albithorax* Rameshkumar & Manickavasagam (male). a) antenna, b) wings, c) mesosoma, d) metasoma.

DISCUSSION

Dicopomorpha Ogloblin constitutes the smallest known insects of the world (male of *D. echmepterygis* Mockford measuring 139 µm in length). All the recorded and described species of the genus from India measure less than 500 µm which makes it one of the most challenging groups in terms of collection and description. Also, the genus shows variation in characters which makes it even more demanding to study the genus collectively from the world. Here, we have described two new species and reported the male of a known species for the first time. The descriptions although based on single specimens but, remarkably show characters that separates them with the already existing species. The photographs are clear enough to record and identify these species when collected again in future.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Research Article
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Psammophilous Grasslands and Other Open Sandy Habitats in the Sandomierz Basin as a Refuge for Threatened Orthoptera in Poland

Rafał BOBREK[1] Tomasz WILK[2]

[1,2]Polish Society for the Protection of Birds, Odrowąża 24, 05–270 Marki, POLAND
e-mails: [1]*rafal.bobrek@gmail.com, [2]tomasz.wilk@otop.org.pl
ORCID IDs: [1]*0000-0002-8304-5583, [2]0000-0002-2837-7219
*Corresponding author

ABSTRACT

Psammophilous grasslands are declining in Europe due to ecological succession and anthropogenic pressures, yet they group many specialised invertebrates of early successional biotopes. We investigated the occurrence of six threatened species of Orthoptera in 135 patches of psammophilous grasslands and other open sand-related habitats (dry heaths, sandy roads, sand pits) located in the Sandomierz Basin (SE Poland). The aim was to verify whether these habitats, and especially the active military range, serve as a refuge for any of the selected species. Localities of *Podisma pedestris* and *Calliptamus italicus* found in the study area covering 0.7% of Poland's territory represented a significant proportion (50 and 18%, respectively) of UTM grid squares known to be currently occupied by these species in the country. In the regional scale of southern Poland, studied habitats also accumulated a large proportion (ca. 17%) of the current localities of *Oedipoda caerulescens*. However, for *Aiolopus thalassinus*, *Psophus stridulus* and *Sphingonotus caerulans* the importance of the Sandomierz Basin as a country wide refuge was rather minor. Five of the six target species were found on the military range, with two (*P. pedestris*, *P. stridulus*) recorded only there. These results indicate that sand-related habitats are important refuges for some threatened orthopterans in the region and should be actively protected.

Keywords: Acrididae, sandy grasslands, heaths, sand pits, military training ground.

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INTRODUCTION

Grasslands are the most biodiverse habitat type for Orthoptera in Europe, being inhabited by 555 species of these insects, i.e. 51.6% of all those found across the continent (Hochkirch et al, 2016). At the same time, this habitat also groups the highest number of European orthopterans threatened with extinction (more than 100 species; Hochkirch et al, 2016). For these reasons, different types of grasslands are a frequent field of orthopterological research (e.g. Bazyluk & Liana, 1970; Krištín, Kaňuch, & Sárossy, 2004; Fartmann, Krämer, Stelzner, & Poniatowski, 2012; Kenyeres, Szabó, Takács, & Szinetár, 2020). In Poland, among grassland habitats, the fauna of Orthoptera inhabiting xerothermic calcareous grasslands is particularly diverse and well-studied (e.g. Bazyluk & Liana, 1970; Liana, 1976, 1978, 1982, 1990, 1994). In contrast, there is significantly less data on other types of open dry ecosystems – psammophilous grasslands and other inland sand-related habitats (Liana, 1990, 1994; Warchałowska-Śliwa, Maryańska-Nadachowska, & Kostia, 1992; Kuřavová, 2014; Krasoń & Wojton, 2021), although they are more widespread and occupy larger areas than xerothermic grasslands (EEA 2021). At the same time, psammophilous grasslands are among the threatened and declining habitats in Europe (Janssen et al, 2016), mostly as a result of vegetation succession or due to being treated as wastelands, which leads to ongoing afforestation or housing development (Liana, 1999, 2002; Krištín et al, 2004; Kujawa-Pawlaczyk, 2010; Trąba & Rogut, 2013). The highly specialised fauna of psammophilous grasslands includes many rare or threatened taxa (Exeler, Kratochwil, & Hochkirch, 2009; Banaszak & Twerd, 2018; Buszko, 2019; Rozwałka, Rutkowski, Sienkiewicz, & Wiśniowski, 2019; Grbić, Hänggi, & Krnjajić, 2021), and this also applies to orthopterans (Krištín et al, 2004). Some species of these insects are associated with the early stages of succession of psammophilous grassland vegetation, so that they can be regarded as bioindicators of the successional changes occurring in these communities (Fartmann et al, 2012). In Poland, species such as *Calliptamus italicus* (L., 1758), *Oedipoda caerulescens* (L., 1758) or *Sphingonotus caerulans* (L., 1767) are among those strongly associated with open sandy habitats (Warchałowska-Śliwa et al, 1992; Liana, 1999, 2002; Bazyluk & Liana, 2000). Taking into account the alarming status of psammophilous grasslands in Europe (Janssen et al, 2016), also the orthopterans of this ecosystem should be of special research interest, as a potentially highly endangered ecological group.

The study was focused on the recognition of the distribution and frequency of occurrence of selected, threatened species of Orthoptera in psammophilous grasslands and other open sand-related habitats (such as dry heaths, sandy roads, sand pits) located in the Sandomierz Basin (south-eastern Poland). The area is known for the occurrence of many rare invertebrates (Krawczyk, Kata, & Nowak, 2011), including orthopterans (Liana, 1999; Krasoń & Wojton, 2021), though generally it has been rather poorly studied in terms of Orthoptera. And the same time it is characterised by the presence of a large area of open sandy biotopes, potentially utilised by highly specialised entomofauna (Nowacki & Pałka, 2015). They are particularly widespread in the central part of the area, where a large military training ground is located (GDOŚ, 2021). On the basis of the above information, we expect that: 1) psammophilous grasslands and other inland sandy habitats in the region studied are inhabited by several rare, specialised species of Orthoptera, 2) this part of the Sandomierz Basin constitutes a refuge of a country-wide importance for threatened orthopterans of dry, sand-related open ecosystems, and 3) the area of the military training ground serves as a regional biodiversity hotspot for this group of insects.

MATERIAL AND METHODS

Study area and sampled habitats

The study was conducted in south-eastern Poland (Fig. 1), covering a fragment of the Sandomierz Basin (specifically, the western and central part of the so-called Sandomierz Forest), located within the boundaries of the Natura 2000 Special Protection Area ‘Puszcza Sandomierska’ PLB180005. This region is bounded to the east and north-east by the San River valley, to the west and north-west by the valleys of the Vistula and Wisłoka rivers, and to the south by the Sub-Carpathian Ice Marginal Valley (Solon et al, 2018). It is characterised by the occurrence of fluvial sands and Pleistocene aeolian formations, forming complex systems of dunes reaching up to 25 m in height, between which there are drainless deflation depressions, often filled with peat and strongly moistened. This creates large environmental contrasts between dry and wet areas located close to each other, which is characteristic of this region. The area is covered by a mosaic of woodlands (mainly pine and pine-oak forests), semi-open and open areas, both farmed and uncultivated (Krawczyk et al, 2011; Kondracki, 2013; GDOŚ, 2021). The area selected for the study comprised a total of 23 UTM (Universal Transverse Mercator) grid squares, covering approximately 2300 km^2 (Fig. 1).

The study covered several types of inland sandy habitats. The vast majority were thermophilic, pioneer, dry sandy (psammophilous) grasslands of the class *Koelerio glaucae-Corynephoretea canescentis*, developed both on dune sands and those not associated with dunes (Kujawa-Pawlaczyk, 2010; Kulpiński & Tyc, 2012). These grasslands were usually created as a result of human activity (deforestration, agricultural management), so they have a secondary character. They are an early stage of succession in vegetation transformations leading to pine forest, usually forming after its cutting or on the edges of forest patches (Kulpiński & Tyc, 2012). Psammophilous grasslands are also commonly formed on post-agricultural areas on sandy soils, as well as accompany the edges of dirt roads or road and railway embankments (Kujawa-Pawlaczyk, 2010; Trąba & Rogut, 2013). Grasslands with various vegetation structure were surveyed, from areas of bare sand with sparse vegetation to late successional stages, with encroaching undergrowth of shrubs and trees (mainly Scots Pine *Pinus sylvestris* L.; Trąba & Rogut, 2013). Moreover, psammophilous grasslands formed in sand pits and on their margins were sampled (Kujawa-Pawlaczyk, 2010; Trąba & Rogut, 2013). In addition, sandy dry heath

Calluno-Ulicetalia communities (Pawlaczyk, 2012) were surveyed, which occurred on an extensive (nearly 2000 ha; GDOŚ, 2021), active military training ground, in a mosaic with psammophilous grasslands, pine forests and woodlots, forest edge and tall-herb thermophilous communities of *Trifolio-Geranietea* and patches of open dune sands (Rapa & Krawczyk, 2020). All these studied habitats belong to nutrient poor, dry, strongly insolated and thermophilic, in this respect ranking among the most extreme habitats found in the Central European lowlands (Kujawa-Pawlaczyk, 2010; Kulpiński & Tyc, 2012).

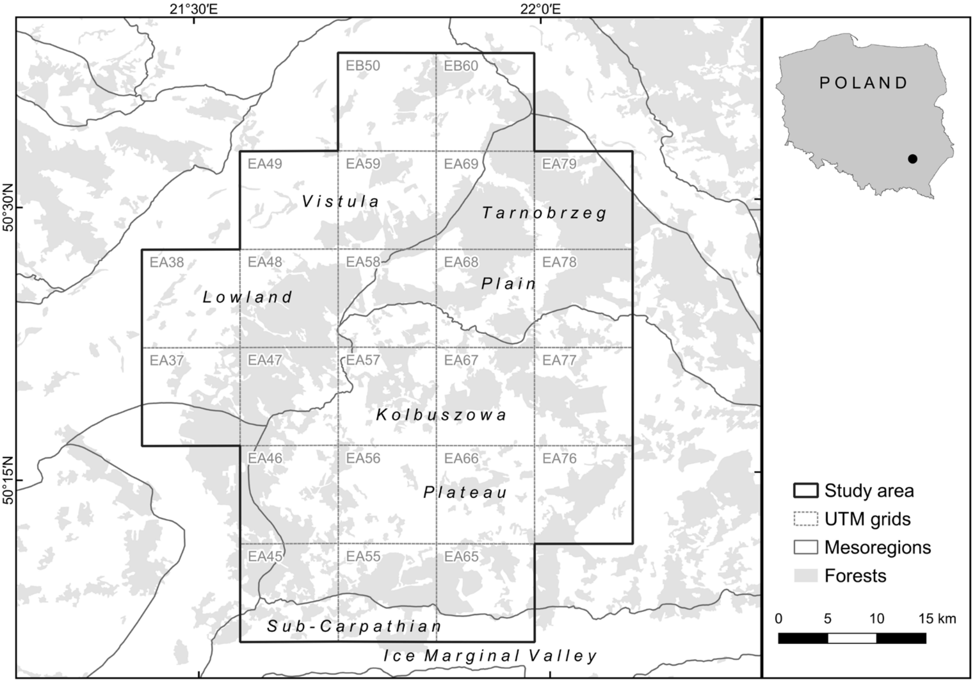

Figure 1. Location of the study area.

Studied species

Sampling was targeted at six selected Orthoptera species belonging to the family Acrididae MacLeay, 1821 (Table 1; taxonomy according to Cigliano, Braun, Eades, & Otte, 2021), found in the open sandy communities described above (Liana, 1990, 1994, 1999; Warchałowska-Śliwa et al, 1992; Bazyluk & Liana, 2000; Kuřavová, 2014). All of these species can be regarded as threatened in Poland, as in the Polish red list of threatened species (Liana, 2002), they were given NT (near threatened) or higher status (Table 1). The occurrence of each of these species has been recorded in the Sandomierz Forest in the past (Bazyluk & Liana, 2000), and some of them also recently (Krasoń & Wojton, 2021).

Table 1. Species of Orthoptera selected for the study. Threat status in Poland is given for each, based on the regional red list of threatened species (Liana, 2002).

No	Species	Threat status
1	*Podisma pedestris* (L., 1758)	VU (vulnerable)
2	*Calliptamus italicus* (L., 1758)	CR (critically endangered)
3	*Aiolopus thalassinus* (Fabr, 1781)	VU (vulnerable)
4	*Psophus stridulus* (L., 1758)	VU (vulnerable)
5	*Oedipoda caerulescens* (L., 1758)	NT (near threatened)
6	*Sphingonotus caerulans* (L., 1767)	NT (near threatened)

Data acquisition and analysis

The study was carried out in 2019 and 2020, with 89% of sites inspected in 2020. Fieldwork was carried out from the last third of June to the second third of August, with the greatest intensity in the last third of July. In each of the 23 UTM grid squares, between 1 and 19 (median=5) patches of sandy habitats were sampled, in which the above-mentioned orthopterans were searched for. Approximately 50% of patches were sampled during the ornithological fieldwork carried out in the area under study. The remaining ones were selected using publicly available satellite and aerial imagery (Google Maps and www.geoportal.gov.pl), as sandy grasslands, due to their unique physiognomy, are usually clearly distinguishable on them. Locations selected in this way were then searched in the field. In 135 sampled habitat patches (localities/sites; Appendix 1), at daytime, adult and larval stages of the studied orthopterans were visually searched for. Insects were identified in the field – with the naked eye, binoculars enabling observation of close objects or from photographs. If necessary, selected individuals were caught and, after species identification, released at the site of capture. Surveys were qualitative (presence/absence) – the abundance of the species in the site was not assessed. Each record was assigned with date, geographical coordinates, UTM grid square, physico-geographical mesoregion and habitat type (psammophilous grassland, dry heath, sand pit, sandy road, railway embankment). This information was collected in a database. The results obtained in the localities selected according to the scheme presented above were used to describe the distribution of the species in the study area and were subjected to quantitative analysis. For each species, the frequency of its occurrence in the studied habitats was calculated as the percentage of sites in which the presence of that species was recorded.

In order to determine the role that the studied region (0.7% of Poland’s area) play in the preservation of each threatened species in the country, a comparison of the results of our study was made with the data of the project of mapping distribution of orthopterans in Poland (Żurawlew et al, 2021). We determined the proportion of UTM grid squares occupied by each species in the study area in relation to squares with an up-to-date (= after 1990) occurrence (i) in the country and (ii) in southern Poland (south of the 50°55’ parallel). Note, however, that the comparative data (Żurawlew et al, 2021) is incomplete, because the mapping project is ongoing.

RESULTS

In 121 of the 135 sites surveyed (90%; Appendix 1), at least one of the six studied Orthoptera species was recorded. This was most often one (84 sites; 62%) or two species (32 sites; 24%), and much less often three, four or five species (5 sites in total; 4%).

Podisma pedestris. A species with a very clustered distribution. Found in three localities (2%; Fig. 2a, 3), located within one compact area – the military training ground in Nowa Dęba (western part of the Tarnobrzeg Plain). Not found in other parts of the study area. The three occupied UTM grid squares found (Appendix 1) represent 50,0% of the squares with known present occurrence of the species, both in the whole country and in southern Poland.

Calliptamus italicus. The second most frequent species, distributed almost throughout the area, although with uneven frequency. It was found in 39 sites (29%; Fig. 2b, 3). There is a noticeable gradient of frequency, decreasing from north to south. It was most frequent in the northern regions: Tarnobrzeg Plain (52% of sites located within this region) and Vistula Lowland (34%), and less frequent in the Kolbuszowa Plateau (24%), especially in its southern parts (Fig. 3). Not recorded in the Sub-Carpathian Ice Marginal Valley. The 22 occupied UTM grid squares found in the study area (Appendix 1) represent 18,3% of the squares with known present occurrence of the species in the whole country and 43,1% of the squares in southern Poland.

Aiolopus thalassinus. The species was recorded only in one locality (frequency of occurrence <1%; Fig. 3), at the northern edge of the Kolbuszowa Plateau (Fig. 2c). Not found in other regions. The single occupied UTM grid square found in the study area (Appendix 1) represent 0,8% of the squares with known present occurrence of the species in the whole country and 5,6% of the squares in southern Poland.

Psophus stridulus. Similarly to the previous species, it was recorded only in one locality (frequency of occurrence <1%, Fig. 3), in the eastern part of the military training ground near Nowa Dęba (Fig. 2d; western part of the Tarnobrzeg Plain). The single occupied UTM grid square found in the study area (Appendix 1) represent 2,1% of the squares with known present occurrence of the species in the whole country and 3,6% of the squares in southern Poland.

Oedipoda caerulescens. The most frequent of all species, found in 115 sites (85%; Fig. 2e, 3). Distributed quite evenly in the whole area, in many parts noted at every sampled site. It occurs in each of the four mesoregions, although in the Tarnobrzeg Plain it was recorded with relatively lower frequency (71%) than in the remaining ones (85–92%; Fig. 3). The 23 occupied UTM grid squares found in the study area (Appendix 1) represent 17,4% of the squares with known present occurrence of the species in southern Poland. Unfortunately, data for the whole country is not available, but the percentage is certainly much lower than for the southern region and probably insignificant.

Sphingonotus caerulans. It was found in 7 sites (5%; Fig. 2f, 3) concentrated in one area. The distinctive centre of occurrence of this species was the military training ground near Nowa Dęba, where 5 out of 7 localities were found. The other two sites were located nearby – on the northern edge of the Kolbuszowa Plateau and in the south-western part of the Tarnobrzeg Plain. In the latter mesoregion the frequency of occurrence reached 29% of sites (Fig. 3). The two occupied UTM grid squares found in the study area (Appendix 1) represent 1,9% of the squares with known present occurrence of the species in the whole country and 6,1% of the squares in southern Poland.

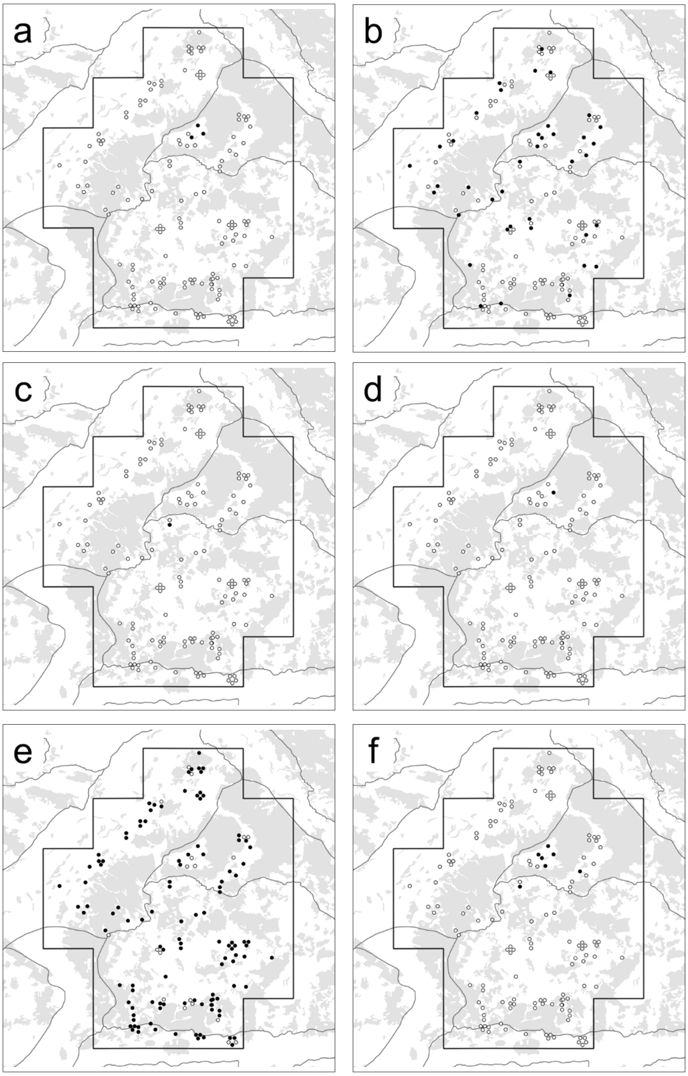

Figure 2. Distribution of each species in the study area – localities where the species was present (solid circles) and those where it was not recorded (empty circles). a) *Podisma pedestris,* b) *Calliptamus italicus,* c) *Aiolopus thalassinus,* d) *Psophus stridulus*, e) *Oedipoda caerulescens,* f) *Sphingonotus caerulans*.

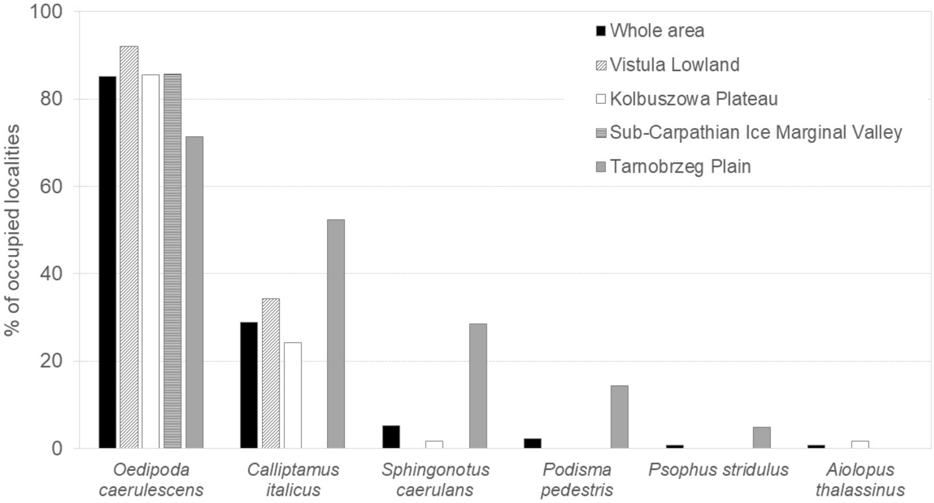

Figure 3. Frequency of occurrence of each species across the study area and in each mesoregion, presented as a percentage of occupied localities.

DISCUSSION

The occurrence of specialised Orthoptera in open sandy habitats, such as psammophilous grasslands and dry heaths, is determined by features such as the large area of exposed, highly insolated soil or sand (suitable for, e.g. egg deposition and development), the presence of low vegetation or the warm and dry microclimate (Maes & Bonte, 2006; Warren & Büttner, 2008; Fartmann et al, 2012; Kenyeres et al, 2020). The presence of all six target threatened Orthoptera species was confirmed in the study area and surveyed types of habitat (cf. hypothesis 1), with *O. caerulescens* being a very widespread species and *C. italicus* being fairly widespread. Both species are distributed quite evenly in the whole studied fragment of the Sandomierz Basin, although the former was generally about three times more frequent than the latter, except for the Tarnobrzeg Plain, where the difference was smaller, only 1.5 times. The remaining four species occurred locally, in small number (*S. caerulans*, *P. pedestris*) or even at single sites (*A. thalassinus*, *P. stridulus*). In the case of *A. thalassinus* this may be due to different larval habitat requirements. This thermophilic and hygrophilic species usually breeds in moist habitats: wet meadows, salt marshes, floodplains of river valleys or near water bodies (Bazyluk & Liana, 2000; Krištín, Kaňuch, & Sárossy, 2007), however, adults also occur in xerophilous and psammophilous grasslands (Bazyluk & Liana, 2000; Krištín et al, 2007), especially when they are located close to the wet habitats (Mielczarek & Grobelny, 2018). That was also the case for the studied locality – the species was encountered in the sand pit. The frequency of occurrence of *A. thalassinus* may be underestimated also due to the fact that the peak of adult abundance occurs in late August and September (Krištín et al, 2007), so it was barely covering with our sampling period. Habitat preferences probably caused also low frequency of occurrence of *P. stridulus,* which was found only at one locality. In lowland Poland it is a species associated mostly with open and ecotone habitats (clearings, heathlands) accompanying pine forests (Liana, 1976; Bazyluk & Liana, 2000), rather than with initial psammophilous grasslands located in an agricultural landscape, where most of the study sites were located. In addition, this species is closely associated with extensive grazing (Rada, Spitzer, Šipoš & Kuras 2017), which is lacking in the research area.

In the case of some species of Acrididae, the studied psammphilous grasslands and other sand-related habitats comprise a significant proportion of currently known localities of these taxa in Poland (*i.e.* UTM grid squares with the occurrence recorded after 1990), so they may be regarded as their refuges (cf. hypothesis 2). Comparison of the results of our research with the data of the project of mapping distribution of orthopterans in Poland (Żurawlew et al, 2021), revealed the remarkable role of the researched region (despite its restricted area) in the preservation of sites of *P. pedestris* and *C. italicus* at the country wide scale. At the regional scale of southern Poland, the sandy habitats of the Sandomierz Basin also proved to be vital as a refuge for *O. caerulescens*. On the other hand, the importance of the region for *A. thalassinus*, *P. stridulus* and *S. caerulans* is lower and does not allow to consider the surveyed area as a refuge for those taxa, especially from a country wide perspective (Żurawlew et al, 2021).

The Tarnobrzeg Plain had the highest species richness among all regions, which was largely due to the location of the military training ground near Nowa Dęba. In its area five of the six target Orthoptera species were found, including two species recorded only here (*P. pedestris*, *P. stridulus*). In addition, the training ground also contained most of the *S. caerulans* sites found during this study. This clearly indicates that the military range is a key refuge for threatened xerophilous orthopterans (cf. hypothesis 3). This may be partly due to its large size (the range is about 30 km^2), as the area and connectivity of sandy habitats positively influences the diversity of threatened invertebrates from different taxonomic groups, including orthopterans (Maes & Bonte, 2006). Some species, such as the extremely xerophilous *S. caerulans* (Bazyluk & Liana, 2000; Krištín et al, 2004), may also be favoured by the specific dry microclimate of the training ground. The natural value of military area is also undoubtedly a consequence of the great variety of sandy habitat types, from pine forests and shrubs, through dry heaths and psammophilous grasslands, to tracts of open sand. Such a mosaic is maintained by military use (e.g. heavy vehicle traffic, explosions; Warren & Büttner, 2008; Gardiner & Benton, 2009; Woodcock & Pywell, 2009), combined with frequent fires (Rapa & Krawczyk, 2020). These inhibit succession, generates local disturbances, and sometimes completely destroy the vegetation cover, which is a necessary condition for the maintenance of early successional biotopes and favourable local vegetation heterogeneity (Maes & Bonte, 2006; Warren & Büttner, 2008). Due to succession-inhibiting land use, the best-developed patches of psammophilous grasslands are often found on active military training grounds (Kulpiński & Tyc, 2012; Janssen et al, 2016). Specific biocoenoses are associated with these regularly disturbed, pioneer habitats of military ranges, including threatened habitat specialists: noctuid moths (Nowacki & Pałka, 2015), orthopterans and beetles (Warren & Büttner, 2008; Gardiner & Benton, 2009) or plants (Rapa & Krawczyk, 2020). It has been suggested that some species (such as *O. caerulescens*) may be dependent on local disturbances destroying vegetation cover and creating a mosaic of vegetation and areas of bare sand (Maes & Bonte, 2006; Warren & Büttner, 2008). Thus, it seems that the continuation of military activities on the training ground near Nowa Dęba is a key condition to maintain the biocoenoses associated with the open habitats of the area.

Psammophilous grasslands are still quite widespread in Poland (Kujawa-Pawlaczyk, 2010; Kulpiński & Tyc, 2012), including the studied region (Trąba & Rogut, 2013). However, they are a subject to many negative pressures (secondary succession, eutrophication, development, afforestation; Liana, 1999, 2002; Exeler et al, 2009; Kujawa-Pawlaczyk, 2010; Janssen et al, 2016; own data), which threaten the conservation status of their fauna, including rare Orthoptera. It is worth emphasizing that vegetation succession occurring in dry grasslands pose a threat especially to orthopterans belonging to the group of habitat specialists (Fartmann et al, 2012), among which endangered species are numerous. And it is known that rapid successional transformations are characteristic for these habitats (Buchholz, 2010; Kulpiński & Tyc, 2012). The most important forms of counteracting negative transformations of these habitats include the use of military training grounds (Janssen et al, 2016) and, in particular, the implementation of active protection measures on the most valuable patches, which inhibit vegetation succession (Buchholz, 2010). The latter include removal of tree and shrub overgrowth, extensive grazing (especially of goats and sheep), hay removal and controlled burning (Maes & Bonte, 2006; Kujawa-Pawlaczyk, 2010; Fartmann et al, 2012; Banaszak & Twerd, 2018; Kenyeres et al, 2020). Proper planning and implementation of such active protection measures, especially creating a complex mosaic of microhabitats and patches of different stages of succession, allows to maintain the high biodiversity of these specific ecosystems (Cremene et al, 2005; Woodcock & Pywell, 2009; Buchholz, 2010). It is also beneficial to restore sandy grasslands that have undergone strong succession as, at least in some cases, this allows for the re-establishment of a species-rich assemblages (Exeler et al, 2009). It should be ensured that an extensive representation of psammophilous grasslands is secured also in the studied fragment of the Sandomierz Basin. Activities aimed at the restoration and maintenance of psammophilous grasslands should be focused primarily in the Tarnobrzeg Plain and Kolbuszowa Plateau, because this is where the greatest number of target Orthoptera species is concentrated. Special care, including entomological and habitat quality monitoring, should be taken over the military training ground near Nowa Dęba, because, as the obtained results show, it plays a key role in preserving the full spectrum of threatened orthopterans of open sandy habitats of the Sandomierz Basin.

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Appendix 1. The list of 135 surveyed sites. For each site the mesoregion, geographical coordinates (X, Y), UTM grid, general habitat type and recorded Orthoptera species (marked ●) are given. Pp - *Podisma pedestris* (L., 1758); Ci - *Calliptamus italicu*s (L., 1758); At - *Aiolopus thalassinus* (Fabr., 1781); Ps - *Psophus stridulus* (L., 1758); Oc - *Oedipoda caerulescens* (L., 1758); Sc - S*phingonotus caerulans* (L., 1767).

Mesoregion	Site no.	X (°E)	Y (°N)	UTM grid	Habitat type	Pp	Ci	At	Ps	Oc	Sc
Kolbuszowa Plateau	1	21.66985	50.17301	EA45	psammophilous grassland					●	
	2	21.65929	50.18314	EA45	sand excavation					●	
	3	21.67025	50.15572	EA45	psammophilous grassland					●	
	4	21.67561	50.15560	EA45	psammophilous grassland					●	
	5	21.66844	50.13835	EA45	psammophilous grassland					●	
	6	21.67004	50.13999	EA45	psammophilous grassland		●			●	
	7	21.63478	50.21418	EA46	psammophilous grassland		●			●	
	8	21.66958	50.21013	EA46	sand excavation					●	
	9	21.67272	50.20789	EA46	psammophilous grassland					●	
	10	21.60466	50.30425	EA47	psammophilous grassland		●				
	11	21.72554	50.18314	EA55	psammophilous grassland					●	
	12	21.75526	50.18446	EA55	psammophilous grassland					●	
	13	21.74854	50.17703	EA55	psammophilous grassland					●	
	14	21.74624	50.17551	EA55	psammophilous grassland					●	
	15	21.81644	50.17738	EA55	sand excavation						
	16	21.81568	50.17474	EA55	psammophilous grassland					●	
	17	21.83550	50.18553	EA55	psammophilous grassland						
	18	21.83877	50.18438	EA55	psammophilous grassland					●	
	19	21.83947	50.18064	EA55	psammophilous grassland						
	20	21.72038	50.14439	EA55	psammophilous grassland		●			●	
	21	21.76005	50.18239	EA55	psammophilous grassland						
	22	21.76359	50.22863	EA56	psammophilous grassland					●	
	23	21.75142	50.27536	EA56	psammophilous grassland						
	24	21.74980	50.27791	EA56	psammophilous grassland					●	
	25	21.74696	50.27753	EA56	psammophilous grassland		●			●	
	26	21.74858	50.27797	EA56	sandy road		●				

Mesoregion	Site no.	X (°E)	Y (°N)	UTM grid	Habitat type	Pp	Ci	At	Ps	Oc	Sc
Kolbuszowa Plateau	27	21.80578	50.28294	EA57	psammophilous grassland		●			●	
	28	21.80859	50.28449	EA57	psammophilous grassland					●	
	29	21.72721	50.34588	EA57	psammophilous grassland		●			●	
	30	21.80927	50.32781	EA57	psammophilous grassland					●	
	31	21.80305	50.29602	EA57	psammophilous grassland		●			●	
	32	21.77902	50.39457	EA58	sand excavation			●		●	●
	33	21.77659	50.39489	EA58	psammophilous grassland		●			●	
	34	21.91110	50.16092	EA65	psammophilous grassland		●			●	
	35	21.89117	50.17225	EA65	psammophilous grassland					●	
	36	21.89479	50.18186	EA65	psammophilous grassland					●	
	37	21.86423	50.17830	EA65	psammophilous grassland					●	
	38	21.90793	50.18804	EA65	psammophilous grassland					●	
	39	21.89303	50.17283	EA65	psammophilous grassland						
	40	21.89832	50.18798	EA65	psammophilous grassland					●	
	41	21.91503	50.16285	EA65	psammophilous grassland					●	
	42	21.88708	50.18038	EA65	psammophilous grassland					●	
	43	21.90850	50.14945	EA65	psammophilous grassland						
	44	21.95497	50.21048	EA66	psammophilous grassland		●			●	
	45	21.93122	50.26060	EA66	psammophilous grassland					●	
	46	21.92162	50.24976	EA66	psammophilous grassland					●	
	47	21.95339	50.25472	EA66	sandy road					●	
	48	21.96134	50.26588	EA66	psammophilous grassland		●			●	
	49	21.89189	50.19396	EA66	psammophilous grassland					●	
	50	21.95418	50.28251	EA67	sand excavation					●	
	51	21.95312	50.28135	EA67	psammophilous grassland					●	
	52	21.94667	50.28446	EA67	psammophilous grassland					●	
	53	21.94848	50.28346	EA67	psammophilous grassland					●	
	54	21.91488	50.29049	EA67	psammophilous grassland					●	
	55	21.87381	50.34276	EA67	psammophilous grassland					●	
	56	21.85045	50.33921	EA67	psammophilous grassland					●	
	57	21.98843	50.20866	EA76	psammophilous grassland		●			●	
	58	22.06205	50.26027	EA76	psammophilous grassland					●	
	59	21.98532	50.26302	EA76	psammophilous grassland					●	
	60	21.99189	50.28582	EA77	psammophilous grassland		●			●	
	61	21.98974	50.28765	EA77	psammophilous grassland					●	
	62	21.99192	50.28827	EA77	psammophilous grassland					●	

Mesoregion	Site no.	X (°E)	Y (°N)	UTM grid	Habitat type	Pp	Ci	At	Ps	Oc	Sc
Sub-Carpathian Ice Marginal Valley	63	21.68992	50.13496	EA45	psammophilous grassland					●	
	64	21.68162	50.13320	EA45	psammophilous grassland					●	
	65	21.67004	50.13560	EA45	psammophilous grassland					●	
	66	21.73335	50.13367	EA55	sand excavation					●	
	67	21.78969	50.12521	EA55	psammophilous grassland					●	
	68	21.85377	50.12316	EA65	sand excavation					●	
	69	21.85412	50.12021	EA65	sand excavation					●	
	70	21.84958	50.11994	EA65	psammophilous grassland					●	
	71	21.86840	50.11859	EA65	psammophilous grassland					●	
	72	21.94590	50.10392	EA65	psammophilous grassland					●	
	73	21.94458	50.10403	EA65	sand excavation						
	74	21.94776	50.10275	EA65	psammophilous grassland					●	
	75	21.94731	50.10142	EA65	psammophilous grassland					●	
	76	21.95408	50.10497	EA65	sand excavation						
Tarnobrzeg Plain	77	21.82746	50.42619	EA58	sandy road, dry heath		●				
	78	21.80467	50.43652	EA58	sandy road, dry heath					●	
	79	21.83099	50.44771	EA58	sandy road, dry heath		●			●	●
	80	21.84051	50.44140	EA58	sandy road, dry heath	●	●				●
	81	21.80819	50.42959	EA58	sandy road, dry heath					●	
	82	21.91978	50.38353	EA68	psammophilous grassland					●	
	83	21.91784	50.38437	EA68	psammophilous grassland					●	
	84	21.92444	50.39827	EA68	psammophilous grassland		●			●	
	85	21.94726	50.41652	EA68	sandy railway embankment					●	●
	86	21.96445	50.40896	EA68	psammophilous grassland		●			●	
	87	21.95872	50.44127	EA68	psammophilous grassland		●				
	88	21.85009	50.42719	EA68	sandy road, dry heath						●
	89	21.87333	50.44773	EA68	sandy road, dry heath	●	●		●	●	●
	90	21.97389	50.47736	EA69	psammophilous grassland, dry heath					●	
	91	21.97505	50.47613	EA69	sandy road		●			●	
	92	21.85845	50.46273	EA69	sandy road, dry heath	●	●			●	●
	93	21.98605	50.42978	EA78	psammophilous grassland		●			●	
	94	21.99293	50.47640	EA79	sand excavation					●	
	95	21.99281	50.47580	EA79	sand excavation						
	96	21.99787	50.47449	EA79	psammophilous grassland						
	97	22.00583	50.45939	EA79	psammophilous grassland		●			●	

Mesoregion	Site no.	X (°E)	Y (°N)	UTM grid	Habitat type	Pp	Ci	At	Ps	Oc	Sc
Vistula Lowland	98	21.51967	50.35577	EA37	psammophilous grassland					●	
	99	21.54590	50.35666	EA37	psammophilous grassland		●			●	
	100	21.53568	50.34510	EA37	psammophilous grassland		●			●	
	101	21.55319	50.42764	EA38	psammophilous grassland		●			●	
	102	21.54102	50.39945	EA38	psammophilous grassland					●	
	103	21.46811	50.39340	EA38	psammophilous grassland		●			●	
	104	21.59592	50.31280	EA47	psammophilous grassland					●	
	105	21.65955	50.33003	EA47	psammophilous grassland					●	
	106	21.63254	50.35385	EA47	psammophilous grassland		●			●	
	107	21.61740	50.34323	EA47	sandy road					●	
	108	21.69930	50.33147	EA47	psammophilous grassland		●			●	
	109	21.58681	50.43488	EA48	psammophilous grassland					●	
	110	21.58422	50.43664	EA48	psammophilous grassland		●			●	
	111	21.57884	50.44897	EA48	psammophilous grassland					●	
	112	21.58420	50.43469	EA48	psammophilous grassland					●	
	113	21.70060	50.50656	EA49	psammophilous grassland					●	
	114	21.69296	50.50246	EA49	psammophilous grassland					●	
	115	21.66004	50.48608	EA49	psammophilous grassland					●	
	116	21.65393	50.48024	EA49	psammophilous grassland		●			●	
	117	21.75789	50.53934	EA59	psammophilous grassland					●	
	118	21.75641	50.53906	EA59	psammophilous grassland						
	119	21.73723	50.53777	EA59	psammophilous grassland					●	
	120	21.72289	50.54069	EA59	psammophilous grassland		●			●	
	121	21.72669	50.52824	EA59	psammophilous grassland		●			●	
	122	21.71137	50.50846	EA59	psammophilous grassland					●	
	123	21.84621	50.59420	EB50	psammophilous grassland						
	124	21.84058	50.59944	EB50	psammophilous grassland		●			●	
	125	21.83410	50.60028	EB50	psammophilous grassland					●	
	126	21.82372	50.56232	EB50	psammophilous grassland		●			●	
	127	21.83676	50.60064	EB50	psammophilous grassland						
	128	21.86904	50.55233	EB60	psammophilous grassland					●	
	129	21.86529	50.55248	EB60	psammophilous grassland					●	
	130	21.86582	50.55391	EB60	psammophilous grassland		●			●	
	131	21.86812	50.55511	EB60	psammophilous grassland					●	
	132	21.86662	50.60121	EB60	psammophilous grassland					●	
	133	21.87176	50.60067	EB60	psammophilous grassland					●	
	134	21.87191	50.59944	EB60	psammophilous grassland					●	
	135	21.86508	50.63023	EB60	sand excavation					●	

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Revision of the Caucasian Myrmeleontoid Lacewings (Neuroptera: Myrmeleontidae, Ascalaphidae, Nemopteridae) Collection of the Georgian National Museum, Identified By P. Esben-Petersen

Ilhama G. KERIMOVA[1]* Viktor A. KRIVOKHATSKY[2] Vera PKHAKADZE[3]
Natalia D. MANDARIA[4] Valera PETROV[5]

[1*]Institute of Zoology, Ministry of Science and Education, A. Abbaszadeh str. 115, passage 1128, block 504, Baku, Az1004, AZERBAIJAN
[1*]Western Caspian University, 31 Istiglaliyyat, Baku 1001, Baku, AZERBAIJAN
[2]Zoological Institute of the Russian Academy of Sciences, Universitetskaya Emb.,1,St. Petersburg,199034, RUSSIA
[3]National Museum of Georgia, Rustaveli Ave. 3, Tbilisi, GEORGIA
[4]Akaki Tsereteli State University, Biology Department, Kutaisi, GEORGIA
[5]National Museum of Georgia, Rustaveli Ave. 3, Tbilisi, GEORGIA
e-mails: [1*]ilkershah@mail.ru, [1*]administration@wu.edu.az, [2]krivokhatsky@yandex.ru, [3]vera_pkhakadze@mail.ru, [4]Natalia_mandaria@yahoo.com, [5]valerapetrovi@mail.ru
ORCID IDs: [1*]0000-0002-0665-131X, [2]0000-0003-3362-3928, [3]0000-0003-4836-0278, [4]0000-0002-0826-3169, [5]0000-0002-2710-6839
*Corresponding author

ABSTRACT

The present work assesses the Tiflis collection (now the collection of the National Georgian Museum in Tbilisi) of Myrmeleontoid lacewings (Neuroptera: Myrmeleontidae, Ascalaphidae, Nemopteridae), identified by the remarkable taxonomist Peter Esben-Petersen at the beginning of the 20th century (Esben-Petersen, 1913). We have confirmed most of his determinations, some of which have been updated in modern accepted combinations. Some of the specimens listed in the original publication were not found in the collection, and some specimens belonging to species not described during Petersen's lifetime were identified or redefined. The total list of 17 described and assigned species in the collection is updated to 20. To facilitate the identification of Caucasian species of the tribe Myrmecaelurini, keys are provided. New synonymy in Nemopteridae established: *Lertha palmonii* Tjeder, 1970: 219 = *Nemoptera extensa* Olivier, 1811 [recently *Olivierina extensa* (Olivier, 1811)] syn. nov.

Keywords: revision, checklist, antlions, owlflies, spoontails.

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INTRODUCTION

Revision and curation of old collections is a necessary task of taxonomists. For example, the revision of long-established identified specimens of the lacewings in a collection can not only confirm their location, but it also allows the systematists to update the taxonomy as needed (McLachlan, 1868; Tjeder, 1952; Pantaleoni, 1991; Krivokhatsky, 1998; Letardi & Pantaleoni, 1996; Whittington, 2002). The National Museum of Georgia in Tbilisi contains specimens collected in the Caucasus during foreign expeditions by famous Georgian and Russian zoologists in the 19th and early 20th centuries. The lacewings (Neuroptera) in this collection were identified by the Danish entomologist Peter Esben-Petersen and kept in good condition in the museum under the supervision of regular employees, including V. Pkhakadze and V. Petrov. The Myrmeleontoid families (Myrmeleontidae, Ascalaphidae, Nemopteridae), to which the present work is dedicated, are stored in five standard boxes with pinned specimen labels, some of which were written by the hand of Esben-Petersen. All three families of myrmeleontoid lacewings Myrmeleontoidea are considered by the authors as distinct families, not integrating them, as proposed by Machado et al, (2018). The classification of Myrmeleontidae is justified by us (Krivokhatsky, 2011), and Ascalaphidae are arranged according to the system of the collection in the Zoological Institute, St. Petersburg, Russian Academy of Sciences. The collection primarily contains 12 species of antlions, 4 species of owlflies, and 2 species of spoon wings, collected from different countries, but mostly in Transcaucasia. The old pinned specimens are the basis of the collection, determined and published by P. Esben-Petersen, 1913. Some specimens are major damaged. Location data and determinations are presented for each species or for small sets of specimens. The names of some species are old, so they are presented in this paper with their currently recognized names. Most specimens had been previously determined by Esben-Petersen and provided with handwritten labels, pinned individually, or with common bottom labels. Some labels are misspelled. Some specimens in this collection are recognized here as synonyms. We present them, as they are given on the labels and in the original text (Esben-Petersen, 1913). Sometimes, among the bottom labels among the species trays of correctly determined specimens, there are some other pinned species. We do not know whether Esben-Petersen mixed all these series himself, or whether the other specimens were added later, but here we also present these specimens. To contrast the identification labels of the various specimens, Esben-Petersen had placed several species in this collection that were from Hugo Theodor Christoph, a collector from Sarepta on the Volga, labeled with “Ch.”, or “Chr.”. Caucasian Neuroptera were collected mostly by Edward Koenig, Konstantin Satunin, Andrey Šhelkovnikov, Boris Uvarov, and Philipp Zaytsev, who used personalized printed labels. Since Esben-Petersen worked during the pregenital period of taxonomy, we made slides of the male genitalia in some cases to clarify the diagnosis of taxa. Additionally, we used for comparison some species from the collection of the Zoological Institute of Russian Academy of Science in St. Petersburg (ZIN) and also made slides of genitalia from specimens in the collection of the Institute of Zoology, National Academy of Sciences of Azerbaijan, Baku (ANAS), as well as from specimens from the original Georgian collection. Information about the world distribution of each species is presented.

RESULTS AND DISCUSSION

The list presented below includes all taxa designated in the collection and/or published by Esben-Petersen.

Order: NEUROPTERA Linnaeus, 1758

Family: MYRMELEONTIDAE Latreille, 1802

Subfamily: PALPARINAE Banks, 1911

Genus: *Palpares* Rambur, 1842

Palpares libelluloides (Linnaeus, 1764)

Distribution: Europe: Spain, South France, Italy, Croatia, Montenegro, Serbia, Albania, Greece, Romania, Bulgaria, Asia: Cyprus, Turkey, Russia (Dagestan), Georgia, Armenia, Azerbaijan, Syria, Israel, NW Iran, Africa: Tunisia, Morocco, and Algeria. Wide spread East-Mediterranean species with two distinctive main parts: South European - North African and Caucasian - Anatolian.

Material examined: ***Azerbaijan**.* *1♀, Castr. limit, Aslanduz, ad. Arax, 12.07.1912, Mus.Caucasus, № 22-12, leg. K.A. Satunin; 4♂♂, 1♀, 1 damaged specimen, Steppe Mugan, prov. Baku, 10.07.1913, Mus.Caucasus, № 58-14, leg. N. Kostin; 1♂, St. v. f. Aljat, ad. lit., Mar. Casp., 21.06.1912, Mus.Caucasus, № 31-12, leg. V. Bankovski; 1♀, Šhachčinar, ad. Mamruch, 07.1916; *1♂, p. Šhachčinar, dist. Karjagin, 1.07.1912, Mus.Caucasus, № 22-12, leg. K.A. Satunin; *1♀, Castr.limit, Šhachčinar, ad. Arax, 5.07.1912, Mus.Caucasus, № 22-12, leg. K.A. Satunin; *1♀, Castr. limit, Šhachčinar, ad. Arax. 9.07.1912. Mus. Caucasus, № 22-12, leg. K.A. Satunin; *1 damaged specimen, Castr. limit, Šhachčinar, ad. Arax, Mus. Caucasus, № 22-12, leg. K.A. Satunin; 1♂, Alexandrovka, Steppe Mugan, 07.1913, Mus.Caucasus, № 10-13, leg. N. Volçanetski; 1♀, Steppe Mugan. prov. Baku, 06-07.1913, Mus.Caucasus, №58-14. leg. N. Kostin; 1♀, St. Mursolini. V.1915; Caucasus, Kreis, Nucha, Without data, leg. E. Koenig; 1♂, Steppe Mugan, prov.Baku, 06-07.1919, Mus.Caucasus, №58-14, leg. N. Kostin; *2♂♂, P. Aslanduz. dist. Karjagin,11.07.1912, Mus.Caucasus, № 22-12, leg. K.A. Satunin; *1♀, Castr. limit, Aslanduz, d. Arax, 12.07.1912, Mus.Caucasus, № 22-12, leg. K.A. Satunin; *1 damaged specimen , P. Aslanduz, dist. Karjagin, 14.07.1912, Mus.Caucasus, № 22-12, leg. K.A. Satunin; 1 damaged specimen, Steppe Mugan, prov. Baku, 10.07.1913, Mus.Caucasus, № 58-14, leg. N. Kostin; *1♀, Chanagei, Karabach, prov. Elisabet, p. V.1908, Mus.Caucasus, № 47-1908, leg. A.A. Florenski; 1♂, Aresh, Caucasus, leg. A. Šhelkovnikov; *1♂, Geok-Tapa, Aresh, Mus.Caucasus, № 46-08, leg. Šhelkovnikov; *1♀, Geok-Tapa, Aresh, 07.1908, Mus.Caucasus, № 46-08, leg. A. Šhelkovnikov; ***Georgia**.* 1♀, 1♂, 1 damaged specimen, Mtschketi, prov. Tiflis, V.1929, leg. Zimin; 1♀, 2 damaged specimens, Tsarsk. Kolodez, dist. Signarch, 8-10.07.1915, Mus. Caucasus, № 94-15, leg. B. Uvarov; ***Armenia**.* 1♀, Karçevan, prov. Erivan, 7.07.1916, leg.Vinokurov; ***Iran.*** 4♀♀, Teheran et. vic., Persia, 21.07.1915, Mus.Caucasus, № 90-15, leg. H.Bocquillon; 1 damaged specimen, Teheran. et. vic., Persia, 4.07.1915, Mus.Caucasus, № 90-15, leg. H.Bocquillon; 1♂, 2♀♀, Teheran et. vic., Persia, 15.07.1915, Mus.Caucasus, № 90-15, leg. H.Bocquillon; 1 damaged specimen, Teheran. et. vic., 12.X.1915, Mus.Caucasus, № 90-15, leg. H.Bocquillon; 1♀, 1 damaged specimen, Karimabad, lac. Urmia, ocr. 17.06.1916, Mus.Caucasus, № 100-16, expedicia Urmiana.

Notes: Among typical specimens of the species, with longitudinal brown stripes along the entire length of the abdomen, there are some specimens with characters that bring them close to *Palpares turcicus* Koçak; the distal part of the abdomen is partially equipped with brown rings. However, small spots on the cubital forks of the hind wing convince us that such specimens belong to the immature individuals of *P. libelluloides*, described from Azerbaijan as *Myrmeleon nordmanni* Kolenati, 1846 (Krivokhatsky et al, 2017). Esben-Petersen knew this synonymous form and cited it in his work (Esben-Petersen, 1913, p. 287). Eight males and females marked in our list with asterisks (*) were examined by Esben-Peterson. The remaining specimens in his Caucasian work were not listed as well as the series of the following species, which are not found in the Caucasus.

Genus: *Parapalpares* Insom, Carfi, 1988

Parapalpares solidus (Gerstaecker, 1893)

Distribution: Iraq, Iran, Turkmenistan, Afghanistan, Pakistan, Oman, India, and Uzbekistan. This South-Palaearctic species is mainly a mountain species from Tourano-Central-Asia. Has never been collected in the Caucasus and records reported from there are erroneous (Kerimova & Krivokhatsky, 2018).

Material examined: ***Iran***. 4♀♀, Teheran et. vic., Persia, 4.07.1915; 1♀, Teheran et. vic., Persia, 21.07.1915, Mus.Caucasus, № 90-15, leg. H. Bocquillon; 1♂, Teheran et. vic., Persia, 15. II.1915, Mus. Caucasus, № 90-15, leg. H. Bocquillon.

Notes: We do not know whether Esben-Petersen himself included the incorrectly defined specimens in the series of *Palpares libelluloides*, or whether someone else did. We do know that Esben-Petersen never published any material on *Parapalpares solidus*, which suggests that he did not add the specimens. All specimens collected by Bocquillon in Persia are typical *P. solidus*.

Subfamily: ACANTHACLISINAE Navás, 1912

Genus: *Acanthaclisis* Rambur, 1842

Acanthaclisis occitanica (Villers, 1789)

Distribution: This is mainly a greater Mediterranean-area species occurring in Morocco, Tunisia, Egypt, Southern Europe, in addition to Anatolia, Caucasus, Israel, Iran, Kazakhstan, Russia, Uzbekistan, Tajikistan, and Kyrgyzstan.

Material examined: ***Georgia***. 1♀, Hort. botan., Tiflis [Tbilisi], 10.07.1912, Mus. Caucas., 47-12, [leg. Ph.A.] Zaitsev; '*Acanthaclisis occitanica* Vill. [E.-Pet. det.]'; 1♀, Tiflis, 11.07.1912, Mus. Caucas., 47-12, [leg. Ph.A.] Zaitsev; 1♀, Tiflis, 20.06.1915; 2 damaged spec., Kodzori, 12.07.1911; ***Azerbaijan***. 2♀♀, 2♂♂, Geok-Tapa, Caucas, 06.1917, [leg. A.] Šhelkov[nikov]; 1♂, Geok-Tapa, Caucas, without data, [leg. A.] Šhelkov[nikov]; 2♀♀, lac. Gok-gol, distr. Elisavetpol, 07.1913, Mus.Caucas. 94-13, [leg. Ph.A.] Zaytsev; 1♂, Adzikent, pr. Elisavetpol, 28.06.1914, Mus.Caucas. 100-14, A. Vasilinin; 2♂♂, Adzikent, pr. Elisavetpol, 23.06.1914, Mus.Caucas. A. Vasilinin; 1♀, Adzikent, pr. Elisavetpol, 21.07.1913, Mus. Caucas. № 100-14, A. Vasilinin; ***Russia***. 1♂, 'Sarepta, *Acanthiaclisis occitanica,* Ch[ristoph].

Notes: This species has a well-known association with Esben-Petersen; a specimen from Sarepta was used by him for comparison. There are 17 specimens of this species in the collection. In his publication, Esben-Petersen (p. 228) writes about the rarity of the species in the Caucasus. In the collection of the Museum there is one female from the Botanical garden in Tiflis, 10. 07. 1912, Zaitzev leg. Thus, the remaining Caucasian findings were not included in the publication (Esben-Petersen, 1913).

Subfamily: MYRMECAELURINAE Esben-Petersen, 1919

Tribe: MYRMECAELURINI Esben-Petersen, 1919

Esben-Petersen worked at a time when the structure of the genitalia was not used in classical neuropteran taxonomy. Since some specimens of this tribe located in the collection required reidentification, we provide here a determination key for species of the Caucasus region according to the male genitalia as well as other external features.

Key to the genera of tribe Myrmecaelurini from the Caucasus Region

1. Males with one pair of hair-pencils on segment VI of the abdomen; ....*Lopezus* Navás, 1913

- *Lopezus fedtschenkoi* (McLachlan, 1875) known in Caucasus only.

- Males with two pairs of hair pencils on the VI and VII segments of the abdomen.....2

2. Wings rounded at ends; Abdomen same length in both sexes; ventral margin of male ectoproct does not extend beyond the line of the VIII sternite of the abdomen; gonarcus tube straight, conical..*Myrmecaelurus* Costa, 1855

- Wings lanceolate; abdomen of the male longer than that of female; Ectoproct of the male with a strongly drawn angle, which protrudes beyond the line of segment VIII of abdomen..... 3

3. Male abdomen much longer than that of female and strongly protrudes beyond ends of folded wings; gonarcus tube straight, conical............*Aspoeckiana* Hölzel, 1969

- Male abdomen slightly longer than that of female and protrudes slightly beyond ends of folded wings; gonarcus tube arcuately curved.............. *Nohoveus* Navás, 1918

Key to species and subspecies of the genus *Aspoeckiana*

1. Frons and vertex yellow with dark spots; ectoprocts of male about 1.5 mm........... ....*Aspoeckiana uralensis* (Hölzel, 1969)..……2

- Frons and vertex red-brown with dark spots; ectoproct over 2 mm.................... ....*Aspoeckiana glaseri* Hölzel,1972

2. Predominant body color is straw yellow; main longitudinal veins dark with some light areas; vein $Sc + R$ light. Forewing 23-25 mm.. ....*Aspoeckiana uralensis jakushenkoi* (Zakharenko, 1983)

- Predominant body color is reddish yellow; Longitudinal veins, including $Sc + R$, yellow, with almost no pronounced darkening; forewing length 18-25 mm................. ....*Aspoeckiana uralensis curdica* Hölzel,1972

Key to species of the genus *Myrmecaelurus*

1. Smaller species with fore wing shorter than 35 mm..2

- Larger species with fore wing length 35-40 mm..4

2. Wing membrane stramineus or yellow; veins wholly yellow; hair pencils dark 3

- Wing membrane transparent; veins pale, with weak shading; hair pencils pale. ....*Myrmecaelurus acerbus* (Walker, 1853)

3. Wings usually transparent with yellowish nuance; immature specimens (var. *derbendicus* Hölzel, 1972) with slight mesh grayish darkening of the membrane along the longitudinal and transverse veins; pronotum with equiform medial and lateral longitudinal dark brown stripes; hair pencils dark yellow.. ....*Myrmecaelurus trigrammus* (Pallas, 1781)

- Wing membranes yellow with ochre points around crossveins; pronotum with wide medial and full narrow lateral longitudinal dark brown stripes; hair pencils brownish...... ....*Myrmecaelurus spectabilis* Navás, 1912

4. Wing membrane light yellow or transparent; the main longitudinal veins are brown; *Rs* of the hind wing is black; pronotum with medial and lateral longitudinal dark brown stripes; hair pencils dark.......................*Myrmecaelurus major* McLachlan, 1875

- The membrane of the wings is lemon yellow; all veins are yellow; pronotum with a full medial longitudinal light brown line and, sometimes, with incomplete lateral ones; hair pencils with silver hairs.........................*Myrmecaelurus solaris* Krivokhatsky, 2002

Key to species of the genus *Nohoveus*

1. Wing membrane in the costal area of the forewing with a number of brown dots around the bases of the transverse veins.. ....*Nohoveus zigan* (Aspöck, Aspöck & Hölzel 1980)

- The wing membrane is completely transparent without a pattern......................... ....*Nohoveus armenicus* (Krivokhatsky, 1993)

Genus: *Myrmecaelurus* A. Costa, 1855

Myrmecaelurus trigrammus (Pallas, 1771)

Distribution: This Ancient-Mediterranean species prefers plain steppe areas of Algeria, Libya, Albania, Hungary, Slovakia, North Macedonia, Serbia, Croatia, Romania, Montenegro, Spain, Italy, Greece, Ukraine, Moldova, Russia, Turkey, Cyprus, Israel, Iran, Azerbaijan, Kazakhstan, Kyrgyzstan and Uzbekistan, (Krivokhatsky, 2011). It is still one of the most common species of antlions in the West Palearctic (Aspöck et al, 2001; Krivokhatsky, 2011).

Material examined: ***Georgia***. *1♂, Mtschet, prov. Tiflis, 10.07.1911; *1♀, Mtschet, prov.Tiflis, 30.07.1911, Mus. Caucas., 36-11, E. Rimanson, *Myrmecaelurus trigrammus* Pall., handwriting bottom lable by E.-Petersen; *1 damaged specimen, Mtschet, prov. Tiflis, 18.07.1915; 1 damaged specimen, Mtschet, prov. Tiflis, 19.07.1913, Mus. Caucas., № 97-13, leg. K.A.Satunin; 1 damaged specimen, Mtschet, prope Tiflis, 9.07.1915. Mus.Caucasus, № 93-15, leg. L.Bančkovski; *1 damaged specimen, Tiflis, Caucasus, 06.1907, Mus.Caucasus, № 52-07; 1♂, Borshom; 1♀, Borjomi, without data of collection; 1♂(?); ***Armenia****.* 1♂, Van et.vic., Armenia, turc, Migri, 19.06.1916; ***Azerbaijan****.* 1♀, p.Karaduly, dist. Dzevat, 2.06.1912, Mus.Caucasus, № 22-12, leg. K.A. Satunin; ***Russia***. *1♀, Volgograd region, Sarepta, Chr. *Myrmecaelurus trigrammus*. [Th. Christoph leg. et det.]; ***Turkey***. 1♀, Zardanec, distr. Olty, pt. Kars [prov. Erzurum], 15. 07. 1908. Mus. Caucas., 47-08, leg. K.A. Satunin, '*Myrmecaelurus major* MacLachl.?', E.-Petersen det. 1 damaged specimen without abdomen, 29. 07. [18]99.

Notes: The entire center of the second box of specimens is occupied by similar specimens under a single Esben-Petersen's label reading '*Myrmeleon trigrammus* Pallas'. Labels '*Myrmecaelurus trigrammus* Pall., are also pinned to some of them. We confirmed a small part of the identifications, but many specimens were reassigned to the closely related species *Myrmecaelurus solaris* Krivokhatsky, 2002, whose area in the Caucasus is in contact with that of *M. trigrammus*. On the other hand, one specimen identified by Petersen presumably as a *Myrmecaelurus major* has been assigned here to *M. trigrammus*. Esben-Petersen lists in his work (Esben-Petersen, 1913, p. 288) 27 specimens, which were mixed with two species, *Myrmecaelurus trigrammus* and synonymous name *Myrmeleon laetus* Kolenati, 1846, *nec* Klug, 1834. It turned out that the second name, not allocated separately by Esben-Petersen, refers here to the species described later, *Myrmecaelurus solaris* Krivokhatsky 2002. All of the 27 specimens of last '*trigrammus'* are marked in both lists with asterisks (*). A pair of specimens from Esben-Petersen's list [1♀. Liškvas, 7.07.1911 (Satunin leg.), 1♀ Geok-Tapa, 07.1908 (Schelkovnikov leg.)] are lost and were not found. For comparison with true *M. trigrammus*, Esben-Petersen had correctly identified specimens of this species from the Volga region (Sarepta), which he had acquired from Christoph. *Myrmecaelurus trigrammus* is a variable species that is difficult to identify; sometimes it is necessary to study the structure of the male's genitalia to make a positive identification.

Myrmecaelurus solaris Krivohatsky, 2002

Distribution: This Irano-Turanian species occurs in Turkmenistan, Tajikistan, Uzbekistan, Kyrgyzstan, Iran, Armenia.

Material examined: ***Armenia****.* 1 ♂, Migri-Sahi, 11.07.; ***Azerbaijan****.* *1 specimen without abdomen, Castr. limit, Veisaly ad. Arax, 19.07.1912, Mus.Caucas, № 47-12, [leg. Ph.A.] Zaitzev, '*Myrmecaelurus trigrammus* Pall. [E.-Pet. det.]'; 1♂, Castr.limit, Dzebrail, ad. Arax. 2.07.1912, Mus.Caucas, [leg. K.A.] Satunin; *1♂, Castr. limit., Djebrail ad. Arax, 2.07.1912, Mus.Caucas, № 22-12, [leg. K.A.] Satunin, '*Myrmecaelurus trigrammus* Pall. [E.-Pet. det.]'; *1♀, Castr.limit, Šachčinar, ad. Arax. 5.06.1912. Mus. Caucasus, № 22-12, leg. K.A. Satunin '*Myrmecaelurus trigrammus* Pall. E.-Petersen det.'; *1 damaged ♀, Castr. limit. Šachčinar, d. Arax. 3.07.1912, Mus.Caucas, 11-12, [leg. K. A.] Satunin '*Myrmecaelurus trigrammus* Pall.'; 1♀, Castr. limit., ad. Arax, 5.07. 1912, [leg. K.A.] Satunin; 1 (damaged) Šachčinar, 3.V.1922, Mus.Caucas, 22-12, [leg. K.A.] Satunin;*1damaged specimen without abdomen, Castr. limit, Karaduly, ad. Arax. 11.07.1912. Mus.Caucasus, №22-12. K.A. Satunin '*Myrmecaelurus trigrammus* Pall.; *1♀, P. Karaduly, distr. Dźevat, 11.07.1912. Mus.Caucasus, №22-12. K. Satunin; 1 damaged specimen without abdomen, Adzikent, 24.07.1914. Mus.Caucasus, 105-14, A. Wasilinin; 1♀, Steppe Mugan, prov. Baku, 4.07.1913, Mus.Caucas. 58-14, N. Kostin; 1 damaged specimen without abdomen, Kaukasus, Kr. Aresch., E. Koenig.; 1♀, Geok-Tapa, Aresh, 07. 1908, [leg. A.] Shelkov[nikov], Mus.Caucas., 46-08, '*Myrmecaelurus trigrammus* Pall. [E.-Pet. det.]'; 1♀, lac. Gök-göl, distr. Elisvetpl [Gok-gol, Ganja distr.], 07.1913, Mus.Caucasus, № 94-13, [leg. Ph.A.] Zaitsev; ***Georgia***. 1♀, Caucasus, Tiflis, E. Koenig; 1 damaged specimen, Hort.botan, Tiflis, 10.07.1912, Mus.Caucasus, № 47-12, [leg. Ph.A.] Zaitsev '*Myrmecaelurus trigrammus* Pall., [E.-Petersen det.]'; 1♀, Strashnyi Okop [close Borshom], 20 IX. 909, anonym; *1♀, Mtschet, prov. Tiflis, 30.07.1911, Mus.Caucasus, № 86-11, E. Rimanson, '*Myrmecaelurus trigrammus* Pall., [E.-Petersen det.]'; 1♂, Tsarski Kolodez, dist. Signach, 10.07.1915; Mus.Caucasus, № 94 - 15, leg. B.Uvarov;.1♂, Mtschet, prov. Tiflis, 30.07.1915, Mus.Caucasus, № 94-15, B. Uvarov; 1♀, Sagaredzo distr., Tiflis, 4.07.1915, Mus.Caucasus, № 94-15, B. Uvarov; 1♂, 1♀, Tsarsk. Kolodez [Royal well], dist. Signach, 7,10.07. 1915, Mus.Caucas., 94-15, B. Uvarov; ***Iran***. 1♀, Teheran. et vic., Persia, 10.07.1915, Mus.Caucasus, № 90-15, [leg. (H.] Bocquillon; 1 damaged specimen, Teheran, et. vic., Persia, 27.V.1915, Mus.Caucasus, № 90-15, [leg. H.] Bocquillon; 1 damaged specimen, Teheran, et. vic., Persia, 18.07.1915, Mus.Caucasus, № 90-15, [leg. H.] Bocquillon; 1♀, 2 damaged specimens, Teheran, et. vic. Persia, 28.V.1915, Mus.Caucasus, № 90-15, [leg. H.] Bocquillon; 1♀, Leškerek, prope Teheran, 27.07.1915, Mus.Caucasus, № 90-15, [leg. H.] Bocquillon; 1♀, Teheran et.vic. Persia, 28.V.1915, Mus. Caucas., 90-15, Bocquillon, with bottom lable '*Myrmecaelurus trigrammus* Pall.' [E.-Pet. det.]; ***Turkey***. 1 damaged specimen, Van et vic., 19.06.1916, Mus.Caucasus, № 111-16, A. Kaznakov; 1♀, Van et vic., 19.06.1916, Mus. Caucas., 111-16, A. Kaznakov; *2 damaged specimens, V. Zardanes, dist. Olty, Pr. Kars, 15.07.1908, Mus.Caucas. 45-08, leg. K.A. Satunin, '*Myrmecaelurus trigrammus* Pall. [E.-Petersen det.]'. ***Russia***. *1♂, damaged, Rutul-Borch, dist. Samur, 30.07.1910, Mus.Caucasus, № 55-10, leg. A. Schelkovnikov '*Myrmecaelurus trigrammus* Pall., E.-Petersen det.

Notes: Esben-Petersen was not familiar with this species, and he mixed some of the specimens he listed with *M. trigrammus*. The specimens of *M. solaris* in the collection show a considerable range of variability, including an increase in melanism in the pronotum pattern. However, *Myrmecaelurus solaris* can always be recognized, taking into consideration its extreme forms. Unlike *M. trigrammus*, which has three complete longitudinal brown stripes on the pronotum, typical individuals of *M. solarius* have only a medial, relatively lighter, pigment strip. In another form, most often in males, lateral stripes extending from the base, not higher than the pronotal fossae, are added to the median. Another variant of melanization is demonstrated by some specimens of *M. solaris* where brown pigment fringes the edges of the pronotum, which should not be confused with full lateral submedian bands on the pronotum of *M. trigrammus*. For all of the above mentioned variants of pronotum melanization, all specimens of *M. solaris* retain other distinct characteristics, including lemon coloration of the membrane of the wings and unpainted light longitudinal and transverse veins, silver hair pencils in males, and a generally a larger body size. In the *M. solaris* series we examined, males were slightly smaller than females. Sometimes, they can fall into the size category of *M. trigrammus*, and then, the entire collection series should be carefully studied. These two similar species may co-occur in some districts although in different localities. For example, in the vicinity of Tbilisi, *M. trigrammus* was repeatedly registered in 'Mtschet, prov. Tiflis', in Tiflis itself, while *M. solaris* is known from other surrounding villages. In Tiflis itself, *M. solaris* was caught only in the Botanical garden. Of particular interest is the fact that both of these species occur together in separate localities in Turkey at the same time. Thus, in V. Zardanes, dist. Olty, Pr. Kars 15.07.1908, two specimens of *M. solaris* were caught by Satunin and identified by E.-Petersen as *M. trigrammus*, and one male of *M. trigrammus* was erroneously identified as *M. major*.

Myrmecaelurus major McLachlan, 1875

Distribution: This species mostly has a East-Mediterranean-North-Turanian distribution (Krivokhatsky, 2011). It has been reported from Armenia, Turkey, Russia, Kazakhstan, Uzbekistan, Turkmenistan, Tajikistan, Iran, and Afghanistan.

Material examined: Absent in the collection.

Notes: A specimen was recorded in the paper (Esben-Petersen 1913:288), but the present specimen in the collection with the bottom and specimen labels definitely refers to *Myrmecaelurus trigrammus* (Pallas, 1771). In his paper, Esben-Petersen (1913:288, 289) indicated a female with pale longitudinal veins from Turkey with a label [Zardanes, 15. 07. 1908 (Satunin leg.)] and wrote: "It is with some hesitation that I refer this not full-colored specimen to the above-named species, but I think I am right in doing so. I have one male in my collection from Van ([Turcish] Armenia) and 1 female from Berlad, Romania". However, two other specimens collected by Satunin in the same place (Zardanes), were determined by Esben-Petersen to be *Myrmecaelurus trigrammus*. We redefined them here as *M. solaris*, not *trigrammus*. *Myrmecaelurus major* was not previously known from the Caucasus, but we recently discovered this species in Armenia (Krivokhatsky et al, 2020).

Myrmecaelurus acerbus (Walker, 1853)

Distribution: Sethian species: India, Afghanistan, Pakistan, Turkmenistan, Tajikistan, Kyrgyzstan, Turkey, Iran, Iraq, Israel, Saudi Arabia.

Material examined: No real *M. atrox* presented in the collection. Collections determined as *Myrmecaelurus atrox* (Walker) occupy the two upper rows of the third box. Esben-Petersen (1913, p. 289) had written, that "it is a very distinct species easily recognizable by its smaller size, by the shape of the wings and by the dark banded nervures". In the collection under the name *Myrmecaelurus atrox*, Esben-Petersen mostly had placed a specimen of *Nohoveus armenicus* (Krivokhatsky, 1994) from Armenia, which had not yet been described. We have already known (Krivokhatsky, 2011: 273-274) that one specimen of *Nohoveus zigan* (Aspöck, Aspöck, & Holzel, 1980), known in his time as *Myrmecaelurus punctulatus* (Steven in Waldheim, 1846), Esben-Petersen, was defined under an erroneous definition of *Myrmecaelurus atrox* Walker. It was 1♂, Sarepta, Coll. Duske from Finnish Museum of Natural History, Helsinki (seen). Based on a number of his works, we suggested that Esben-Petersen, judging by the description and the cited material (Esben-Petersen, 1913: 289), mixed this species with *Nohoveus armenicus* (Kriv.) and brought it under the name *Myrmecaelurus atrox* Walk. for several places in Armenia (where it was *N. armenicus*), and here also referred specimens (really *N. zigan*) from the mouth of the river Kuma, from Sarepta, and the floodplain of the river Ural (Bostanzhoglo coll.). The present collection that we have studied shows that under the name *Myrmecaelurus atrox* E.-Pet., nec Walker, representatives of not only the genus *Nohoveus*, but also the genus *Aspoeckiana* are hiding. Both genera, Nohoveus and Aspoeckiana, reliably differ mostly according to the male genitalia; we distinguished females or broken specimens from Azerbaijan and Armenia by more fuzzy species characteristics of the head and pronotum patterns and coloration of wing venation.

Genus: *Nohoveus* Navás, 1918

*Nohoveus armenicus (*Krivokhatsky, 1993)

Material examined: ***Armenia***. 1♂ with broken wings, Takältu, decliv. Ararat, 21.06.1911, Mus.Caucas. K. Satunin, as 'Myrme*caelurus atrox* Walk. [E.-Petersen det.];

Notes: Determination of the male was confirmed after the preparation of genitalia, which clearly show relatively elongated ectoprocts and a straight gonarcus tube.

Genus: *Aspoeckiana* Hölzel, 1969

Aspoeckina uralensis jakushenkoi (Zakharenko, 1983)

Material examined: ***Azerbaijan***. 1 damaged specimen without abdomen, Ordubad, prov. Erivan, 25.06.1911; Mus. Caucas., 22. II, K. Satunin; '*Myrmecaelurus atrox* Walk. [E.-Petersen det.]; 1♂, 6 damaged specimens, Ordubad. 20-29.06.1911, K. Satunin; ***Russia***. 1♀, Steppae ad.fl. Kuma. Ciscaucasia, Mus.Caucas. 30-06. '*Myrmecaelurus atrox* Walk. [E.-Petersen det.]; 3 damaged specimens, Steppae ad. fl. Kuma. Ciscaucasia, Mus.Caucas. 30-06.

Notes: The subspecific position of the female from the mouth of the Kuma is doubtful; perhaps it belongs to the nominative subspecies.

Genus: *Lopezus* Navás, 1913

Lopezus fedtschenkoi (McLachlan, 1875)

Distribution: Algeria, Tunisia, Ukraine, Russia (Dagestan), Turkey, Saudi Arabia, Iraq, Iran, Kazakhstan, Turkmenistan, Uzbekistan, Tajikistan, Kyrgyzstan, Afghanistan, Pakistan, China, Mongolia. Sakharo-Gobian species, preferring sandy deserts.

Material examined: ***Russia***. 1♀, 2 damaged specimens, without abdomen, Steppae, ad. fl. Kuma, Mus. Caucas., 30-06.

Notes: Three damaged specimens listed in publication on p. 289, complete with common label '*Myrmecaelurus fedtschenkoi* M.L.*'* placed in the top of the first box. All three specimens belong to the typical, non-striped morph of the species.

The indicated old location (the mouth of Kuma River) confirmed by modern collection finds: (Krivokhatsky & Khabiev, 2016: 46).

Genus: *Cueta* Navás, 1911

Cueta lineosa (Rambur, 1842)

A thorough study of more than 50 specimens by A. Krivokhatsky from Armenia, Azerbaijan, Iran, Albania, and Russia made it possible to come to the conclusion that although *C. anomala* and *C. lineosa* are outwardly very similar, the first species reliably differ from the second one only by the structure of the male genitalia. Therefore, it is quite understandable why G. Holzel considered the females of *C. anomala* and *C. albanica* to be synonyms of *C. lineosa*. Krivokhatsky separated female specimens in a large series of *C. anomala* from Transcaucasia, (Armenia: river Araks, between Nyuvadi and Syurtui, 3 IX 1932, I. Rodionov; Azerbaijan: Geoktape, Elizavetpol province, 24 July 1901, R. Schmidt), reliably compared with the types of *C. anomala* and *C. albanica* (Krivokhatsky, 2011).

Females of *C. lineosa* on the membrane of the forewing have an oblique brown streak running from a bright dot on the regma to the apical area of the wing along a row of stepped veins; the females of *C. anomala*, as well as the males of both species, do not have such a streak; they usually retain only a dot on the regma. The Eastern Mediterranean *C. anomala* and the Iranian-Turanian *C. lineosa* are sympatrically distributed. All indications of the location of *C. lineosa* in Russia (Luppova, 1987) are erroneous (Krivokhatsky, 2011).

Distribution: North Africa, the Middle East, Turkey, Azerbaijan, Kazakhstan, Iran, Turkmenistan, Uzbekistan, Afghanistan, Pakistan (Aspöck et al., 2001; Krivokhatsky, 2011). Wide spread (Krivokhatsky, 2009: 114), Ancient Mediterranean species.

Material examined: ***Azerbaijan**.* 2 damaged specimens without abdomen (the wing pattern corresponds to ♀), Geok-Tapa, Aresch, Shelkovnikov, 07. 1908, Mus.Caucas. 46.08., ‘*Nesoleon oulianini* M. L.’[E.-P. det.]; 2 damaged specimens, Castr.limit. Altar. ad.Arax. 20.07.1912, Mus Caucasus, 22-12, [K.A.] Satunin; 4♂, 1♀, Šhachčinar, 3.07.1912, Mus Caucas., 22-12, [leg. K.A.] Satunin; 1♀, Šhachčinar, 5.07.1912, Mus Caucas., 22-12, [leg. K.A.] Satunin, ‘*Nesoleon oulianini* M. L. [E.-P. det.]’; 1♂, Steppa Mugan, prov. Baku, 06 - 07. 1913, Mus.Caucas. 58 - 14. N. Kostin.

Notes: The full third row of the second box of 10 specimens is accompanied by a common label *Nesoleon vulianini* M.L.’. This grammatical error leads us to assume that the collection, after being processed by Esben-Petersen, was exaggerated.

Subfamily: NEMOLEONTINAE Banks, 1911

Genus: *Distoleon* Banks, 1910

Distoleon tetragrammicus (Fabricius, 1798)

Distribution: Europe, Russia, Caucasus, Israel, Syria, Turkey, Iraq, N Iran. European-Mediterranean species.

Material examined: ***Georgia**.* 1♀, damaged specimen, Tbilisi, v. Silva Chudatovi, IV.1938, A. Vash[agidze leg], ‘*Myrmeleon europeos’*, anonym det. (after bottom lable ‘*Myrmeleon europaus*’); 1♂, Tiflis. 12.07.1912. Mus. Caucas., 47-12 P.A. Zaitsev; 2 damaged specimens, Mtschet, prov. Tiflis, 22.06. 1912; 1 damaged specimen, Caucasus, Tiflis, leg. E.Koenig; 1♀, Tiflis, 21.07.1907, Mus.Caucasus, № 61-02. leg. Ph.A.Zaytsev; 1 damaged specimen, Tiflis. 4.07.1912. Mus.Caucasus, № 47-12, leg. Ph.A.Zaitsev;1♀, Borjom, 5.07.1910, leg. Winogradow - Nikitin); 1♂, 1 damaged specimen, Teliani, prope Telavi, Kachetia, 10.07.1907, leg. N.L.Fursov.; ***Azerbaijan**.* 1♀, Castr. limit., Karad[uly] ad. Arax. 29.06.1912, Mus. Caucas, 22-12, leg. K.A.Satunin 1♂, Steppe Mugan. prov.Baku. 06-07.13. N.Kostin; 1 damaged specimen, 1♀, Geok-Tapa, Aresh, 07. 1908, Shelkov[nikov], Mus. Caucas., 46-08, ‘*Formicaleon tetragrammicus* Fabr.’, Esben-Petersen det.; 1♀, Adjikent, Pr. Elisavetpol, 23.07.1912, Mus. Caucas., 8-12, Vasilinin; 5♀, Adjıkent, Elisavetpol, 26.07.1913, Mus. Caucas., 6-13, Vasilinin; 1 damaged specimen, Geok-Tapa, Aresh, 6.07.1915; 1 damaged specimen, Geok-Tapa, Aresh, 07.1908, leg. A.Šhelkovnikov, Mus.Caucasus, № 46-08; 1 damaged specimen, Geok-Tapa, Caucasus, № 40-15; 1♂, Geok-Tapa, Caucasus, A.Šhelkovnikov, Mus.Caucasus, №40, 3.06.1915; 2♀♀, 1 damaged specimen, Geok-Tapa, Caucasus, leg. A.Šhelkovnikov; 1 damaged specimen, Geok-Tapa, dist. Aresh, 6.07.1915.

Notes: Two last full rows of the third box occupied by identified *Formicaleon tetragrammicus*, labelled with the name in an obsolete combination, like in Esben-Petersen publication. Only one specimen placed in second position after bottom label ‘*Myrmeleon europaus’* (sic!) and added with other wrong spelling label ‘*Myrmeleon europeos’* (sic!).

Genus: *Neuroleon N*avás 1909

Neuroleon nemausiensis (Borkhausen, 1791)

Distribution: Ancient-Mediterranean species, distributed in Europe, Northern Africa and West Asia.

Material examined: ***Georgia***. 1 damaged specimen, Tiflis, Caucasus, 10.07.1913, leg. K.A. Satunin; ***Turkey***. 1 specimen without abdomen, distr. Artvin, Chod-Salačur, 26.07.1911, Mus. Caucas., 74 - 11, leg. J. Voronov, ‘*Neuroleon nemausiensis* Borkh.’ [E.-P. det.].

Notes: Esben-Petersen (p. 291) discussed the significant variability of the species from east to west with a large number of synonyms, not dividing them into subspecies. We propose that Turkish damaged specimen with the bottom label ‘*Neuroleon nemausiensis* Borkh.’ belongs to *Neuroleon nemausiensis ssp. nemausiensis* Borkh.

Genus: *Macronemurus* Costa, 1855

Macronemurus bilineatus Brauer, 1868

Distribution: Nemoral-steppe, Euxine-Black-sea species.

Material examined: Absent.

Notes: E.-Petersen gives one specimen (without abdomen) from Zardanes, 15.07.1908 (Satunin leg.) for the Georgian collection. Unfortunately, the specimen is lost now.

Genus: *Creoleon* Tillyard, 1918

Creoleon plumbeus (Olivier, 1811)

Distribution: Czech Republic, Hungary, Romania, Montenegro, Albania, Macedonia, Bulgaria, Greece, Crete, Ukraine, Moldova, Russia, Turkey, Cyprus, Syria, Israel, Georgia, Armenia, Azerbaijan, Iraq, Iran, Kazakhstan, Turkmenistan, Uzbekistan, Afghanistan, Tajikistan, Kyrgyzstan. A broad Eastern-ancient-Mediterranean species, distributed from the Eastern Mediterranean to Central Kazakhstan and Tajikistan (Krivokhatsky, 2011).

Material examined: ***Georgia****.* *1♂, Hort.botan. Tiflis. 10.07.1912, Mus.Caucasus, 47-12, Zaitsev, ‘*Creagris plumbeus* Oliv. [E.-Pet. det.]‘; 2 damaged specimens, Hort.botan. Tiflis. 26.07.1912, Mus. Caucasus, № 47-12, leg. Ph.Zaitsev; 3♂, Sagaredjo, distr. Tiflis, 4.07.1915.; ***Azerbaijan****.* *1♂,1♀, 2 damaged specimens, Castr.limit, Šhachčinar. ad. Arax. 3.07.1912; *1♀, Castr. limit, Dzebrail, ad. Arax, 2.07.1912, Mus. Caucas, 2-12, [leg. K.A.] Satunin; 1♀, Sachčinar ad Arax, 3.07.1912, Mus. Caucas, 2-12, Satunin; 1♂, Sachčinar ad Arax, [p. Šhachčinar, dist. Karjagin], 3-10.07.1912, Mus. Caucas, 2-12.

[leg. K.A.] Satunin; 1♂, Petropavlovsk, Steppa Mugan, 07.1913, Mus. Caucas, № 141-13, M. Volčanetski; 2 damaged specimens, Castr. limit, Šhachčinar, ad.Arax, 22.07.1912.; ***Turkey***. *1♀, v. Ardanuç, 21.07.1908, Mus. Caucas, 45-08, [leg. K.A.] Satunin, '*Creagris plumbeus* Oliv. [E.-Pet. det.]; ***Russia.*** 1♀, West Caucasus, [Krasnodar Reg.], Utch-Dere, E. Koenig.

Notes: It is clearly seen that in the row behind the bottom label '*Creagris plumbeus* Ol.' in the third box there are several specimens with very long abdomens characteristic of males of this common species. Of the dozen specimens listed in the article (marked with an asterisk *), we found only two, Kosmalian (1500 m altitude), 20. 07. 1906 and Geok-Tapa (Ares), 07. 1908 (Schelkovnikov leg.), but the collection itself is much richer.

Subfamily: MYRMELEONTINAE Latreille, 1802

Genus: *Euroleon* Esben-Petersen, 1919

Euroleon nostras (Geoffroy in Fourcroy, 1785)

Distribution: Widespread in Europe. Ukraine, Moldova, European Russia, Turkey, Georgia, Armenia, Azerbaijan. West Palaearctic nemoral species.

Material examined: ***Georgia***. 1♀, damaged spec., Tbilisi, Hortus botanicus, 11.07.1912, Mus. Caucas, 47-12, [leg. Ph.] Zaytsev, '*Myrmeleon europaus'* M.Lachl.' Esben-Petersen det., bottom label - *'Myrmeleon europaus* M.L.; ***Azerbaijan***. 1 damaged specimen, Geok-Tapa, Aresh, 07.1915.; ***Turkey***. 1♂, damaged specimen, Ani, prov. Kars [Turkish], 14.07.1914, Mus.Caucas., 133-14, Loris.-Kalantar.

Notes: The pair of specimens with a common bottom label *Myrmeleon europeus* is followed by a double row of *Creagris plumbeus*. The first of them added with the individual Esben-Petersen identification label «*Myrmeleon europas* M.L.», but the second one appeared in the collection in the Soviet period and really belongs to Distoleon tetragrammicus. Esben-Petersen's Caucasian publication (p. 290) also indicates two samples from Tiflis, but the second is now replaced by a later one, which was later, determined incorrectly. It is surprising that there are no representatives of the true genus *Myrmeleon* in the collection and the Caucasian list.

Family: ASCALAPHIDAE Lefébvre, 1842

In the publication about the collection of the Caucasian Museum, there is no information about the owlflies, identified by Esben-Petersen, but there are labels with his own handwritten definitions in the collection.

Subfamily: HAPLOGLENIINAE Burmeister, 1839

Genus: *Idricerus* McLachlan, 1871

Idricerus sogdianus McLachlan, 1875

Distribution: Kazakhstan, Kyrgyzstan, Uzbekistan, Turkmenistan, Tajikistan, Afghanistan, India. Turano -Turkestanian species does not occur in the Caucasus.

Notes: Two specimens with the bottom label '*Idricerus sogdianus* M. L.' is preceded by a small collection of ascalaphids. Both specimens, including one much-destroyed specimen with handwritten label ‘*Idricerus sogdianus* MacLachl., det. Esb.-Petersen’, belongs to *Bubopsis hamatus* (Klug). The determination was checked against specimens of *I. sogdianus* from Tajikistan and Turkmenistan and a series of *B. hamatus* from the ZIN collection collected in all areas. The redetermination was confirmed by us, despite the fact that the damaged specimen is superficially similar to *I. sogdianus,* in part because of its damage.

Material examined: Absent.

Subfamily: ASCALAPHINAE Lefèbvre, 1842

Genus: *Bubopsis* Mac Lachlan, 1898

Bubopsis hamatus (Klug, 1834)

Distribution: Turkey, Azerbaijan, Georgia, Russia, Kazakhstan, Kyrgyzstan, Iran, Israel, Jordania, Syria, UAE. East-Mediterranean species.

Material examined: ***Azerbaijan***. 1 damaged specimen, Kaukasus. Kr. Aresch. 30.06.1899. E. Koenig, ‘*Idricerus sogdianus* MacLachl., det. Esb.-Petersen’; 1♀, Geok-Tapa, Aresh, 07.1908, Mus. Caucasus, № 46-08, [A.] Šhelkov[nikov].

Notes: Both specimens are in the first row of the box immediately behind the bottom label of a representative of another subfamily «*Idricerus sogdianus*».

It is noteworthy that a synonym for this species, *Ascalaphus forcipatus* Eversmann 1850, has been described from the southern Caucasus.

Genus: *Deleproctophylla* Lefebvre, 1842

Deleproctophylla variegata (Klug, 1845)

Distribution: Greece, Cyprus, Caucasus, Russia, Kazakhstan, Kyrgyzstan, Uzbekistan, Iran. Ancient-Mediterranean species.

Material examined: ***Azerbaijan***. 1 damaged specimen, Chanagei, Karabagh, prov. Elisavetpol, V.1908. Mus.Caucasus, № 47-08, leg. A.A. Florensk.; 2 damaged specimens, Geok-Tapa, Aresh, 07.1908, Mus.Caucasus, № 46-08, A. Šhelkovnikov, det. Esben Petersen; 2♀♀, Aresh, Caucasus, Mus. Caucasus, № 40-15, leg. A. Šhelkovnikov.

Notes: All specimens are placed after bottom label ‘*Theleproctophylla variegata* Klug.’ and three of them have handwriten labels ‘*Theleproctophylla variegata* Klug., det. Esben Petersen’.

Genus: *Libelloides* Schäffer, 1763

Libelloides macaronius kolyvanensis (Laxmann, 1842)

Distribution: Europe, Caucasus, Turkey, Iran, Middle Asia. East Mediterranean widespread steppe and semi-desert form (Krivokhatsky 1998: 430).

Material examined: ***Georgia***. 1♀, Mtschet, prope. Tiflis, 17.07.1915, Mus.Caucasus, № 93-15, leg. L.Bančkovski; 1♀, Mtschet, prope. Tiflis, 8.07.1915, Mus.Caucasus, № 94-15, leg. B.Uvarov; 1♂, Mtschet, prope. Tiflis. 29.06.1915, Mus.Caucasus, № 93-15, leg. L.Bančkovski; 3♀,Tsarsk. Kolodez, dist. Signach, 10.07.15. Mus.Caucasus. № 94-15. leg.B.Uvarov; 1♂, 6♀, Mtschet, prope. Tiflis. 29.06.1915, Mus.Caucas., 93-15, L. Bančkovski; 1♀, Mtschet, prope. Tiflis, 21.06.1915, Mus.Caucasus, № 94-15, leg. L.Bančkovski; 1♀, Mtschet, prope. Tiflis, 21.07.1915, Mus.Caucasus, № 94-15, leg. B.Uvarov; 1♀, Mtschet, prope. Tiflis, 8.07.1915, Mus. Caucasus. № 93-15, leg.B.Uvarov; ***Azerbaijan****.* 1♂, Helenendorf [Göygöl], 10.05. 1910, anonym coll.; 2♂♂, Elisavetpol, Caucasus, 12.V.[1]911, leg. A.Wasilinin; 1♀, without head, Lenkoran, Mai 1910, anonym coll.; ***Iran****.* 1♀, Cheregirduk, deşt. Urmia, 06.1916, Mus. Caucas, № 94-15. [leg. B.] Uvarov, exp. Urmiana; 1♀, Cherrri-Qirdjk, dest, 06.1916, Mus.Caucas, 100-16. exp. Urmia, [leg. B. Uvarov]; ***Turkmenistan****.* 1♀, Sarykamyś et vicina, 7.07.1914, Mus. Caucas, 49-14, Poltoratski; ***Russia****.* 1♀, [Dagestan], Kasumkent, *Ascalaphus kolyvanensis* Chr[istoph].

Notes: Almost all material consists of yellow specimens belonging to typical morph (Krivokhatsky et al., 2018: 54). There are no specimens determined by Esben-Petersen himself, only one sample provided with a label handwritten by Christoph.

Libelloides lacteus (Brullé,1832)

Distribution: Albania, Bulgaria, Greece, Italy, Croatia, Serbia, Montenegro, France, North Macedonia, Turkey. East-Mediterranean-Anatolian species.

Material examined: ***Turkey****.* 1♀, Artvin, Caucasus, 14.06.1914, leg. Petrosjan.

Notes: The drawing of Brulle type, female (1832), is very schematic. Our female is identical in color and wing shape to the male from southern Bulgaria depicted by Popov (2004).

Libelloides hispanicus ustulatus (Eversmann, 1850)

Distribution: Georgia, Armenia, Azerbaijan, Turkey, Russia. Kura-Araxian-Anatolian subspecies of disjunctive Mediterranean species.

Material examined: ***Georgia****.* 2♀♀, 1♂, Borjomi, Transcaucasus, [P.] Winogradow, 05.07.1911 (all specimens are yellow); 1♂, Borjomi, Likani, A.Wasilinin, 04.06.1912 (white morph).

Notes: There are no known handwritten labels by Esben-Petersen under specimens of that species. The taxon is represented in nature by two co-inhabiting color morphs with a white and yellow wing pattern background. Both morphs are in the collection.

Family: NEMOPTERIDAE Burmeister,1839

Genus: *Nemoptera* Latreille, 1802

Nemoptera sinuata Olivier, 1811

Distribution: Bulgaria, North Macedonia, Greece, Lebanon, Syria, Turkey, Southern Russia, Armenia, Azerbaijan*.* East-Mediterranean species.

Material examined: ***Armenia****.* 1♀, 1 damaged specimen, pag. Liškvas, distr. Zangezur, 8.07.1911, Mus.Caucasus, № 22-11, leg. K.A. Satunin; 1♀, pag. Liškvas distr. Zangezur, 8.07.1911, Mus.Caucasus, № 22-11. leg. K.Satunin; 1♀, Karchevan, prov. Erivan, 7.07.1916, Mus.Caucasus, № 152-11, leg. Vinokurov; ***Turkey****.* 1♀, Artvin, distr. Batum, 07.1913, 20-14. Petrosjan; ***Iran****.* 11♀♀, 3♂♂, 6 damaged specimens, Benarve, Sausdinan, 10.06.1916, Mus.Caucasus, № 100-16, expedisia Urmiana.

Notes: These insects occupy two rows in the fourth box under a single common bottom label: *Nemoptera sinuate.*

Genus: *Lertha* Navás 1910

Lertha extensa (Olivier, 1811)

Distribution: Turkey, Armenia, Azerbaijan, Iran. Irano-Anatolian species.

Material examined: ***Azerbaijan***. 1♀, Ordubad, prov. Erivan, 24.06.1911, Mus. Caucas., 22-11, K. Satunin; ***Iran***. 7♂♂, 4♀♀, 1 damaged specimen, Karim-abad, Lac. Urmia coc, 17.06.1916, Mus.Caucasus, № 100-16, expedisia Urmiana; 5♂♂, 1♀, 2 damaged specimens, Leškerek, prov. Teheran, V.1916.

Notes: This species, as well as the family as a whole, is absent from Esben-Petersen's publication (1913). Nevertheless, in the work of O. Martynova (1930), we have found an almost complete list of specimens of this species, corresponding to the above. According to published information, all of them belong to the ZIN collection and identified by Dr. Kolbe at her request. Moreover, for this article, she made drawings of the males' genital segments and the color variation of the apical plates of the hind wing in specimens from the Urmia expedition. It is noteworthy that all of studied specimens are characterized by the presence of the distinct apical white round spot on the apical extension of the hind wing. The views of modern researchers in respect to the separateness of *Olivierina extensa* and *Lertha palmonii* Tjeder, 1970 on this basis are not in alignment (Dobosz & Ábrahám 2009:124). Here we add a couple more names from the old Navás catalog (1910), *Kirbynia extensa* from Iraq, Iran and Arax and *K. litigiosa* from Arax summarized in synonyms by O. Martynova (1930). According to the ideas of both of these authors, typical *Olivierina extensa* is distinguished by a pronounced apical white spot on the hind wing. At the same time, we note that this spot is characteristic of almost all individuals of the Transcaucasian and Anatolian populations of the early 20th century, as well as those collected 100 years later (Krivokhatsky, Karagyan, Ghrejyan, & Kalashian, 2019). Specimens with dark apical plates on the hind wings and missing white spots were attributed by us to geographical variability (sbsp.), but if we take into account the drawings of the hind wings from the works of Alexandrova-Martynova (1930) and Tjeder (1970), they should be attributed to the infraspecific variability (var.) of one species. Moreover, the brown form attributed by Tjeder to *Nemoptera extensa*, the type of which is lost, has not been proven. To conclude, we establish a synonym here: *Lertha palmonii* Tjeder, 1970: 219 = *Nemoptera extensa* Olivier, 1811 [recently *Olivierina extensa* (Olivier, 1811)] syn. nov. The most secure are full rows of lacewings and mantispids, which will be discussed separately.

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Two New Records of Fruit Fly (Diptera: Tephritidae) for the Fauna of Türkiye

Mehmet YARAN[1*] Vedat GÖRMEZ[2] Mürşit Ömür KOYUNCU[3] Gülcan TARLA[4]

[1,3]Gaziantep University, Faculty of Science and Art, Department of Biology, Gaziantep, 27310, TÜRKİYE
[2,4]Uşak University, Faculty of Agriculture, Department of Plant Protection, Uşak, 64000, TÜRKİYE
e-mails: [1*]yaran@gantep.edu.tr, [2]vgormez@gantep.edu.tr, [3]mursitkoyuncu@gantep.edu.tr [4]gulcan.tarla@usak.edu.tr
ORCID IDs: [1]0000-0002-2151-5471, [2]0000-0001-8136-8226, [3]0000-0002-0789-6925 [4]0000-0003-4025-3806
*Corresponding author

ABSTRACT

Fruit flies (Diptera: Tephritidae) one of the most important Diptera families with number of high pest species. In this study, caper fly *Capparimyia savastani* (Martelli, 1911) and rose hip fly *Rhagoletis alternata* (Fallén, 1811) were determined for the first time from Türkiye. In the paper, also wing figures of each species were given.

Keywords: Caper fly, *Capparimyia savastani, Rhagoletis alternata,* rose hip fly.

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INTRODUCTION

Fruit flies (Diptera: Tephritidae) are one of the most economically important Diptera families in agriculture. Some species of Tephritidae can damage vegetables, flower crops and fruits. They limit agricultural production in some countries because of the strict trade quarantines imposed to prevent their spread (Norrborm, 2004). Tephritidae includes more than 5000 valid species worldwide. In Türkiye, 175 species of fruit flies have been reported up to date by several authors (Kütük, Bayrak, & Hayat, 2011, 2012; Korneyev, et al, 2013; Kütük, Yaran, Hayat, Koyuncu, Görmez, & Aytekin, 2013; Yaran & Kütük, 2012, 2014, 2015, 2016; Yaran, Kütük, Görmez, & Koyuncu, 2018a, 2018b; Çalışkan Keçe, Çatal, & Ulusoy, 2019; Görmez & Kütük, 2020; Kütük & Yaran, 2020; Yaran & Görmez, 2020; Yaran, Görmez & Koyuncu, 2021; Görmez & Kütük, 2022; Kütük, Junaid, Akpınar, Görmez, & Can, 2022).

Genus *Capparimyia* Bezzi, 1920 revised by De Meyer & Freidberg (2005) and provided eight species mainly distributed in Africa. *Capparimyia savastani* (Martelli, 1911) (Caper fly) is distributed mainly in countries bordering the Mediterranean region. The caper fly larvae develop in both flower buds and fruits of *Capparis* spp. (Capparidaceae) (De Meyer & Freidberg, 2005). Genus *Rhagoletis* Loew, 1862 includes 73 valid species. *Rhagoletis* species feed on fruits, and some species of them are important economically and known as pest species for apples, blueberries, cherries, walnuts and tomatoes (Boller & Prokopy, 1976). Larvae of the *Rhagoletis alternata* (Fallén, 1814) (Diptera: Tephritidae) develops in hips of several rose species (Vaupel et al, 2007).

This paper provides new faunistic records for two fruit fly species in Türkiye. In the paper, wing figures and zoogeographic distribution of each species were given.

MATERIAL AND METHOD

Adult *Capparimyia savastani* sample was collected from cultivated fruits of *Capparis* spp.. The sample was identified according to key for genus which provided by De Meyer & Freidberg, 2005. The adult *Rhagoletis alternata* samples were collected from the rose hip by swepping insect net. Samples were identified according to key for *Rhagoletis* which was provided by Korneyev, Mishustin, & Korneyev, 2017. All materials were deposited in the Gaziantep University, Biology Department, Entomology Laboratory, Gaziantep, Türkiye (GUGT).

RESULTS

Capparimyia savastani (Martelli, 1911) (Fig. 1a)

Distribution: Algeria, Cyprus, Egypt, France, Greece, Israel, Italy (Sicily), Lebanon, Libya, Malta, Morocco, Spain, Tunisia, Iran, Jordan, Oman, Pakistan, Yemen and United Arab Emirates (UAE) (Donati & Belcari, 2003; De Meyer & Freidberg, 2005; Merz, Van Aartsen, White, & Van Harten, 2006; Miranda, Terrassa, & Miquel, 2008;

Papachristos, Milonas, & Papasotirioy, 2009; Ghahari, 2013; Moussa & Yammouni, 2014; El Harym & Belqat, 2017; Demetriou & Kryfos, 2020; Mohamadzade Namin & Roberts, 2020).

Material examined: Türkiye, Aydın, Germencik, Tekin Village, Magnesia Antic theatre, 37° 50' 58" N, 27° 31' 11'' E, 53 m, 10.09.2010, 1 ♀; Det: Yaran M. (Leg. Tarla G.)

Rhagoletis alternata (Fallén, 1814) (Fig. 1b)

Distribution: Andorra, Austria, Belgium, Britain, Bulgaria, Czech Republic, Danish mainland, Finland, French mainland, Germany, Hungary, Lithuania, Norwegian mainland, Poland, Romania, Russia (European and Asian parts), Slovakia, Sweden, Ukraine; Kazakhstan, Kyrgyzstan, Japan (Korneyev et al., 2017).

Materials examined: Türkiye, Bayburt, Kop Mountain, 40° 01' 56'' N, 40° 30' 57'' E, 2433 m, 08.07.2021, 1 ♀; Ardahan, Çamlıçatak, 41° 05' 35'' N, 42° 49' 05'' E, 2034 m, 08.07.2021, 1 ♂, 1 ♀. Det: Yaran M. (Leg. Yaran M. Görmez V. and Koyuncu M. Ö.)

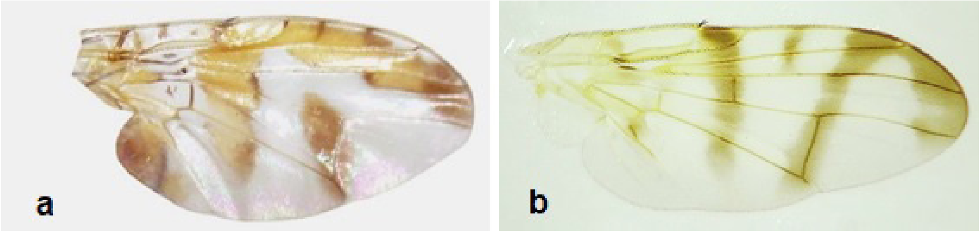

Figure 1. a) Wing of *Capparimyia savastani* (♀), b) Wing of *Rhagoletis alternata* (♀)

DISCUSSION

Capparis spp. are widespread plant species in Türkiye. But up to date, *Capparimyia savastani* didn't record from Türkiye. Recently, Demetriou, & Kryfos, (2020) recorded from Cyprus and Mohamadzade Namin & Roberts, (2020) recorded from UAE. Together with this study, the genus *Capparimyia* and *Capparimyia savastani* were recorded first time for the fauna of Türkiye. Genus *Rhagoletis* known with 5 species in Türkiye (Pakyürek, 2006; Kütük & Özaslan, 2006; Koçak & Kemal, 2013). Yıldırım, Civelek, Çıkman, Dursun, & Eskin, (2010) reported *Utetes magnus* Fischer, 1958 (Braconidae) as parasitoid of *Rhagoletis alternata* for the first time from Türkiye. However, authors didn't mention about collection of *R. alternata.* This species is new for fauna of Türkiye.

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Wing Morphometrics of Forensic Important Fly Species in Kelantan, Malaysia

Helmi HADI

Forensic Science Program, School of Health Sciences, University Sains Malaysia, 16150
Kubang Kerian, Kelantan, MALAYSIA
e-mail: helmi_mhp@usm.my
ORCID ID: 0000-0002-3225-8327

ABSTRACT

This research discusses the possibility of determining the species and sex of necrophagous flies by examining their wing morphology. Fresh beef meat (500 g) was placed in both rural and urban areas of Kelantan, Malaysia, and third instar maggots were collected for rearing from the third to the seventh day. Adult flies were killed with chloroform and their species and sexual status were determined using standard procedures. After detaching their wings, the morphology was documented using a Leica MC 170HD digital camera attached to a Leica stereo microscope. Thirteen wing landmarks were chosen for geometric morphometric analysis. Principal Component Analysis (PCA), canonical variate analysis (CVA), centroid size variation, and the unweighted pair-group method with arithmetic averages (UPGMA) were used to analyse the wing morphology of *Chyrsomya megacephala* (Fabricius, 1794), *Chrysomya rufifacies* (Macquart, 1843), and *Sarcophaga ruficornis* (Fabricius, 1794) The results indicate that sex and fly species can be distinguished based on wing morphology, and that CVA is more effective at species differentiation than sex grouping using Malahanobis distances, with a P value of 0.0001. When comparing different species and sexes of the fly, CVA demonstrates distinct clustering. Identification of necrophagous fly wings is a useful alternative tool for fly classification.

Keywords: Principal Component Analysis (PCA), Canonical variate analysis (CVA), *Chyrsomya megacephala , Chrysomya rufifacies, Sarcophaga ruficornis,* necrophagous fly, species identification

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INTRODUCTION

Necrophagous insects are particularly useful during forensic investigations as they help in estimating the post-mortem interval (PMI) in a scientifically acceptable manner whenever decomposing dead bodies are found at the crime scenes. These flies usually populate the dead body within hours of the body being exposed to the environment, provided the ambience is conducive for the fly to oviposit. Several types of research have been conducted on the necrophagous fly species in Malaysia (Abidin, 2013; Abu Bakar and Zuha, 2016; Chin et al, 2007; Kavitha et al, 2013; Mahat et al, 2009; Mahat et al, 2014; Morry, 2007; Pritam and Jayaprakash, 2009; Ting, 2005). Reports have been published summarizing the necrophagous fly species populating cadavers in different periods in Malaysia such as between 1972-2002 (Lee et al, 2004) and 2005 to 2010 (Kavitha et al, 2013) and in Thailand between 2002 to 2006 (Sukontason et al, 2007).

Morphometrics is quantitatively researching the relationship between biological shape, variation and covariation of shape and other abiotic or biotic factors (Webster and Sheets, 2010). Landmark based Geometric Morphometric (GMM) analysis has been conducted on butterfly wings (Roggero and Entreves, 2005; Breuker et al, 2010), various non-necrophagous fly wings (Klingenberg et al, 1998; Haas and Tolley, 1998; Hall et al, 2014; Pepinelli et al, 2013; Sadeghi and Kiany, 2012), Psychodidae (De la Riva et al, 2001), cricket wings (Klingenberg et al, 2010), culicid mosquito (Sanchez et al, 2017) and necrophagous fly wings (Vásquez and Liria, 2012; Macedo, 2016; Nuñez-Rodríguez and Liria, 2017; Sontigun et al, 2017).

There are various GMM methods for explaining the research data. The use of centroid size (CS) measurement for measuring allometry is useful when measuring the overall GMM landmark configuration provided that all specimens have the same landmark coordinates (Webster and Sheets, 2010). Principal Component Analysis is a useful tool for exploring the shape differences present in a landmarked sample (Webster and Sheets, 2010). The first principal component (PC) shows the most variation of the data followed by the second PC which is orthogonal to the first direction to showcase the most variation (Webster and Sheets, 2010). Canonical variates analysis (CVA) gives a graph output with specimens ordered along the created output. The differences between PCA and CVA are that CVA assumes that the specimens are grouped before the analysis is carried out and tests how well the data or shape in the pre-defined groups support the findings. The CVA tests assume that the data is multivariate and normally distributed and the groups share a common structure (Webster and Sheets, 2010).

Morphometric studies on categorising necrophagous fly species wings have been conducted in Venezuela (Vásquez and Liria, 2012; Nuñez-Rodríguez and Liria, 2017), Brazil (Macedo, 2016) and Thailand (Sontigun et al, 2017). Table 1 summarises the previous research on necrophagous fly species. There are various methods for recording insect wings for morphometric analysis. Wing harvesting could be destructive or non-destructive. Destructive techniques include removing the wings and flattening the wings to view under microscopes (Johnson et al, 2013; Bubliy et al, 2008; Demayo et al, 2011).

Table 1. Previous GMM research on necrophagous fly wing morphology of forensic interest.

Author	Type of species and amount studied (no. of female/male if indicated)	Amount of fly samples landmarked	No of geometric morphometric landmark	Type of Geometric morphometric analysis
Vásquez and Liria, 2012	*C. megacephala* (57) *C. albiceps* (111)	168	8	Procrustes superimposition and PCA
Macedo, 2016	*C. albiceps* (55) *C. megacephala* (42) *H. segmentaria* (42)	139	13	PCA, DA, CVA
Nuñez-Rodríguez and Liria, 2017	*L. cuprina* (21f/19m) *C. megacephala* (21f/19m) *C. albiceps* (35f/35m)	150	8	Procrustes superimposition, CS variation, CVA, SShD,
Sontigun et al, 2017	*C. megacephala* (29f/24m) *C. chani* (23f/17m) *C. pinguis* (10f/29m) *C. rufifacies* (22f/25m) *C. villeneuvi* (16f/23m) *C. nigripes* (15f/17m) *L. cuprina* (14f/15m) *L. papuensis* (23f/9m) *L. porphyrina* (7f/11m) *L. sinensis* (4f/4m) *H. ligurriens* (18f/14m) *H. pulchra* (3f/0m)	372	19	Procrustes superimposition, CVA, CS, SShD, DA with cross validation, UPGMA
Grzywacz, Ogiela and Tofilski, 2017	*A. nebulosa* (0f/14m) *E. cyanicolor* (0f/40m) *G. maculata* (0f/17m) *H. impuncta* (0f/35m) *M. levida* (0f/163m) *M. urbana* (0f/14m) *H. dentipes* (0f/318m) *M. domestica* (0f/46m) *N. cornicina* (0f/30m) *P. pallida* (0f/45m) *P.lardarius* (0f/8m) *S. calcitrans* (0f/32m) *T. simplex* (0f/28m)	790	15	MANOVA, CVA
Szpila, Żmuda, Akbarzadeh, & Tofilski, 2019	*C. rohdendorfi* (3f/7m *C. subalpina (3f/7m)* *C. vicina 85f/130m)* *C. vomitoria (38f/38m)* *C. albiceps (38f/24m)* *C. megacephala (49f/46m)* *C. mortuorum* (6f/27m) *L. ampullacea* (12f/19m) *L. Caesar* (40f/88m) *L. illustris* (8f/9m) *L. sericata* (72f/98m) *L. silvarum* (16f/27m) *P. Regina* (21f/9m) *P. terraenovae* (32f/12m)	968	15	PCA, MANCOVA, CVA, UPGMA
Jos, Jos, & Martínez-s, 2020	*C. vicina* (50f/50m) *C. vomitoria* (50f/50m) *C. albiceps* (50f/50m) *C. megacephala* (50f/50m) *L. caesar* (50f/50m) *L. sericata* (50f/50m)	600	17	PCA, Procustes ANOVA,
López-garcía, Angell and Martín-vega, 2020	*L. varipes* (20f/24m) *P. flavipes* (1f/5m) *P. vulgaris* (2f/6m) *P. casei* (24f/14m) *P. megastigmata* (12f/12m) *P. nigrimana* (26f/39m) *P. latipes* (5f/12m) *P. litigate* (5f/5m) *S. nigriceps* (7f/12m) *C. furcate* (1f/2m) *T. cynophile* (0f/5m)	239	14	PCA, CVA, DA, UPGMA

PCA Principal component analysis, DA Discriminant function analysis, CVA Canonical variates analysis, SShD Sexual shape dimorphism, CS Centroid size, UPGMA, Unweighted pair group method with arithmetic averages.

Non-destructive methods include orientating the insects so that the photographed wings are perpendicular compared to the microscope (Hall et al, 2014). Other non-destructive method includes the fabrication of a wing-capturing device (Perrard et al, 2012). A research on the accuracy of the different registering modalities (pinned, scanned and detached wings) has been conducted on dragonfly wings (Johnson et al, 2013). It is crucial to standardise the wing harvesting and capturing procedure as differences will introduce errors (Johnson et al, 2013).

MATERIALS AND METHODS

Sample collection and preparation

This study was conducted between 24 March - 24 May 2016 in the rural and urban areas of Kota Bharu district (6° 8N, 102° 15E). Two fresh beef samples each weighing 500 g from animals that have been slaughtered two to four hours earlier were placed in a rural area of Dewan Beta and within the compounds of Universiti Sains Malaysia Kelantan. A slotted plastic basket with few bricks were placed on top of the basket to avoid interference from carnivores. The slots permitted free entry and exit of adult flies. A plastic sheet was placed covering the top half of the basket to protect the meat from the rain. The above setup and data collection were repeated another two times one week apart at the same location with new beef meat. Daily observation was conducted twice, and maggots were collected from the second day to the eighth day. Maggots collected twice a day were reared in suitably labelled ventilated containers. Weather data was collected from the local weather station (less than 2 KM from the study site). The temperature for the entire research period was recorded every day and it ranged between 24°C to 35°C.

Maggots were reared in 14 transparent plastic containers containing dry soil and a piece of decomposing meat. The top of the container was covered with gauze and secured with rubber bands to allow for ventilation while preventing the flies from escaping. Once the adult flies emerge from the pupae, a chloroform-soaked cotton ball was added to the plastic container and the container is closed tight with a wrapper for the chloroform to act on the flies. Species and the sex of the adult flies were noted based on available guidelines (Szpila, 2007) and the wings (left and right) were then removed carefully with tweezers for analysis. Damaged wings were excluded from the analysis. The removed wings were taped onto A4 papers with clear cellophane tape. The sample of flies studied during this research is shown in Table 2. The A4 papers were then scanned with an Epson L210 printer at 1200 dpi into jpeg images. Each wing image was cropped with Adobe® Photoshop® CS6 software for GMM analysis.

Geometric morphometric analysis in MorphoJ

Collected images were utilised to build a tps file by using TpsUtil V 1.74 software (Rohlf, 2005) before being landmarked in TpsDig2 V 2.30 software (Rohlf, 2005). Type I landmarks were placed on 13 points on the wing's venation. Type I landmarks are easily identified structures such as the intersection of two meeting points (Bookstein, 1997). Fig. 1. shows the GMM landmark location on the wing veins adopted for analysis as detailed in previous research (Rahman, 2015). Each wing image was duplicated further and was landmarked three times by the same individual to reduce the measurement error (Arnqvist and Martensson, 1998). The TPS file created was uploaded into MorphoJ V 1.06d software (Klingenberg, 2011) and the raw landmarks were aligned and superimposed with the Procrustes Fit function based on the centroid size. The PCA and CVA were conducted on the species while Procrustes ANOVA was conducted on the centroid size and shape variation of the dataset. The CVA was conducted in order to identify the important features which can discriminate all the groups.

Table 2. Total number of specimens examined.

Fly specieS	Total no of specimens (male/female)
Chrysomya megacephala	136 (29/107)
Chrysomya rufifacies	97 (27/70)
Sarcophaga ruficorniS	50 (0/50)

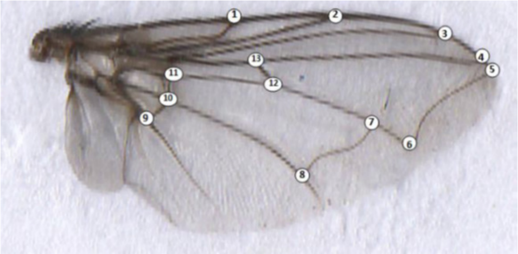

Figure 1. Wing of *Chrysomya megacephala* showing the 13 landmarks adopted for the morphometric analysis based on previous research (Rahman, 2015).

Size, shape, sex and phenetic relationship analysis

SPSS V 22.0 (IBM Corp. Armonk, NY) was utilised for the wing size, sex and sex difference based on fly species. The wing size was estimated with the Kruskal-Wallis H-test by utilising the mean centroid size, followed by the Mann-Whitney *U*-test (significance level of 0.05). The sex differences were tested with the Mann-Whitney *U*-test (significance level of 0.05). Boxplots of the centroid size by species and sex were produced to show the differences in the relative centroid size of the species.

In order to examine the phenetic relationship among the three fly species based on morphology, the unweighted pair-group method with arithmetic averages (UPGMA) was performed on the Procrustes Coordinates of the average dataset exported from MorphoJ in PAST V 3.17 (Hammer et al, 2001). The UPGMA dendrogram by Euclidian distance was constructed showing the relationship between the species analysed.

Sexual dimorphism

The sexual shape dimorphism (SShD) between each species for both males and females were tested by Mann-Whitney *U*-test in SPSS V 22.0 with a significance level of 0.05. The shape differences for females and males was analysed by Mahalanobis distances (with 10,000 permutations) was conducted in MorphoJ. Results are shown in Table 6.

RESULTS

Three species of necrophagous flies (n= 283) of *Chrysomya megacephala* (CM), *Chrysomya rufifacies* (CR) and *Sarcophaga ruficornis* (S) were collected from the reared maggots. Most of the species collected for the research were female flies (n= 227) compared to males (n= 56). No *Sarcophaga ruficornis males* were observed in the study (Table 2).

From the GMM PCA test, the Eigenvalues (Table 3) show that the top four Principal Components (PCs) account for 62.8% variation. The scatterplot graph of PC1 vs. PC2 of the wings are shown in Fig. 2 with the fly wing morphology at different ends (indicated by dotted box) of the x- and y-axes indicated by the selected dataset. The data for the male population (left polygon) and female population (right polygon) is shown for all fly species.

Table 3. Table showing Eigenvalues, percentage of variance and cumulative percentage variation of the Principal Components.

PC	Eigenvalues	% Variance	Cumulative %
1.	0.00027173	27.967	27.967
2.	0.00013921	14.328	42.294
3.	0.00011011	11.333	53.627
4.	0.00008891	9.151	62.778

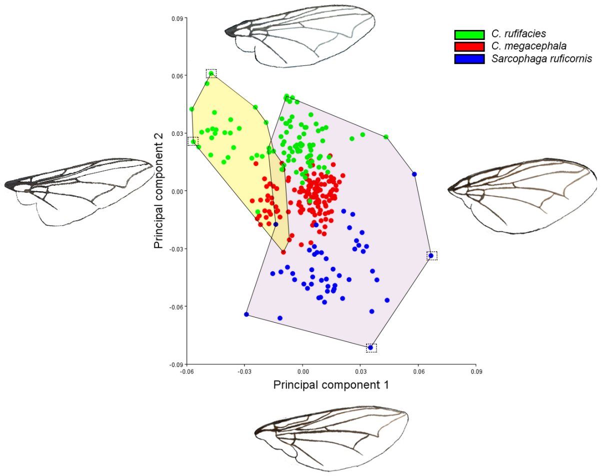

Figure 2. Scatterplot graph for PC1 and PC2 with the fly wing morphology which explains 42.3% of variance. The male fly population is shown in the left (yellow) polygon and female in the right (purple) polygon. The Rectangle box outline shows the fly morphology at extreme ends of the scatterplot.

Results for the size and shape are reported in Tables 4 and 5. Based on Table 4, the main effect of sex for size was almost 1% of the total sum of squares with a P-value of 0.0097. Differences in the fly species were larger and explained 60% with a significant P-value. The individual effect is lower compared to the fly species at 39%. For size variation, the fly species show the highest difference compared to the individual effect with sex having the smallest influence. Results for the shape (Table 5) mimic those for the size with the exception the sex being higher at 11% with a significant P-value. The shape variation for the fly species and fly wings were 50% and 38% which was slightly lower compared to the centroid size variation.

The CVA indicates that the species and the sex are clearly distinct from each other (Fig. 3). Mahalanobis distances from Table 6 range from 3.60 (CM, (male vs. female)) to 16.05 (CR (male) vs S, (female)). All permutation tests indicate that the mean shapes vary among taxa with P <0.0001 in pairwise permutation tests (10000 permutation rounds) for Mahalanobis distances among groups. The Procrustes distances among groups range from 0.0269 (CM, (male vs. female)) to 0.1115 (CR (male) vs S, (female)). The scatter plot of CV shows a marked concentration at high CV1 scores for the Chrysomyinae subfamily. The shape of the wings differs in morphology on the CV1 axes and differs in size on the CV2 axes (Fig. 3).

Table 4. Centroid size variation.

Effect	Explained SS (%)	SS	MS	df	F	*p*
Sex	0.959	61769.922	61769.922	1	6.78	0.0097
Fly species	59.571	3835404.079	1917702.040	2	210.54	<0.0001
Fly wings (individual)	39.470	2541231.903	9108.358	279		
Total	100	6438405.904				

SS sum of squares, MS mean squares, df degree of freedom, F F statistics, p paramatic p-value.

Table 5. Shape variation.

Effect	Explained SS (%)	SS	MS	df	F	P	Pillai tr.	*p*
Sex	11.801	0.077	0.003	22	86.76	<.0001	0.76	<0.0001
Fly species	50.249	0.327	0.007	44	184.71	<.0001	1.68	<0.0001
Fly wings (individual)	37.950	0.247	0.000	6138				
Total	100	0.650						

SS sum of squares, MS mean squares, df degree of freedom, F F statistics, p paramatic p-value, Pillai tr. Pillai's trace.

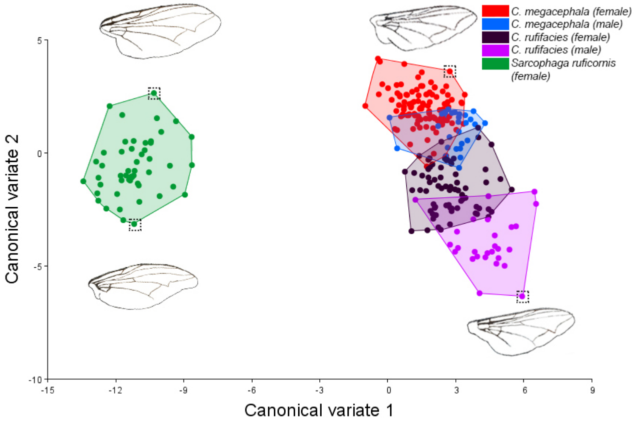

Figure 3. Canonical variate analysis of necrophagous fly wings. The fly wing shapes show the shapes for CV1 scores of -15 and +9 and CV2 scores of -10 and +5 (all other CV scores kept at value 0). The various wing morphology nearest to the dotted square box is displayed as well.

Table 6. Mahalanobis distances among groups for the specimens analysed with the sex (male/female). The p-values for all attributes were highlight significant (p<0.0001) with 10,000 permutation rounds in MorphoJ.

	CR (f)	CR (f)	S (f)	CM (m)
CR (f)	4.035			
S (f)	13.021	14.042		
CM (m)	3.602	4.848	14.410	
CR (f)	6.919	4.422	16.048	5.700

CR *C. rufifacies*, CM *C. megacephala*, S *Sarcophaga ruficornis*, f female, m male.

The centroid size among species were significantly different. Fig. 4. shows the boxplot with the centroid size of wings for the sex of each species. Only female S were available in this research. The Kruskal-Wallis Chi-square test is 114.026, *df* = 2, *P* = <0.001). There is no significant difference between males and females as the P value was > 0.05 (at 0.366). Post-hoc analysis was conducted between groups on the mean centroid size and the Mann-Whitney *U*-test between CM vs CR (7528.000), CR vs S (7153.000) and CM vs S (1275.000) were highly significant (P = <0.001). The UPGMA phenetic relationship between the samples revealed that the Chrysomyinae subfamily pairing close together compared to S (Fig. 5). The above results agree with the CV1 vs CV2 results in Fig. 3 wherein samples from the Chrysomyinae subfamily overlap compared to S.

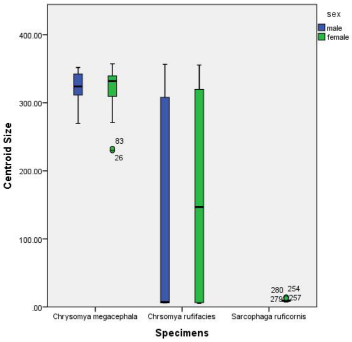

Figure 4. Boxplot showing the centroid size of wings for each fly specimen. Only female *Sarcophaga ruficornis* were collected in this research thus it could not be utilized for classifying between sexes. The mean centroid size Mann-Whitney U-test was highly significant at p <0.001.

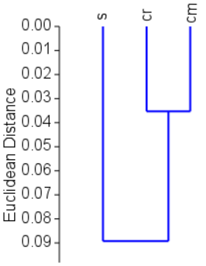

Figure 5. UPGMA dendrogram showing the fly wing morphology relationship construction based on Euclidean distances between specimens of *Chrysomya megacephala* (CM), *Chrysomya rufifacies* (CR) and *Sarcophaga ruficornis* (S).

DISCUSSION

Species-level identification of necrophagous species is fundamental for determining the time of death of an individual. CM and CR are the most common species in Kelantan followed by S. The amount and distribution of fly species obtained from this research are similar to the fly population available from other previous research (Lee et al, 2004; Mahat et al, 2009; Mahat et al, 2014; Morry, 2007) and case studies (Kavitha et al, 2013) in Malaysia. Based on previous findings (Kavitha et al, 2013), the majority of fly infestation on cadavers in Malaysia is single fly species (92.5%) while only 7.5% are double fly species. According to Kavitha et al. (2013) also, CR and CM were the most abundant species in Malaysia accounting for 82.8% of species recovered. These findings are replicated here and the most dominant species were CM and CR. No other species were recorded. *Sarcophaga ruficornis* only accounted for 17.7% of the total flies found which was slightly higher compared to the 10% documented previously (Kavitha et al, 2013). Despite the geographical proximity of Malaysia and Thailand, the variety of necrophagous fly species in Thailand (Sukontason et al, 2007; Zajac et al, 2016) is more compared to species found in Malaysia (Kavitha et al, 2013; Lee et al, 2004). Majority of the maggots obtained and reared were females. It is unknown why females were observed more than male flies. There were no male *Sarcophaga ruficornis* flies recovered from the reared specimens. A total of 227 females were collected compared to 56 males. From the reared specimens obtained, female flies were significantly more compared to male flies by almost three times. It is unsure why there is a small number of male flies for *C. megacephala* and *C. rufifacies* and why no males were obtained from the *Sarcophaga ruficornis* maggots reared. The lack or little male flies observed may be due to female maggots being oviposited more on the meat. There does not seem to be dominant fly sex trend based on previous findings (Nuñez-Rodríguez and Liria, 2017; Sontigun et al, 2017).

From Fig. 2 and Table 3, the first four PC cumulative variation value 62.8%. The cumulative variation values for PC 5 to PC 22 amount to 37.2%. The fly morphology difference for PC1 against PC2 is shown in Fig. 2 for the extreme ends of the PC axes. Figure 2 shows that the wing morphology of male flies tend to be narrower and longer particularly between the intersection of veins Costa and Media (landmark 5) and Intersection of crossvein media-cubitus and vein cubitus (landmark 8). The PCA results show that there is a distinct difference between the wing shape morphology of the three different fly species recorded. Differences also extend into the sex morphology. However, as there are no male S flies collected, it is unknown how the wing morphology of the wings would differ further.

The CVA (Fig. 3) show the grouping of males and females Chrysominae flies and the difference in the grouping of the S flies. The species variation is closer compared to the sex variation as the clustering of both CM and CR were adjacent. The Kruskal-Wallis test followed by the post-hoc Mann-Whitney *U*-test show significant differences when analysing the mean centroid size of each species. However, there is no significant difference when comparing the wing size intra-species. The results for the CM wings obtained are similar compared to the results by Sontigun et al. (2017) in Thailand Some of the CM wings were collected from Phatthalung and Trang which is nearer to Kota Bharu. However, they noted that there is a significant difference in the wing size variation for CR (Sontigun et al, 2017). The CR fly population is only available in Chiang Mai which is further north compared to Trang and Phatthalung (Sontigun et al, 2017) which is nearer to Kota Bharu. It seems that the CR population is available sporadically and may not be present throughout Thailand. It also has to be noted that S is not available anywhere in Thailand. The *Sarcophaga* genus may be present in Thailand but was not observed in the research (Sontigun et al, 2017).

The use of fly wing morphology for species identification in a forensic investigation has not yet been explored by researchers and practitioners. Previous researches indicate that wing venation can help determine the species of a fly (Nuñez-Rodríguez and Liria, 2017; Sontigun et al, 2017). It is usually easier to recover the whole fly sample from a crime scene compared to just the wings which are quite transient. The wing morphology may be useful in additional information on species identity for cases where flies obtained from a crime scene are severely damaged. When a dead fly degrades over time, the body of the fly is susceptible to fungus in humid conditions and withers in dry conditions. In this situation, the wings are the only organ available to identify the species of the fly. The overlap of the species clusters in the canonical variates analysis (Fig. 3) indicates that it is harder to differentiate between sexes of the same species compared to species that are different. While differences do exist, there is an overlap between the groups that have to be considered.

Wing morphometric analysis on the sex and species of forensic important fly species in Malaysia could be conducted with the aid of geometric morphometrics. The shape differences are useful for class classifying the fly species which populates the cadaver. If any fly wings were to be found at the crime scene, then the fly wing morphology is useful in determining the type of species which populates the cadaver. However, it has to be noted that acquiring fly wings may be difficult compared to the whole fly as it is quite small and light enough to be dispersed by the wind.

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Research Article
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New Records of Species *Taeniopteryx hubaulti* Aubert, 1946 and *Taeniopteryx schoenemundi* (Mertense, 1923) (Plecoptera: Taeniopterygidae) in Serbia

Predrag SIMOVIĆ[1] Vladica SIMIĆ[2] Djuradj MILOŠEVIĆ[3] Ana PETROVIĆ[4]*

[1,2,4]Institut of Biology and Ecology, Faculty of Science, University of Kragujevac, Radoja Domanovića 12, Kragujevac, SERBIA
[3]Department of Biology and Ecology, Faculty of Sciences and Mathematics, University of Niš, Niš, SERBIA
e-mails: [1]predrag.simovic@pmf.kg.ac.rs; [2]vladica.simic@pmf.kg.ac.rs, [3]djuradj.milosevic@pmf.edu.rs, [4]*ana.petrovic@pmf.kg.ac.rs
ORCID IDs: [1]0000-0001-6723-8230, [2]0000-0001-6787-9783, [3]0000-0002-5328-3898, [4]*0000-0002-4908-8176
*Corresponding author

ABSTRACT

Species *Taeniopteryx hubaulti* Aubert, 1946 and *T. schoenemundi* (Mertense, 1923) have been emphasized as endangered or vulnerable species in several European countries due to their local and limited distribution. More intensive research of these species on the territory of Serbia was conducted from 2011 to 2022. *Taeniopteryx hubaulti* was registered in seven localities. Five of these localities (Brusnička and Jerma rivers, the spring of the Raška River, and two localities on Grza River, spring and tufa barriers) were recorded as new sites of this species in Serbia. Two localities (Barska River and Lisinski Stream) had already been known from previous studies. The species *T. schoenemundi* was recorded in four new localities (Dulenska, Visočica, Temštica, and Trnavska rivers). Both species were found in waters with high oxygen concentration, relatively uniform low water temperature, and variable values of hardness, electrical conductivity, and pH values, with a small amount of inorganic nutrients. Considering the rare occurrence and prominent importance of these species as biological indicators of water quality and climate change, their findings have a great deductive value. This research represents an important contribution to the insight into the distribution of *T. hubaulti* and *T. schoenemundi* species.

Keywords: Stoneflies, distribution, endangered species, climate change, karst rivers.

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INTRODUCTION

Taeniopterygidae (early or winter stoneflies), a small family within the order Plecoptera, is an ecologically significant component of the entomofauna of lotic waters worldwide, characterized by a high degree of endemism and narrow ecological requirements (Koese, van Maanen, & Boumans, 2014; Fochetti, 2020a). The global diversity of the family Taeniopterygidae includes 104 known species classified into 12 genera, out of which 48 species inhabit European waters (Fochetti & de Figueroa, 2007; DeWalt & Ower, 2019). The genus *Taeniopteryx* Pictet, 1841, the only genus within the subfamily Taeniopteryginae, shows a holarctic distribution (Zwick, 2000; Zhiltzova, 2006) and comprises ten species in European freshwater (Guareschi, Ramos-Merchante, Ruiz-Delgado, & Mellado-Díaz, 2018). However, some of these species have become extinct from their habitats (Lock & Goethals, 2008, Kosterin, Akimbekova, & Dubatolov, 2017), while others are given the status endangered or vulnerable due to local and limited distribution (Lubini, Knisp, Sartori, Vicentini, & Wagner, 2012; Petrović, Simić, Milošević, Paunović, & Sivec, 2014; Tyufekchieva, Evtimova, & Murányi, 2019). The genus *Taeniopteryx* includes typical winter, cold-stenothermal species, with an early flight period from January to April, according to the altitude (Fochetti & Paolo, 1996; Zwick & Hohmann, 2003; Rupprecht, 2014). In European waters, larvae begin to appear in late September and mostly behave as shredders (Harper, Lauzon, & Harper, 1991; Lock & Goethals, 2008; Cummins, 2019).

In some countries, there are numerous studies on the distribution of species of the genus *Taeniopteryx* (Zhiltzova, 2006; Kovács & Murányi, 2008; Bojková, Soldán, Špaček, & Straka, 2011; Tyufekchieva et al, 2013). However, many authors believe that these species have not been adequately studied, blaming their typical winter life cycle and inaccessibility of habitats for it (Koese et al, 2014; Guareschi et al, 2018). Nevertheless, due to their extreme sensitivity to elevated temperatures, these species are useful indicators of climate change. In addition, they are considered as good bioindicators of environmental quality, as they respond to habitat change and pollution (Puig, Ubero-Pascal, Amore, & Fochetti, 2011; Guareschi et al, 2018; Fochetti, 2020b).

The diversity and distribution of the order Plecoptera in aquatic ecosystems on the territory of Serbia were recently studied by Petrović et al (2014). However, as a separate entity, the family Taeniopterygidae has not been the subject of scientific research yet. According to literature data, three species of the genus *Taeniopteryx* have been recorded in Serbia: *T. nebulosa* (Linnaeus, 1758), *T. hubaulti* Aubert, 1946, and *T. schoenemundi* (Mertense, 1923) (Petrović et al, 2014). Based on previous data on their distribution in aquatic ecosystems of Serbia, mutually isolated populations of the species *T. nebulosa* were recorded in the Timok Basin (the Trgoviški Timok) (Simić,1993) and the West Morava (the Lomnička River) (Konta, 1997), and the South Morava (the Vlasinska River) Basins (Paunović, Jakovčev-Todorović, Simić, Stojanović, & Petrović, 2006).

Species *T. schoenemundi* was recorded almost 30 years ago at only one site in the South Morava Basin (the Manojlovica River) (Simić, 1996); while *T. hubaulti* was registered in the West Morava Basin (the Barska River and the Lisinki Stream), in the area of the Kopaonik National Park (Konta, 1997; Petrović et al, 2014). In Serbia, the species of *T. hubaulti* and *T. nebulosa* are on the list of strictly protected species (Anonymous, 2010). Although *T. schoenemundi* is not included in the list of protected species by any Serbian regulative, efforts should be directed towards its conservation. Moreover, there are already reports about population decline in Germany and Italy or the extinction of local populations in Switzerland and Belgium (Lock & Goethals, 2008; Fochetti, 2020b). This paper provides data on new findings of species *T. hubaulti* and *T. schoenemundi* in the aquatic ecosystems of Serbia, together with key environmental factors affecting their biogeographical distribution in current aquatic ecosystems.

MATERIAL AND METHODS

Study area

The primary data source for this study was hydrobiological surveys in Serbia that were conducted every three years during different seasons from 2011 to 2022 in 71 localities in the Morava basin, including the West, South, and Great Morava rivers and their tributaries. The list and details about investigated localities can be found in Simić et al (2022). Additionally, this study included seasonal investigations of aquatic macroinvertebrates in 20 karst rivers of Serbia that were conducted over four years from 2019 to 2022. The study encompassed various lotic karst habitats (springs, mountainous rivers, streams, and tufa barriers). Within all investigated localities, target species were found in the following 11: Barska River, Lisinski Stream, spring of Raška River, Brusnička River, and Trnavska River (the West Morava basin); Dulenska River, Grza River (two localities: spring and tufa barriers) (the Great Morava basin); Temštica, Visočica, and Jerma rivers (the South Morava basin) (Fig. 1). Data for the geographical coordinates, altitude, and type of substrates of each locality are presented in Table 1.

Table 1. Sampling sites of species *Taeniopteryx hubaulti* and *T. schoenemundi* in Serbia.

Abbreviation	River/localites	Geographical coordinates	Altitude	Substrate
BA	Barska River	43°17’41.2”N 20°45’31.6”E	1346 m	cobbles, gravel, sand
LI	Lisinski Stream	43°16’54.4”N 20°45’02.0”E	1170 m	cobbles, gravel, sand
JE	Jerma River	42°57’06.2”N 22°36’37.7”E	554 m	large stones, cobbles, gravel, and sand
BR	Brusnička River	43°25’05.9”N 20°22’28.1”E	794 m	cobbles, gravel, and sand
GRS	Spring of Grza River	43°53’49.2” N 21°38’45.0”E	432 m	large stones, cobbles, gravel, moss, macrophytes
GRT	Tufa barriers of Grza River	43°53’48.4”N 21°38’42.8”E	411 m	mosses on tufa, gravel, macrophytes
RA	Spring of Raška River	43°06’57.4”N 20°22’13.7”E	739 m	large stones, cobbles, gravel, sand, moss
DU	Dulenska River	43°53’38.7”N 21°00’50.1”E	338 m	cobbles, gravel, sand
VI	Visočica River	43°09’23.9”N 22°48’27.2”E	694 m	cobbles, gravel, sand
TE	Temštica River	43°17’48.0”N 22°36’22.7”E	509 m	large stones, cobbles, gravel, sand
TR	Trnavska River	43°17’13.9”N 20°34’31.9”E	528 m	cobbles, gravel, sand

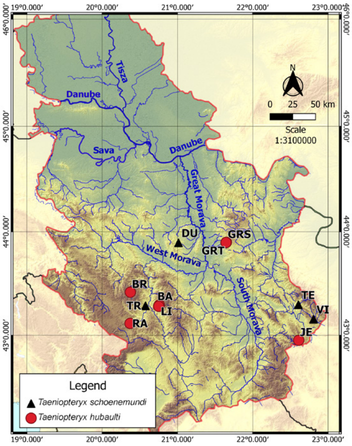

Figure 1. The geographical distribution of *Taeniopteryx hubaulti* and *T. schoenemundi* in Serbia: (●) *T. hubaulti*: the Jerma River (JE), the Brusnička River (BR), the spring of the Raška River (RA), and the spring and tufa barriers of the Grza River (GRS and GRT), the Barska River (BA), the Lisinki Stream (LI); (▲) *T. schoenemundi*: the Dulenska River (DU), the Visočica River (VI), the Temštica River (TE) and the Trnavska River (TR).

Sample collection and processing

At each site, six subsamples of macrozoobenthos were collected from the most common substrate types with a 0.0625 m^2 Surber sampler of 250 mm mesh, according to the standard EN 10970 (2012). The subsamples were merged into a single sample. The samples were fixed with 4% formaldehyde solution and stored at the Institute of Biology and Ecology, Faculty of Science, University of Kragujevac, Serbia. From the entire macrozoobenthos sample, genus *Taeniopteryx* specimens were separated for further identification and taxonomic studies. Collected specimens were analyzed under a NIKON SMZ 800 stereomicroscope with a MOTIC camera and Nikon Eclipse E100 microscope. The material was identified according to established entomological keys (Aubert, 1959; Hynes, 1967; Raušer, 1980; Zwick, 2004). Quantitative estimation of macrozoobenthos was based on numerical counting, i.e., units per square meter (ind. per m^2). Simultaneously with collecting benthic invertebrate samples in the field, the following physical and chemical parameters were measured: water temperature (°C), pH (1-14), oxygen concentration (mg L^{-1}), saturation (%), conductivity (μs cm^{-1}), nitrate, ammonia and phosphate concentration (mg L^{-1}) and water hardness (CaCO3 mg L^{-1}) according to EN ISO 5667 (2017).

RESULTS

New records of *T. hubaulti* larvae on the territory of Serbia were recorded at the following localities: the Jerma River (the South Morava Basin), the Brusnička River, the spring of the Raška River (the West Morava), and the Grza River (spring and tufa barriers) (the Great Morava). During these investigations, two previously recorded findings in the area of NP Kopaonik (the Barska River and the Lisinski Stream) were confirmed (Fig. 1). During the research, new findings of *T. schoenemundi* were recorded in four new localities: the Dulenska River (the Great Morava Basin), Visočica and Temštica rivers (the South Morava), and the Trnavska River (the West Morava).

The springs of karst rivers had higher number of individuals of *T. hubaulti* in comparison to other study sites. In the spring of Grza River 45 and 24 ind./m^2 were recorded, while 21 and 16 ind./m^2 were recorded in the spring of Raška River. Similarly, in the locality on the Jerma River 16 ind./m^2 were detected. In other localities, small populations were recorded with less than 11 ind./m^2 (Table 2). The number of *T. schoenemundi* individuals found at investigated sites ranged from 3 to 16 individuals per m^2 (Table 3). In a comprehensive study of the genus *Taeniopteryx* performed in this work, several preferences according to substrate type were observed. The high number of individuals of *T. hubaulti* in springs can be attributed to a greater variety of substrates. These localities are characterized by substrate with stones, cobbles, and specialized vegetation, especially mosses. The types of substrate in other localities consisted mostly of stones, cobbles, gravel, and, less frequently, tufa with mosses (Table 1). The highest number of individuals of *T. schoenemundi* was on the Temštica River (16 ind./per m^2), which is characterized by a substrate consisting of large stones, cobbles, and gravel. In general, based on our results, *T. schoenemundi* prefers mineral substrate without vegetation (Table 1).

Table 2. The number of individuals per m^2 and environmental parameters of *Taeniopteryx hubaulti* localities in Serbia.

River/localties	**BA**	**LS**	**JE**	**BR**	**GRS**		**GRT**	**RA**	
Date	16.08.’11.	17.08.’11.	02.11.’11.	15.10.’12.	24.10.’20.	25.02.’22.	24.10.’20.	31.10.’21.	17.02.’22.
Number of individuals per m^2	11	3	16	5	45	24	3	16	21
Water temperature (°C)	12	13.1	8.2	9.4	10	8.9	10.2	10.7	4.6
Conductivity (µS cm^{-1})	80	120	390	710	500	480	490	360	170
Hardness (mg L^{-1})	40	60	200	350	230	240	240	180	80
pH	6.7	7.92	6.90	7.86	6.88	7.24	7.56	7.39	7.44
O_2 (mg L^{-1})	9.40	9.10	11.10	10.98	10.16	-	9.92	10.29	-
O_2 (%)	91.5	95.5	105.7	84.3	101.2	-	99.1	100.8	-
NO_3 (mg L^{-1})	5.6	6	3	<3	<3	5	3.5	3.2	<3
PO_4 (mg L^{-1})	0.08	0.09	0.10	0.10	0.08	<0.06	0.22	0.07	<0.06
NH_4 (mg L^{-1})	0.3	0.24	0.07	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03

Table 3. The number of individuals per m^2 and environmental parameters of *Taeniopteryx schoenemundi* localities in Serbia.

River/localites	**DU**	**VI**		**TE**		**TR**
Date	29.10.’11.	02.11.’11.	25.12.’13.	22.10.’21.	22.03.’22	16.02.’22.
Number of individuals per m^2	3	11	5	16	5	8
Water temperature (°C)	5.9	7.8	6	5.8	4.9	6.2
Conductivity (µS cm^{-1}).	460	240	180	80	100	400
Hardness (mg L^{-1})	230	130	90	40	50	190
pH	9.09	7.78	8.11	7.63	8.04	7.68
O_2 (mg L^{-1})	11.36	11.31	9.93	10.71	-	11.43
O_2 (%)	95.6	105.4	100.8	96.5	-	103.5
NO_3 (mg L^{-1})	6.7	<3	<3	4.9	<3	4.5
PO_4 (mg L^{-1})	0.29	0.10	0.12	0.14	<0.06	0.06
NH_4 (mg L^{-1})	0.09	<0.03	<0.03	<0.03	0.05	<0.03

Findings of *T. hubaulti* species were recorded at altitudes ranging from 411 m a.s.l. (the Grza River - tufa barriers) to 1346 m a.s.l. (the Barska River), and *T. schoenemundi* species at altitudes from 338 m a.s.l. (the Dulenska River) to 694 m a.s.l. (the Visočica River). *Taeniopteryx hubaulti* and *T. schoenemundi* species were recorded in waters with relatively uniform high values of oxygen concentration (> 9.1 mg L^{-1}) and low temperature at all localities (< 13.1 °C). However, values of hardness, electrical conductivity, and pH varied significantly. In all localities where both species were recorded, the amounts of inorganic nutrients were small (Table 2 and 3). During our investigations, both species, as typically cold stenotherms, usually occur during autumn and winter. Specifically, the species *T. schoenemundi* was observed to occur from October to April, while the species *T. hubaulti* was observed to occur earlier, from mid-August to March (Table 2 and 3). These observations of earlier occurrence of species *T. hubaulti* were recorded at higher altitudes in the watercourses of the Kopaonik National Park (Barska River and Lisinski Stream).

CONCLUSIONS AND DISCUSSION

The main environmental factors determining the distribution of the abundance of *Taeniopteryx* species in rivers are altitude, type of substrate, oxygen saturation, the solubility of oxygen in water, and temperature (Tyufekchieva et al, 2013).

Species *T. hubaulti* is a western Palearctic species with fragmented and localized areal in European freshwaters (Zhiltzova, 2006). According to recent studies, *T. hubaulti* is widely spread across Central Europe (Bojková et al, 2011), while there are only a few findings in eastern and southern Europe, including the Balkan Peninsula (Tyufekchieva et al, 2019; Ridl et al, 2018). *Taeniopteryx hubaulti* inhabits clean and cold streams in mountainous and sub-mountainous regions in the rhithral zones (Bojková et al, 2011; Graf et al, 2022). Previous studies on Serbian freshwater systems confirmed the distribution of *T. hubaulti* in regions of upper and middle rhithral, altitudes between 1000 and 1500 m a.s.l. (Petrović, 2014). However, in our research, the species was found at a lower altitude, in a zone between 400 and 800 m a.s.l., similar to the latest findings in Macedonia by Slavenska-Stamenković et al (2016). Studies conducted in the National Park Plitvice Lakes, located in the karst part of northwestern Dinarides (in the mountainous part of Croatia), recorded the presence of *T. hubaulti* on several locations between 390 and 720 m a.s.l. (Korana River, springs, and rhithral of the Bjela and the Crna rivers) (Popijač & Sivec, 2009; Ridl et al, 2018). The species was not recorded on the tufa barriers of the previously mentioned rivers. In Bulgaria, Tyufekchieva et al (2013) described the findings of this species above 1000 m a.s.l. This species is widely studied in the mountainous parts of the Czech Republic, between 320 and 900 m a.s.l. (Bojková et al, 2011). In Switzerland, it was recorded at an altitude between 700 and 1400 m a.s.l. (Lubini, 2012), whilst in Spain and France between 1400 and 1600 m a.s.l. (Puig, 1984; Guareschi et al, 2018).

In our research, *T. hubaulti* was found in various biotopes such as springs, streams, small and medium-sized rivers with stones, and tufa barriers. According to recent studies, the species was recorded in the area of the springs and the upper flows of streams (epirhithral and metarhithral), in turbulent and fast-flowing water with few specialized macrophytes on mineral substrates, mainly consisting of stones, cobbles, and gravel (Guareschi et al., 2018; Ridl et al., 2018). In our research, the type of substrate in investigeted habitats (stones, cobbles, and gravel with or without specialized vegetation) directly correlates with findings in similar studies (Tyufekchieva et al, 2013; Ridl et al, 2018). The exception is the habitats of tufa barriers for which there is no literature evidence.

Although widespread throughout Central Europe and the Balkans, populations of *T. schoenemundi* count only a few specimens (Angersbach, Stein, & Wolf, 2010). In the east, the distribution area extends to Poland, Slovakia, and Romania, while in the south and the west, it occurs in the Iberian Peninsula, Italy, the southwestern France (Berthelemy & Whytton Da Terra, 1980; Vincon & Pardo, 2004; Enting, 2005; Graf et al, 2022). This species was found in a wide range of altitudes, from 100 to 1900 m with the largest number of finds in the hyporhithral and epipotamal water zones, with rocky and gravelly bases (Angersbach et al, 2010; Manko, 2011; Tyufekchieva et al, 2013). However, based on the results obtained in our research, it can be concluded that the species, in addition to hyporhithrals (the Dulenska River), also inhabit metarhithral, being supported by Tyufekchieva et al. (2013) research.

To this date, the presence of the species *T. schoenemundi,* on the territory of Serbia has been recorded only in the Manojlovica River, in the South Morava Basin (Simić, 1996). Simić (1996) and Paunović et al (2006) stated the presence of *Taeniopteryx* sp. in the area of Vlasina, the landscape of exceptional features.

On the territory of Serbia, both investigated species were found in waters with high oxygen concentration above 9 mg L^{-1} and oxygen saturation above 80%, which is in correlation with the research from other areas. In Bulgaria, the studied species were not found in waters with dissolved oxygen below 7 mg L^{-1} and 70% saturation (Tyufekchieva et al, 2013). In Croatia, Ridl et al (2018) found *T. hubaulti* species in waters with oxygen concentration from 7.6 to 14.1 mg L^{-1}, and saturation between 65 and 121%. In the Netherlands, *T. schoenemundi* was recorded in waters where oxygen conditions always remain relatively high, i.e., above 8 mg L^{-1} (Koese et al, 2014).

Almost all authors state that *T. hubaulti* prefers cold, clean waters of the rhithral type (Tyufekchieva et al, 2013; Schmidt-Kloiber & Hering, 2015; Graf et al, 2022). According to our research, the maximum recorded water temperature at which *T. hubaulti* was found was 13.1 °C. According to the literature, the best growth is achieved at temperatures below 10 °C (Graf et al, 2022), which corresponds to some findings. Koese et al (2014) states that the limiting factor for the survival of *T. schoenemundi* is 20 °C. While *T. hubaulti* is a typical indicator species of oligosaprobic waters, *T. schoenemundi* can tolerate mildly polluted waters and live in β-mesosaprobic waters corresponding to individual findings in epipotamal water zones (Tyufekchieva et al, 2013).

Recognition of conservation and vulnerability of these species has been acknowledged by many countries, considering habitat quality change and high levels of running water pollution (sewage, agricultural and industrial waste) and other human activities along with climate change as major factors causing decline and loss of their populations (Angersbach et al, 2010; Fochetti, 2020b). Based on previous findings on the territory of Serbia, species *T. hubaulti* has been marked as critically endangered (Petrović, 2014), and it is on the list of strictly protected species (Anonymous, 2010). *Taeniopteryx hubaulti* is classified as endangered in Bulgaria (Tyufekchieva et al, 2019), vulnerable in Switzerland (Lubini, 2012), while in Spain, it is considered as a moderately vulnerable and relict species (Puig et al, 2011; Guareschi et al, 2018). In the Czech Republic, it is considered by most authors as a vulnerable species (Bojková, Komprdová, Soldán, & Zahrádková, 2012; Bojková & Soldán, 2013). However, given a large number of new sites and the wider distribution in the Czech Republic, its vulnerability category has been put into question, and some authors have suggested that the Near Threatened category (NT) would better describe its current status (Bojková et al, 2011). In the Bulgarian Red List, *T. schoenemundi* is classified as vulnerable species (Tyufekchieva et al, 2019; Tyufekchiev & Rimcheska, 2019), while in Slovakia is critically endangered (Manko, 2011). In Germany, *T. schoenemundi* is considered an endangered species, with the threat of extinction in most parts of the country (Zwick, 1992; Enting, 2005). In Italy, sites of this species have been reduced to small, isolated relict populations threatened with extinction (Fochetti, 2020a). In most of Belgium and Switzerland, it is considered extinct because the rivers in which they were once recorded are now loaded with various types of pollution, resulting in the non-survival of sensitive Plecoptera species (Lock & Goethals, 2008; Lock & Oosterlynck, 2012; Lubini, 2012).

Some of these new habitats in Serbia are protected to various degrees. Localities on the Visočica and the Temštica rivers are located within the Stara Planina Nature Reserve, while the locality on the Brusnička River is part of the Golija Nature Park. The Barska River and the Lisinski Stream are in the area of National Park Kopaonik. The Jerma River belongs to the category of Special Nature Reserve. Species of the genus *Taeniopteryx* are on the Austrian list of sensitive macroinvertebrate taxa (Moog, 2002), as their biological characteristics and ecological preferences determine their vulnerability to freshwater ecosystem modification and climate change (Fochetti, 2020b; Graf et al, 2022). This point out their bioindicator significance. Since the studied species require specific ecological conditions, any direct or indirect negative anthropogenic influence may affect their survival in their microhabitats. This is especially true of the karst of the Grza River, which is in danger of pollution due to excessive and unplanned construction of recreational settlements and due to the so-called mass tourism. This also applies to the Brusnička River, where a small hydroelectric power plant was built. Future research will show whether these factors affect the population of species of the genus *Taeniopteryx*. During the systematic research of the rivers of NP Kopaonik, from 2016 to 2021 (during September), no records of the species *T. hubaulti* were made. However, we noticed various habitat modifications caused by intensive urbanization processes. Along with investigated species, it was discovered that some of these researched localities are habitats of many other critically endangered, endangered, and vulnerable aquatic macroinvertebrate species (eg. *Thremma anomalum* (Mclachlan, 1876), *Helichopsyche bacescui* (Orghidan & Botosaneanu, 1953), *Drusus discolor* (Rambur, 1842), *Amphinemura sulcicoris* (Stephens, 1836)), which according to the local red list have conservation priority.

Insufficient literature data on these species could be explained by their winter life span or the inaccessibility of their habitats. It has been noted that the most frequent findings of these species were in the period from late October to mid-April (Zhiltzova et al, 2006; Tyufekchieva et al, 2013), which correlates with our research, with small deviations in the NP Kopaonik rivers. Therefore, future studies should include a larger number of study sites and microhabitats in higher lentic habitats, primarily during the winter months. The current study represents an important contribution to the evidence on the distribution of two species of the genus *Taeniopteryx*, but also their preferences for habitat and environmental factors in current aquatic ecosystems in Serbia. Due to their rare occurrence, these species are important biological indicators of water quality and climate change, and therefore their findings are always of great value. Further study analysis of the species would contribute to a better understanding of the real status and distribution of these species, which would precisely indicate its category of endangerment, and thus the appropriate necessary protection measures.

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A Comparison of Methods for Investigating the Quantitative Relationships Between *Empoasca onukii* Matsuda (Hemiptera: Cicadellidae) and its Natural Enemies

Shiyan CHEN[1] Junjie CAI[2] Honghao CHENG[3] Xiaomeng WU[4]
Yunding ZOU[5] Shoudong BI [6*]

[1,3,6]College of Science, Anhui Agricultural University, Hefei 230036, CHINA
[2,4,5]College of Resources and Environment, Anhui Agricultural University, Hefei 230036, CHINA
e-mails: [1]shiyanchen112@163.com, [2]15240010575@163.com, [3]1587229816@163.com,
[4]1020130931@qq.com, [5]658789314@qq.com, [6*]bishoudong@163.com
ORCID IDs: [1]0000-0003-4459-7411, [2]0000-0003-3147-9600, [3]0000-0003-3121-0716,
[4]0000-0001-5493-6400, [5]0000-0001-8069-7909, [6]0000-0002-1113-0105
*Corresponding Author

ABSTRACT

To systematically study the quantitative relationship between natural enemies and pests, this paper used grey relational analysis method, angular cosine coefficient method, fuzzy similarity priority ratio method and correlation coefficient method to analyze the closeness of the quantitative relationship between natural enemies and *Empoasca onukii* Matsuda in “Anjibaicha”, “Huangshandayezhong” and “Longjing 43” tea plantations. The conclusions obtained by the grey relational analysis method were used as a criterion to compare the sum of the rankings of the top three natural enemies, *Plexippus paykulli*, *Tetragnatha squamata* and *Ebrechtella tricuspidata*, thus comparing and discussing the similarities and differences between the conclusions obtained by the four research methods. The angular cosine coefficient method and grey relational analysis method yielded no major differences in conclusions, followed by the correlation coefficient method, with the fuzzy similarity priority ratio method yielding more varied results. According to the ranking analysis of the close relationship between the number of *E. onukii* and its natural enemies, *Tetragnatha squamata*, *Hylyphantes graminicola* and *Ebrechtella tricuspidata* are the first three natural enemies closely related to the number of *E. onukii*. This paper is an attempt to compare the consistency of research results of various research methods, which provides a reference for selecting research methods in analyzing the quantitative relationship between natural enemies and pests.

Keywords: Tea plantation, Natural enemies, *Empoasca onukii*, Quantitative relationship, Research method.

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INTRODUCTION

With the widespread consumption of tea as a beverage, the economic benefits of tea trees are gradually coming to the fore and the conservation of tea plantation ecosystems is being emphasized, while the yield and quality of tea are affected by many aspects, an important one being the direct harmful effects of pests (Yao, Li, & Xie, 2020; Wu, Tian, & Chen, 2020). The study of the relationship between pest and natural enemy populations is useful for analysing the behavioural habits and predation preferences of individuals in a population at a particular time (e.g. peak season), and is also important for evaluating the effects of various factors (e.g. the environment) on individuals or populations of pests and natural enemies. The study of the dynamics of the larger pattern as a whole is extremely useful in analysing the associations between different species and the extent and patterns of their adaptive capacity to external uncontrollable factors. The quantitative relationship between natural enemies and target pests in tea plantations is related to many factors, one of these factors is the magnitude of the predatory (or parasitic) effect of natural enemies on the target pest (Liu, Zhang, Bi, Yu, & Li, 2019). In recent years, Bi, Zou, Chen, Meng, & Wang (2000) studied several natural enemies closely related to *Aphis gossypii* (Glover) using the grey system analysis method. Cheng et al. (2018) analyzed several natural enemies closely related to *Frankliniella intonsa* (Trybom) and *Brevipalpus obovatus* (Donnadieu) using the method of geostatistics and grey relational analysis. Lin et al (2013) studied the relationship between planthoppers and natural enemies by grey system analysis. The degree of spatial aggregation of *Lygus lucorum* (Meyer-Dür) and its natural enemies was studied by Zhou et al (2019a) using blocked quadrat variance analysis and grey relational analysis. Gong et al (2019) studied the natural enemies most closely related to *E. onukii* in plum gardens based on the fuzzy similarity priority ratio method. They used fuzzy similarity priority ratio method, correlation coefficient method, angular cosine coefficient method and grey system analysis method to analyze the quantitative relationships between natural enemies and various pests, and their results are helpful for the conservation and utilization of natural enemies in pest control. Few studies have been conducted on the quantitative relationship between the same pest and its natural enemies using multiple methods. *E. onukii* is one of the main pests in tea plantations, and *Clubiona japonicola* Boes. et Str, *Oxyopes sertatus* L. Koch and *Tetragnatha squamata* Karsch are the first three natural enemies that are most closely related spatially to *E. onukii* (Sun et al, 2021). In this paper, we studied the quantitative relationships between *E. onukii* and its natural enemies in “Anjibaicha”, “Huangshandayezhong” and “Longjing 43” tea plantations. The results of the four methods, namely, grey relational analysis method, angular cosine coefficient method, fuzzy similarity priority ratio method and correlation coefficient method, were analyzed and compared to provide reference for the selection of research methods for the relationship between natural enemies and pest populations.

MATERIALS AND METHODS

Field investigation

A total of three tea plantations were surveyed in the Science and Technology Demonstration Park of Anhui Agricultural University (31°56′N, 117°12′E). The tea tree varieties in these three tea plantations are “Anjibaicha”, “Huangshandayezhong” and “Longjing 43”, with a total area of 0.2 hm^2. The survey period was from May 23, 2021 to September 17, 2021. The frequency of survey was about once every 10 days, 8 times in total. The three tea plantations were not connected to each other and were managed according to conventional measures. No chemical pesticides, ploughing and hoeing to loosen the soil before spring, appropriate irrigation in the morning and evening in summer according to weather conditions, light pruning after the end of autumn tea or before the sprouting of spring tea, clearing the garden in winter, cutting out diseased and weak branches, sweeping up dead branches and leaves on the ground and removing weeds from the tea garden.

According to the parallel jumping method, three rows were randomly selected in the tea garden, and a 2 m long sample square was taken at a distance of 1 m from each row of tea trees, with each sample square having an area of 2 m^2. 10 sample squares were selected for each row, and a total of 30 sample squares were taken. A visual survey was carried out by taking 10 leaves at random from each sample plot and observing the species of pests and natural enemies that adhered firmly to the tea trees. A solution of 1000 times laundry detergent was sprayed onto an enamel tray (40 cm long and 30 cm wide), and the branches of the tea trees in the sample plots were tapped to make the insects fall off and adhere to the tray. The species and number of pests and natural enemies in the tray were counted visually and recorded. Carry charts of common pest and spider species in tea plantations and compare them meticulously with the bugs in the tray for species identification in terms of shape, colour, size, etc. Some species that could not be identified were numbered, stored in special bottles and brought back to the laboratory for observation and identification.

Analysis methods

Grey relational analysis method

$$\xi_{ij} = \frac{\min\min|Y_i(k)-X_j(k)|+\rho\max\max|Y_i(k)-X_j(k)|}{|Y_i(k)-X_j(k)|+\rho\max\max|Y_i(k)-X_j(k)|}$$

The above equation ρ is the resolution coefficient, it’s usually between 0 and 1, this paper takes $\rho=0.8$. $\min\min|Y_i(k)-X_j(k)|$ is the 2-level minimum difference, and $\max\max|Y_i(k)-X_j(k)|$ is the maximum difference of 2 levels (Deng, 1990).

From the above equation, the grey relational coefficient between the number of natural enemies and pest is $R(Y_i,X_j)=\frac{1}{n}\sum r_{ij}(k)$. The higher the grey relational coefficient between natural enemies and pest populations, the closer the relationship between natural enemies and pest populations (Legendre & Fortin, 1989).

Angular cosine coefficient method

The angular cosine coefficients between the number of pest and its natural enemies were analyzed using the angular cosine coefficient method (Xu, 2002). The higher the value of the angular cosine coefficient, the closer the relationship between the numbers of the two species. The formula for the angular cosine coefficient is

$$\alpha_{ij} = \frac{\sum_{k=1}^{n} x_{ik} \cdot x_{jk}}{[(\sum_{k=1}^{n} x_{ik}^2) \cdot (\sum_{k=1}^{n} x_{jk}^2)]^{1/2}}$$

where x_{ik} and x_{jk} are the number of individuals of species i and j at the k_{th} sample.

Fuzzy similarity priority ratio method

The similarity priority ratio is a way to perform a metric in which a pair of samples is analyzed with a fixed sample, so that the one between the two that is more similar to the fixed sample is discussed (Lu, Yu, & Li, 2006; Zhao & Yang, 2005). If the paired samples are samples M_i and M_j, respectively, and the fixed sample is M_k, then the fuzzy similarity priority ratio Z_{ij} is

(1) If Z_{ij} is between [0, 0.5], it means that M_j has priority over M_i;

(2) If Z_{ij} is between [0.5, 1.0], it means that M_i has priority over M_j;

(3) If $Z_{ij} = 1$, then M_i has priority over M_j; if $Z_{ij} = 0$, then M_j has priority over M_i; if $Z_{ij} = 0.5$, then M_i and M_j do not have much difference in priority.

Comparisons between samples M_i and samples M_j and fixed samples M_k are mostly made using the Hemming distance $\beta_{ij} = \dfrac{d_{ki}}{d_{ki} + d_{kj}}$.The fuzzy similarity priority ratio analysis in this paper is performed by the DPS software.

Correlation coefficient method

The correlation coefficient is a quantity used to analyze the degree of linear correlation between two or more variables and can be expressed by r. There are various ways to define it, and different ways can be chosen according to different research objects, the most common one is Pearson correlation coefficient.

The definition formula is

$$r(X,Y) = \frac{\mathrm{Cov}(X,Y)}{\sqrt{\mathrm{Var}[X]\mathrm{Var}[Y]}}$$

Where $\mathrm{Cov}(X,Y) = \sum (X - \overline{X})(Y - \overline{Y})$ is the covariance of X and Y, $\mathrm{Var}[X] = \sum (X - \overline{X})^2$

is the variance of X and $\mathrm{Var}[Y]=\sum(Y-\overline{Y})^2$ is the variance of Y. The absolute value of the r value is between 0 and 1. Usually, the closer r is to 1, the stronger the correlation between the two quantities X and Y. Conversely, the closer r is to 0, the weaker the correlation between the two quantities X and Y.

RESULTS

The top 11 natural enemies in the tea plantations of “Anjibaicha”, “Huangshan-dayezhong” and “Longjing 43” were *Tetragnatha squamata*, *Tetragnatha maxillosa*, *Hylyphantes graminicola, Xysticus ephippiatus, Ebrechtella tricuspidata, Theridion octomaculatum, Clubiona japonicola, Clubiona reichlini, Oxyopes sertatus, Plexippus paykulli* and *Plexippus setipes*. Therefore, these 11 species were selected as the main natural enemies to study their close relationship with *E. onukii*, and their population dynamics are listed in Table 1.

Table 1. Population dynamics of *E. onukii* and its natural enemies in the three tea plantations (individual/30 quadrats).

Tea plantation	Date	Population[1]											
		Y	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}
Anjibaicha	5-23	69	3	4	15	2	13	0	1	0	0	7	2
	6-4	166	2	13	0	5	2	0	0	0	0	4	0
	6-20	134	8	9	0	7	1	1	5	3	3	2	0
	7-8	441	65	4	46	13	34	60	29	132	42	32	0
	8-10	246	27	13	19	36	7	34	24	102	19	32	42
	8-22	245	20	3	26	27	17	12	30	89	45	22	13
	9-6	193	20	8	21	38	19	28	11	38	56	19	22
	9-17	30	9	5	10	7	11	11	15	4	12	12	10
Huangshan dayezhong	5-23	106	8	1	14	3	8	3	3	0	2	4	1
	6-4	513	14	12	3	10	14	12	5	7	1	13	0
	6-20	507	10	4	7	2	8	6	32	18	2	17	0
	7-8	371	19	24	33	56	30	26	10	23	26	29	0
	8-10	755	31	25	28	29	23	30	20	65	22	30	26
	8-22	348	34	10	44	19	17	37	31	57	22	24	17
	9-6	264	35	0	28	18	10	21	24	28	30	13	39
	9-17	28	8	0	10	9	9	17	20	7	37	6	15
Longjing 43	5-23	267	9	1	4	2	7	0	2	0	0	5	0
	6-4	576	4	0	1	7	5	3	2	2	0	0	5
	6-20	641	2	1	8	7	5	0	2	12	2	7	0
	7-8	866	30	8	46	49	22	21	11	38	30	37	0
	8-10	679	27	8	25	18	17	36	14	36	37	27	24
	8-22	543	34	12	31	23	12	33	35	27	51	28	2
	9-6	270	16	0	13	21	10	15	16	14	128	22	13
	9-17	15	12	0	15	6	5	15	10	14	23	7	14

[1] Y: *Empoasca onukii*; X_1: *Tetragnatha squamata*; X_2: *Tetragnatha maxillosa*; X_3: *Hylyphantes graminicola*; X_4: *Xysticus ephippiatus*; X_5: *Ebrechtella tricuspidata*; X_6: *Theridion octomaculatum*; X_7: *Clubiona japonicola*; X_8: *Clubiona reichlini*; X_9: *Oxyopes sertatus*; X_{10}: *Plexippus paykulli*; X_{11}: *Plexippus setipes* (The same below)

Results of the grey relational analysis method

The grey relational coefficients between the number of *E. onukii* and its natural enemies obtained by grey relational analysis method are listed in Table 2. The top three natural enemies that were closely related to the population of *E. onukii* were *Tetragnatha squamata* (X_1, 0.855), *Plexippus paykulli* (X_{10}, 0.846) and *Hylyphantes graminicola* (X_3, 0.826) in "Anjibaicha" tea plantation; *Plexippus paykulli* (X_{10}, 0.853), *Ebrechtella tricuspidata* (X_5, 0.814) and *Tetragnatha maxillosa* (X_2, 0.813) in "Huangshandayezhong" tea plantation; and *Ebrechtella tricuspidata* (X_5, 0.875), *Tetragnatha squamata* (X_1, 0.841) and *Hylyphantes graminicola* (X_3, 0.825) in "Longjing 43" tea plantation. Based on the sum of the grey relational coefficient of the same natural enemy in the three tea plantations, the top three natural enemies that were closely related to the population of *E. onukii* were *Plexippus paykulli* (X_{10}, 2.521), *Tetragnatha squamata* (X_1, 2.490) and *Ebrechtella tricuspidata* (X_5, 2.488).

Table 2. Grey relational coefficient and serial number between *E. onukii* and its natural enemies in the three tea plantations.

Tea plantation	Project[1]	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}
Anjibaicha	A	0.855	0.774	0.826	0.771	0.799	0.764	0.802	0.799	0.762	0.846	0.700
	B	1	7	3	8	6	9	4	5	10	2	11
Huangshan dayezhong	A	0.795	0.813	0.728	0.774	0.814	0.769	0.754	0.794	0.708	0.853	0.700
	B	4	3	9	6	2	7	8	5	10	1	11
Longjing 43	A	0.841	0.790	0.825	0.816	0.875	0.763	0.756	0.823	0.740	0.822	0.686
	B	2	7	3	6	1	8	9	4	10	5	11
Total grey relational coefficient		2.490	2.376	2.378	2.361	2.488	2.296	2.312	2.417	2.211	2.521	2.086
Total serial number		2	6	5	7	3	9	8	4	10	1	11

[1] A: Grey relational coefficient; B: Serial number of the grey relational coefficient

Results of the angular cosine coefficient method

The angular cosine coefficients between the number of target pest and its natural enemies and their serial numbers are listed in Table 3. In 2021, the top three natural enemies closely related to the population of *E. onukii* were *Tetragnatha squamata* (X_1, 0.941), *Plexippus paykulli* (X_{10}, 0.927), and *Hylyphantes graminicola* (X_3, 0.923) in "Anjibaicha" tea plantation; *Plexippus paykulli* (X_{10}, 0.933), *Ebrechtella tricuspidata* (X_5, 0.876) and *Tetragnatha maxillosa* (X_2, 0.865) in "Huangshandayezhong" tea plantation; *Ebrechtella tricuspidata* (X_5, 0.916), *Clubiona reichlini* (X_8, 0.866) and *Xysticus ephippiatus* (X_4, 0.856) in "Longjing 43" tea plantation. According to the total angular cosine coefficient, the top three natural enemies that were closely related to the population of *E. onukii* were *Plexippus paykulli* (X_{10}, 2.707), *Ebrechtella tricuspidata* (X_5, 2.682) and *Clubiona reichlini* (X_8, 2.640) in the combined analysis of the three tea plantations.

Table 3. The angular cosine coefficient and serial number between *E. onukii* and its natural enemies in the three tea plantations.

Tea plantation	Project[1]	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}
Anjibaicha	C	0.941	0.741	0.923	0.787	0.891	0.902	0.891	0.922	0.836	0.927	0.571
	D	1	10	3	9	7	5	6	4	8	2	11
Huangshan dayezhong	C	0.844	0.865	0.748	0.729	0.876	0.817	0.786	0.852	0.596	0.933	0.584
	D	5	3	8	9	2	6	7	4	10	1	11
Longjing 43	C	0.826	0.773	0.842	0.856	0.916	0.751	0.673	0.866	0.504	0.848	0.498
	D	6	7	5	3	1	8	9	2	10	4	11
Total angular cosine coefficient		2.611	2.379	2.513	2.371	2.682	2.469	2.350	2.640	1.935	2.707	1.652
Total serial number		4	7	5	8	2	6	9	3	10	1	11

[1] C: Angular cosine coefficient; D: Serial number of the angular cosine coefficient

Results of the fuzzy similarity priority ratio method

The similarities between the numbers of target pest and natural enemies in the three tea plantations and their serial numbers are listed in Table 4. The top three natural enemies closely related to the numbers of *E. onukii* were *Tetragnatha squamata* (X_1), *Hylyphantes graminicola* (X_3) and *Theridion octomaculatum* (X_6) in "Anjibaicha" tea plantation; *Clubiona reichlini* (X_8), *Xysticus ephippiatus* (X_4) and *Oxyopes sertatus* (X_9) in "Huangshandayezhong" tea plantation; *Tetragnatha squamata* (X_1), *Hylyphantes graminicola* (X_3) and *Oxyopes sertatus* (X_9) in "Longjing 43" tea plantation. A comprehensive analysis showed that the top three natural enemies that were closely related to the population of *E. onukii* were *Tetragnatha squamata* (X_1), *Clubiona reichlini* (X_8) and *Hylyphantes graminicola* (X_3).

Table 4. The similarity and serial number between *E. onukii* and its natural enemies in the three tea plantations.

Tea plantation	Project[1]	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}
Anjibaicha	E	30	60	32	43	43	34	41	35	48	38	55
	F	1	11	2	7	8	3	6	4	9	5	10
Huangsha dayezhong	E	37	52	40	35	47	43	43	31	36	39	49
	F	4	11	6	2	9	7	8	1	3	5	10
Longjing 43	E	27	63	31	33	45	37	41	33	32	33	50
	F	1	11	2	6	9	7	8	4	3	5	10
Total similarity		94	175	103	111	135	114	125	99	116	110	154
Total serial number		1	11	3	5	9	6	8	2	7	4	10

[1] E: Similarity; F: Serial number of the similarity

Results of the correlation coefficient method

The correlation coefficients between the numbers of *E. onukii* and its natural enemies in the three tea plantations and their serial numbers are listed in Table 5. The top three natural enemies that were closely related to the number of *E. onukii* were *Tetragnatha squamata* (X_1, 0.910), *Clubiona reichlini* (X_8, 0.895) and *Theridion* *octomaculatum* (X_6, 0.839) in “Anjibaicha” tea plantation; *Plexippus setipes* (X_{11}, 0.734), *Tetragnatha maxillosa* (X_2, 0.715) and *Clubiona reichlini* (X_8, 0.606) in the tea plantation of “Huangshandayezhong”; *Ebrechtella tricuspidata* (X_5, 0.640), *Xysticus ephippiatus* (X_4, 0.606) and *Tetragnatha maxillosa* (X_2, 0.573) in “Longjing 43” tea plantation. According to the sum of the correlation coefficients of the same natural enemies in the three tea plantations, the top three natural enemies that were closely related to *E. onukii* were *Clubiona reichlini* (X_8, 2.072), *Ebrechtella tricuspidata* (X_5, 1.799), and *Tetragnatha squamata* (X_1, 1.659).

Table 5. Correlation coefficient and serial number between *E. onukii* and its natural enemies in the three tea plantations.

Tea Plantation	Project[1]	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}
Anjibaicha	G	0.910	0.058	0.796	0.381	0.677	0.839	0.688	0.895	0.605	0.759	0.095
	H	1	11	4	9	7	3	6	2	8	5	10
Huangshan dayezhong	G	0.382	0.715	0.108	0.274	0.482	0.297	0.174	0.606	0.290	0.290	0.734
	H	5	2	11	9	4	6	10	3	7	8	1
Longjing 43	G	0.367	0.573	0.517	0.606	0.640	0.266	0.053	0.571	0.191	0.510	0.233
	H	7	3	5	2	1	8	11	4	10	6	9
Total correlation coefficient		1.659	1.230	1.422	1.261	1.799	1.402	0.915	2.072	0.124	0.980	0.596
Total serial number		3	7	4	6	2	5	9	1	11	8	10

[1] G: Correlation coefficient; H: Serial number of correlation coefficient

Comparison of the results of the four methods

The results obtained from the four methods are listed in Table 6, and the differences in the results of the other three methods are compared using the results of the grey relational analysis method as a control.

Table 6. The results of the four methods.

Tea plantation	Method[1]	The top three natural enemies closely related to *E. onukii*		
		1	2	3
Anjibaicha	I	X_1	X_{10}	X_3
	J	X_1	X_{10}	X_3
	K	X_1	X_3	X_6
	L	X_1	X_8	X_6
Huangshandayezhong	I	X_{10}	X_5	X_2
	J	X_{10}	X_5	X_2
	K	X_8	X_4	X_9
	L	X_{11}	X_2	X_8
Longjing 43	I	X_5	X_1	X_3
	J	X_5	X_8	X_4
	K	X_1	X_3	X_9
	L	X_5	X_4	X_2

[1] I: Grey relational analysis method; J: Angular cosine coefficient method; K: Fuzzy similarity priority ratio method; L: Correlation coefficient method (The same below)

The differences between the results of the four methods were analysed and compared for the same tea plantation. Of the 11 natural enemies in “Anjibaicha” tea plantation, the top three natural enemies that were closely related to the number of *E. onukii* were selected for comparison. The results of the grey relational analysis method were *Tetragnatha squamata* (X_1), *Plexippus paykulli* (X_{10}) and *Hylyphantes graminicola* (X_3), and the results of the angular cosine coefficient method were also in the order of these three species, which were identical to the top three natural enemies in the grey relational analysis method. The results of the fuzzy similarity priority ratio method were *Tetragnatha squamata* (X_1), *Hylyphantes graminicola* (X_3) and *Theridion octomaculatum* (X_6), with two species identical to the grey relational analysis method. The results of the correlation coefficient method were *Tetragnatha squamata* (X_1), *Clubiona reichlini* (X_8) and *Theridion octomaculatum* (X_6), with only one species identical to the grey relational analysis method. A comprehensive analysis shows that the results of the angular cosine coefficient method and the grey relational analysis method are the same for the top 3 natural enemies; the results of the fuzzy similarity priority ratio method differ more from the grey relational analysis method, and the results of the correlation coefficient method differ most from the grey relational analysis method.

In the same analysis, it can be concluded that in the tea plantation of "Huangshandayezhong", the top three natural enemies were the same in the angular cosine coefficient method and the grey relational analysis method; in the comparison between the results of the correlation coefficient method and the grey relational analysis method, only one of the top three natural enemies was the same, so it is more different from the results of the grey relational analysis method; while in the comparison between the results of the fuzzy similarity priority ratio method and the grey relational analysis method, the top three natural enemies are all different, so it is the most different from the results of the grey relational analysis method.

For "Longjing 43" tea plantation, the results of the angular cosine coefficient method, the fuzzy similarity priority ratio method and the correlation coefficient method were all the same for only one of the top three natural enemies compared to the results of the grey relational analysis method, and all differed significantly from the results of the grey relational analysis method.

A comprehensive analysis of the results of the relationship between the number of *E. onukii* and its natural enemies in the three tea plantations

To comprehensively analyze the differences of the results obtained by the four methods in the three tea plantations, the ranking of the natural enemies in relation to the number of *E. onukii* in the three tea plantations was analyzed and compared, and the results of the comprehensive analysis of Tables 2-5 are listed in Table 7. The results of the grey relational analysis method were used as the basis for comparison, and the results obtained by the four research methods were comprehensively analyzed according to the changes in the ranking of the natural enemies.

The sum of the top three natural enemies (*Plexippus paykulli*, *Tetragnatha squamata* and *Ebrechtella tricuspidata*) that were closely related to *E. onukii* by the grey relational analysis method was used as the basis for comparison, and the greater the sum of the resulting rankings, the greater the variation from the results obtained by the grey relational analysis method. The sum of the rankings of the grey relational analysis method was 6, the angular cosine coefficient method was 7, the fuzzy similarity priority ratio method was 14, and the correlation coefficient method was 13. The comparison was then based on the sum of the rankings of the top 5 natural enemies of the grey relational analysis method, which was 15 for the grey relational analysis method, 15 for the angular cosine coefficient method, 19 for the fuzzy similarity priority ratio method, and 18 for the correlation coefficient method. The difference between the results obtained by the angular cosine coefficient analysis and the grey relational analysis method was small, followed by the correlation coefficient method, and the fuzzy similarity priority ratio method had a large difference in the results. The sum of the frequencies of the top three natural enemies obtained by the four methods in the three tea plantations was compared (up to 12 times), *Tetragnatha squamata* (X_1) for 6, *Hylyphantes graminicola* (X_3) for 5, *Ebrechtella tricuspidata* (X_5) for 5, *Tetragnatha maxillosa* (X_2) for 4, *Clubiona reichlini* (X_8) for 4, *Plexippus paykulli* (X_{10}) for 4, *Xysticus ephippiatus* (X_4) for 3, *Theridion octomaculatum* (X_6) for 2, *Oxyopes sertatus* (X_9) for 2, *Plexippus setipes* (X_{11}) for 1, *Clubiona japonicola* (X_7) for 0. Therefore, *Tetragnatha squamata* (X_1), *Hylyphantes graminicola* (X_3) and *Ebrechtella tricuspidata* (X_5) were the three natural enemies most closely related to the number of *E. onukii*.

Table 7. Comparison of the results of the four analysis methods.

Method	Total serial number of natural enemies in the three tea plantations											Remarks	
	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	Sum of the serial numbers of X_{10}, X_1 and X_5	Sum of the serial numbers of X_{10}, X_1, X_5, X_8 and X_3
I	2	6	5	7	3	9	8	4	10	1	11	6	15
J	4	7	5	8	2	6	9	3	10	1	11	7	15
K	1	11	3	5	9	6	8	2	7	4	10	14	19
L	3	7	4	6	2	5	9	1	11	8	10	13	18

DISCUSSION

Generally, the higher the number of pests and the more adequate the sample, the more accurate the results obtained and the closer the relationship between the number of pests and natural enemies reflected in the real situation (Zou, 1997). The relationship between natural enemies and pests is an interdependent and mutually constraining relationship, which is expressed in the number of ups and downs, not a linear relationship, so the results of using the correlation coefficient method are not accurate enough. The fuzzy similarity priority ratio is a form of fuzzy metric, which is calculated to get the ordinal number of the sample, the smaller the ordinal number value of the sample, the more similar the sample is to the fixed sample. The degree of influence of each factor on the sample is different, so it is necessary to give certain weights to each factor, so that the results obtained will be more in line with the actual situation. However, assigning weight is highly subjective and depends on professional knowledge. The angular cosine coefficient method solves the problem that the two are linearly related. Grey relational analysis method can solve the problem of both linear and non-linear relationship. Therefore, it is a more ideal analysis method, so this paper compares the similarities and differences of several analysis results based on the analysis results of grey relational analysis method. This paper can provide reference for similar analysis.

For the closeness of the quantitative relationship between natural enemies and target pest, in the case of tea plantations, the main factor is the magnitude of the predatory (or parasitic) effect of natural enemies on the target pest. Therefore, the study of the quantitative relationship between natural enemies and pest is of great importance and is one of the main research directions in insect ecology (Veres, Petit, Conord, & Lavigne, 2013). Zhou et al (2019b) analyzed and compared the dominant species of natural enemies of *Breuipalpus oboyats* Donnadieu in “Wuniuzao” and “Baihaozao” tea plantations by using the geostatistical method combined with the ecological niche method. Cheng et al (2022) used the geostatistical method and the angular cosine coefficient method to identify the natural enemy species which are close to Ricanidae in spatial relationship. Zhang et al (2021) studied the relationship between natural enemies and *Breuipalpus oboyats* Donnadieu in time and space by niche analysis and geostatistics combined with angular cosine coefficient method. Qian et al (2019) creatively combined the angular cosine coefficient with niche similarity coefficient and the range of spatial dependence of semivariogram theoretical model in geostatistics to analyze the relationship of neutral insects, mosquitoes and natural enemies. All the above studies involve the four methods mentioned in this paper to study the relationship between natural enemies and pests in tea plantations, which provide a scientific reference for effective pest control. In addition, the geostatistical method mentioned above is a method for studying the spatial relationship between pests and natural enemies, and can accurately reflect the spatial predation preferences of natural enemies on pests. The method is applicable to the analysis of spatial relationships between natural enemies and pests at the largest scale (or range) of tea plantations, tea farms and tea hills in any orientation, as well as to the study of spatial relationships between natural enemies and pests in homogeneous and non-homogeneous habitats, provided that the distances between sampling squares are equidistant. The ecological niche method reflects the temporal following relationship between pests and natural enemies, the greater the temporal ecological niche overlap index, the closer the natural enemies follow the pests in time. Combining these methods with the methods mentioned in this paper for studying quantitative relationships provides a more comprehensive assessment of the dominant species of natural enemies of pests from three aspects: spatial, temporal and quantitative. The four methods studied in this paper are important models that have been creatively proposed by many ecologists and mathematicians based on their own findings and the conclusions obtained by others, contributing to the development and progress of ecology.

CONCLUSIONS

The grey relational analysis method, angular cosine coefficient method, fuzzy similarity priority ratio method and correlation coefficient method were used to study the quantitative relationship between natural enemies and *E. onukii* in "Anjibaicha", "Huangshandayezhong" and "Longjing 43" tea plantations. The results of the four methods were ranked in terms of the closeness of the relationship between natural enemies and *E. onukii*, and the top three natural enemies derived from the grey relational analysis method, *Plexippus paykulli* (X_{10}), *Tetragnatha squamata* (X_1), and *Ebrechtella tricuspidata* (X_5), were used as control specimens. The results of the angular cosine coefficient analysis method and the grey relational analysis method were less different, followed by the correlation coefficient method, and the results of the fuzzy similarity priority ratio method were more different. Based on the sum of the number of occurrences of natural enemies in the top three positions in the four methods, it was concluded that *Tetragnatha squamata*, *Hylyphantes graminicola* and *Ebrechtella tricuspidata* were the top three natural enemies that were closely related to the number of *E. onukii.*

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A Further Contribution to the Aphid (Hemiptera:Aphidoidea) Fauna of Turkey Including a Description of a New Host Plant Associations and Colony Appearances

Gazi GÖRÜR[1*] Özhan ŞENOL[2] Hayal AKYILDIRIM BEĞEN[3]
Gizem BAŞER[4] Bengisu Venhar AKÇAY[5]

[1,2,4,5]Department of Biotechnology, Faculty of Arts and Sciences, Niğde Ömer Halisdemir University, Niğde, TURKEY
[2]Vocational School of Health Services, Artvin Çoruh University, Artvin, TURKEY
e-mails: [1*]gazigorur@yahoo.com, [2]shenol_euzhan@hotmail.com, [3]h.akyildirim@artvin.edu.tr, [4]gizemli_baser@hotmail.com, [5]bengisuakcy@gmail.com
ORCID IDs: [1*]0000-0001-5713-418X, [2]0000-0002-7747-0866, [3]0000-0003-2028-5827, [4]0000-0002-4588-786X, [5]0000-0002-4187-7322
*Corresponding Author

ABSTRACT

Aphids fascinated scientists due to their unique life cycles, being a one of the important agricultural pest worldwide, overcoming various applied control strategies and also relatively benefited from global climate change. The importance of aphid studies in developing countries like a Turkey requires additional attention because the effects of aphids on agricultural production. In this aspect, 3 genera, *Anthemidaphis* Tashev, 1967, *Olegia* Shaposhnikov, 1979; *Pentalonia* Coquerel, 1859 and 15 aphid species added to Turkey aphid fauna which are; *Anthemidaphis oligommata* Tashev 1967, *Aphis (Aphis) coreopsidis* (Thomas, 1878), *Aphis* (*Aphis*) *narzikulovi* Szelegiewicz, 1963, *Aphis (Aphis) pseudeuphorbiae* Hille Ris Lambers, 1948, *Aphis (Aphis) picridis* (Börner, 1950), *Chaitophorus clarus* Tseng & Tao 1936, *Chaitophorus parvus* Hille Ris Lambers, 1935, *Dysaphis* (*Pomaphis*) *gallica* (Hille Ris Lambers, 1955), *Melanaphis sorghi* Theobald, 1904, *Metopolophium* (*Metopolophium*) *festucae* Theobald, 1917, *Olegia ulmifoliae* (Aoki, 1973), *Schizaphis* (*Schizaphis*) *nigerrima* (Hille Ris Lambers, 1931), *Semiaphis heraclei* (Takahashi, 1921), *Semiaphis pastinacae* Börner, 1950, *Pentalonia nigronervosa* Coquerel, 1859. Recorded species were evaluated for host plant relations, colony appearances, also ant attendance and new interactions observed. Number of the listed aphid species for Turkey aphid fauna increased to 631 with these new records.

Keywords: Aphid, Host Plant Relations**,** New record, Turkey.

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INTRODUCTION

World aphid fauna is recently represented by 5600 valid species in 703 genera placed in 30 subfamilies (Favret, 2022). Up to date, 616 aphid species in 166 genera placed in 7 families and 19 subfamilies were listed for Turkey aphid fauna (Görür, Şenol, Akyıldırım Beğen, & Akyürek, 2022). Turkey's unique floristic richness, both having a great agricultural fields and variability of the agricultural products, showing a different climatic conditions in same season among 7 geographically distinctive regions and a location at the junction of the Africa, Asia and Europe give a chance to inhabit a very diverse aphid fauna. In spite of a remarkable number of study was conducted during last 20 years, there are still poorly investigated areas waiting for to be studied. With these studies, considerable number of aphid species have been added Turkey aphid fauna, and some new host plant associations have also been detected (Görür, Zeybekoğlu, Akyürek, Işık, & Akyıldırım, 2009; Görür, Şenol, Akyıldırım, & Demirtaş, 2014; Görür, Şenol, Gezici, & Parmaksız, 2018; Kök & Özdemir, 2021; Şenol, Görür, Başer, & Akyıldırım Beğen, 2021; Patlar, Oğuzoğlu, Avcı, & Şenol, 2021; Kök & Kasap, 2022). Despite the recent additions, there was an interesting result that total aphid fauna of Turkey was sampled on only about 1100 plant species while there are more than12.000 plant species with a 31% endemism ratio (Görür, Şenol, Akyıldırım Beğen, & Akyürek, 2020). Recent climatic changes are going to influence the biodiversity of the Turkey as well as the whole earht, and thus it is getting much more crucial area to observe current composition of the Aphid fauna of Turkey. Perzanowska, Korzeniak, & Chmura, (2019) strongly indicated that invasion of exotic plant species is one of the most important threats to ecosystems as a result of global climatic changes, any possible changes in plant community most probably going to affect aphid species diversity as all aphid species are obligate phytophagous. In addition to the possible changes in aphid community in certain region, agricultural importance of the aphid species also is going to increase as aphid species benefited from global climatic changes. Aphids have become important pest globally by causing severe damages either directly or indirectly on economically crucial crops as they are obligate phytophagous insect. Direct damage can be caused by aphids alone, whereas indirect damage includes pathogen-virus transmission and inducing sensitivity to other stress factors. Both adult and nymph aphids can suck plant sap causing direct feeding damage to crops from seedlings to grain fill. The infestation of large aphid population might cause to seedling death, stunting, tiller or flower deformation and decreased seed number and size, finally limiting crop production (van Emden & Harrington, 2017). Possible increase in economic importance, extending geographical invasion and host plant range of aphid species were attracted researchers to carry out more study at the different regions. The importance of the determination of the current composition of Turkey aphid fauna increases since a higher importance of the agricultural production in the development of Turkey and being a country that is going to be affected adversely from global climate change. In this aspect, this study aimed to search aphid fauna of Antalya, Karaman and Muğla provinces to figure out any possible contribution the Turkey aphid fauna.

MATERIAL AND METHODS

This study was conducted in Antalya, Muğla and Karaman provinces from March 2020 to October 2021. Samples were identified with Olympus BX51 microscope according to key proposed by Blackman & Eastop (2022) and current species status was checked by Favret (2022). For each defined species, worldwide and Turkey distribution, host plant relations, colony appearances were derived and compared with published studies (Holman, 2009; Blackman & Eastop, 2022; Favret, 2022; Görür et al., 2022). Some certain morphological characters which are considered mostly acceptable distinguishing features related with host plant utilization were measured for each determined species as much as possible. Measured characters are; Body length(BL), Total antennal length (TAL), VIth antennal segment processuc terminalis/ VIth antennal segment Base (PT/BASE), Length of the ultimate rostral segment VI+V(URS IV+V) (Blackman & Eastop, 2022). The voucher samples were stored at the Biotechnology Department of the Nigde Ömer Halisdemir University. General information and observed characteristics of the defined species were provided.

RESULTS AND DISCUSSION

As a result of the evaluation of about 100 sampling from various host plants, 3 genera and 15 aphid species determined as new records for Turkey aphid fauna. Colony appearances of the 13 species among 15 aphid species on the recorded host plant were provided (Fig. 1). Each new recorded aphid species features were given individually as follows.

Aphidoidea Latreille, 1802

Aphididae Latreille, 1802

Aphidinae Latreille, 1802

Aphidini Latreille, 1802

***Anthemidaphis* Tashev 1967**

Anthemidaphis oligommata Tashev 1967

General features, host plants and distribution: Colony appearances (colour) has not been indicated in literature so far, adult apterae individual's BL was about 1.5 mm. Mainly colonize on roots of the *Anthemis tinctoria*, also sampled on subterranean parts of *Achillea* sp. (*Achillea millefolium*). Palaearctic (Europe) originated, distributed in Bulgaria, France, Italy-Sicily, Poland, Slovakia, Spain (Holman, 2009; Blackman & Eastop, 2022).

Material examined: 10 apterae individuals (♀) were collected on the stem of the *Anthemis tinctoria*, which was the first record on aerial part of the *A. tinctoria*, from Antalya on I.VI. 2020. Adult apterae individuals were olive green that was the first time colony appearances was detected, visited by ants, BL was 1.3 mm, (TAL) was 0.85mm, PT/Base was 1.33 mm, URSIV+V was 0.1 mm. Morphological appearances of the mounted specimen on permanent slides were similar to *Sipha* spp. Genera

Anthemidaphis consist of 2 species (*A. ligusticae, A. oligommata*), it was the first time species belongs to this genus was recorded from Turkey, thus *Anthemidaphis* was also recorded new genera for Turkey aphid fauna.

Figure 1. Colony appearances of the determined aphid species; a) *Aphis coreopsidis* on *Hibiscus syriacus*, b) *Aphis narzikulovi* on *Nepeta nuda*, c) *Aphis picridis* on *Helminthothecia (Picris) echioides*, d) *Aphis (Aphis) pseudeuphorbiae* on *Euphorbia amygdaloides*, e) *Melanaphis sorghi* on *Triticum* sp., f) *Schizaphis nigerrima* on *Cynodon dactylon*, g) *Metopolophium festucae* on *Triticum* sp., h) *Semiaphis heraclei* on *Petroselinum crispum*, i) *Pentalonia nigronervosa* on *Musa sapientum*, j) *Chaitophorus clarus* on *Populus tremulae*, k) *Chaitophorus parvus* on *Salix viminalis*, l) *Olegia ulmifoliae* on *Ulmus* sp.

Aphis Linnaeus, 1758

Aphis (Aphis) coreopsidis (Thomas, 1878) Tupelo-blackjack aphid

General features, host plants and distribution: Mainly feed on stems and leaves of host plants, host alternates between *Nyssa sylvatica*. (as a primary host plants) and various members of Compositae/Asteracea (*Bidens*, *Clibadium*, *Eupatorium*, *Sonchus*), Malvaceae (*Hibiscus*, *Sida*) and Lamiaceae (*Blephilia*, *Nepeta*) as a secondary host plants (Holman, 2009; Blackman & Eastop, 2022). Adult apterae individuals are yellowish green, BL was about 1.5-1.8 mm. Neotropical (Central and South America) originated, distributed in Hawaii, presumably in Ghana, Uganda, Saudi Arabia (Blackman & Eastop, 2022).

Material Examined: 12 apterae individuals (♀) were collected on leaves and stem of the *Hibiscus syriacus* from Muğla-Milas on V.VII.2020. and stem of the *Sonchus* sp. mixed colony with *Uroleucon* *sonchi* from Antalya-Alanya on XVI.III. 2020. Adult apterae individuals are pale green, yellowish (Fig.1a), BL 1.1 was mm, (TAL) was 0.72mm, PT/Base was 2.43 mm, URSIV+V was 0.1 mm.

Aphis (*Aphis*) *narzikulovi* Szelegiewicz, 1963

General features, host plants and distribution: Mostly feed on *Schizonepeta (Nepeta) multifida*, also feed on various members of Lamiaceae, apterae individuals are dark green, 0.8-1.7 mm. Palearctic (Mongolia) originated, there has been no further information provided for distribution (Holman, 2009; Blackman & Eastop, 2022).

Material examined: 8 apterae individuals (♀) were sampled on *Nepeta nuda* for the first time that is an endemic plant species for Turkey flora from Muğla-Milas on VII.VI.2020. Adult apterae individuals were dull green (Fig.1b) mixed with *Aphis nasturtii* and visited by ants, BL 1.1 was mm, (TAL) was 0.86mm, PT/Base was 3.7 mm, URSIV+V was 0.15 mm.

Aphis (Aphis) picridis (Börner, 1950), Yellow oxtongue aphid

General features, host plants and distribution: Feed mainly on leaves and root collar of *Picris* spp. and *Reichardia picroides*. Palaearctic (Europe) originated, distributed in south, central and eastern Europe, Iran, Algeria (Holman, 2009; Blackman & Eastop, 2022; Dransfield, 2022). Adult apterae individuals were bright yellow and BL was about 1.1-1.8 mm.

Material examined: 12 apterae individuals (♀) were sampled on young shoots and undersides of leaves of *Helminthothecia (Picris) echioides* from Antalya-2016 Expo area on IV.III. 2021. Adult apterae individuals were light-dull green, bluish (Fig.1c), heavily colonized the sampled host plant, BL was 1.24 mm, (TAL) was 0.59mm, PT/Base was 2.44 mm, URSIV+V was 0.12 mm.

Aphis (Aphis) pseudeuphorbiae Hille Ris Lambers, 1948

General features, host plants and distribution: Feed mainly on *Euphorbia lanata* and *E. macroclada*. The Middle East originated, distributed in Israel and Jordan (Holman, 2009; Blackman & Eastop, 2022). There was no clear information provided for colony colour, probably wax dusted, adult apterae individuals were 1.4-1.8 mm.

Material examined: 15 apterae individuals (♀) were collected on stem and undersides of leaves of *Euphorbia amygdaloides* from Karaman-Ayrancı on XXVIII.V.2020. Adult apterae individuals were black, grey blackish with whitish wax (Fig.1d), which was the first obvious colony appearances provided, visited by ants, BL was 1.35 mm, (TAL) was 0.89mm, PT/Base was 1.54 mm, URSIV+V was 0.13 mm.

Melanaphis van der Goot, 1917

Melanaphis sorghi Theobald, 1904

General features, host plants and distribution: Feed mainly on members of Poaceae, especially *Sorghum bicolor*, also sometimes on other grasses or cereals (*Eleusine*, *Panicum*, *Saccharum*, *Zea*). Adult apterae individuals were white or yellow, BL was 1.1-2.0 mm. Palearctic (Europe) originated, distributed in Europe, Africa, east and south-east Asia, the southern USA and Mexico (Blackman & Eastop, 2022; Dransfield, 2022).

Material examined: 20 adult apterae individuals (♀) were collected on leaves of the *Triticum* sp. (that was the first record from *Triticum* sp.) from Antalya-Döşemealtı on XVIII.IX.2020, from Muğla-Ortaca on IV.VI.2020 and also on *Elytrigia repens* from Muğla-Köyceğiz on IV.VI.2020. Adult apterae individuals were small, brownish-reddish, heavily colonized the host plant and visited by ants(Fig.1e), BL was 1.05 mm, (TAL) was 0.79 mm, PT/Base was 3.25 mm. Even presence of the species in Turkey were given as *M. sacchari* (Özdemir & Kök, 2021), detailed identification showed that *M. sorghi* is distinct species clearly from *M. sacchari* in Turkey.

Schizaphis Börner, 1931

Schizaphis (*Schizaphis*) *nigerrima* (Hille Ris Lambers, 1931)

General features, host plants and distribution: Adult apterae individuals were black, BL was 1.7-2.3 mm. Palaearctic (Europe) originated, distributed in Europe and Iran. Mainly feed on upper sides of leaves of *Festuca pratensis*, *Alopecurus pratensis, Calamagrostis purpurea, Calamagrostis stricta, Alymus* spp. yellowing the tips (Holman, 2009; Blackman & Eastop, 2022).

Material examined: 20 adult apterae individuals (♀) were collected on upper sides of the leaves of *Agropyron repens* from Antalya Centrum on VII.VIII.2020 and *Elytrigia repens* from Antalya-Gazipaşa on XXIII.VIII.2020, it was the first time this species was recorded on both host plants. Adult apterae individuals were dark grey, light brownish (Fig.1f), BL was 1.1 mm, (TAL) was 0.79mm, PT/Base was 2.33 mm, URSIV+V was 0.07 mm.

Macrosiphini Wilson, 1910

Dysaphis Börner, 1931

Dysaphis (*Pomaphis*) *gallica* (Hille Ris Lambers, 1955), Ivy-leaved toadflax aphid

General features, host plants and distribution: Feed on mainly *Antirrhinum majus*, C*ymbalaria muralis, Veronica anagallis-aquatica* as a secondary host plants while feed possibly on *Cotoneaster tomentosus* or *Malus* spp. as a primary host plants even it is not confirmed. Palearctic (Mediterranean part of the Europe) originated, distributed in Europe and possibly recorded from Pakistan (Blackman & Eastop, 2022; Dransfield, 2022).

Material examined: 7 adult apterae individuals (♀) were collected on base of leaves and inside slightly curled leaves of the *Veronica anagallis-aquatica* that was growing by the water from Muğla-Köyceğiz-Yuvarlak Çay on XXX.III.2021. Adult apterae individuals were pale green, yellowish, it was not easily recognized on host plant, BL was 1.2 mm, (TAL) was 0.83mm, PT/Base was 3.27 mm, URSIV+V was 0.13 mm, host plant was not heavily colonized by *D. gallica*.

Metopolophium Mordvilko, 1914

Metopolophium (*Metopolophium*) *festucae* Theobald, 1917

General features, host plants and distribution: Feed mainly on various members of Poaceae, adult apterae individuals are yellowish green to green or salmon pink, BL was 1.4-2.2 mm. Palearctic (Europe) originated, distributed in Europe, Iran, Central Asia, New Zealand (Holman, 2009; Blackman & Eastop, 2022)

Material examined: 15 adult apterae individuals (♀) were collected on leaves of the *Triticum* sp. from Antalya-Side on III.III.2021. Adult apterae individuals were pale green (Fig.1g), BL was 2.12 mm, (TAL) was 1.28mm, PT/Base was 4.77 mm, URSIV+V was 0.11 mm.

Semiaphis van der Goot, 1913

Semiaphis heraclei (Takahashi, 1921)

General features, host plants and distribution: Adult apterae individuals were pale grey-green to yellow-green, BL was about 2.1 mm. Palearctic (South and South east) originated, distributed in Algeria, India, Pakistan, Japan, Hawaii. Feed mainly on stems or curled leaves of various Umbelliferae/Apiaceae (*Angelica, Apium*, *Bupleurum*, *Carum*, *Changium, Cnidium, Coriandrum*, *Cryptotaenia*, *Daucus*, ***Glehnia***, *Heracleum*, ***Ligusticum***, *Oenanthe,Osmorhiza*, ***Seseli***, *Torilis*) as a secondary host plant while mostly feed on *Lonicera* spp as a primary host plant (Holman, 2009; Blackman & Eastop, 2022).

Material examined: 12 adult apterae individuals (♀) were sampled on young shoots and receptacle of the *Petroselinum crispum* from Muğla-Köyceğiz on V.VI.2020. Adult apterae individuals were dull green, bluish (Fig.1h), BL was 1.5 mm, (TAL) was 0.44mm, PT/Base was 2.33 mm, URSIV+V was 0.07 mm.

Semiaphis pastinacae Börner, 1950

General features, host plants and distribution: Adult apterae individuals were grey-green to yellow-green, BL was about 1.3 mm. Palaearctic (Europe) originated, distributed in central Europe, characteristically downwardly rolled leaflets of *Pastinaca sativa*. Possible host alternation between *P. sativa* as a secondary host plant and *Lonicera* spp. as a primary host plant needs confirmation (Holman, 2009; Blackman & Eastop, 2022).

Material examined: 15 adult apterae individuals (♀) were sampled on flowers of the *Pastinaca sativa* from Muğla-Ula on VII.IV.2020 and Muğla-Milas on VII.V.2020. Small adult apterae individuals are green, pale green, visited by ants, BL was 1.6 mm, (TAL) was 0.5mm, PT/Base was 2.17 mm, URSIV+V was 0.08 mm, heavily colonized the host plant.

Pentalonia Coquerel, 1859

Pentalonia nigronervosa Coquerel, 1859

General features, host plants and distribution: Adult apterae individuals were reddishbrown, brown, black, BL was 1.2-1.9 mm. Mostly feed on *Colocasia antiquorum, Alpinia versicolor*, *Curcuma* sp., *Eletteria cardamomum*, *Maranta zebrina*, *Maranta* sp., *Musa sapientum* (Holman, 2009; Blackman & Eastop, 2022) it was often found living under the old leaf bases, usually ant-attended. Ethiopian (Madagascar-east Africa) originated, it is widespread through all tropical and subtropical parts of the world, in Europe, Australia and North America.

Material examined: 10 adult apterae individuals (♀) were sampled on the old leaf bases near the root of *Musa sapientum* from Antalya-Gazipaşa on III.IX.2021. Adult apterae individuals were orange-brown, blackish (Fig.1i), visited by ants, BL was 1.25 mm, (TAL) was 0.95mm, PT/Base was 7.00 mm, URSIV+V was 0.15 mm. *Pentalonia* was also recorded as new genera for Turkey aphid fauna.

Chaitophorinae Börner, 1949

Chaitophorini Wilson, 1910

Chaitophorus Koch, 1854

Chaitophorus clarus Tseng & Tao 1936

General features, host plants and distribution: There has been no clear information provided for the colony appearance (probably pale colored) and biology of the species. Recorded on *Populus simonii*, record also was given on *Acer villosum* even that it has not been confirmed. Palaearctic (China) originated, recorded from China and probably from India (Holman, 2009; Blackman & Eastop, 2022).

Material examined: 10 adult apterae individuals (♀) were sampled on leaves of the *Populus tremula* from Karaman-Bucakkısla on XXXI.VIII.2021. Adult apterae individuals were green, pale green with reddish-orange head (Fig.1j), produced reasonable amount of honeydew, visited by ants, BL was 1.47 mm, (TAL) was 0.53mm, PT/Base was 2.29 mm, URSIV+V was 0.09 mm. All these features were observed for the first time for *C. clarus*.

Chaitophorus parvus Hille Ris Lambers, 1935

General features, host plants and distribution: Feed basically on undersides of leaves of *Salix repens*, *S. viminalis* and *S. purpurea lambertiana*. Palaearctic (Europe) originated, widely distributed in Europe. Adult apterae individuals were black, body length was about 1.2-1.8. Even sometimes synonymized with *S. niger*, it has been considered as a distinct species (Holman, 2009; Blackman & Eastop, 2022).

Material examined: 20 apterae individuals (♀) were collected on leaves of the *Salix viminalis* from Muğla-Akyaka on VI.V.2020. Adult apterae individuals were brown-blackish (Fig.1k), heavily colonized the whole plant, intensively attended by ants, BL was 1.38 mm, (TAL) was 0.56mm, PT/Base was 2.43 mm, URSIV+V was 0.1 mm.

Phylloxeroidea Herrich-Schaeffer, 1854

Phylloxeridae Herrich-Schaeffer, 1854

Phylloxerinae Herrich-Schaeffer, 1854

Phylloxerini Herrich-Schaeffer, 1854

Olegia Shaposhnikov, 1979

Olegia ulmifoliae (Aoki, 1973)

General features, host plants and distribution: Adult apterae individuals in gall formed on the mid ribs of the leaves of *Ulmus japonica* (show monoecious holocyclic life cycle on same host plant) were yellowish, also record was given on *U. davidiana*, BL was 1.0-1.7 mm. Palaearctic (Japan) originated, distributed in Japan, east Siberia. (Holman, 2009; Blackman & Eastop, 2022).

Material examined: 10 adult apterae individuals (♀) were sampled in a pseudo gall formed on the mid ribs of through undersides curled leaves of the *Ulmus* sp. were whitish, yellow (Fig.1l), BL is

1.92 mm, (TAL) is 0.52mm, PT/Base is 2.67 mm. Specimens were collected from Karaman-Akköprü on XXVIII.V.2020 and from Muğla-Ortaca on III.VI.2020. *Olegia* consists of only 1 species (*Olegia ulmifoliae*), as it is the first time this species recorded from Turkey, *Olegia* also was recorded as new genera for Turkey aphid fauna.

CONCLUSION

Aphids fascinate researchers for a long time due to the existence of the unique cyclical parthenogenetic reproduction, showing higher adaptability to various ecological conditions throughout phenotypic plasticity, extending distribution area, host plant usage and causing a considerable amount of decrease in agricultural production (Dixon, 1998; Kindlmann, Dixon, & Michaud, 2009; Vilcinckas, 2016). An importance of aphids is mostly being ignored by most of the researchers and farmers as they tried to link aphid damages with their size. In fact, aphids might look tiny and delicate, but do not be deluded by their minute appearance as they can cause considerable amount of damage if they are not managed properly. Their damages cannot be neglected as they cause about 40-45 % yield losses in developing countries and 30-35 % yield losses in developed countries (Ruberson, 1999). As Turkey is one of the country where properly detecting a presence of the aphid species will play important role in agricultural production. Moreover, geographically separated regions might have their own aphid composition related with agricultural richness and altitude differences. Study area includes Antalya, Karaman and Muğla Provinces. Antalya province is located in the Mediterranean Region, Muğla province is located in Aegean and transition zone between Aegean and Mediterranean region and Karaman province located in the Inner Anatolia and transition zone between Inner Anatolia and Mediterranean region. Antalya and Muğla provinces has higher forested area in Turkey, and Karaman is characterized by step vegetation. Despite these particular characteristics of study area make them suitable for aphid species, there are less study were conducted to determine aphid fauna (Güleç, 2011; Saraç, Özdemir, & Karaca, 2015; Tıraş & Yaşar, 2017). Recently Şenol et. al, (2021, 2022) were recorded 14, Başer et. al, (2021, 2022) were recorded 3 aphid species from study area. Results presented here were the preliminary findings of the ongoing project and number of the aphid species listed for Turkey aphid fauna was increased to 631, and genera to 169 with these new records. In addition to new records, both undefined colony appearances of the detected species and aphid-host plant interactions were determined for the first time. Colony appearances of the *Anthemidaphis oligommata*, *Aphis (Aphis) pseudeuphorbiae*, *Chaitophorus clarus* were described for the first time from study area. Moreover, *Nepeta nuda*, which is an endemic plant species to Turkey flora, was added to host plant list of the *Aphis* (*Aphis*) *narzikulovi*. *Triticum* sp. was added to host plant list of the *Melanaphis sorghi*. Both *Agropyron repens* and *Elytrigia repens* were added to host plant range of the *Schizaphis* (*Schizaphis*) *nigerrima* from Turkey. Findings of the presented study strongly supported the approaches that it was highly possible to record a lot of aphid species- new records, describe a new aphid host plant interactions and defining colony appearances as a result of the regular field works at the different regions and locality of Turkey.

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Research Article
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The Research Hotspots and Frontiers of Black Soldier Fly during 1994-2021: A Bibliometric Analysis

Shuang LIU[1] Huilin LANG[2] Jianwei HAO[3*]
Wenfeng HU[4] Songling LI[5]

[1,2]Institute of Loess Plateau, Shanxi University, Wucheng Road 92, Xiaodian District 030006, Taiyuan, CHINA
[3]Department of Biological Science and Technology, Jinzhong University, Jinzhong 030600, CHINA
[4]College of Food Science, South China Agriculture University, Guangzhou 510642, CHINA
[5]Qinghai Academy of Agricultural and Forestry Sciences, Xining 810000, CHINA
e-mails: [1]liushuang@sxu.edu.cn, [2]1986833496@qq.com, [3*]1346994217@qq.com
[4]johnwfhu@qq.com; [5]465883114@qq.com
ORCID IDs: [1]0000-0001-8148-1974, [2]0009-0003-7839-2876, [3]0009-0002-9390-0149,
[4]0009-0004-9290-6674; [5]0009-0001-1334-5257
*Corresponding author

ABSTRACT

Black soldier fly (BSF) studies have attracted increasing scientific attention because of their role in determining sustainability. However, there exists rare quantitative analyses of the scientific literature on research of BSF, especially for visual analyzing from the systematical and bibliometric perspective has not been reported. Therefore, the investigation on the temporal development, scientific collaboration, research hotspots and frontiers in terms of BSF over the last 28 years (1994-2021) is executed for the first time. Combined with the Web of Science Core Collection database, this study collected 914 literatures from 1994 to 2021 that were conducted co-occurrence, co-citation and cluster analysis via CiteSpace software. The main findings are as follows: Firstly, research on BSF has advanced rapidly during the recent five years and Italy, USA and China were the three core force leaders countries with the high publications, centrality and cooperation degrees. In addition, Huazhong Agr Univ showed the core force ranked the third in the world of BSF research, which had high publications and strong cooperation with international institutions. Furthermore, in terms of keyword co-occurrence, time-zone and burst analysis delineated biological characteristics, application and management of BSF larvae were the main research themes. And early studies concentrated on BSF development and breeding, and current hotspots include exploiting BSF larvae as animal feed and recycling organic wastes. Additionally, based on co-cited references cluster analysis, it was concluded that the bioconversion and animal feed using the BSF larvae will still be the focus research fields in the near future, and the technical improvement on the efficiency of bioconversion, integrating the healthy, safety and eco-friendly that future studies should focus on, supporting policy making that indicate the prospective research field on BSF and it's applications.

Keywords: Hermetia illucens, Knowledge Mapping, Evolution of Frontiers, CiteSpace

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INTRODUCTION

Rapid worldwide population increase will reach 10 billion by 2050. This rise in global population is causing deep changes from industrialization to urbanization that have not been witnessed in a century, yet it is required to concern about the energy crises and environmental challenges (Zhang et al, 2021). Because of the predicted 58% and 70% increases in milk and meat consumption in 2050 compared to 2010 due to rising per capita income, there would be a massive protein shortage in the world by that year (Makkar, Tran, Heuzé, & Ankers, 2014; Finley, 2020). The vegetable and meat expected to increased 265 million per year, to meet this demand, global feed and food productions would need to increase to 25—70% from existing land and energy resources (Liland et al, 2021; Traksele et al, 2021). The increased demand for meat, milk, and animal byproducts, animal products and livestock output are predicted to increase, as is livestock manure and food waste (Melikoglu, Lin, & Webb, 2013; Makkar, Tran, Heuzé, & Ankers, 2014; Kim et al, 2021), which will result in more serious environmental problems.

Presently, the standard techniques of composting, co-composting, or anaerobic digestion currently only process 20 to 30% of animal and poultry manure (Awasthi et al, 2019). Then using the productions as animals' feed, soil organic fertilizer, or the methane energy production. However, these techniques take a long time, produce low-quality goods, and are constrained by land availability and expensive initial investment costs (Fan, Yang, Fang, & Zhang, 2019). For the food waste, most was treated by landfill disposal and incineration, a small part was treated by anaerobic digestion (Kim et al, 2021). The livestock manure and food waste treated both by traditional method or unprocessed could induce environmental problems, such as pose risks to water, soil and air quality, and the spread of pathogens (Kong et al, 2018; Ravindran et al, 2019). Therefore, there's an increasing demand for better management of livestock manure and food waste. The use of insects, such as the black soldier fly to treat excess manure and recycle nutrients into the food system is therefore gaining popularity.

It is common in tropical and temperate locations, although the black soldier flies (BSF), (*Hermetia illucens* L.) (Diptera: Stratiomyidae) may also be found in a wide range of climates (warm, tropical and subtropical) around the globe (Rozkosny, 1983; Gold, Tomberlin, Diener, Zurbrügg, & Mathys, 2018; Lalander, Diener, Zurbrügg, & Vinnerås, 2019). Organic waste is the primary food source for all four stages of the BSF life cycle: egg, larva, pupa, and imago (Li et al, 2011). According to the relevant literature, the researches about BSF mainly including the following aspects: (*İ*) BSF larvae can consume various decaying organic wastes (e.g., livestock manures, food and kitchen waste, and plant materials etc.) and convert these waste into organic fertilizer containing the antimicrobial peptide activity that can inhibit pathogens (Lalander et al, 2013; Li & Zhao, 2015; Elhag et al, 2017) and with lower content of heavy metal and pathogens in their growing substrate (Wynants et al, 2019; Wu, Liang, Wang, Xie, & Xu, 2021; Wang, Wu, Cai, Geng, & Xu, 2021). Therefore, the BSF shall be deemed as a continuable tool of animal manure management. (*İİ*) The BSF larvae with the high protein content which can be used as high-quality good nutrition source of animal feed that can better instead of the traditional feed, especially used for feeding the fish (Gao et al, 2019; Lalander, Diener, Zurbrügg, & Vinnerås, 2019). (*iii*) The BSF larvae can collect lipids required for biodiesel production, which is superior to the oil crops that are currently utilized as biodiesel sources due to the BSF's shorter life cycle, greater fertility, and smaller acreage requirements for production (Li et al, 2015; Wang & Shelomi, 2017; Kim et al, 2021).

In the past two decades, there are many research publications on BSF. However, in some degree, it is not easy for researchers to quickly grasp the hotspots and frontiers in the future research trend and direction. Bibliometrics, a new data-driven method (Broadus, 1987; Qin et al, 2021), has assumed an ever-increasing importance in scientific study in recent years (Aleixandre-Benavent, Aleixandre-Tudó, Castelló-Cogollos, & Aleixandre, 2017). For example, it can be used to provide data on the most productive authors, institutions, and countries, in terms of identifying the collaboration between authors, institutions, and countries (Li & Zhao, 2015), determining the of research areas and recognizing the future research trends and fields, and depicting the hotspots and frontiers (Zhi et al, 2015; Zhang, Zhang, Shi, & Yao, 2017). Given that the large mounts of literature need to be analyzed, the computer analysis and mapping software are required, such as the VOSviewer, CitNetExplorer, HistCite and CiteSpace etc. CiteSpace is the current mainstream software due to its' easy operation system and multi-function (Chen, 2006).

Therefore, we use CiteSpace tools and methodology to conduct a bibliometric study of BSF research published between 1994 and 2021 to gain insights into the future of BSF research by evaluating BSF research. The current research hotspots were investigated using the frequency and interactive to analyze the top authors's situation and cooperation, and main institutions and countries/regions. Furthermore, it is possible to identify changes in the BSF's dynamic variation and trend by examining keywords in reference citations and the current research focuses and knowledge gaps. The examination of evolution and the identification of hotspots offers a comprehensive view of the evolution of this issue. Filling the gaps in BSF knowledge, findings and recommendations from the BSF research are provided herein.

METHODS

Data collection and processing

As a component of the Web of ScienceCore Collection (WoSCC), SCI-expand was used to collect data on English-language literature for this investigation. The WoS Core Collection was selected since it contains the most complete database and high-quality research data, and the most influential and relevant journal with a lengthy history (Harzing & Alakangas, 2016; Olawumi & Chan, 2018; Qin et al, 2021). An advanced search was performed in the WoS Core Collection database, the retrieval parameter as Topic (TS) = “black soldier fly” was set to search for literature in the Title, Abstract, Author Keywords and Keywords Plus fields during Jan 1, 1994 to Dec 31, 2021. As a result, a total of 953 records were obtained, after eliminating the duplicate records, 914 records were ultimately obtained, which including 775 articles, 79 reviews and 40 proceedings papers. Fig. 1 summarizes the acquired search results.

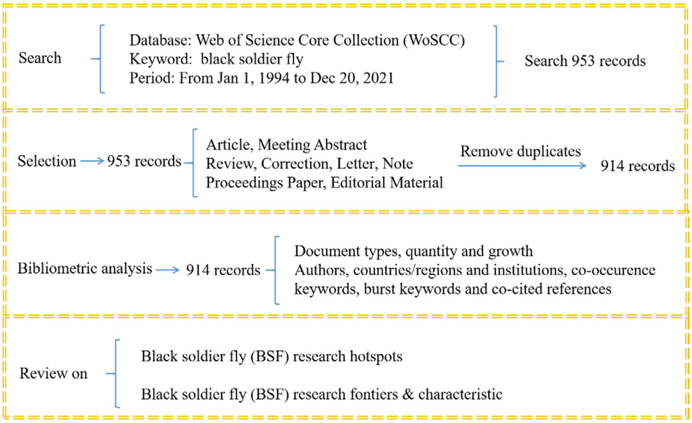


Figure 1. Document selection and flow diagram of BSF.

Bibliometric analysis

Visualization of knowledge can be done with CiteSpace, a software application (Chen, 2004). It can be using for analyzing research hotspots and identifying frontier, and future trend in visualization forms, which based on the co-occurrence analysis, co-citation analysis, and institution and author collaboration analysis (Chen, 2016). Thus, the gathered WoSCC documents were further examined in this study via CiteSpace 5.8 R3. The pruning method was used for the minimum spanning tree and pruning sliced networks, and the thresholds of C, CC, and CCV were set to (2, 2, 20), (4, 3, 30), and (4, 3, 30), where C represents the frequency or lowest cited occurrence, CC represents the co-citation times or collinearity in a particular time slice, and CCV represents the co-citation or collinearity rate (Yang & Meng, 2019). In the visualization analysis, the objects are depicted as nodes, which might represent a keyword, an author, an institution, or a country; the larger the node, the higher the frequency of occurrence of the keyword (Yang & Meng, 2019). A co-citation relationship between two nodes is denoted by a line that connects the two nodes; the line's color signifies the first time that the two nodes are mentioned together, and the line's length and thickness reflect the strength of the link that exists between the two nodes (Xie, 2015; Liu, 2017).

RESULTS AND DISCUSSION

Documents types and quantity analysis

From 1994 to 2021, the WoSCC database yielded a total of 914 documents. The total accumulated quantity of the documents are accumulated year by year (red line) and the quantity of the documents for each year (blue histogram) are summarized (Fig.

2a). Since 1994, the number of publications in the BSF sector has steadily increased, reaching 914 pieces at the end of the year (Fig. 2a). A total of 15 documents were published prior to 2008, indicating that the BSF field was still in its early stages of development. After that, compared to prior years, there was a modest development from 2008 to 2016. This stage saw the publication of a total of 89 documents. For the period from 2017 to 2021, the number of documents published increased significantly, accounting for 88.62% of the whole number, compared to the previous year. According to these findings, BSF is getting more and more popular among academics across the globe.

A total of seven sorts of documents were categorized according to the type of document collected, which included a book chapter and a letter (Fig. 2b). The "article" type came out on top with 84.79 % of the vote, while the "review" type came in second with 8.64 % of the vote. Note that the proportion of "review" type BSF field papers was higher than other categories such as proceeding papers, early access material and editorial content. Researchers in the field of BSF have begun to summarize and draw conclusions based on the current research status, indicating that the field's development and progress have been promoted. This also suggests that future publications will probably concentrate on BSF research.

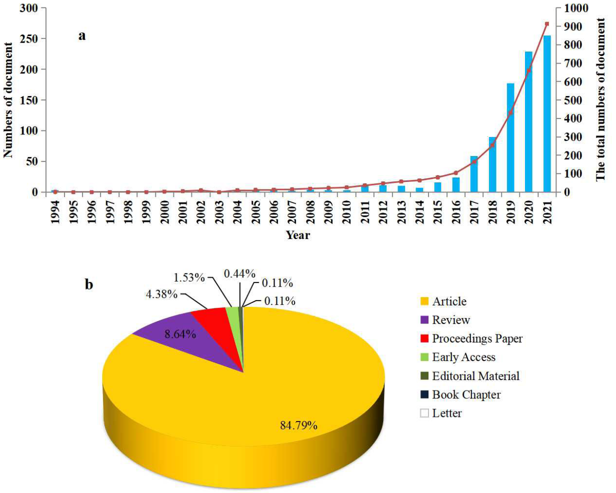


Figure 2. Distribution of the number of BSF-related publications (a) and proportion of publication types (b) during the years of 1994-2021.

The characteristics' analysis of BSF research

The characteristics of author group

A total of 467 authors were found to be involved in BSF research. The top 20 conductive authors are summarized according to numbers (Table 1). Jeffery K Tomberlin was the author with the highest number of publications (52), accounting for 3.43%, followed by Ziniu Yu (34, 2.24%), Laura Gasco (33, 2.18%), Longyu Zheng

(30, 1.98%), and Jibin Zhang (29, 1.91%). As shown in Table 1, The high-yield authors mainly came from Italy, USA and China. Furthermore, Ziniu Yu, Longyu Zheng and Jibin Zhang, three of high conductive authors were all from China, but degrees of centrality were low (0.01, 0.01 and 0.02 respectively). The highest degrees of centrality is 0.13, 0.10 and 0.05 for Jeffery K Tomberlin, Laura Gasco and Chrysantus M Tang, respectively (Table 1), which indicating these authors have a pivotal influence in the BSF research field.

According to author co-appearance analysis, the network has 467 nodes, 1230 lines, and 0.0113 density (Fig. 3). The academic teams of Yu, Zheng, Zhang, and Cai have the most publications and collaboration degrees (52 and 55, respectively) of any of the academic teams in this analysis, who are in close contact with BSF research (Fig. 3; Table 1). The academic teams of Gai, Schiavone, Olivotto, Dabbou, Biasato, and Randazzo are in constant communication and cooperation with the research teams of Gasco, who has the third-highest production and the highest degree of interaction (33, 33). Tang is the seventh most prolific author (20, 18), and the academic research teams in close contact and collaboration are Vanloon, Ekesi, Subramanian, Dicke, and Khamis (Fig. 3; Table 1). It is worth noting that there are primarily three large cooperation author groups, namely Tomberlin, Gasco, and Tang, and that the inter-cooperation of the groups is much stronger, whereas their academic connections are very weak based on the author groups.

Table 1. The performance of top 20 most productive authors.

Rank	Author	Publications	Centrality	Cooperation degree	Proportion (%)
1	JEFFERY K TOMBERLIN	52	0.13	55	3.43
2	ZINIU YU	34	0.01	43	2.24
3	LAURA GASCO	33	0.1	33	2.18
4	LONGYU ZHENG	30	0.01	31	1.98
5	JIBIN ZHANG	29	0.02	24	1.91
6	MINMIN CAI	20	0	20	1.32
7	CHRYSANTUS M TANGA	20	0.05	18	1.32
8	FRANCESCO GAI	19	0	18	1.25
9	ACHILLE SCHIAVONE	19	0	17	1.25
10	JOOP J A VAN LOON	18	0.02	17	1.19
11	IKE OLIVOTTO	16	0.01	16	1.06
12	QING LI	14	0	16	0.92
13	SUNDAY EKESI	13	0	15	0.86
14	SEVGAN SUBRAMANIAN	13	0	15	0.86
15	MARCEL DICKE	13	0	15	0.86
16	SIHEM DABBOU	12	0	15	0.79
17	ILARIA BIASATO	12	0	15	0.79
18	WU LI	11	0	15	0.73
19	BASI LIO RANDAZZO	11	0	15	0.73
20	FATHIYA M KHAMIS	11	0	15	0.73

Figure 3. Authors of BSF-related stuides from 1994 to 2021.

The characteristics of different institutions

Findings from this search were depicted in Fig. 4 (320 institutions were found). Since there are 320 nodes and 620 links on the BSF research institution map, it may be concluded that the majority of BSF research institutions are involved in independent research (Fig. 4). It is shown in Table 2 that the top twenty most productive institutions are. Among them, the Texas A&M Univ ranked first with 69 publications, accounting for 5.49%, followed by Huazhong Agr Univ (55, 4.38 %), Univ Turin (32, 2.55%), and Wageningen Univ & Res (32, 2.55%), with the degree of centrality is 0.30, 0.11, 0.14 and 0.13 respectively. The highest centrality indicating these institutions have a pivotal impact in the BSF research field. However, the publications of the other institutions are ranging from 27 to 12, and all the degree of centrality were lower than 0.10 (Table 2). A visual network analysis of co-occurrence of the academic cooperation of institutions showed that the Texas A&M Univ, Huazhong Agr Univ, Univ Turin and Wageningen Univ & Res are closely relevant to international institutions with the cooperation degrees of 44, 26, 25 and 22 respectively. The CNR and Wageningen Univ both ranked 5th in the cooperation network, the other institutions with the publications below or equal to 21 ranked 6th, 7th, 8th and 9th in the cooperation network (Fig. 4; Table 2).

Table 2. The performance of top 20 most productive institutions.

Rank	Institution	Publications	Centrality	Cooperation degree	Proportion (%)
1	Texas A&M Univ	69	0.30	44	5.49
2	Huazhong Agr Univ	55	0.11	26	4.38
3	Univ Turin	32	0.14	25	2.55
4	Wageningen Univ & Res	32	0.13	22	2.55
5	CNR	27	0.01	17	2.15
6	Wageningen Univ	26	0.05	17	2.07
7	Univ Padua	21	0.03	16	1.67
8	Northwest A&F Univ	20	0.00	15	1.59

table continued

Rank	Institution	Publications	Centrality	Cooperation degree	Proportion (%)
9	Univ Modena & Reggio Emilia	18	0.01	15	1.43
10	Univ Milan	17	0.03	15	1.35
11	Inst Marine Res	17	0.03	14	1.35
12	Int Ctr Insect Physiol & Ecol	15	0.01	14	1.19
13	Univ Politecn Marche	14	0.01	14	1.11
14	Eawag Swiss Fed Inst Aquat Sci & Technol	14	0.07	13	1.11
15	Univ Udine	14	0.00	13	1.11
16	Swedish Univ Agr Sci	14	0.02	13	1.11
17	Univ Parma	13	0.02	13	1.04
18	Chinese Acad Sci	13	0.03	13	1.04
19	Katholieke Univ Leuven	13	0.02	13	1.04
20	Univ Ghent	12	0.01	13	0.96

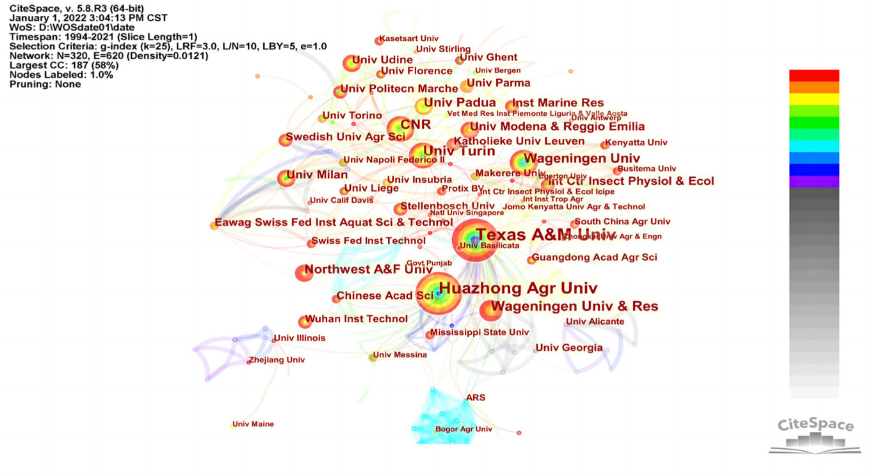


Figure 4. Institutions of BSF-related stuides from 1994 to 2021.

The characteristics of different countries/regions

BSF-related articles have been submitted by researchers in 85 different countries throughout the world. Countries and regions with the most productive economies are listed in Table 3. Italy published the most publications (161), accounting for 11.54%, higher than that of USA (155, 11.11%) and China (the third, 147, 10.54%), followed by the Netherlands (80, 5.73%), Germany (53, 3.80%) and Belgium (53, 3.80%). Italy, USA and China with the degree of centrality is 0.29, 0.15 and 0.13 respectively, indicating that these three country have a pivotal impact in the BSF research field. Although the publications of GERMANY (53 publications), KENYA (32 publications), ENGLAND (29 publications) and SOUTH AFRICA (25 publications) was not so abundant, but the degree of centrality is 0. 11, 0.14, 0.14 and 0.10, which means these countries also have a pivotal impact in the BSF research field (Table 3; Fig. 5). However, the publications of NETHERLANDS, BELGIUM, SWITZERLAND and MALAYSIA were abundant enough (ranging from 29 to 80 publications), all the degree of centrality were lower than 0.10 the centrality (ranging from 0.05 to 0.09). The cooperation degrees of ITALY, USA and CHINA were 37, 31 and 30 respectively, indicating they all have strong cooperation with other countries/regions (Table 3; Fig. 5). Although the KENYA, ENGLAND and SOUTH AFRICA have higher centrality values, the cooperation degrees were only ranging from 19 to 22, which indicating that these countries also need to improve their cooperation with other countries. Developing countries/regions account for only six of the top twenty countries/regions, lagging behind developed countries/regions somewhat. Therefore, researchers in developing countries shall work along with those in developed ones to improve their research capabilities in BSF.

Table 3. The performance of top 20 most productive countries.

Rank	Country	Publications	Centrality	Cooperation degree	Proportion (%)
1	ITALY	161	0. 29	37	11.54
2	USA	155	0. 15	31	11.11
3	PEOPLES R CHINA	147	0. 13	30	10.54
4	NETHERLANDS	80	0. 08	29	5.73
5	GERMANY	53	0. 11	28	3.80
6	BELGIUM	53	0. 06	26	3.80
7	SWITZERLAND	38	0. 09	25	2.72
8	MALAYSIA	34	0. 05	23	2.44
9	KENYA	32	0. 14	22	2.29
10	ENGLAND	29	0. 14	22	2.08
11	AUSTRALIA	28	0.02	22	2.01
12	SPAIN	27	0. 03	21	1.94
13	INDONESIA	26	0.01	21	1.86
14	POLAND	25	0.00	20	1.79
15	SOUTH KOREA	25	0.00	20	1.79
16	CANADA	25	0. 02	20	1.79
17	SOUTH AFRICA	25	0. 10	19	1.79
18	NORWAY	25	0.00	19	1.79
19	TAIWAN	24	0. 02	19	1.72
20	BRAZIL	23	0. 02	18	1.65

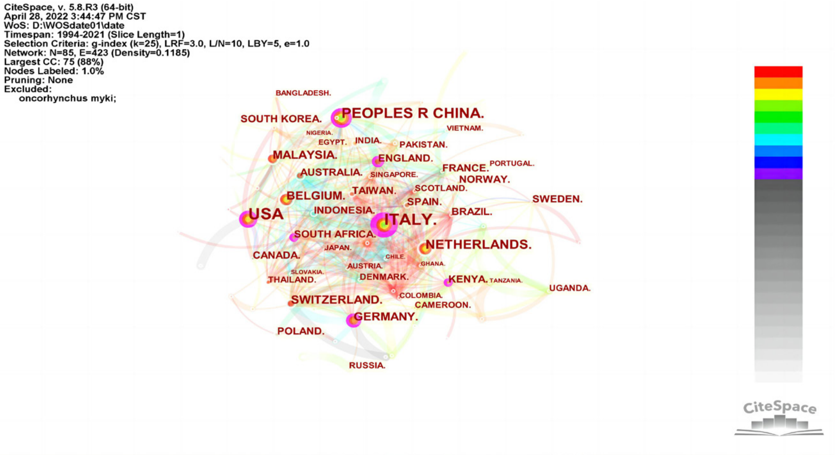


Figure 5. Countries of BSF-related studies from 1994 to 2021.

Hotspots and frontiers of BSF Research

Keywords co-occurrence

Keywords can not only reflect the hot spots and topics but also reflect the direction and the development of research frontiers over a period. Fig. 6 showed the keywords co-occurrence networks for three time periods. A total of 438 keywords were obtained. The first time period from 1994 to 2007 network consisted of 38 nodes and 114 links, and only 38 keywords were obtained in this time period. The keywords of “diptera”, “fly”, “fly diptera” and “hermetia illucen” had a total frequency of 10. The second high frequency keyword was “larvae (Frequency=6)” (Fig. 6a; Table S1). The second time period from 2008 to 2016 network consisted of 173 nodes and 699 links, and a total of 173 keywords were obtained in this time period. The keywords of “black soldier fly”, “flies diptera”, “stratiomyidae”, “diptera”, “hermetia illucen”, “fly”, “hermetia illucens diptera” and “diptera stratiomyidae” had a total frequency of 85. The second high frequency word was “larvae (Frequency=21)” (Fig. 6b; Table S1). However, the third time period from 2017 to 2021 network consisted of 327 nodes and 1887 links, and a total of 334 keywords were obtained in this time period. The keywords of “black soldier fly”, “hermetia illucen”, “diptera stratiomyidae” and “diptera” had a total frequency of 493. The followed keywords of “growth performance”, “growth” and “performance” had a total frequency of 243. Other high frequency keywords were “insect (119)”, “food (115)”, “feed (107)”, “larvae (103)”, “protein (88)”, “rainbow trout (77)”, “fish meal (72)” (Fig. 6c; Table S1).

Table S1. The performance of top 20 keywords for 1994 to 2007, 2008 to 2016 and 2017 to 2021 time periods.

1994-2007				2008-2016				2017-2021			
NO.	Freq.	Keyword	Cent.	NO.	Freq.	Keyword	Cent.	NO.	Freq.	Keyword	Cent.
1	6	larvae	1.06	1	21	black soldier fly	0.40	1	176	black soldier fly	0.05
2	4	diptera	1.02	2	21	larvae	0.31	2	144	hermetia illucens l.	0.02
3	4	management	0.03	3	15	flies diptera	0.20	3	125	growth performance	0.07
4	2	behavior	0.29	4	14	stratiomyidae	0.15	4	119	insect	0.03
5	2	fly	0.11	5	12	oviposition	0.16	5	115	food	0.03
6	2	milk	0	6	11	escherichia coli	0.18	6	107	feed	0.03
7	2	hermetia illucen	0	7	11	diptera	0.18	7	103	larvae	0.06
8	2	pupae	0	8	10	postmortem interval	0.01	8	88	protein	0.05
9	2	fly diptera	0	9	8	hermetia illucen	0.16	9	77	rainbow trout	0.05
10	1	atlantic salmon	0.15	10	7	growth	0.05	10	72	fish meal	0.03
11	1	acid	0.15	11	6	fly	0.05	11	71	tenebrio molitor	0.04
12	1	diet	0.15	12	6	hermetia illucens diptera	0.09	12	66	conversion	0.05
13	1	bacteria	0.01	13	5	manure	0.01	13	65	growth	0.05
14	1	calliphora erythrocephala	0.01	14	5	meal	0.05	14	64	escherichia coli	0.05
15	1	bovine manure	0.01	15	5	rainbow trout	0.10	15	60	diet	0.04
16	1	insect	0.01	16	5	life history	0.07	16	55	diptera stratiomyidae	0.04
17	1	destruction	0.01	17	4	carrion	0	17	53	performance	0.02
18	1	oviposition	0	18	4	food	0.03	18	53	diptera	0.02
19	1	fish	0	19	4	diptera stratiomyidae	0.04	19	51	prepupae	0.02
20	1	feed	0	20	4	conversion	0.01	20	49	waste	0.02

Freq.= Frequency; Cent.= Centrality

From the analysis above, we found that during the whole time (from 1994 to 2021), the keywords both focused on “larvae” and “diptera”, “fly diptera”, “hermetia illucen”, “black soldier fly”, “fly” and “diptera stratiomyidae” etc. that related to “black soldier fly”, which have high frequencies and represent the research on biological characteristics of BSF and the application of it’s larvae, especially on BSF growth and breeding including “oviposition”, “growth”, “pupae”, “prepupae” and “life history” (Fig. 6; Table S1). In addition, from other keywords such as “protein”, “food”, “feed”, “meal”, “fish meal” and “rainbow trout” that with relatively high frequency, we found that more and more researches focused on the applications of BSF larvae, especially on the nutrition of BSF as the feed of animals, especially as fish feed (Fig. 6b, c; Table S1). Notably, other keywords related to BSF larvae applications including “manure”, “dairy manure”, “waste”, “food waste”, “conversion”, “nutrient digestibility” and “biodiesel production”, which indicated that the applying the BSF to deal with organic waste (food and manure) and to produce the biodiesel attract researchers’ attention.

Keywords time-zone visualization

The analysis of changes of keywords over time (1994-2021) (Fig. 7; Table S2) was performed to determine the overall trend of BSF research. The results showed that there were various high frequency hot keywords in BSF research, with the exception of the period between 1995 and 2006. The following is evidenced by the findings that are presented in Fig. 7: (*İ*) The focus on BSF development and breeding shows that BSF research is biological. For instance, Tomberlin, Sheppard, & Joyce (2002) fed BSF three different larval diets to study preimaginal development and adult life-history features. Diet had little effect on preimaginal development or survival past the prepupal stage, according to the findings. However, all diets had lower adult emergence rates than the wild population. It took 40 to 43 days from egg to adult for individuals raised at 27°C, with the larval stage lasting between 22 and 24 days. Furthermore, 96% of larvae reached the prepupal stage, however only 21-27% of adults emerged from the eggs. (*İİ*) Studies on the usage of BSF larvae in various applications have been rising since 2007, notably with regard to the management of organic waste and as animal feed. For instance, Wang & Shelomi (2017) studied BSF’s that are capable of efficiently converting diverse organic resources, including food waste and manure, into insect biomass. Researchers Murawska and colleagues (2021) discovered that supplementing soybean meal protein with BSF larvae meal in broiler chicken diets at a percentage of more than 50% deteriorated carcass quality and meat sensory quality. (*İİİ*) New research focus at BSF include biodiesel, essential oils, greenhouse gas emissions, antibacterial peptides, and genes that confer resistance to antibiotics. For example, when employing non-catalytic transesterification to extract BSF larval lipids, Jung et al (2022) obtained 94.1 wt% biodiesel, and the fuel characteristics of BSF larvae were in line with Korea and EU biodiesel fuel specifications. Through *Hermetia illucens* larvae, Matos et al (2021) found that methane emissions from the bioconversion of animal dung were 86% lower than those from controls. Fig. 7 also shows that, the number of studies on BSF is increasing significantly since 2007. As research has become more in-depth, studies on BSF have become increasingly detailed, diverse, and systematic.

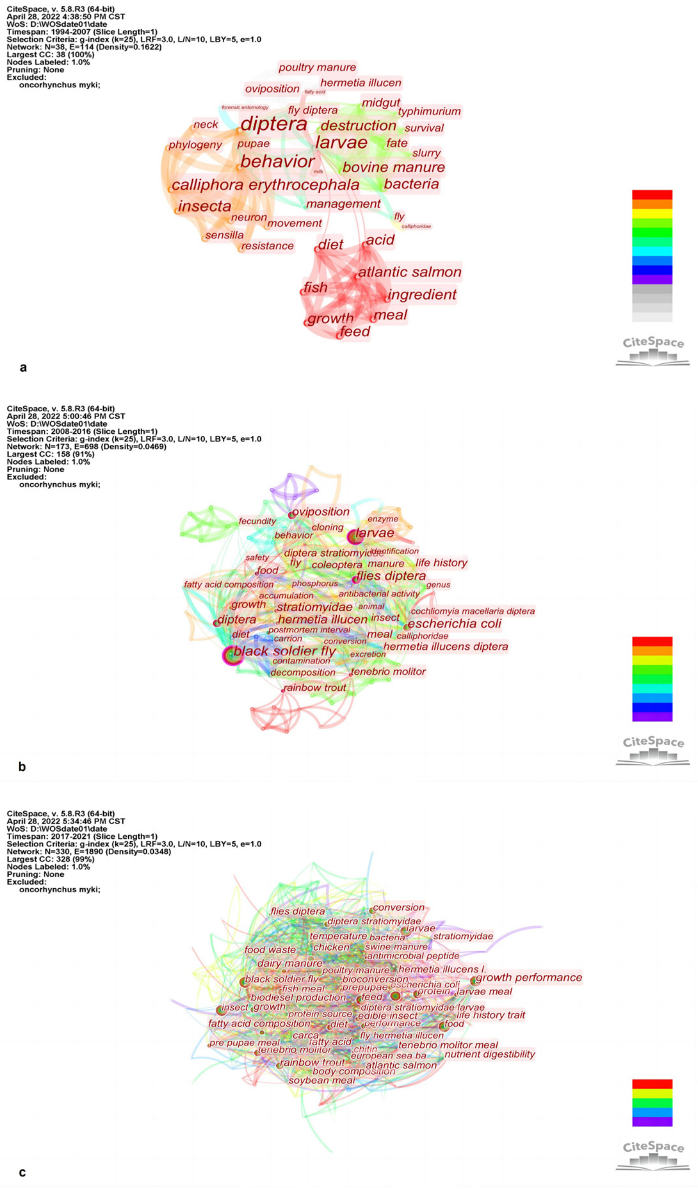


Figure 6. Keywords co-occurrence maps of publications in BSF-related studies for different time periods. a) Initial budding phase from 1994 to 2007. b) Primary growth phase from 2008 to 2016. c) Rapid development phase from 2017 to 2021.

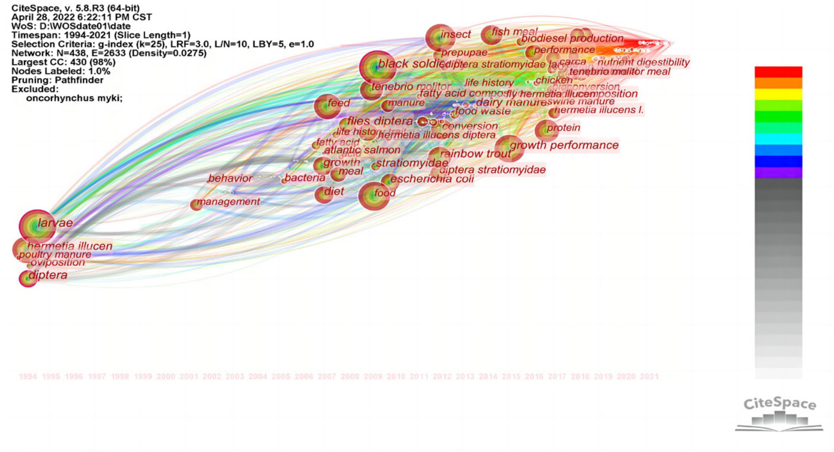


Figure 7. The time-zone view of WoS BSF hotspots keywords from 1994 to 2021.

Table S2. The information related to the keywords from 1994 to 2021.

Freq.	Cent.	Keyword	Year
197	0.16	black soldier fly	2009
154	0.05	hermetia illucen	1994
130	0.16	larvae	1994
127	0.06	growth performance	2015
122	0.04	insect	2012
119	0.03	food	2009
110	0.03	feed	2007
88	0.03	protein	2017
82	0.07	rainbow trout	2012
75	0.04	tenebrio molitor	2009
75	0.09	escherichia coli	2010
73	0.03	fish meal	2014
73	0.07	growth	2007
70	0.04	conversion	2012
68	0.15	diptera	1994
65	0.04	diet	2007
59	0.04	diptera stratiomyidae	2012
55	0.02	performance	2016
53	0.02	prepupae	2012
51	0.09	flies diptera	2008
50	0.02/0.01	manure/waste	2010/2009
46	0	nutritional value	2018
41	0.07/0.04/0.02	stratiomyidae/meal/management	2009/2007/2001
40	0.02	larvae meal	2017
39	0.01/0.01	pre pupae meal/digestibility	2018/2017
38	0.01	chitin	2017
37	0.03	hermetia illucens l.	2017
34	0.03	edible insect	2017

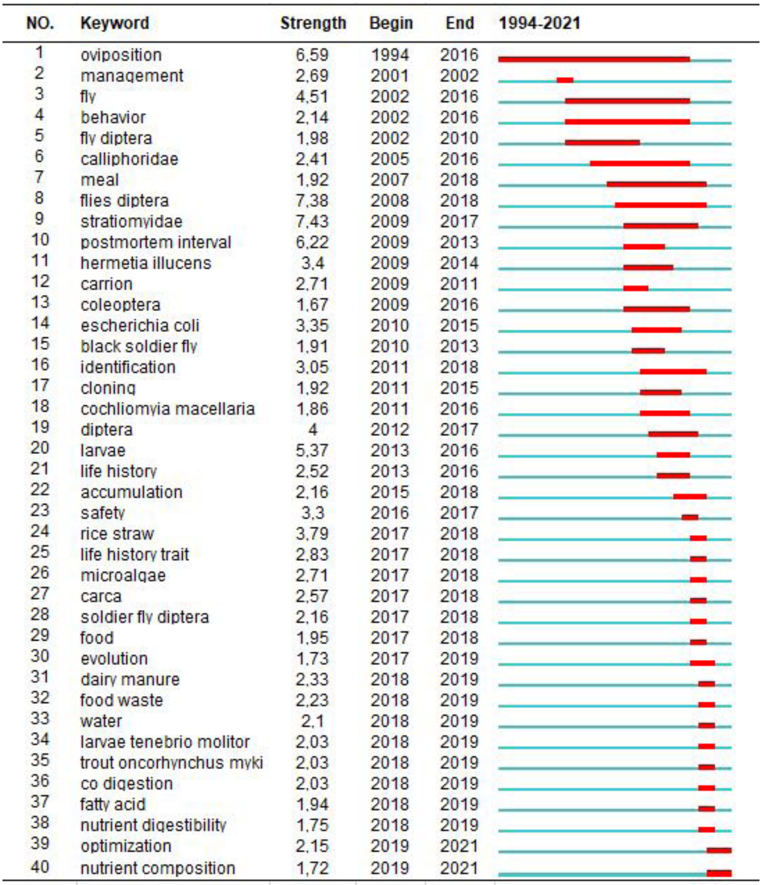
table continued

Freq.	Cent.	Keyword	Year
33	0.01/0.01	extraction/chicken manure	2017/2011
30	0.02/0.01	protein source/soybean meal	2018/2017
27	0.02/0.01/0.01/0.01/0.03/0.02	quality/reduction/replacement/bioconversion/life history trait/fatty acid	2018/2018/2018/2017/2008/2007
26	0.01/0.05	life cycle assessment/food waste	2018/2012
25	0.05	oviposition	1994
23	0.03/0.06/0.05	body composition/biodiesel production/bacteria	2016/2015/2004
22	0.04/0.04	temperature/acid	2017/2007
20	0.03/0.03/0.01	diptera stratiomyidae larvae/lipid/diversity	2015/2011/2011
18	0.03/0.02/0.04/0.02/0.02/0.05	nutrient digestibility/oil/chicken/chitosan/postmortem interval/atlantic salmon	2018/2018/2016/2016/2015/2009/2007
17	0.01/0.03	european sea ba/life history	2019/2013
16	0.01/0.01/0.05/0.05	impact/expression/dairy manure/hermetia illucens diptera	2019/2015/2013/2009
15	0.01/0.02/0.02/0.01/0.01/0.01	musca domestica/nutritional composition/carca/heavy metal/amino acid/animal feed	2019/2018/2017/2017/2016/2013
14	0/0.01/0.02/0.07	nitrogen/gut microbiota/decomposition/behavior	2017/2011/2009/2002
13	0.01/0/0.02/0.02	efficiency/insect meal/tenebrio molitor meal/swine manure	2020/2018/2017/2015

Freq.= Frequency; Cent.= Centrality

Burst keywords analysis

The analysis of burst keywords can quantitatively the focus and emerging hotspots and research frontiers. The burst keywords with the strength citation bursts implies the focus intensity of the field. We found that studies related to biological characteristics of BSF were the main topics during the whole time (1994-2021) especially before 2018, among which “oviposition” (strength: 6.59 in 1994-2016), “behavior” (strength: 2.14 in 2002-2016), “black soldier fly” (strength: 1.91, 2010-2013), “soldier fly diptera” (strength: 2.16, 2017-2018) ,“larvae”(strength: 5.37 in 2013-2016) and “life history” (strength: 2.52 in 2013-2016), “life history trait” (strength: 2.83 in 2017-2018),“escherichia coli”(strength: 3.35 in 2010-2015) received major focus. The applications of BSF larvae were also the research hotspots and frontiers, especially using as animal feed, the keywords “meal” with strength of 1.92 during 2007-2018, and studies related to other applications such as “rice straw”, “microalgae”, “food”, and “dairy manure”, “food waste”, “nutrient digestibility”, “nutrient composition” and “optimization” were the emerging active topics, especially in the last five years from 2017 to 2021 (Fig. 8). In addition, some new burst keywords emerged as the active topics, such as “accumulation” (strength: 2.16) emerged in 2015-2018, and “safety” (strength: 3.3) emerged in 2016-2017.



NO.	Keyword	Strength	Begin	End	1994-2021
1	oviposition	6,59	1994	2016	
2	management	2,69	2001	2002	
3	fly	4,51	2002	2016	
4	behavior	2,14	2002	2016	
5	fly diptera	1,98	2002	2010	
6	calliphoridae	2,41	2005	2016	
7	meal	1,92	2007	2018	
8	flies diptera	7,38	2008	2018	
9	stratiomyidae	7,43	2009	2017	
10	postmortem interval	6,22	2009	2013	
11	hermetia illucens	3,4	2009	2014	
12	carrion	2,71	2009	2011	
13	coleoptera	1,67	2009	2016	
14	escherichia coli	3,35	2010	2015	
15	black soldier fly	1,91	2010	2013	
16	identification	3,05	2011	2018	
17	cloning	1,92	2011	2015	
18	cochliomyia macellaria	1,86	2011	2016	
19	diptera	4	2012	2017	
20	larvae	5,37	2013	2016	
21	life history	2,52	2013	2016	
22	accumulation	2,16	2015	2018	
23	safety	3,3	2016	2017	
24	rice straw	3,79	2017	2018	
25	life history trait	2,83	2017	2018	
26	microalgae	2,71	2017	2018	
27	carca	2,57	2017	2018	
28	soldier fly diptera	2,16	2017	2018	
29	food	1,95	2017	2018	
30	evolution	1,73	2017	2019	
31	dairy manure	2,33	2018	2019	
32	food waste	2,23	2018	2019	
33	water	2,1	2018	2019	
34	larvae tenebrio molitor	2,03	2018	2019	
35	trout oncorhynchus myki	2,03	2018	2019	
36	co digestion	2,03	2018	2019	
37	fatty acid	1,94	2018	2019	
38	nutrient digestibility	1,75	2018	2019	
39	optimization	2,15	2019	2021	
40	nutrient composition	1,72	2019	2021	

Figure 8. The top 40 keywords with the strongest citation bursts from 1994 to 2021.

Co-cited references timeline visualization

The BSF research literature has grown since 2007, mainly focusing on cluster #0, bioconversion; cluster #1, insect meal; cluster #2, meat quality; cluster #3, lipids; cluster #4, contaminants; cluster #5, organic solid waste; cluster #6, biodiesel; cluster #7, waste conversion (Fig. 9). Lee, Chen, & Tsai (2016) reported that the values of the silhouettes for each cluster were greater than 0.5, indicating that the results were reliable and significant. Therefore, the results in Table 4 were reliable and significant. The largest cluster, #0, has a size of 134 and a Silhouette value of 0.776; the LLR algorithm's label is a bioconversion. Thomas Spranghers and Yu-Shiang Wang are the most notable authors, as they focus their study on the bioconversion of various organic wastes by BSF larvae. Spranghers et al (2017), for instance, used BSF larvae to convert four types of organic waste (chicken feed, vegetable waste, biogas digestate, and restaurant waste) and concluded that protein content and quality were high and comparable for prepupae reared on different substrates, suggesting that BSF could be an interesting source of protein for animal feeds. Wang & Shelomi (2017) evaluate the literature on BSFs that are capable of efficiently converting a wide range of organic resources, including food waste and manure, into insect biomass. The results revealed that BSF larvae have 42% crude protein and 29% fat, but do not concentrate pesticides or mycotoxins. BSF are already cultivated and approved for use as animal feed, albeit subject to regional legal constraints. "insect meal" (#1) and "lipids" (#3) were the most recent clusters, while "organic solid waste" (#5) was the oldest. In addition, the "insect meal (#1)" cluster was the most recent and relatively larger (98), therefore we concentrated on examining it. The most notable writers are Makkar and Renna, whose study focuses on the ability of BSF larvae to serve as animal feed. For instance, Makkar, Tran, Heuzé, & Ankers (2014) investigated the nutritional value of black soldier fly larvae and its application as a substitute for soymeal and fishmeal in the diets of chickens, pigs, fish species, and ruminants. The results indicated that the crude protein and lipid contents are high, with unsaturated fatty acid concentrations ranging from 19 to 37 percent, confirming that the palatability of BSF larvae as an alternative feed for animals is excellent and that it can replace 25 to 100 percent of soymeal or fishmeal, depending on the animal species. Renna et al (2017) evaluated the suitability of BSF larvae meal as an ingredient for rainbow trout diets. The results indicated that a partially defatted BSF larvae meal can be used as a feed ingredient in trout diets up to 40 percent without affecting survival, growth performance, condition factor, somatic indices, dorsal fillet physical quality parameters, or intestinal morphology of the fish. To mitigate the reported deleterious effects of insect meal on the fatty acid content of dorsal muscle, however, more research into specialized feeding procedures and diet compositions is required.

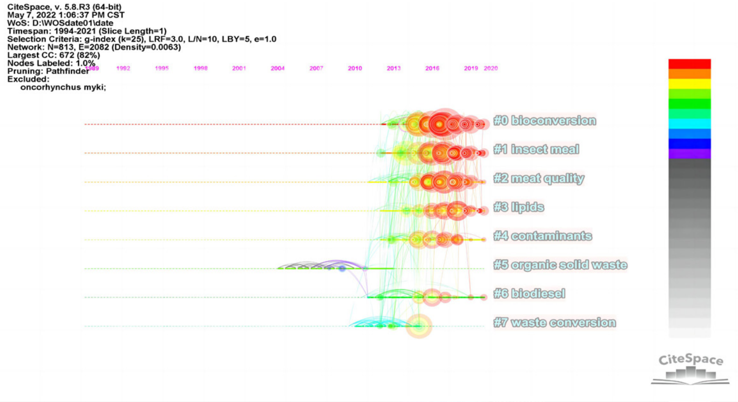


Figure 9. Timeline view analysis of co-cited references cluster.

Highly co-cited references

The 10 top co-cited references with different emphasis can be obtained from Fig. 10

and Table 5. The first and second highly cited references were published by Spranghers et al (2017) and Wang & Shelomi (2017) in cluster #0, with a citation counts of 205 and 152 respectively. The third most highly cited reference was published by Makkar, Tran, Heuzé, & Ankers (2014) in cluster #1, with a citation counts of 135, followed by Barragan-Fonseca, Dicke, & van Loon (2017), Liland et al. (2017), Lalander, Diener, Zurbrügg, & Vinnerås (2019), Xguyen, Tomberlin, & Vanlaerhoven (2015), Meneguz et al (2018), Surendra, Olivier, Tomberlin, Jha, & Khanal (2016) and Renna et al (2017), with the citation counts ranging from 103 to 125. There are eight references belong to cluster #0, and two references belong to cluster #1, therefore, these references were mainly divided into two categories, i.e. bioconversion and insect meal, which was in accordance with the results in the co-citation cluster timeline analysis. Firstly, using the BSF larvae to converse different organic waste attracted widespread attention. Secondly, researches related on the ability of using BSF larvae as the animal feed. Therefore, the bioconversion and animal feed using the BSF larvae will still be the focus research fields and will be continuously improved the research depth especially on the technical improvement on the efficiency of bioconversion.

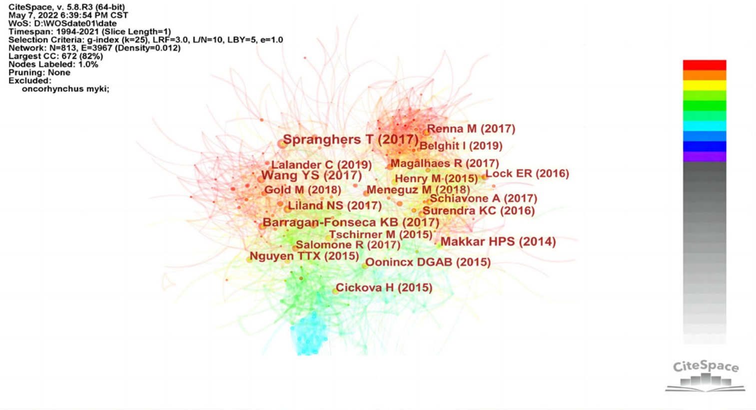


Figure 10. Network of co-cited references from 1994 to 2021.

SUMMARY AND CONCLUSIONS

This is the first time that scientometric analysis has been used to provide a complete overview of the black soldier fly (BSF) and its progress, hotspots, and future research directions. It is clear that research on BSF has progressed swiftly in the last five years, since the number of publications on this issue has increased significantly since 2007, with most of these articles coming in the form of Article and Review. The top 20 authors in BSF related studies also have high cooperation with other authors. The countries with the most publications and centrality were Italy, the United States, and China. With a significant number of publications and close ties to foreign institutions, Huazhong Agr Univ in China was the driving force behind BSF research in China. In terms of keyword co-occurrence and time-zone analysis delineated two main research themes during the study period: biological characteristics, application and management of BSF larvae. Research on BSF larvae as animal feed and organic waste conversion were found to be two of the most popular topics in recent studies, according on a burst keyword analysis. Finally, using co-cited references cluster analysis, the eight greatest clusters could be identified. The top six clusters were bioconversion, insect meal, meat quality, lipids, contaminants and organic solid waste. Therefore, the bioconversion and animal feed using the BSF larvae will still be the focus research fields in the near future, and the technical improvement on the efficiency of bioconversion, integrating the healthy, safety and eco-friendly will attract more attentions. In addition, it could aid in the formulation of policies indicating the future direction of BSF research and it's applications.

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Research Article
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Beta-cyfluthrin-Induced Alterations in the Total and Differential Haemocytes Count in the Red Cotton Bug, *Dysdercus koenigii* (Fabricius,1775)

Panmei LANBILIU[1] Roopa Rani SAMAL[2] Kungreiliu PANMEI[3]
Sarita KUMAR[4*]

[1234]Insect Pest and Vector Laboratory, Department of Zoology, Acharya Narendra Dev College (University of Delhi) New Delhi-110019, INDIA
e-mails: [1]lanbiliupans@gmail.com, [2]rupasamal29@gmail.com, [3]kungreiliu@gmail.com, [4*]sarita.sanjay90@gmail.com, [4*]saritakumar@andc.du.ac.in
ORCID IDs: [1]0000-0003-3791-6832, [2]0000-0001-8268-3279, [3]0000-0002-1828-0675, [4*]0000-0002-1066-3057
*Corresponding author

ABSTRACT

Dysdercus koenigii is a major global pest of cotton that causes severe economic loss. Among several control measures, pyrethroids are frequently used toxicants because of high efficacy at low dosages and relative safety. Since haemocytes are biomarkers of the physiological response and immunity of insect which determine the insecticide efficacy, the current study assessed the effect of a pyrethroid, β-cyfluthrin, on the total and differential haemocyte counts of *D. koenigii*. Haemolymph was collected from the fifth instars after the topical application of β-cyfluthrin (0.8, 1.6, 3.2, 6.4 and 12.8 mg/L) on the thoracic tergum. The haemolymph of control nymphs revealed 5270 haemocytes/mm^3 which decreased instantly by 1.4-3.1-fold on β-cyfluthrin exposure; more reduction observed at lower dosages. Increase in exposure duration and β-cyfluthrin dosages fluctuated the count considerably, eventually raising them at lower dosages and diminishing at higher dosages. Among five kinds of haemocytes recorded in the haemolymph, the β-cyfluthrin exposure increased %prohaemocytes count; diminished %granulocytes and %plasmatocytes count while spherulocyte and oenocyte counts were inconsistent. The alterations in haemocyte counts indicate the immunity response trigger in *D. koenigii* due to β-cyfluthrin-induced stress. Further investigations may decipher the mechanisms involved and help to formulate the strategies for its management in fields.

Keywords: Biomarkers, DHC, Haemolymph, Pyrethroid, Red Cotton Bug, THC.

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INTRODUCTION

Cotton is one of the most important fibers and cash crops of India responsible for the industrial and agricultural economy of the country (Pal, Bhattacharya, & Sahani, 2020). India accounts for about 25% of the total global cotton production. In India, the majority of cotton is produced in Madhya Pradesh, Maharashtra and Gujarat under central cotton-growing zones; while the southern zone comprises Andhra Pradesh, Karnataka and Tamil Nadu and the northern zone includes Punjab, Haryana and Rajasthan.

The major cotton losses have been attributed to the immense infestation by diverse pests and diseases affecting the crop quantity as well as quality. Among these pests, Red cotton bug, *Dysdercus koenigii* (Hemiptera: Pyrrhocoridae) is regarded as one of the major global pests causing severe economic loss. Immature nymphs as well as adults capable of rapid dispersal, suck the moisture and oil contents from the cotton leaves and seeds at a widespread scale reducing the crop yield extensively (Sahayaraj & Fernandez, 2017; Gupta, Shazad, & Kumar, 2019; Saeed & Abbas, 2020; Karar et al, 2020; Karar et al, 2021).

Despite the implementation of multifarious control interventions to alleviate *Dysdercus* infestation, chemical insecticide-based interventions are the predominant and most preferred means due to their rapid action and toxic effects. A wide range of chemical groups of pesticides such as organochlorines, organophosphates, carbamates, pyrethroids, avermectins, spinosyns and neonicotinoids etc., have been used in the fields (Saeed et al, 2018). Toxicity of chlorpyrifos, deltamethrin, lufenuron, flucycloxuron (andalin), chlorfenpyr and thiamethoxam has been demonstrated against *D. koenigii* (Khan & Qamar, 2011; Saeed, Naqqash, & Jaleel, 2016; Jameel & Jamal, 2017). Despite control attempts and studies with diverse chemicals and alternate measures to chemicals, pyrethroids are still preferred because of their efficacy at low dosages and relative safety (Mohd, Nasreen, Snigdha, & Altaf, 2021).

The β-cyfluthrin (cyano-(4-fluoro-3-phenoxyphenil)-methyl-3-(2,2-dichhloroethenyl)-2,2-dimethyl-cyclopropane carboxylate) is a non-systemic second generation of pyrethroid causing contact as well as stomach toxicity in insects (Chawla et al, 2018). Though it has been investigated against a wide variety of pests on cotton, corn, sunflower and soybean crops (Athanassiou et al*,* 2004a; Athanassiou, Papagregoriou, & Buchelos, 2004b; Addy-Orduna*,* Zaccagnini, Canavelli, & Mineau, 2011), reports of its efficacy against *D. koenigii* are limited. Our previous studies have implicated the toxic and growth regulatory effects of β-cyfluthrin on *D. koenigii* (Lanbiliu, Samal, Panmei, & Kumar, 2020). Nevertheless, effect of this chemical insecticide on the physiological functions of *D. koenigii* which can affect their reproductive fitness, metabolism and immunity has not been studied. The haematological studies play a significant factor and biomarker in the field of insect physiology as their haemolymph acts as a transport system for nutrients, hormones and metabolic waste, as well as contains elements of the immune system i.e., phagocytic haemocytes (Richards & Davies, 1977; Sarwar, Ijaz, Sabri, Yousaf, & Mohsan, 2018). Besides that, haemocytes perform several other vital functions of the body, such as food storage, connective tissue formation and cellular defense (Wigglesworth, 1959; Sapcaliu et al, 2009). Insecticide exposure can affect the insect haemocytes altering their physiological functions (Sarwar et al, 2018). Thus, a number of total and differential haemocytes present under various stress conditions can provide information about the insects' physiological conditions, virulence and immunity (Rizwan-ul-Haq, Sabri, & Rashid, 2005).

Thus, present studies were conducted to assess the effects of β-cyfluthrin on the structure and count of total and differential haemocytes in *D. koenigii*. It is proposed that this study will help to understand the physiological response of the pest to insecticide exposure which may assist in optimizing the β-cyfluthrin concentration to be tested in fields as a growth regulatory intervention measure.

MATERIAL AND METHODS

Rearing of *Dysdercus koenigii*

Nymphs and adults of *D. koenigii*, procured from the Insect Reproduction Laboratory, Deshbandhu College, University of Delhi, India; were reared in the Insect Pest and Vector Laboratory, Acharya Narendra Dev College, University of Delhi, India. The culture was maintained under the controlled conditions of 28 ± 2 °C, 80 ± 5% RH (Relative Humidity), and 14 h of light and 10 h of darkness. Adults and different nymphal stages were kept in separate sterilized glass jars of 1L capacity, containing sterilized cotton seeds and cotton swabs soaked in autoclaved water so as to minimize the risk of infection in the insects (Gupta et al, 2019). The food was changed on alternate days while the jars were changed twice a week in order to maintain hygienic conditions throughout the mass rearing of the culture.

Insecticide taken into consideration

The technical grade of β-cyfluthrin (99% purity) was procured from M/s Sigma-Aldrich. Desired concentrations were prepared in acetone (eMerck) from a stock of 1% solution and stored at 4 °C.

Collection of haemolymphs

Based on our previous study which demonstrated the toxic efficacy of the β-cyfluthrin against 5th instar nymphs of *D. koenigii* (Table 1), the current study was conducted on the newly emerged 5th instar nymphs with the concentrations ranging from 0.8 mg/L-12.8 mg/L (Lanbiliu et al, 2020).

The individual insect was topically subjected to different concentrations (0.8 mg/L-12.8 mg/L) of β-cyfluthrin on the thorax tergum with the help of a micropipette (10 μL) at the rate of 1 μL/insect. Each concentration was replicated 4 times to reduce error. The control was run simultaneously. The haemolymph of the control and treated *D. koenigii* nymphs was collected immediately at 0 min followed by collection after 30 min and 60 min of β-cyfluthrin exposure (Perveen & Ahmad, 2017). One of the antennae of the nymphs was dissected with the help of a fine blade and the maximal haemolymph was forced out of the body by applying dorsoventral pressure on the insect's abdomen with a fine microtip forceps (Barakat, Meshrif, & Shehata, 2002).

Table 1. Percent mean mortality of 5th instar nymphs of *Dysdercus koenigii* treated with β-cyfluthrin.

β -cyfluthrin (mg/L)	Total Nymphal mortality after 24 h
Control	0.00 ± 0.00 a
0.8	0.66 ± 0.33 b
1.6	1.66 ± 0.33 b
3.2	4.66 ± 0.33 c
6.4	17.66 ± 0.33 d
12.8	21.66 ± 0.88 d

Notes: 75 insects treated in 3 replicates of 25 each; Values are mean ± Standard Error of Mean (SEM) (Lanbiliu et al, 2020); Values in each row followed by different letters are significantly different $p < 0.05$, one-way ANOVA followed by Tukey's all pairwise multiple comparison test.

Total and differential haemocyte count in 5th nymphal instar of *Dysdercus koenigii*

The total haemocytes in the nymphs were counted by using a Neubauer haemocytometer. Collected haemolymph was sucked into a Thoma white cell pipette up to the mark 0.5. The tip of the pipette was cleaned and haemolymph was diluted 20X with diluting fluid (1ml of 0.3% of gentian violet, 1mL of glacial acetic acid in 100 mL of distilled water) by drawing it up to the mark 11. The contents were mixed thoroughly by slightly rotating the pipette (Sarwar et al, 2018). The first few drops were discarded to negate the error and the Neubauer chamber was charged with the content. Haemocytes were counted in four corner squares of the Neubauer chamber (1mm^2) and the total circulating haemocytes in cubic millimetre (mm^2) was calculated using the following formula (Jones, 1962).

$$\frac{\text{(Haemocyte in four square (1mm}^2\text{) x Dilution x depth factor of chamber)}}{\text{(No.of squares counted)}}$$

Differential haemocyte count (DHC) was carried out by following the Battlement method (Parveen & Ahmad, 2017). A thin film smear of *D. koenigii* haemolymph was prepared by drawing a slide across the haemolymph-containing slide at an angle of 45º. The smear was air-dried at room temperature and was added with Leishman stain for 20 min. Subsequently, the slide was washed carefully with distilled water to remove excess stain and dried again at room temperature. The haemocytes were scrutinized under the microscope (Nikon ECLIPSE E100) and at least 100 cells of different categories were counted from random areas (Jones, 1967). The percentage of different cell types was calculated in order to assess the physiological impact of β-cyfluthrin on *D. koenigii*.

Statistical analysis

The data obtained were subjected to analysis of variance (ANOVA). The means were compared by Tukey's all pairwise multiple comparison test for statistical significance at $p < 0.05$.

RESULTS

Total haemocytes count in *Dysdercus koenigii* nymphs

The topical application of β-cyfluthrin on 5th instar nymphs of *D. koenigii* decreased the total haemocytes significantly ($p < 0.05$) instantly in comparison to control (Table 2, Fig. 1). As the exposure duration increased to 30 min, the haemocytes count increased on exposure to 0.8-3.2 mg/L β-cyfluthrin but decreased on treatment with 6.4 and 12.8 mg/L ($p < 0.05$) than recorded in the control. Further, increase in the exposure time by 30 min, the haemocyte count decreased significantly with an exception of 0.8 mg/L (Table 2).

The exposure with 0.8 mg/L β-cyfluthrin reduced the THC in *D. koenigii* nymphs by 3.1-fold at 0 min ($p > 0.05$) while increased it by 1.0-fold and 1.5-fold after 30 and 60 min, respectively. Likewise, at 1.6 mg/L, the THC decreased by 3.4-fold at 0 min ($p < 0.05$) but increased by 1.2-fold after 30 min. However, the haemocyte count decreased again by 2.0-fold after 60 min of exposure (Table 2). As the exposure concentration increased to 3.2 mg/L, the THC decreased by 1.4-fold at 0 min, increased by 1.2-fold after 30 min and again decreased by 2.9-fold at 60 min ($p < 0.05$). Increasing the β-cyfluthrin concentration further, a significantly reduced THC ($p < 0.05$) was observed irrespective of the duration of exposure (Table 2, Fig. 1).

Table 2. Effect of topical application of β -cyfluthrin on the total haemocyte count of the 5th instar nymphs of *Dysdercus koenigii*.

β -cyfluthrin (mg/L)	Total haemocyte count		
	T1	T2	T3
Control	5270 ± 34.20 a	5270 ± 34.20 a	5270 ± 34.20 a
0.8	1675 ± 11.53 b (-3.1)*	5340 ± 24.94 a (+1.0)	7707.5 ± 23.34 b (+1.5)
1.6	1540 ± 23.42 b (-3.4)	6475 ± 41.00 b (+1.2)	2635 ± 2.70 c (-2.0)
3.2	3810 ± 13.37 c (-1.4)	6545 ± 7.14 b (+1.2)	1815 ± 5.32 d (-2.9)
6.4	4295 ± 20.07 d (-1.2)	2250 ± 18.58 c (-2.3)	2075 ± 4.08 e (-2.5)
12.8	3655 ± 2.70 e (-1.4)	3285 ± 10.75 d (-1.6)	2110 ± 4.44 e (-2.5)
r	+0.185**	-0.682	-0.574

Insects treated in 4 replicates; Values are mean ± Standard Error of Mean (SEM) T1: 0 minutes of exposure; T2: 30 minutes of exposure; T3: 60 minutes of exposure; Values in each column followed by different letters are significantly different $p < 0.05$, one-way ANOVA followed by Tukey's all pair wise multiple comparison test.

*Figures in parentheses indicates the fold changes with respect to control ("+" indicates increased fold changes and "-" indicates decreased fold changes). "r" indicates the correlation coefficient; **In r values, "+" sign indicates direct relation between the concentration and total number of haemocytes whereas "-" sign indicate inverse relation between concentration and total number of haemocytes.

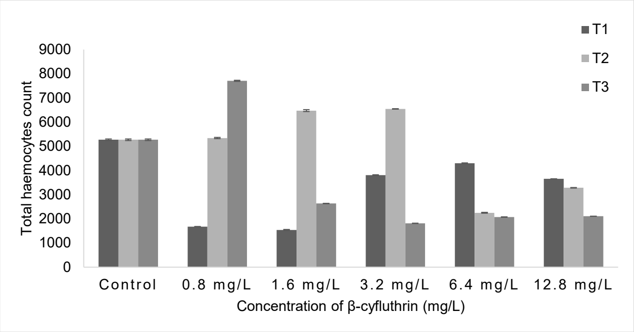

Figure 1. Comparative total haemocyte count of *Dysdercus koenigii* on exposure to various concentrations of β-cyfluthrin under control conditions.
*T1: 0 minutes of exposure; @T2: 30 minutes of exposure; #T3: 60 minutes of exposure

Differential haemocytes count in *Dysdercus koenigii* nymphs

The haemolymph of the red cotton bug recorded five kinds of haemocytes; phagocytic granulocytes (GR – 55%), adhering plasmatocytes (PL – 18%), stem cells - prohaemocytes (PR – 16%), phenoloxidase-containing oenocytes (OE – 11%), and refractile spherulocytes (SP – 0%); in order of their decreasing abundance (Table 3; Fig. 2).

Table 3: Effect of instant topical application of β -cyfluthrin on the differential haemocyte count of the 5th instar nymphs of *D. koenigii*.

β -cyfluthrin (mg/L)	Differential haemocyte count after 0 min				
	GR (%)	PL (%)	PR (%)	OE (%)	SP (%)
Control	55 ± 2.75 a	18 ± 0.90 a	16 ± 0.80 a	11 ± 0.55 a	0 ± 0 a
0.8	37 ± 1.85 b (-1.5)*	16 ± 0.80 b (-1.1)	43 ± 2.15 bc (+2.7)	02 ± 0.10 b (-5.5)	02 ± 0.10 b (0)
1.6	36 ± 1.80 b (3.2)	12 ± 0.60 c (-1.5)	39 ± 1.95 b (+2.4)	13 ± 0.65 c (+1.2)	0 ± 0 a (0)
3.2	45 ± 2.25 c (12.8)	16 ± 0.80 b (-1.1)	26 ± 1.30 d (+1.6)	12 ± 0.60 a (+1.1)	01 ± 0.05 c (0)
6.4	52 ± 2.60 a (-1.1)	15 ± 0.75 b (-1.2)	19 ± 0.95 e (+1.2)	10 ± 0.50 d (-1.1)	04 ± 0.20 d (0)
12.8	30 ± 1.50 d (-1.8)	15 ± 0.75 b (-1.2)	48 ± 2.40 c (+3)	07 ± 0.35 e (-1.6)	0 ± 0 a (0)
r	-0.284**	-0.191	0.395	-0.132	0.038

Insects treated in 4 replicates; Values are mean ± Standard Error of Mean (SEM) GR-granulocyte, PL-plasmatocyte, PR-prohaemocyte, OE-oenocyte, SP-spherulocyte; Values in each column followed by different letters are significantly different $p < 0.05$, one-way ANOVA followed by Tukey's all pairwise multiple comparison test. *Figures in parentheses indicate the fold changes with respect to control ("+" indicates increased fold changes and "-" indicates decreased fold changes). "r" indicates the correlation coefficient; **In r values, "+" sign indicates direct relation between the concentration and differential haemocytes whereas "-" sign indicates inverse relation between concentration and differential haemocytes.

The β-cyfluthrin exposure decreased the granulocyte count significantly at each dose. A reduction of 1.5-1.8-fold was recorded with 12.8 mg/L β-cyfluthrin exposure. A similar trend was recorded in the plasmatocytes count in *D. koenigii* haemolymph. On the other hand, prohaemocytes increased at all β-cyfluthrin dosages with the highest percentage rise with 12.8 mg/L. The oenocytes, nevertheless, decreased drastically with 0.8 mg/L β-cyfluthrin on all the exposure time as compared to control but increased at higher dosages. In contrast, spherulocytes increased with β-cyfluthrin exposure, the maximum increase was recorded at 6.4 mg/L (Table 3, Fig. 2). A similar trend of results was observed after 30 min of exposure with a decrease in granulocytes and plasmatocytes percentage with the increase in the concentration as compared to control (Table 4, Fig. 3). However, a significant decrease in prohaemocyte percentage was observed with a significant increase in higher concentration (12.8 mg/L). At 60 min of exposure, a significant decrease in granulocytes percentage was observed at 1.6 mg/L which further decreased drastically at higher concentration (12.8 mg/L) (Table 5; Fig. 4). Spherulocyte percentage was observed to increase in the nymph at all exposure times (0, 30 and 60 min).

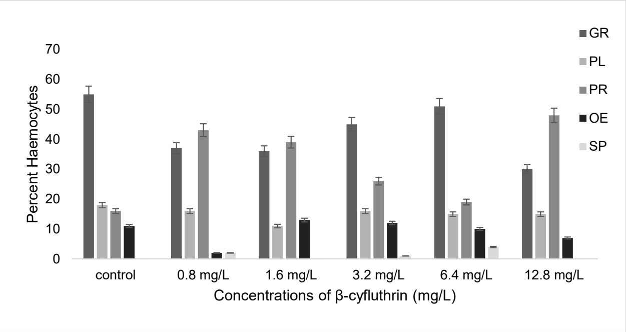

Figure 2. Graph depicting the Differential haemocyte count (DHC) of the *Dysdercus koenigii* on immediate exposure to different concentrations of β-cyfluthrin.

*GR-granulocyte, PL-plasmatocyte, PR-prohaemocyte, OE-oenocyte, SP-spherulocyte

Table 4. Effect of topical application of β -cyfluthrin on the differential haemocyte count of the 5th instar nymphs of *D. koenigii* after 30 minutes.

β -cyfluthrin (mg/L)	Differential haemocyte count after 30 min				
	GR (%)	PL (%)	PR (%)	OE (%)	SP (%)
Control	55 ± 2.75 a	20 ± 1.00 a	15 ± 0.75 a	10 ± 0.50 a	0 ± 0 a
0.8	43 ± 2.15 b (-1.3)*	13 ± 0.65 b (-1.5)	34 ± 1.70 b (+2.3)	08 ± 0.40 b (-0.4)	02 ± 0.10 b (0)
1.6	41 ± 2.05 b (-1.3)	13 ± 0.65 b (-1.5)	34 ± 1.70 b (+2.3)	12 ± 0.60 c (+2.3)	0 ± 0a (0)
3.2	45 ± 2.25 b (-1.2)	19 ± 0.95 a (-1.1)	23 ± 1.15 c (+1.5)	11 ± 0.55cd (+1.5)	02 ± 0.10 b (0)
6.4	45 ± 2.25 b (-1.2)	17 ± 0.85 c (-1.2)	25 ± 1.25 c (+1.7)	10 ± 0.50 d (0)	03 ± 0.15 c (0)
12.8	32 ± 1.60 c (-1.7)	17 ± 0.85 c (-1.2)	39 ± 1.95 d (+2.6)	12 ± 0.60 c (+1.2)	0 ± 0 a (0)
r	0.768**	0.130	0.552	0.493	-0.054

Insects treated in 4 replicates; Values are mean ± Standard Error of Mean (SEM) GR-granulocyte, PL-plasmatocyte, PR-prohaemocyte, OE-oenocyte, SP-spherulocyte; Values in each column followed by different letters are significantly different $p < 0.05$, one-way ANOVA followed by Tukey's all pairwise multiple comparison test. *Figures in parentheses indicate the fold changes with respect to control ("+" indicates increased fold changes and "-" indicates decreased fold changes). "r" indicates the correlation coefficient; **In r values, "+" sign indicates direct relation between the concentration and differential haemocytes whereas "-" sign indicates inverse relation between concentration and differential haemocytes.

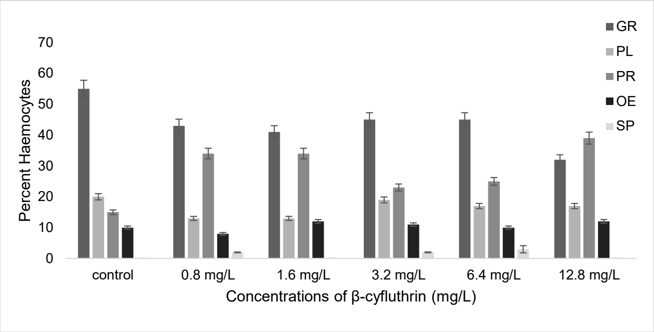

Figure 3. Graph depicting the Differential haemocyte count (DHC) of the *Dysdercus koenigii* on exposure to different concentrations of β-cyfluthrin for 30 minutes.

*GR-granulocyte, PL-plasmatocyte, PR-prohaemocyte, OE-oenocyte, SP-spherulocyte

Table 5. Effect of topical application of β -cyfluthrin on the differential haemocyte count of the 5th instar nymphs of *D. koenigii* after 60 minutes.

β -cyfluthrin (mg/L)	Differential haemocyte count after 30 min				
	GR (%)	PL (%)	PR (%)	OE (%)	SP (%)
Control	55 ± 2.75 a	20 ± 1.00 a	15 ± 0.75 a	10 ± 0.50 a	0 ± 0 a
0.8	43 ± 2.15 b (-1.3)*	13 ± 0.65 b (-1.5)	34 ± 1.70 b (+2.3)	08 ± 0.40 b (-0.4)	02 ± 0.10 b (0)
1.6	41 ± 2.05 b (-1.3)	13 ± 0.65 b (-1.5)	34 ± 1.70 b (+2.3)	12 ± 0.60 c (+2.3)	0 ± 0a (0)
3.2	45 ± 2.25 b (-1.2)	19 ± 0.95 a (-1.1)	23 ± 1.15 c (+1.5)	11 ± 0.55cd (+1.5)	02 ± 0.10 b (0)
6.4	45 ± 2.25 b (-1.2)	17 ± 0.85 c (-1.2)	25 ± 1.25 c (+1.7)	10 ± 0.50 d (0)	03 ± 0.15 c (0)
12.8	32 ± 1.60 c (-1.7)	17 ± 0.85 c (-1.2)	39 ± 1.95 d (+2.6)	12 ± 0.60 c (+1.2)	0 ± 0 a (0)
r	0.768**	0.130	0.552	0.493	-0.054

Insects treated in 4 replicates; Values are mean ± Standard Error of Mean (SEM) GR-granulocyte, PL-plasmatocyte, PR-prohaemocyte, OE-oenocyte, SP-spherulocyte; Values in each column followed by different letters are significantly different $p < 0.05$, one-way ANOVA followed by Tukey's all pairwise multiple comparison test. *Figures in parentheses indicate the fold changes with respect to control ("+" indicates increased fold changes and "-" indicates decreased fold changes). "r" indicates the correlation coefficient; **In r values, "+" sign indicates direct relation between the concentration and differential haemocytes whereas "-" sign indicates inverse relation between concentration and differential haemocytes.

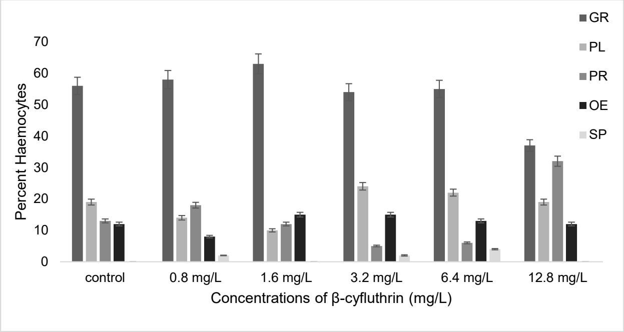

Figure 4. Graph depicting the Differential haemocyte count (DHC) of the *Dysdercus koenigii* on exposure to different concentrations of β-cyfluthrin for 60 minutes.

*GR-granulocyte, PL-plasmatocyte, PR-prohaemocyte, OE-oenocyte, SP-spherulocyte

DISCUSSION

Red cotton bug, *D. koenigii* which is one of the major insect pests of various crops, especially cotton, has caused immense crop yield reduction qualitatively as well as quantitatively. Despite the use of various intervention measures, synthetic pyrethroids are the major control agents for these pests on account of their efficacy and comparative safety to other conventional insecticides. Among various pyrethroids, β-cyfluthrin displays promising effects against various insect pests. Our earlier study has shown the control efficacy of β-cyfluthrin against *D. koenigii* (Lanbiliu et al, 2020). However, continued and persistent use of these insecticides can result in development of resistance in insects against these toxicants. Since, haemocytes perform various vital physiological functions of the body and play a significant role in the cellular defense, the current study evaluated the effect of β-cyfluthrin on the insect's haemocyte count to understand their involvement in immunity of insect (Cho & Cho, 2019).

The current study revealed a total of 5270 haemocytes/mm^3 in the fifth instar nymphs of an Indian strain of *D. koenigii.* In comparison, reports have demonstrated a much higher count of 8450 cell/mm^3 (Sarwar et al, 2018) and 17000 cell/mm^3 (Rizwan-ul-Haq et al, 2005) in the adults of two Pakistan Strains of *D. koenigii*. A much higher THC of 11623.67 cell/mm^3 has also been reported by Kumar, Kumari, & Verma (2019) in red cotton bug. The topical treatment of *D. koenigii* fifth instar nymphs with β-cyfluthrin decreased the total haemocyte count instantly. However, increase in the treatment duration to 30 min and 60 min; and increasing dosages of β-cyfluthrin, the pattern of total haemocyte count fluctuated considerably. At a lower dosage (0.8 mg/L) of β-cyfluthrin, the THC which decreased instantly by 3.1-fold increased gradually after 30 and 60 min of exposure. In contrast, the exposure concentration of 1.6 and 3.2 mg/L β-cyfluthrin decreased THC in *D. koenigii* by 1.4-3.4-fold (1540 cell/mm^3, 3810 cell/mm^3) at 0 min, subsequently increased by 1.2-fold (6475 cell/mm^3, 6545 cell/mm^3) after 30 min and again decreased by 2.0-2.9-fold (2635 cell/mm^3, 1815 cell/mm^3) after 60 min. These results indicate the latent effects of the higher dosages of β-cyfluthrin which though initially were less effective in comparison to the lower dosages but imparted much more effects as the treatment duration increased.

The continued decrease in the haemocyte count in *D. koenigii* after the application of 6.4 and 12.8 mg/L β-cyfluthrin; 4295 cell/mm^3, 3655 cell/mm^3, respectively at 0 min (2250 cell/mm^3, 3285 cell/mm^3) after 30 min and (2075 cell/mm^3 and 2110 cell/mm^3) 60 min was alike to the pattern observed with the treatment of *D. koenigii* with imidacloprid 20SL (Sarwar et al, 2018). Comparable results have been observed in *D. koenigii* adults on exposure to another pyrethroid, deltamethrin @250 mL/acre (Sarwar et al, 2018). They recorded a significant reduction in THC by 32.8% at 0 min followed by a gradual increase in the count by 3.3%, and 8.9% after 30 and 60 min of deltamethrin treatment. The contrary counts have been recorded by Rizwan-ul-Haq et al (2005) on the application of imidacloprid 25 WP to adult *D. koenigii* demonstrating an initial decrease in THC which continued to decrease till 30 min but then increased after 60 min of exposure. Nevertheless, treatment with another neonicotinoid, acetamiprid

20% SL, increased the total haemocyte count immediately, while decreased after 30 min and increased again after one hour.

Several other reports in other insects have revealed different patterns in total haemocyte count on treatment with xenobiotics. Kumar et al (2019) showed decreased haemocyte count in *D. koenigii* after treatment with *Aspergillus niger* while Fatima et al (2016) noticed increased total haemocytes count (20650 cell/mm^3, 10222 cell/mm^3) just after the application of thiacloprid and imidacloprid in 5th instar larvae of *Helicoverpa armigera*. The application of carbamates and pyrethroids on *Tryporyza* sp. and *Schistocera gregaria* Forsk induced an immediate rise in the total haemocyte count (Alhariri & Suhail, 2001).

It has been reported that the number of haemocytes in insects fluctuates depending upon their immunity level and thus indicates the response to the external insecticidal stress (Perveen & Ahmad, 2017). An instant decline in the haemocyte count of *D. koenigii* on β-cyfluthrin treatment suggest the decreased defensive action of haemocytes due to stress induced by β-cyfluthrin. However, as the exposure time increased, the haemocytes multiplied gradually to combat the induced stress. It is proposed that the higher dosages of β-cyfluthrin could not raise the haemocyte count considerably probably because of the toxic effects leading to the higher nymphal mortality. The reports have revealed that haemocyte count in insects responds to different insecticides variably leading to enhanced immunity; since they differ in their mitotic division under normal conditions (Perveen & Ahmad, 2017).

The investigations on the differential haemocytes count in *D. koenigii* showed five types of haemocytes; phagocytic granulocytes - GR (55%), adhering plasmatocytes - PL (18%), prohaemocytes - PR (16%), phenoloxidase-containing oenocytes - OE (11%), and refractile spherulocytes – SP (0%); in order of their decreasing abundance. Similar proportion of haemocytes were observed by Mannakkara (2022), in rice brown planthopper (*Nilaparvata lugens*) development stages revealing PLs, GRs and PRs as the most abundant cells in the haemolymph while SPs being the scarcest. In comparison, Rizwan-ul-Haq et al (2005) reported plasmatocytes as the most abundant cells (39.75%) in adult *D. koenigii* followed by 32% GR, 22% PR, 4.25% OE and 2% SP.

Current studies showed differential effects on different haemocytes of *D. koenigii* on exposure to β-cyfluthrin which increased the prohaemocytes% while reduced GR% and PL% at higher dosages. On the other hand, the impact of β-cyfluthrin on SP and OE was inconsistent at different concentrations. Comparable results were reported in *D. koenigii* 5th nymphal instar on exposure to juvenile-hormone analogue farnesol (Kumar, Shazad, Kayesth & Gupta, 2022). They observed an increase in percent of prohaemocytes in farnesol-treated nymphs at the dosages ranging from 0.05 μg/μL to 0.2 μg/μL.

Alike to our results, Sarwar et al (2018) demonstrated reduced count of granulocytes in adult *D. koenigii* after the application of three pyrethroids; deltamethrin, lambda-cyhalothrin and cyfluthrin 20EC. Likewise, imidacloprid 25 WP exposure alleviated plasmatocytes, granulocytes, oenocytes, spherulocytes to 28.25%, 24.50%, 2.25%, and 1.25%, respectively from 39.75%, 32%, 4.25% and 2%; while increased prohaemocyte from 22% to 32.75% in adult *D. koenigii* (Rizwan-ul-Haq et al, 2005). In contrast, the acetamiprid 20% SL exposure reduced the PL (35%), GR (23%) and PR (18.25%); while rest two haemocytes, OE and SP rose to 7.5% and 3.5%, respectively (Rizwan-ul-Haq et al, 2005), alike to the results when *D. koenigii* was exposed to 12.8 mg/L β-cyfluthrin which reduced the GR (30%) and PL (15%) while increased the PR (48%) counts.

A rapid decline in all cell types with total elimination of prohaemocytes while the continuous reduction in granulocytes and plasmatocytes was reported in *D. koenigii* after topical application of plumbagin (Saxena & Tikku, 1990). Comparable results were demonstrated by Kumar et al (2019) in *D. koenigii* on exposure to *A. niger* reporting granulocytes and plasmatocytes as the most negatively affected cells with a drastic reduction in comparison to control. Teleb (2011) reported an increased percentage of prohaemocytes, oenocytes, plasmatocytes and granulocytes in *S. gregaria* on the application of Nomolt® (Teflubenzuron), while the reduced number of spherulocytes.

Plasmatocytes and granulocytes are considered the main haemocytes in cell-mediated immunity being an active participants in the recognition of foreign agents while the rest of the haemocyte types interact with them and contribute to the immune response (Kwon, Bang, & Cho, 2014). Major immune functions in Lepidoptera and some Coleoptera are also imparted by granulocytes and plasmatocytes by encapsulation and phagocytosis of xenobiotic agents (Manachini, Arizza, Parrinello, & Parrinello, 2011; Lavine & Strand, 2002). The fluctuating number of the haemocytes indicates the immunity response in *D. koenigii* on exposure to β-cyfluthrin. The decrease in granulocyte count in *D. koenigii* indicates the probable active participation of the primary haemocytes in countering the action of xenobiotic. On the other hand, an increase in prohaemocyte due to β-cyfluthrin exposure may be attributed to their stem cell property leading to active mitotic divisions as an immune response caused by xenobiotic stress. Further investigations may help in deciphering the mechanisms associated with the impact of β-cyfluthrin on the haemocytes of *D. koenigii* which may assist in the formulation of strategies to optimize the dosage of toxicant with increased efficacy in the fields.

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CONFLICT OF INTEREST

We declare that there is no conflict of interest.

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Contributions to the Dolichopodidae (Insecta: Diptera) Fauna of Muğla Provinces with the Six New Records for Turkey

Alper TONGUÇ[1*] Ömer YÜZER[2]

[1*]Molecular Biology and Genetics Department, Faculty of Science, Muğla Sıtkı Kocman University, Muğla, TURKEY
[2]Doğuş University, Vocational School, Medical Laboratory Techniques, Ümraniye, İstanbul; TURKEY
e-mails: [1*]alpertonguc@mu.edu.tr, [2]oyuzer@dogus.edu.tr
ORCID IDs: [1*]0000-0002-2613-3114, [2]0000-0003-0209-324X
*Corresponding author

ABSTRACT

In this study, we report the Turkey fauna of long-legged flies and new records of six species (Diptera: Dolichopodidae) collected from Muğla province during the 2018-2019 field trips. These newly recorded species are *Acropsilus niger* (Loew, 1869), *Hercostomus kravchenkoi* (Grichanov and Freidberg, 2018), *Sybistroma leptocerca* (Stackelberg, 1949), *Oncopygius magnificus* (Loew, 1873), *Medetera glaucella* (Kowarz, 1877), and *Syntormon submonilis* (Negrobov, 1975). In total, 107 species from 10 subfamilies and 35 genera of Dolichopodidae are known from Muğla. With these records, the number of the Turkey Dolichopodidae species has reached 224.

Keywords: Dolichopodidae, new records, fauna, Muğla.

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INTRODUCTION

Dolichopodidae, or long-legged flies, are one of the biggest families in Diptera. Their body length changes between 0,8-9 mm with a slender build. The species of the family are diagnosed with wing venation, metallic colorations, especially blue-green with bronze reflections, sexual characters, and antenna shapes. The adults are found in aquatic and semiaquatic areas, grasslands, marshlands, coastal dunes, woodlands and heathland. Some adult Dolichopodidae species feed on agricultural pests and play an important role in ecosystems (Grichanov & Brooks, 2017).

Muğla is located in the Aegean Region of Turkey, where the Aegean Sea meets the Mediterranean Sea, and it contains 13 districts, some of which include popular touristic areas. Muğla also contains a wide variety of ecosystems. It has very abundant plants, especially endemic ones (Yeşilyurt & Akaydın, 2012). Some authors have conducted comprehensive investigations of the Dolichopodidae fauna of Muğla until recently. Up to now, 86 species have been identified in the province (Grichanov, Tonguç, Civelek, Vikhrev, Özgül, & Dursun, 2007; Naglis, Tonguç, & Barták, 2021).

The aim of this paper is to contribute to the distribution of long legged flies in the Muğla province of Turkey.

MATERIAL METHOD

1166 adult dolichopodid specimens (730 males, 436 females) were collected using a net and aspirator in 13 districts of Muğla province (Menteşe, Ula, Köyceğiz, Ortaca, Marmaris, Datça, Dalaman, Fethiye, Seydikemer, Yatağan, Kavaklıdere, Milas, Bodrum) between May-September 2018 and May-August 2019. The collected specimens were killed in jars containing ethyl-acetate. They were preserved in insect envelopes or jars with %70 ethyl alcohol in the survey area. Some of the Dolichopodidae specimens brought to the laboratory were pinned, whereas the others were protected in different sizes of glass or plastic jars. All of the specimens have been stored in insect collection boxes that are in Muğla Sıtkı Koçman University, Faculty of Science, Department of Molecular Biology and Genetics Laboratory of Molecular Ecology. In the results section, a faunistic list and new records of species distribution belonging to the Palaearctic and Turkey distribution, is given for Turkey and Muğla.

RESULTS

Diaphorinae

Acropsilus niger (Loew, 1869)

Material examined: Muğla, Köyceğiz, Kaptan choltry, 36° 59' N / 28° 38' E, 14 m., 10.08.2018, 1 ♂.

Palaearctic distribution: Bulgaria, Romania, Russia, Europe, Algeria, Tunisia (Grichanov, 2007).

Turkey distribution: New record for Turkey

Chrysotus cilipes Meigen, 1824

Material examined: Muğla, Kavaklıdere, 37° 27' N / 28° 20' E, 711 m, 08.07.2019, 1 ♂.

Palaearctic distribution: Abkhazia, Armenia, Austria, Azerbaijan, Belgium, Czech Republic, Denmark, England, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Lithuania, Luxembourg, Madeira, Netherlands, Norway, Poland, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Russia, Turkey, Ukraine (Yang et al, 2006; Grichanov, 2007).

Turkey distribution: Artvin, Rize (Tonguç, Grichanov, & Naglis, 2016).

Chrysotus femoratus Zetterstedt, 1843

Material examined: Muğla, Kavaklıdere, Dokuzçam village 37° 24' N / 28° 29' E, 643 m, 08.07.2019, 1 ♂.

Palaearctic distribution: Austria, Belgium, Czech Republic, Denmark, England, Estonia, Finland, France, Georgia, Germany, Greece, Hungary, Ireland, Italy, Latvia, Moldova, Netherlands, Norway, Poland, Romania, Slovakia, Russia, Sweden, Switzerland, Turkey, Ukraine (Yang, Zhu, Wang, & Zhang, 2006, Grichanov, 2007).

Turkey distribution: Erzurum, Hakkari (Tonguç et al, 2016).

Chrysotus laesus (Wiedemann, 1817)

Material examined: Muğla, Dalaman, Akkaya, Gizli valley 36° 50' N / 28° 49' E, 207 m, 13.05.2019, 1 ♂.

Palaearctic distribution: Armenia, Austria, Belarus, Belgium, Bulgaria, Czech Republic, Denmark, England, Estonia, Finland, France, Georgia, Germany, Hungary, Italy, Kazakhstan, Moldova, Netherlands, Norway, Poland, Romania, Russia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey, Ukraine (Yang, Zhu, Wang, & Zhang, 2006; Grichanov, 2007).

Turkey distribution: Kars (Tonguç et al, 2016).

Dolichopodinae

Gymnopternus celer (Meigen, 1824)

Material examined: Muğla, Marmaris, Günlük National Park, 36° 50' N / 28° 17' E, 17 m, 06.07.2018, 41 ♂♂, 33 ♀♀; Köyceğiz, Kaptan choltry 36° 59' N / 28° 38' E, 103 m, 09.07.2019, 6 ♂♂, 1 ♀.

Palaearctic distribution: Austria, Belgium, Bosnia and Herzeginova, Bulgaria, Crotia, Czech Republic, Denmark, England, Estonia, Finland, France, Germany, Hungary, Italy, Ireland, Kazakhstan, Luxembourg, Macedonia, Moldova, Netherlands, Norway, Poland, Romania, Russia, Slovakia, Slovenia, Sweden, Switzerland, Turkey, Ukraine (Yang et al, 2006; Grichanov, 2007).

Turkey distribution: Çanakkale (Tonguç et al, 2016).

Hercostomus fulvicaudis (Walker, 1849)

Material examined: Muğla, Seydikemer, Tylos, Gizli valley Restaurant 36° 33' N / 29° 26' E, 651 m, 12.05.2019, 1 ♂, 3 ♀♀.

Palaearctic distribution: Austria, Belgium, Czech Republic, England, France, Germany, Holland, Sweden, Poland, Romania, Slovakia, Turkey, Ukraine, Turkmenistan, Tajikistan (Yang et al, 2006; Grichanov, 2007).

Turkey distribution: Afyonkarahisar, Bursa, Kütahya (Tonguç, 2020).

Hercostomus gracilis (Stannius, 1831)

Material examined: Muğla, Fethiye, Çenger village, 36° 46' N / 29° 08' E, 560 m, 08.08.2018, 2 ♂♂, 1 ♀; Kavaklıdere, 37° 27' N / 28° 20' E, 711 m, 08.07.2019, 1 ♂; Kavaklıdere, Dokuzçam village 37° 24' N / 28° 29' E, 643 m, 08.07.2019, 1 ♂.

Palaearctic distribution: Armenia, Austria, Belgium, Bulgaria, Czech Republic, Denmark, England, France, Germany, Greece, Holland, Hungary, Italy, Poland, Russia, Spain, Sweden, Tajikistan, Turkmenistan Turkey, Ukraine (Yang et al, 2006; Grichanov, 2007).

Turkey distribution: Afyonkarahisar, Aydın, Balıkesir, Burdur, Bursa, Kütahya, Uşak (Tonguç, 2020).

Hercostomus kravchenkoi (Grichanov and Freidberg, 2018)

Material examined: Muğla, Fethiye, Çenger village, 36° 44' N / 29° 05' E, 160 m, 08.08.2018, 2 ♂♂.

Palaearctic distribution: Israel (Grichanov & Freidberg, 2018)

Turkey distribution: New record for Turkey

Hercostomus stroblianus Becker, 1917

Material examined: Muğla, Köyceğiz, Ağla road 6-7. km, 37° 01' N / 28° 44' E, 42 m, 10.08.2018, 1 ♂, 3 ♀♀.

Palaearctic distribution: Georgia, Hungary, Romania, Russia, Turkey (Grichanov, 2007).

Turkey distribution: Kırklareli (Tonguç, 2020).

Sybistroma leptocerca (Stackelberg, 1949)

Material examined: Muğla, Köyceğiz, Ağla road 6-7. Km, 37° 01' N / 28° 44' E, 42 m, 10.08.2018, 1 ♂; Seydikemer, Dont village, 36° 51' N / 29° 37' E, 1236 m, 28.08.2019, 1 ♂, 1 ♀; Kavaklıdere, Dokuzçam village, 37° 24' N / 28° 29' E, 697 m, 05.07.2018, 1 ♂.

Palaearctic distribution: Iran, Tajikistan (Grichanov & Kazerani, 2014)

Turkey distribution: New record for Turkey

Sybistroma sphenopterus (Loew, 1859)

Material examined: Muğla, Menteşe, Yılanlı, Eşkiyalar, 37° 15' N / 28° 31' E, 1066 m, 08.07.2019, 1 ♂, 1 ♀.

Palaearctic distribution: Austria, Czech Republic, Germany, Hungary, Italy, Poland, Romania, Turkey (Yang et al, 2006; Tonguç et al, 2016).

Turkey distribution: Bursa, Çanakkale, Kars, Kırklareli, Kocaeli, Kütahya (Tonguç, 2020).

Medeterinae

Medetera glaucella Kowarz, 1877

Material examined: Muğla, Seydikemer, Tylos, Gizli valley Restaurant, 36° 33' N / 29° 26' E, 651 m, 12.05.2019, 1 ♂, 2 ♀♀; Seydikemer, Kavaklı, 36° 41' N / 29° 25' E, 545 m, 12.05.2019, 1 ♂, 1 ♀.

Palaearctic distribution: Austria, Bulgaria, Czech Republic, France, Germany, Netherlands, Poland, Romania, Slovakia, Sweden (Yang et al, 2006).

Turkey distribution: New record for Turkey

Neurigoninae

Oncopygius magnificus Loew, 1873

Material examined: Muğla, Köyceğiz, Ağla, 37° 01' N / 28° 44' E, 665 m, 13.05.2019, 4 ♂♂, 2 ♀♀.

Palaearctic distribution: Albania, Austria, Greece, Hungary, Italy, Romania, Slovenia (Yang et al, 2006).

Turkey distribution: New record for Turkey

Peloropeodinae

Chrysotimus molliculus (Fallen, 1823)

Material examined: Muğla, Kavaklıdere, Dokuzçam, 37° 24' N / 28° 29' E, 697 m, 05.07.2018, 7 ♂♂, 2 ♀♀; Köyceğiz, Ağla, 37° 01' N / 28° 45' E, 794 m, 10.08.2018, 1 ♂; Yuvarlak stream, Defne restaurant, 36° 56' N / 28° 48' E, 214 m, 10.08.2018, 1 ♀; Ula, Ula pond, Yaraş turnout, 37° 09' N / 28° 26' E, 807 m, 10.07.2019, 2 ♀♀.

Palaearctic distribution: Austria, Belgium, Czech Republic, Denmark, England, France, Germany, Hungary, Ireland, Netherlands, Norway, Poland, Romania, Russia, Slovenia, Sweden, Switzerland, Turkey, Ukraine (Yang et al, 2006).

Turkey distribution: Kırklareli (Tonguç, 2020).

Peloropeodes acuticornis (Oldenberg, 1916)

Material examined: Muğla, Köyceğiz, Ağla road 6-7. km, 37° 01' N / 28° 44' E, 42 m, 10.08.2018, 1 ♂; Köyceğiz, Ağla village, 37° 01' N / 28° 45' E, 796 m, 09.07.2019, 2 ♂♂; Köyceğiz, Ağla, 37° 01' N / 28° 44' E, 595 m, 09.07.2019, 3 ♂♂; Marmaris, Marmaris National Park, 36° 50' N / 28° 17' E, 1 m, 20.05.2019, 7 ♂♂, 7 ♀♀; Marmaris-Datça road, 36° 45' N / 27° 51' E, 56 m., 20.05.2019, 10 ♂♂, 5 ♀♀; Kavaklıdere, Dokuzçam village, 37° 24' N / 28° 29' E, 643 m, 08.07.2019, 1 ♂.

Palaearctic distribution: France, Greece, Romania, Russia, Turkey (Grichanov, 2007).

Turkey distribution: Kütahya, Muğla, Samsun (Naglis et al, 2021).

Sciapodinae

Sciapus maurus Parent, 1930

Material examined: Muğla, Fethiye, Çenger village, 36° 44' N / 29° 05' E, 160 m, 08.08.2018, 1 ♂, 2 ♀♀; Çenger village, 36° 43' N / 29° 05' E, 378 m, 08.08.2018, 8 ♂♂, 5 ♀♀.

Palaearctic distribution: Algeria, Belgium, Israel, Turkey (Grichanov, 2007).

Turkey distribution: Antalya, Uşak (Tonguç & Barlas, 2020).

Sympycninae

Syntormon submonilis Negrobov, 1975

Material examined: Muğla, Fethiye, Çengelköy, 36° 44' N / 29° 05' E, 107 m, 11.05.2019, 1 ♂, 1 ♀.

Palaearctic distribution: Russia (Grichanov, 2007).

Turkey distribution: New record for Turkey

Teuchophorus bisetus Loew, 1871

Material examined: Muğla, Köyceğiz, Kaptan choltry, 36° 59' N / 28° 38' E, 14 m, 10.08.2018, 8 ♂♂, 4 ♀♀; 09.07.2019, 3 ♂♂, 2 ♀♀; Marmaris, Günlük National Park, 36° 50' N / 28° 17' E, 17 m, 06.07.2018, 11 ♂♂, 9 ♀♀.

Palaearctic distribution: Israel, Iraq, Tajikistan, Turkey, Uzbekistan (Grichanov, 2007).

Turkey distribution: Afyonkarahisar, Aydın (Naglis et al, 2021).

Teuchophorus chaetifemoratus Pollet & Kechev, 2007

Material examined: Muğla, Menteşe, Eşkiyalar, 37° 15' N / 28° 31' E, 1058 m, 05.07.2018, 1 ♂, 1 ♀; Fethiye, Çenger village, 36° 44' N / 29° 05' E, 160 m, 08.08.2018, 1 ♂, 3 ♀♀; Köyceğiz, Ağla, 37° 01' N / 28° 45' E, 794 m, 10.08.2018, 1 ♂, 1 ♀, Ortaca, Günlükbaşı, 36° 52' N / 28° 44' E, 45 m, 22.05.2018, 1 ♂, 2 ♀♀; Fethiye, Faralya, 36° 29' N / 29° 08' E, 316 m, 27.08.2019, 1 ♂; Kavaklıdere, 37° 27' N / 28° 20' E, 711 m., 08.07.2019, 6 ♂, 3 ♀♀; Marmaris, 36° 53' N / 28° 16' E, 213 m., 06.07.2020, 11 ♂♂, 11 ♀♀; Marmaris-Datça road, 36° 45' N / 27° 51' E, 56 m, 20.05.2019, 9 ♂♂, 10 ♀♀, Menteşe, Akyer, 37° 18' N / 28° 30' E, 1101 m, 05.07.2018, 1 ♂.

Palaearctic distribution: Bulgaria, Turkey (Tonguç et al, 2016)

Turkey distribution: Kütahya, Uşak (Tonguç et al, 2016).

Teuchophorus simplex Mik, 1880

Material examined: Muğla, Yatağan, Çamlıca, 37° 13' N / 27° 59' E, 647 m, 04.07.2018, 1 ♂.

Palaearctic distribution: Austria, Belgium, Bulgaria, Czech Republic, England, France, Germany, Greece, Hungary, Netherlands, Poland, Sweden, Turkey (Yang et al, 2006; Grichanov, 2007).

Turkey distribution: Kırklareli (Tonguç, 2020).

50 species, 22 genera, and 9 subfamilies [Diaphorinae (5 species), Dolichopodinae (20 species), Hydrophorinae (2 species), Medeterinae (1 species), Neurigoninae (1 species), Peloropeodinae (2 species), Rhaphiinae (2 species), Sciapodinae (4 species), Sympycninae (13 species)] were determined as a result of identification of collected specimens from the research area.

6 species, namely *Acropsilus niger* (Loew, 1869), *Hercostomus kravchenkoi* (Grichanov & Freidberg, 2018), *Sybistroma leptocerca* (Stackelberg, 1949), *Oncopygius magnificus* (Loew, 1873), *Medetera glaucella* (Kowarz, 1877), and *Syntormon submonilis* (Negrobov, 1975) are new records for Turkey. It is worth noting that *Hercostomus kravchenkoi* and *Syntormon submonilis* Negrobov, 1975 were collected for the first time after their identification. On the other hand, 14 species are new records for Muğla. A total of 86 species were recorded in the previous studies from Muğla. With these new records, the fauna of Muğla has increased to 107 species [Achalcinae (1 species), Diaphorinae (15 species), Dolichopodinae (32 species), Hydrophorinae (8 species), Medeterinae (8 species), Neurigoninae (1 species), Peloropeodinae (4 species), Rhaphiinae (4 species), Sciapodinae (8 species), Sympycninae (26 species) (Table 1)].

As a consequence of this study, the number of the Turkey Dolichopodidae species has reached 224.

Table 1. The number of already-known and newly-added species from Muğla provinces.

Subfamily	Species	Faunistic Notes
Achalcinae	*Achalcus flavicollis* (Meigen, 1824)	Naglis et al, 2021
Diaphorinae	*Acropsilus niger* (Loew, 1869)	New record for Turkey
	Argyra argyria (Meigen, 1824)	Naglis et al, 2021
	Argyra leucocephala (Meigen, 1824)	Naglis et al, 2021
	Argyra vestita (Wiedemann, 1817)	Naglis et al, 2021
	Asyndetus latifrons (Loew, 1857)	Tonguç et al, 2016
	Asyndetus separatus (Becker, 1902)	Naglis et al, 2021
	Chrysotus cilipes Meigen, 1824	New record for Muğla
	Chrysotus larachensis Grichanov, Nourti & Kettani, 2020	Naglis et al, 2021
	Chrysotus dorli Negrobov, 1980	Naglis et al, 2021
	Chrysotus femoratus Zetterstedt, 1843	New record for Muğla
	Chrysotus laesus (Wiedemann, 1817)	New record for Muğla
	Chrysotus suavis (Loew, 1857)	Naglis et al, 2021
	Diaphorus varifrons Becker, 1918	Grichanov et al, 2007
	Diaphorus winthemi Meigen, 1824	Naglis et al, 2021
	Trigonocera rivosa Becker, 1902	Naglis et al, 2021

Table 1. Continued.

Subfamily	**Species**	**Faunistic Notes**
Dolichopodinae	*Dolichopus cilifemoratus* Macquart, 1827	Tonguç et al, 2016
	Dolichopus diadema Haliday, 1832	Tonguç et al, 2016
	Dolichopus excisus Loew, 1859	Tonguç et al, 2016
	Dolichopus griseipennis Stannius, 1831	Grichanov et al, 2007
	Dolichopus latilimbatus Macquart, 1827	Naglis et al, 2021
	Dolichopus sabinus Haliday, 1838	Naglis et al, 2021
	Dolichopus signifer Haliday, 1832	Tonguç et al, 2013
	Dolichopus strigipes Verall, 1875	Naglis et al, 2021
	Gymnopternus blankaartensis (Pollet, 1990)	Naglis et al, 2021
	Gymnopternus celer (Meigen, 1824)	New record for Muğla
	Hercostomus apollo (Loew, 1869)	Tonguç et al, 2013
	Hercostomus chetifer (Walker, 1849)	Grichanov et al, 2007
	Hercostomus convergens (Loew, 1857)	Tonguç et al, 2016
	Hercostomus fulvicaudis (Walker, 1849)	New record for Muğla
	Hercostomus gracilis (Stannius, 1831)	New record for Muğla
	Hercostomus kravchenkoi (Grichanov & Freidberg, 2018)	New record for Turkey
	Hercostomus longiventris (Loew, 1857)	Grichanov et al, 2007
	Hercostomus nanus (Macquart, 1827)	Grichanov et al, 2007
	Hercostomus phoebus Parent, 1927	Grichanov et al, 2007
	Hercostomus stroblianus Becker, 1917	New record for Muğla
	Hercostomus thraciensis Kechev & Negrobov, 2015	Naglis et al, 2021
	Hercostomus tongucii Naglis & Negrobov, 2017	Naglis & Negrobov, 2017
	Ortochile nigrocoerulea Latreille, 1809	Naglis et al, 2021
	Poecilobothrus principalis (Loew, 1861)	Naglis et al, 2021
	Poecilobothrus regalis (Meigen, 1824)	Grichanov et al, 2007
	Sybistroma impar (Rondani, 1843)	Tonguç et al, 2013
	Sybistroma leptocerca (Stackelberg, 1949)	New record for Turkey
	Sybistroma lorifer (Mik, 1878)	Naglis et al, 2021
	Sybistroma nodicornis Meigen, 1824	Naglis et al, 2021
	Sybistroma sphenopterus (Loew, 1859)	New record for Muğla
	Tachytrechus beckeri Lichtwardt, 1917	Grichanov et al, 2007
	Tachytrechus notatus (Stannius, 1831)	Grichanov et al, 2007
	Tachytrechus tessallatus (Macquart, 1842)	Naglis et al, 2021

Table 1. Continued.

Subfamily	**Species**	**Faunistic Notes**
Hydrophorinae	*Aphrosylus venator* Loew, 1857	Naglis et al, 2021
	Hydrophorus balticus (Meigen, 1824)	Grichanov et al, 2007
	Hydrophorus praecox (Lehmann, 1822)	Naglis et al, 2021
	Liancalus virens (Scopoli, 1763)	Grichanov et al, 2007
	Orthoceratium lacustre (Scopoli, 1763)	New record for Muğla
	Orthoceratium sabulosum (Becker, 1907)	Naglis et al, 2021
	Thinophilus flavipalpis (Zetterstedt, 1843)	Naglis et al, 2021
	Thinophilus quadrimaculatus Becker, 1902	Naglis et al, 2021
Medeterinae	*Cyrturella albosetosa* (Strobl, 1909)	Naglis et al, 2021
	Medetera flavipes Meigen, 1824	Grichanov et al, 2007
	Medetera glaucella Kowarz, 1877	New record for Turkey
	Medetera pallipes (Zetterstedt, 1843)	Naglis et al, 2021
	Medetera petrophiloides Parent, 1925	Naglis et al, 2021
	Medetera signaticornis Loew, 1957	Naglis et al, 2021
	Medetera truncorum Meigen, 1824	Grichanov et al, 2007
	Thrypticus bellus Loew, 1869	Grichanov et al, 2007
Neurigoninae	*Oncopygius magnificus* Loew, 1873	New record for Turkey
Peloropodinae	*Chrysotimus molliculus* (Fallen, 1823)	New record for Muğla
	Micromorphus albipes (Zetterstedt, 1843)	Tonguç et al, 2016
	Peloropeodes acuticornis (Oldenberg, 1916*)*	Naglis et al, 2021
	Vetimicrotes mediterraneus (Becker, 1918	Naglis et al, 2021
Rahphiinae	*Rahphium appendiculatum* Zetterstedt, 1849	Naglis et al, 2021
	Rahphium brevicorne Curtis, 1835	Naglis et al, 2021
	Rahphium fascipes (Meigen, 1824)	Naglis et al, 2021
	Rhaphium micans (Meigen, 1824)	Tonguç et al, 2016
Sciapodinae	*Sciapus aberrans* Becker, 1918	Naglis et al, 2021
	Sciapus adana Grichanov & Negrobov, 2014	Naglis et al, 2021
	Sciapus euchromus (Loew, 1857)	Naglis et al, 2021
	Sciapus flavicinctus (Loew, 1857)	Naglis et al, 2021
	Sciapus heteropygus Parent, 1926	Tonguç et al, 2016
	Sciapus holoxanthos Parent, 1926	Grichanov et al, 2007
	Sciapus maurus Parent, 1930	New record for Muğla
	Sciapus tenuinervis (Loew, 1857)	Naglis et al, 2021

Table 1. Continued.

Subfamily	Species	Faunistic Notes
Sympycninae	*Campsicnemus crinitarsis* Strobl, 1906	Naglis et al, 2021
	Campsicnemus curvipes (Fallén, 1823)	Naglis et al, 2021
	Campsicnemus magius (Loew, 1845)	Naglis et al, 2021
	Campsicnemus simplicissimus Strobl, 1906	Naglis et al, 2021
	Campsicnemus umbripennis Loew, 1856	Grichanov et al, 2007
	Lamprochromus bifasciatus (Macquart, 1827)	Naglis et al, 2021
	Lamprochromus kowarzi Negrobov &Tshalaja, 1988	Naglis et al, 2021
	Lamprochromus speciosus (Loew, 1871)	Tonguç et al, 2016
	Sympycnus annulipes (Meigen, 1824)	Tonguç et al, 2016
	Sympycnus simplicipes Becker, 1908	Naglis et al, 2021
	Sympycnus pulicarius (Fallén, 1823)	Grichanov et al, 2007
	Syntormon denticulatum Zetterstedt, 1843	Grichanov et al, 2007
	Syntormon metathesis (Loew, 1850)	Naglis et al, 2021
	Syntormon mikii Strobl, 1899	Naglis et al, 2021
	Syntormon pallipes (Fabricius, 1794)	Grichanov et al, 2007
	Syntormon submonilis Negrobov, 1975	New record for Turkey
	Syntormon triangulipes Becker, 1902	Naglis et al, 2021
	Teuchophorus bisetus Loew, 1871	Naglis et al, 2021
	Teuchophorus calcaratus (Macquart, 1828)	Naglis et al, 2021
	Teuchophorus chaetifemoratus Pollet & Kechev, 2007	New record for Muğla
	Teuchophorus cristulatus Meuffels & Grootaert, 1992	Tonguç et al, 2016
	Teuchophorus monacanthus Loew, 1859	Tonguç et al, 2016
	Teuchophorus simplex Mik, 1880	New record for Muğla
	Thrypticus caeruleus Negrobov & Naglis, 2020	Negrobov & Naglis, 2020
	Thrypticus kechevi Negrobov & Naglis, 2020	Negrobov & Naglis, 2020

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