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A Bioinformatics Study to Detect the Genetic Characteristics of *Vespa* Hornets (Hymenoptera: Vespidae)

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ABSTRACT

Hornets especially from genus *Vespa* can attack honey bee colonies, causing economic damages to beekeeping. Also, these hornets can cause damages to some crops. Basically, *Vespa* hornets occur in different parts of the world including Asia, North Africa, and Europe. Recently, the hornet, *Vespa velutina*, has invaded Europe and considered as a serious pest to beekeeping. This study aimed to investigate the genetic similarities between five *Vespa* hornets: *V. orientalis*, *V. ducalis*, *V. mandarinia*, *V. affinis*, and *V. velutina* depending on analyzing the sequences of mtDNA utilizing bioinformatics. The phylogenetic relationships, segments from the enzymatic digestion, open reading frames, and number of shared gene cluster families were investigated to detect the genetic similarities. Also, specific primers to discriminate between these hornets were designed and tested in silico. The potential infection of these hornets with similar pathogens was emphasized in light of the obtained results.

Key words: Honey bees, invasion, predators, genes, bioinformatics.

INTRODUCTION

Hornets belong to *Vespa* (Hymenoptera, Vespoidea, Vespidae) are considered as pests to honey bees and may cause damages to some crops. There are about 22 *Vespa* hornets (Archer, 2012), and these hornets are differed in their geographical distribution. The oriental hornets, *Vespa orientalis* Linnaeus, 1771, are existed in some Asian countries, North Africa, and some European countries, and accidentally found in Mexico (Dvořák, 2006). Some hornet species distribute in Asia, e.g. Japan, China, Korea, including; the black-tailed hornet *Vespa ducalis* Smith, 1852 (predator to immature stages of paper wasp), the giant hornet *Vespa mandarinia* Smith, 1852 (a serious pest to agriculture in Japan, Matsuura & Sakagami, 1973), the lesser banded hornet *Vespa affinis* Linnaeus, 1764 (predator to wasps and bees), and the Asian hornet *Vespa velutina* Lepeletier, 1836 (predator to honey bees, Tan et al, 2007). Recently, *V. velutina* was recorded in Iberian Peninsula (López, González, & Goldarazena, 2011) and has invaded Europe (Grosso-Silva & Maia, 2012; Monceau, Bonnard, & Thiéry, 2014).

Previous studies on the oriental hornets have focused on many aspects including behavioral aspects and nest building (Ishay, 1976; Kugler, Motro, & Ishay, 1979; Ishay et al, 1995), body properties and pheromones (Ishay, Ikan, & Bergmann, 1965; Ikan, Gottlieb, Bergmann, & Ishay, 1969; Raina & Singh, 1996), venom components (Edery, Ishay, Lass, & Gitter, 1972; Rosenberg, Ishay, & Gitter, 1977; Schoeters & Billen, 1995; Haim, Rimon, Ishay, & Rimon, 1999). Some studies have focused on their damages to honey bee colonies (Taha, 2014) or crops (Al-Mahdawi & Al-Kinani, 2011), and potential control strategies (Bacandritsos, Papanastasiou, Saitanis, & Roinioti, 2006; Mahdi, Glaiim, & Ibrahim, 2008) including possible biological trends (Haddad, Fuchs, Haddaden, & Kopelke, 2005) and honey bee response (Papachristoforou et al, 2008; Papachristoforou, Rortais, Sueur, & Arnold, 2011). Studies on other *Vespa* hornets have concentrated on similar aspects, for examples mating behavior of *Vespa ducalis* (Takahashi, Akimoto, Hasegawa, & Nakamura, 2002), Venom of *Vespa affinis* (Sukprasert et al, 2013), behaviors and control of *Vespa velutina* (Abrol, 1994), and response of bees to *Vespa mandarinia* attack (Sugahara & Sakamoto, 2009).

The mtDNA of some *Vespa* species have been sequenced including: *V. mandarinia* (Chen, Wei, & Liu, 2016), *V. orientalis* (Haddad et al, 2017), *V. affinis* (Okuyama, Martin, & Takahashi, 2017), *V. ducalis* (Kim, Jeong, Jeong, Kim, & Kim, 2017), and *V. velutina* (Takahashi, Okuyama, Minoshima, & Takahashi, 2018). The phylogenetic relationships between *Vespa* have been studied based on morphological characters (Perrard, Pickett, Villemant, Kojima, & Carpenter, 2013) while few studies have focused on the genetic characteristics of these hornets. Indeed, bioinformatics can be effectively used to investigate the genetic relationships between organisms (Abou-Shaara & Bayoumi, 2019). Therefore, this study aimed to investigate the genetic relationships between some *Vespa* hornets based on the mtDNA sequence with comparing genetic characteristics. This study expands the knowledge about genetic characteristics of *Vespa* hornets.

METHODS

Sequences of *Vespa* hornets

The mtDNA sequences were downloaded from the National Center for Biotechnology Information (<https://www.ncbi.nlm.nih.gov>) and utilized in this study. The sequences were to *Vespa orientalis* (NCBI Reference Sequence: NC_034003.1, 16101 bp), *Vespa ducalis* (GenBank: KX950825.1, 15779 bp), *Vespa affinis* (NCBI Reference Sequence: NC_039134.1, 19109 bp), *Vespa velutina* (GenBank: AP017943.2, 16416 bp), and *Vespa mandarinia* (NCBI Reference Sequence: NC_027172.1, 15902 bp).

Phylogenetic tree

The sequences were aligned using ClustalW, then maximum likelihood method and the Jukes-Cantor model (Jukes & Cantor, 1969) was used to construct the phylogenetic tree. This analysis was performed using MEGA7 (Kumar, Stecher, & Tamura, 2016).

Digest

The available restriction enzymes at Genome Compiler 2.2.88 (<http://www.genomecompiler.com>) were used to digest the full sequences of *Vespa* hornets, and then simulated on gel using NEB 100bp ladder. The resulted fragments were compared to detect similarity aspects according to Abou-Shaara (2019a; 2019b).

Open reading frames

For each *Vespa* species, open reading frames (ORFs) were identified using Genome Compiler 2.2.88 with start codons of ATG or GTG or TTG or CTG and minimum length of amino acids of 100, considering all frame types. The identified ORFs were compared between studied hornets to check the similarities.

Shared gene cluster

The number of shared gene clusters between the studied hornets was identified using OrthoVenn (<http://www.bioinfo-genome.net>) utilizing proteins downloaded from Uniprot according to Abou-Shaara (2019a; 2019b).

Primers to discriminate between *Vespa* hornets

Primers were designed using Genome Compiler 2.2.88 utilizing the option of auto primer design. Primers were designed to amplify the region between 3000 to 3100 nt. The designed primers were tested using PCR simulation of SnapGene 4.2.6 software (from GSL Biotech; available at snapgene.com) to ensure the amplification of the target sequences.

RESULTS AND DISCUSSION

Phylogenetic tree

The phylogenetic tree (Fig. 1) shows the close relationships between *V. velutina*, *V. mandarinia* and *V. ducalis* while no close relationship was detected between *V.*

orientalis and *V. affinis*. Previous studies support these findings, the phylogenetic analysis based on 45 morphological characters and molecular analysis showed the presence of close relationships between *V. mandarinia* and *V. ducalis* while the other three *Vespa* species were placed in different groups (Perrard et al, 2013). Based on the nucleotide sequences of the 13 protein-coding genes, close relationships between *V. mandarinia*, *V. ducalis* and *V. affinis*, were found (Okuyama et al, 2017; Takahashi et al, 2018), and between *V. mandarinia* and *V. ducalis* (Kim et al, 2017; Zhang, Huang, Chen & Li, 2018). On the contrary with the current study, a close relationship was found between *V. mandarinia* and *V. orientalis* based on the un-gapped sequences of a multiple sequence alignment (Haddad et al, 2017), while *V. orientalis* and *V. velutina* were placed in close groups based on the nucleotide sequences of the 13 protein-coding genes (Takahashi et al, 2018). The present and previous studies confirm the high relatedness between *V. mandarinia*, and *V. ducalis* while the relationships between other *Vespa* species showed variation in relation to the analysis method. It is clear that the analysis method can result in different phylogenetic relationships between *Vespa* hornets.

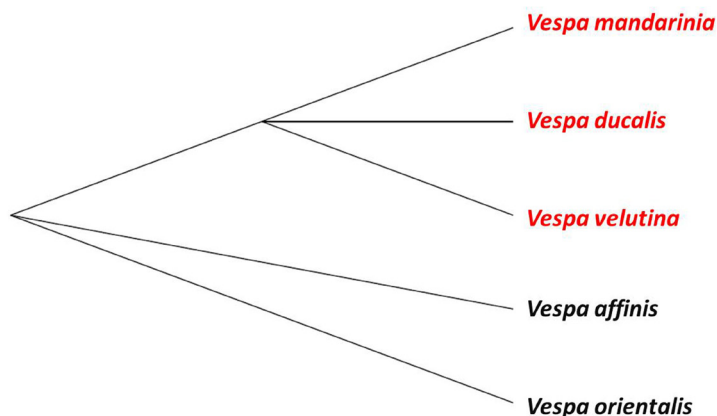


Fig. 1. The phylogenetic relationships between *Vespa orientalis*, *Vespa ducalis*, *Vespa affinis*, *Vespa velutina*, and *Vespa mandarinia*.

Digest

The fragments resulted from the enzymatic digestion of the mtDNA sequences of *Vespa* hornets are shown in Fig. 2. The number of fragments was 15, 14, 17, 16, and 21 for *V. orientalis*, *V. ducalis*, *V. affinis*, *V. velutina* and *V. mandarinia*, respectively. The number of similar fragments between *V. mandarinia* and the other hornets was 6, 4, 6, and 9 fragments with *V. velutina*, *V. affinis*, *V. ducalis*, and *V. orientalis*, respectively. The closely related species *V. mandarinia*, *V. velutina*, and *V. ducalis* as shown from the phylogenetic tree had the same number of similar fragments. This supports the genetic relationships between these three hornets. In general, the presence of similar fragments among *Vespa* hornets is anticipated because they belong to the same family and genus.

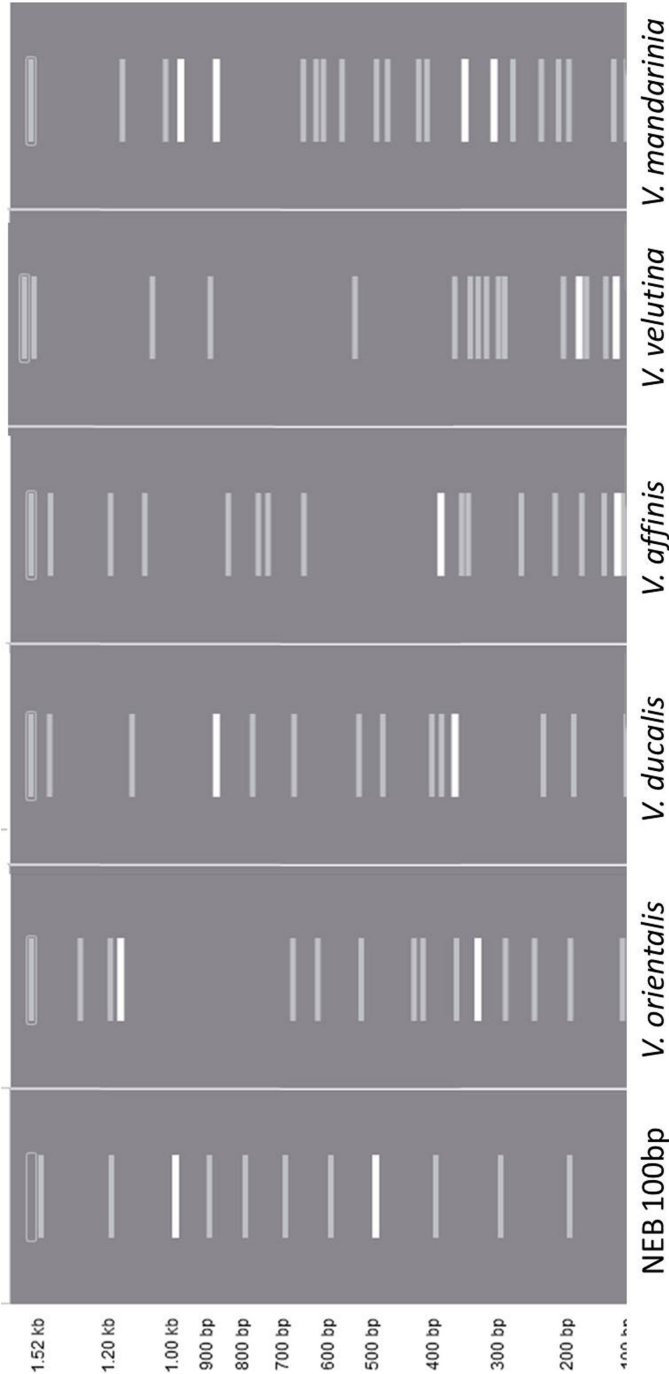


Fig. 2. Gel simulation to the resulted fragments from the enzymatic digestion of mtDNA sequences of *Vespa orientalis*, *Vespa ducalis*, *Vespa affinis*, *Vespa velutina*, and *Vespa mandarinia* using Genome Compiler.

Open reading frames

The detected ORFs were somewhat similar between *V. mandarinia*, *V. velutina*, and *V. ducalis* unlike *V. orientalis* and *V. affinis* (Fig. 3). This finding supports the phylogenetic relationships detected based on the phylogenetic tree, and suggests the low number of ORFs with 100 amino acids in the studied hornets.

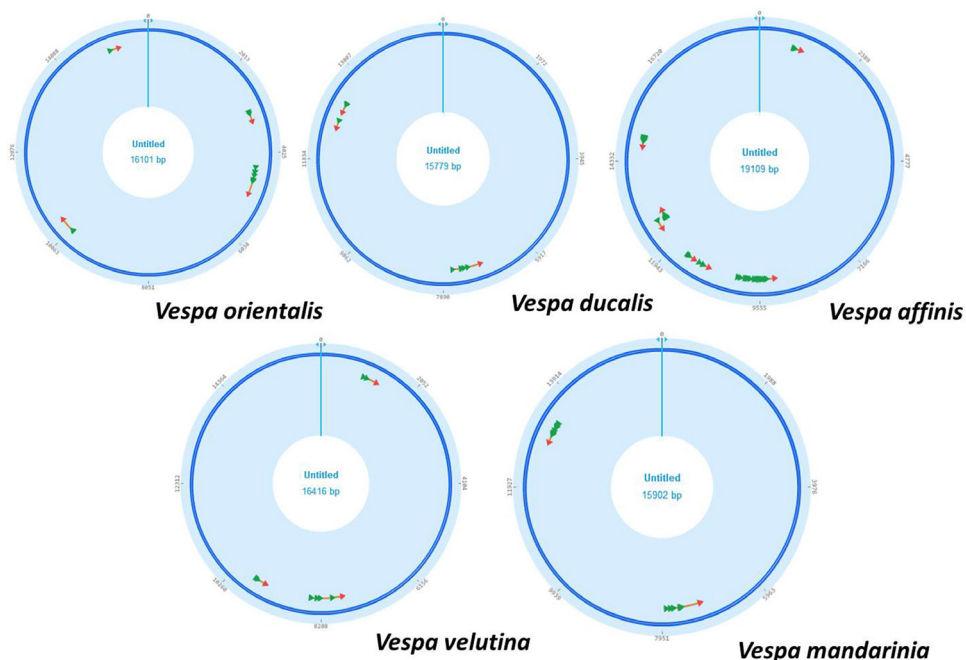


Fig. 3. Open reading frames using Genome Compiler for *Vespa orientalis*, *Vespa ducalis*, *Vespa affinis*, *Vespa velutina*, and *Vespa mandarinia*.

Functional analysis

The shared gene cluster families were 491 among all the five hornets (Fig. 4), and this because all hornets belong to the same family and genus. Indeed, *V. mandarinia*, *V. velutina*, and *V. ducalis* shared 112 gene cluster families (Fig. 4). This supports their close relationships than the other two hornet species. Few shared gene cluster families (<38) were detected between *V. orientalis* and *V. affinis* and the other three hornet species, suggesting lacking of high genetic similarities.

Primers to discriminate between *Vespa* hornets

Specific primers were designed to each hornet (Table 1), and tested against amplified region (Fig. 5). These primers can clearly discriminate between these five species, as each primer can give bands only with the target species while no bands can be obtained with the non-target species.

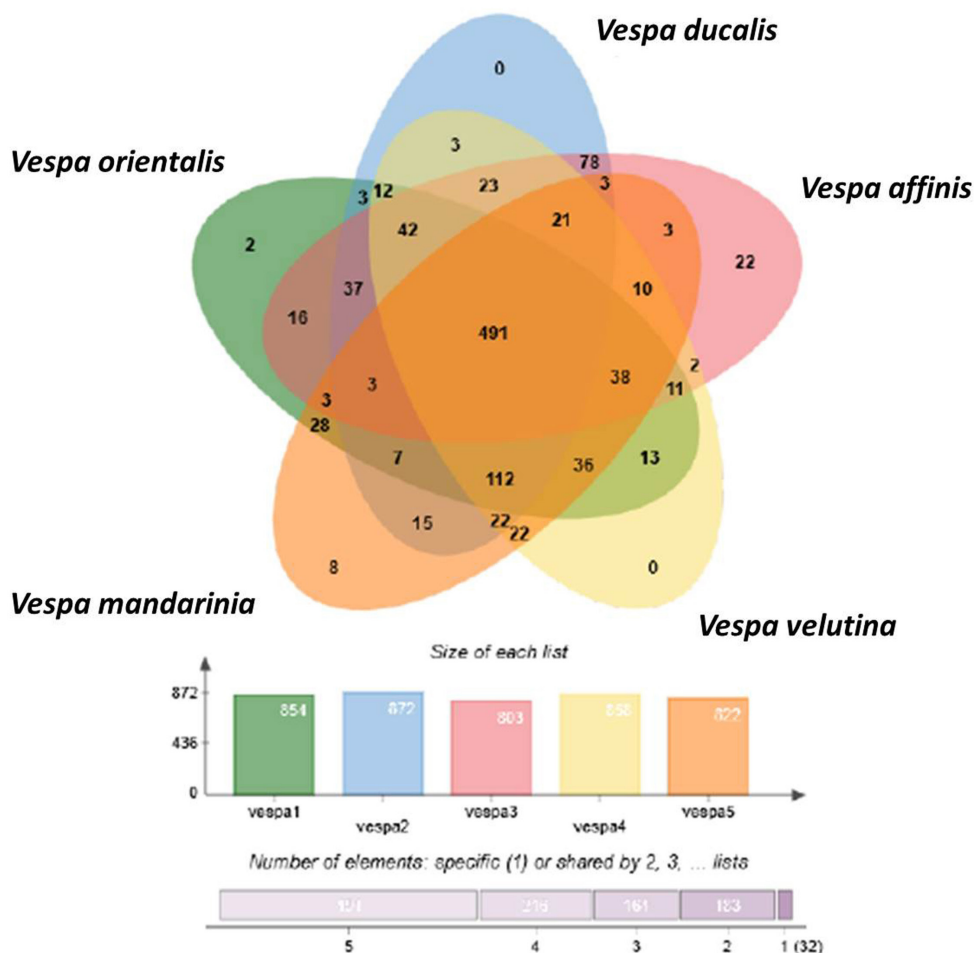


Fig. 4. Gene clusters produced to *Vespa orientalis*, *Vespa ducalis*, *Vespa affinis*, *Vespa velutina*, and *Vespa mandarinia*.

CONCLUSION

The study confirms the close genetic relationships between three *Vespa* species: *V. velutina*, *V. mandarinia* and *V. ducalis* utilizing different analytical techniques. Due to the high genetic similarities detected in this study, it is anticipated that these hornets may be infected with similar pathogens and can host similar pathogens, points which worth further investigations. Especially, some viruses including Israeli acute paralysis virus, deformed wing virus and Moku virus were detected in *Vespa* hornets (Yañez, Zheng, Hu, Neumann, & Dietemann, 2012; Garigliany et al, 2017; Forzan, Sagona, Mazzei, & Felicioli, 2017). Also, the study presented specific primers to discriminate between the five *Vespa* species: *V. orientalis*, *V. ducalis*, *V. affinis*, *V. velutina*, and *V. mandarinia*.

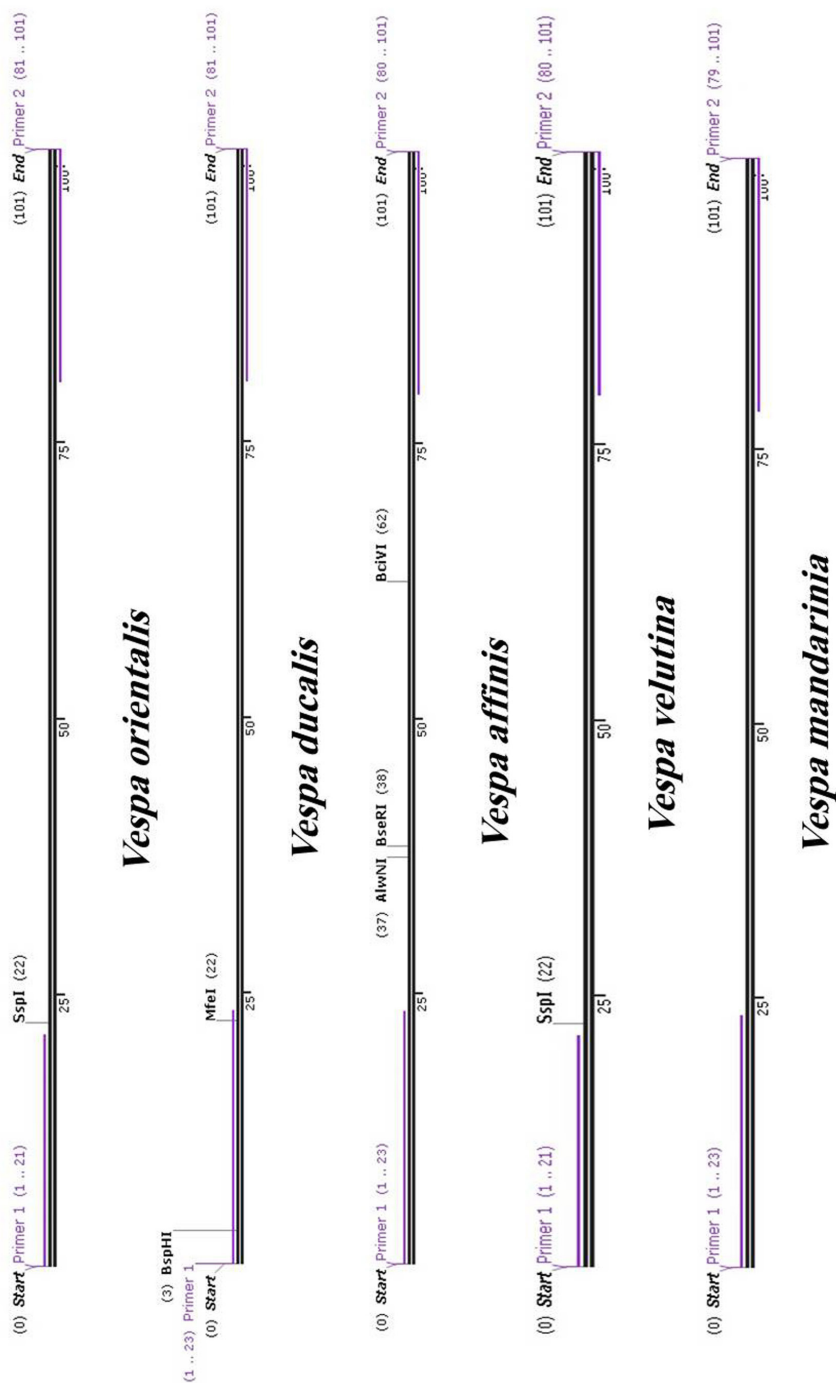


Fig.5. PCR simulation for the amplified regions using the specific primers to *Vespa orientalis*, *Vespa ducalis*, *Vespa affinis*, *Vespa velutina*, and *Vespa mandarinia* using SnapGene.

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Table 1. Specific primers to identify *Vespa orientalis*, *Vespa ducalis*, *Vespa affinis*, *Vespa velutina*, and *Vespa mandarinia*.

Species	Type	Sequences
<i>Vespa orientalis</i>	Forward 5'	TGAAATTAGGTAGGTATGGAA
	Reverse 5'	TCCTCCTACAATTCTAATGT
<i>Vespa ducalis</i>	Forward 5'	CTTCATGAAATGTTATCTCATCA
	Reverse 5'	AATTCGTTGTGATATAAATGA
<i>Vespa affinis</i>	Forward 5'	TTAATTACAAGAACTTTATCGG
	Reverse 5'	AAATCAACGGCAGGAGAATTGT
<i>Vespa velutina</i>	Forward 5'	TAAAAATTCAATTTACTTCAA
	Reverse 5'	GTAATCTGAGTAACGTCGTGGG
<i>Vespa mandarinia</i>	Forward 5'	CGAATTATCTTATATAAATTTTT
	Reverse 5'	TTAGTAGTATAGGGAATTTCTTG

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Five New Records for Turkish Robber Flies (Diptera: Asilidae) Fauna

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ABSTRACT

We here record five robber fly (Asilidae) species [*Lasiopogon cinctus* (Fabricius, 1781), *Stenopogon mediterraneus* Lehr, 1963, *Saropogon longicornis* (Macquart, 1838), *Machimus arthriticus* (Zeller, 1840), *Machimus gonatistes* (Zeller, 1840)] belonging to three subfamilies and four genera for the first time in Turkey. While all species are reported from Turkish Thrace, one of is also from Anatolia. Distributional data and taxonomical remarks with photographs of male genitalia and habitus for each species are given.

Key words: Asilidae, robber flies, new records, Turkish Thrace, Turkey.

INTRODUCTION

Asilidae is one of the largest families of Diptera with 7104 species and 89 subspecies (Geller-Grimm, 2015). The members of this family are commonly named as robber flies. The adult and larvae of robber flies are predacious in nature. They feed on various arthropods, mainly insects, and so play an important role in keeping the balance of insect populations (Lehr, 1988). Adult robber flies attack wasps, bees, dragonflies, grasshoppers, other flies, and some spiders (Shurovnekov, 1962). These flies are known to have a large variety of species in mostly dry and sunny habitats (Theodor, 1980).

Considering the species diversity of family, as well as the biodiversity potential of Turkey, our knowledge about Turkish robber fly fauna is still insufficient. Until 1981, there was limited information about the species living in Anatolia, obtained especially from studies conducted by foreign researchers such as Séguy (1927), Engel (1930), Jansens (1968a, 1968b) and Tsacas (1968). The first checklist of the family in Anatolia was published by Giray in 1981. In his work, he summarized all data published in the past as well as his own unpublished findings and reported 131 species in Turkey. After 13 years, the robber fly fauna of Erzurum and adjacent provinces were evaluated by Hayat and Alaoglu in their three articles (Hayat & Alaoglu, 1994, 1996a, 1996b). Also, three new records for Turkey were reported by Hayat & Özbek (1994). Weinberg & Hayat (1997) published a checklist of Turkish robber flies including 180 species. Then, two new species were described by Hasbenli & Geller-Grimm (1999). Hayat & Özbek (1999) reported two new records of genus *Dasypogon*. Also another six species were recorded by Durmuş (1999) for the first time from Turkey at his MSc thesis. In the same year, Tomasovic (1999a, 1999b) reported three species from Turkey for the first time. Bosak & Hradsky (2001) published a comprehensive study on Asilidae fauna in Turkey. They recorded 24 species of totally 102 records as new species from the country and stated 210 species living in Turkey. Six species were recorded for the first time from Turkey by Çalışkan (2003). Hasbenli, Candan, & Alpay (2006a) described a new species from the genus *Leptogaster*. Additionally, three new records, one of them from the genus *Erax* and the others from *Neoitamus*, were published by Hasbenli, Bayrakdar, & Alpay (2006b) and Hasbenli & Bayrakdar (2006). In the following year, 3 species belonging to the Leptaogastrinae subfamily were recorded by Aral-Alpay, Aktaş, & Hasbenli (2007) for the first time. Two years later, two *Dioctria* species were reported for the first time by Bayrakdar & Hasbenli (2009) and a new species belonging to *Mesoleptogaster* was described by Hasbenli & Çağlar (2009). Çalışkan (2010) reported *Pamponerus germanicus* as a new record for the country. Aral-Alpay (2011) stated that one species of *Machimus*, four species of *Tolmerus* and one of *Eutolmus* were reported for the first time from Turkey in her PhD thesis. The last faunistic data on Turkish robber flies fauna was published by Hasbenli, Çiftçi, & Çağlar (2020). In this study, they described the new genus *Tanap* and the new species *Tanap cinar* Hasbenli & D. Çiftçi, 2020 belonging to the Dioctriinae subfamily from Turkey.

The latest version of the “Catalogue of World Robber flies” lists 60 genus, 228 species and 9 subspecies for Turkey (Geller-Grim, 2015). However, the following seven species reported in different studies from Turkey; *Leptogaster fumipennis* Loew, 1871,

Leptogaster truncata Theodor, 1980, *Leptogaster montana* Theodor, 1980 (Aral-Alpay et al, 2007), *Mesoleptogaster cappadocia* Hasbenli & Çağlar, 2009 (Hasbenli & Çağlar, 2009), *Pamponerus germanicus* (Linnaeus, 1758) (Çalışkan, 2010) and *Machimus aberrans* (Schiner, 1868), *Tolmerus lesinensis* Palm, 1876 Loew (Aral-Alpay, 2011) are not in this catalog.

In this study, we report five species for the first time in Turkey. Thus, Turkish robber flies are represented by 63 genera, 241 species and 9 subspecies, together with the seven species that were not included in the list before, a new genus and a new species reported by Hasbenli et al (2020) and our new records.

MATERIAL AND METHODS

The specimens were collected with entomological net from meadows, fields, rivers edges, bridges, forests and residential areas in Turkish Thrace in 2002, 2003, 2005, 2018 and 2019.

A total of 342 specimens, 174 males and 168 females, were examined. All specimens are preserved in the Entomology Collection of Eskişehir Osmangazi University, Arts and Science Faculty, Eskişehir, Turkey. Material was studied according to methods described by Theodor (1976), with the aid of a stereomicroscope (Leica MZ 16) and photographed by Leica DFC 490 imaging system. Identifications were made using the keys and descriptions by Weinberg & Bachli (1995), Lehr (1963, 1984, 1986, 1992), Theodor (1976, 1980), Cannigs (2002) and Astakhov (2015). The nomenclature follows that of Geller-Grimm, Dikow, & Lavinge (2020).

RESULTS AND DISCUSSION

Lasiopogon cinctus (Fabricius, 1781), *Stenopogon mediterraneus* Lehr, 1963, *Saropogon longicornis* (Macquart, 1838), *Machimus arthriticus* (Zeller, 1840), and *Machimus gonatistes* (Zeller, 1840) are reported for the first time from Turkey. For all species, details of examined material, distribution data and brief descriptions of them are given. In addition, our observations and taxonomic evaluations of the specimens are presented under the title of "Remarks".

STENOPOGONINAE

Lasiopogon Loew, 1847

Lasiopogon cinctus (Fabricius, 1781) (Fig. 1A-E; Fig. 2)

Material examined: Tekirdağ, Malkara, Çınarlıdere, 102 m, 40°46'N, 27°03'E, 24.04.2005, 7 ♂♂, 1 ♀; Tekirdağ, Şarköy, Gölcük, 120 m, 40°41'N, 27°05'E, 24.04.2005, 5 ♂♂, 2 ♀♀.

Distribution: Austria, Belgium, Belarus, Bulgaria, Czech Republic, Denmark, Finland, France, Greece, Germany, Hungary, Italy, Norway, Poland, Romania, Soviet Union (North European territory, Central European territory, South European territory), Spain, Sweden, Switzerland, Netherlands, United Kingdom, former Yugoslavia (Geller-Grimm, 2015; Sakhvon & Caxboh, 2015).

Description

Body: 8-10 mm, Wings: 6-8 mm

Head: Vertex has a wide opening that is half the width of the head. The facial gibbosity is large, the face and frons are brownish, the facial beard black, spread to leave small gaps in the upper and lateral parts of the face. The lower half of the head and especially the posterior part of the eyes are grayish white and long thin white pubescent. The upper half behind the head is brownish, the hairs behind the eyes at the top of the head are long and curved forward. In the vertex, the hairs are long and extend into 2 segments of the antenna. Proboscis is shiny black, the palls are short, thin and black. The antenna is grayish brown and black. Segment 1 is longer than segment 2 and segments 1+2 is longer than half of the 3rd segment. Segments 1 and 2 carry black hairs. Stylus thick.

Thorax: Mesonotum is brownish yellow, humeral callus, button-shaped prominent, thin, small, black-haired. The brown band extending from the humeral calluses towards the posterior reaches the scutellum by bending. The brown band in the middle extends to the transverse suture. Setae: 2 Presutural, 3 supraalar, 1 strong long, 2 weak short postalar, dorsocentral 5 pairs strong, long, black, 2 pairs of these setae before the suture, (Additionally there are also 2 smaller setae in front of these). Pleura grayish brown, 6-7 weak setae on the upper part of the rear edge of the Mesopleura. Metapleura 7-9 setae in a row. Scutellum is grayish brown, bare and flat, half disc shaped. There are 10-12 setae in different thickness and length.

Legs: are black. Coxae gray hairs anteriorly, bare posteriorly, whitish yellow hairs at the bottom of fore and middle femur, black hairs at fore and middle tibia, and all the setae are black. White hairs in the ventral of hind femur. The hind tibia has short white hairs and with some black setae. In hind legs, coxal peg present.

Wings: with light brown veins.

Abdomen: is brown or black. Tergites are grayish at the posterior and lateral edges. The anterior is black, dark brown, near the middle area rectangle. Bristle are gray-white. It becomes sparse on the sides from 1st to 4th tergite. In the first three segments, hairs are longer than others. Hypopygium of male and ovipositor of female in bright black. Male hypopygium has dense black hairs on it, female ovipositor carries short acanthoforites with 13 spines (on the left side 6 spines and right side 6 spines separate and a weak spinose in the middle).

Genitalia: Male genitalia is wide and rotated 180°. The two halves of epandrium are completely separate. Apex of the aedeagus is a trifid shaped. Apodeme of hypandrium and gonocoxite complex is very long and strong, spatula shaped. Apical lobes of gonocoxite are broadly rounded. Form of dististylus apex is like a finger with processes and longer than its width.

Remarks

Lasiopogon cinctus are known to be widespread especially in Balkan countries and the European continent. The presence of the species in Turkish Thrace is an expected result. We examined totally 15 specimens (12 male and 3 female) of this

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species collected two different sites in Tekirdağ provinces. Morphological features of our specimens mostly confirm to description of this species in Lehr (1984) and Cannings (2002). So far it is known that there are two species of the genus *Lasiopogon* living in Turkey (Geller-Grimm, 2015). *L. cinctus* is the third species of *Lasiopogon* recorded from Turkey.

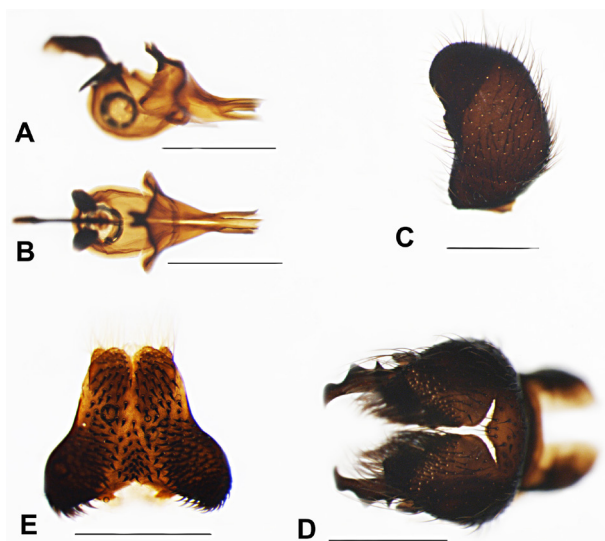


Fig. 1. Male genitalia of *Lasiopogon cinctus* (Fabricius, 1781). a. aedeagus (lateral), b. aedeagus (dorsal), c. epandrium, d. gonocoxite and dististylus, e. subepandrial sclerite (Scale 0,5 mm).



Fig. 2. *Lasiopogon cinctus* (Fabricius, 1781). Male (Scale 5 mm).

Stenopogon* Loew, 1847**Stenopogon mediterraneus* Lehr, 1963 (Fig. 3A-E; Fig. 4)**

Material examined: Çanakkale, Gelibolu, Yeniköy, 43 m, 40°37'N, 26°55'E, 05.07.2003, 1 ♀; Edirne, Uzunköprü, Karapınar, 56 m, 41° 6'N, 26°38'E, 06.07.2003, 8 ♂♂, 8 ♀♀; Edirne, Değirmenyeni, 58 m, 41°43'N, 26°33'E, 07.07.2003, 4 ♂♂, 4 ♀♀; Edirne, Keşan, Çobançeşme, 93 m, 40°56'N, 26°39'E, 06.07.2003, 1 ♂, 2 ♀♀; Edirne, Uzunköprü, Kırçasalih, 84 m, 41°22'N, 26°47'E, 06.07.2003, 1 ♂, 2 ♀♀; Kırklareli, Kocahıdır, 100 m, 41°39'N, 26°55'E, 07.07.2003, 4 ♂♂; Tekirdağ, Malkara, 151, m, 40°52'N, 26°56'E, 05.07.2003, 14 ♂♂, 10 ♀♀; Tekirdağ, Çınarlıdere, 102 m, 40°46'N, 27° 3'E, 04.06.2002, 1 ♂.

Distribution: Albania and Greece (Geller-Grimm, 2015).

Description

Body: 21-26 mm, Wings: 12,5-15 mm

Head: Head is circular, all setae and hairs are yellow. Antennae; segment 1 and segment 2 are red, segment 3 is dark red or dark red in all segments in some specimens. Segment 1 is longer than 2, segment 3 is slightly longer than 1 + 2.

Thorax: is yellowish. the humeral callus is reddish, brown; and some specimens is slightly dark, median stripe brown. Setae: 8-10 humeral, 6-9 posthumeral, 4-5 notopleural, 10-14 supraalar, 6-8 postalar, 14-16 dorsocentral, 6-9 sucutellar (The setae are aligned to the right and left sides of the back edge of the sucutellum).

Legs: The anterior femora is with a half black band along the femur on both sides, from the femoral base to the apical. The inner band is smaller. The middle leg femur carries a black band tapering from the base to 3/2 apical. Hind femora with a black band along the femora on the outer lateral surface of the femora in male. The black band of the femora sometimes may be shorter, and not extend to the apical in female.

Abdomen: is yellowish brown, some of the specimens' tergites are red anteriorly, dark brown posteriorly in male. Hypopygium red or dark reddish. The last 2-3 segments and ovipositor are dark reddish brown in female.

Genitalia: Male genitalia is yellow and reddish, bristle color is yellow. Hypandrium has got two lobes that are separated on both sides of the apical part. These parts are yellow hairy.

Remarks

The most important taxonomic character used to distinguish between the species of *Stenopogon* is the structure of the hypandrium. According to Lehr (1963), the apex of the hypandrium of *Stenopogon mediterraneus* have two lobes as in *S. callosus* (Pallas, 1818), but the lobes in the hypandrium of *S. mediterraneus* are slightly curved on both sides. In present study, we examined 33 males and also 27 females from eight different localities. The hypandrium structures of our male specimens are similar to original description (Fig. 3E). Furthermore, we observed light yellow and slightly dark specimens in color and also have color differences between female and male individuals.

The species was described by Lehr (1963) in Albania and it is known to exist only in Macedonia outside of the type locality (Adamovic, 1966). So, our record is the eastern

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boundary of the distribution area of the species. However, 18 species of this genus are known to exist in Turkey and this number has increased to nineteen with our record.

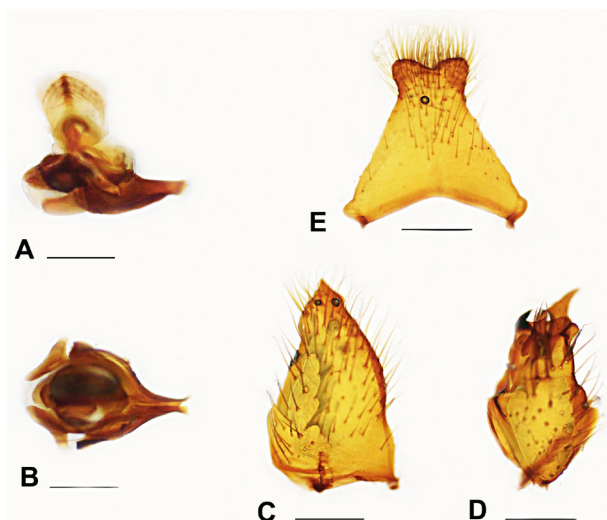


Fig. 3. Male genitalia of *Stenopogon mediterraneus* Lehr, 1963. a. aedeagus (lateral), b. aedeagus (dorsal), c. epandrium, d. gonocoxite and dististylus e. hypandrium (Scale 0,5 mm).



Fig. 4. *Stenopogon mediterraneus* Lehr, 1963. Male (Scale 5 mm).

DASYPOGONINAE

Saropogon Loew, 1847

Saropogon longicornis (Macquart, 1838) (Fig. 5A-E; Fig. 6)

Material examined: Kırklareli, Kofcaz, Kocatarla, 389 m, 41°56'N, 27° 03'E, 28.05.2002, 2 ♂♂.

Distribution: Egypt, Iran, Israel (Ghahari, Hayat, Lavigne, & Ostovana, 2014; Geller-Grimm, 2015)

Description

Body: 11-12 mm, Wings: 9 mm

Head: facial tomentum yellow, facial beard yellow, frons with gray tomentum, black in the middle, in the form of a strip. Ocellar tubercle is black, with two reddish spot behind the ocellar tubercle. Occipital setae are black on the sides, yellowish in the middle. The antennae are cylindrical, thin and significantly longer over the head. Segment 1 is longer than 2, and the segment 3 is 1.5 times longer than 1 + 2. Style fused with segment 3, a sensillum is situated in the dorsal cavity. Segment 1, 2 and base of segment 3 is reddish, apical part is more or less black. Palps are black.

Thorax: pronotum is black in color with black and black - yellow setae. Mesonotum is black. It carries a gray band from the humeral callus to the scutellum on both sides. This band reaches the back of the suture. Very small black hairs cover mesonotum. The posterior area is bare. Pleura is black, mesopleura dorsally has gray striped-shaped tomentum. Postnotal fan has a row of reddish black setae. Setae: 1-2 (short, reddish black) humeral, 3 notopleural, 2 supraalar (reddish black), 2 postalar, 2-3 dorsocentral (very short), 4 scutellar (black). The wings are brownish yellow, anteriorly dark. M3 cell open, anal cell closed.

Legs: Coxa and trochanter are black, femur base and half is colored black. The fore and middle femora and tibia are light red or orange, base of the fore and middle basitarsus is yellow, dark anteriorly. Hind femora red apically, dark posteriorly. The base of the hind tibia is red, black apically, tarsals are black.

Abdomen: all tergites are black and covered with small black hairs. Posterior of the sternite 2-3 is slightly reddish in the form of a thin band.

Genitalia: Aedeagus is conical, curved on both sides at the tip. Epandrium, incompletely divided. Apical processes of the proctiger are not curved. The apical processes from the gonocoxite is long and pointed, the ventral process are triangular. Hypandrium at the base is wide, triangular and with setae.

Remarks

Saropogon longicornis (Macquart, 1838) is known to date only in Iran, Israel and Egypt. In this study, it is reported for the first time from the European continent. Individuals of the species were caught in a single locality in Kırklareli province.

According to Engel (1930) and Theodor (1980), *S. longicornis* mesonotum: medially black, humeral calli, lateral stripe and posterior part reddish, with greyish tomentum in the anterior part to the suture or slightly beyond. *Legs*: coxae black, fore and mid trochanters yellowish-red, hind trochanters black or also partly yellowish. *Abdomen*: tergites 1-7 reddish-yellow in male, with lateral black spots of varying size on tergites or spots absent. Some sternites reddish - yellow. Hypopygium black laterally, reddish-yellow dorsally in the middle. *Male genitalia*: Dististylus slender, tapering, strongly curved, with pointed apex. Proctiger with broad, rounded, inward-curved lateral posterior processes with setae before the apex and with two rounded processes.

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In this study, some differences in body structure and male genitalia were observed in the two *S. longicornis* specimens examined by us, as follows: mesonotum: there is a gray band from the humeral callus to the scutellum on both sides. This band reaches the back of the suture. Very small black hairs cover mesonotum. The posterior area is bare. Legs: coxa and trochanter are black, femur base and half is colored black. The fore and middle femora and tibia are light red or orange, base of the fore and middle basitarsus is yellow, anteriorly dark. Hind femora red apically, dark posteriorly. The base of the hind tibia is red, black apically, tarsals are black. Abdomen: all tergites black. Male genitalia: Apical processes of the proctiger are not curved. Dististylus slender, tapering, uncurved, with pointed apex. Coloration of the legs is similar to *Sarapogon eucerus*. The typical structure of the aedeagus, the shape of the epandrium and hypandrium are consistent with the *Sarapogon longicornis* stated by Theodor (1980). Theodor (1980) states that variations can be seen in body color and male sexual organs of *Sarapogon* species living in different habitats. Likewise, we think that the observed differences in body coloring of our samples can be considered as variation.

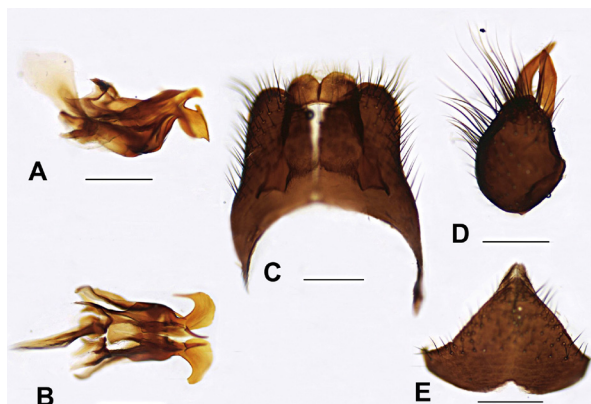


Fig. 5. Male genitalia of *Sarapogon longicornis* (Macquart, 1838). a. aedeagus (lateral), b. aedeagus (dorsal), c. epandrium, d. gonocoxite and dististylus, e. hypandrium (Scale 0,5 mm).



Fig. 6. *Sarapogon longicornis* (Macquart, 1838). Male (Scale 5 mm).

ASILINAE***Machimus* Loew, 1849*****Machimus arthriticus* (Zeller, 1840) (Fig. 7A-E; Fig. 8)**

Material examined: Kırklareli, Vize, Pazarlı, 241 m, 41°36'N, 27°43'E, 09.07.2003, 1 ♂; Tekirdağ, Çerkezköy, Kapaklı, 105 m, 41°20'N, 27°55'E, 06.08.2019, 3 ♂♂, 1 ♀; Kocaeli, Kandıra, Şeyhtimarı, 41°01'N, 30°13'E, 78 m, 15.06.02, 1 ♂; Kocaeli, Merkez, Eşme, 40°44'N, 30°14'E, 7 m, 19.06.02, 1 ♂; Kocaeli, Kocaeli University Campus, 40°47'N, 29°56'E, 287 m, 19.06.02, 1 ♂.

Distribution: Austria, Belarus, Bulgaria, Denmark, Finland, France, Germany, Italy, The Netherlands, Norway, Poland, Romania, Russia, Spain, Sweden, Switzerland, United Kingdom (Geller-Grimm, 2015; Sakhvon & Caxboh, 2015).

Description

Body: 14-15 mm, Wings: 8-9 mm

Head: Face has gray-white tomentum. Behind the head, some specimens have white setae, some have a strong number of black setae among white setae, or very few (one or few more) among the white setae. Genae and postgenae beard is completely white, facial gibbosity is more or less rusty yellow in color, black hairs are seen towards the upper edge. Palps are black. Antennae: segment 1 is longer than segment 2, segment 3 is approximately 1+ 2 tall.

Thorax: Mesonotum yellowish-brown. Median stripe is brown, wide anteriorly and reached to the scutellum. Setae: 1-2 posthumeral, 2 notopleural, 2 supralar, 3 postalar (1 setae is white), 6 pairs dorsocentral setae, the last two pairs, pass the transversal suture, scutellar 2. Hairs color is white.

Legs: The dorsal of the femur and tibia are black and the ventral is brownish. Fore coxae covered with thick and long whitish hairs. Fore femora, carries a row of spine ventrally.

Abdomen: There are white setae on the posterior edges of the tergites. All short hairs are white. The male hypopygium is smaller than the second abdominal segment. It is colored with dark red - brown. It's covered with white hair.

Genitalia: Aedeagus curved at the base. Dististliys shape is characteristic. It is curved at the base, very short, with wide and angular at the apex. Gonocoxite broadly rounded, with a small, pointed process in the middle of the apical margin.

Remarks

Machimus arthriticus is widespread in Europe and also spread Bulgaria and Romania in the Balkan Peninsula. In this study, we found the species in two localities from Kırklareli and Tekirdağ provinces in the Thrace region, and also in three localities in Kocaeli province, east of the Bosphorus. So, its record in the east of the Bosphorus indicates that it can be spread to the western parts of Anatolia. Zeller (1840) compared *M. arthriticus* with *Asilus genualis*, the synonym of *M. rusticus* (Meigen, 1820). The same researcher stated that although this species is significantly smaller than *M.*

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rusticus as body, its body form, color and feathering are similar to it. Schiner (1862) described this species as *Epitriptus arthriticus*. Zeller (1840) and Schiner (1862) stated that the male genitalia of this species is smaller than the second abdominal segment in the length and the legs are black generally but its basal part is red brown. Similarly, the length of the genitalia of our male specimens is observed to be smaller than the second abdominal segment. However, the femurs of the legs are black in the dorsal and brown in the ventral. Additionally, in our collection it was observed that the setae behind the head were white in some individuals, or some of with strong black setae among the white setae, and others with black setae between the white seta and feathers. It is thought that the difference in leg colors should be compared with other populations in Palearctic and that the difference in seta colors could be the variation of the species. On the other hand, male genitalia structures of the specimens are compatible with the descriptions of Theodor (1976) and Lehr (1992).

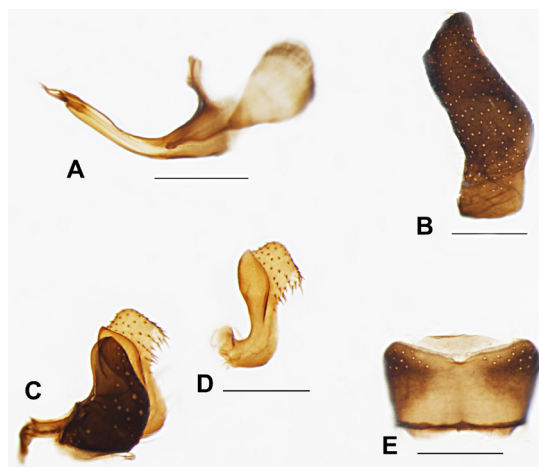


Fig. 7. Male genitalia of *Machimus arthriticus* (Zeller, 1840). a. aedeagus (lateral), b. epandrium, c. gonocoxite and dististylus, d. dististylus, e. hypandrium (Scale 0,5 mm).



Fig. 8. *Machimus arthriticus* (Zeller, 1840). Male (Scale 5 mm).

***Machimus gonatistes* (Zeller, 1840) (Fig. 9A-F; Fig. 10)**

Material examined: Edirne, Karaağaç, 58 m, 41°40'N, 26°31'E, 31.05.2002, 4 ♂♂; 07.07.2003, 1 ♂; Edirne, Enez, Kocaali, 86 m, 41°39'N, 26°20'E, 19.06.2018, 2 ♂♂; İstanbul, Arnavutköy, Çilingir, 129 m, 41°11'N, 28°41'E, 22.05.2002, 1 ♂; İstanbul, Terkos Lake, 15 m, 41°21'N, 28°36'E, 23.05.2002, 1 ♂; İstanbul, Çatalca, Akalankalfa, 117 m, 41°15'N, 28°25'E, 3.06.2018, 2 ♂♂.

Distribution: Austria, Belarus, Bulgaria, Denmark, Finland, Greece, Germany, Hungary, Italy, Kazakhstan, Morocco, North Africa, Poland, Romania, Russia, Switzerland, Tunisia, former Yugoslavia (Geller-Grimm, 2015; Sakhvon & Caxboh, 2015).

Description

Body: 18-23 mm, Wings: 12-14 mm

Head: Face has gray tomentum, face beard has white setae on bottom, and black on top. Facial gibbosity is large, the gena towards the palps are black with 2-3 black hairs, palp is black and hairs are black. The antennae are black, with gray tomentum and lateral surface of the 1st and 2nd segment of antennae are gray tomentum. Segment 1 has strong black and weak white hairy. Segment 1 is also longer than segment 2. Segment 3 is around 1 + 2 long. Style is long. Vertex is with long, black strong hairy. A row of black hairs up to the antennae on both sides. Behind the vertex and posterior lateral of the eyes have white hairs, strong black hairs on both sides.

Thorax: Acrostichal hairs short, extend to the front of mesonotum. Humeral callus has white and black hairs. 1-2 posthumeral, 2 notopleural, 2-3 supraalar (1 white), 2 postalar (1 black, 1 white), 8-9 pairs dorsocentral (5 pairs after transversal suture), 5-6 scutellar, the scutellum has long, black and white hairs. Thorax and pleura has gray tomentum, post notal fan with one row, long white hair, in mesopleuron with 1 strong black seta ventrally.

Wings and veins are brown. In legs, coxa has white hairs, hairs on all legs are white, setae are black. Reddish brown in a small area at basal part of tibia.

Abdomen: in the posterior of tergites, setae are yellowish white, hairs are white, posteriorly and laterally with gray tomentum.

Genitalia: Hairs color are yellowish-white. Lateral progs of the aedeagus are curved. The apical of the epandrium is straight and wide. The dististylus is curved, its base is narrow, it extends towards the apical and the apex is round and has a small protrusion.

Remarks

Machimus gonatistes is known as a steppe species. It has a wide distribution area in the Palearctic Region and its presence in the Balkan Peninsula is also known (Richter, 1964; Geller-Grimm, 2015). Therefore, it is not surprising that the species is determined in Thrace. The species was only in five localities in the broad study area. This situation is thought to be due to the habitat preference of the species. The morphological characteristics of the 11 males examined in the study generally confirm to its descriptions in Theodor (1976) and Lehr (1992). However, we observed remarkable differences that there was no posterior branch of both wing M3 cells of a sample captured in Istanbul

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Gaziosmanpaşa. Similarly, supraalar and postalar setae are black in the specimens collected from Istanbul Gaziosmanpaşa, whereas these setae are yellow in individuals collected from Edirne. We think that the difference in the vein can be an anomaly and there is a variation in the differences in the setae coloration.



Fig. 9. Male genitalia of *Machimus gonatistes* (Zeller, 1840). a. aedeagus (lateral), b. end of the aedeagus (dorsal), c. epandrium, d. gonocoxite and dististylus, e. dististylus, f. hypandrium (Scale 0, 5 mm).

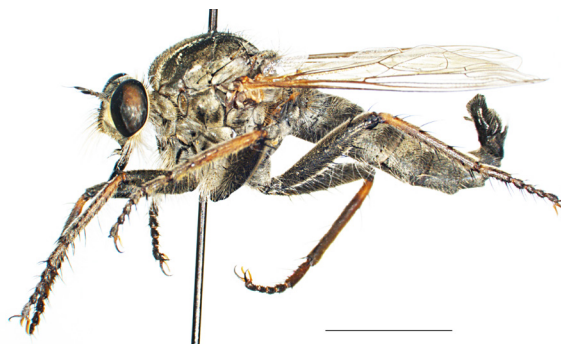


Fig. 10. *Machimus gonatistes* (Zeller, 1840). Male (Scale 5 mm).

As a result, 4 species are found in the Thrace Region and one species in both Thrace and Anatolian sides in the study. Undoubtedly, new studies are needed to determine the existence and distribution of these species in Anatolia. Based on the results of the study, we think that it is possible to discover many new records or perhaps new species in future studies on Turkish robber fly fauna.

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Ethology of *Proctacanthus gracilis* Bromley, 1928 (Diptera: Asilidae) in Northeastern Florida, U.S.A.

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ABSTRACT

Proctacanthus gracilis Bromley, 1928 forages primarily from vegetation, capturing prey in flight, and immobilizing them in flight or on the ground. Identified prey is in eight insect orders (Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Neuroptera, Odonata, and Orthoptera), with Orthoptera making up 63.0%. Mating occurs in the tail-to-tail position and oviposition is in the ground. This species exhibits a distinct daily rhythm of activity for feeding and mating. Grooming behavior resembles that described for other species of robber flies. Morphology, habitats and distribution in Florida, resting behavior, and predators and parasites also are discussed.

Key words: Behavior, robber flies, morphology, prey, Diptera, Asilidae.

INTRODUCTION

Proctacanthus gracilis Bromley, 1928 is a Nearctic species with distribution in the southeastern United States in Alabama, Florida, Georgia, Mississippi, and South Carolina (Fisher & Wilcox, 1997; Geller-Grimm, 2019). This paper provides detailed information on the ethology of this species in the Moses Creek Conservation Area (MCCA) in St. Augustine, St. Johns County, in Northeastern Florida, U.S.A. Information also is provided on morphological characteristics that distinguish *P. gracilis* from other species of *Proctacanthus*, in particular in the MCCA.

Proctacanthus gracilis is 23-34 mm in length and its body is reddish in ground color with a pale yellowish to yellowish-brown pruinescence (Fig. 1). The proboscis is slender and the mystax is white, sometimes with a few black setae above. The wings are nearly hyaline with a reddish tinge and veins, and extend to at least abdominal segment 6. The legs are reddish with black bristles.



Fig. 1. Male *Proctacanthus gracilis* with adult ant lion prey (Photograph: D.S. Dennis, 29.07.2011, 10:47 AM).

MATERIALS AND METHODS

Records in the Florida State Collection of Arthropods (FSCA) indicate that *P. gracilis* occurs from about mid-May through early October in Florida. In the MCCA the majority of observations were made during June through August over nine field seasons: 02.06.2011-17.08.2011; 09.07.2012; 04.06.2013-15.08.2013; 26.05.2014-29.08.2014; 16.06.2015-24.07.2015; 23.05.16-28.07.2016; 07.07.2017-23.08.2017; 14.06.2018-24.07.2018; and 28.06.2019-06.08.2019. Some years had shorter periods of study because of inclement weather and low populations of flies.

The identification of *P. gracilis* was confirmed using Hine (1911) and the description in Bromley (1928), and comparing specimens with those in the FSCA. Most of the latter specimen identifications were by C.H. Martin (1958, 1960) and J. Wilcox (1967, 1979), and one by S.W. Bromley (1949). The identifications were confirmed by Riley Nelson (Brigham Young University, Provo, Utah in 1989) who has extensively studied species in the genus *Proctacanthus*.

The author studied *P. gracilis* when it was most abundant in mowed scrub vegetation communities in the MCCA. Observations involved an average of 3 individuals per

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day, each for up to 5 hours and 55 minutes. Total number of hours of observations equaled approximately 112, not including the many hours searching for individuals or populations of *P. gracilis* to observe.

Proctacanthus gracilis was studied by the author sitting on the ground or standing and observing single flies for as long as possible in order to collect information on their various behaviors and diurnal activities. The author also slowly walked through a study area and observed the activities of a number of flies, primarily to collect prey and to locate mating pairs and ovipositing females.

Collected prey was placed in glass vials with a label indicating the sex of the predator, date, time, and location. The author sent prey that he could not identify to the U. S. Department of Agriculture, Agricultural Research Service, Systematic Entomology Laboratory (SEL), Beltsville, Maryland, U.S.A. for identification. Prior to shipment, prey was measured with a clear, plastic ruler to the nearest 0.5 mm.

While in the field, a hand held Taylor thermometer and/or a Cooper-Atkins DPP400W Digital Thermometer were used to take air, surface and subsurface ground temperatures. A Dwyer Hand-Held Wind Meter measured wind speed.

RESULTS AND DISCUSSION

Morphology

Bromley (1928) described *P. gracilis* based on specimens from Georgia and indicated that they are 26.0-30.0 mm in length. The author measured 10 males and females from the MCCA and females range in size from 23.0-31.0 mm (average 27.4 mm) in length, and males range from 24.0-30.0 mm (average 27.1 mm) in length. The lengths of the specimens in the MCCA and the FSCA are similar, although two females in the latter were 33 and 34 mm long.

Bromley (1928) indicated that the hairs of the palpi, mystax, beard, and post-genae are "...nearly white with a very pale yellowish tinge." The mystax of some of the specimens from the MCCA and in the FSCA have a few black bristles/hairs above, and/or one to two black, subvibrissal (oral margin) bristles/hairs, one of which is usually smaller than the other. The postgenae bristles/hairs are pure white to a pale yellowish white.

Bromley (1928) described *P. gracilis* with a, "Thorax reddish in ground color...and covered...with pale yellowish bloom....Ground color of abdomen reddish, but covered with pale yellowish bloom and fine pubescence. "

The reddish brown ground color on the thorax is particularly prevalent anteriorly on either side of the dorsal stripes. This color often extends to the postpronotal lobe (postpronotum), mesothoracic spiracle, and anepisternum, which are partly to completely reddish brown. The scutellum also may be reddish brown.

The "bloom" or pruinescence, is yellowish to yellowish brown. In the field, when *P. gracilis* is in the sun and viewed from the side, some of their bodies look yellowish to

yellowish green, in particular in front of the wings and ventrolaterally. Also, on some specimens in the MCCA and FSCA, the disc of the scutellum (in particular anteriorly) is silverish white and the sides of the thorax may be glistening yellowish-silverish white.

Hine (1911) did not describe in detail the markings [i.e., stripes (lines) and lateral spots] on the dorsum of the thorax of the sixteen species of *Proctacanthus* that he dealt with, but often called them, "...the usual markings..." For most of these species the thorax is dark and often a uniform color, and the stripes and spots are not clearly seen or are indistinct unless magnification is used (e.g., in the MCCA, *P. fulviventris*, *P. heros*, and *P. rufus*). At least the outlines of the stripes and spots of only a few species can be seen without magnification (e.g., in the MCCA, *P. brevipennis*, *P. gracilis*, and *P. longus*).

Bromley (1928) stated that the thorax of *P. gracilis* has a "...dark red median line and vittae..." The specimens in Florida usually have distinct median and paramedian stripes of the scutum and lateral spots (Fig. 2; terminology follows Geller-Grimm, 2020). The stripes are wider anteriorly and narrow posteriorly, with the posterior 1/4-1/3 split. The median stripe is light red to dark reddish brown, becoming lighter posteriorly. The paramedian stripe is usually a lighter color should than median stripe, but has a darker outer margin that is similar in coloration to the median stripe. Each tip of the paramedian stripe has either a separate or attached, widely separated reddish-brown stripe that often has an irregular shaped edge (the acrostichal bristles are generally on the outer edge of each stripe). On *P. gracilis*, these stripes are widely separated posteriorly and may be called the postsutural acrostichal stripes. Cannings (2002) called the paired "...stripes on the scutum to be the acrostichal stripe..."

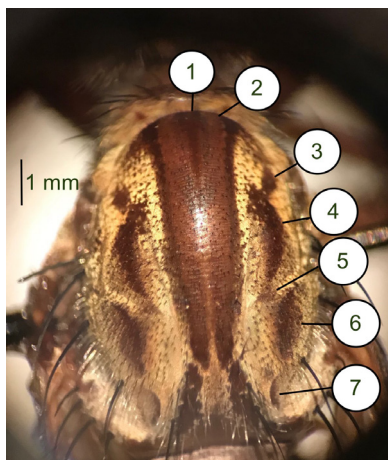


Fig. 2. Dorsal view of *Proctacanthus gracilis* thorax showing stripes and spots (Photograph: J.G. Dennis, 06.01.2020, 10:49 AM). Number designations: 1, Median stripe of scutum; 2, Paramedian stripe of scutum; 3, Posthumeral spot; 4, Presutural spot; 5, Sutural spot; 6, Postsutural spot; and 7, Prescutellar spot and postsutural acrostichal stripe.

The dorsal thoracic spots on *P. gracilis* are reddish brown and may be very dark. The posthumeral spot is often broadly "U" shaped (in particular on the asilids in the

MCCA), merged with the presutural spot and extend laterally to the outer margin of the paramedian stripe. The presutural spot may be triangular or elongate and have an outer lighter margin. If elongate, the presutural spot is usually slightly concave towards the paramedian stripe. The presutural spot also may be only slightly separated from or partially merged with the postsutural spot. The sutural spot is very small, indistinct, or absent. If present, the sutural spot is close to the paramedian stripe, light reddish brown, circular to more elongate or triangular. The postsutural spot is usually somewhat shaped like a triangle with a lighter margin, although it may consist of a convex curved stripe with a lighter margin. The prescutellar spot is indistinct to dark reddish brown and shaped like a small triangle or crescent. Sometimes, on either side of the paramedian stripe, there is a light reddish-brown to light blackish area that blends into the spots and makes them difficult to see.

In addition to the pale setae on the coxae of the legs of *P. gracilis* mentioned by Bromley (1928), the coxae may have one to six (usually 1 or 2) medial black bristles, one of which is usually longer and larger than the others. Some specimens may have one to four medial pale bristles instead of the black bristles or the pale bristles are below the black bristles.

Bromley (1928) described, *P. gracilis* with "...an obscure brownish area in the center of each segment forming a broken median line along the dorsum of the abdomen." This median line is faint brownish to dark reddish brown.

In Hine's (1911) key to the species of *Proctacanthus*, *P. gracilis* would key to the last couplet 15 with the smaller *P. brevipennis* (Wiedemann, 1828). The two species can be distinguished from each other as follows, (1) *P. gracilis* has palpi with all or almost all white hairs (bristles) and *P. brevipennis* palpi have black hairs; (2) *P. gracilis* has reddish femora and *P. brevipennis* has black femora or a black anterior stripe (in particular on the fore femora); and (3) *P. gracilis* thoracic stripes and spots are reddish brown and *P. brevipennis* stripes are black and the spots are light gray to dark black.

Hine (1911) indicates that *P. brevipennis* has the "...mesothoracic dorsum with the usual markings very plainly differentiated..." Like *P. gracilis*, the stripes narrow from anterior to posterior, but the median stripe is dark black, the paramedian stripe is lighter black with a darker margin, and the postsutural acrostichal stripe is reddish brown to black and connected to or blends into each side of the paramedian stripe. Anteriorly on either side of the stripes, the thorax is light reddish to reddish-orange/brown and extends to at least half of the postpronotal lobe. The mesothoracic spiracle is reddish brown to brown and the anepisternum is partially to completely black. The posthumeral spot is cup shaped with the "hollow" facing the paramedian stripe, reddish brown to black, and connected to the lighter margin of the presutural spot. The latter has a dark black, elongate area surrounded by a light to dark gray margin. The sutural spot, if present, is light gray and oval; it often blends into the presutural and postsutural spots. The postsutural spot is light gray to brownish and may have a darker convex, crescent-shaped area. The prescutellar spot is generally triangular, dark brown to black with a posterior shiny grayish area that extends onto the postalar callus.

Habitat and distribution in Florida

In the MCCA, as part of vegetation management, the St. Johns River Water Management District mows and roller drum chops scrub, scrubby flatwoods, and sandhill communities. Roads are usually mowed every year in the fall and other areas are mowed or rolled drum chopped every few years, depending on the height of vegetation.

Proctacanthus gracilis occurs in the mowed scrub communities and in mowed roads (Fig. 3) that pass through these communities with the vegetation shown in Table 1. In both of these habitats, 15 cm to 1 m tall grasses (Poaceae), sedges and nutrushes (Cyperaceae), and rushes (Juncaceae) are dominant. In the mowed scrub communities these plants are generally in areas that vary in size from approximately 30-276 m² and are surrounded by other plants, in particular fetterbush (Ericaceae, *Lyonia lucida* (Lam.) K. Koch], scrub oaks (Fagaceae, *Quercus* spp.), and some saw palmetto [Arecaceae, *Serenoa repens* (W. Bartram) Small].



Fig. 3. *Proctacanthus gracilis* habitat in road in mowed scrub vegetation community (Photograph: D.S. Dennis, 08.08.2013, 11:10 AM).

Proctacanthus brevipennis (Dennis, 2012), *P. fulviventris* Macquart, 1850 (Dennis, 2015), and *P. longus* (Wiedemann, 1821) (Dennis, 2019) also occur in the MCCA in mowed and roller drum chopped scrub communities. Bromley (1928) said that *Proctacanthus* "...inhabit dry fields or pastures, several being restricted to dry sandy plains." Hull (1962) commented that *Proctacanthus* are found in "...rank grassland and shrubs on the edges of woodlands in swampy country and some prefer sandy river banks."

Material examined: Bromley (1950) reported *P. gracilis* occurring in Florida in Hillsborough (County), Live Oak (Suwanee County), Sanford (Seminole County), and Wakulla (County). The FSCA has specimens of *P. gracilis* collected from the following locations in Florida with the indicated date of collection, collector, and sex of the robber fly, if known: ALACHUA COUNTY: 21.06.1954 (H.A. Denmark; 1 ♂ taken in dense woods), 24.06.1954 (H.V. Weems, Jr.; 1 ♀), 13.05.1955 (H.V. Weems, Jr.; 3 ♂♂), 04.07.1955 (H.V. Weems, Jr.; 1 ♂, 3 ♀♀), 24.07.1976 (1 ♂), 9.08.76 (1 ♂) (both collected by Lynn Dubose in CO₂ Bated South, Insect Flight Trap); Junction SR 225 & SR 340, 31.08.1976 (Lynn Dubose; 1 ♀); Gainesville, ? 08.1955 (J.D. Morrison, Jr.; 1 ♂), 08.10.1955 (F.L. Wilson; 1 ♀), 24.08.1957 (H.V. Weems, Jr.; 1 ♂), 25.06.1958 (H.V. Weems, Jr.; 1 ♀ In Wooded Ravine), 05.08.1962 (R.E. Woodruff; 1 ♂); 5.6 km NE Gainesville, Airport Area, 16.06.1991 (Lloyd R. Davis, Jr.; 4 ♂♂, 8 ♀♀; 1 ♂ with Orthoptera, Tettigoniidae prey), 16.06.1991 (Sara L. Davis; 2 ♂♂, 1 ♀). Montecocha, 08.06.1977 (1 ♂), 22.06.1977 (4

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♂♂), 29.06.1977 (1 ♂), 20.07.1977 (2 ♀♀), 03.08.1977 (1 ♂) (all collected by Jerry F. Butler in an Insect Flight Trap). BREVARD COUNTY: Melbourne, 09.07.1951 (Price, Beamers, Wood; sex unknown since abdomen missing). DUVAL COUNTY: Jacksonville, 21.06.1958 (Ribble; 1 ♀), 22.06.1963 (F.J. Santana; 1 ♀); NAS Cecil Field, Jacksonville, 08.08.1963 (F.J. Santana; 1 ♂). HILLSBOROUGH COUNTY: U.S.F. Campus, 25.05.1985 (1 ♀), 30.05.1985 (1 ♂), 13.06.1985 (1 ♂) (all collectors unknown). LEVI COUNTY: 30.06.56 (H.V. Weems, Jr.; 1 ♂, 1 ♀); Rosewood, 19.08.1991 (Lloyd R. Davis, Jr.; 1 ♂, 3 ♀♀). LIBERTY COUNTY: Torreya State Park, 04.07.1965 (H.V. Weems, Jr.; 1 ♂). OKALOOSA COUNTY: Choctawhatchee Bay, Niceville, 12.08.1959 (Bill Platt; 1 ♂ Caught on Screen). PUTNAM COUNTY: 06.07.1956 (H.V. Weems, Jr.; 1 ♀); Grandin, 19.08.1991 (2 ♂♂), 26.06.1992 (2 ♂♂) (both collected by Lloyd R. Davis, Jr.); 5.6 km NE of Grandin, 30.08.1991 (Lloyd R. Davis, Jr.; 1 ♀). SANTA ROSA COUNTY: 02.08.1955 (F.W. Mead; 1 ♀). ST. JOHNS COUNTY: 03.09.1955 (H.V. Weems, Jr.; 2 ♂♂).

Table 1. Vegetation in communities in which *Proctacanthus gracilis* was studied in the Moses Creek Conservation Area.

Vegetation Type ^c	Vegetation Community	
Family/Genus/Species/Common Name	Mowed Scrub Community	Mowed Vegetation in Roads in Mowed Scrub Community
Aquifoliaceae		
<i>Ilex opaca</i> Alton var. <i>opaca</i> /American holly	X	-
Arecaceae		
<i>Serenoa repens</i> (W. Bartram) Small/saw palmetto	X	-
Asteraceae		
<i>Carphephorus corymbosus</i> (Nutt.) Torr. & A. Gray/ coastalplain chaffhead (Florida paintbrush)	X	-
<i>Eupatorium</i> sp./fennel (thoroughwort)	X	X
<i>Erechtites hieraciifolius</i> (L.) Raf. ex DC./American burnweed (fireweed)	X	-
<i>Liatris tenuifolia</i> Nutt./ shortleaf gayfeather	X	X
<i>Pityopsis graminifolia</i> (Michx.) Nutt./narrowleaf silkgrass	X	-
Cyperaceae		
<i>Carex</i> sp./sedge	-	X
<i>Cyperus</i> spp./flatsedge	X	X
<i>Rhynchospora colorata</i> (L.) H. Pfeiff./starrush whitetop	-	X
<i>Rhynchospora</i> spp./beaksedge	X	-
<i>Scleria</i> sp./nutrush	X	X
Dennstaedtiaceae		
<i>Pteridium aquilinum</i> L. (Kuhn) var. <i>pseudocaudatum</i> (Clute) Clute ex A. Heller/tailed bracken	X	-
Ericaceae		
<i>Ceratiola ericoides</i> Michx./Florida rosemary (sand heath)	X	-
<i>Lyonia lucida</i> (Lam.) K. Koch/ fetterbush	X	-
Eriocaulaceae		
<i>Lachnocaulon</i> sp./bogbutton	X	-

Table 1. Continued.

Vegetation Type ^c	Vegetation Community	
Family/Genus/Species/Common Name	Mowed Scrub Community	Mowed Vegetation in Roads in Mowed Scrub Community
Fabaceae		
<i>Galactia elliotii</i> Nutt./Elliott's (white) milkpea	X	X
Fagaceae		
<i>Quercus</i> spp./scrub oaks	X	-
Haemodoraceae		
<i>Lachnanthes caroliana</i> (Lam.) Dandy/Carolina redroot	X	X
Hypoxidaceae		
<i>Hypoxis juncea</i> Sm./fringed yellow stargrass	-	X
Juncaceae		
<i>Juncus</i> sp./rush	X	-
Pinaceae		
<i>Pinus clausa</i> (Chapm. ex Engelm.) Vasey ex Sarg./sand pine	X	X
<i>Pinus elliotii</i> Engelm./slash pine	X	-
Poaceae		
<i>Andropogon glomeratus</i> (Walter) Britton et al. var. <i>glaucopsis</i> (Elliott) C. Mohr/purple bluestem	X	X
<i>Andropogon virginicus</i> L. var. <i>glaucus</i> Hack./chalky bluestem	-	X
<i>Aristida spiciformes</i> Elliott / bottlebrush threeawn	X	-
<i>Aristida stricta</i> Michx./wiregrass	X	-
<i>Sorghastrum secundum</i> (Elliott) Nash/lopsided Indiangrass	X	-
<i>Axonopus</i> sp./carpetgrass	-	X
Polygalaceae		
<i>Polygala lutea</i> L./orange milkwort	X	X
Saururaceae		
<i>Saururus cernuus</i> L./lizard's tail	X	X
Smilacaceae		
<i>Smilax auriculata</i> Walter/earleaf greenbrier vine	X	-
<i>Smilax bona-nox</i> L./saw greenbrier vine	X	-
Xyridaceae		
<i>Xyris</i> sp./yelloweyed grass	X	X

X = present; — = not present.

Resting behavior

Proctacanthus gracilis only rest on live vegetation, typically 5 cm-1 m above the ground with an average of 35 cm ($n=20$). They usually rest with their bodies parallel to the vegetation (e.g., grass stem) and vertical to the ground. Although sometimes they will straighten their hind legs and have their bodies at a 45° angle so that it looks like they are leaning forward. If they rest in a more horizontal position they may extend their fore legs so that their bodies are at a 45° angle with the abdomen almost touching the vegetation that they rest on. They usually remain in these positions for 19.0-33.5 minutes (average 24.4 minutes, $n=5$). Although one female remained vertical to the ground holding onto a dead sedge stalk for 1 hour and 43.5 minutes, with only four slight adjustments in her position and one short (20 cm) flight to another stalk during this period of time.

When resting, in particular in shade of surrounding vegetation, *P. gracilis* will usually remain stationary. A few move their heads in response to other insects flying by and one male groomed (four times) his fore tarsi and head with his fore tibiae and proximal part of the fore tarsi.

When *P. gracilis* are resting in shade they will often flatten themselves against the substrate that they are on. As their bodies become exposed to the sun, they will make other postural adjustments, including facing the sun or elevating one side of their bodies to the sun. These postural adjustments have been shown to maintain the body temperature of many other species of robber flies (Dennis & Lavigne, 1975; Morgan, Shelly, & Kimsey, 1985; Morgan & Shelly, 1988), including *P. brevipennis* (Dennis, 2012), *P. fulviventris* (Dennis, 2015), and *P. longus* (Dennis, 2019) in the MCCA.

If it starts to rain when *P. gracilis* is resting on vegetation stalks, they will hold their bodies closer to the stalk.

While resting and feeding, *P. gracilis* often expel drops of creamy white or white drops of liquid from their anus. Generally more (three to four) or larger drops are expelled during feeding than when resting.

Abdominal pumping of the first two abdominal segments was observed in one female while resting. Pumping of the first three abdominal segments was observed in one male during feeding. In the laboratory, Morgan and Shelly (1988) observed *Promachus giganteus* Hine, 1911 pumping haemolymph into the abdomen to regulate thoracic temperatures. *Proctacanthus gracilis* may exhibit similar behavior in the field to regulate its body temperature. Musso (1968) and Lavigne & Holland (1969) attributed abdominal pumping or contractions during feeding to the injection of proteolytic enzymes into prey and food pumping.

Foraging and feeding behavior

Proctacanthus gracilis almost always forage from the tops and sides of live and dead vegetation. Only one female foraged from the ground. When foraging, their bodies are either horizontal or parallel to the vegetation or they are at a 30-45° angle with the fore legs extended. The latter position is particularly common when the asilids face the sun, which presumably allows them to better see potential prey because of

backlighting. *Proctacanthus brevipennis* (Dennis, 2012), *P. fulviventris* (Dennis, 2015), *P. longus* (Dennis, 2019), *P. nearno* (Lavigne & Dennis, 1979), and *P. micans* (Dennis & Lavigne, 1975) have similar foraging positions.

Proctacanthus gracilis make three types of foraging flights: flights that are not directed at potential prey (i.e., orientation flights); flights directed at potential prey without coming into contact with them (i.e., investigatory flights); and flights when they capture and release potential prey or capture prey. After making these flights *P. gracilis* would sometimes re-land at their original foraging position or within 5-15 cm of their original position (n=8), but most of the time they would re-land 20.0 cm-6.1 m (average 1.2 m; n=35) from their original position.

Proctacanthus gracilis will often remain in the same location for 2-11 minutes (average 6.6 minutes; n=6) between making orientation flights and moving to new locations. Flights to new locations are direct and do not involve weaving in and out of vegetation.

Investigatory flights are for distances of 5.0-60.0 cm (average 11.4 cm; n=18) to the side of or in front of a foraging position, and 10.0-50.0 cm (average 27.8 cm; n=17) above the ground. Both male and female *P. gracilis* would often follow grasshoppers until they landed on vegetation or on the ground and then the robber fly would stop pursuing the potential prey. This indicates that visual stimuli, including prey movement, are required to elicit predatory response from *P. gracilis*. Lavigne & Holland (1969) found that the robber flies they studied reacted to lure motion and size.

Foraging flights when *P. gracilis* capture and release prey are also made to the side of or in front of their foraging positions. These flights are made for distances of 5.0-61.0 cm (average 32.8 cm; n=4) and 15.0-30.0 cm (average 23.8; n=4) above the ground.

Proctacanthus gracilis captures all of its prey in the air when the prey are within 30.0-150 cm (average 65.8 cm; n=8) behind, in front of, or to the side of their foraging location and 7.5-91.4 cm (average 33.4 cm; n=8) above the ground. *Proctacanthus brevipennis* (Dennis, 2012), *P. fulviventris* (Dennis, 2015), and *P. longus* (Dennis, 2019) captured all of their prey in flight; whereas, *P. micans* (Dennis and Lavigne, 1975), and *P. nearno* (Lavigne and Dennis, 1979) captured most of their prey in flight. *Proctacanthus micans* also captured prey on vegetation and *P. nearno* captured some prey as they were landing on the ground.

When capturing large prey, such as adult grasshoppers, *P. gracilis* would either immediately insert its proboscis and then fall into vegetation holding onto prey with all six tarsi or fall into vegetation and insert its proboscis while holding onto prey with the tarsi. The asilids would hold onto the captured prey for up to seven minutes before standing up on the vegetation. One male asilid fell into vegetation holding onto the grasshopper prey with all six tarsi and then released the grasshopper without inserting its proboscis.

After capturing small prey, such as grasshopper nymphs or some Diptera, *P. gracilis* would hover, hold onto the prey with all six tarsi, insert its proboscis, and then land on vegetation. They insert the proboscis most frequently in the side or back of the prey's head. They inserted the proboscis into the side or dorsal surface of the thorax in a few prey captures. In one adult grasshopper the proboscis was inserted in the

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side of the neck; and in another adult grasshopper the proboscis was inserted in the side of the abdomen just posterior of the hind leg.

Male and female *P. gracilis* captured prey that are approximately the same length. Average prey length for males is 16.6 mm (n=19) with a range of 10.5-35.0 mm. For females the average prey length is 16.2 mm (n=27) with a range of 9.0-33.0 mm. The overall mean prey length is 16.4 mm with a predator to prey ratio of 1.7:1.0 which indicates that *P. gracilis* is between 1.5 to 2 times larger than its prey. Mean predator to prey ratios for *P. brevipennis* (Dennis, 2012), *P. fulviventris* (Dennis, 2015), *P. longus* (Dennis, 2019), and *P. micans* (Dennis and Lavigne, 1975), are 3.0:1.0, 1.9:1.0, 1.7:1.0, and 2.0:1.0, respectively. Mean predator to prey ratios for other species of robber flies range from 0.9:1.0 to 8.4:1.0 (Dennis, 2016), with a mean of 2.8:1.0.

Proctacanthus gracilis moves to a new location up to eight times while feeding on prey. When it moves, it is to a location that averages 95.2 cm (range 2.5 cm to 4.6 m; n=23) from the previous location.

During feeding, *P. gracilis* either does not manipulate small prey (e.g., grasshopper nymphs and Diptera) or it manipulates them up to two times with all six tarsi in a hover above the feeding site. It also manipulates large prey (e.g., adult grasshoppers) up to two times while holding the prey against vegetation and crawling on or moving the prey with a combination of tarsi, before reinserting its proboscis.

When *P. gracilis* is feeding, prey less than 13.5 mm long (e.g., *Apis mellifera* L., 1878) generally hang free from the asilid's proboscis without support by the tarsi or vegetation. Prey around 18.8 mm long [smaller *Orphulella pelidna* (Burmeister, 1838); Acrididae] would hang free or be supported by a combination of tarsi. For longer prey greater than approximately 24.5 mm [e.g., *Spharagemon marmorata* (Harris, 1841); Acrididae], an asilid would use its body to hold prey against vegetation while grasping the vegetation with its tarsi or would hold vegetation with a combination of tarsi and support the prey with the rest of its tarsi.

Nine complete *P. gracilis* feedings were observed and ranged from 17-355 minutes (5 hours and 55 minutes) with an average of 167 minutes (2 hours and 47 minutes). The time spent feeding correlates with prey length. One asilid fed on an unidentified grasshopper nymph, with a length of 13 mm, for 49 minutes; whereas, *P. gracilis* fed on adult grasshoppers with lengths ranging from 20.5-33.0 mm, for 105-355 minutes (average about 258 minutes; n=5). For a number of other species of robber flies, the time spent feeding usually depends on prey length (Dennis, 2019).

At the completion of feeding, *P. gracilis* discards prey in one of three ways: (1) it pushes prey off its proboscis with the fore tarsi while still at the feeding site (n=1); (2) it allows prey to drop off its proboscis at the feeding site (n=4); or (3) it drops prey in flight as it moves to a new location (n=6). The latter is the most common way of discarding prey. Other species of *Proctacanthus* use similar methods to discard prey (Dennis, 2019). In addition, *P. fulviventris* (Dennis, 2015), *P. longus* (Dennis, 2019), and *P. micans* (Dennis & Lavigne, 1975) drop prey while hovering at the feeding site; and *P. micans* pushes prey off its proboscis with the fore tarsi during its flight to a new location.

Because of the length of feedings and flight speeds flown to new locations after feeding, inter-feeding times (time between feedings) were difficult to obtain. Thus, only one inter-feeding time of 66.5 minutes, was obtained.

The theoretical number of prey an individual *P. gracilis* can feed on in one day can be calculated if one assumes that: (1) it continually forages and feeds between 9:00 AM and 3:00 PM (the time when individuals were found with prey), and (2) it captures and feeds on prey every 233.5 minutes (the average feeding time and one inter-feeding time). Therefore, over a 6-hour period (360 minutes) an individual can feed on approximately 1 to 2 prey. Species of *Proctacanthus* that generally feed on smaller prey and/or have shorter feeding and inter-feeding times can feed on an estimated 3 to 8 prey per day (Dennis, 2012, 2015, 2019; Dennis & Lavigne, 1975). Dennis (2016) reported that other investigators estimate robber flies feed on 1 to 35 prey per day.

Prey

Proctacanthus gracilis feed on Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Neuroptera, Odonata, and Orthoptera (Table 2). However, the majority of prey is Orthoptera (63.0%) with each of the other orders making up 2.2-8.5% of the prey. Bromley (1950) reported *P. gracilis* preying on honeybees (*Apis mellifera*) in a bee yard in Live Oak, Florida, during August. Fattig (1945) also reported *P. gracilis* feeding on honeybees in Georgia in August and on the robber fly *Megaphorus clausicellus* (Macquart, 1850) (as *Mallophora clausicella*).

Dennis (2012) reported *P. brevipennis* feeding on six insect orders (Coleoptera, Diptera, Hymenoptera, Isoptera, Lepidoptera, and Orthoptera) with most prey Coleoptera (59.7%) and Hymenoptera (17.7%). *Proctacanthus fulviventris* (Dennis, 2015) fed on only Diptera (12.0%) and Hymenoptera (88.0%), and *P. longus* (Dennis, 2019) fed on Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Neuroptera, and Orthoptera with most prey Diptera (43.3%) and Orthoptera (24.3%).

The following is a list of prey taken by *P. gracilis* with the number and sex of the predator following the prey record.

COLEOPTERA, Cicindelidae: *Cicindela hirtipes* LeConte, 1875, 11.06.2013 (1 ♀), 12.06.2013 (1 ♂). Elateridae: *Blauta cribraria* (Germar, 1843), 17.06.2014 (1 ♀). DIPTERA, Asilidae: *Efferia tabescens* 12.07.2017 (1 ♂); *Proctacanthus gracilis* Bromley, 1928, 05.08.2014 (1 ♂). Tabanidae: *Tabanus* sp. poss. *gracilis* Wiedemann, 1828, 12.06.2014 (1 ♀). Unidentified Family: 23.05.2016 (1 ♂). HEMIPTERA, Homoptera, Cicadidae: *Cicadetta calliope floridensis* (Davis, 1920), 08.08.2011 (1 ♀), 17.06.2013 (1 ♀). HYMENOPTERA, Apidae: *Apis mellifera* L., 1878, 03.06.2016 (1 ♂). Vespidae: *Vespula maculifrons* (Buysson, 1905), 12.07.2011 (1 ♂). Unidentified Family: 12.06.2014 (1 ♀). LEPIDOPTERA, Unidentified: moth, 23.05.2016 (1 ♂). NEUROPTERA, Myrmeleontidae: *Myrmeleon carolinus* Banks, 1943, 29.07.2011 (1 ♂). ODONATA, Libellulidae: *Erythrodiplax minuscula* Rambur, 1842, 24.07.15 (1 ♀). ORTHOPTERA, Acrididae: *Achurum carinatum* (Walker, 1870), 05.08.2014 (1 ♂); *Amblytropidia mysteca* (Saussure, 1861), 03.06.16 (1 ♀); *Chortophaga australior* Rehn and Hebard, 1911, 01.06.2016 (1 ♀); *Orphulella pelidna* (Burmeister, 1838),

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05.07.2011 (1 ♀), 14.07.2011 (1 ♀), 09.07.2012 (1 ♀), 16.07.2013 (1 ♂), 25.07.2013 (1 ♀), 30.07.2013 (1 ♀), 15.07.2014 (1 ♂), 22.07.2014 (1 ♀), 23.07.2014 (1 ♀), 29.07.2014 (1 ♂), 02.07.2015 (1 ♀), 10.07.2015 (1 ♂), 13.07.2015 (1 ♂), 15.07.2016 (1 ♀), 20.07.2017 (1 ♂); *Spharagemon cristatum* Scudder, 1875, 02.06.2011 (1 ♀); *Spharagemon marmorata* (Harris, 1841), 09.07.2015 (1 ♂); unidentified, 03.06.11 (1 ♀), 15.07.2014 (1 ♀), 22.07.2014 (1 ♀), 16.06.2015 (1 ♂), 01.08.2017 (1 ♀); unidentified nymphs, 31.07.2013 (1 ♀), 08.08.2013 (1 ♀), 09.08.2013 (1 ♂). Tettigoniidae: unidentified, 01.08.2014 (1 ♀). UNIDENTIFIED Order: 05.07.11 (1 ♀), 17.06.13 (1 ♂).

Table 2. Number and percent composition of orders of prey taken by *Proctacanthus gracilis*.

Order	Male		Female		Total	
	Number	Percent	Number	Percent	Number	Percent
Coleoptera	1	5.3	2	7.4	3	6.5
Diptera	3	15.7	1	3.7	4	8.8
Hemiptera	0	0	2	7.4	2	4.3
Hymenoptera	2	10.5	1	3.7	3	6.5
Lepidoptera	1	5.3	0	0	1	2.2
Neuroptera	1	5.3	0	0	1	2.2
Odonata	0	0	1	3.7	1	2.2
Orthoptera	10	52.6	19	70.4	29	63.0
Unidentified	1	5.3	1	3.7	2	4.3
Totals	19	100.0	27	100.0	46	100.0

Male and female *P. gracilis* generally feed on the same insect orders, although more prey insects are usually collected for females than for males. Many investigators have reported collecting more female robber flies with prey than males with prey (Dennis, 2016).

Mating behavior

Male *P. gracilis* perform searching flights for receptive females with which to mate. Searching flights consist of males flying in an erratic pattern or weaving in and out of vegetation. Sometimes, depending on the distance flown, there would be four to five slight vertical undulations. When actively searching, males would make a flight every 10-75 seconds (average approximately 38 seconds; n=18) for distances of 1.5-10.1 m (average 4.2 m; n=29), at 10.0-30.0 cm (average 15.3 cm; n=23) above the ground. Male searching flights have been described for *P. brevipennis* (Dennis, 2012), *P. fulviventris* (Dennis, 2015), *P. longus* (Dennis, 2019), and *P. micans* (Dennis and Lavigne, 1975).

Proctacanthus gracilis usually initiates matings in-flight when a male lands on the dorsum of a female's thorax and clasps the female's genitalia from below. Then the pair fall to the ground or into vegetation where they straighten out in the tail-to-tail position. One male landed on the dorsum of a female shortly after she landed on vegetation and clasped her genitalia. Then the pair straightened out in the tail-to-tail position. Some males had trouble clasping females in flight and were not successful

until after the pair fell to the ground or into vegetation. Even then the males would often take a few seconds to clasp the female's genitalia. One male unsuccessfully tried to clasp a female's genitalia for 205 seconds before the female flew off.

After mating begins, the pair sometimes flies to a vertical position on a grass stem in the shade of surrounding vegetation, approximately 30-60 cm above the ground. Either both sexes hold onto the stem or one holds onto the stem with its head up and the other hangs free with its head down.

When one mating pair had been in the tail-to-tail position for 39 minutes, another male landed on the female (Fig. 4). He then proceeded to try and mate with the female and in the process clasped and unclasped both the mating male's and female's genitalia. After 27.5 minutes, the second male flew off.



Fig. 4. Mating pair of *Proctacanthus gracilis* in tail-to-tail position with another male attempting to mate with the female (Photograph: D.S. Dennis, 08.08.2013, 10:59 AM).

The asilids' wings are generally closed during mating. One male had his left wing slightly open during mating. Another male opened and closed his wings slightly after approximately 112 minutes of mating, and after 118 minutes of mating opened and closed his wings to a 45° angle and then a 30° angle. The female of this same mating pair held onto a grass stem below the male and after both 93.5 and 114 minutes of mating released the grass stem, swung up and grasped the male so that she briefly faced the male and then resumed her position holding onto the grass stem below the male. The female unsuccessfully tried to grab the male again after 125 and 135.5 minutes of mating, after which the male released the female and both asilids flew off.

During mating, *P. gracilis* usually move very little, except when exposed to the sun. Then they relocate to a position in the shade where it is slightly cooler. Mating occurs when the air temperature at the height of mating in the sun range from 30.0-36.0°C (average 33.7°C; n=10) and in the shade range from 30.5-34.0°C (average 32.8°C; n=7).

At the completion of mating, male *P. gracilis* unclasp the female's genitalia and both asilids fly off or the pair fly into the air in the tail-to-tail position and then separate.

The author observed four complete matings of *P. gracilis* that lasted 66.0 to 135.5 minutes with an average of 94.0 minutes. *Proctacanthus brevipennis* mated for 78.0 to 111.0 minutes with an average of 90.0 minutes (Dennis, 2012); *P. fulviventris* mated for 30.0 to 63.5 minutes with an average of 40.6 minutes (Dennis, 2015); *P. longus* mated for 106.0 to 116.0 minutes with an average of 111.0 minutes (Dennis, 2019); and *P. micans* mated for 23.0 to 66.0 minutes with an average of 42.0 minutes (Dennis and Lavigne, 1975).

Oviposition behavior

One oviposition was observed at 11:03 AM (19.07.2011) in a road that consisted of compacted sugar sand (fine silt made up of ultrafine mineral sand mixed with a large percentage of organic granules). The female was in the shade of surrounding vegetation and inserted her ovipositor in the sand approximately 6 mm. She had her wings closed over her slightly curved abdomen and oviposited for 53 seconds. Then the female quickly withdrew her ovipositor from the sand and swept the sand with the tip of her ovipositor for 27 seconds, and flew off. Sand was collected and examined for eggs, but none were found.

The air temperature above the oviposition site was 30.0°C; the sand surface temperature was 29.5°C, and just below the surface, the temperature was 29.0°C.

At a later date (15.08.2014), a female was observed probing the ground with her ovipositor at 11:01 AM when the sky was overcast. The air temperature was 29.5°C and the surface temperature of the ground was 32.5°C.

Grooming behavior

Proctacanthus gracilis, like *Holopogon snowi* (Dennis, 2018) and *H. phaeonotus* (Dennis, 2014), did not frequently groom themselves. This may be because these asilids occupy various heights on vegetation and do not frequently land on or spend much time on the ground. When *P. gracilis* did groom it was in much the same way as reported for other species of *Proctacanthus* and other robber flies (Dennis, 2019). They always use the fore legs to groom their heads, and the hind legs to groom their wings and abdomen. Grooming of the head follows feeding, and grooming of the abdomen follows mating, although *P. gracilis* also frequently groom while resting and between foraging flights.

Grooming of the head is sometimes preceded by rubbing together of the fore tarsi. When grooming the head, *P. gracilis* uses the distal part of the fore femora (Fig. 5), the entire tibiae, and the proximal part of the tarsi, or only the tibiae and/or tarsi. Also, they often turn their head while grooming.

Proctacanthus gracilis sometimes rub the hind tarsi together before curving the abdomen down and grooming 1/4 to 1/2 of the abdomen and 1/4 to 1/3 of the wings. One female pulled her abdomen down to a 90° angle with her hind legs before grooming her abdomen and wings. Grooming of the wings and abdomen always proceeded from anterior to posterior with the hind tibiae and/or tarsi. When the wings are closed, only the top surface is groomed; when the wings are spread at a 30-45° angle to the body, both the tops and bottoms of the wings are groomed outward.



Fig. 5. Male *Proctacanthus gracilis* grooming head (Photograph: D.S. Dennis, 28.08.2014, 9:01 AM).

Daily rhythm of activity

Proctacanthus gracilis exhibit a diurnal or daily rhythm of activity between 9:00 AM and 3:00 PM for feeding and mating, with most activity between 9:00 AM and 12:00 noon (84.8% of feedings and 92.4% of matings). As indicated above, only one oviposition was observed at 11:03 AM.

The number of feeding *P. gracilis* peaked in the morning between 10:00-11:00 AM (37.0%) and then tapered off until the last feedings were observed between 1:00-3:00 PM (4.4%). The number of mating *P. gracilis* initially peaked between 9:00-10:00 AM (38.5%) and continued through the peak feeding period between 10:00-11:00 AM (38.5%). There was a second small mating peak in the afternoon between 2:00-3:00 PM (7.7%).

In the MCCA and a nearby storm water drainage basin, the peak *P. brevipennis* period of feeding (10:00-11:00 AM) was before the peaks for mating (1:00-2:00 PM) and ovipositing (2:00-3:00 PM) (Dennis, 2012). For *P. fulviventris* these behaviors in the MCCA had similar patterns and all peaked between 10:00-11:00 AM, with ovipositing having the largest peak (Dennis, 2015). *Proctacanthus longus* had a peak period of feeding from 10:00-11:00 AM, which was between the peak periods of mating (9:00-10:00 AM) and ovipositing (11:00 AM-12:00 noon) (Dennis, 2019).

Förster, Nitabach, & Holmes (2011) reported that, "Insects display an impressive variety of daily rhythms, which are most evident in their behaviour. Circadian timekeeping systems that generate these daily rhythms of physiology and behaviour all involve three interacting elements: the timekeeper itself (i.e., the clock), inputs to the clock through which it entrains and otherwise responds to environmental cues such as light and temperature, and outputs from the clock through which it imposes daily rhythms on various physiological and behavioural parameters." Lavigne, Dennis, & Gowen (2000) indicated that a number of environmental variables (e.g., temperature, wind, cloudy weather) affect the behavior of robber flies, with temperature one of the most important.

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Temperature appears to affect the behavior of the different species of *Proctacanthus* studied in the MCCA. With the lower average spring temperatures (Table 3), *P. brevipennis* has peak periods of feeding, mating and ovipositing that occur throughout the day. Whereas, as the average temperature increases during the summer, in particular in the afternoon, *P. fulviventris*, *P. gracilis*, and *P. longus* exhibit the major peak periods of these behaviors in the morning.

Table 3. Months with most behavioral observations, peak periods of behaviors, and air temperatures for *Proctacanthus brevipennis*, *P. fulviventris*, *P. gracilis*, and *P. longus* in the MCCA.

Species	Months With Most Behavioral Observations	Major Peak Periods of Behaviors	Air Temperature (°C)	
			Range	Average (n)
<i>P. brevipennis</i>	April through May	10:00 AM-3:00 PM	17.5-35.8	30.3 (42)
<i>P. fulviventris</i>	June through August	10:00-11:00 AM	27.8-39.0	32.6 (104)
<i>P. gracilis</i>	June through August	9:00-11:00 AM	28.0-36.0	32.5 (21)
<i>P. longus</i>	June through July	9:00 AM-12:00 noon	28.0-37.0	31.8 (54)

Depending on the species, the behaviors include feeding, mating, and ovipositing.

Predators and parasites

One male *P. gracilis* captured a female in flight and inserted his proboscis between the female's dorsal and left posterolateral side of her thorax. Cannibalism is often reported for robber flies (Lavigne et al, 2000).

A female *Promachus bastardii* (Macquart, 1838) was found feeding on a male *P. gracilis*.

Campsomeris plumipes (Drury, 1770) (Scoliidae) wasps repeatedly hover around *P. gracilis* while they are on vegetation feeding on prey. This causes the robber flies to move up to 1.5 m from their previous location to avoid the wasps. One female *P. gracilis* feeding on a grasshopper (*Spharagemon cristatum*) moved eight times.

There are a number of ants (Formicidae, *Formica* spp. and *Solenopsis invicta* Buren, 1972) in the same habitats as *P. gracilis*. Sometimes the ants disturb the asilids while they are feeding, causing the asilids to move to a new location.

Mites are found on the sides of the thorax of *P. gracilis*. According to Lavigne et al (2000), mites are often found on the thoraxes of robber flies.

The six-lined racerunner [*Cnemidophorus sexlineatus* (Linnaeus, 1766)] is a common lizard in the MCAA. One crawled under a female *P. gracilis* resting on vegetation and this resulted in the female flying to a new location. Although racerunners are known to be insectivorous, none preyed on *P. gracilis*.

CONCLUSIONS

The stripes and spots on the mesonotum are one morphological characteristic that can be used to separate *P. gracilis* from other species of *Proctacanthus* in the MCCA. *Proctacanthus gracilis* rests on live vegetation and forages from both live and dead vegetation. They capture prey in flight, and prey consists of Othoptera (63.0%), Diptera (8.8%), Coleoptera and Hymenoptera (each 6.5%), Hemiptera and

unidentified (each 4.3%), and Lepidoptera, Neuroptera and Odonata (each 2.2%). During feeding, *P. gracilis* crawls on prey and manipulates it with a combination of tarsi or manipulates it while hovering above the feeding site. Males search for receptive females with which to mate, but there is no courtship prior to mating. Mating occurs in the tail-to-tail position. Females oviposit in the ground. Peak period for feeding and mating is from 9:00 to 11:00 AM. Grooming is in much the same manner as other asilids. *Proctacanthus gracilis* exhibits cannibalism and is preyed upon by *Promachus bastardii*. Mites are found on the sides of the thorax of *P. gracilis*.

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Morphometric Analysis of the Oil Palm Pollinating Weevil, *Elaeodobius kamerunicus* (Faust, 1878) (Coleoptera: Curculionidae) from Oil Palm Plantations in Malaysia

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ABSTRACT

The pollinating weevil, *Elaeodobius kamerunicus* was introduced to Malaysia as an oil palm pollinator agent in 1981. After the introduction, the weevil has distributed widely in the oil palm plantation around Malaysia. This study aims to investigate the morphometric differences in *Elaeodobius kamerunicus* population within Malaysia, as well as from its originating country, Cameroon. A total of 540 weevils were collected from eight localities in Malaysia, and 60 from Cameroon, Africa. The five morphological measurements were taken ie. total body length (TL), elytra length (EL), pronotum length (PL), body width (BW) and the snout length (SL) using the Portable Capture Pro v2.1 Dinolite. The one-way ANOVA indicated significant differences amongst the five morphological characteristics between the weevil populations for both males and females from the eight localities ($P < 0.05$), respectively. For the male and female populations at different localities, the Principal Component Analysis explained 79.0% and 76.6%, respectively. Moreover, the Canonical Discriminant Function explained 95.5% and 90.2% of the variance for male and female population, respectively. Both the result of PCA and CDA conducted showed little variation between the eight localities for male and female weevil populations in Malaysia respectively. This study showed slight differences in morphometric characters for the weevil population in Malaysia.

Key words: *Elaeis guineensis*, *Elaeodobius kamerunicus*, principal component analysis, canonical discriminant analysis, morphological characters.

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INTRODUCTION

The oil palm, *Elaeis guineensis* (Arecales: Arecaceae), a monoecious plant that contains both male and female flowers on a single tree is one of the most extensively cultivated plantation crops in many parts of the world (Sambathkumar & Ranjith, 2011). *Elaeis guineensis* are known to be most important industrial crop in Malaysia. It originated from West Africa and being introduced to Malaysia as landscape plants in early 19 centuries. Then, oil palm has become as one of the most importance industrial crop and powered the economic growth of Malaysia. Today, approximately 5.9 million hectares of land in Malaysia is under oil palm cultivation; producing 19.86 million tonnes of palm oil and 2.32 tonnes of palm kernel oil (Parvez et al., 2020). Malaysia is one the largest producers and exporters of palm oil in the world, accounting for 11% of the world's oils & fats production and 27% of export trade of oils & fats (Malaysian Palm Oil Council).

Until the late 1970s, due to poor yield and fruit developments, hand-pollination or assisted pollination had to be carried out by most plantations, especially for younger palms (Hussein, Lajis, & Ali, 1991). In the early 1980s, the oil palm pollinating weevil, *Elaeidobius kamerunicus* (Coleoptera: Curculionidae), was introduced into the plantations to overcome the problem of inconsistencies in oil palm pollination (Syed, Law, & Corley, 1982). It is well known as one of the main pollinating insects in oil palm plantations.

Elaeidobius kamerunicus has an important role in pollinating oil palm plants. Pollination occurs because this beetle is attracted by the scent of flowers, especially male flowers that are stronger in aroma than female flowers. When perched on male flowers, pollen will stick to his body. Then when perched on a receptive (blooming) female flower, pollen will be released from the beetle and pollinate female flowers (Setyamidjaja, 2006). In addition, this beetle is harmless and does not damage other plants, because this beetle can only eat and reproduce in oil palm male flowers (Syed, 1982). The study by Luqman et al. (2018) reported that the abundance of *E. kamerunicus* are highest in the oil palm plantation due to the presence of more male flowers of oil palm where they live, feed and breed as they are highly dependent on them.

Through its introduction, oil palm pollination significantly improved resulting in better fruit set levels and oil extraction ratios and hence increased yields (Caudwell, Hunt, Reid, & Mensah, 2003). The introduction of the oil palm pollinating weevil into Malaysia has saved the industry an estimated US\$ 60 million annually, mostly due to the discontinuation of assisted pollination practices (Hussein et al, 1991; Caudwell et al, 2003). However, there has been a growing concern regarding the periodic occurrence of poor pollination and yield loss in certain Malaysian oil palm plantations (Teo, 2015). In several areas in Indonesia, the fruit set formation was so poor that they had to reintroduce the assisted pollination program as a solution (Prasetyo, Arif, & Hidayat, 2012; Prasetyo, 2013). Factors that could contribute to these problems are, (1) poor pollination activity due to the low weevil population (Prasetyo, Purba, & Susanto, 2014) (2) inbreeding depression (3) parasitism by female nematode *Elaeolenchus*

parthenonema (Nematoda: Anandranematidae) (Rao & Law, 1998; Poinar, Jackson, Bell, & Wahid, 2002) (4) changing diets of predators favoring to prey on oil palm pollinating weevil (Muhammad-Luqman, Izfa-Riza, Idris, & Dzulhelmi, 2017) and (5) weather change (Dhileepan, 1994; Prasetyo, Supriyanto, Susanto, & Purba, 2010; Melendez & Ponce, 2016). According to Nurul Fatihah et al., (2019), the amount of rainfall was found to positively correlate with the *E. kamerunicus* population.

The oil palm pollinating weevil is dark brown to black and small with the average length size of the adult weevil is between 1.8 to 4.0 mm (O'Brien & Woodruff, 1986). Males have short rostrum, are hairier, having wing cases with small tubercles and are generally larger (3-4 mm), while the females have longer rostrum, less hair, absence of small tubercles on wing cases and smaller in size (2-3 mm) (Aisagbonhi et al, 2004; Kurniawan, 2010; Ayuningsih, 2013). The duration for the oil palm pollinating weevil to develop from an egg to larva I stage, followed by larva II, then larva III before forming a pupa, then becoming an adult, covers 10-25 days and the life span of the weevil is between 15-17 days (Syed et al, 1982; Herlinda, Pujiastuti, Adam, & Thalib, 2006; Meliala, 2008; Tuo, Koua, & Hala, 2011). According to Fitriani, Bakti, Prasetyo, & Rozziansha (2018), the span for the egg to develop into larva I took about two days, to larval II about two days, to larval III about five days, and from larval III to adults in two days, while the adult took about 10-14 days for living.

Morphometric measurements in insects are important tools to evaluate variations because the exoskeleton can be easily measured and physical distortions rarely occur as in the soft parts of other animal groups (Thiago, Femanda, & Marconi, 2011). The analysis of their shape and size should be one of the earliest parameters to be established before other factors are examined (Adams & Rolhlf, 2000). A study by Siti-Nurlydia et al., (2018), successfully proved that *Rhynchophorus vulneratus* and *R. ferrugineus* are morphologically distinct species based on the morphometric study. For a commodity industry, monitoring one of the main oil palm pollinating weevils is extremely important, especially when its productivity relies on the ecological services provided by these insects. Slight differences in their morphometric characteristics are one of many indications on the possible effects of the local environmental conditions, climatic situation and gene pool on the weevils (Inward, Davies, Pergande, Denham, & Vogler, 2011). This could further impact their life cycle, reproduction and general behavior (Den Boer, 1985; Matalin, 2007; Sukhodolskaya, 2016).

After more than 35 years from the first introduction of weevils in Malaysia, prolonged exposure to biotic and abiotic factors, different farming practices in various oil palm plantations may have directly and indirectly affected the morphological characteristics of the oil palm pollinating weevil (Nurul Fatihah et al., 2019). Therefore, in recent years, Nurul Fatihah et al., (2019) has conducted a study on the morphological difference of *E. kamerunicus* at three different locations (countries), namely Malaysia, Liberia, and Indonesia. The results by Nurul Fatihah et al., (2019) suggested that the separation of *E. kamerunicus* existed among the three different countries.

In consideration of the Malaysian population, this study aims to investigate the morphometric differences and variations among the weevil population in oil palm

plantation around Malaysia, ie. Perlis, Pahang, Perak, Terengganu, Johor, Selangor, Sabah and Sarawak.

MATERIAL AND METHODS

Samples of weevils were collected from eight different oil palm plantations in Malaysia (Fig. 1), which planted with seed-derived commercial DxP materials, aged between seven to 12 years after planting. The *E. kamerunicus* samples are also collected from Cameroon Africa and treated as an outgroup (Table 1). The spikelets of fully anthesized flowers occupied by weevils were sampled.

Sixty (60) weevils consisting of thirty males and thirty female individuals were taken from each population, respectively from the fully anthesized spikelet. Morphometric characters, treated as a variable was measured using portable capture digital microscope (portable capture Pro v2.1 Dinolite). The five variables measured were total body length (TL), elytra length (EL), pronotum length (PL), body width (BW) and the snout length (SL) (Nurul-Fatihah et al., 2019). One-way ANOVA tests were calculated to determine the presence of significant difference amongst the five morphological characters between the male and female weevil populations from the eight localities in Malaysia. Following that, the male and female weevils were pooled while maintaining their morphological characters and localities. A t-test was then run to determine whether there were significant differences between the five morphological characters, regardless of the sex of the weevils in Malaysia. Principal component analysis and canonical discriminant analysis were then applied to confirm if each of the weevil population from the eight localities forms distinct units. The analyses were done using the MINITAB version 17 for Principal Component Analysis (PCA) and SPSS version 12 to run Canonical Discriminant Analysis (CDA).

RESULTS AND DISCUSSION

A total of 540 weevils (270 males and 270 females) were successfully measured according to the five variables, ie TL: Total body length; EL: Elytra length; HL: Pronotum length; BW: Body width; SL: Snout length. The mean of each variable, the male and female for each location are presented in Table 2. The weevils' sample from Perlis showed the smallest in all the morphological characters while the largest was from the Johor population (Figs. 2-6). There was a significant difference in all measured characters amongst the weevil populations, both in male and female, from the different localities (One-way ANOVA; $P < 0.05$) (Table 3).

Based on the sex of the weevil, there was a difference in the morphometric evaluation (Table 4). The morphological size variation between the male and female weevils was discernible merely on their appearance. This is following the report by Nurul Fatimah et al., (2019) which morphometric characters were significantly contributed to the separation of sexes in *E. kamerunicus*.

Morphometrical characters are influenced by the interaction between environmental conditions and the genetic population (Zahiri, Sarafrazi, Salehi, & Kunkel, 2006). However, the previous study on insects with specific host plants showed that the phenotype of each individual was affected far more strongly by its host plant than by the genetic differences among populations (Wool & Hales, 1997). In the case of the oil palm, the weevil populations exhibited many similarities in their morphological characters. The variation in the beetle's body form and size is highly likely to be related to the specific habitat demands on their physiological and behavioral characteristics (Den Boer, 1986). Intraspecific sexual dimorphism is a phenomenon widely distributed among animals (Fairbairn, 1997; Blanckenhorn, 2005). In many beetles, female tends to be much larger than male, a reason generally associated with the reproductive features where the dimorphism is conspicuous (Teder & Tammaru, 2005), although there were no significant differences in body size between sexes (e.g. Ferreira, Pires, Guedes, Mendes, & Coelho, 2006; Brygadyrenko & Slynko, 2015). On the contrary, the male of the oil palm pollinating weevil is much larger than the female in body size except the snout length. The female weevils have longer snouts that could be associated with a better protrusion to assist in placing the eggs deeper into the inflorescence during the anthesis of the male flower.

To evaluate the existence of differences in the morphological characters of the weevil populations from the eight localities in Malaysia and Cameroon as an outgroup, multivariate examination, Principal Component Analysis (PCA) and Canonical Discriminate Analysis (CDA) were implemented. Canonical Discriminate Analysis is based on original variables extracted from linear combinations aimed at quantifying the relationship between categorical variables. Meanwhile, PCA aims at identifying and explaining the maximum number of variances in the multivariate hyperspace of the data set (Huntley, 2011; Sorbolini et al, 2016). Most of the total variance was accounted for the first two principal components analysis (PCA) for the score plot graph.

Based on PCA, four principal components were extracted, with the first two components accounting for most of the total variance. Moreover, the five variables were well represented in the first two principal components based on their loading values. Both the male and female weevil populations for Principal Component 1 (PC1) generally showed high positive loading values for all parameters (Table 5 and Table 6). The range of loading values for the male weevil population is between 0.022 to 0.819 with the highest eigenvalue of 2.846 and explained by 57.0% variation as the main contributor. Principal Component 2 (PC2) accounted for only 22.1% of the variance, with an eigenvalue of 1.103. Based on the two extracted principal components, a total cumulative variation of 79.1% was achieved (Table 5). The female weevil population showed a similar trend, whereby the Principal Component 1 (PC1) with a range of loading values from 0.009 to 0.807 resulted in the highest eigenvalue at 2.967 associated with a 59.3% total variation. Principal Component 2 (PC2) had an eigenvalue of 0.862 accounting for only 17.2% of the variance. Based on the extracted principal components, a cumulative variation at 76.6% was achieved (Table 6).

The scatter plot showed that male weevil populations within the eight localities from Malaysia clustered together but formed a distinct cluster with weevils from Cameroon (Fig. 7). Meanwhile, non-distinguishable clusters were observed with the female weevil populations (Fig. 8).

Based on CDA, five canonical functions were extracted. For the male weevil populations, Canonical Discriminant 1 (CD1) had an eigenvalue of 3.918 with the range of loading values between 0.046 to 9.155 accounting for 86.6% of the variance, while Canonical Discriminant (CD2) with an eigenvalue of 0.400 accounted for 8.9%. The two canonical discriminants explained by 95.5% of the total variance (Table 7).

The scatter plot generated between CD1 and CD2 revealed a clear separation between the male weevil populations from Malaysia and Cameroon in Africa (Fig. 9). For the female weevils, the Canonical Discriminant 1 (CD1) had an eigenvalue of 2.927, accounting for 69.9% of the variance, while Canonical Discriminant 2 (CD2) with an eigenvalue of 0.851 represented 20.3% of the total variance. The two canonical discriminants together covered 90.2% of the total variance (Table 8). The scatter plot of the female weevil populations based on the component scores showed clear clustering between the populations from Malaysia and Cameroon (Fig. 10).

In this study, both the result of PCA and CDA conducted showed little variation between the eight localities for male and female weevil populations in Malaysia respectively. Weevil population from Cameroon, Africa was treated as an outgroup and was distinctly separated from the weevil populations in Malaysia. The properties of soil, plant cover and anthropogenic impact in ecosystems are some factors that may influence the morphology in ground beetles (Blake, Foster, Eyre, & Luff, 1994; Giglio, Giulianini, Zetto, & Talarico, 2011; Sukhodolskaya, 2013). However, there was no indication of soil properties or palm age to have affected the weevil size as shown in the weevil population. This shows healthy traits and that the morphological characters are stable (Schluter, 2000; Schauble, 2004).

CONCLUSIONS

The present study allows morphometric characters of male and female weevil populations to be evaluated, to reveal their reaction towards the present condition in oil palm plantations. It is evident that there is very little variation in the body size of the weevils between the different localities in Malaysia, however, a distinct separation is observed with the weevil population from Cameroon, Africa. This is an indication that the weevil population in Malaysia exhibit slight differences in their morphological characters. Future studies should focus on management practices and other possible biotic factors (ie. parasitism, nematode infestation) that may lead to body size variation.

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Table 1. Sampling locations in Malaysia.

	Localities	GPS Coordinates	Soil type
	Malaysia		
L1	FELDA Chuping 2, Perlis	6°30'14" N, 100°11'50" E	Mineral
L2	FELDA Jerantut, Pahang	4°21'22" N, 102°23'34" E	Mineral
L3	MPOB Teluk Intan, Perak	4°02'51" N, 101°01'08" E	Shallow peat
L4	MPOB Hulu Paka, Terengganu	4°33'14" N, 103°11'16" E	Mineral
L5	Yong Peng, Johor	2°04'11" N, 103°04'09" E	Mineral
L6	UPM Plantation, Selangor	2°59'45" N, 101°42'56" E	Mineral
L7	MPOB Lahad Datu, Sabah	5°06'12" N, 118°37'05" E	Mineral
L8	MPOB Sessang, Sarawak	1°55'35" N, 111°13'32" E	Deep peat
	Africa		
	Cameroon, West Africa	7°22'11" N, 12°20'41" E	Kaolisol

Table 2. The average values in millimeters (mean \pm standard deviation) of the morphometric characters of male and female weevils in eight different localities in Malaysia and one locality from Africa.

State	Perlis		Pahang		Perak		Terengganu		Johor	
Gender	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
TL	2.62 \pm 0.32	2.46 \pm 0.16	3.07 \pm 0.38	2.70 \pm 0.17	3.28 \pm 0.38	2.92 \pm 0.20	3.05 \pm 0.25	2.56 \pm 0.19	3.45 \pm 0.38	3.03 \pm 0.20
EL	1.75 \pm 0.24	1.62 \pm 0.13	2.09 \pm 0.30	1.81 \pm 0.13	2.24 \pm 0.32	1.94 \pm 0.17	2.11 \pm 0.14	1.80 \pm 0.16	2.43 \pm 0.30	2.11 \pm 0.16
PL	0.63 \pm 0.12	0.65 \pm 0.10	0.74 \pm 0.13	0.69 \pm 0.11	0.80 \pm 0.13	0.78 \pm 0.08	0.69 \pm 0.26	0.60 \pm 0.13	0.77 \pm 0.17	0.72 \pm 0.12
BW	0.81 \pm 0.07	0.77 \pm 0.08	0.93 \pm 0.09	0.82 \pm 0.07	1.00 \pm 0.08	0.91 \pm 0.06	1.11 \pm 0.12	0.99 \pm 0.13	1.12 \pm 0.14	0.98 \pm 0.08
SL	0.76 \pm 0.12	1.01 \pm 0.06	0.81 \pm 0.13	1.15 \pm 0.08	0.80 \pm 0.12	1.19 \pm 0.07	0.89 \pm 0.13	1.10 \pm 0.16	0.84 \pm 0.11	1.21 \pm 0.09
State	Selangor		Sabah		Sarawak		Cameroon			
Gender	♂	♀	♂	♀	♂	♀	♂	♀		
TL	3.22 \pm 0.36	2.84 \pm 0.16	3.06 \pm 0.39	2.82 \pm 0.22	3.38 \pm 0.21	2.94 \pm 0.20	3.60 \pm 0.18	2.92 \pm 0.14		
EL	2.11 \pm 0.32	1.85 \pm 0.10	2.12 \pm 0.31	1.92 \pm 0.15	2.35 \pm 0.21	1.93 \pm 0.13	2.57 \pm 0.18	2.02 \pm 0.09		
PL	0.86 \pm 0.21	0.78 \pm 0.11	0.69 \pm 0.13	2.01 \pm 0.15	0.78 \pm 0.17	0.81 \pm 0.12	0.82 \pm 0.07	0.80 \pm 0.18		
BW	1.07 \pm 0.13	0.92 \pm 0.08	1.05 \pm 0.17	0.93 \pm 0.12	1.04 \pm 0.11	0.89 \pm 0.08	1.51 \pm 0.06	1.26 \pm 0.05		
SL	0.81 \pm 0.13	1.20 \pm 0.11	0.77 \pm 0.10	1.10 \pm 0.10	0.73 \pm 0.09	1.09 \pm 0.14	1.09 \pm 0.11	1.30 \pm 0.08		

TL: Total body length; EL: Elytra length; HL: Pronotum length; BW: Body width; SL: Snout length.

Table 3. One-way ANOVA between localities and sex of weevils with each of five morphological variables in Malaysia. Significant differences were only shown for morphological characters for male and female weevil populations between the eight localities in Malaysia respectively.

Variables	Male					Female				
	df	SS	MS	F	P	df	SS	MS	F	P
TL	7	13.9	1.986	17.08	P < 0.0001	7	7.38	1.054	29.42	P < 0.0001
EL	7	8.90	1.271	17.00	P < 0.0001	7	4.19	0.598	29.39	P < 0.0001
PL	7	1.10	0.157	5.34	P < 0.0001	7	1.07	0.153	12.50	P < 0.0001
BW	7	2.22	0.32	23.32	P < 0.0001	7	1.01	0.14	12.89	P < 0.0001
SL	7	0.53	0.075	5.63	P < 0.0001	7	1.17	0.167	20.96	P < 0.0001

TL: Total body length; EL: Elytra length; PL: Pronotum length; BW: Body width; SL: Snout length.

Table 4. Comparison on morphological variables between male and female weevils using t-test.

Variables	t	df	P-value
Total body length	11.26	398.9	<0.0001
Elytra length	11.13	384.5	<0.0001
Body width	1.98	429.6	0.0489
Pronotum length	29.17	478.0	<0.0001
Snout length	9.61	442.9	<0.0001

Table 5. Coefficient component principal of male weevils using Principal Component (PC) analysis.

Parameter	PC1	PC2	PC3	PC4
TL	0.562	-0.241	0.118	-0.267
EL	0.520	-0.044	0.524	-0.296
PL	0.354	-0.459	-0.754	-0.019
BW	0.490	0.298	0.022	0.819
S	0.219	0.800	-0.376	-0.412
Eigenvalue	2.8475	1.1026	0.7199	0.3264
% Variation	57.0	22.1	14.4	6.5
% Cumulative Variation	57.0	79.0	93.4	99.9

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Table 6. Coefficient component principal of female weevils using Principal Component (PC) analysis.

Parameter	PC1	PC2	PC3	PC4
TL	0.540	-0.215	-0.356	0.009
EL	0.498	0.212	-0.594	0.021
PL	0.351	-0.807	0.339	0.018
BW	0.407	0.378	0.470	0.685
S	0.413	0.339	0.430	-0.728
Eigenvalue	2.9669	0.8616	0.6017	0.5358
% Variation	59.3	17.2	12.0	10.7
% Cumulative Variation	59.3	76.6	86.6	99.3

Table 7. Coefficient component principal of male weevils using Canonical Discriminant (CD) analysis.

Parameter	CD1	CD2	CD3	CD4	CD5
TL	-1.991	1.922	-1.837	-1.872	-9.155
EL	0.860	-0.598	1.790	0.493	4.554
PL	1.572	-0.592	1.410	2.108	7.415
BW	0.935	-0.283	-0.393	-0.526	-0.046
S	0.536	-0.202	0.513	0.558	-0.350
Eigenvalue	3.918	0.400	0.102	0.075	0.029
% Variation	86.6	8.9	2.3	1.7	0.6
% Cumulative Variation	86.6	95.5	97.7	99.4	100.0
Canonical Correlation	0.893	0.535	0.304	0.264	0.167

Table 8. Coefficient component principal of female weevils using Canonical Discriminant (CD) analysis.

Parameter	CDA1	CDA2	CDA3	CDA4	CDA5
TL	-0.865	0.847	0.371	-0.371	-2.761
EL	0.228	-0.003	0.426	-0.442	1.693
PL	0.500	0.284	-0.919	0.321	2.205
BW	1.022	-0.355	-0.084	-0.272	-0.169
S	0.302	0.070	0.669	0.775	-0.032
Eigenvalue	2.927	0.851	0.270	0.133	0.008
% Variation	69.9	20.3	6.5	3.2	0.2
% Cumulative Variation	69.9	90.2	96.7	99.8	100.0
Canonical Correlation	0.863	0.678	0.461	0.342	0.086

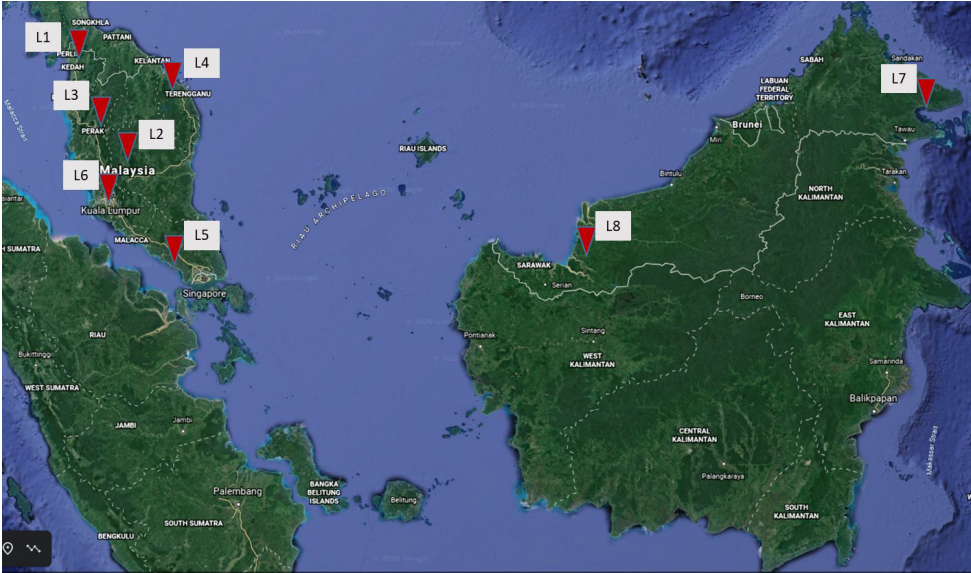


Fig. 1. Eight sampling locality located in Malaysia.

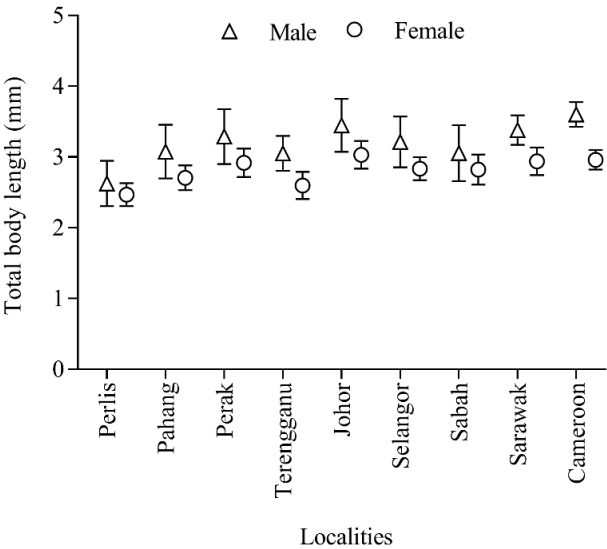


Fig. 2. Mean and standard deviation on total body length of the male and female weevil populations in eight different localities.

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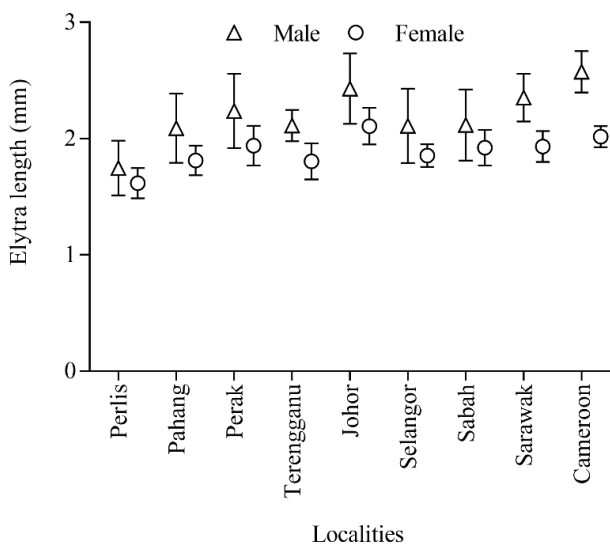


Fig. 3. Mean and standard deviation on elytra length of the male and female weevil populations in eight different localities.

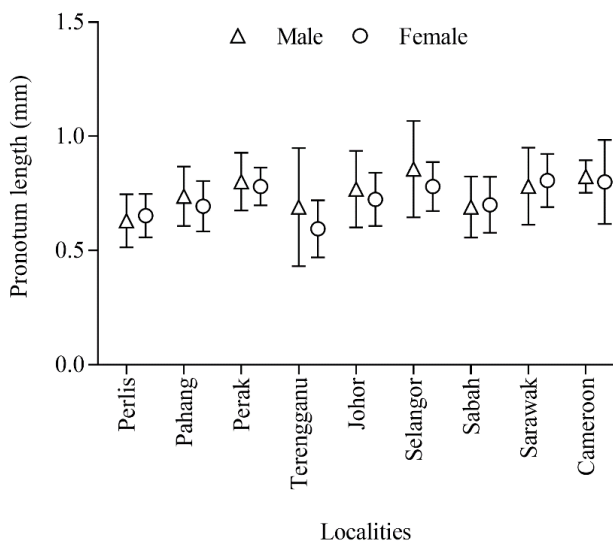


Fig. 4. Mean and standard deviation on pronotum length of the male and female weevil populations in eight different localities.

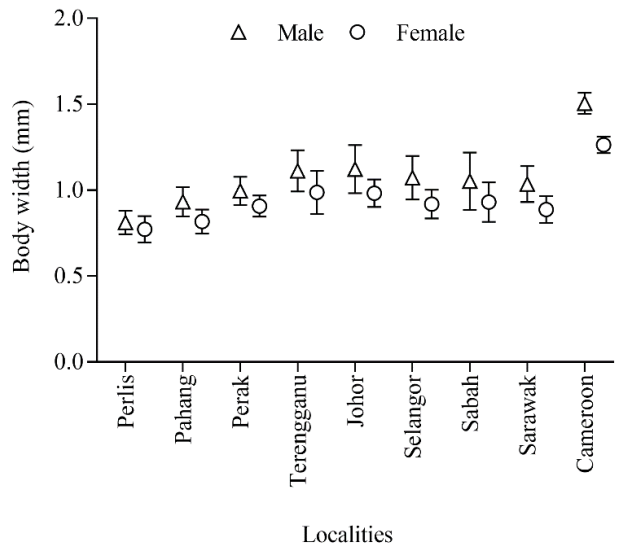


Fig. 5. Mean and standard deviation on body width of the male and female weevil populations in eight different localities.

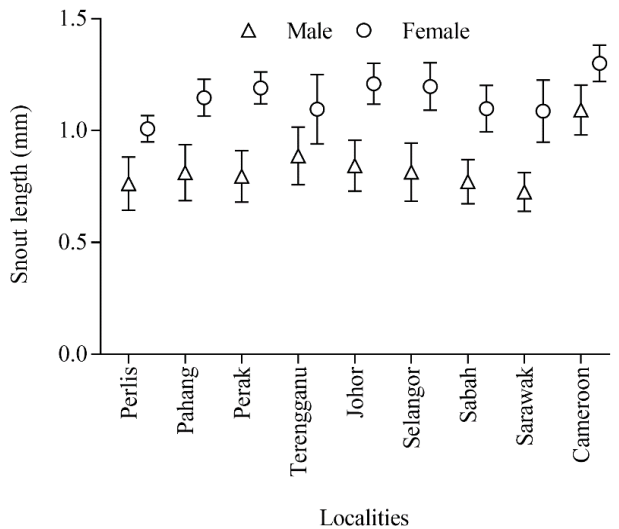


Fig. 6. Mean and standard deviation on snout length of the male and female weevil populations in eight different localities in Malaysia and one locality in Africa.

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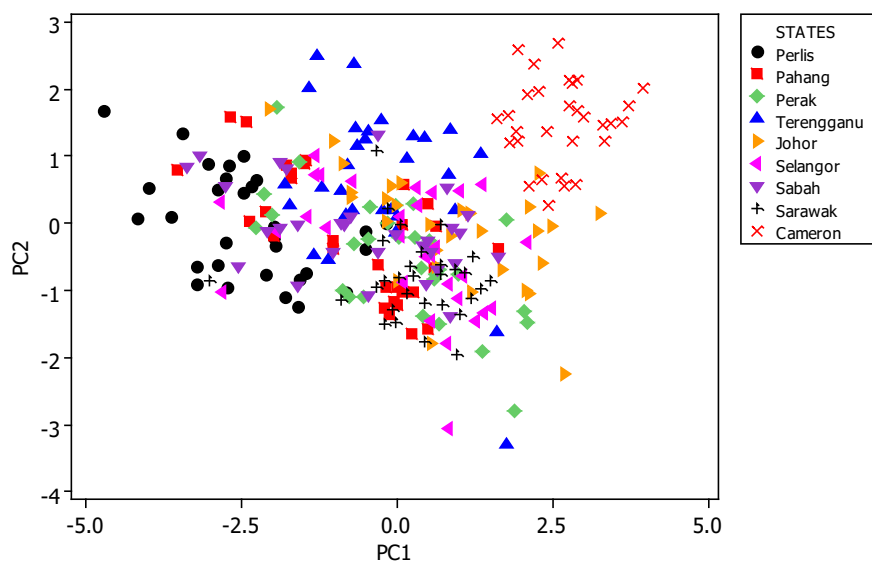


Fig. 7. Scatter plot based on the Principal Component Analysis of Principal Component 1 against Principal Component 2 for male weevil population at eight localities in Malaysia and one locality in Africa.

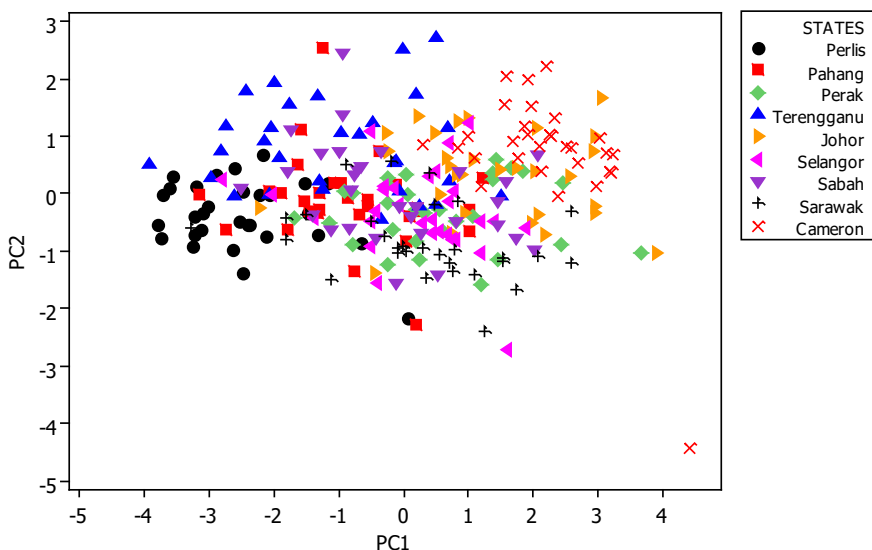


Fig. 8. Scatter plot based on the Principal Component Analysis of Principal Component 1 against Principal Component 2 for female weevil population at eight localities in Malaysia and one locality in Africa.

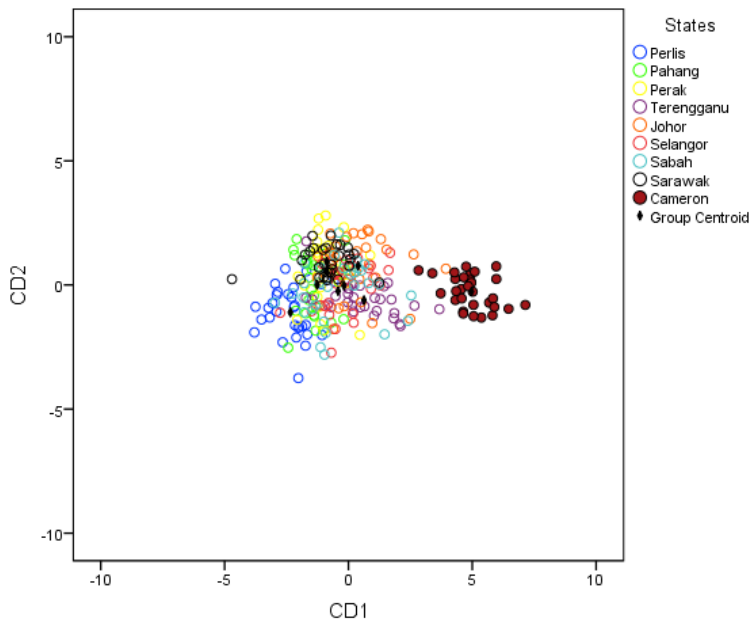


Fig. 9. Scatter plot based on the Canonical Discriminant analysis of Canonical Discriminant 1 against Canonical Discriminant 2 for male weevil population at eight localities in Malaysia and one locality in Africa.

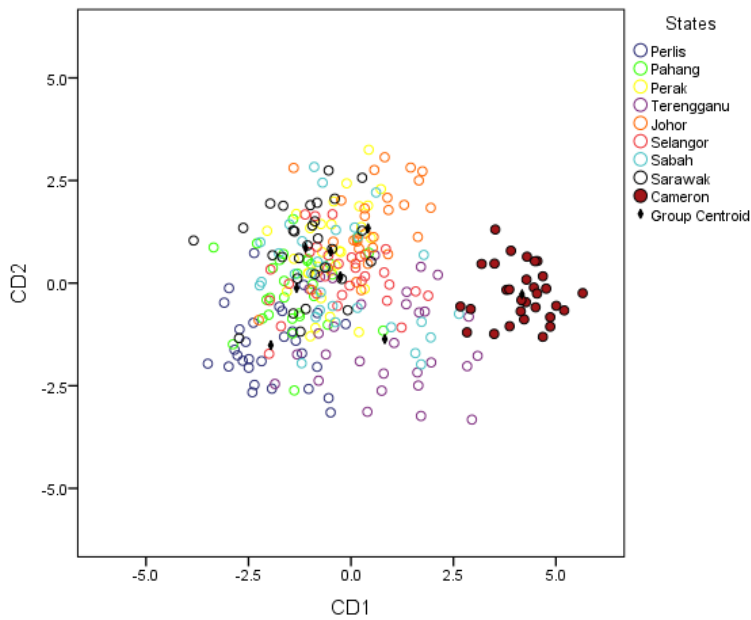


Fig. 10. Scatter plot based on the Canonical Discriminant analysis of Canonical Discriminant 1 against Canonical Discriminant 2 for female weevil population at eight localities in Malaysia and one locality in Africa.

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New Species of *Aphelinoidea* Girault (Hymenoptera: Chalcidoidea: Trichogrammatidae) along with Key from India

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ABSTRACT

Two new species of *Aphelinoidea* Girault (Hymenoptera: Trichogrammatidae), *A. almoraensis* Ikram & Khan sp. nov and *A. rudrapurensis* Ikram & Khan sp. nov. are being described from India . A key to the Indian species of *Aphelinoidea* Girault and re-description of *Aphelinoidea gwaliorensis* Yousuf & Shafee are also provided. *Aphelinoidea gwaliorensis* Yousuf & Shafee is being revalidated.

Key words: *Aphelinoidea*, Chalcidoidea, new species, India.

INTRODUCTION

Aphelinoidea a cosmopolitan genus of the family Trichogrammatidae. Some members of which are known as egg parasitoids of various leafhoppers (Hemiptera: Cicadellidae) and thus they can be important for biological control (Trjapitzin 1995; Walker, Zareh, Bayoun, & Triapitsyn, (1997); Walker, Bayoun, Triapitsyn, & Honda, (2005); Bayoun, Walker, & Triapitsyn, (2008). Walker, Bayoun, Triapitsyn & Honda (2005) reviewed the species of egg parasitoids attacking *N. tenellus* in California. Triapitsyn (2018) synonymized *A. gwalioensis* Yousuf & Shafee under *A. (Lathromeroides) neomexicana* (Girault), and *A. retiruga* Lin, was synonymized under *A. (Aphelinoidea) longiclavata* Yousuf & Shafee. So, presently the genus *Aphelinoidea* Girault includes 39 described (including three Indian species: *A. gwalioensis* Yousuf & Shafee (revalidated), *A. yousufi* Khan & Anis, *A. longiclavata* Yousuf, & Shafee) valid species in the world (Noyes, 2019). Here we have described two new species *A. almoraensis* Ikram, & Yousuf sp. nov and *A. rudrapurensis* Ikram & Yousuf sp. nov. of the genus *Aphelinoidea* Girault collected from Uttarakhand region in India. An updated key to the Indian species of *Aphelinoidea* Girault and re-description of *Aphelinoidea gwalioensis* Yousuf & Shafee are also provided. *Aphelinoidea gwalioensis* Yousuf & Shafee is being revalidated, which was synonymized with *Aphelinoidea (Lathromeroides) neomexicana* (Girault, 1915) by Triapitsyn (2018).

MATERIAL AND METHODS

All specimens were collected from the forestry and adjoining agro-forestry areas of Haryana, Uttarakhand and Punjab (India) by sweep net. Screened out specimens from insect samples were preserved in 70% alcohol. Later specimens were dissected and kept in a drop of euparal under coverslips. Only body lengths of specimens were measured in millimeters, rest parts of measurements were taken from the divisions of a linear scale micrometer placed in the eye piece of a Nikon Digital Sight attached with Optiphot Microscope, at 10X, 20X and 40X (objective lens) for slide-mounted parts. Scales are placed on photographs of slide mounted parts and measurement was taken with the help of NIS-ELEMENT software in micrometer (μm). Photographs of slide-mounted specimens were taken with digital camera "Nikon Digital Sight attached with Optiphot Microscope (Japan)" fitted over a compound microscope (Leica's Leitz Labor Lux S). All the specimens including types are deposited at NFIC (National Forest Insect Collection), Forest Protection Division, Forest Research Institute, Dehradun, Uttarakhand (India).

The following abbreviations are used: OOL= Ocello-ocellar length; POL= Post-ocellar; C1 & C2= Club segments 1 & 2; FWW= Fore wing width; STV= Stigmal vein; MV= Marginal vein; PM= Pre marginal vein.

RESULTS

Taxonomy

Key to Indian species of *Aphelinoidea* Girault, based on females

1. Fore wings less than 2.5X as long as wide2
 - Fore wings 2.5X as long as wide; marginal fringe about one fifth of wing width; antennae with scape more than 3X as long as wide, pedicel about 2X as long as wide1. *A. gwaliorensis* Yousuf & Shafee
2. Antennae with club about 4X as long as wide3
 - Antennae with club always less than 4X as long as wide4
3. Fore wings with discal setae restricted to apical half of disc; antennae with scape more than four times as long as2. *A. yousufi* Khan & Anis
 - Fore wings with disc densely setose after venation, covering two third of disc3. *A. longiclavata* Yousuf & Shafee
4. Antennae with club less than 3X as long as wide, scape more than 3.5 times as long as wide; fore wings with marginal fringe about one-sixth of wing width4. *A. almoraensis* sp. n.
 - Antennae with club more than 3X as long as wide; scape less than 3.5X as long as wide; fore wings with marginal fringe about than one-tenth of wing width5. *A. rudrapurensis* sp. n.

Aphelinoidea gwaliorensis Yousuf & Shafee (Figs. 1-5)

Aphelinoidea gwaliorensis Yousuf & Shafee, 1985: 303.

Aphelinoidea gwaliorensis Yousuf & Shafee, 1988: 105.

Aphelinoidea (*Lathromeroides*) *neomexicana* (Girault): Trjapitzin 1995: 301. Syn. quoted by Triapitsyn, 2018: 61.

Female. Length. 0.50 mm. Head with fronto-vertex light yellow; facial area from ventral margin of toruli to mouth margin of the eye gena light brown; eyes and ocelli red; Mandibles golden yellow with tip brown. Antennae with scape and pedicel light brown except club with brownish. Mid-lobe of mesoscutum light golden yellow except light dorsellum and propodeum parts with light yellow. Fore and hind wings hyaline except infuscation beneath marginal vein.

Head: (facial view) (Fig. 1) 1.14X as broad as long (176: 154). Eyes 1.4X as long as malar space; mandibles with tri-denticles. Antennae (Fig. 2) with scape 3.52X as long as broad (67: 19); pedicel 2X as long as broad (44: 22); one anellus present; funicle absent; club three-segmented (C1, C2 and C3), about 2.3X as long as broad (90: 40).

Mesosoma: (Fig. 4) Midlobe of mesoscutum about 1.2X wider than long (92: 78) with 2 pairs of setae present on dorsal surface; midlobe of scutellum 1.5X wider than long (92: 61) with 2 pairs of setae, dorsellum 0.61X as long as propodeum (13: 21). Fore wings (Fig. 3), 2.6X as long as broad (501: 194); disc densely setose; STV rudimentary.

Metasoma: (Fig. 5) longer than mesosoma; ovipositor very long, arising from base of 2nd tergite of mesothorax and cover the entire length of gaster slightly exserted, 2.8X as long as hind tibia (230: 82).

Male. Unknown.

Host. Unknown.

Material examined: INDIA: Haryana: Karnal, 1♀ (on slide), 03.09.2014; Yamunanagar, Kamain chowk, 1♀ (on slide), 16.02.2015, by sweeping, S. Khan; Yamunanagar, Bhelpur, 1♀ (on slide), 09.iii.2016, R.B. Singh, by sweeping; Hisar, Alipur, 1♀ (on slide), 30.12.2015; Bhiwani, Raigarh, 1♀ (on slide), 30.12.2015, by sweeping, S. Khan. PUNJAB: Taran Tarn, Aladdinpur, 1♀ (on slide), 11.09.2013; Jalandhar, Jandusingha, 1♀ (on slide), 11.09.2013, S. Khan, by sweeping.

Distribution: India: Andhra Pradesh, Arunachal Pradesh, Haryana (New record), Himachal Pradesh, Jammu & Kashmir, Madhya Pradesh, Odisha, Punjab (New record), Uttar Pradesh, West Bengal.

Discussion: *Aphelinoidea gwaliorensis* was described by Yousuf & Shafee (1985), based on female characters. During present study additional morphometric characters (Midlobe of mesoscutum about 1.2X wider than long (92: 78) with 2 pairs of setae present on dorsal surface; midlobe of scutellum 1.5X wider than long (92: 61) with 2 pairs of setae, dorsellum 0.61X as long as propodeum (13: 21) have been studied.

Aphelinoidea gwaliorensis Yousuf & Shafee was synonymized with *Aphelinoidea* (*Lathromeroides*) *neomexicana* (Girault, 1915) by Triapitsyn (2018) but several specimens of *A. gwaliorensis* collected during the present study, have been studied in detail and following key characters make it distinct from *A. (Lathromeroides) neomexicana*. Therefore *A. gwaliorensis* being treated as valid distinct species and hence, it is being revalidated as *Aphelinoidea gwaliorensis* Yousuf & Shafee, 1985.

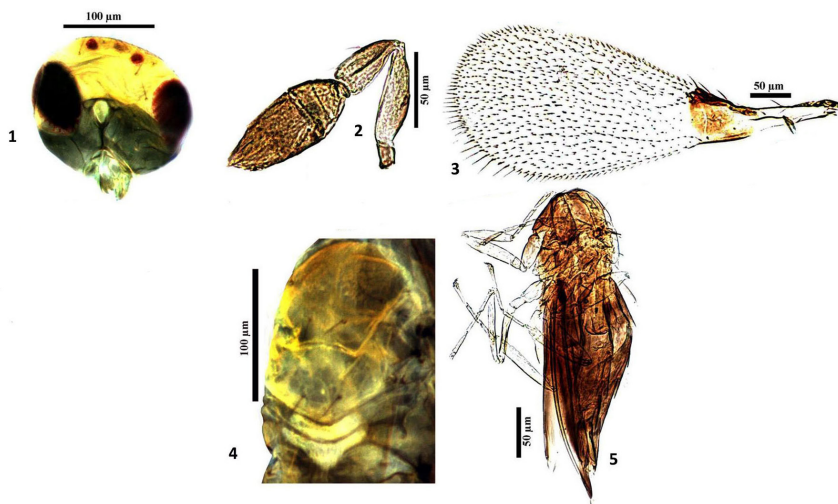


Fig. 1-5. *Aphelinoidea gwaliorensis* Yousuf & Shafee (Female). 1. Head, 2. Antenna, 3. Fore wing, 4. Mesosoma, 5. Ovipositor & hind tibia.

New Species of *Aphelinoidea* Girault

Table 1. Comparative characters of *A. (Lathromeroides) neomaxicana* Girault and *A. gwaliorensis* Yousuf & Shafee.

Key characters	<i>A. (Lathromeroides) neomaxicana</i> (Girault, 1915)	<i>A. gwaliorensis</i> Yousuf & Shafee (1985) (n=5)
Body size	0.60-1.10 mm	0.50-0.56 mm
Body colour	Face brown, Mesoscutum mostly orange; gaster generally dark brown or sometimes with several pale or orange transverse strips.	Face dark yellow with pale yellow vertex; gaster dark yellow with brown transverse strips.
Antennae (Length/ width)	Club: 2.2-3.1×	2.1-2.6×
	Pedicel: 3×	2.0-2.5×
	Scape: About 4×	3.7-4.4×
Fore wings (Length/ width)	2.3-2.4× as long as wide	2.5-2.7× as long as wide
Ovipositor: Hind tibia	About 4×	2.5-3.0×
Ovipositor: Meta tibia	3.0-3.9×	2.8-3.0×
Genitalia arising from	2 nd tergite of mesothorax	3 rd tergite of mesothorax

Aphelinoidea almoraensis sp. nov. (Figs. 6-11)

Holotype, 1♀ (on slide), INDIA: Uttarakhand, Almora, 06.02.2018, R. B. Singh, by sweeping.

Paratype: 1♀ (on slide) same data as holotype.

Description

Female. Length 0.85 mm. Head with vertex pale yellow; gena brownish; eyes and ocelli red. Mandibles dark brown. Antennae with scape, pedicel and club light brown. Mesosoma with pronotum, mesoscutum and scutellum brown; metanotum and propodeum pale brown. Gaster uniformly dark brown with several pale transverse stripes.

Head: (facial view) (Fig.6) 1.14X as broad as high (153: 134); front-vertex with prominent setae; vertex with rugose sculpture. Eyes reddish, about 1.55X as long as malar space (70: 45); mandible with three denticles (Fig. 10). Antennae (Fig. 7) with scape broad at the base, 3.8X as long as broad (65: 17); pedicel 2X as long as broad (44: 22); one anellus present; club three-segmented (C1, C2 and C3), 2.7X as long as broad (92: 34), C3 longest, more than 2X longer than pedicel.

Mesosoma: Fore wings (Fig. 11), hyaline except infuscation beneath venation, 2.4X as long as broad (484: 201), fore wings disc with dens setose not arranged in rows, STV rudimentary and marginal fringe short. Hind wings about 11X as long as wide (396: 37) with 3 rows of discal setae, marginal fringe long, about 1/5th of wing width.

Metasoma: Gaster longer than mesosoma; ovipositor 1.3X as long as hind tibia (175: 131) (Figs. 8, 9).

Host: Unknown

Distribution: INDIA: Uttarakhand.

Etymology: The name of the species is derived from collection site 'Almora' district in Uttarakhand.

Remarks: *Aphelinoidea almoraensis* sp. nov. is very close to *Aphelinoidea longiclavata* Yousuf & Shafee (1988a) but can be separated by having antennae with scape 3.8X as long as broad; pedicel 2X as long as broad; club 2.7X as long as broad. Fore wings, 2.4X as long as broad.

In *Aphelinoidea longiclavata*, antennae with scape 4X as long as broad; pedicel 2.5X as long as broad; club long, about 4X as long as broad. Fore wings, slightly more than 2X as long as broad.

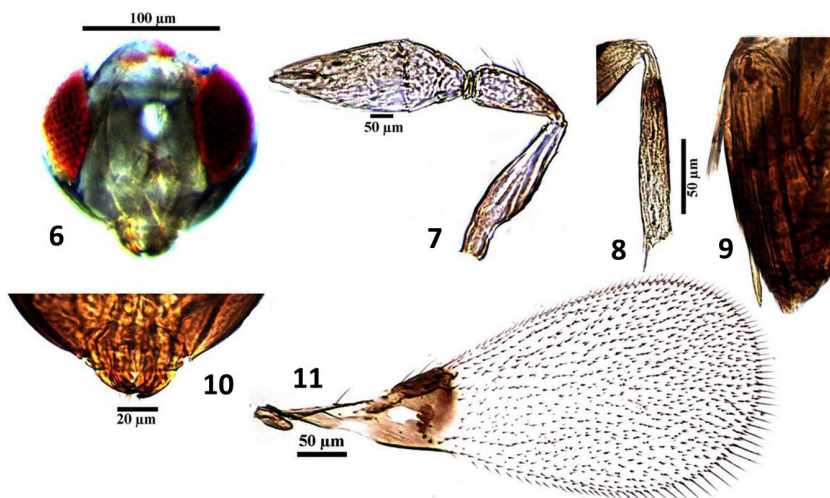


Fig. 6-11. *Aphelinoidea almoraensis* sp. nov. (Female) 6. Head, 7. Antenna, 8. Hind tibia, 9. Ovipositor, 10. Mandibles, 11. Fore wing.

***Aphelinoidea rudrapurensis* sp. nov. (Figs. 12-19)**

Holotype, 1♀ (on slide), INDIA: Uttarakhand, Udham Singh Nagar. Rudrapur, 06.02.2018, R. B. Singh, by sweeping.

Description

Female. Length. 0.79 mm. Head with fronto-vertex bright yellow; facial area from ventral margin of toruli upto mouth margin of the eye gena light brown; eyes and ocelli red; Mandibles golden yellow with tip brown. Antennae with scape, pedicel and club brown. Midlobe of mesoscutum light golden yellow, except dorsellum and propodeum parts with light yellow. Fore and hind wings hyaline except light infuscation beneath venation.

Head: (facial view) (Fig. 12) 1.2X as broad as high (191: 162). Eyes 2.3X as long as malar space; mandibles with tri-denticles (Fig. 14). Antennae (Fig. 13) with scape broad at the base, 3.25X as long as broad (78: 24); pedicel 2.4X as long as broad (53: 22); one anellus present; funicle absent, club three-segmented (C1, C2 and C3), 3.3X as long as broad (119: 36), C3 longest.

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Mesosoma: (Fig. 15) Midlobe of mesoscutum 1.14X wider than long (124: 108) with 2 pairs of setae present on dorsal surface; midlobe of scutellum 1.52X wider than long (116: 76), having 2 pairs of setae, dorsellum as long as propodeum. Fore wings (Fig. 16), 2.30X as long as broad (578: 251); disc densely setose, STV rudimentary; marginal fringe about 1/10th of FWW. Hind wings (Fig. 17) 11X as long as wide (452: 41), 2 rows of setae present. Metasoma: Gaster longer than mesosoma; ovipositor very long, arising from base of and cover the entire length of gaster and slightly exserted, 2.3X as long as hind tibia (421: 181) (Figs. 18-19).

Host: Unknown

Distribution: Uttarakhand.

Etymology: The name of the species is derived from collection site 'Rudrapur' district in Uttarakhand.

Remarks: *Aphelinoidea rudrapurensis* sp. nov. is very similar to *Aphelinoidea yousufi* Khan & Anis (2016) but can be separated by having antennae with scape 3.25X as long as broad; club 3.3X as long as broad. Midlobe of mesoscutum 1.14X wider than long; midlobe of scutellum 1.5X wider than long. Fore wings, 2.30X as long as broad; disc densely setose upto the margin of stigmal vein. Ovipositor very long, arising from base, and cover the entire length of gaster and slightly exserted, 2.3X as long as hind tibia.

In *Aphelinoidea yousufi* antennae with scape broad at the base, 4.44X as long as broad, club about 4X as long as broad. Midlobe of mesoscutum 1.23X wider than long; midlobe of scutellum 2.25X wider than long. Fore wings, 2.2X as long as broad; discal setae restricted to apical half. Ovipositor short, arising from IIIrd of the gaster, about 1.47X as long as hind tibia.

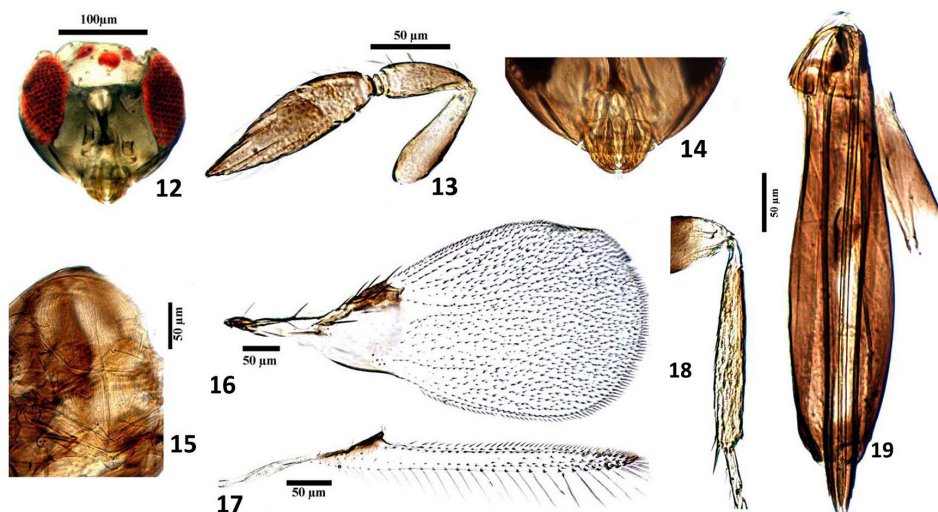


Fig. 12-17. *Aphelinoidea rudrapurensis* sp. nov. (Female) 12. Head, 13. Antenna, 14. Mandibles, 15. Mesosoma, 16. Fore wing, 17. Hing wing, 18. Hind tibia, 19. Ovipositor.

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A New Species of *Trichogramma* Westwood (Chalcidoidea: Trichogrammatidae) from Unknown Lepidopterous Egg on *Populus deltoides* from India

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ABSTRACT

The genus *Trichogramma* Westwood comprises as egg parasitoids attacking lepidopterous insect pests of important forestry and agro-forestry tree species. Survey was conducted in forest areas of Thano range, Dehradun, India, for collection of parasitized eggs. In the present paper, a new species *Trichogramma hayati* sp. nov. is described from an unknown lepidopterous egg from poplar tree.

Key words: Egg parasitoid, biological control, *Trichogramma*, Hymenoptera, poplar.

INTRODUCTION

Trichogramma species are well known natural egg parasitoids which have been used as biocontrol agents against various insect pests of important forest tree species including the key defoliators of teak, poplar and shisham. Nagarkatti & Nagaraja (1977) compiled the information on 48 species of *Trichogramma*. Yousuf, Joshi, & Kulkarni (2004) enlisted 142 species of *Trichogramma*, distributed worldwide. About 250 species of genus *Trichogramma* have been recorded all over the world of which twenty eight species have been recorded from India (Yousuf, Ikram, & Faisal, 2015). Recently, some researchers have also made contribution in taxonomic study of *Trichogramma* (Khan, Yousuf, Ikram, & Singh, 2017; Khan, Yousuf, & Ikram, 2018; Yousuf, Khan, & Ikram, 2018) from India. Currently, *Trichogramma* comprises worldwide 251 species with 29 species from India including the species described in this paper from unknown lepidopterous egg on *Populus deltoides*.

MATERIAL AND METHODS

Survey of important forestry and agro-forestry areas of Thano range, Dehradun (Uttarakhand), India was conducted for collection of egg bunches of insects. Collected samples of eggs were reared for the emergence of egg parasitoids (Fig. 3.). Specimens (♂♂ & ♀♀) of *Trichogramma* were emerged out from unknown lepidopterous egg on *Populus deltoides*. Emerged *Trichogramma* specimens were preserved in 70% ethanol, after following the course of dehydration the specimens were dissected out in clove oil under microscope and dissected body parts were mounted on a slide in drop of Euparal. All measurements and photographs were taken from Leica wild M10-MDG. Type specimens have been deposited in the National Forest Insect Collection, Forest Entomology Discipline, Forest Protection Division of the Forest Research Institute, Dehradun, India.

The following abbreviations are used in the text:

DEG - Dorsal expansion of gonobase; CS - Chelate structure; GF - Gonoforceps; RS1-Radial sector 1.

Trichogramma hayati sp. nov.

Description

Male: Body length 0.52 mm. Body honey yellow to dark brown; ocelli and eyes dark red. Antennae light to dark brown except scape pale yellow. Mesosoma (Fig. 1D) light brown, forewing (Fig. 1C) hyaline, lightly infuscated behind stigmal vein and sub marginal vein, RS1 vein track having 4 setae. Legs light yellow, genitalia light to dark brown.

Head wider than long (219: 149) (Fig. 1A); eye length slightly shorter than malar sulcus (93:87); mandible three denticles; scape 3.6×(84:23) as long as wide (Fig. 1B); pedicel 2.1×(42: 20) as long as wide; 35 to 40 pointed flagellar hairs, longest hair of antennae about 2.7× (77:28) maximum width of flagellum.

Mesosoma: Fore wing about 2× (540:283) as long as wide (Fig. 1C); discal setae arranged in rows, RS1 vein track with 4 setae, marginal fringe on tornus long, about

A New Species of Trichogramma Westwood (Chalcidoidea: Trichogrammatidae)

1/8 (32: 283) of forewing width.

Metasoma: Genitalia with DEG having broadly rounded lobes with notches (Fig. 1E); GF below the level of CS. Aedeagus longer than apodemes, both together slightly shorter than entire; about 1/2 of hind tibia (Fig. 1F).

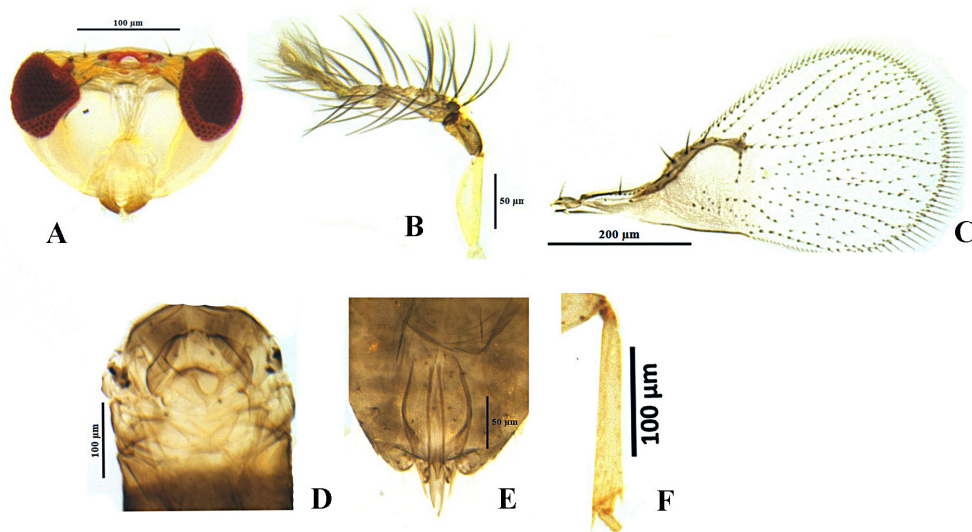


Fig. 1A-F. *Trichogramma hayati* sp. n. (Male). A. Head, B. Antenna, C. Forewing, D. Mesosoma, E. Genitalia, F. Hind tibia.

Female: Body length 0.53mm. Body yellow to dark brown in ocelli and eyes dark red. Antennae yellowish. Forewing (Fig. 2C) hyaline, lightly infuscated behind stigmal vein and sub marginal vein, with RS1 having 5 setae. Legs light yellow, ovipositor dark brown in colour.

Head 1.3× longer than wide (230: 175) (Fig. 2A); eye 1.2× shorter than malar sulcus (117:101); mandible tridentate; antennal scape about 3.4× as long as wide (206:60) (Fig. 2B); pedicel 2.3× as long as wide (91:39); funicle two and club single segment 3× (177:74) as long as wide.

Mesosoma: 2.1× (553:263) as long as wide (Fig. 2C); discal setae arranged in rows, with vein track RS1 having 5 setae, marginal fringe on tornus, about 1/7 (36: 263) wing width.

Metasoma: Ovipositor (Fig. 2D) slightly longer than hind tibial length (219: 201) (Fig. 2E).

Host: Unknown lepidopterous egg

Material examined: Holotype ♂. INDIA: UTTARAKHAND: Dehradun, Thano range, 29.09.2019, coll. A. K. Mishra. Paratype 2♂♂, ♀♀ same data as holotype.

Distribution: India: Uttarakhand

Etymology: The species is named for Dr Mohd. Hayat, Aligarh Muslim University,

Aligarh, in recognition of his contribution to the taxonomy of Chalcidoidea.

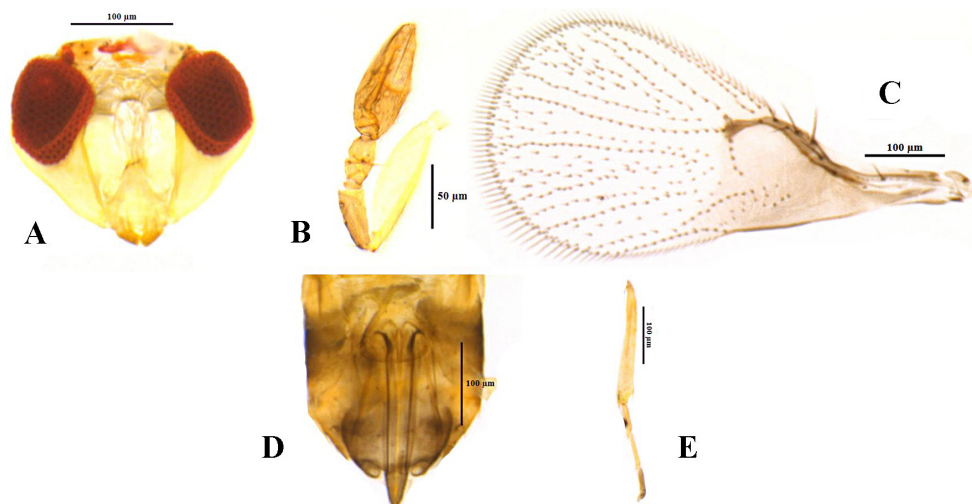


Fig. 2A-E. *Trichogramma hayati* sp. n. (Female). A. Head, B. Antenna, C. Forewing, D. Ovipositor, E. Hind tibia.



Fig. 3. A. Lepidopterous egg on poplar leaf, B. Parasitized egg with emergence hole.

DISCUSSION

T. hayati sp. nov. is very close to *T. evanescens* Westwood but can be separated by having its antennal scape $3.6\times$ as long as wide; pedicel $2.1\times$ as long as wide; fore wings with RS1 having 4 setae in males and 5 setae in females; Male genitalia with tip of DEG reaching the base of CS; basal notch of DEG broadly rounded and reaching almost side lobes of Genital capsule; central ridge restricted up to mid of GC; GF below the level of CS. In *T. evanescens*, antennal scape $4\times$ as long as wide; pedicel $1.5\times$ as long as wide; forewing with RS1 having 5 setae; DEG reaching beyond level of CS; basal notch of DEG narrowly rounded and not reaching wall of GC; Central ridge reaching beyond mid of GC; GF far below level of CS.

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First Checklist of *Tachysphex* Kohl, 1883 (Hymenoptera: Crabronidae) of Tunisia with New Distributional Data

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ABSTRACT

This study is based on the material of the genus *Tachysphex* Kohl, 1883 (Crabronidae: Hymenoptera) collected from different localities of Tunisia between 2017 and 2019. In this study, a total of 38 species and subspecies are recorded. Among them, *Tachysphex mauretanus* Puławski, 1971 and *T. sericans gracilis* Puławski, 1971 are new records for Tunisian fauna. New provincial records are cited for the majority of newly collected species. Digital photographs for general view of newly collected species are provided. Distribution in Tunisia and in the world of identified taxa is given.

Keys words: Hymenoptera, Crabronidae, *Tachysphex*, checklist, new records, Tunisia.

INTRODUCTION

The genus *Tachysphex* Kohl, 1883 belongs to the family Crabronidae and to the tribe Larrini Latreille, 1810. The world fauna of this genus is presented by 453 species; thus, presenting approximately 34% and 9.36% of species of the tribe Larrini and of the subfamily Crabroninae respectively (Puławski, 2020). *Tachysphex* species are found all over the world. They are terricolous, digging their nests in the ground and the female supply its nests generally by prey of orthopteroids species (Bohart & Menke, 1976). Prey of Palearctic *Tachysphex* are members of the families Acrididae, Tettigoniidae and Mantidae and genera of *Oecanthus* Audinet-Serville, 1831 and *Ectobius* Stephens, 1835 (Puławski, 1971).

Systematic studies of the genus started with Kohl (1885) who studied Palearctic fauna by describing 23 species. Additional studies over the genus appeared later in time, especially that of de Beaumont (1936) who revised this genus in France by dividing it into groups of species to facilitate its study. The same author (1940, 1947a) studied in details the Egyptian fauna and his studies are considered as the first and the only one treating properly the fauna of a single Northern African country. However, Puławski in his review of the genus in Eastern and Western Palearctic region (1971) and in Africa and Arabia (2007) described new species from North Africa and cited several new records from North African countries. In this sense and with the exception of some records included in older labours such as these of Graeffe (1906) and of von Schulthess (1926) and in the recent studies Puławski (1971, 2007), the Tunisian fauna of *Tachysphex* looks like to be still poorly investigated and a focused over this area study lacks at present. This paper aims to establish a complete list of *Tachysphex* species in Tunisia as well as to contribute to the knowledge of this fauna in the country with original data.

MATERIAL AND METHODS

The checklist is established on records mentioned in different sources of literature and on materials newly collected using entomological nets in Tunisia during spring and summer between 2017 and 2019. Collecting localities with their geographical coordinates are presented in Table 1. For each taxon, current name species, distribution in Tunisia and in the world is presented. Species and subspecies valid names are followed by a reference to the original description, including the name under which it was originally described and the year and page of description. 'Tunisia (no specific locality)' is cited if the province is not mentioned.

Newly collected specimens were identified firstly by the first author using keys proposed in the literature mainly Puławski (1971, 2007) and then confirmed or re-identified by Dr. Jakub Straka (Charles University, Faculty of Science, Department of Zoology, Czech Republic) and Dr. Toshko Ljubomirov (Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences Bulgaria). Identified materials are deposited a part in the Collection Ben Khedher at the Laboratory of Entomology and Insect Ecology in Regional Research Centre for Horticulture and

First Checklist of Tachysphex Kohl, 1883 of Tunisia

Organic Agriculture (CRRHAB) in Chott Meriem, Sousse, Tunisia and a part in Atatürk University Biodiversity Science Museum (ABBM), Erzurum, Turkey.

Table 1. Collection localities for Tachysphex Kohl, 1883 wasps in Tunisia during 2017-2019 years, with geographical coordinates.

Province	Locality	Latitude	Longitude	Climate
North east region				
Nabeul	Mida, Libna	36°42'27.5"N	10°55'34.3"E	Sub Humid-Semi Arid
North west region				
Beja	South Mjez Elbeeb	36°38'14.7"N	9°35'54.3"E	Semi Arid
	Slouguia	36°35'24.6"N	9°31'8.9"E	
	Testour, Chambou	36°32'47.5"N	9°24'26.0"E	
	Testour, Elghanima	36°33'08.8"N	9°23'35.2"E	
Le Kef	Western Le Kef, Semmena	36°6'11.6"N	8°43'21.3"E	Semi Arid
Siliana	Kisra	35°47'46.9"N	9°21'10.0"E	Semi Arid
East center region				
Sousse	Bouficha, Salloum	36°18'12.3"N	10°28'29.1"E	Semi Arid- Arid
	Kalaa Kbira, Belaoum	35°54'14.0"N	10°23'37.5"E	
Mahdia	Ksour Essef, Alya	35°19'26.5"N	11°02'32.6"E	Semi Arid- Arid
	Ksour Essef, Salakta	35°22'11.4"N	11°01'53.1"E	
	Ksour Essef, Route Ksour Essef-Sidi Alouane	35°23'54.9"N	10°58'23.5"E	
	Sidi Alouane, Ghlalba	35°22'49.6"N	10°49'50.6"E	
	Sidi Alouane, Rmadhnia	35°21'23.9"N	10°51'16.3"E	
	Sidi Alouane, West Oued Beja, Ecole primaire Amel	35°20'39.8"N	10°50'25.6"E	
	Sidi Alouane, Saada	35°22' 0.2"N	10°49'52.1"E	
	Sidi Alouane, Saguiet Khadem	35°22'36.2"N	10°52'30.9"E	
	Sidi Alouane, Aouled Kloula	35°22'9.4"N	10°51'22.6"E	
	Sidi Alouane, Sidi Alouane 2	35°22'04.4"N	10°55'16.3"E	
	Sidi Alouane, Lemsanaa	35°19'44.5"N	10°54'51.3"E	
	Sidi Alouane, Oued Beja	35°20'34.0"N	10°53'19.9"E	
	Zelba	35°13'48.8"N	10°51'30.1"E	
	Rejich	35°26'35.8"N	11°00'51.4"E	
	Eljem, Achaba	35°18'53.3"N	10°47'31.1"E	
	Eljem, Aouled Lahmar	35°19'59.0"N	10°47'18.9"E	
	Elejem, Tielsa	35°20'44.2"N	10°47'20.6"E	
	Eljem, Touahria	35°20'04.4"N	10°47'43.9"E	
	Eljem, Zghabna	35°20'57.6"N	10°49'09.0"E	
	Eljem, Ababsa	35°22'20.4"N	10°47'16.7"E	
	Eljem, Athamnia	35°20'20.5"N	10°46'54.3"E	
	Eljem, Zouazi	35°20'11.1"N	10°49'00.0"E	

Table 1. Continued.

Province	Locality	Latitude	Longitude	Climate
West center region				
Kairouan	Chbika, Aouled Zair	35°36'42.4"N	9°53'29.8"E	Semi Arid- Arid
	South Kairouan, Ragada	35°34'46.2"N	10°3'06.1"E	
	South Kairouan, Barouta	35°34'28.1"N	10°2'44.3"E	
Sidi Bouzid	East Sidi Bouzid, Elhachria	34°53'56.2"N	9°26'11.6"E	Arid
	West Sidi Bouzid, Zaafrja	35°01'39.8"N	9°17'18.3"E	
	Rgueb, Aouled Ayouni	34°49'14.5"N	9°51'7.7"E	
	Aouled Hafouz center	35°5'07.4"N	9°53'55.2"E	
Kasserine	Sbeitla, Athar, CFPA	35°13'18.7"N	9°05'26.8"E	Semi Arid- Arid
	Sbeitla, Athar, Oued Nakhil	35°14'41.6"N	9°05'40.5"E	
	Sbeitla, Athar, Route Fej Ettin	35°13'20.07"N	9°05'58.7"E	
South region				
Tozeur	Hamma Ejrid	33°59'52.9"N	8°9'57.9"E	Dry
	Temghza, Cascade 2	34°22'34.0"N	7°54'42.7"E	
	Dguech, Elmanachi	33°58'41.5"N	8°12'33.1"E	
	Nefta, Nefta oasis	33°52'39.1"N	7°52'34.9"E	
	Tozeurcenter, Tibebsa oasis	33°55'27.3"N	8°8'44.5"E	
Kebili	Janouara, RassElain	33°42'02.3"N	8°59'26.4"E	Dry
	South Kebili, Errahmat	33°39'01.8"N	8°58'27.4"E	
Tataouine	North Tataouine, Oued Tlelet	33°2'41.8"N	10°28'24.0"E	Dry

RESULTS

Family Crabronidae Latreille, 1802

Subfamily Crabroninae Latreille, 1802

Tribe Larrini Latreille, 1810

Genus *Tachysphex* Kohl, 1883

Tachysphex adjunctus Kohl, 1885

Tachysphex adjunctus Kohl, 1885: Verhandlungen der kaiserlich-königlichen Zoologisch-Botanischen Gesellschaft in Wien, 34: 362.

Distribution in Tunisia: Nabeul (Puławski, 1971).

General distribution: Algeria, France, Morocco, Portugal, Spain, Tunisia (Puławski, 2020).

Tachysphex agnus Puławski, 1971

Tachysphex agnus Puławski, 1971. Państwowe Wydawnictwo Naukowe. Wrocław: 152.

Distribution in Tunisia: Medenine (Puławski, 1971); Gabes, Gafsa, Jendouba, Kairouan, Kasserine, Medenine, Tataouine, Tozeur (Puławski, 2007).

First Checklist of Tachysphex Kohl, 1883 of Tunisia

General distribution: Algeria, Egypt, Israel, Morocco, Saudi Arabia, Tunisia, Turkey, Yemen (Gadallah, 2020).

***Tachysphex albocinctus* (Lucas, 1849) (Fig. 1A)**

Tachytes albocincta Lucas, 1849. Exploration scientifique de l'Algérie pendant les années 1840, 1841 et 1842 publiée par ordre du gouvernement et avec le concours d'une commission académique, Sciences physiques, Zoologie, 3. Histoire naturelle des animaux articulés, 3. Insectes: 250.

Material examined. Kairouan: South Kairouan, Barouta, 95 m, 27.8.2018, 1 ♀; Mahdia: Eljem, Athamnia, 70 m, 22.7.2019, 1 ♀; Tlelsa, 70 m, 13.8.2019, 2 ♂♂, Ksour Essef, Alya, 1 m, 03.8.2018, 2 ♂♂, Salakta, 1 m, 03.8.2018, 1 ♂, Sidi Alouane, Saada, 74 m, 18.6.2019, 1 ♂, 1 ♀, Zelba, 49 m, 17.6.2019, 2 ♂♂; Tataouine: North Tataouine, Oued Tlelet, 170 m, 12.5.2018, 2 ♀♀.

Distribution in Tunisia: Gabes, Jendouba, Kebili, Tozeur (Puławski, 2007).

General distribution: Africa to Southwestern Europe, western and central Asia to India, United Arab Emirates, Yemen (Gadallah 2020).

***Tachysphex atlanteus* de Beaumont, 1955**

Tachysphex atlanteus de Beaumont, 1955: Bulletin de la Société des Sciences Naturelles et Physiques du Maroc, 34: 177.

Distribution in Tunisia: Kasserine (Puławski, 2007).

General distribution: Morocco, Tunisia (Puławski, 2007).

***Tachysphex brevipecten* de Beaumont, 1955 (Fig. 1B)**

Tachysphex brevipecten de Beaumont, 1955. Bulletin de la Société des Sciences Naturelles et Physiques du Maroc, 34: 179.

Material examined. Kasserine: Sbeitla, Athar, Route Fej Ettin, 545 m, 05.9.2018, 1 ♂; Tozeur: Nefta, Nefta oasis, 56 m, 28.6.2018, 1 ♂.

Distribution in Tunisia: Tozeur (Puławski, 1971); Sfax, Tozeur (Puławski, 2007).

General distribution: North Africa to Jordan, United Arab Emirates (Gadallah, 2020).

***Tachysphex brevipennis* Mercet, 1909 (Fig. 1C)**

Tachysphex brevipennis Mercet, 1909: Boletín de la Real Sociedad Española de Historia Natural, 9: 197.

Material examined. Mahdia: Ksour Essef, Route Ksour Essef - Sidi Alouane, 33 m, 01.7.2019, 1 ♀, Sidi Alouane, Ghlalba, 09.9.2019, 1 ♀; Tozeur: Tozeur center, Tibebsa oasis, 35 m, 27.6.2018, 1 ♀.

Distribution in Tunisia: Sfax (de Beaumont, 1947a); Gabes, Gafsa, Kasserine, Kebili, Medenine, Tozeur (Puławski, 2007).

General distribution: Africa, Arabian Peninsula, Iberian Peninsula, Southwestern Europe north to Hungary and Romania, Azerbaijan and Turkey to Israel, Transcaspia, Pakistan and India (Gadallah, 2020).

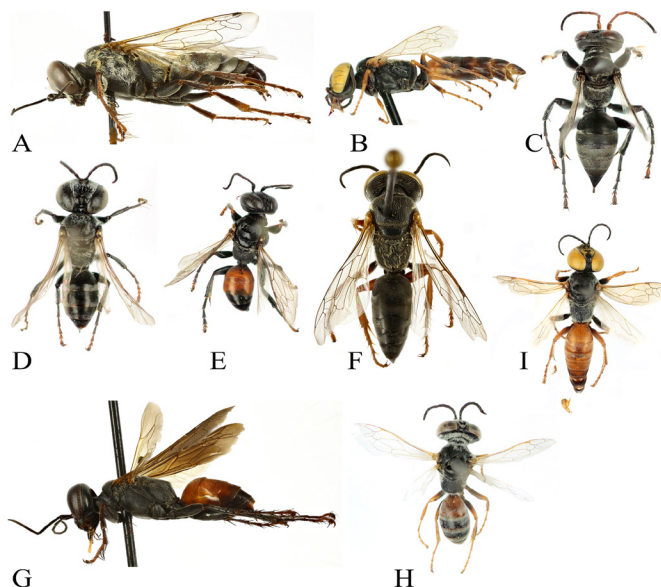


Fig. 1. General view of: A- *Tachysphex albocinctus* (♀); B- *Tachysphex brevipecten* (♂); C- *Tachysphex brevipennis* (♀); D- *Tachysphex consocius* (♀); E- *Tachysphex denisi* (♀); F- *Tachysphex erythropus* (♀); G- *Tachysphex gracilicornis* (♀); H- *Tachysphex gujaraticus* (♂); I- *Tachysphex incertus* (♂).

***Tachysphex brullii* (F. Smith, 1856)**

Tachytes Brullii F. Smith, 1856. Catalogue of hymenopterous insects in the collection of the British Museum. Part IV. Sphegidae, Larridae, and Crabronidae: 296.

Distribution in Tunisia: Kairouan (von Schulthess, 1926).

General distribution: Albania, Austria, Azerbaijan, Bulgaria, Croatia, France, Greece, Hungary, Iran, Italy, Portugal, Romania, Spain, Russia, Switzerland, Tunisia, Turkey, Ukraine, Yugoslavia (Puławski, 2020).

Remark: This species was identified from Tunisia by von Schulthess (1926) as the synonym *Tachysphex rufipes* (von Aichinger, 1870).

***Tachysphex consocius* Kohl, 1892 (Fig. 1D)**

Tachysphex consocius Kohl, 1892: Annalen des kaiserlich-königlichen naturhistorischen Hofmuseums. Wien, 7: 217.

Material examined. Le Kef: Western Le Kef, Semmena, 555 m, 25.4.2018, 1 ♂; Kebili: South Kebili, Errahmat, 30 m, 17.5.2018, 1 ♀, 18.5.2018, 1 ♂.

Distribution in Tunisia: Tozeur (Puławski, 1971, 2007).

General distribution: Africa, Arabian Peninsula (Oman, Saudi Arabia, United Arab Emirates), Southern Europe to Central Asia, India, Sri Lanka (Gadallah, 2020).

***Tachysphex costae* (De Stefani Perez, 1882)**

Tachytes costae De Stefani Perez, 1882: Il Naturalista Siciliano, 1: 42.

Distribution in Tunisia: Nabeul (Puławski, 2007).

General distribution: Mediterranean basin, north to southern France, Romania and Hungary, East to Kazakhstan, Transcaspian region, Western Pakistan, China, South to South Africa, Arabian Peninsula (Oman, United Arab Emirates, Yemen) (Gadallah, 2020).

***Tachysphex denisi* de Beaumont, 1936 (Fig. 1E)**

Tachysphex denisi de Beaumont, 1936: Annales de la Société Entomologique de France, 105: 197.

Material examined. Siliana: Kisra, 809 m, 27.4.2018, 2 ♀♀.

Distribution in Tunisia: Nabeul (Puławski, 1971).

General distribution: Algeria, Canary Islands, Egypt, France, Libya, Morocco, Portugal, Spain (Puławski, 2020).

***Tachysphex detritus* Arnold, 1924**

Tachysphex schönlandi var. *detritus* Arnold, 1924: Annals of the Transvaal Museum, 11: 69.

Distribution in Tunisia: Medenine (Puławski, 1971, 2007).

General distribution: Arabian Peninsula (Bahrain, United Arab Emirates), South Africa to North Africa, Israel, Tajikistan, Turkmenistan (Gadallah, 2020).

Remark: This species was identified by Puławski (1971) from Tunisia as the synonym *Tachysphex maidli* de Beaumont, 1940.

***Tachysphex erythropus* (Spinola, 1839) (Fig. 1F)**

Lyrops erythropus Spinola, 1839. Annales de la Société Entomologique de France, 7: 479.

Material examined. Kasserine: Sbeitla, Athar, CFPA, 560 m, 03.9.2018, 1 ♂; Kebili: Janouara, Rass Elain, 56 m, 16.5.2018, 1 ♀; Mahdia: Eljem, Athamnia, 70 m, 22.7.2019, 1 ♂, Ksour Essef, Alya, 1 m, 03.8.2018, 3 ♂♂, Salakta, 1 m, 03.8.2018, 1 ♂, Rejich, 3 m, 18.9.2018, 1 ♂, Sidi Alouane, Lemsanaa, 41 m, 01.6.2018, 1 ♂, Saada, 74 m, 18.6.2019, 2 ♂♂; Nabeul: Mida, Libna, 20 m, 05.9.2017, 1 ♂; Sidi Bouzid: Rgueb, Aouled Ayouni, 142 m, 18.7.2017, 1 ♂, West Sidi Bouzid, Zaafrja, 394 m, 25.7.2018, 3 ♂♂.

Distribution in Tunisia: Kairouan (von Schulthess, 1926); Jendouba, Kebili (Puławski, 2007).

General distribution: Africa, Arabian Peninsula (Oman, Saudi Arabia, United Arab Emirates), Southern Europe to Central Asia, India, China (Gadallah, 2020).

Remark: This species was identified by von Schulthess (1926) from Tunisia as the synonym *Tachysphex heliopolites* Morice, 1897.

***Tachysphex fugax* (Radoszkowski, 1877)**

Tachytes fugax Radoszkowski, 1877. Izvestiya Imperatorskobo Obshchestva Lyubiteley Estestvoznaniya, Antropologii i Étnografii pri Imperatorskom Moskovskom Universitete, 26: 30.

Distribution in Tunisia: Bizerte, Kasserine, Kebili, Sfax, Tozeur (Puławski, 2007).

General distribution: Africa, Southern Europe, Middle East and southern Caucasus, Kazakhstan, Tajikistan, Turkmenistan, Uzbekistan (Puławski, 2007).

***Tachysphex fulvitaris* (A. Costa, 1867)**

Tachytes fulvitaris A. Costa, 1867. Annuario del Museo Zoologico della R. Università di Napoli, 4: 86.

Distribution in Tunisia: Jendouba (Puławski, 2007).

General distribution: Africa, Azerbaijan, Europe, Kazakhstan, Middle East, Pakistan, Russia, Transcaspiya (Puławski, 2020).

***Tachysphex gracilicornis* Mercet, 1909 (Fig. 1G)**

Tachysphex gracilicornis Mercet, 1909. Boletín de la Real Sociedad Española de Historia Natural, 9: 194.

Material examined. Beja: Slouguia, 60 m, 17.8.2018, 1 ♀; Kairouan: Chbika, Aouled Zair, 136 m, 29.8.2018, 2 ♀♀, South Kairouan, Ragada, 92 m, 28.8.2018, 1 ♂; Kasserine: Sbeitla, Athar, Oued Nakhil, 562 m, 04.9.2018, 1 ♂, 1 ♀, Route Fej Ettin, 545 m, 05.9.2018, 1 ♀; Mahdia: Eljem, Tlelsa, 70 m, 13.8.2019, 1 ♂, 1 ♀, Ksour Essef, Alya, 1 m, 03.8.2018, 2 ♀♀, Sidi Alouane, Sidi Alouane 2, 69 m, 09.8.2018, 1 ♂, 2 ♀♀, Zelba, 49 m, 11.8.2018, 1 ♂; Sidi Bouzid: East Sidi Bouzid, Elhachria, 326 m, 24.7.2018, 3 ♀♀; Tozeur: Hamma Ejrid, 52 m, 27.6.2018, 1 ♀.

Distribution in Tunisia: Gafsa (von Schulthess, 1926); Kairouan (Puławski, 1971); Bizerte, Gabes, Jendouba, Kairouan, Medenine, Tataouine, Tozeur, Zaghuan (Puławski, 2007).

General distribution: Morocco to Mediterranean coast of Egypt and Sinai, south to Mauritania (Puławski, 2007).

Remark: This species was identified by von Schulthess (1926) from Tunisia as the synonym *Tachysphex eduardi* Morice in E. Saunders, 1910.

***Tachysphex gracilitarsis* Morice, 1910**

Tachysphex gracilitarsis Morice in E. Saunders, 1910. The Transactions of the Entomological Society of London, 1910: 527.

Distribution in Tunisia: Kairouan, Sfax (von Schulthess, 1926).

General distribution: Algeria, Libya, Morocco, Spain, Tunisia (Puławski, 2020).

***Tachysphex graecus* Kohl, 1883**

Tachysphex graeca Kohl, 1883. Deutsche Entomologische Zeitschrift, 27: 174.

Distribution in Tunisia: Kairouan (von Schulthess, 1926).

First Checklist of Tachysphex Kohl, 1883 of Tunisia

General distribution: Azerbaijan, Bulgaria, Greece, Iraq, Israel, Lebanon, Tunisia, Turkey, Yugoslavia (Puławski, 2020).

***Tachysphex grandissimus* Gussakovskij, 1933**

Tachysphex grandissimus Gussakovskij, 1933. Travaux de l'Institut Zoologique de l'Académie des Sciences de l'URSS, 1: 382.

Distribution in Tunisia: Kebili, Tozeur (Puławski, 2007).

General distribution: Arabian Peninsula (Kuwait, Saudi Arabia, United Arab Emirates), Sahara from Morocco to Egypt in the North, and from Mali to Chad in the south, India, Iran, Israel, Pakistan, Tajikistan, Turkmenistan (Gadallah, 2020).

***Tachysphex gujaraticus* Nurse, 1909 (Fig. 1H)**

Tachysphex gujaraticus Nurse, 1909. The Journal of the Bombay Natural History Society, 19: 516.

Material examined. Tozeur: Nefta, Nefta oasis, 56 m, 28.6.2018, 1 ♂.

Distribution in Tunisia: Kebili (Puławski, 2007).

General distribution: Arabian Peninsula (Oman, Saudi Arabia, United Arab Emirates, Yemen), North Africa south to Mali, Nigeria and Sudan, India, Israel, Sri Lanka, Tajikistan, Turkmenistan (Gadallah, 2020), Iran (Fallahzadeh et al, 2018).

***Tachysphex horus* de Beaumont, 1940**

Tachysphex horus de Beaumont, 1940. Bulletin de la Société Fouad I^{er} d'Entomologie, 24: 168.

Distribution in Tunisia: Tataouine (Puławski, 2007).

General distribution: Arabian Peninsula (Oman, Yemen, Saudi Arabia), Burkina Faso, Egypt, Israel, Tunisia (Gadallah, 2020).

***Tachysphex incertus* (Radoszkowski, 1877) (Fig. 1I)**

Tachytes incerta Radoszkowski, 1877. Izvestiya Imperatorskobo Obshchestva Lyubiteley Estestvoznaniya, Antropologii i Étnografii pri Imperatorskom Moskovskom Universitete, 26: 28.

Material examined. Beja: Mjez Elbeb, South Mjez Elbeb, 53 m, 17.8.2018, 1 ♂, Testour, Chambou, 81 m, 16.8.2018, 1 ♂.

Distribution in Tunisia: Sfax, Tozeur (von Schulthess, 1926); Ben Arous (de Beaumont, 1947b); Jendouba, Tozeur (Puławski, 2007).

General distribution: Arabian Peninsula (Oman, United Arab Emirates, Saudi Arabia), Europe north to southern France, northern Italy, Hungary, Slovakia, Ukraine, Russia, Africa south to southern Egypt to Mali, Asia north to Turkey, Kazakhstan, east to Pakistan (Gadallah, 2020).

Remark: This species was identified by von Schulthess (1926) and de Beaumont (1947b) from Tunisia as the synonym *Tachysphex pygidialis* Kohl, 1883.

***Tachysphex julliani* Kohl, 1883 (Fig. 2A)**

Tachysphex julliani Kohl, 1883. Deutsche Entomologische Zeitschrift, 27: 177.

Material examined. Tozeur: Temeghza, Cascade 2, 247 m, 27.6.2018, 1 ♂.

Distribution in Tunisia: Jendouba, Monastir, Sfax, Tozeur (Puławski, 2007).

General distribution: Algeria, Arabian Peninsula (Oman, Saudi Arabia, United Arab Emirates, Yemen), Bulgaria, Cyprus, Egypt, France, Greece, Iran, Israel, Italy, Kazakhstan, Lebanon, Libya, Morocco, Pakistan, Portugal, Spain, Tajikistan, Tunisia, Turkey, Turkmenistan, Ukraine, Uzbekistan (Gadallah, 2020).

***Tachysphex mauretanus* Puławski, 1971 (Fig. 2B)**

Tachysphex mauretanus Puławski, 1971. Państwowe Wydawnictwo Naukowe, Wrocław: 366.

Material examined. Mahdia: Sidi Alouane, Lemsanaa, 41 m, 26.5.2018, 1 ♀.

General distribution: Algeria, Morocco (Puławski, 2007).

Remark: This species is new for Tunisian fauna.

***Tachysphex mediterraneus* Kohl, 1883**

Tachysphex mediterranea Kohl, 1883. Deutsche Entomologische Zeitschrift, 27: 173.

Distribution in Tunisia: Jendouba (Puławski, 2007).

General distribution: Europe north to southern France, Hungary and Rostov area in Russia, Africa south to northern South Africa, Israel, Turkey, India, Iran, Azerbaijan, Kazakhstan, Sri Lanka, Tajikistan, Turkmenistan, Uzbekistan (Puławski, 2007).

***Tachysphex mocsaryi* Kohl, 1884**

Tachysphex mocsaryi Kohl, 1884. Verhandlungen der kaiserlich-königlichen zoologisch-botanischen Gesellschaft in Wien, 33: 367.

Distribution in Tunisia: Tunisia (no specific locality) (de Beaumont, 1947b).

General distribution: West Africa, Mediterranean area to southern Central Asia (Gadallah, 2020).

Remark: This species was cited by de Beaumont (1947b) from Tunisia as the synonym *Tachysphex mocsaryi maroccanus* de Beaumont, 1947.

***Tachysphex mycerinus* de Beaumont, 1940 (Figs. 2C-D)**

Tachysphex mycerinus de Beaumont, 1940. Bulletin de la Société Fouad I^{er} d'Entomologie, 24: 166.

Material examined. Kairouan: Chbika, Aouled Zair, 136 m, 29.8.2018, 1 ♀, South Kairouan, Ragada, 92 m, 28.8.2018, 1 ♂.

Distribution in Tunisia: Tozeur (de Beaumont, 1947a); Tataouine (Puławski, 2007).

General distribution: Morocco, to Egypt, south to Mali and Niger, Israel, Jordan (Puławski, 2007), Arabian Peninsula (Saudi Arabia, United Arab Emirates) (Gadallah, 2020).

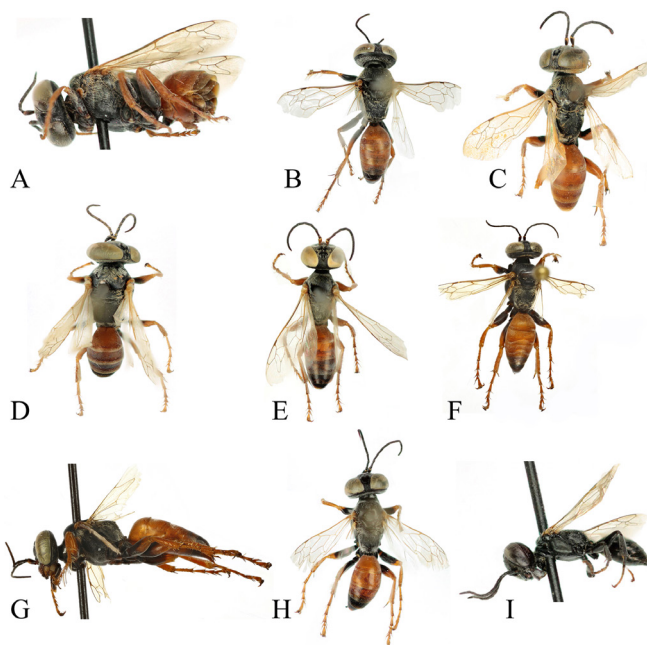


Fig. 2. General view of: A- *Tachyspex julliani* (♂); B- *Tachyspex mauretanus* (♀); C- *Tachyspex mycerinus* (♀); D- *Tachyspex mycerinus* (♂); E- *Tachyspex palopterus* (♀); F, G- *Tachyspex panzeri* (♀); H- *Tachyspex sericans gracilis* (♀); I- *Tachyspex unicolor* (♂).

***Tachyspex nitidior* de Beaumont, 1940**

Tachyspex nitidior de Beaumont, 1940. Bulletin de la Société Fouad I^{er} d'Entomologie, 24: 175.

Distribution in Tunisia: Bizerte, Gabes, Jendouba (Puławski, 2007).

General distribution: Mediterranean area to Central Asia and Mongolia, India, Korea, Senegal, United Arab Emirates (Gadallah, 2020).

***Tachyspex nitidus* (Spinola, 1805)**

Astata nitida Spinola, 1805. Faunnae [sic] Ligusticae fragmenta. Decas prima. Genuae, Typis Petri Cajetani Api: 17.

Distribution in Tunisia: Gafsa, Kairouan, Kasserine, Sfax, Tunis (von Schulthess, 1926); Nabeul, Tunis (Puławski, 1971); Gabes, Medenine, Tataouine, Tozeur (Puławski, 2007).

General distribution: Arabian Peninsula (Kuwait, Oman, United Arab Emirates, Saudi Arabia), Canary Islands, Europe, Israel, Kazakhstan, North Africa, Syria, Turkmenistan, Uzbekistan (Gadallah, 2020).

***Tachyspex notogoniaeformis* Nadig, 1933**

Tachyspex notogoniaeformis Nadig, 1933. Jahresbericht der Naturforschenden Gesellschaft Graubündens, 71: 80.

Distribution in Tunisia: Gafsa (de Beaumont, 1947b)

General distribution: Morocco to Libya (Puławski, 2007).

***Tachyspex palopterus* (Dahlbom, 1845) (Fig. 2E)**

Tachytes palopterus Dahlbom, 1845. Hymenoptera Europaea praecipue borealia; formis typicis nonnullis Specierum Generumve Exoticorum aut Extraneorum propter nexum systematicus associatis; per Familias, Genera, Species et Varietates disposita atque descripta. Tomus: Sphecx in sensu Linneano. Fascicle 3: 470.

Material examined. Kasserine: Sbeitla, Athar, CFPA, 560 m, 03.9.2018, 1 ♀; Kebili: South Kebili, Errahmat, 30 m, 17.5.2018, 1 ♀; Mahdia: Rejich, 3 m, 18.9.2018, 1 ♀; Sidi Bouzid: Aouled Hafouz center, 207 m, 26.7.2018, 1 ♀; Tozeur: Hamma Ejrid, 52 m, 27.6.2018, 3 ♂♂.

Distribution in Tunisia: Tunisia (no specific locality) (de Beaumont, 1940, 1947a).

General distribution: Arabian Peninsula (Oman, Saudi Arabia, United Arab Emirates, Yemen), North Africa, to Gambia, Israel, Niger, Sudan (Gadallah, 2020).

***Tachyspex panzeri* (Vander Linden, 1829) (Figs 2F-G)**

Tachytes panzeri Vander Linden, 1829. Nouvelles Mémoires de l'Académie Royale des Sciences et Belles Lettres de Bruxelles, 5: 22.

Material examined. Beja: Mjez Elbeb, South Mjez Elbeb, 53 m, 17.8.2018, 3 ♂♂, Slouguia, 60 m, 17.8.2018, 2 ♂♂, Testour, Chambou, 81 m, 16.8.2018, 2 ♂♂, 3 ♀♀, Elghanima, 84 m, 16.8.2018, 1 ♂; Kairouan: Chbika, Aouled Zair, 136 m, 29.8.2018, 1 ♂, 3 ♀♀; South Kairouan, Barouta, 95 m, 27.8.2018, 1 ♀, Ragada, 92 m, 28.8.2018, 1 ♂, 3 ♀♀; Kasserine: Sbeitla, Athar, CFPA, 560 m, 03.9.2018, 2 ♂♂, 1 ♀, Route Fej Ettin, 545 m, 05.9.2018, 4 ♂♂, 5 ♀♀, Oued Nakhil, 562 m, 04.9.2018, 1 ♀; Kebili: South Kebili, Errahmat, 30 m, 17.5.2018, 1 ♀, 18.5.2018, 1 ♂; Mahdia: Eljem, Ababsa, 70 m, 14.8.2019, 4 ♂♂, 2 ♀♀, Achaba, 101 m, 02.7.2019, 4 ♂♂, Aouled Lahmar, 99 m, 18.6.2019, 1 ♂, 27.8.2019, 5 ♂♂, 2 ♀♀, Athamnia, 70 m, 22.7.2019, 5 ♀♀, 28.8.2019, 5 ♂♂, 2 ♀♀, Tlelsa, 70 m, 13.8.2019, 4 ♂♂, 6 ♀♀, Touahria, 74 m, 22.7.2019, 3 ♂♂, 4 ♀♀, 26.8.2019, 11 ♂♂, 1 ♀, Zghabna, 74 m, 12.8.2019, 5 ♂♂, 5 ♀♀, Zouazi, 74 m, 16.6.2019, 5 ♂♂, 5 ♀♀, Ksour Essef, Alya, 1 m, 03.8.2018, 1 ♂, Route Ksour Essef - Sidi Alouane, 33 m, 01.7.2019, 4 ♂♂, 3 ♀♀, Rejich, 3 m, 18.9.2018, 10 ♂♂, 7 ♀♀, Sidi Alouane, Ghlalba, 48 m, 19.6.2019, 2 ♂♂, 2 ♀♀, 09.9.2019, 2 ♂♂, Rmadhnia, 74 m, 03.9.2019, 4 ♂♂, 2 ♀♀, West Oued Beja, Ecole primaire Amel, 74 m, 22.6.2019, 6 ♂♂, 5 ♀♀, 10.9.2019, 3 ♂♂, 1 ♀, Saada, 74 m, 18.6.2019, 7 ♂♂, 3 ♀♀, 04.9.2019, 2 ♂♂, Saguiet Khadem, 53 m, 29.6.2019, 4 ♂♂, 2 ♀♀, 24.7.2019, 7 ♂♂, 1 ♀, 02.9.2019, 1 ♂, 1 ♀, Sidi Alouane 2, 69 m, 09.8.2018, 2 ♂♂, Oued Beja, 75 m, 21.5.2017, 1 ♂, 1 ♀, Zelba, 49 m, 11.8.2018, 1 ♂, 17.6.2019, 8 ♂♂, 4 ♀♀, 03.7.2019, 5 ♂♂, 2 ♀♀, 23.7.2019, 11 ♂♂, 4 ♀♀; Sidi Bouzid: West Sidi Bouzid, Zaafrina, 394 m, 25.7.2018, 1 ♂, East Sidi Bouzid, Elhachria, 326 m, 24.7.2018, 1 ♀; Siliana: Kiswa, 809 m, 27.4.2018, 2 ♀♀; Sousse: Bouficha, Salloum, 5 m, 11.9.2017, 1 ♀, Kalaa Kbir, Belaoum, 45 m, 29.7.2017, 1 ♂, 1 ♀; Tataouine: North Tataouine, Oued Tlelet, 170 m, 12.5.2018, 1 ♀; Tozeur: Dguech, Elmanachi, 63 m, 29.6.2018, 2 ♂♂, Hamma Ejrid, 52 m, 27.6.2018, 3 ♀♀, Nefta, Nefta oasis, 56 m, 28.6.2018, 1 ♀, Temeghza, Cascade 2, 247 m, 27.6.2018, 2 ♀♀.

Distribution in Tunisia: Tunis (Graeffe, 1906); Kairouan, Monastir, Tozeur, Tunis (von Schulthess, 1926); Tozeur (Puławski, 1971); Gabes, Gafsa, Jendouba, Kasserine, Kebili, Medenine, Monastir, Nabeul, Sfax, Sousse, Tozeur (Puławski, 2007).

General distribution: Albania, Algeria, Armenia, Austria, Belgium, Botswana, Bulgaria, Burkina Faso, Canary Island, Chad, China, Croatia, Cyprus, Czech Republic, Egypt, Ethiopia, France, Gambia, Germany, Greece, Hungary, India, Iran,

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Israel, Italy, Kazakhstan, Latvia, Libya, Lithuania, Mali, Malta, Mauritania, Mongolia, Morocco, Netherlands, Niger, Pakistan, Poland, Portugal, Romania, Russia, Senegal, Slovakia, Spain, Sri Lanka, Sudan, Tajikistan, Tunisia, Turkey, Turkmenistan, Ukraine, Uzbekistan, former Yugoslavia, Arabian Peninsula (Bahrain, Oman, Qatar, Saudi Arabia, United Arab Emirates, Yemen) (Gadallah, 2020).

Remark: This species was identified by von Schulthess (1926) and Puławski (1971) from Tunisia as the synonym *Tachysphex panzeri oraniensis* (Lepeletier de Saint-Fargeau, 1845).

***Tachysphex psammobius* (Kohl, 1880)**

Tachytes psammobius Kohl, 1880. Zeitschrift des Ferdinandeums für Tirol und Vorarlberg, 3: 235.

Distribution in Tunisia: Tunis (von Schulthess, 1926).

General distribution: Azerbaijan, Canada, Europe, Iran, Kazakhstan, Russia, Syria, Tajikistan, Turkey, Turkmenistan, Uzbekistan (Puławski, 2020).

***Tachysphex pseudofasciatus* Puławski, 2007**

Tachysphex pseudofasciatus Puławski, 2007. Proceedings of the California Academy of Sciences (Fourth Series) 58, Supplement 1: 506.

Distribution in Tunisia: Tozeur (Puławski, 2007).

General distribution: Algeria, Egypt, Israel, Tunisia (Puławski, 2007).

***Tachysphex schmiedeknechti* Kohl, 1883**

Tachysphex schmiedeknechti Kohl, 1883. Deutsche Entomologische Zeitschrift, 27: 170.

Distribution in Tunisia: Kebili, Tozeur (Puławski, 2007).

General distribution: Arabian Peninsula (Oman, Saudi Arabia, United Arab Emirates, Yemen), Africa south to Ghana and Kenya, Cyprus, Greece, Iran to Central Asia, southern Spain, Syria, Turkey, India (Gadallah, 2020).

***Tachysphex sericans gracilis* Puławski, 1971 (Fig. 2H)**

Tachysphex sericans gracilis Puławski, 1971. Państwowe Wydawnictwo Naukowe, Wrocław: 326.

Material examined. Kebili: South Kebili, Errahmat, 30 m, 17.5.2018, 2 ♂♂, 1 ♀.

Remark: This subspecies is new for Tunisian fauna.

General distribution: Algeria, Egypt (Puławski, 2020).

***Tachysphex speciosissimus* Morice, 1897**

Tachysphex speciosissimus Morice, 1897. The Transactions of the Entomological Society of London, 1897: 309.

Distribution in Tunisia: Gabes (Puławski, 2007).

General distribution: North Africa, Israel, Syria, Iran, Pakistan (Puławski, 2007).

***Tachysphex tarsinus* (Lepeletier de Saint-Fargeau, 1845)**

Tachytes tarsinus Lepeletier de Saint-Fargeau, 1845. Histoire naturelle des Insectes. Hyménoptères. Tome Troisième: 243.

Distribution in Tunisia: Tozeur (Puławski, 2007).

General distribution: Algeria, Austria, Belarus, Bulgaria, China, Croatia, Czech Republic, Egypt, France, Germany, Greece, Hungary, Iran, Israel, Italy, Kazakhstan, Lebanon, Libya, Mauritania, Morocco, Poland, Portugal, Russia, Slovakia, Slovenia, Spain, Switzerland, Tajikistan, Tunisia, Turkey, Ukraine, Arabian Peninsula (Oman, Saudi Arabia, United Arab Emirates) (Gadallah, 2020).

***Tachysphex unicolor* (Panzer, 1809) (Fig. 2I)**

LARRA unicolor Panzer, 1809. Faunae Insectorum Germanicae initia oder Deutschlands Insecten. Hundert und sechsten Hefts: 16.

Material examined. Mahdia: Sidi Alouane, Aouled Kloula, 64 m, 06.4.2018, 1 ♂.

General distribution: Afghanistan, China, Europe, Kazakhstan, North Africa, Lebanon, Mongolia, Palestine, Russia, Turkey, Turkmenistan (Puławski, 2020).

DISCUSSION

In this checklist 38 species and subspecies of the genus *Tachysphex* are presented in Tunisia. Because of its complexity and the problematic that it poses in systematical studies and to facilitate its study, the genus *Tachysphex* is divided into species groups (de Beaumont, 1936; Puławski, 1971, 2007). According to Puławski (2007), there are 18 *Tachysphex* species groups. Taxa present in this study belong to 11 species groups (Table 2). *T. panzeri* and *T. pompiliformis* species groups are the most diversified with 11 and 12 taxa respectively.

T. mauretanus Puławski, 1971 and *T. sericans gracilis* Puławski, 1971 are newly recorded from Tunisia. Geographically, these two taxa are distributed in Algeria and Morocco for the first species and in Algeria and Egypt for the second (Puławski, 2020). Therefore, Tunisia is added as a third distribution area for these two taxa.

According to de Beaumont (1940, 1947a) *T. palopterus* (Dahlbom, 1845) was recorded in Tunisia but without specific locality. With this study, the presence of this species is confirmed with six new provincial records (Kasserine, Kebili, Mahdia, Sidi Bouzid and Tozeur provinces). While, *T. mocsaryi* Kohl, 1884 was cited in Tunisia by de Beaumont (1947b) under the synonym *T. mocsaryi maroccanus* but without a specific locality; further study is so needed to confirm its presence in Tunisian territory.

The fauna of Tozeur province located in southern Tunisia is the most diversified with 17 records followed by Kebili with 11, Kairouan and Jendouba with 10 records (Fig.3). In other provinces, records vary between one and nine records with three provinces (Mannouba, Ariana, Ben Arous) are without records.

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Number of species and subspecies presented in this checklist estimated by 38 species can be considered relatively low compared with that of other countries in North Africa such as the Egyptian fauna of *Tachysphex* estimated by 56 species (Roche, 2007). In addition to that, the absence or the reduced number of records in provinces suggests to conduct other entomological surveys to contribute better to the knowledge and evaluate this fauna in Tunisia. Discover of new country and provincial records and new species for the science is highly possible.

Table 2. Distribution of species and subspecies in species groups.

Species group	Species and subspecies
<i>Tachysphex albocinctus</i> group	<i>T. albocinctus</i>
<i>Tachysphex bicolor</i> group	<i>T. adjunctus</i> , <i>T. denisi</i> , <i>T. graecus</i>
<i>Tachysphex brevipennis</i> group	<i>T. brevipennis</i>
<i>Tachysphex brullii</i> group	<i>T. brullii</i>
<i>Tachysphex erythropus</i> group	<i>T. costae</i> , <i>T. detritus</i> , <i>T. erythropus</i> , <i>T. grandissimus</i>
<i>Tachysphex geniculatus</i> group	<i>T. horus</i>
<i>Tachysphex julliani</i> group	<i>T. julliani</i> , <i>T. mauretanus</i>
<i>Tachysphex panzeri</i> group	<i>T. atlanteus</i> , <i>T. brevipecten</i> , <i>T. gracilicornis</i> , <i>T. gracilitarsis</i> , <i>T. incertus</i> , <i>T. mocsaryi</i> , <i>T. mycerinus</i> , <i>T. notogoniaeformis</i> , <i>T. pallopterus</i> , <i>T. panzeri</i> , <i>T. sericans gracilis</i>
<i>Tachysphex plicatus</i> group	<i>T. mediterraneus</i>
<i>Tachysphex pompiliformis</i> group	<i>T. agnus</i> , <i>T. consocius</i> , <i>T. fugax</i> , <i>T. fulvitaris</i> , <i>T. gujaraticus</i> , <i>T. nitidus</i> , <i>T. nitidior</i> , <i>T. psammobius</i> , <i>T. pseudofasciatus</i> , <i>T. speciosissimus</i> , <i>T. tarsinus</i> , <i>T. unicolor</i>
<i>Tachysphex schmiedeknechti</i> group	<i>T. schmiedeknechti</i>
Total	11 species groups 38 species and subspecies

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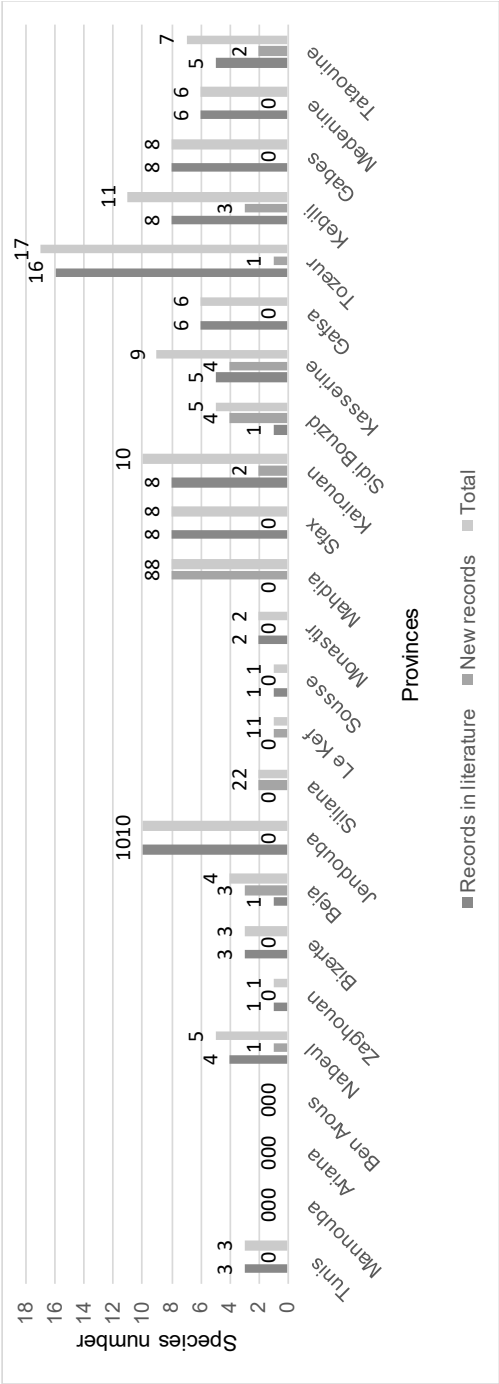


Fig. 3. Records in literature and new records number per province

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Mitochondrial Genomic Analysis of the Tea Geometrid, *Ectropis grisescens* Warren, 1894 (Lepidoptera: Geometridae) and Its Phylogenetic Implications

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ABSTRACT

Ectropis grisescens Warren, 1894 is a major pest species only feeding on tea plants but lacks sufficient molecular data to clarify its genetic characters. Complete mitochondrial genome of the tea geometrid *Ectropis grisescens* is sequenced for the first time. The mitogenome of *E. grisescens* is 16,575 bp in length, with an A+T content of 81.2%. The typical set of 37 genes (13 PCGs, 22 tRNA genes and two rRNA genes) are all identified. Most PCGs have standard ATN start codons and TAN stop codons. Most tRNA genes exhibit cloverleaf secondary structures, whereas the dihydrouridine (DHU) arm of *trnSer* (AGN) is reduced into a small loop. Poly-T stretch, tandem repeats and stem-loop (SL) structures are predicted in the control region, which is long (1550 bp) and highly biased toward A+T nucleotides (92.3%). The mitochondrial phylogenetic analyses using the Bayesian inference (BI) and maximum likelihood methods (ML) consistently recover the generic relationships within Geometridae. *E. grisescens* is correctly grouped with its congener and the monophyly of Ennominae and Larentiinae are both supported.

Key words: Lepidoptera, Geometridae, *Ectropis grisescens*, Mitochondrial genome.

INTRODUCTION

Mitochondrial genome (mitogenome) has become one of the most popular molecules used in recent phylogenetic studies. The advantages of using mitogenomic data in phylogenetic studies include low cost, sufficient genetic information for higher taxa, and low rate of rearrangement, etc (Boore, 1999). Meanwhile, the mitochondrial cytochrome oxidase subunit I (*cox1*) gene is widely used for DNA barcoding in species delimitation and intraspecific genetics (Hebert, Cywinska, Ball, & deWaard, 2003). Structures of animal mitogenomes are usually conserved with 37 typical set of genes, including 13 protein-coding genes (PCGs), 22 transfer RNA (tRNA) genes and two ribosomal RNA (rRNA) genes (Simon, Buckley, Frati, Stewart, & Beckenbach, 2006).

Lepidoptera is the second largest insect order with about 15,7000 species, and many of them are major economic pests (Stork, 2018; Mullen & Zaspel, 2019). Geometridae is one of the largest families in Lepidoptera, comprising over 21,000 species usually harmful to the agriculture and forestry and about 2000 of which are found in China (Scoble, 1999; Gu & Xin, 2018). Among the considerable large number of geometrids, only 12 species have sequenced mitogenomes available from GenBank. The limited mitogenomes are out of proportion to the large number of species in Geometridae.

Ectropis grisescens Warren, 1894 also known as the tea geometrid, is an economically important pest species in Asia and is especially widely distributed in the tropical and subtropical areas of China (Warren, 1894; Jiang et al, 2014). Larvae of *E. grisescens* prefer to damage the bud and leaves of the tea plants, causing considerable economic loss in the tea industry of China (Ge, Yan & Xiao, 2015). Accumulation of genetic data of *E. grisescens* is warranted to find new control strategies for this pest from a molecular view. However, the mitogenome of *E. grisescens* is still undetermined, hindering our understanding of the relationship between *E. grisescens* and other lepidopteran pests. In this study, the complete mitogenome of *E. grisescens* is sequenced and described for the first time. The mitogenomic structure, nucleotide compositions, codon usages of PCGs, secondary structures of tRNA genes and the control region are investigated to better understand the genetic characters of this important pest.

MATERIALS AND METHODS

Sampling and DNA extraction

Adult specimens of *E. grisescens* were collected from Wolong Town, Qionglai City, Sichuan Province of China in May of 2019. The specimens were identified by Sichuan Academy of Agricultural Sciences and preserved in 100% ethanol. Total genomic DNA was extracted using the E.Z.N.A.® Tissue DNA Kit (OMEGA, America) and stored at -20 °C until used for mitogenome sequencing.

Mitogenome sequencing, assembly and annotation

After DNA extraction, 1.0 µg of purified DNA was fragmented and used to construct Illumina TruSeq short-insert libraries (insert size = 450 bp) following the manufacturer's instructions, then sequenced on the Illumina Hiseq 4000 (Shanghai BIOZERON Co., Ltd). Raw reads were filtered prior to assembly. The reads with adaptors, with a low quality score below 20, with over 10% "N" bases, or with very few nucleotides were removed. High-quality reads were used for de novo and reference-guided assembling according to the following steps: 1) Firstly, the filtered reads were assembled into contigs using SOAPdenovo2.04 (Luo et al, 2012); 2) Secondly, the contigs were aligned to the reference mitogenome of *Abraxas suspecta* Warren, 1894 (GenBank accession number KY095828) using BLAST; the aligned contigs (≥80% similarity and query coverage) were ordered according to the reference mitogenome; 3) Finally, the clean reads were mapped to the assembled draft mitogenome to calibrate the wrong bases, and the gaps were filled by GapFiller v2.1.1 (<https://sourceforge.net/projects/gapfiller/>).

The complete mitogenome sequence of *E. griseascens* was deposited into GenBank under the accession number MN792921. Location and secondary structures of the tRNA genes were predicted by MITOS (Bernt et al, 2013). PCGs and rRNA genes were annotated using homology alignments. Open reading frames of PCGs were examined and adjusted by ORF finder (<https://www.ncbi.nlm.nih.gov/orffinder/>). CGView Server (http://stothard.afns.ualberta.ca/cgview_server/) was used to illustrate the mitogenome of *E. griseascens* (Grant & Stothard, 2008). Nucleotide composition and codon usage were calculated by MEGA v.6.0 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013). The two formulas AT-skew = $[A-T]/[A+T]$ and GC-skew = $[G-C]/[G+C]$ were used in the composition skew analysis (Perna & Kocher, 1995). Secondary structures in the control region were predicted by Tandem Repeats Finder (<http://tandem.bu.edu/trf/trf.advanced.submit.html>) and DNAMAN v6.0.3.

Phylogenetic analyses

PCGs derived from 13 moths of Geometridae, including *E. griseascens* sequenced in this study, were used in the phylogenetic analyses (Table 1). Another moth, *Cameraria ohridella* Deschka & Dimic 1986 from family Gracillariidae (GenBank accession number KJ508042) was used as the outgroup. Each of the 13 PCGs was respectively aligned by MAFFT, then concatenated into a combined dataset using SequenceMatrix v1.7.8 (Kato & Standley, 2013). PartitionFinder v2.1.1 was used to determine the optimal nucleotide substitution models and partitioning schemes with the Bayesian Information Criterion (BIC) and a greedy search algorithm (Lanfear, Frandsen, Wright, Senfeld, & Calcott, 2016). Two phylogenetic inferences were conducted, including Bayesian inferences (BI) and Maximum likelihood (ML) analysis. BI analysis was conducted by MrBayes v3.2.6, running 20 million generations, sampling every 1000 generations and executing one cold chain and three hot chains with a burn-in of 25% trees (Ronquist & Huelsenbeck, 2003). All runs of BI analysis were examined by Tracer v.1.5 (Rambaut & Drummond, 2019). ML analysis was performed with RAxML v8, with 1000 bootstrap replicates (Stamatakis, 2014). Both BI and ML trees were depicted with FigTree v1.4.2.

Table 1. Species of Lepidoptera used in the phylogenetic study.

Family	Species	GenBank accession number
Geometridae	<i>Abraxas suspecta</i>	KY095828
	<i>Apocheima cinerarium</i>	KF836545
	<i>Biston panterinaria</i>	JX406146
	<i>Biston perclara</i>	KU325536
	<i>Biston suppressaria</i>	KP278206
	<i>Biston thibetaria</i>	KJ670146
	<i>Celenna</i> sp.	KM244697
	<i>Dysstroma truncata</i>	KJ508061
	<i>Ectropis grisescens</i>	KY095828
	<i>Ectropis obliqua</i>	KX827002
	<i>Jankowskia athleta</i>	KR822683
	<i>Operophtera brumata</i>	KP027400
	<i>Phthonandria atrilineata</i>	EU569764
Gracillariidae	<i>Cameraria ohridella</i>	KJ508042

RESULTS AND DISCUSSION

Mitogenome structure and nucleotide composition

The complete mitogenome of *E. grisescens* is a double-strand circular molecule with a length of 16,575 bp, longer than all available mitogenomes of Geometridae. The typical set of 37 genes, including 13 PCGs, 22 tRNA genes and two rRNA genes were all identified in the mitogenome. Among these genes, nine PCGs and 14 tRNA genes are encoded by the majority strand (J-strand); the remaining four PCGs, eight tRNA genes and two rRNA genes are on the minority strand (N-strand) (Fig. 1, Table 2). The mitochondrial gene arrangement of *E. grisescens* differs from the putative ancestral mitogenome of *Drosophila yakuba* Burla, 1954 by showing the *trnMet-trnIle-trnGln* tRNA cluster, which is a very common rearrangement pattern in Lepidoptera (Chen & Du, 2016). The mitogenome of *E. grisescens* has 41 overlapping nucleotides in seven gene pairs; the longest overlap is found between *trnPhe* and *nad5*, which is 17-bp long. There are also 238 intergenic nucleotides (IGNs) scattered in 13 gene gaps, indicating a loose mitogenomic structure of *E. grisescens*.

The whole mitogenome of *E. grisescens* is biased toward A+T nucleotides (81.2%) with a positive AT-skew and negative GC-skew as commonly found in most other insects (Table 3) (Wei et al, 2010). The A+T contents are similarly high in the concatenated PCGs, PCGs on J-strand, PCGs on N-strand, tRNA genes and rRNA genes (Table 3). All codon positions of the concatenated PCGs have reversal of strand

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asymmetry (negative AT skew and positive GC-skew), whereas tRNA genes and rRNA genes exhibit a positive AT skew and negative GC-skew. In the 37 mitochondrial genes, the A+T content is highest in *trnGlu* (92.3%) and lowest in *cox1* (72.0%).

Table 2. Mitochondrial genome structure of *Ectropis grisescens*.

Gene	Position (bp)	Size (bp)	Direction	Intergenic nucleotides	Anti- or start/stop codons	A+T%
<i>trnMet</i> (M)	1–69	69	Forward	0	CAT	73.9
<i>trnIle</i> (I)	70–137	68	Forward	0	GAT	77.9
<i>trnGln</i> (Q)	135–203	69	Reverse	–3	TTG	84.1
<i>nad2</i>	268–1269	1002	Forward	64	ATA/TAA	84.0
<i>trnTrp</i> (W)	1276–1344	69	Forward	6	TCA	78.3
<i>trnCys</i> (C)	1337–1404	68	Reverse	–8	GCA	83.8
<i>trnTyr</i> (Y)	1405–1471	67	Reverse	0	GTA	79.1
<i>cox1</i>	1479–3009	1531	Forward	7	CGA/T	72.0
<i>trnLeu2</i> (UUR)	3010–3076	67	Forward	0	TAA	73.1
<i>cox2</i>	3077–3758	682	Forward	0	ATG/T	76.8
<i>trnLys</i> (K)	3759–3830	72	Forward	0	CTT	73.6
<i>trnAsp</i> (D)	3833–3898	66	Forward	2	GTC	87.9
<i>atp8</i>	3899–4066	168	Forward	0	ATT/TAA	91.1
<i>atp6</i>	4060–4737	678	Forward	–7	ATG/TAA	79.1
<i>cox3</i>	4741–5529	789	Forward	3	ATG/TAA	72.9
<i>trnGly</i> (G)	5532–5597	66	Forward	2	TCC	90.9
<i>nad3</i>	5595–5951	357	Forward	–3	ATA/TAA	82.9
<i>trnAla</i> (A)	5955–6018	64	Forward	3	TGC	82.8
<i>trnArg</i> (R)	6019–6082	64	Forward	0	TCG	76.6
<i>trnAsn</i> (N)	6083–6147	65	Forward	0	GTT	78.5
<i>trnSer1</i> (AGN)	6148–6213	66	Forward	0	GCT	80.3
<i>trnGlu</i> (E)	6282–6346	65	Forward	68	TTC	92.3
<i>trnPhe</i> (F)	6349–6414	66	Reverse	2	GAA	83.3
<i>nad5</i>	6398–8152	1755	Reverse	–17	ATC/TAA	81.3
<i>trnHis</i> (H)	8153–8220	68	Reverse	0	GTG	89.7
<i>nad4</i>	8221–9559	1339	Reverse	0	ATG/T	80.5
<i>nad4l</i>	9559–9849	291	Reverse	–1	ATG/TAA	85.6
<i>trnThr</i> (T)	9856–9920	65	Forward	6	TGT	80.0
<i>trnPro</i> (P)	9921–9986	66	Reverse	0	TGG	83.3

Table 2. Continued.

Gene	Position (bp)	Size (bp)	Direction	Intergenic nucleotides	Anti- or start/stop codons	A+T%
<i>nad6</i>	9989–10522	534	Forward	2	ATA/TAA	85.6
<i>cytb</i>	10,564–11,715	1152	Forward	41	ATG/TAA	75.6
<i>trnSer2</i> (UCN)	11,714–11,779	66	Forward	–2	TGA	81.8
<i>nad1</i>	11,812–12,750	939	Reverse	32	TTG/TAA	77.2
<i>trnLeu1</i> (CUN)	12,751–12,819	69	Reverse	0	TAG	82.6
<i>rrnL</i>	12,820–14,194	1375	Reverse	0	–	84.5
<i>trnVal</i> (V)	14,195–14,260	66	Reverse	0	TAC	83.3
<i>rrnS</i>	14,261–15,025	765	Reverse	0	–	85.4
Control region	15,026–16,575	1550	–	0	–	91.9

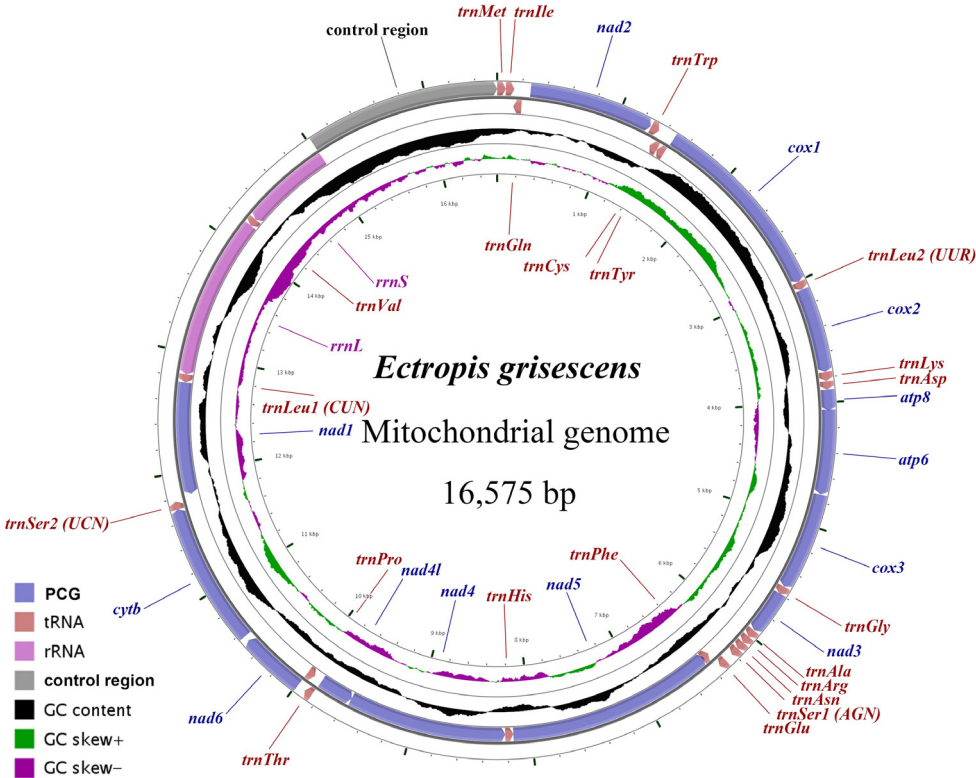


Fig. 1. Mitochondrial map of *Ectopis grisescens*. Genes outside the map are transcribed clockwise; genes inside the map are transcribed counterclockwise. GC content and GC skew are shown as inside circles. GC content and GC skew are plotted as the deviation from the average value of the entire mitogenome sequence.

PCGs, tRNA and rRNA genes

No rearrangement or variations were found in the 13 PCGs of *E. grisescens*. Most PCGs start with the standard ATN codon, including ATA start codon in *nad2*, *nad3* and *nad6*, ATG in *cox2*, *cox3*, *cytb*, *atp6*, *nad4* and *nad4l*, ATT in *atp8* and ATC in *nad5* (Table 2). However, *cox1* uses the special start codon CGA, and *nad1* uses another special start codon TTG. Ten of the PCGs have the complete stop codon TAA, whereas *cox1*, *cox2* and *nad4* contain an incomplete stop codon T, which is common in other lepidopteran species (Garey & Wolstenholme, 1989). The relative synonymous codon usage (RSCU) of the 13 PCGs indicate that TTA (Leu), TCT (Ser) and CGA (Arg) are the most frequently used codons whereas CTG (Leu), CCG (Pro) and AGG (Ser) are the least (Fig. 2).

The regular set of 22 tRNA genes are all found in the mitogenome, while the ancestral *trnIle-trnGln-trnMet* tRNA cluster is rearranged into *trnMet-trnIle-trnGln*. The 22 tRNA genes have a total length of 1471 bp and an A+T content of 81.6%; each tRNA gene ranges in size from 64 to 72 bp (Table 2). Twenty-one of the tRNA genes can fold into typical cloverleaf secondary structures (Fig. 3), however, the TΨC arms of *trnPhe* and *trnThr* are somewhat reduced, and the dihydrouridine (DHU) arm is reduced into a small loop, which is very common in other metazoans (Garey & Wolstenholme, 1989). In these tRNA genes, 12 mismatched base pairs are detected and all of them are G-U pairs. The anticodon (AC) arm of *trnSer2* forms two loops, which is uncommon in other lepidopteran species. The anticodons of the 22 tRNA genes of *E. grisescens* are all identical with other sequenced lepidopterans (Yang, Wei, Hong, Jiang, & Wen, 2009).

Two rRNA genes were identified in the conserved locations between *trnLeu1* and the control region of *E. grisescens*, with a total length of 2140 bp and an A+T content of 84.8% (Table 3). The large ribosomal RNA (*rrnL*) gene is 1375-bp long, with an A+T content of 84.5%. The small ribosomal RNA (*rrnS*) gene is 765-bp in size with an A+T content of 85.4%.

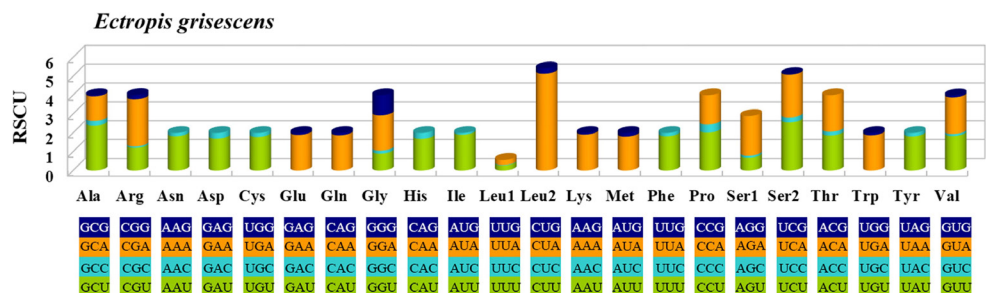


Fig. 2. Relative synonymous codon usage (RSCU) of PCGs in *Ectropis grisescens*. Full codon families are indicated below the X-axis.

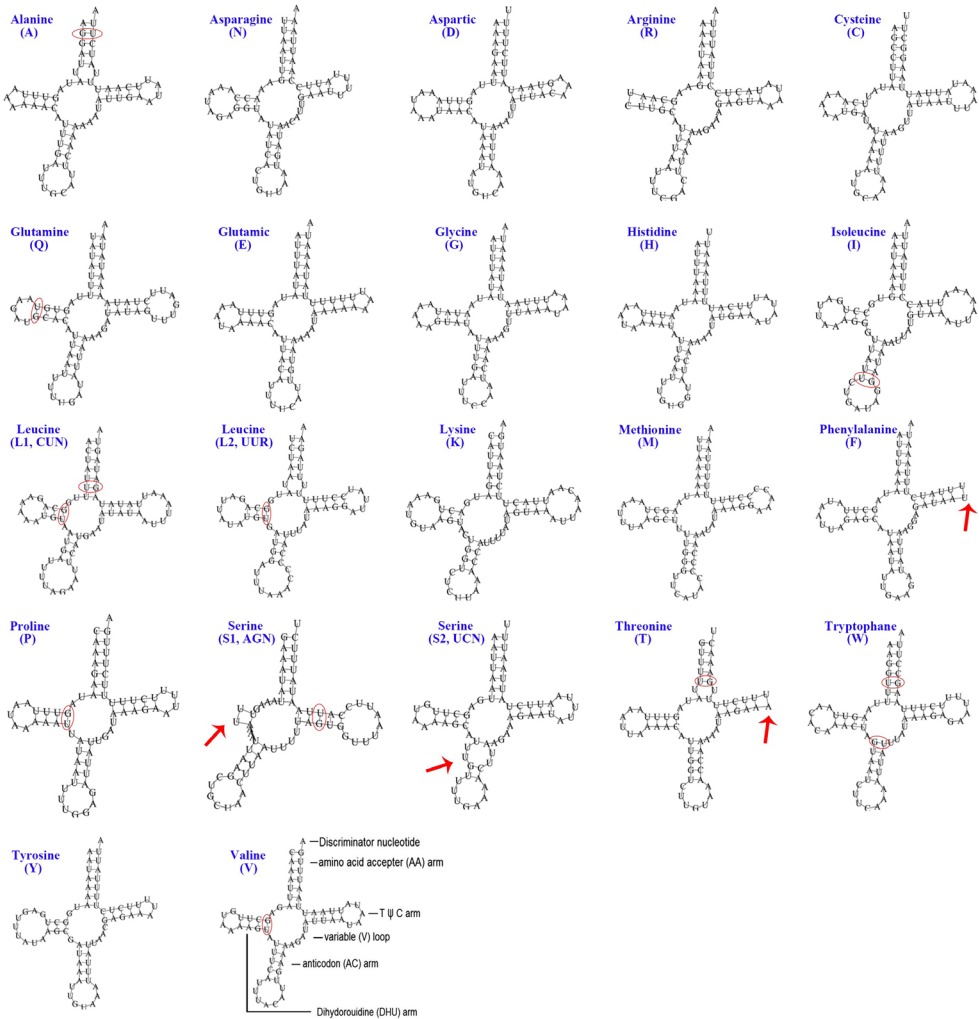


Fig. 3. Secondary structures of tRNA genes in the mitogenome of *Ectropis grisescens*. The tRNA genes are labelled with their corresponding amino acids. Structural elements are illustrated as for trnV. Modified arms are indicated with red arrows. Mismatched pairs are indicated with red circles.

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Table 3. Nucleotide composition of the mitogenome of *Ectropis grisescens*.

Regions	A%	T%	G%	C%	A+T%	G+C%	AT Skew	GC Skew
Whole mitogenome	41.2	40.0	7.7	11.1	81.2	18.8	0.015	-0.181
PCGs	34.4	44.4	11.1	10.1	78.8	21.2	-0.127	0.047
1 st codon position	36.3	44.6	9.7	9.4	80.9	19.1	-0.103	0.016
2 nd codon position	34.3	44.5	11.9	9.3	78.8	21.2	-0.129	0.123
3 rd codon position	32.6	44.1	11.6	11.7	76.7	23.3	-0.150	-0.004
PCGs-J	35.9	41.9	10.1	12.1	77.8	22.2	-0.077	-0.090
1 st codon position	37.3	40.6	11.2	10.9	77.9	22.1	-0.042	0.014
2 nd codon position	32.6	42.5	11.1	13.8	75.1	24.9	-0.132	-0.108
3 rd codon position	37.7	42.5	8.1	11.7	80.2	19.8	-0.060	-0.182
PCGs-N	32.0	48.5	12.5	7.0	80.5	19.5	-0.205	0.282
1 st codon position	43.6	39.7	6.7	10.0	83.3	16.7	0.047	-0.198
2 nd codon position	38.9	42.8	5.5	12.8	81.7	18.3	-0.048	-0.399
3 rd codon position	41.1	45.0	3.3	10.6	86.1	13.9	-0.045	-0.525
tRNA genes	41.6	40.0	8.3	10.1	81.6	18.4	0.020	-0.098
tRNA genes-J	41.9	38.6	10.1	9.4	80.5	19.5	0.041	0.036
tRNA genes-N	41.2	42.5	5.2	11.1	83.7	16.3	-0.016	-0.362
rRNA genes	43.9	40.9	4.9	10.3	84.8	15.2	0.035	-0.355
Control region	41.4	50.5	3.0	5.1	91.9	8.1	-0.099	-0.259

Control region

The longest non-coding sequence of the *E. grisescens* mitogenome is the control region located between *rrnS* and *trnMet* as in other lepidopterans (Fig. 1, Table 2). The control region of *E. grisescens* is 1550-bp in size, longer than all sequenced control regions of Geometridae. The A+T content of the control region is 91.9%, which is very high but still lower than *trnGlu* (92.3%).

As the most variable region in a mitogenome, the control region of *E. grisescens* contains some secondary structures (Fig. 4): a poly-T leading stretch (15,069-15,088) near the 5' end; seven complete and a partial tandem repeats (15,095-16,502) with a consensus size of 192 bp; three continuous stem-loop (SL) structures (16510-16575) near the 3' end of the control region. The large tandem repeat region is the main reason for the large size of the control region in *E. grisescens*. The tandem repeats and SL structures can act as regulators during the mitogenomic replication and transcription processes (Crozier & Crozier, 1993). However, exact functions of the control regions of Lepidoptera are still unclear.

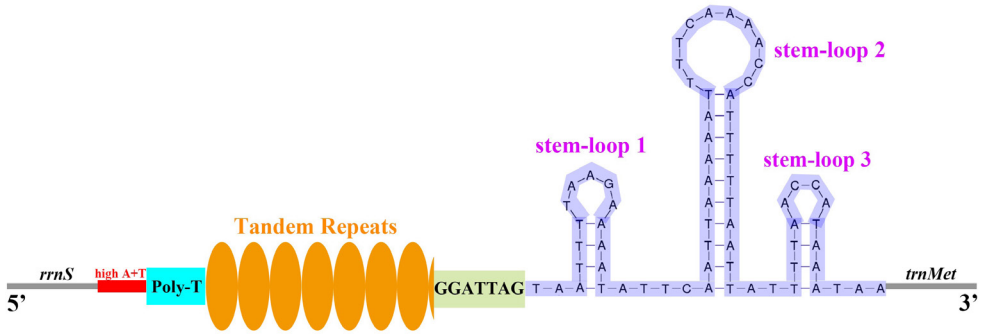


Fig. 4. Predicted structural elements in the control region of *Ectropis grisescens*. Poly-T stretch is indicated with blue box; tandem repeat units are indicated by orange ellipses; stem-loop structures are depicted showing their nucleotides. Other sequences with high A+T content are shown as red lines.

Phylogenetic analyses

The two phylogenetic trees respectively generated by the BI and ML analyses have almost identical topological structures (Figs. 5-6). The overall generic relationships recovered by both phylogenetic inferences are (((((*Biston* + (*Apocheima* + *Jankowskia*)) + *Ectropis*) + *Abraxas*) + *Celenna*) + *Phthonandria*) + (*Dysstroma* + *Operophtera*).

In both trees, the two subfamilies Ennominae and Larentiinae are recovered as monophyletic with high support (posterior probability = 1, bootstrap value = 100). The four species of genus *Biston* are grouped together, but *B. panterinaria* is clustered with *B. thibetaria* in the BI tree and with *B. perclara* in the ML tree. In *Ectropis*, *E. grisescens* sequenced herein is correctly supported as the sister group of *E. obliqua*. The sister group relationships are recovered between genera *Apocheima* and *Jankowskia*, and also between *Dysstroma* and *Operophtera*. In Ennominae, *Phthonandria* is supported as the basal group of its subfamily, but this conclusion is only reliable before further mitogenomes of Ennominae are sequenced.

The phylogenetic results of this study are generally identical with previous mitochondrial phylogenetic studies (Liu, Xue, Cheng, & Han, 2014; Xu, Chen, Wang, Peng, & Li, 2016; Sun et al, 2017; Li, Wang, Chen, & Han, 2018; Zheng et al, 2018). The mitogenomic data has been widely used in Lepidoptera during recent years and considerable efforts have been spent on the molecular phylogeny of diverse lepidopterans, however, very few attention was paid for Geometridae, leaving only nine genera with sequenced mitogenomes, which is far from inferring a robust mitochondrial phylogeny of Geometridae. Additional sampling and mitogenome sequencing of each geometrid genus are vital works to clarify the phylogeny and evolution of this biodiverse and economically important insect group.

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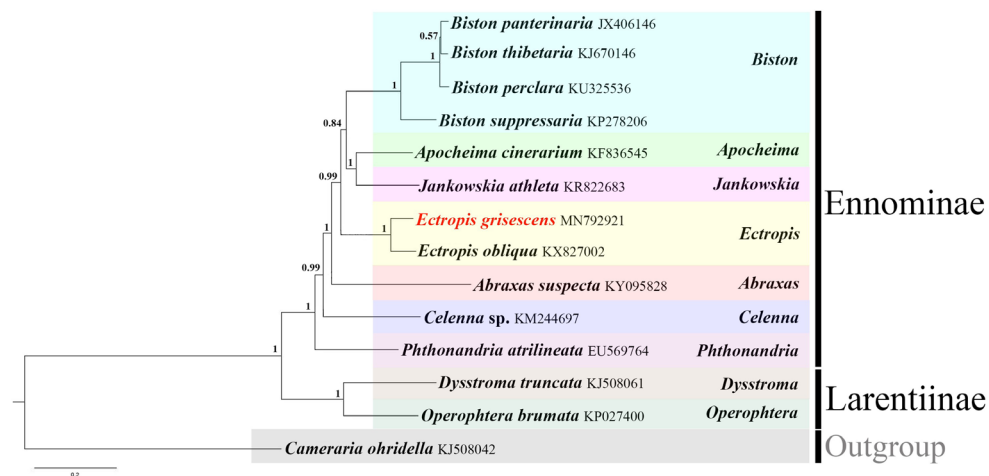


Fig. 5. Phylogenetic relationships within Geometridae inferred by Bayesian inference. Numbers at the nodes are posterior probabilities. The subfamily names are listed after the species. The tree was rooted with the outgroup *Cameraria ohridella*.

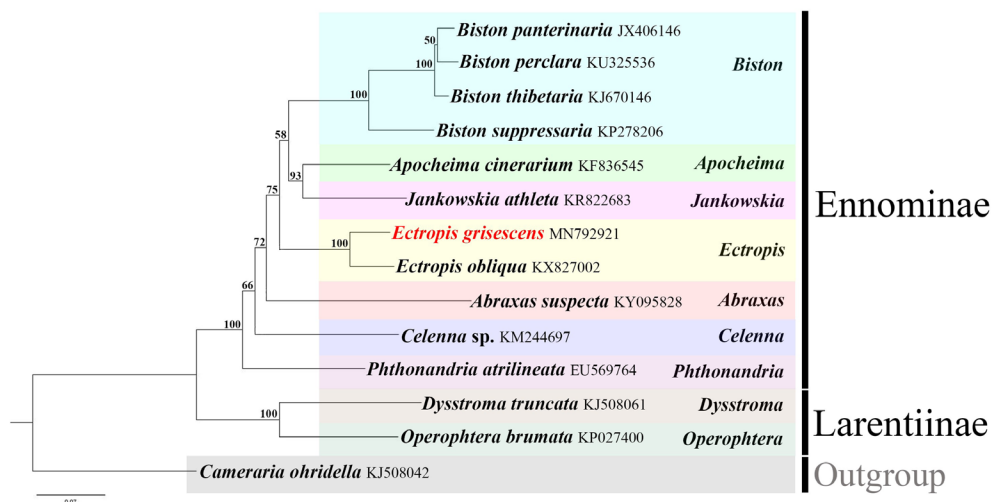


Fig. 6. Phylogenetic relationships within Geometridae inferred by maximum likelihood analysis. Numbers at the nodes are bootstrap values. Subfamily names are shown after the species. The tree was rooted with the outgroup *Cameraria ohridella*.

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Example:

Sphex oxianus Gussakovskij, 1928

Distribution: Central and South West Asia, Afghanistan, Iran, Israel, Turkey (Bohart and Menke, 1976; Menke and Pulawski, 2000; Kazenas, 2001), Turkey: Artvin (De Beaumont, 1967).

Material examined: Ankara, Altındağ, Çubuk Dam Lake, 900 m, 29.06.1998, 1 ♂; Kalecik, 600 m, 24. 07. 2001, 2 ♀♀, Kalecik, 800 m, 25. 07. 2001, 3 ♀♀

Host plant: *Echinophora* sp.

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Book

Steinmann, H.A. & Zombori, L. (1985). *An atlas of insect morphology*. (2nd ed.). Akadémiai Kiadó, Budapest, Hungary.

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