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Cover image: A female Anterhynchium gibbifrons sp. n. (Courtesy: Prof. Seike Yamane)

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First report of Western Flower Thrips, Frankliniella occidentalis (Pergande) (Thripidae: Thysanoptera) from India- A Potential Havoc to Indian agriculture

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Abstract

Western Flower thrips (WFT), Frankliniella occidentalis (Pergande) is being reported for the first time from India based on specimens collected on tomato. It is the most economically important species in the insect Order Thysanoptera damaging wide variety of crops as a sucking pest and vector for plant viruses.

Key words: WFT, pest, vector, new record, India.

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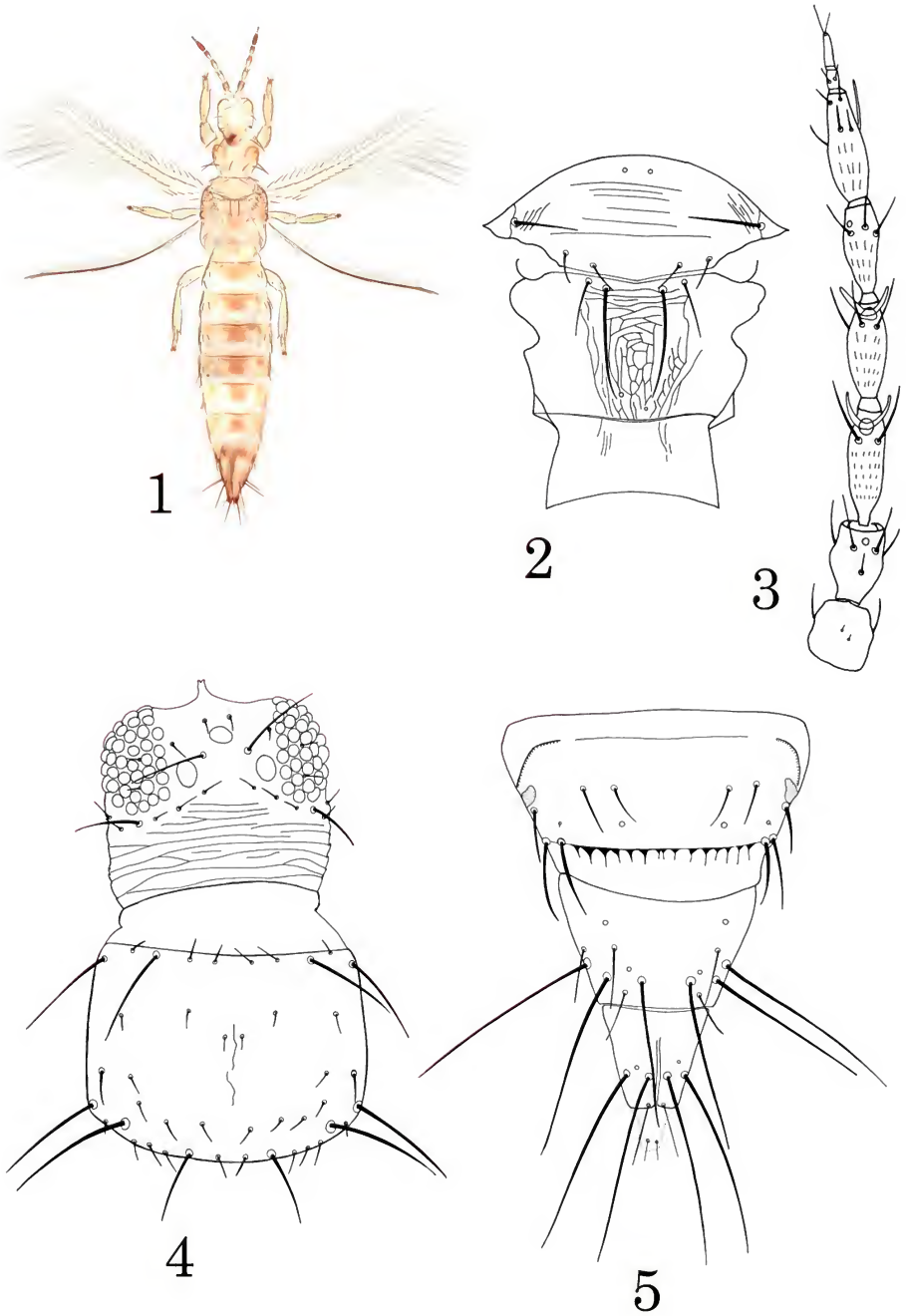
Introduction

Western Flower thrips (WFT), Frankliniella occidentalis (Pergande) is highly polyphagous and the most destructive pest species in the order Thysanoptera (Mound, 2002). WFT causes direct feeding damage to a wide variety of agricultural and horticultural crops across the globe (Rugman-Jones et al. 2010). Apart from direct feeding damage, WFT is one of the important vector of tospoviruses (family Bunyviridae, genus Tospoviruses) causing high economic loss worldwide (Wijkamp et al. 1995). So far, fourteen species of thrips have been reported as vectors for tospoviruses. Out of these fourteen species, F. occidentalis is responsible to transmit as many as five species of tospoviruses (Riley et al. 2011). At present, data on exact economic loss caused by WFT is not available. However, Rugman-Jones et al. (2010) has reported that WFT transmitted Tomato spotted wilt virus (TSWV) alone cause >$1billion economic loss worldwide in early 1990s.The species has been identified using available taxonomic key (Mound and Marullo, 1996, Cavalleri & Mound, 2012).

The objective of this paper is to report the first record of Frankliniella occidentalis from India. Earlier report of this species from India (Kulkarni, 2010) has been found unreliable (EPPO, 2013). The presence of this economically important species in India is a serious problems and a concern for quarantine authorities. Considering its economic importance as a serious pest and vector of tospoviruses, occurrence of F. occidentalis in other parts of India need systematic monitoring.

Frankliniella occidentalis (Pergande) (Figs. 1-5)

Female macroptera: Body pale yellow with brown patches on abdominal tergites; legs yellow antennal segments I yellow, II brown III yellow with brown shade at apex, IV–V yellow basally and brown apically, VI–VIII brown; fore wings shaded (Fig. 1). Head with transverse lines of sculpture; three pairs of ocellar setae present, pair III well-developed and arising between parallel tangent of fore and hind ocelli; six pairs of fine postocular setae, pair IV distinctively longer than others (Fig.4). Eyes without pigmented facets. Antennae 8-segmented, segments III and IV each with a forked sense cone; pedicel of segment III simple (Fig.3). Pronotum with four small setae between the major anteromarginal setae. Mesonotum with faint transverse line of sculpture, anteromedian campaniform sensilla present. Metanotum with irregularly reticulate posteromedially; paired campaniform sensilla present; two pairs of setae arising at anterior margin (Fig.2). Fore wings with two complete rows of setae. Abdominal tergite VIII with irregular comb of microtrichia; S1 setae on IX longer than tergite X (Fig.5).



Figures 1–5. Frankliniella occidentalis. 1. Whole insect, female; 2. Meso-metanota; 3. Antenna; 4. Head & pronotum; 5. Tergites VIII–X.

Material studied. India, Karnataka, Bangalore, 4 females, 2-xi-2014, tomato plantation, Vikas (Reg. No. 5961/H17 to 5964/H17). All specimens have been deposited in the National Zoological Collections (NZC), Zoological Survey of India, Kolkata, India.

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Ants (Hymenoptera: Formicidae) and their aphid partners (Homoptera: Aphididae) in Mashhad region, Razavi Khorasan Province, with new records of aphids and ant species for Fauna of Iran

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Abstract

A survey of ant-aphid associations was conducted by collecting and identifying samples of ants and aphids found together on aphid host plants in Mashhad region, Razavi Khorasan province of Iran. As a result, a total of 21 ant species representing 13 genera and 3 subfamilies and 26 aphid species belonging to 13 genera from 37 host plant species were collected and identified. Among the recorded ant species, the genus Crematogaster with four species had the highest species richness. The three most frequent aphid attendant ants were Lepisiota nigra (Dalla Torre, 1893), Tapinoma erraticum (Latreille, 1798) and Crematogaster inermis Mayr, 1862 associated with 11, 10 and 9 aphid species, respectively. Eleven ant species were recorded from the colonies of one aphid species. Among the recorded ants, the species Crematogaster sordidula (Nylander, 1849) is new to Iranian ant fauna. This record increases the recorded ant-fauna of Iran to over 171 species. Among the identified aphid species, Aphis craccivora Koch, 1856 had the highest ant attraction. Also, Aphis salicariae Koch, 1855; Chaitophorus hillerislambersi Pintera 1987; Chaitophorus israeliticus Eastop and Hille Ris Lambers, 1976; Cinara maghrebica Mimeur, 1934 and Schizaphis nigerrima (Hille Ris Lambers, 1931) are first records for aphid fauna of Iran. The aphids, their attendant ants, and host plants collected in this study are given. Findings of this preliminary study indicated that much more detailed study should be conducted to investigate aphid-ant mutualistic associations in Iran.

Key words: Mutualistic insects, Myrmecophilous aphids, new record, Iran.

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Introduction

Family Formicidae with approximately 15,000 species worldwide is considered one of the most successful taxa after their arising in the Mid-Cretaceous about 120 million years ago (Ward, 2007). They thrive in most ecosystems, and may form 15-25% of the terrestrial animal biomass (Schultz, 2000). Their social organization and behaviors, ability to change different habitats and exploit the useful resources have made them successful and survive in diverse environments. Most species are omnivorous and combine predation with feeding on sugary fluids from plants, aphids and other hemipterans. Among the phloem-sucking insects, aphids with over 4,500 species world-wide (Remaudie`re and Remaudie`re, 1997) have many species that are strongly host-specific (Dixon, 1987) and many are tended by ants. The relationship between aphids and ants is generally thought to be mutualistic, as both partners seem to benefit from their association. By attending aphid colonies, ants gain a rich source of carbohydrates from honeydew which is thought to result in higher colony growth rates (e.g. Cushman and Beattie, 1991). In turn, ants often act as guards and decrease the impact of predators and parasitoids on the fitness of their hosts (El-Ziady and Kennedy, 1956). Also, ant-tended aphids live longer, mature earlier, have higher rate of reproduction in comparison with

those not tended by ants (Flatt and Weisser, 2000). However, interactions between ants and aphids range from mutualistic to antagonistic (Stadler and Dixon, 2005, 2008; Billick et al., 2007).

The mutualistic relationship between ants and aphids has been the subject of many studies on various aspects of this phenomenon. Unfortunately, this subject in Iran has been ignored so far, and more importantly, the fauna of Iran has not been adequately studied systematically.

Paknia et al. (2008) reviewed the literature and provided the first checklist of Iranian ant fauna. Since that, several faunistic studies have been performed in some parts of Iran (e.g. Mossadegh et al., 2008; Ghahari et al., 2009; Rafinejad et al., 2009; Paknia et al., 2010; Radchenko and Paknia, 2010; Firouzi et al., 2011; Mohammadi et al., 2012; Gholami et al., 2012; Hossein Nezhad et al., 2012; Shiran et al., 2013). As a result, the number of ant species reported from Iran has reached over 218 species. As many parts of the country have not been explored, more new records is expected to be discovered by further works. Except a recent study by Shiran et al. (2013), there is no report on aphids and their mutualistic ants in Iran. To fulfill this gap and provide a base for further studies on interactions between aphids and ants, the present study was conducted in some parts of Razavi Khorasan province, NE Iran.

Materials and Methods

During the growing season of the year 2013, a variety of cultivated and wild plants inside and around the agricultural ecosystems in Mashhad region, Razavi Khorasan province of Iran were visited and sampled for aphids and their attendant ants. Because host identity is important in identifying aphids, sampling was mostly done by visual inspection of plants, and the aphids and their attendant ants were removed using soft brush and forceps. Geoposition coordinates were recorded with a hand-held GPS unit. Collected materials were preserved in ethanol (75%) in small glass vials and transferred to the laboratory for processing and identification. Aphids were identified by Łukasz Depa. The resources applied for identification were the host-based keys by Blackman and Eastop (1994, 2006) and also keys by Heie (1986) and Nieto Nafria et al. (2002, 2005).

Ants were identified by Nihat Aktac using mainly Agosti and Collingwood (1987); Collingwood and Agosti (1996); Czechowski et al. (2002); Dlussky (1967, 1969); Aktac and Radchenko (2002); Karaman and Aktac (2013); Seifert (1988, 1992) and materials compared with N.A. Collection.

Aphid names were updated with reference to Aphid Species File (Favret, 2009), ant names were checked with Bolton's Catelogue (2015), and host names were checked with the USDA Plants database (USDA, NRCS, 2009).

Voucher specimens of ants and aphids were deposited in Insect and Mite Collection of Plant Protection Department, Ferdowsi University of Mashhad, Iran. Also, some specimens of ants are held at the department of Zoology, Faculty of Science, Trakya University, Edirne, Turkey and of aphid specimens are deposited in the collection of the Departament of Zoology, University of Silesia in Katowice, Poland.

Results and Discussion

21 ant species were found to be attending 26 aphid species on 37 host plants. Among the determined ants and aphid species, the ant species Crematogaster sordidula and also, five aphids namely, Aphis salicariae Koch, 1855; Chaitophorus hillerislambersi Pintera, 1987; Chaitophorus israeliticus Eastop and Hille Ris Lambers, 1976; Cinara maghrebica Mimeur, 1934 and Schizaphis nigerrima (Hille Ris Lambers, 1931) are reported for the first time from Iran. Below is the list of ants attending aphid colonies on their various host plants found in the study area.

I. Subfamily Dolichoderinae

Tapinoma erraticum (Latreille, 1798)

Material examined: Molkabad (36° 0'2.09"N, 59°35'35.59"E), 17♀♀ associated with Aphis pomi De Geer, 1773 on Malus sp. (Rosaceae), 15-4-2013; 6♀♀, 12-4-2013; 7♀♀ associated with Aphis fabae Scopoli, 1763 on Elaeagnus angustifolia L. (Elaeagnaceae), 12-4-2013; Mashhad-Toos (36°25'20.14"N, 59°28'57.74"E),

5♀♀ associated with Aphis fabae Scopoli, 1763 on Elaeagnus angustifolia L. (Elaeagnaceae), 25-4-2013; 11♀♀ associated with Aphis craccivora Koch, 1856 on Alhaji psoudo-alhaji, 25-4-2013; 5♀♀ associated with Aphis craccivora Koch, 1856 on Kochia sp. (Amaranthaceae), 25-4-2013; 7♀♀ associated with Aphis craccivora Koch, 1856 on Robinia pseudoacacia L. (Fabaceae), 25-4-2013; Mashhad-Toroq (36°13'6.29"N, 59°40'24.02"E), 5♀♀, associated with Aphis craccivora Koch, 1856 on Robinia pseudoacacia L. (Fabaceae), 16-5-2013; Molkabad (35°59'51.76"N, 59°35'23.06"E), 6♀♀ associated with Brachycaudus amygdalinus Schout., 1905 on Prunus persica (L.) Stokes (Rosaceae), 27-4-2013; Mashhad-Toos (36°25'20"N, 59°28'57"E), 9♀♀ associated with Pterochloroides persicae Cholodkovsky, 1899 on Prunus persica (L.) Stokes (Rosaceae), 23-6-2013; Aman abad (35°59'51.76"N, 59°35'23.06"E), 3♀♀ associated with Aphis sp. on Lepidium draba L. (=Cardaria draba) (Brassicaceae), 16-4-2013; Aman abad, 4♀♀ associated with Aphis sp. on Prunus dulcis (Mill.) D.A.Webb (=Prunus amygdalus Batsch) (Rosaceae), 16-4-2013; Mashhad-Grab (36°23'56.16"N, 59°39'11.31"E), 6♀♀ associated with Aphis sp. on Carthamus lanatus L. (Asteraceae), 23-5-2013; Molkabad (35°59'51.76"N, 59°35'23.06"E), 7♀♀ associated with Aphis craccivora Koch, 1856 on Robinia pseudoacacia L. (Fabaceae), 10-5-2013; Toroq (36°13'6.29"N, 59°40'24.02"E), 6♀♀ associated with Eulachnus tuberculostemmatus Theobald, 1915 and Cinara maghrebica Mimeur 1934 on Pinus eldarica Medw (Pinaceae), 16-5-2013; Mashhad-Ferdowsi University (36°18'19.03"N, 59°31'44.71"E), 12♀♀ associated with Brachycaudus tragopogonis Kaltenbach, 1843 on Tragopogon sp. (Asteraceae), 28-5-2013; Mashhad-Toos (36°25'20"N, 59°28'57"E), 7♀♀ associated with Chaitophorus israeliticus Hille Ris Lambers, 1960 on Salix babylonica L. (Salicaceae), 23-6-2013.

Distribution in Iran: Northern and southern parts of Iran (Paknia et al., 2008).

II. Subfamily Formicinae

Camponotus turkestanicus Emery, 1887
Material examined: Mashhad-Shahid Shaabani Blv. (36°26'27.58"N, 59°30'13.29"E), 13♀♀ associated with Aphis salicariae Koch, 1855 on Carduus pycnocephalus L. (Asteraceae), 25-5-2013. The range of this aphid species and its host plants need further investigation.

Distribution in Iran: Northeast of Iran (Paknia et al., 2008).

Cataglyphis aenescens (Nylander, 1849)
Material examined: Mashhad-Toos (36°25'20.14"N, 59°28'57.74"E), 9♀♀ associated with Pterochloroides persicae Cholodkovsky, 1899 on Prunus persica (L.) Stokes (Rosaceae), 23-6-2013; Mashhad-Toos, 4♀♀ associated with Macrosiphum euphorbiae Thomas, 1878 on Sonchus arvensis L. (Asteraceae), 23-6-2013.

Distribution in Iran: Northern and northeast of Iran (Paknia et al., 2008).

Cataglyphis nodus (Brulle, 1833)
Material examined: Bozveshk (36° 4'23.37"N, 59°28'32.27"E), 3♀♀ associated with Aphis gossypii Glover, 1877 on Prunus dulcis (Mill.) D.A.Webb (=Prunus amygdalus Batsch) (Rosaceae), 16-5-2013; same locality, 3♀♀ associated with Aphis gossypii Glover, 1877 on Prunus cerasus L. (Rosaceae), 16-5-2013.

Distribution in Iran: Northern and southern parts of Iran (Paknia et al., 2008; Shiran et al., 2013).

Formica cunicularia Latreille, 1798
Material examined: Mashhad-Ferdowsi University campus (36°18'26.73"N, 59°31'38.65"E), 5♀♀ associated with Aphis acetosae L., 1761 on Rumex sp., 6-4-2013; same locality, 5♀♀ associated with Acyrthosiphon rubi Narzikulov, 1957 on Sonchus sp. (Asteraceae), 6-4-2013; Mashhad-kohsangi (36°16'57.35"N, 59°33'46.54"E), 6♀♀ associated with Acyrthosiphon pisum Harris, 1776, Brachycaudus tragopogonis Kaltenbach, 1843 and Cinara sp. Curtis, 1835 on Calendula officinalis L. (Asteraceae), 19-5-2013; same locality, 9♀♀ associated with Aphis craccivora Koch, 1856 on Ligustrum vulgare L. (Oleaceae), 19-5-2013.

Distribution in Iran: Northern parts of Iran (Paknia et al., 2008).

Lasius alienus (Foerster, 1850)
Material examined: Mashhad-kohsangi (36°16'57.70"N, 59°33'49.79"E), 3♀♀ associated with Periphyllus bulgaricus Tashev, 1964 on Acer sp. (Aceraceae), 19-5-2013.

Distribution in Iran: North-west of Iran (Paknia et al., 2008).

Lasius turcicus Santschi, 1921
Material examined: Mashhad-Ferdowsi University campus (36°18'19.03"N, 59°31'44.71"E), 6♀♀ associated with Acyrthosiphon gossypii Mordvilko,1914 on Lepidium draba L. (=Cardaria draba) (Brassicaceae), 6-4-2013; same locality, 8♀♀ associated with Chaitophorus hillerislambersi Pintera, 1987 on Populus alba L. (Salicaceae), 7-5-2013; Bozveshk (36°4'20.89"N, 59°26'35.38"E), 8♀♀ associated with Brachycaudus amygdalinus Schout.,1905 on Prunus armeniaca L. (Rosaceae), 16-5-2013; Mashhad-kohsangi (36°17'0.34"N, 59°33'43.85"E), 10♀♀ associated with Aphis craccivora Koch, 1856 on Kochia sp. L. (Amaranthaceae), 19-5-2013; Mashhad-Ferdowsi University campus (36°18'19.03"N, 59°31'44.71"E), 9♀♀ associated with Aphis craccivora Koch, 1856 on Hibiscus syriacus L. (Malvaceae), 2-7-2013.

Distribution in Iran: Northern parts of Iran (Paknia et al., 2008).

Lepisiota dolabellae (Forel, 1911)
Material examined: Mashhad-kohsangi (36°16'57.35"N, 59°33'46.54"E), 6♀♀ associated with Acyrthosiphon pisum Harris, 1776, Brachycaudus tragopogonis Kaltenbach, 1843 and Cinara sp. on Calendula officinalis L. (Asteraceae), 19-5-2013.

Distribution in Iran: Northern parts of Iran (Paknia et al., 2008); Brazjan in South of Iran (Ghahari et al., 2011).

Lepisiota frauenfeldi (Mayr, 1855)
Material examined: Beheshte-reza (36° 9'58.60"N, 59°42'11.21"E), 5♀♀ associated with Pterochloroides persicae Cholodkovsky, 1899 on Ulmus sp. L. (Ulmaceae), 1-5-2013.

Distribution in Iran: Zanjan (http://www.antweb.org/iran.jsp).

Lepisiota nigra (Dalla Torre, 1893)
Material examined: Molk abad (36° 0'2.09"N, 59°35'35.59"E), 7♀♀ associated with Aphis fabae Scopoli, 1763 on Elaeagnus angustif L. (Elaeagnaceae), 15-4-2013; Arefi (36° 7'22.20"N, 59°31'1.12"E), 7♀♀ associated with Brachycaudus helichrysi Kalt., 1843 and Hyalopterus amygdali Blanchard, 1840 on Prunus dulcis (Mill.) D.A.Webb (=Prunus amygdalus Batsch) (Rosaceae), 15-4-2013; Bozveshk (36° 4'43.81"N, 59°25'50.65"E), 5♀♀ associated with Aphis gossypii Glover, 1877 on Prunus dulcis (Mill.) D.A.Webb (=Prunus amygdalus Batsch) (Rosaceae), 16-5-2013; Beheshte-reza (36° 9'58.60"N, 59°42'11.21"E), 6♀♀ associated with Brachycaudus tragopogonis Kaltenbach, 1843 on Tragopogon sp. (Asteraceae), 17-4-2013; Mashhad-kohsangi (36°16'59.11"N, 59°33'48.46"E), 8♀♀ associated with Chaitophorus israeliticus Hille Ris Lambers, 1960 on Salix babylonica L. (Salicaceae), 19-5-2013; Beheshte-reza (36°9'58.60"N, 59°42'11.21"E), 4♀♀ associated with Pterochloroides persicae Cholodkovsky, 1899 on Ulmus sp. (Ulmaceae), 1-5-2013; Mashhad-Ferdowsi University campus (36°18'19.03"N, 59°31'44.71"E), 11♀♀ associated with Aphis sp. on Tamarix sp. (Tamaricaceae), 27-5-2013; same locality, 3♀♀ associated with Macrosiphon rosae L., 1758 on Rosa sp. (Rosaceae), 27-5-2013; Mashhad-Tollab (36°18'9.63"N, 59°39'24.59"E), 10♀♀ associated with Periphyllus bulgaricus Tashev, 1964 on Acer sp. (Aceraceae), 28-5-2013; Mashhad-Shahid Shafei (36°22'9.78"N, 59°33'12.43"E), 14♀♀ associated with Schizaphis nigerrima Hille Ris Lambers, 1931 on Sorghum vulgare Pers. (Poaceae), 23-6-2013.

Distribution in Iran: Fars province (Mohammadi et al., 2012).

Plagiolepis pallescens Forel, 1889
Material examined: Mashhad-Kohsangi (36°16'57.35"N, 59°33'46.54"E), 3♀♀ associated with Acyrthosiphon pisum Harris, 1776,

Brachycaudus tragopogonis Kaltenbach, 1843 and Cinara sp. on Calendula officinalis L. (Asteraceae), 19-5-2013; Mashhad-Toos (36° 4'20.89"N, 59°26'35.38"E), 3♀♀ associated with Callaphis juglandis Goeze, 1778 on Juglans sp. (Juglandaceae), 23-6-2013.

Distribution in Iran: Northern parts of Iran (Paknia et al., 2008); Dezful (Shiran et al., 2013).

Plagiolepis pygmaea (Latreille, 1798)
Material examined: Mashhad-Tollab (36°18'10.13"N, 59°39'15.53"E), 5♀♀ associated with Aphis craccivora Koch, 1856 on Fraxinus sp. (Oleaceae), 1-5-2013; Bozveshk (36° 4'16.47"N, 59°27'13.21"E), 15♀♀ associated with Aphis craccivora Koch, 1856 on Glycirizia glabra L. (Fabaceae), 16-5-2013.

Distribution in Iran: Ahvaz (Ghahari et al., 2009).

Proformica pilosiscapa Dlussky, 1969
Material examined: Bozveshk (36° 4'22.70"N, 59°28'24.67"E) (10♀♀), associated with Brachycaudus amygdalinus Schout., 1905 on Pistacia terebinthus L. (Anacardiaceae), 16-5-2013.

Distribution in Iran: Kaleibar (East Azarbaijan Province) (Ghahari et al., 2011).

III. Subfamily Myrmicinae

Aphaenogaster kurdica Ruzsky, 1905
Material examined: Beheshte-reza (36°10'1.24"N, 59°42'12.07"E), 8♀♀, associated with Brachycaudus helichrysi Kalt., 1843 on Malcolmia africana (L.) R. Br. (Brassicaceae), 24-4-2013.

Distribution in Iran: Northern part of Iran, Golestan province (Paknia et al., 2008)

Crematogaster inermis Mayr, 1862
Material examined: Mashhad-Tollab (36°18'24.35"N, 59°39'1.79"E), 6♀♀ associated with Acyrthosiphon gossypii Mordvilko, 1914 on Hibiscus syriacus L. (Malvaceae), 1-5-2013; Mashhad-Tollab, 6♀♀ associated with Aphis craccivora Koch, 1856 on Robinia pseudoacacia L. (Fabaceae), 1-5-2013; Mashhad-Tollab, 6♀♀ associated with Aphis craccivora Koch, 1856 on Cydonia oblonga Mill. (Rosaceae), 28-5-2013; Mashhad-Ferdowsi University campus (36°18'19.03"N, 59°31'44.71"E), 3♀♀ associated with Aphis craccivora Koch, 1856 on Hibiscus syriacus L. (Malvaceae), 2-7-2013; Bozveshk (36° 4'23.37"N, 59°28'32.27"E), 6♀♀ associated with Callaphis juglandis Goeze, 1778 on Juglans sp. L. (Juglandaceae), 16-5-2013; Bozveshk, 6♀♀, associated with Aphis gossypii Glover, 1877 on Prunus cerasifera Ehrh. (Rosaceae),16-5-2013; Mashhad-kohsangi (36°16'59.11"N, 59°33'48.46"E), 3♀♀, associated with Chaitophorus israeliticus Hille Ris Lambers, 1960 on Salix babylonica L. (Salicaceae), 19-5-2013; Mashhad-kohsangi, 6♀♀, associated with Acyrthosiphon pisum Harris, 1776, Brachycaudus tragopogonis Kaltenbach, 1843 and Cinara sp. on Calendula officinalis L. (Asteraceae),19-5-2013; Mashhad-Kalat road (36°34'31.22"N, 59°48'16.03"E), 2♀♀, associated with Aphis pseudocardui Theobald, 1915, on Carthamus oxycantha M. Bieb. (Asteraceae), 4-7-2013.

Distribution in Iran: Northern parts of Iran (Paknia et al., 2008).

Crematogaster schmidti (Mayr, 1853)
Material examined: Mashhad-kohsangi (36°16'59.11"N, 59°33'48.46"E), 3♀♀ associated with Chaitophorus israeliticus Hille Ris Lambers, 1960 on Salix babylonica L. (Salicaceae), 19-5-2013; Mashhad-Ferdowsi University campus (36°18'19.03"N, 59°31'44.71"E), 3♀♀, associated with Aphis craccivora Koch, 1856 on Hibiscus syriacus L. (Malvaceae), 2-7-2013; Mashhad-Kalat road (36°34'31.22"N, 59°48'16.03"E), 2♀♀ associated with Aphis pseudocardui Theobald, 1915 on Carthamus oxycantha M. Bieb. (Asteraceae), 4-7-2013.

Distribution in Iran: Northern parts of Iran (Paknia et al., 2008).

Crematogaster sordidula (Nylander, 1849)
Diagnostic characters: Head smooth and shiny with long setae, distance between the setae less

than their length; CI ≤ 95; clypeus shiny, medially smooth; mandibles smooth and shiny with appressed pubescence, masticatory border with five teeth, first tooth biggest and sharply pointed than the rests; antennae with abundant, long sub erected setae; alitrunk shiny dorsally, sides irregular sculptured with more than 6 long setae; propodeal spines long, about two times longer than their width at the base, divergent in dorsal view; petiole with 4, postpetiole with more than 6 sub erect long setae; legs with scattered sub erect setae; dorsal surface of gaster with densely long setae, distance between their bases equal to their length.

Material examined: Mashhad-Mahammadabad (36°29'17.04"N, 59°27'40.50"E), 18♀♀ associated with Brachycaudus cardui Linnaeus, 1758 on Cirsum arvense (L.) Scopoli (Asteraceae), 27-5-2013.

Distribution in Iran: The first record from Iran.

Crematogaster subdentata Mayr, 1877
Material examined: Mashhad-Shahid Abaspour (36°16'15.38"N, 59°39'28.49"E), 16♀♀ associated with Aphis craccivora Koch, 1856 on Vitis sp. L. (Vitaceae), 19-4-2013; Mashhad-Grab (36°23'56.16"N, 59°39'11.31"E),14♀♀, associated with Aphis craccivora Koch, 1856 on Carduus pycnocephalus L. (Asteraceae), 23-5-2013; Mashhad-Tollab (36°18'11.83"N, 59°39'16.88"E), 12♀♀ associated with Aphis craccivora Koch, 1856, Morus alba L. (Moraceae), 1-5-2013; Bozveshk (36°4'16.47"N, 59°27'13.21"E), 5♀♀ associated with Aphis craccivora Koch, 1856 on Glycirizia glabra L. (Fabaceae), 16-5-2013; Mashhad-Kohsangi (36°16'57.70"N, 59°33'49.79"E), 7♀♀ associated with Periphyllus bulgaricus Tashev, 1964 on Acer sp. L. (Aceraceae), 19-5-2013; Mashhad-Tollab (36°18'11.83"N, 59°39'16.88"E), 9♀♀ associated with Tuberculatus maximus Hille Ris Lambers, 1974, Ulmus sp. L. (Ulmaceae), 2-6-2013.

Distribution in Iran: Northern parts of Iran (Paknia et al., 2008).

Messor orientalis (Emery, 1898)
Material examined: Mashhad-kohsangi (36°16'59.11"N, 59°33'48.46"E), 3♀♀ associated with Chaitophorus israeliticus Hille Ris Lambers, 1960 on Salix babylonica L.(Salicaceae), 19-5-2013.

Distribution in Iran: Zanjan (Hossein-Nezhad et al., 2012).

Monomorium nitidiventre Emery, 1893
Material examined: Beheshte-reza (36°10'0.89"N, 59°42'12.82"E), 3♀♀ associated with Aphis craccivora Koch, 1856 on Chenopodium album L. (Chenopodiaceae), 24-4-2013.

Distribution in Iran: Southern parts of Iran (Paknia et al., 2008).

Tetramorium chefteki Forel, 1911
Material examined: Mashhad-Tollab (36°18'10.13"N, 59°39'15.53"E), 3♀♀ associated with Aphis craccivora Koch, 1856 on Fraxinus sp. (Oleaceae), 1-5-2013.

Distribution in Iran: Northern parts of Iran (Paknia et al., 2008).

From a biogeographical point of view, it is expected that the Iranian fauna would be much more diverse than those of its neighbors largely due to its geographic positioning between three distinct biogeographic realms, the Palaearctic, Afrotropical and the Oriental. However, still the number of insects especially ants recorded from Iran is much less than those of its neighbors (Shiran et al., 2013). Most probably due to the fact that the Iranian fauna has been poorly investigated and many areas have been sampled only sporadically. This preliminary research on aphid-ant association and their host plants in NE Iran have added five new records of aphid species and one new record of ant species to Iranian fauna. Previously about 480 aphid species were known from Iran (Alikhani et al., 2010). By the present study, the Iranian aphid fauna has at least 485 species. The occurrence of the new records of some aphids in Iran show a range extension and provides an important baseline for studying changes in the distribution of these important species which might be a result of climatic change.

Aphid species reported here exhibited a range of ant tending. Three species of ants, namely Lepisiota nigra, Tapinoma erraticum and Crematogaster inermis were found tending more than 9 species of aphids. It seems that these three ant species most possibly have an important role in dispersion of aphids from one plant to other ones in the region. Among the determined ant-aphid associations, Aphis craccivora had the greatest variety of ant tending it. We have no a clear answer to give why A. craccivora has the highest ant attraction as the studies of the various researchers indicated how highly dynamic the mutualistic relations between aphid and ant species. Several different factors might influence this relation such as density of ants as well as aphids, host plants species and its features, climatic conditions and seasonal differences (Depa and Wojciechowski, 2009). Of the four ant species found at colonies of Chaitophorous israeliticus two of them, Messor orientalis and Crematogaster schmidti, were exclusive to it, not found tending any other aphids.

The interaction between ants and hemipterans has been the subject of many studies on various aspects of this phenomenon (Stadler and Dixon 2005, 2008). Unfortunately, this topic in Iran has been limited to a few studies. In the only study of the mutualistic association between ants and aphids on different host plants in Iran, Shiran et al. (2013) reported 20 different species of ants associated with 33 aphid species. Dezhakam and Soleyman-Nejadian (2002) stated that the symbiotic ant Crematogaster antaris Forel, interfere with the performance of two encyrtid parasitoids Anagyrus agraensis (Saraswat) and A.dactylopii (Howard) on N. viridis. Also, Mossadegh et al. (2008) reported that the ants in the colony of Nipaecoccus viridis (Newstead) in Dezful citrus orchards have a negative influence on biological control of this pest, by preventing feeding and subsequently reproduction of the released Crypt beetles, Cryptolaemous montrouzieri Mulsant.

The present study surveyed the ant-aphid association on aerial parts of the host plants. So, the underground living aphid-ant interactions remain unexplored. Depa and Wojciechowski (2009) investigated root aphid-ant interaction and discussed morphological, behavioral and ecological interactions. This interesting subject needs further studies and discussion in the frame of mutualistic relations. It is expected that these preliminary results stimulate further studies in this context and provide a base for further studies on different interactions between aphids and their attendant ants which has been ignored so far in Iran.

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A revision of genus Toxorhynchites Theobald, 1901, in the South-East Asian countries, with description of a new species Toxorhynchites (Toxorhynchites) darjeelingensis from West Bengal, India (Diptera, Culicidae)

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Abstract

The genus Toxorhynchites (Tribe Toxorhynchitini), comprising 89 species worldwide which are organized under four subgenera (Afrorhynchus, 19 species; Ankylorhynchus, 4 species; Lynchiella, 16 species and Toxorhynchites, 50 species), is characterized by mosquitoes that do not feed on blood; instead they subsist on variety of plant juices and have their mouth parts commensurately designed. Globally there are about 3,543 species of mosquitoes (Family: Culicidae), of which nearly 3,061 species are culicines under Subfamily Culicinae that is further divided into eleven tribes one of which is Toxorhynchitini Lahille, 1904, represented by a solitary genus Toxorhynchites Theobald, 1901. Species of the subgenus Toxorhynchites alone are prevalent in the southeastern Asian countries (Indonesia, 12 species; India, 9 species; Thailand, 8 species; Bangladesh, 2 species; Sri Lanka, 2 species; DPR Korea, 1 species; Myanmar, 1 species; and Nepal, 1 species). A taxonomic comparison is made amongst all taxa endemic to these countries. Ironically no species of Toxorhynchites has ever been reported from Bhutan, Maldives and Timor-Leste. Toxorhynchites (Tox.) splendens is the most common species amongst all and has so far been recorded from only seven countries including India which is a home for as many as ten species, including the current Tx. (Tox.) darjeelingensis sp. n. collected from the foothills of Darjeeling Himalayan Mountains in the West Bengal State. Toxorhynchites (Tox.) darjeelingensis sp. n. is described, with a comparison offered with its closest allies, i.e., Tx. bengalensis, Tx. splendens and Tx. tyagii.

Keywords: Toxorhynchites darjeelingensis sp. n., Culicinae, mosquitoes, Southeast Asia.

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Introduction

Mosquitoes (Family Culicidae; Order Diptera) are highly diverse creatures, represented by a monophyletic taxon (Wood and Borkent, 1989; Miller et al., 1997; Harbach and Kitching, 1998). Culicidae is a large and abundant group of strikingly varied species (3,543) (Harbach and Howard, 2007; Tyagi et al., 2015) that occur throughout tropical and temperate regions of the world, and well beyond the Arctic Circle. Most of the mosquito species of the world (3,061) belong to subfamily Culicinae which is subdivided into 11 tribes, including Toxorhynchitini that is represented by single genus, Toxorhynchites. The species under Toxorhynchitini, characterized by an evenly rounded scutellum, make a very special group of mosquitoes, next to Anophelini in the evolutionary tree, which are generally large sized mosquitoes and essentially vegetarian, deriving their nutrition requirements from plants only with an exquisitely designed mouthparts for the purpose. The genus comprises 89 species which are organized under four subgenera, namely, Afrorhynchus (19 species), Ankylorhynchus (4 species), Lynchiella (16 species), and Toxorhynchites (50 species). Toxorhynchites species are endemic to Asia, and the southeast region comprising eleven countries, where as many as 24 species occur (Tyagi et al., 2015). The larvae are predatory and opportunistically feed

on other mosquito larvae, and thus have the potential of a biological agent for controlling obnoxious mosquitoes particularly the dengue/ chikungunya vectors, Aedes aegypti and Ae. albopictus (Collins and Blackwell, 2000).

Genus Toxorhynchites Theobald, 1901

Adults of Toxorhynchites species are large and colourful, with their body covered with green, purple or red iridescent scales. The distal half of the proboscis is slender and bent sharply downwards and backwards. The scutellum is evenly rounded (as in Anopheles and Bironella) and the posterior margin of the wing is distinctly emarginated opposite the termination of vein CuA. Larvae have mouth brushes composed of about 10 broad, flat filaments that are used to capture prey, and the dorsal and ventral abdominal setae occur in groups and large sclerotized plates. The comb and pecten are both absent. The Toxorhynchites larvae are found in plant cavities, mainly tree holes and bamboo stumps, but sometimes in littered tin boxes as well as earthen pots and even physiologically active phytotelmata such as insectivorous pitcher plants, like Nepenthes species (Tsukamoto, 1989), although Munirathinam et al. (2014) who reported 128 species belonging to all the three tribes, Anophelini, Toxorhynchitini and Culicini, from a variety of plant materials in certain regions of Western Ghats, did not report such a breeding behavior of a Toxorhynchites species (Table 1). The larvae of all species are predators. They feed mainly on other mosquito larvae, including their own kind when other species are few or absent. The adults are basically forest mosquitoes. Male and female both feed exclusively on nectar and sugary substances. Due to non-blood sucking behaviour, they are not of medical importance. However, the larvae of a few species have been used with some success to control medically important mosquitoes whose immature co-inhabit with that of Toxorhynchites in plant cavities and artificial containers.

Subgenus Toxorhynchites Theobald, 1901

(1) Distribution of species:

The subgenus Toxorhynchites, comprising 24 species disseminated over eight countries, viz., Indonesia (12), India (9), Thailand (8), Bangladesh (2), Sri Lanka (2), DPR Korea (1), Myanmar (1) and Nepal (1), is endemic to the south-east Asian countries (Table 2). Ironically, Bhutan, Maldives and Timor-Leste are yet to record any species despite abundant sylvatic environment.

(ii) Taxonomic characteristics

The subgenus Toxorhynchites is one of the four subgenera grouped under the genus by the same name. It is characteristically endemic to south-east Asian countries, albeit hitherto unreported from Bhutan, Maldives and Timor-Leste. Indonesia leads with species diversity enlisting 12 species, followed by India boasting of ten species, two of which having been recently discovered by the scientists of Centre for Research in Medical Entomology. Some of the salient and distinguishing subgeneric characters are offered in Table 3, whereas the species-wise distinguishing characteristics have been given in Table 4.

Identification Key to the Adults of Toxorhynchites Species

Adults of species under the subgenus Toxorhynchites, species of which are organized under seven groups, can be distinguished from those of other subgenera in the following key characteristics:

1. Tarsi entirely dark; small and slender species.....**Tx. minimus (Theobald)**
- Some tarsal segments with white markings; large species.....2

2(1)Abdominal tergites VI-VIII with lateral tufts 3
- Abdominal tergites VI-VIII weakly or without lateral tufts.....17

3(2)Mesonotum with conspicuous border of white or pale golden scales usually extending over the wing roots.....4
- Mesonotum without conspicuous border of pale scales.....11

4(3)Proboscis with distinct median pale band or with dorsomedian pale spot.....5
- Proboscis dark.....7

5(4)Proboscis with distinct median pale band; lateral tufts on tergum VI with golden

scales, VII & VIII with brilliant orange scales.....**Tx. sunthorni Thurman**

- Proboscis with dorsomedian pale spot.....6

6(5) Lateral tufts on abdominal segment VII dark with bluish black and VIII with dark golden setae.....**Tx. bickleyi Thurman**

- Proboscis dark with few pale scales dorsally at base; Lateral tufts on abdominal segments VI & VIII with golden and VII black setae..... .....**Tx. speciosus (Skuse)**

7(4) Each abdominal segment with two creamy yellow bands (one broad and another thin line); lateral tufts on tergum VI-VIII orange and black; Knee spots of all legs peacock blue..... .....**Tx. quasiferox (Leicester)**

- Without this combination.....8

8(7) Mid and hind tarsomeres 3-5 complete white or dark scales.....9

- Mid tarsomeres 2-4 white; tarsi 5 dark scales; lateral tufts on tergum VI & VIII orange and VII dark setae.....**Tx. manopi Thurman**

9(8) Midtarsomeres 3-5 complete white; abdominal segments III & V with incomplete medial pale bands; lateral tufts on tergum VI pale yellow; VII golden & VIII orange setae..... .....**Tx. edwardsi (Barraud)**

- Mid tarsomeres 3-5 dark scales.....10

10(9) Lateral tufts on tergum VI & VII black & VIII with orange setae..... .....**Tx. tyagii Krishnamoorthy et al.**

- Lateral tufts on tergum VI with three-fourth golden yellow one-fourth black setae, VII deep blue green and VIII pale yellow setae..... .....**Tx. darjeelingensis Tyagi et al.**

11(3) Lateral tufts present on tergum VII &VIII and no tufts on tergum VI.....12

- Lateral tufts present on tergum VI-VIII.....13

12(11) Lateral tufts on tergum VII & VIII orange setae; mid tarsomeres 2 & 4 basal half white and tarsi 3 complete white scales.....**Tx. sumatranus (Brug)**

- Lateral tufts on tergum VII & VIII black setae; mesonotum with narrow broad decumbent greenish scales becoming broader and bluish laterally.....**Tx. amboinensis (Doleschall)**

13(11) Lateral tufts on tergum VI & VII with orange (or) dark brown setae.....14

- Lateral tufts on tergum VI-VIII with black and orange (or) white and black setae.....15

14(13) Lateral tufts on tergum VI -VIII with orange setae; fore and hind tarsi 3-5 black; mid tarsi 2-4 white and mid tarsi 5 black scales...**Tx. auranticauda Lane**

- Lateral tufts on tergum VI white and dark brown and VII & VIII dark brown setae; mid tarsi 1-5 with white banding on basal half.....**Tx. bengalensis Rosenberg and Evenhuis**

15(13) Lateral tufts on tergum VI white, VII & VIII black setae; tarsomere 5 of all legs entirely dark scales; all femora have three rows of short black spines..... .....**Tx. magnificus (Leicester)**

- Lateral tufts on tergum VI-VIII with black and orange setae.....16

16(15) Lateral tufts on tergum VI & VII black setae; VIII orange setae; sub basal on mid tarsomere 1 with one-fourth and 2 with half white scales..... .....**Tx. inornatus (Walker)**

- Lateral tufts on tergum VI with yellow and black setae; VII with black and VIII with orange setae; mid tarsomeres 1-5 with white scales; fore and hind tarsomeres 3-5 with dark scales..... .....**Tx. splendens (Wiedemann)**

17(2) Lateral tufts on tergum VI-VIII weakly developed; VI & VIII with pale yellow and VII with black setae; tarsomeres 5 of all legs with pale and dark scales.....**Tx. albipes (Edwards)**

- Lateral tufts on tergum VI-VIII without tufts; proboscis dark or with pale band.....18

18(17) Proboscis with brown scales apically and violet tinge on basal part; a ring of silvery scales at the site of the bent.....**Tx. christophi (Portschinsky)**

- Proboscis without pale ring.....19

19(18) Abdominal tergum with complete or incomplete bands.....20

- Abdominal tergite V-VII with narrow incomplete basal pale bands; fore and mid tarsomeres 2-4 with pale scales.....**Tx. gravelyi (Edwards)**

20(19) Abdominal tergites all with basal bands.....21

- Abdominal tergites few (II – VI) with basal bands….....24

21(20) First joints of palpi a little shorter; third a little longer than second; venter of abdomen without median purple strip.....**Tx. klossi (Edwards)**

- Abdominal tergites with narrow blue or honey yellow bands.....22

22(21) Abdominal tergites each with narrow basal blue band; sub-basal white ring on tarsal segment 1 of all legs 23

- Abdominal tergites each with rose purple scales, banded with honey yellow expanding laterally into triangular patches; mid and hind tarsomeres dark covered with brilliant metallic scales..... .....**Tx. metallicus Leicester**

23(22) Mid tarsomeres 2-5 white scales; sternite IV with large median purple spot.....**Tx. leicesteri Theobald**

- Mid tarsi 4 and large part of 5 white scales; sternite IV with purple scales in middle.....**Tx. kempi (Edwards)**

24(20) Abdominal tergites II-VI with small lateral yellowish scales; tergite I with deep blue scales dorsally; sternites IV yellowish scales interrupted medially by purple scales; ocular setae (4pairs) amber to brownish; basal half of mid tarsi 1 & 2 with white band…..... .....**Tx. acaudatus (Leicester)**

- Abdominal tergites II-V with small lateral white scales; tergite I with golden scales dorsally; sternites IV with silvery white scales interrupted medially by brownish scales; ocular setae (3pairs) dark brown; basal one-fourth of mid tarsi 1 & 2 with white band.....**Tx. coeruleus (Brug)**

Toxorhynchites (Toxorhynchites) darjeelingensis Tyagi et al., sp. n.

urn:lsid:zoobank.org:act:16DC9A08-2E48-4D23-9B90-E4E37DBAEE5D

Materials and Methods

Larvae and pupae of Tx. (Tox.) darjeelingensis, sp. n. were collected from Ghadhalar Kurthi, Matha Bhanga block, Cooch Behar district in the foothills of Darjeeling mountains (West Bengal, India), during May-June 2012. Specimens were collected from littered battery chambers, having a capacity of 4 lit., at an altitude of 100-150m. Specimens were individually reared to the adult stage for species identification, using keys by Barraud (1934). Chaetotaxy of the associated larval and pupal exuviae were examined following Harbach and Knight (1980).

Three legs from one side of one paratype specimen were used for molecular analysis. From the homogenized material a whole genomic DNA was extracted following the techniques described by Dhananjeyan et al. (2010). The genomic DNA isolated was used to amplify the mitochondrial Cytochrome C Oxidase subunit I (COI) gene following Simon et al. (1994). The amplified PCR product was visualized on a 1.2% agarose gel using a gel documentation system (Vilber Lourmet, France) (Fig. 1). The product was sequenced commercially (Eurofins India Pvt. Ltd., 183, Gayathri Tech Park, EPIP – II Phase Whitefield, Bangalore-560066, Karnataka, India).

The nomenclature and chaetotaxy used in the description of new species, Toxorhynchites (Tox.) darjeelingensis, were described following Harbach and Knight (1980, 1982) and Bickley and Ward (1989).

Description

Female: Wing: 5.7mm, proboscis 5.9mm, fore femur 4.1mm, abdomen 5.3mm. Head: (Fig. 2): Integument blackish, scales of vertex light brown and with broad distinct violet orbital line; proboscis bluish; maxillary palpus bluish purple, scattered pale scales on dorsum, comprised of 4 palpomeres with equal length; antennal pedicel with a large conspicuous dense patch of silvery scales, scales of flagellomeres 1–6 dense with many small hairs. Thorax (Fig. 2): Integument dark brown or blackish, mesonotum densely covered with rather dull bronzy scales with bluish-green tinge, scales slightly narrower on disc than on sides, with whitish yellow scales with blue tinge patches over wing root to scutellum; antepronotum with 6-8 minute hairs along with bluish scales, postpronotum with silvery scales along with 4-5 setae, pleural and coxal scales silvery; one weak lower mesepimeral seta and usual row of caudal mesepimeral setae. Abdomen (Fig. 3): Terga largely bluish or greenish, tergum I with blue-green scales in the middle and brownish yellow scales laterally; tergum II with blue-green scales in

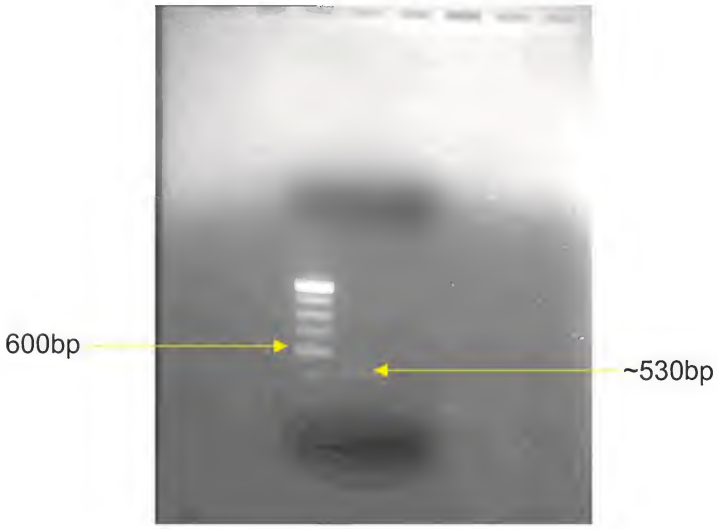

Figure 1. Gel picture showing the amplification of ~530bp amplicon of Tx. darjeelingensis sp. n.

the middle and golden scales laterally; terga III & V with deep green scales in center and apico-lateral broad golden scales visible from above; tergum IV with deep-green scales; with 75% of golden yellow and 25% of black lateral hair-tufts; tergum VII with 90% deep blue-green and remaining yellow hair tufts; tergum VIII with a bunch of pale yellow setae; sterna I & II completely with broad white scales; sternum III mainly broad white scales with narrow median dark line; sternum IV completely broad black-scaled; creamy white scales are present in major area in sternum V and VI along with black scales forming a 'V' shape medially; sternum VII with black scales forming narrow line in the median, remaining area with broad white scales; sternum VIII mainly black-scaled with few scattered white scales, and with golden yellow tuft. Legs (Fig. 4): Fore femur with wide basal yellow ring, mid femur black with pale scales, hind femur 70% with golden yellow, remaining black; fore and hind tibiae dark, mid-tibia with white longitudinal stripe; fore tarsomere 1 mainly pale, narrowly dark at base, fore tarsomere 2 with ⅓ white basally; mid tarsomere 1 with broad basal pale band, mid tarsomere 2 entirely pale; hind tarsomere 1with ¼ whitish yellow basal band, hind tarsomere 2 with broad basal pale band, tarsomere 3–5 of all legs dark.

Male: Generally similar to female. Head: Integument blackish; maxillary palpus slightly longer than proboscis; antenna verticillate, flagellomeres 1 with few white scales and numerous black scales. Legs: All tibia dark; fore tarsus completely dark; mid femora with a longitudinal pale line, mid tarsomere 1,2 with basal pale band and 3-5 completely dark; hind tarsomere 1 with few scattered white scales posteriorly, 2 with broad white band and 3-5 completely dark. Genitalia (Fig. 5): Gonocoxite 0.55 mm, gonostylus 0.52 mm, gonostylar claw 0.05 mm. Gonocoxite with numerous microsetae; gonostylus with a single sub apical gonostylar claw; medial margin of gonostylus with about 14 micro-setae distributed evenly from base to apex. Basal mesal lobe (BML) with one stout and long seta length 0.30 mm, medial surface with numerous short simple setae, less than half length of longest. Tergum IX with about 25 simple setae arranged on dorsolateral and lateral margins.

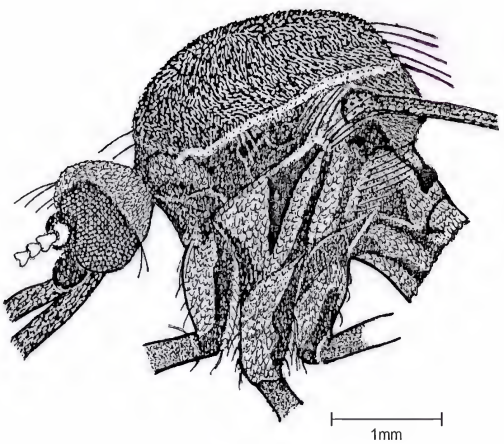

Figure 2. Head and thorax of holotype female of *Toxorhynchites darjeelingensis*, sp.n.

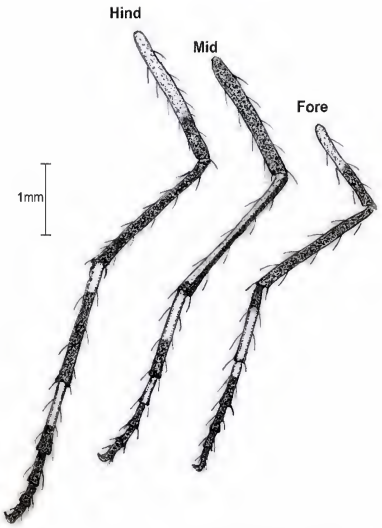

Figure 4. Legs of *Toxorhynchites darjeelingensis*, sp.n.

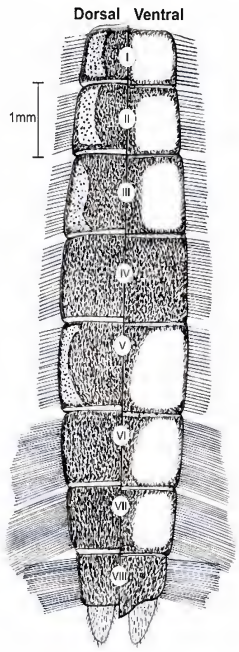

Figure 3. Abdomen of *Toxorhynchites darjeelingensis*, sp.n.

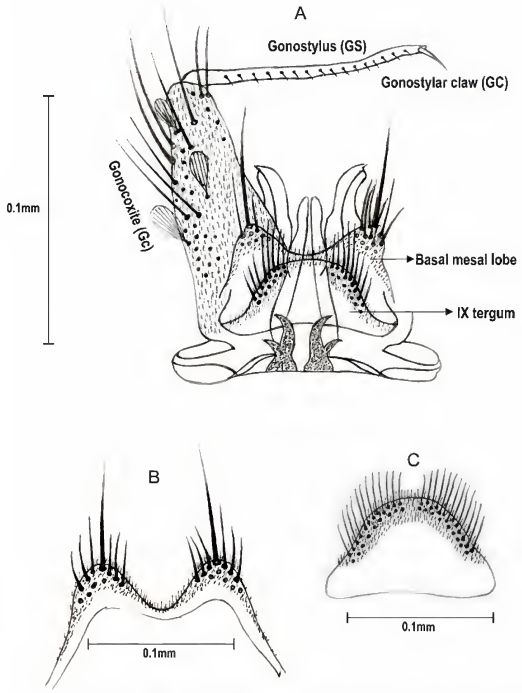

Figure 5. Male genitalia of *Toxorhynchites darjeelingensis*, sp.n. A. Dorsal aspect; B. basal mesal lobe, C. tergum IX

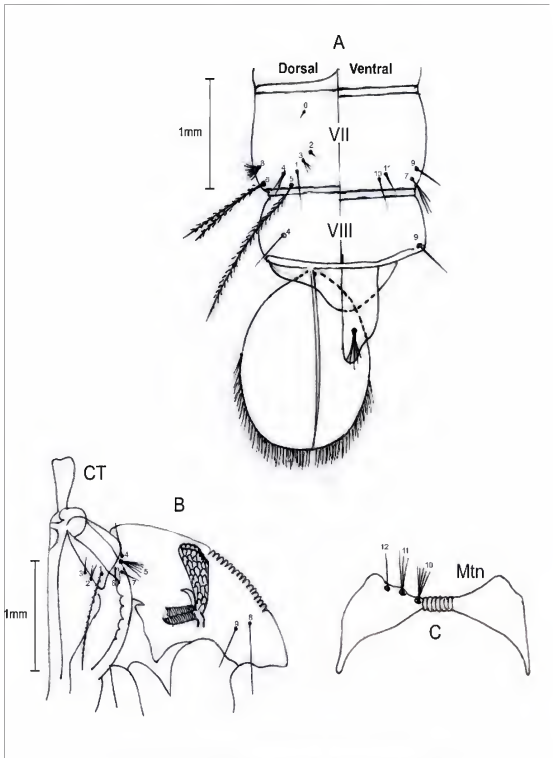

Figure 6. Pupa of *Toxorhynchites darjeelingensis*, sp.n.
A, terminal abdominal segments (VII & VIII); B, cephalothorax (CT); C, metanotum (Mtn)

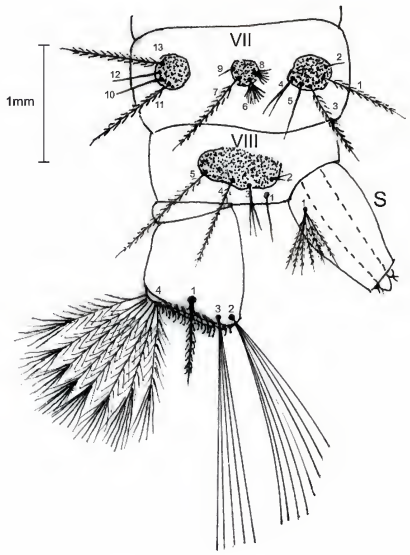

Figure 7. Terminal abdominal segments of the larva of *Toxorhynchites darjeelingensis*, sp.n.

Pupa: Abdomen 5.1 mm, trumpet 0.9 mm, paddle 1.4 mm. Chaetotaxy as an illustrated (Fig.6), and the range of variation shown in Table 5. Cephalothorax: Moderately pigmented; seta 1-CT single, very long, barbed; seta 2-CT with 2 branched; setae 3-4, 6-9-CT single, seta 5-CT with 5-7(7) branches; seta 10-CT with 4,5 (5) branches, seta 11-CT with 3,4 (3) branches and seta 12-CT with 1,2 (1) branches. Trumpet: Dark orange-brown, heavily pigmented, almost twice as long as wide at apex. Abdomen: Bright brown, moderately pigmented, large setae darker than integument; seta 6-VII single, bifid at the center; seta 7-VII single, forked at the center; paddle pigmented, more or less rounded, very wide, width about 0.65 of length, and similar as in Tx. tyagii, midrib complete, distal 0.57 of outer and inner margins with long fine hair-like spicules; setae 1,2-Pa absent.

Larva: Head 1.21 mm, siphon 0.87 mm, saddle 0.76 mm. Chaetotaxy as illustrated (Fig.7) and the range of variation shown in Table 6. Antenna: Concolorous with head capsules. Thorax: Setae, tubercles and plates strongly pigmented; seta 7-P,T double, barbed, stiff; seta 10-P,M,T single, thin, barbed; seta 13-M with 2 branches, stiff and barbed. Abdomen: Setae 10,12,13-I on single plate, seta 11-I on separate plate; seta 3-I slightly longer than setae 1- I and 4-I; seta 3-II double, barbed, 3-III-V single, long, barbed; setae6-II-V and 7-I –IV double, long, barbed; setae 1,3-VII long, strongly barbed; seta 1-VIII distinctly separate from large sclerotized plate and without tubercle; seta 2-VIII simple with two branches; seta 3-VIII origin with single at the end with three branches; setae 4,5-VIII single, long, barbed. Siphon: Index about 1.68; seta 1-S with 6 branched. Segment X: Uniformly darkly pigmented; saddle with long spicules on caudolateral margins; ventral brush (seta 4-X) with 8 pairs of setae.

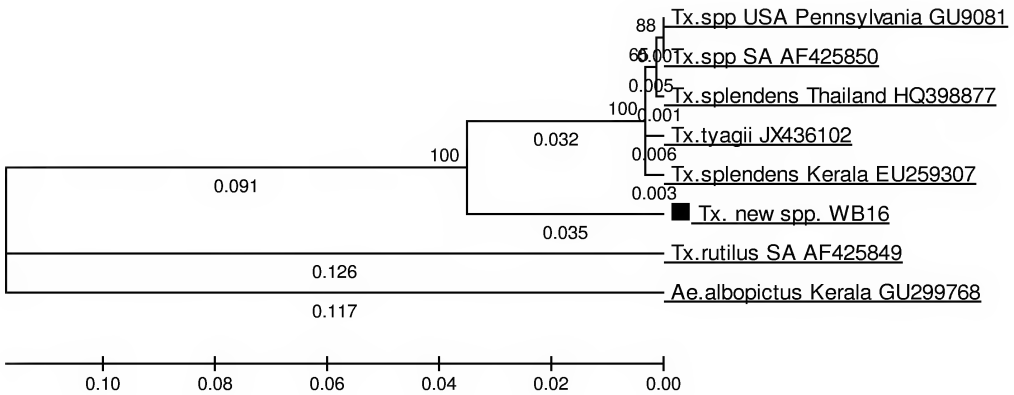

Fig. 8. Phylogenetic tree showing the positioning of Toxorhynchites darjeelingensis sp. n. (WB16) along with other associated taxa of the genus Toxorhynchites

Molecular characterization

For phylogenetic analysis mitochondrial Cytochrome C Oxidase subunit I gene sequences of six species of Toxorhynchites were included that were retrieved from GenBank. The GenBank Accession Numbers of each sequence is shown in the phyletic tree. Of the six sequences utilized for the phylogenetic tree construction, 2 sequences belonged to Tx. splendens from Thailand (HQ398877) and India (EU259307). Two sequences of Toxorhynchites genus, which are not identified to species level; one collected in Pennsylvania, USA (GU908123) and another collected in South Africa (AF425850). The fifth CO1 gene sequence belong to Tx. tyagii (JX436102), isolated in Nilgiri hills, Tamil Nadu, India and identified and reported as a new species by CRME, India (Krishnamoorthy et al., 2013). The 6th sequence is of Tx. rutilus (AF425849) from South Africa. The COI gene sequence of Aedes albopictus (GU299768) from Kerala, India has been included in the

Phylogenetic tree construction as an outgroup.

The evolutionary history of Toxorhynchites darjeelingensis sp. n. (WB16) was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches.

The Phylogenetic tree was linearized assuming equal evolutionary rates in all lineages (Tekezaki et al., 2004). The tree is drawn to scale, with branch lengths (next to the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree.

The evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980) and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 392 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 software (Tamura et al., 2007).

Type material: Holotype female (coded A#1793) with associated larval (Le#851) and pupal (Pe#829) exuviae mounted on microscopic slides with the following collection data: INDIA, West Bengal, Jalpaiguri district, Darjeeling hills, 21st May 2012, collected as larva from littered battery chambers at an altitude of 150m, collected by R. Govindarajan, deposited in the CRME Museum, ICMR, Madurai, Tamil Nadu, India. The allotype male (coded A#1794) with associated larval (Le#852) and pupal (Pe#830) exuviae, and 2 paratype males with Le and Pe mounted on microscopic slides, have the collection data same as that of the holotype. All are also deposited in the CRME Museum.

Distribution: Known only from the type locality in Jalpaiguri district, West Bengal, India.

Bionomics: The immature stages of Tx. darjeelingensis sp. n. were collected in a littered battery chambers, having a capacity of 4 lit., at an altitude of 100-150m from Ghadhalar Kurthi, Matha Bhanga block, Cooch Behar district in the foothills of Darjeeling mountains (West Bengal, India) in May-June, 2012. Larvae of Tx. darjeelingensis were found in association with Armigeres (Leicesteria) magnus, Stegomyia (Heteraspidion) annandalei, Aedes albopictus and Tripteroides (Rachionotomyia) aranoides.

Etymology: This species is designated name after the place of its discovery, the Darjeeling hills in West Bengal State, India.

Discussion

Adults of Toxorhynchites (Tox.) darjeelingensis sp. n. are superficially similar to Tx. (Tox.) bengalensis, Tx. (Tox.) splendens and Tx. (Tox.) tyagii. However, the diagnostic characters for larva, pupa, adult (mesonotum, abdomen, wing, legs and male genitalia) of these species clearly distinguish Tx. darjeelingensis sp. n. from the rest as shown in Table 6. These distinguishing characters are summarized below:

(i) Adult mesonotum with broad pale yellow scales over wing root to scutellum are present in Tx. darjeelingensis and Tx. tyagii but absent in Tx. bengalensis and Tx. splendens.

(ii) The lateral tufts of VI-Te in Tx. darjeelingensis is two-third deep blue, with remaining black but in Tx. tyagii it is two-third black and remainder only is yellow; white and dark brown in Tx. bengalensis, and yellow and black in Tx. splendens.

(iii) Adult of Tx. darjeelingensis is a rather small sized mosquito, next to Tx. minimus.

(iv) In male genitalia, BML with one stout apical seta present in Tx. splendens and Tx. darjeelingensis compared to two stout apical seta present in Tx .bengalensis and Tx. tyagii.

(v) The pupa of Tx. darjeelingensis can easily be separated on the basis of 10-C with 5 branches, whereas others have lesser (Tx. bengalensis) and more (Tx. tyagii) (cf. Table 6).

(vi) Larva of Tx. darjeelingensis appears closer to Tx. splendens and Tx. tyagii, but the new species can be quickly distinguished on the basis of seta 7-M with 3 branches, and 13-M double in thoracic region.

Table 1. Preference of different Toxorhynchites (Tox.) species in selecting habitats for breeding in the South-East Asia Region countries

S.No	Species / Habitat	Bamboo Stump	Coconut husks	Discarded container	Discarded battery	Leaf axil	Metal barrels / tin	Mud pot	Pitcher plants	Rocky pool	Sintex Tank	Small wells	Tree hole	Resting collection
1	Tx. acaudatus								●					
2	Tx. albipes												●	
3	Tx. amboinensis	●												
4	Tx. auranticauda			●									●	
5	Tx. bengalensis	●											●	
6	Tx. bickleyi													●
7	Tx. coeruleus								●				●	
8	Tx. christophi												●	
9	Tx. edwardsi		●										●	
10	Tx. gravelyi	●											●	
11	Tx. inornatus		●	●			●		●		●	●	●	
12	Tx. kempi	●												
13	Tx. klossi												●	
14	Tx. leicesteri	●												
15	Tx. magnificus	●											●	
16	Tx. manopi													●
17	Tx. metallicus	●											●	
18	Tx. minimus	●											●	
19	Tx. quasiferox	●							●					
20	Tx. speciosus			●			●		●		●	●	●	
21	Tx. splendens	●		●		●		●		●	●		●	
22	Tx. sumatranus								●					
23	Tx. sunthorni													●
24	Tx. tyagii						●							
25	Tx. darjeelingensis sp.n.				●									

Table 2. Geographical distribution of taxa under subgenus Toxorhynchites

Sl. No.	Species	Country	Describing valid authority
1	Tx.(Tox.) acaudatus	Indonesia	Leicester, 1908
2	Tx.(Tox.) albipes	India, Thailand	Edwards, 1922
3	Tx.(Tox.) amboinensis	Indonesia	Doleschall, 1857
4	Tx. (Tox.) auranticauda	Indonesia	Lane, 1992
5	Tx.(Tox.) bengalensis	Bangladesh	Rosenberg and Evenhuis, 1985
6	Tx.(Tox.) bickleyi	Thailand	Thurman, 1959
7	Tx. (Tox.) coeruleus	Indonesia	Brug, 1934
8	Tx.(Tox.) christophi	DPR Korea	Portschinsky, 1884
9	Tx.(Tox.) edwardsi	India	Barraud, 1924
10	Tx.(Tox.) gravelyi	India, Thailand	Edwards, 1921
11	Tx.(Tox.) inornatus	Indonesia	Walker, 1865
12	Tx.(Tox.) kempi	India, Indonesia	Edwards, 1921
13	Tx(Tox.) klossi	India	Edwards, 1921
14	Tx.(Tox.) leicesteri	Thailand	Theobald, 1904
15	Tx.(Tox.) magnificus	Thailand	Leicester, 1908
16	Tx.(Tox.) manopi	Thailand	Thurman, 1959
17	Tx(Tox.) metallicus	India, Indonesia	Leicester, 1904
18	Tx.(Tox.) minimus	India, Indonesia and Sri Lanka	Theobald, 1905
19	Tx.(Tox.) quasiferox	Indonesia	Leicester, 1908
20	Tx.(Tox.) speciosus	Indonesia	Skuse, 1889
21	Tx.(Tox.) splendens	Bangladesh, India, Indonesia, Nepal Myanmar, Sri Lanka and Thailand	Wiedemann, 1819
22	Tx.(Tox.) sumatranus	Indonesia	Brug, 1939
23	Tx.(Tox.) sunthorni	Thailand	Thurman, 1959
24	Tx.(Tox.) tyagii	India	Krishnamoorthy et al., 2013

Table 3. Characters of subgenera under Toxorhynchites

S. No.	Afrorhynchus Ribeiro	Ankylorhynchus Lutz	Lynchiella Lahille	Toxorhynchites Theobald
1	Mesokatepisternum with a small patch of golden scales; scales on forecoxa all or almost all golden; laterotergite with few or no scales	Mesokatepisternum without golden scales; scales on forecoxa all white; laterotergite densely clothed with scales	ibid.	ibid.
2	Male midungues small, equal and simple; gonostylus widened at middle; gonostylar claw long; dorsal bridge of aedeagus wide; paraproct appearing divided into a proximal and a distal portion by a narrow unsclerotized transverse band	Male midungues unequal, one of them toothed and stronger; gonostylar claw small; dorsal aedeagus bridge narrow; paraproct without unsclerotized transverse band	ibid.	ibid.
3	-	Female antenna subplumose, with long verticilate hairs; maxillary palpus about as long as proboscis, with 3 distinct palpomeres, of which the apical one is the longest, pointed and directed upward	ibid.	ibid.
4	-	-	Female antenna normal, not subplumose; maxillary palpus obviously shorter than proboscis	ibid.
5	-	-	Female maxillary palpus about 0.67 – 0.75 length of proboscis, with 3 distinct palpomeres, of which the second is the longest	ibid.
6	-	-	-	Female maxillary palpus about 0.25 of proboscis with only 2 distinct palpomeres and shorter

Table 4. Important characters for each species

S.No	Species	Head	Thorax	Abdomen		Legs		Male genitalia
1	Tx. acaudatus	ocular setae 4 pairs with amber to brownish		VI-VIII with tufts	tergites II -VI with lateral yellow scales; tergite I with deep blue; sternite IV yellow scales interrupted medially by purple scales	tarsi with white markings	basal 1/2 of mid tarsi 1 & 2 with white band	Basal mesal lobe with 3 stout apical seta; medial margin of gonostylus with numerous microsetae from apex to just above middle
2	Tx. albipes			VI-VIII with weak tufts	VI & VIII pale yellow, VII -dark	tarsi with white markings	tarosomeres 5 of all legs pale and dark	IX-Te narrow, apical border not produced into lobes, lateral plate with few small obvious teeth; gonostylus with few microsetae
3	Tx. amboinensis		mesonotum without border of broad pale yellow scales over wing roots to scutellum	VI-VIII with tufts	VI-without tuft, VII & VIII- dark	tarsi with white markings		Basal mesal lobe with 1 stout apical seta; few short mesal hairs in gonostylus from middle to apex
4	Tx. auranticauda		mesonotum without border of broad pale yellow scales over wing roots to scutellum	VI-VIII with tufts	VI - VIII with orange	tarsi with white markings	midtarsi 2-4 white,t5 black	not available
5	Tx. bengalensis		mesonotum without border of broad pale yellow scales over wing roots to scutellum	VI-VIII with tufts	VI- white and dark brown, VII & VIII- dark brown	tarsi with white markings		Basal mesal lobe with 2 stout apical setae; gonostylus with few microsetae restricted to apical 1/3
6	Tx. bickleyi	proboscis with dorsomedian pale spot	mesonotum with border of broad pale yellow scales over wing roots to scutellum	VI-VIII with tufts	VII- dark with bluish black, VIII-dark golden	tarsi with white markings		not available
7	Tx. coeruleus	ocular setae 3 pairs with dark brown		VI-VIII without tufts	tergites II-V with lateral white scales; tergite I with golden scales; sternite IV with silver white scales interrupted medially by brownish scales	tarsi with white markings	basal 1/4 of mid tarsi 1 & 2 with white band	Basal mesal lobe with 2 stout apical seta; gonostylus with numerous microsetae extending from apex to near base
8	Tx. christophi	proboscis with a ring of silvery scales at site of bend		VI-VIII without tufts		tarsi with white markings		Basal mesal lobe with 1 stout apical seta; gonostylus without microsetae; aedeagus flask shaped; IX - Te concave, two broad projections with 17-19 hairs

9	Tx. edwardsi		mesonotum with border	VI-VIII with tufts	VI- pale yellow, VII-golden, VIII - orange; III & V segment with incomplete medial pale bands	tarsi with white markings	mid tarsomeres 2-5 white	not available
10	Tx. gravelyi			VI-VIII without tufts	V-VII with narrow incomplete basal bands	tarsi with white markings	mid tarsomeres 2-4 pale	IX-Te with a pair of submedian lobes, more pronounced and pointed; lateral plate without obvious teeth; gonostylus with few microsetae
11	Tx. inornatus		mesonotum without border of broad pale yellow scales over wing roots to scutellum	VI-VIII with tufts	VI & VII- black, VIII- orange	tarsi with white markings		Basal mesal lobe with 2-3 stout apical setae; IX-Te truncate with a pair of 12-16 bristles each; lateral plate without obvious teeth; gonostylus with numerous short microsetae from basal 1/4 to apex
12	Tx. kempi			VI-VIII without tufts		tarsi with white markings	mid tarsi 4 and large part of 5 white	IX-Te narrow, submedian hairy lobes not well developed with 10-12 long hairs; Basal mesal lobe with 2 stout apical setae, gonostylus with five microsetae in a row before the tip; lateral plate with a small number of minute teeth
13	Tx. klossi	first joints of palpi little shorter, third little longer than second		VI-VIII without tufts	abdominal tergites all with basal bands	tarsi with white markings		IX-Te broader, less emarginate, gonostylus with hair like terminal spine
14	Tx. leicesteri			VI-VIII without tufts	sternite IV with large median purple spot	tarsi with white markings	mid tarsomeres 2-5 white	not available
15	Tx. magnificus		mesonotum without border of broad pale yellow scales over wing roots to scutellum	VI-VIII with tufts	VI -white, VII & VIII-black	tarsi with white markings	all femora with three rows of shot black spines	apparently very simple, the claspers being composed of a basal piece with simple hinged hook at the end
16	Tx. manopi		mesonotum with border of broad pale yellow scales over wing roots to scutellum	VI-VIII with tufts	VI & VIII-orange, VII-dark	tarsi with white markings	mid tarsi 2-4 white,t5 dark	not available
17	Tx. metallicus			VI-VIII without tufts	abdominal tergites with rose purple, banded with honey yellow	tarsi with white markings	mid and hind torsomeres dark covered with metallic scales	not available

18	Tx. minimus					tarsi entirely dark		IX-Te strongly produced in middle into a shield like plate with hairs; Basal mesal lobe with 1 stout apical setae; gonostyle about length of coxite; lateral plate narrow, with few minute blunt teeth near apex
19	Tx. quasiferox		mesonotum with border of broad pale yellow scales over wing roots to scutellum	VI-VIII with tufts	VI-VIII with orange and black; each segment with two creamy yellow bands	tarsi with white markings		not available
20	Tx. speciosus	proboscis with dorsomedian pale spot	mesonotum with border of broad pale yellow scales over wing roots to scutellum	VI-VIII with tufts	VI- with golden, VII-dark , VIII- with golden	tarsi with white markings		not available
21	Tx. splendens		mesonotum without border of broad pale yellow scales over wing roots to scutellum	VI-VIII with tufts	VI- yellow and black, VII-dark, VIII- orange	tarsi with white markings	fore and hind tarsomeres 3-5 dark	Basal mesal lobe with 1 stout apical seta; gonostylus with few microsetae restricted to distal half
22	Tx. sumatranus		mesonotum without border of broad pale yellow scales over wing roots to scutellum	VI-VIII with tufts	VI- without tuft, VII-brilliant orange, VIII- brilliant orange	tarsi with white markings	midtarsi 3 complete white	not available
23	Tx. sunthorni	proboscis with median pale band	mesonotum with border of broad pale yellow scales over wing roots to scutellum	VI-VIII with tufts	VI- golden scales, VII-brilliant orange, VIII- brilliant orange	tarsi with white markings		not available
24	Tx. tyagii		mesonotum with border of broad pale yellow scales over wing roots to scutellum	VI-VIII with tufts	VI- yellow and black, VII-black, VIII- orange	tarsi with white markings		Basal mesal lobe with 2 stout apical seta; gonostylus with few microsetae from base to apex
25	Tx. darjeelingensis		mesonotum with border of broad pale yellow scales over wing roots to scutellum	VI-VIII with tufts	VI- golden yellow and black, VII-deep blue green, VIII- pale yellow	tarsi with white markings		Basal mesal lobe with 1 stout apical seta; gonostylus with numerous microsetae distributed evenly from base to apex

Table 5. Chaetotaxy of the pupa of Toxorhynchites darjeelingensis sp. n.

Seta No	Cephalothorax	Abdominal Segments								
		I	II	III	IV	V	VI	VII	VIII	IX
0			1	1	1	1	1	1	1	-
1	1(L,B)	m(F)	m	1-2(1) L,B	1 L,B	1 L,B	1	1	-	3-5(5)
2	2	1-3(2)	1	1	1	1	1	1	-	-
3	1	1	1	1L,Wb	2-5(4)	1,2(2)	2-4(3)	2-4(3)	-	-
4	1	2-8(5)	3-6(5)	3-7(4)	2-7(6)	4-7(5)	2-4(3)	2,3(2)	1	-
5	5-7 (6)	1-3(3)	1L,Wb	1L,Wb	1 L,B	1 L,B	1 L,B	1L,B	-	-
6	1	1L,B	1L,Wb	1L,Wb	1L,Wb	1 L,B	1 L,B	1 Bf	-	-
7	1	1-4(3)	1-5(3)	1-4(2)	2-4(3)	2-5(3)	2-4(3)	1	-	-
8	1L	0	1	1	1	1	6,7(7)	8-10(8)	-	-
9	1	1	1	1	1	1	1	1,2(1)	1	-
10	4,5 (5)	-	-	1-2(1)L	1L	1 L	1L	1	-	-
11	3,4 (3)	-	1	1	1	1	1	1	-	-
12	1,2(1)	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	1	-	-
L- long; Wb- Weakly barbed; B- barbed, Bf- Bifid; F- fanlike setae, m- multiple (more than 10 branches)										

Table 6. Chaetotaxy of the larva of Toxorhynchites darjeelingensis sp. n.

Seta No	Head	Antenna	Thorax			Abdominal segments								
			Pro-(P)	Meso (M)	Meta (T)	I	II	III	IV	V	VI	VII	VIII	X
0	-	-	M,d	-	-									
1	1	4,5(5)d	1	1	1 (L,B)	1 (L,B)	1 (B)	1 (B)	1 (B)	1 (B)	1 (B)	1 L,B	1,2(1)	1 (B)
2	1	1	1,2(2)	1 (L)	2,3(2)	2	1	1	1	1	1	1	2,3(2)	8,9(8)
3	1	1	5,6(6)	1	m,d	2 (B)	2(L,B)	1 (L,B)	1,2(1) (L,B)	1 (L,B)	1 (B)	1 L,B	3-5(3)	4-6(5)
4	1 (L)	1	m, d	1	m,d	2(L,B)	2(L,B)	2(L,B)	1,2(1) (L,B)	1 (B)	5	2	1(L,B)	8,9 (8)(B)
5	m,d	1	1 (B)	1 (L,B)	m,d	4-6(4)	4,5(5)	4,5(4)	4,5(4)	2(L,B)	2	1	1(L,B)	-
6	1 (L)	1	4-6(4)	1 (Sf,B)	1 (Sf,B)	2 (B)	2(L,B)	2(L,B)	2(L,B)	2(L,B)	1(L,B)	m	-	-
7	1 (L)	-	2 (Sf,B)	3,4(3)	2 (Sf,B)	2(L,B)	2(L,B)	2(L,B)	2(L,B)	1(L,B)	1(L,B)	1 (B)	-	-
8	1 (L)	-	4,5(4) (L,B)	m	m,d	-	1	1	1	1	m	m	-	-
9	4-6(4)(B)	-	1 (Sf,B)	1 (Sf,B)	1 (Sf,B)	2	2	2	1	1	1	1	-	-
10	7-9(8)	-	1 (L,B)	1 (L,B)	1 (L,B)	1	1,2 (2)(L,B)	2(L,B)	2(L,B)	1 (B)	1 (L)	1	-	-
11	5,6 (5)	-	1	1	2	2(L,B)	1,2 (2)(L,B)	2 (B)	2(L,B)	1(L,B)	1(L,B)	1 L,B	-	-
12	4(B)	-	1 (L)	1 (L)	1 (L)	m,d	2,3(2)	3,4(4)	3,4(4)	3,4(4)	1	1	-	-
13	-	-	-	2 (Sf, B)	1 (Sf,B)	1 (L,B)	1 (B)	1 (B)	1 (B)	1 (B)	1 (B)	1 L,B	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L-long seta; B-barbed; m- multiple (more than 10 branches); d- dendritic; Sf- Stiff. 1-S with 6,7(6)(Sf, B)														

Table 7. Morphological characters of Tx. darjeelingensis sp. n. compared with Tx. bengalensis, Tx. tyagii and Tx. splendens

Species	Tx. bengalensis	Tx. tyagii	Tx. splendens	Tx. darjeelingensis
Mesonotum	No broad pale yellow scales over wing roots to scutellum	Broad pale yellow scales over wing roots to scutellum	No broad pale yellow scales over wing roots to scutellum	Broad pale yellow scales over wing roots to scutellum
Abdomen terga	Lateral tufts of VI-Te with long white and dark brown tufts; VII & VIII-Te with dark brown tufts	lateral tuft of VI-Te, 0.75 with black remaining yellow; VII-Te black; and VIII-Te orange	lateral tuft of VI-Te yellow and black; VII-Te black; and VIII-Te orange	lateral tuft of VI-Te 75% deep blue remaining black; VII-Te 90% deep-green remaining yellow; and VIII-Te yellow
Wing size	7.3mm	7.5mm	8.5mm	5.7mm
Legs	Mid and hind femora with metallic purple scales dorsal, and yellow scales ventral; tibiae with purple scales	Hind femur black; mid femur black with pale areas, fore femur with wide basal ring; all tibiae dark	Hind femur mainly pale golden, purple dorsally on distal ½, fore and mid femora purple, pale golden ventrally and posteriorly; fore tibia purple; mid tibia mainly pale golden, purplish at base and apex; hind tibia purple, with greenish reflections.	Hind femur 70% with golden yellow, remaining black, fore femur with wide basal ring, mid femur black with pale scales; fore and hind tibia dark, mid-tibia with white longitudinal strips
Male genitalia	Basal mesal lobe with 2 stout apical seta; gonostylus with few microsetae restricted to apical 1/3	Basal mesal lobe with 2 stout apical seta; gonostylus with few microsetae from base to apex	Basal mesal lobe with 1 stout apical seta; gonostylus with few microsetae restricted to distal half	Basal mesal lobe with 1 stout apical seta; gonostylus with numerous microsetae distributed evenly from base to apex.
Larva	Seta 7-M 4,5 branches, and 13-M multiple branches; seta 1-S with 10 branches	Seta 7,13-Mwith 5 branches; seta 1-S with 7 branches	Seta 7-P,M and 13-M double; seta 1-S with 7 branches	Seta 7-M with 3 branches and 13-M double, stiff with barbed; seta 1-S with 6 branches
Pupa	10-CT-12-CT with 2 branches; Seta 8- VII with 8 branches	10-CT with 11, 11-CT with single and 12-CT with 2 branches; Seta 8-VII with 10 branches	10-CT with 5, 11-CT with 2 and 12-CT with 4 branches; Seta 8-VII with 4 branches	10-CT with 5, 11-CT with 3 and 12-CT with single branches; Seta 6-VII with 8 branches

(vii)In Tx. darjeelingensis seta 1-VIII of abdominal segment arises from outside the large sclerotized plate and is without the basal tubercle. In the rest of species under discussion the 1-VIII originates from within the periphery of sclerotized plate and is embedded in a tubercular structure.

(viii)The molecular analysis of Toxorhynchites sp. n. (WB16) alludes its affinity with Tx. splendens rather than Tx. rutilus. The species analyzed are positioned in three separate branches in the phylogenetic tree. Toxorhynchites rutilus has branched much earlier (branch length 0.126). On the other hand, Tx. darjeelingensis sp.n. has branched out (0.035) much lately but slightly before Tx. splendens and Tx. tyagii clustered branch (0.032).

These differences in various morphological structures as well as the branching time of different Toxorhynchites species clearly indicates that each species has evolved separately at different points of time; thus confirming Tx. darjeelingensis to be clearly a distinct and hitherto undescribed species.

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Coprophilic dipteran community associated with horse dung in Malaysia

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Abstract

Blood-sucking flies are known as vectors of pathogens to farm animals worldwide. However, little is known about dipteran biodiversity associated with horse dung in Malaysia which could serve as vectors. Thus, a field trip to a horse farm located in Tanjung Rambutan, Perak, peninsular Malaysia was conducted in 2010. We examined adults and larvae of dipterans associated with 100 horse dung pats. A total of 1480 dipteran specimens from nine families were collected including Sphaeroceridae, Muscidae, Sarcophagidae, Calliphoridae, Sepsidae, Tabanidae, Ulidiidae, Dolichopodidae and Milichiidae. The lesser dung fly (Sphaeroceridae) which consisted of four species was the most abundant dipteran followed by sarcophagids and muscids. Seven species of muscids were collected from the horse farm and three of them were haematophagic namely Musca conducens Walker, 1859, Musca ventrosa Wiedemann, 1830 and Stomoxys calcitrans (Linnaeus, 1758). Fly larvae collected from the dung were raised to adult stage and subsequently identified as M. conducens and Neomyia gavisa Walker, 1859. We reported new locality records for three species of Muscidae in Malaysia namely Pyrellia proferens Walker, 1859, Lispe kowarzi Becker, 1903 (new to peninsular) and N. gavisa Walker, 1859.

Keywords: Coprophilic Diptera, horse dung, Malaysia, new record, Pyrellia proferens, Lispe kowarzi, Neomyia gavisa.

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Introduction

The Diptera, or known as two-winged flies, is one of the largest orders of Insecta and can be found almost ubiquitously on Earth (Triplehorn and Johnson, 2005). Members of Diptera play variety of roles in ecosystem either as pollinators, nutrient recyclers, or carrion decomposers (Borkent and Harder, 2007; Hirabayashi and Wotton, 1998; Putman, 1978). However, there are many dipteran species of economic importance which are responsible for loss of agricultural products and commodity (White and Elson-Harris, 1992; Spencer, 1973). Most importantly, flies have been incriminated either as biological or mechanical vectors to wide range of infectious diseases that are prevalent in both developed and developing countries (Gubler, 2002).

Other than ticks (Acari: Ixodida), Diptera is considered a significant group of arthropods in term of medical and veterinary importance (Cleton et al., 2012). Flies are found accountable for the transmission of variety of pathogens namely viruses, bacteria, protozoans and helminthes (Gestmann et al., 2012), which are responsible for the spread of infectious diseases such as malaria, lymphatic

filariasis, dengue, yellow fever, West Nile fever, and chikungunya (transmitted by nematocerans such as mosquitoes), leishmaniasis, onchocerciasis, loiasis (transmitted by sand flies, black flies and deer flies, respectively) (Service, 2012). Other members of Diptera such as house flies (Musca domestica L.), face flies (Musca autumnalis De Geer), dog dung flies (Musca sorbens Wiedemann), and blow flies (Lucilia spp.) are a source of nuisance to humans and animals (Graczyk et al., 2001). Some species may act as the causative agent to myiasis either obligatory or facultative on animal hosts (James, 1947; Hall and Wall, 1995). Besides, there are many dung-frequenting and carrion-breeding dipterans which may spread pathogenic organisms to the surrounding environment, including human dwelling (Howard, 2001).

Other than the diseases mentioned above, veterinary important Diptera have been reported to impose a serious economic loss to the agricultural sector (Bowman, 2014). For instance, In the United States, flies had impacted the cattle production substantially by losing $2,211 million per year (Taylor et al., 2012a). The stable fly, with just 200,000 flies emerging from an average sized winter hay feeding site could reduce annual milk production of 50 dairy cows by an estimated 890 kg (Taylor et al., 2012b).

In Malaysia, Reid (1953) published the first note on the local house flies and blow flies and indicated their important roles as potential mechanical transmitter of pathogens causing human diseases. Subsequently, Sulaiman et al. (1988) demonstrated that the cyclorrhaphan flies collected from four sites in Malaysia are carriers of human parasitic helminths such as Ascaris lumbricoides, Trichuris trichiura, and filariform larva of hookworm (Necator americanus). Sulaiman et al. (2000) trapped four species of synantrophic flies in downtown Kuala Lumpur viz. Chrysomya megacephala, Chrysomya rufifacies (= Achoetandrus rufifacies), M. domestica and M. sorbens. A total of 18 bacterial species was isolated from these specimens, with Burkholderia pseudomallei, the causative pathogen for melioidosis, had been reported for the first time. Nazni et al. (2005) examined bacteria from the house flies and found 10 different species of bacteria (e.g., Bacillus sp., Staphylococcus sp., Proteus sp., Escherichia sp., Klebsiella sp. etc.) isolated from feces, vomitus, external surfaces and internal organs of house flies. However, newly emerged house fly did not harbor any bacteria. The importance of house fly wings in mechanical transmission of Vibrio cholerae was assessed and the results revealed that the wings did not play an important role in pathogen transmission (Yap et al., 2008). Nazni et al. (2013) carried out a study on house fly to establish whether the house flies can transmit the H1N1 virus mechanically. The findings indicated that the persistency of H1N1 virus on fly legs could be detected up to 24 hours either in chilled or actively flying flies. However, virus was not found in the vomitus, feces and the external body of the flies.

Recently, filth fly surveys were conducted in Malaysia by Nurita and Hassan (2013), Khoso et al. (2015) and Heo et al. (2010) where they found a very high diversity of filth flies in the region. Nurita and Hassan (2013) recorded eight dipteran species breeding in the solid waste namely: M domestica, M. sorbens, Synthesiomyia nudiseta Van der Wulp, Hydrotaea chalcogaster (Wiedemann), C. megacephala, Lucilia cuprina (Wiedemann), Hemipyrellia ligurriens (Wiedemann) and Sarcophaga sp.; whereas Heo et al. (2010) recorded 16 families of Diptera from the cow dung. These results showed that landfills and animal farms can act as major breeding grounds for filth flies.

From the above stated data, it is clear that little is known about the coprophilic dipteran community associated with horse manure in Malaysia, particularly the blood sucking flies that might feed on horses. Hence, there is a need to identify the veterinary important Diptera in the horse farm, as the baseline data would be of paramount important in terms of risk management and in vector control programs in future. The present study was aimed to determine the diversity and abundance of coprophilic Diptera associated with horse manure, and to identify the veterinary and medically important dipterans in a horse farm in Malaysia.

Materials and Methods

Horse farm located in Tanjung Rambutan, Perak, Malaysia was selected as the study site (4°41'13" N 101°09'31" E, ~89 m a.s.l.) located approximately 212 km northwest

from the capital city, Kuala Lumpur (Fig. 1). A total of four visits to the horse farm were conducted from 8-11 November 2010. Duration and time of fly collection was 5 hours per visit from 9 am to 2 pm. The mean temperature and humidity during the survey period was 28.92 ± 2.36 °C and 73.88 ± 10.72%, respectively. The weather conditions during the four-day field trials were mostly cloudy and all weather data was obtained from the nearest weather station in Ipoh, Perak.

We examined a total of 100 horse dung pats scattered in an area of approximately 5,900 m^2. The age of horse dung examined was from fresh to 3-day old. Each dung pat was examined for the presence of adult flies and dung-breeding larvae. Adult flies either resting on or swarming around the dung pats were collected using a sweep net or transparent plastic bags, which were then transferred to a killing jar which contained cotton balls soaked with ethyl acetate. A pair of forceps was used to examine and recover fly larvae from the dung pats. Any larvae or soil arthropods observed in the dung were then collected and preserved in vials (diameter: 25 mm; height: 85 mm) containing 70% ethanol with label. Several larvae from the same batch were also collected and placed in a transparent plastic container (diameter: 44 mm; height: 57 mm; volume: 70 ml) together with a small amount of horse manure which served as food source for rearing purposes. The screw cap of the rearing container was removed and replaced with a breathable paper towel which had been tighten with a rubber band around the lid. Few drops of water were added ad-libidum to maintain the moisture of the horse manure throughout the rearing periods. All preserved and rearing specimens were then transferred to parasitology laboratory at the Faculty of Medicine, Sungai Buluh Campus, Universiti Teknologi MARA.

The emerging adult flies from rearing containers were prompted for species identification under a stereomicroscope (Olympus SZX7, Japan) by using the keys provided in Triplehorn and Johnson (2005), Emden (1965) and Kurahashi et al. (1997). Several adult specimens from the families Calliphoridae, Muscidae and Sarcophagidae were sent to the third author (Kurahahsi, H.) while specimens of Sphaeroceridae were sent to the fourth author (Hayashi, T.) for species confirmation. Other soil arthropods recovered from horse dung were processed for preservation and identified by using respective taxonomical keys to the lowest taxon. Collembola, ants and centipedes were preserved in vials filled with 70% ethanol while Coleoptera were pinned and labelled. For acari processing, the mite specimens were placed in lactophenol (50% lactic acid, 25% phenol crystal, and 25% distilled water) for clearing purposes. They were left in clearing medium for a week and then transferred on slides by using a probe. Hoyer's medium was used as the mounting fluid and then the specimens were covered with thin cover slips. The slides were then placed in a drying oven at 40°C for a week. The mites were identified to family level based on Krantz and Walter (2009) whereas genus of Macrochelidae was confirmed using the key of Emberson (1980).

Diversity and abundance data of the collected dipterans was calculated for ecological indices (Dominance, Species Richness, Simpson's Index, Shannon-Wiener Index, and Evenness) for the study period. Dominance (D_i) was calculated according to the equation:

$$D_i = \frac{n_i}{N} \times 100$$

Where n_i is the number of individuals collected during the study period, and N is the total number of specimen collected. Species dominance of all families or species were classified according to Tischler's scale: eudominant 10%≤ D_i ≤100%, dominant 5%≤ D_i ≤10%, subdominant 2%≤ D_i ≤5%, recedent 1%≤ D_i ≤2%, and subrecedent 0%≤ D_i ≤1% (Tischler, 1949).

Species richness (S) is the total of different species presented in the sample while Simpson's Index (D) measured both richness and proportion of each species and is calculated by using the formula:

$$D = \sum_{i=1}^{S} P_i^2$$

Where P_i is the proportion of species i. In brief, Simpson's index is the sum of proportion of each species in the community and represented the probability of two randomly selected individuals in the community belong to the same species. Shannon-Wiener Index (H') is

similar with Simpson's Index where the measurement takes species richness and proportion of species into account, and is calculated by the following formula:

$$H' = -\sum_{i=0}^{n} P_i(\ln P_i)$$

In general, Shannon-Wiener Index is the negative sum of multiply products between species proportion (P_i) and natural log of species proportion ($\ln P_i$). Evenness (E) is an indicator of similarity in abundance of different species. Evenness is measured on the scale from 0 to 1 where zero represents more variation in communities whereas one represents complete evenness. Evenness is defined as:

$$E = \frac{H'}{\ln S}$$

Evenness is the number obtained via dividing the value of Shannon-Wiener Index by natural log of species richness (S).

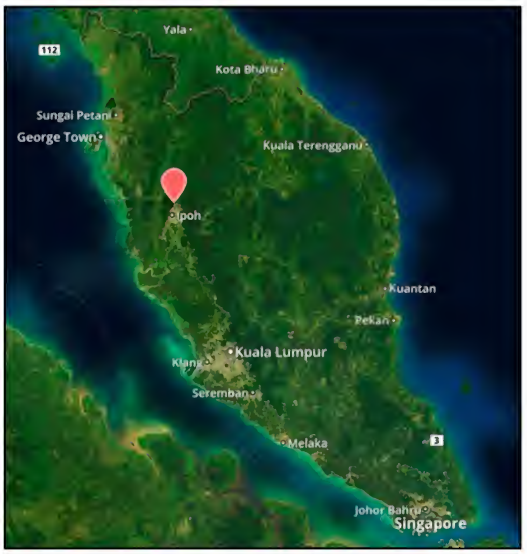

Fig. 1. Location of the study site at peninsular Malaysia is indicated by a pink arrow (map created using www.mapbox.com)

Results

A total of 1,480 adult specimens from nine families of Diptera were collected from the horse farm. The families consisted of Sphaeoceridae (four species), Muscidae (seven species), Sarcophagidae (two species), Calliphoridae (two species), Sepsidae (two species), Tabanidae (one species), Ulidiidae (one species), Dolichopodidae (one species) and Milichiidae (one species) (Table 1). Throughout the study period, the most abundant dipterans caught were Sphaeroceridae (93.78%), followed by Sarcophagidae (3.04%), Muscidae (2.30%), Calliphoridae (0.50%), Sepsidae and Ulidiidae (0.10% each) and the least were Tabanidae, Dolichopodidae and Milichiidae (0.06% each) (Fig. 2).

We collected 34 individual of muscids which consisted of seven species (Fig. 3). The species of muscid flies according to their decreasing abundance were as follows: M. domestica (32.4%), Musca conducens Walker (26.50%), Lispe kowarzi Becker (23.50%), Pyrellia proferens Walker (5.90%), Neomyia gavisa Walker (5.90%), Musca ventrosa Wiedemann (2.90%) and Stomoxys calcitrans (2.90%). Out of seven species recovered, three species were blood sucking muscids (M. conducens, M. ventrosa and S. calcitrans), with M. conducens being the most abundant haematophagic species in the horse farm.

Three species of Muscidae were also recorded for the first time in Malaysia namely L. kowarzi (new in peninsular Malaysia), P.

proferens, and N. gavisa (Fig. 4). These adult flies were collected on or around the horse dung pats, indicating their coprophilic behavior in nature. We hereby provide locality notes for all three species:

Specimens examined:

Lispe kowarzi Becker, 1903
MALAYSIA: Perak: Tanjung Rambutan 4°41'13" N 101°09'31"E, ~89 m a.s.l. 9.xi.2010, C.C. Heo, 4 males, 4 females.

Pyrellia proferens (Walker, 1859)
MALAYSIA: Perak: Tanjung Rambutan 4°41'13" N 101°09'31"E, ~89 m a.s.l. 10.xi.2010, C.C. Heo, 1 male, 1 female.

Neomyia gavisa (Walker, 1859)
MALAYSIA: Perak: Tanjung Rambutan 4°41'13" N, 101°09'31"E, ~89 m a.s.l. 10.xi.2010, C.C. Heo, 1 female.

The most abundant Diptera caught in this study was the lesser dung flies (Sphaeroceridae), which constituted about 94% of total specimen collected. This family was observed to frequent on almost every horse dung scattered in the farm, and usually seen in a large group resting on dung surfaces. Four species of sphaerocerids were collected and identified: Norrbomia tropica Duda, Coproica rufifrons Hayashi, Coproica coreana Papp and Coproica aliena Papp (Fig. 5). On the other hand, two species of flesh flies (Sarcophagidae) were found in the farm namely Parasarcophaga taenionota (Wiedemann) and Liopygia ruficornis (Fabricius), and two species of blow flies (Calliphoridae) were identified associated with horse manure, the oriental latrine fly, Chrysomya megacephala Fabricius and the hairy maggot blow fly, Achoetandrus rufifacies (Macquart). These are common blow fly species found in Malaysia and were known to visit decomposing organic matter. Other than that, we also collected specimens from the families Sepsidae, Tabanidae, Ulidiidae (Physiphora sp.), Dolichopodidae and Milichiidae (Milichiella sp.). Note that the tabanid fly is also a blood-sucking species, however, it was rarely seen in the farm.

In addition to Diptera, there were many other taxa associated with horse feces. Examination into the dung pats revealed springtails (one family), beetles (four families), mites (three families), centipedes (one order), ants and spider (one family). However, no earthworm was collected. A butterfly (Lepidoptera: Lycanidae) was also observed to frequent on dung surface. Table 2 listed the families of non-Diptera taxa associated with horse dungs.

Results of ecological indices obtained from formulas stated in methodology were presented in Table 3 and 4. Among dipteran families, family Sphaeroceridae was the most dominant family. Simpson's Index was 0.88 while Shannon-Wiener Index was 0.31. The abundance of all the families collected was dissimilar and skewed, resulted in an uneven distribution among families (E= 0.14). In terms of Family level, only Muscidae was analyzed. Musca domestica, M. conducens and L. kowarzi were all classified in the eudominant group. Simpson's Index and Shannon-Wiener Index were 0.26 and 1.54, respectively, indicated a more equal proportion among species. This observation was strengthened with a higher evenness index (E=0.80).

Discussion

Animal dung pats are patchy but resourceful ephemeral microhabitats which host a vast diversity of species (Hanski and Koskela, 1977). The adults and larvae of Coleoptera and Diptera are considered to play a major role in the dung utilization. However, little is known about the coprophilous fly species compared to studies conducted on dung beetles (Hammer, 1941; Nichols et al., 2007).

Sphaeroceridae are very small, black or brown flies that can be identified by the swollen hind tarsi. They can be found in humid and swampy places near excrement and often aggregated in large number on various mammalian dung piles (Laurence, 1955; Triplehorn and Johnson, 2005). Unexceptionally, the most abundant Diptera collected on horse dung in this study was sphaerocerids. Similar observation was made in U.K. on stable manure and two species of sphaerocerids were considered as potential pests (Hussey, 1957). This finding was in agreement with Bai and Sankaran (1977) where sphaerocerids were found breeding in bovine manure in India. Similar results were demonstrated in North America where

sphaerocerids were collected from cattle droppings (Blume, 1985). Other than cattle and horse manures, sphaerocerids can be found in poultry manure (Hulley, 1986). Sphaerocerids, together with acarid mites were also reported as the first colonizer on poultry manure (Stoffolano and Geden, 1987). The cosmopolitan genus Coproica is known to be endemic on various kinds of pasture dung whereas some species breed in decaying vegetable material. In fact, most larvae actually feed on microbial layer developing on the decaying matter (Papp, 2008). However, during the present study, we were not able to collect any sphaerocerid larva in the horse manure. Perhaps it was due to the smaller sample size or other abiotic factors (e.g., condition of horse manure or dung management strategies employed by the farm).

Table 1. Family and species of Diptera recovered from horse dung

Family	Species
Sphaeroceridae	Norrbomia tropica Duda, 1923 Coproica rufifrons Hayashi, 1991 Coproica coreana Papp, 1979 Coproica aliena Papp, 2008
Muscidae	Musca domestica vicina Macquart, 1850 Musca conducens Walker, 1859 Musca ventrosa Wiedemann, 1830 Stomoxys calcitrans (Linnaeus, 1758) Pyrellia proferens Walker, 1859 Lispe kowarzi Becker, 1903 Neomyia gavisa Walker, 1859
Sarcophagidae	Parasarcophaga taenionota (Wiedemann, 1819) Liopygia ruficornis (Fabricius, 1794)
Calliphoridae	Chrysomya megacephala Fabricius, 1794 Achoetandrus rufifacies (Macquart, 1842)
Sepsidae	Unidentified sp. 1 Unidentified sp. 2
Tabanidae	Unidentified sp.
Ulidiidae	Physiphora sp.
Dolichopodidae	Unidentified sp.
Milichiidae	Milichiella sp.

Table 2. Other arthropods associated with horse dungs

Class	Order	Family	Subfamily / Species
Insecta	Collembola	Hypogastruridae	Ceratophysella sp.
	Coleoptera	Scarabaeidae	Onthophagus sp. Aphodiinae
		Hydrophilidae	Unidentified sp.
		Histeridae	Unidentified sp.
		Staphylinidae	Staphylininae
	Hymenoptera	Formicidae	Odontoponera sp.
Arachnida	Mesostigmata	Macrochelidae	Macrocheles sp.
		Uropodidae	Unidentified sp.
		Parasitidae	Unidentified sp.
	Araneae	Lycosidae	Unidentified sp.
Chilopoda	Geophilomorpha	Unidentified	Unidentified sp.

Table 3. Standard ecological indicators of Order Diptera found on horse dung

Family	Number	Dominance	Tischler's scale	P_i	P_i^2	$P_i \ln(P_i)$
Sphaeroceridae	1387	93.7	Eudominant	0.937	0.878	-0.061
Sarcophagidae	45	3.0	Subdominant	0.030	0.001	-0.106
Muscidae	34	2.3	Subdominant	0.023	0.001	-0.087
Calliphoridae	7	0.5	Subrecedent	0.005	0.000	-0.025
Sepsidae	2	0.1	Subrecedent	0.001	0.000	-0.009
Ulidiidae	2	0.1	Subrecedent	0.001	0.000	-0.009
Tabanidae	1	0.1	Subrecedent	0.001	0.000	-0.005
Dolichopodidae	1	0.1	Subrecedent	0.001	0.000	-0.005
Millichidae	1	0.1	Subrecedent	0.001	0.000	-0.005
Total	1480	100.0		1.000		
Ecological Indicators			**Value**			
Species Richness (S)			9			
Simpson Index (D)			0.880			
Shannon-Wiener Index (H')			0.312			
Evenness (E)			0.142			

Table 4. Standard ecological indicators of Family Muscidae found on horse dung

Species	Number	Dominance	Tischler's scale	P_i	P_i^2	$P_i \ln(P_i)$
M. domestica	12	35.3	Eudominant	0.382	0.146	-0.368
M. conducens	9	26.5	Eudominant	0.265	0.070	-0.352
L. kowarzi	8	23.5	Eudominant	0.235	0.055	-0.340
P. proferens	2	5.9	Dominant	0.059	0.003	-0.167
N. gavisa	1	2.9	Subdominant	0.029	0.001	-0.104
M. ventrosa	1	2.9	Subdominant	0.029	0.001	-0.104
S. calcitrans	1	2.9	Subdominant	0.029	0.001	-0.104
Total	34	100.0		1.000		
Ecological Indicators			**Value**			
Species Richness (S)			7			
Simpson Index (D)			0.256			
Shannon-Wiener Index (H')			1.538			
Evenness (E)			0.790			

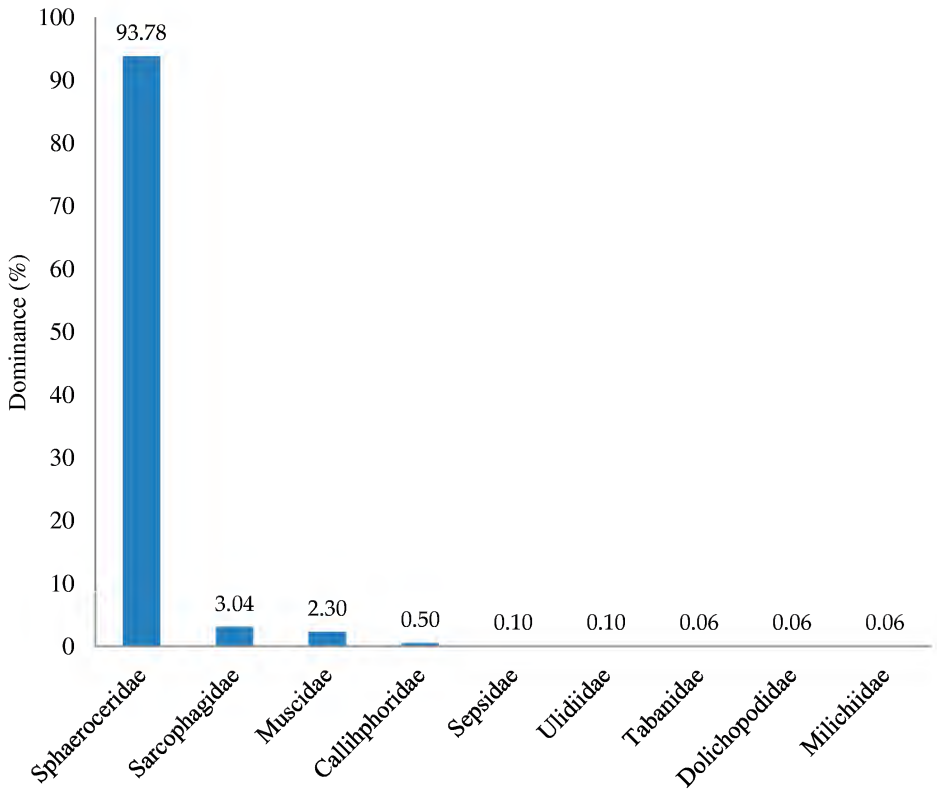

Fig. 2. Abundance of Diptera collected according to family (in percentage)

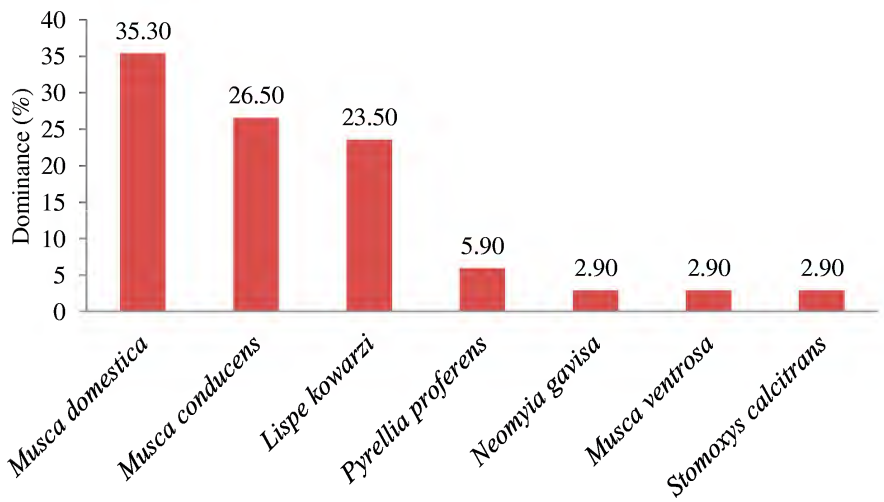

Fig. 3. Species dominance of seven species of family Muscidae collected from cow dung

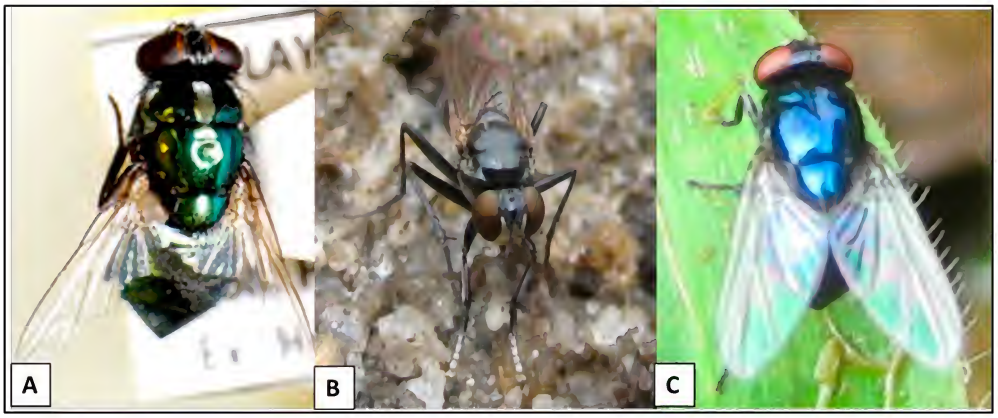

Fig. 4. Newly recorded Muscidae from peninsular Malaysia. A. Pyrellia proferens; B. Lispe kowarzi; C. Neomyia gavisa

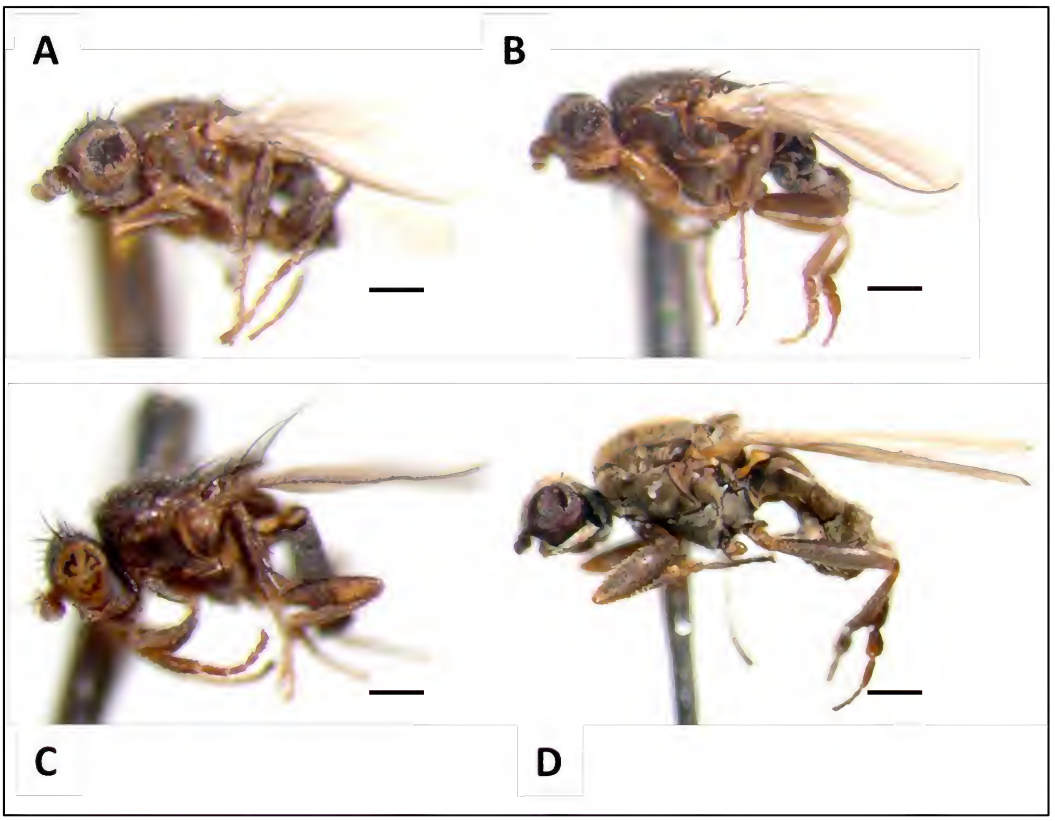

Fig. 5. Sphaeroceridae collected from horse dung. A. Coproica aliena; B. Coproica coreana; C. Coproica rufifrons; D. Norrbomia tropica. Scale bar = 0.5 mm for all figures

We collected four species of blood-sucking flies (three in Muscidae and one in Tabanidae) during the study period. Among these, M. conducens was the most abundant (26.5% of total muscid caught), followed by M. ventrosa and S. calcitrans (both were equally abundant), and lastly a Tabanus sp. As discussed earlier, the blood sucking Diptera are considered medical and veterinary important pests due to their potential in causing and transmitting vector-borne pathogens. Musca conducens was incriminated as the vector for stephanofilariasis, which had been reported to cause chronic eosinophilic dermatitis in scrotal area among Charolais bulls in France (Watrelot-Virieux and Pin, 2006) and transmit Stephanofilaria assamensis in India and Russia (Patnaik and Roy, 1969; Johnson et al., 1981) and Stephanofilaria kaeli in peninsular Malaysia (Fadzil, 1973). Interestingly, we collected 13 larvae of M. conducens from horse manures and reared them to the adult stage, out of these four males and nine females emerged (male: female ratio 1:2).

Musca ventrosa was found in fresh markets in Malaysia, albeit its population was low (Khoso et al., 2015). Nazni et al. (2007) conducted a study on distribution of diurnal and nocturnal dipterous flies in Putrajaya, Malaysia and collected six specimens of M. ventrosa out of 1,534 flies (0.04%) during the day. Despite feeding on wounds, sores and bites inflicted by other insects, these flies also frequent cow dung and their larvae breed in it (Patton, 1922). So far, little literature is available on the status of M. ventrosa as a vector for veterinary diseases although it is a haematophagous species (Sucharit and Tumrasvin, 1981). In our study, we collected a single adult M. ventrosa. Due to their low population, their feeding impact on horses seems to be limited.

Adults of S. calcitrans suck blood and inflict painful bites and thereby causing trouble to the confined livestock such as cattle and horses (Service, 2012). The fly causes interference with the normal feeding activity of cattle which could result in weight loss, decreased milk production, and possibly anemia. Moreover, stable flies have been found responsible for the mechanical transmission of several livestock diseases such as equine infectious anemia and bovine anaplasmosis (Bay and Harris, 1988). Likewise, we collected only one adult S. calcitrans, which may suggest limited vectorial capacity due to their low population density.

Heo et al. (2010) conducted a study on cow dung Diptera in Kuala Lumpur, Malaysia and found Musca inferior Stein to be the most abundant (37%) blood-sucking muscid associated with cattle manure, followed by S. calcitrans (10.3%), M. ventrosa (9.4%), M. crassirostris (3.4%) and Haematobia sp. (0.8%). In comparison with the present study, the results showed that M. ventrosa and S. stomoxys were present in both study sites and were of intermediate abundance either on cattle or horse manures. However, we did not collect M. inferior, M. crassirostris and Haematobia from the horse dung. Additionally, the most abundant non-biting Diptera associated with cattle dung was Sepsidae, which was different in the current study. A comparison of dipteran composition with Heo et al. (2010) is presented in Table 5.

Although cattle and horses are herbivores, there are some remarkable differences in the diversity of arthropod assemblages on their wastes. The differences in results of arthropod communities between cattle and horse dungs could be due to two main reasons: biotic and abiotic factors. Biotic factors include the differences in microbiome in the animal dung (Dowd et al., 2008; Girija et al., 2013; Costa and Weese, 2012), which may affect directly the behavior of arthropods (Ezenwa et al., 2012) or perhaps the subsequent colonization events (Shade et al., 2013). The microbiome on the dung could be the direct result from the animal intestine itself or the contamination from the external environment (Girija et al., 2013). Besides, arthropod visiting the dung could also deliver and contribute microbes to it (Estes et al., 2013) which consequently initiate a change in arthropod successional sequence either due to the exploration of new resources (Pechal et al., 2014; Finley et al., 2015) or interkingdom signaling through volatile organic compounds released by bacteria (Tomberlin et al., 2012). Moreover, ecological interactions such as mutualism, competition, predation and parasitism could shape the structures and

functions of the microbial and arthropod communities in the dung ecosystem (Valiela, 1974). The abiotic factors include the differences in spatial and temporal factors (e.g., geographical locations of the study sites and timing of study), weather factors (e.g., differences in temperature and relative humidity, event of rains), and most notably, is the difference in the physical structure and chemical composition between cattle and horse manures. Cattle dungs had more moisture content (31%) than horse (27%) (Akhtar et al., 2013). Besides, horse dungs were more alkaline (pH 9.4) compared to cattle (pH 5.2). The percentage of organic carbon, C:N ratio, total nitrogen and concentration of inorganic nitrogen were generally higher in cow dung in comparison with horse manure (Ajwa and Tabatabai, 1994).

Table 5. Comparison of dipteran composition of cow and horse dungs.

Result	Cow dung (modified from Heo et al., 2010 with correction on the species of the Diptera larva collected)	Horse dung
Location of study	Sentul Timur, Kuala Lumpur (3°11' N 101°41' E)	Tanjung Rambutan, Perak (4°41'13" N 101°09'31" E)
Total family collected	16	9
Most abundant Diptera	Sepsidae (43.8%)	Sphaeroceridae (93.8%)
Total species collected in Muscidae	12	7
Total blood sucking species	6	4
Percentage of haematophagous muscid collected	51.5%	29.4%
Most dominant haematophagous muscid	M. inferior	M. conducens
Diptera larvae collected from dung	M. inferior Stein Psychodidae	M. conducens N. gavisa

Neomyia spp. is common muscids to frequent on animal dung. Their larvae are found to be saprophagous, coprophagous (Couri et al., 2006) and also serves as food source to many other carnivorous larvae living in the dung (Emden, 1965). The larvae of Neomyia and other dung fauna (e.g., dung beetles and earthworms) play a vital role in dung decomposition and nutrient recycling (Holter, 1977, 1979; Sommer et al., 2001). In Malaysia, six species of Neomyia have been documented to date namely Neomyia rufitarsi (Stein), Neomyia coerulea Wiedemann, Neomyia coeruleifrons (Macquart), Neomyia diffidens (Walker), Neomyia lauta Wiedemann and Neomyia indica Robineau-Desvoidy (Emden, 1965; Heo et al., 2010). With the finding of N. gavisa during the present study, the number of species of Neomyia in Malaysia has increased to seven. Neomyia gavisa was recorded in Sichuan Province, China as dominant species and can be seen all year round (Wang and Feng, 2008). To date, no literature is available about the role of Neomyia in disease transmission.

Pyrellia proferens, a new record from Malaysia, was previously documented from India, Myanmar and Indonesia. The adults can be found on flowers and on decaying animal matters, especially on dungs. The larvae live in animal excrement and feed on carrion (Emden, 1965).

Lispe spp. are shore-living mucids and their larvae are aquatic in the environment (Shinonaga and Kano, 1989; Pont et al., 2012). The adults Lispe are known to be highly predacious to small insects and potentially useful in biological control of black fly (Werner and Pont, 2006). We had a chance to collect L. kowarzi through this study and it is therefore newly recorded in peninsular Malaysia. It was previously recorded in Kota

Kinabalu, Sabah, Malaysian Borneo (Vikhrev, 2012a). In general, this species is widely spread in South Palaearctic and Oriental region (Tumrasvin and Shinonaga, 1982; Ebejer and Gatt, 1999; Bharti, 2008; Vikhrev, 2012a). It is interesting to note that this species could be the predator to other small insects associated with horse manure. So far, six species of Lispe (Lispe pacifica Shinonaga and Pont, Lispe assimilis Wiedemann, Lispe pectinipes Becker, Lispe manicata Wiedemann, Lispe orientalis Wiedemann) have been recorded in Malaysia, including the newly recorded L. kowarzi (Shinonaga and Pont, 1992; Nazni et al., 2007; Kurahashi and Shinonaga, 2009; Chew et al., 2012; Vikhrev, 2012b).

House flies, M. domestica, are responsible for the spread of infectious diseases such as typhoid, dysentery, diphtheria, leprosy, tuberculosis, intestinal parasitic infections in humans and are found to be mechanical vectors for various pathogenic bacteria, viruses and protozoans (Greenberg, 1973; Chaiwong et al., 2014). Blow flies such as C. megacephala and A. rufifacies may act as facultative myiasis agents on the wounds of animals inflicted by injuries or insect bites (Sukontason et al., 2005). Moreover, C. megacephala has been shown to have greater chances of finding helminth ova attached to their external surfaces compared to M. domestica. A study conducted in Ethiopia demonstrated that A. rufifacies acted as a vector of at least five helminthes parasites and four species of protozoan parasites (Getachew et al., 2007). It is pertinent to mention here that these two species of blow flies are forensically important in Malaysia and other adjacent countries and their larvae are frequently encountered in forensic cases (Sukontason et al., 2002; Lee et al., 2004; Chen et al., 2004; Wang et al., 2008).

Parasarcophaga taenionota and L. ruficornis have been found to breed on human and animal carcasses in Thailand and Malaysia (Sukontason et al., 2007; Tan et al., 2010; Kumara et al., 2012) and are used by forensic entomologists in determining the minimum post-mortem interval (mPMI) of a corpse. In medicine, the larvae of L. ruficornis have been documented to cause myiasis in the vagina of a comatose woman in Thailand (Sucharit et al., 1981). Both P. taenionota and L. ruficornis had been recently collected at several fresh markets in Malaysia, indicating their synantrophic behaviors that could contribute as potential mechanical vectors of pathogens (Khoso et al., 2015). Moreover, Heo et al. (2010) collected L. ruficornis from cattle droppings, implying its coprophilous behavior.

Dung beetles (Coleoptera: Scarabaeidae) play a crucial role in dung decomposition and improve nutrient recycling and soil structure (Hanski and Cambefort, 1991). Both adults and larvae are dung feeders, and through the process of feeding, dung beetles facilitate a range of ecosystem services such as secondary seed dispersal, bioturbation, parasite suppression, plant growth enhancement, and soil fertilization (Nichols et al., 2008). Higher diversity of dung beetles could accelerate nitrogen and carbon transfer from the grass-produced dung to the soil (Yoshihara and Sato, 2015). Predators such as rove beetles (Staphylinidae), hister beetles (Histeridae), centipedes, spiders and ants were also found in the horse manures. These predators were searching for fly eggs or insect larvae that were residing in the dung (Triplehorn and Johnson, 2005). Predatory staphylinids inflict heavy mortality on fly eggs, maggots, pupae and adults (Valiela, 1969). In tropical rain forest in Sarawak (Malaysian Borneo), Hanski (1983) revealed 60 Scarabaeinae and four Hybosorinae (necrophagous subfamily of Scarabaeidae), about 20 Hydrophilidae, six Histeridae and more than 150 Staphylinidae from dung and carrion. The water scavenging beetles (Hydrophilidae) are coprophagous, but their larvae are predators (Bøving and Henriksen, 1938).

Apart from Insecta, we also collected mites (Acari: Mesostigmata) from the family Macrochelidae, Parasitidae and Uropodidae, with macrochelids being the most abundant inhabitant of the horse dung. The result of the present study is similar to the earlier study by Axtell (1963) where Macrochelidae, Uropodidae, Parasitidae, Oribatidae and Laelapidae were collected from 211 samples of domestic animal manures (e.g. cattle, horses, sheep, chickens and ducks). Mesostigmata are free-living predators and known to predate on soil nematodes, Collembola and insect larvae (Koehler, 1999). Macrochelidae are active consumers of eggs and larvae of synantrophic Diptera and they might be useful as biological control for dung breeding flies (Krantz, 1983). Macrochelid mite can be found especially on

coprophagous Scarabaeidae (e.g., Onthophagus sp. and Aphodius sp.) (Glida and Bertrand, 2002). Both Parasitidae and Uropodidae are predacious on other soil arthropods and are closely associated with dung beetles through phoresy (Mašán and Halliday, 2009).

Numerous individuals of collembolans (Hypogastruridae: Ceratophysella sp.) were collected from the horse dung where they occurred in large aggregation. The members of Hypogastruridae feed on the decaying plant materials (Greenslade and Ireson, 1986). They may also be feeding on nematodes and other microscopic animals associated with decomposition (Chernova et al., 2007). It should be noted that Ceratophysella spp. are beneficial as they contribute to the decomposition of organic matter (e.g., carrion and dung) and enhance nutrient recycling in agricultural land (Zhang et al., 2012).

In conclusion, four species of blood sucking flies were identified in the horse farm in Malaysia with M. conducens being the most abundant haematophagic muscid. The most populous non-biting Diptera in the farm was the sphaerocerids. Proper management of animal manure is highly recommended to reduce population of blood sucking muscids and tabanids. Annual trapping and phenological studies of horse dung Diptera should be carried out to draw a clearer picture of the bionomics of veterinary important flies, as well as those dung decomposers who play a vital ecological role in dung ecosystem. Spatial-temporal distribution of coprophagous dipterans communities and their ecosystem functions should be better understood for more efficient Integrated Pest Management (IPM) programs. We also suggest further studies on vectorial capacity and efficiency of certain dung associated Diptera (e.g. M. ventrosa) to determine their possibility in transmitting medical and veterinary pathogens.

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First report of Aphid, Liosomaphis ornata Miyazaki, 1971 (Hemiptera: Aphididae) from India

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Abstract

First occurrence of the aphid, Liosomaphis ornata Miyazaki (Hemiptera: Aphididae) making colonies on Berberis lycium Royle (Berberidaceae) from Naggar, Kullu, Himachal Pradesh, India is reported. The viviparous female is re-described with the help of photographs and measurements. A key to the species of Liosomaphis Walker occurring in India is provided.

Keywords: Aphid, Berberis, New record, Liosomaphis, India.

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Introduction

Berberis lycium Royle (Family: Berberidaceae) is an evergreen shrub present in Himalayan region. It is a medicinal plant, known as Indian berberry in English, Kashmal or Kasmal in Hindi and Ishkeen in Urdu (Sood et al., 2012). B. lycium is native to Nepal and is distributed in various parts of the world. It occurs abundantly in the Himalayan regions of India and Pakistan. In India, it has been found in Himachal Pradesh, Jammu and Kashmir, Uttar Pradesh, Sikkim, Madhya Pradesh and Tamil Nadu.

The aphid genus Liosomaphis Walker, (1868) belongs to the tribe Macrosiphini of subfamily Aphidinae (Hemiptera: Sternorrhyncha: Aphididae) with Aphis berberidis Kaltenbach, (1843) as a type species. This genus is distributed in Australia, North America, China, Europe, India, Japan, Nepal, New Zealand, Pakistan and east Siberia (Blackman and Eastop, 2006). So far six species have been described under this genus (Remaudière and Remaudière, 1997), out of which three species have been recorded from India. These species are Liosomaphis atra Hille Ris Lambers, L. berberidis (Kaltenbach) and L. himalayensis Basu. In India, L. atra has been found to feed on B. aristata and B. asiatica, while L. himalayensis was recorded infesting B. aristata, B. asiatica, B. umbellata and B. wallichiana. L. berberidis was recorded on B. lyceum and B. umbellate (Raychaudhuri, 1983). (L. atra Hille Ris Lambers has been recorded on Berberis spp. in East Asia (India, Pakistan, China) while L. berberidis (Kaltenbach) has been recorded on undersides of leaves of Berberis and Mahonia from Europe, India, and introduced to North America, Australia and New Zealand. L. himalayensis Basu is known from India and China on Berberis spp., and L. ornata Miyazaki is known to occur in Japan and China on Berberis spp.). Liosomaphis atra, L. berberidis and Myzus persicae have been reported from B. lyceum.

In the present paper, we report the occurrence of L. ornata for the first time from India. A redescription of the species in detail is included, along with photographs of the mounted specimen. A key based on apterous viviparous females of Liosomaphis species in India is also provided.

Materials and methods

The specimens were collected during surveys from Naggar, Kullu, Himachal Pradesh, India. Nymphs and viviparous apterous females were collected directly from

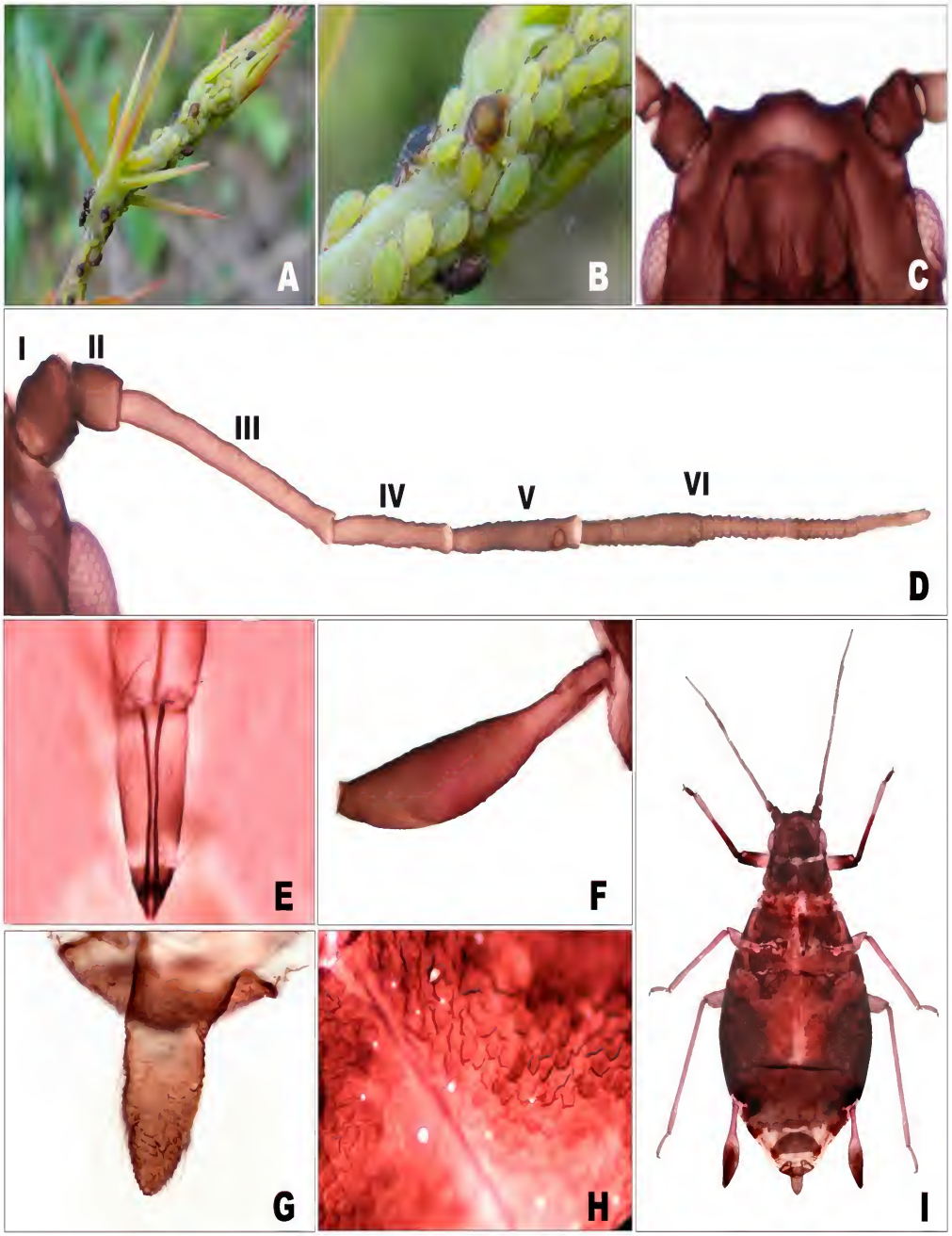

Fig. 1. Liosomaphis ornata A, Colony infesting Berberis lyceum; B, Nymphs (greenish yellow) and completely grown up female (brown); C, Head; D, Antenna; E, Rostrum; F, Siphunculus; G, Cauda; H, Abdominal pattern; I, Habitus.

the tender shoots and buds of Berberis lycium plant (Fig. 1). The specimens thus collected were preserved in 70% ethanol and slides were prepared by following standard procedures (Eastop and van Emden, 1972). Photographs of the mounted aphids were captured with Nikon DS-Vi1 Camera and measurements of different body parts of aphids are taken as suggested by Martin (1983) and Blackman and Eastop (2000) are given in millimetres. All the specimens examined were deposited at the Division of Insect Systematics, Indian Council of Agricultural Research- National Bureau of Agricultural Insect Resources (ICAR-NBAIR), Bangalore, Karnataka, India.

Genus Liosopmaphis Walker

Liosomaphis Walker, 1868, Zoologists, 2(3): 1119.

TYPE - SPECIES: Aphis berberidis Kaltenbach 1843.

Characters of the genus

Head with dorsum slightly wrinkled, lateral frontal tubercles low and diverging, and median frontal prominence distinct; dorsal cephalic hairs short with incrassate apices. Antennae usually 6-segmented, sometimes 5-segmented, much shorter than body, in apterae 0.33-0.50 and in alatae 0.50-0.90 X body; segment I scabrous wholly on inner margin; secondary rhinaria absent in apterae but in alatae circular, flat rhinaria present on segments III, IV and V, those on dorsum of head; processus terminalis (terminal process) nearly as long as to almost 2.0 X base of last segment. Ultimate rostral segment about 0.75-0.90 X hind tarsal segment 2 and bears 2-3 secondary hairs. Midthoracic furca in apterae with separate arms. Abdominal tergum in apterae pale or variably pigmented, corrugated or even papillated but tergum of 7th and 8th segments and near bases of siphunculi spinulose. Siphunculi nearly subcylindrical basad, distinctly clavate on distal 0.50-0.75 portion and then gradually tapers to a small but distinct flange with 4-5 rows of pre-apical spinulose striae which sometimes join to form small cells, smooth to distinctly rugose, much longer than cauda. Cauda elongate with blunt apex, with or without constriction basally but bears 4-9 hairs. Hairs on subgenital plate arranged in more than two rows. Femora sparsely imbricated at least at tip; tibiae smooth; first tarsal chaetotaxy 3,3,3.

Discussion

Börner (1952) and Shaposhnikov (1964) recognize two subgenera, Liosomaphis and Elatobium, under the genus Liosomaphis. But the majority of workers are of the opinion that Liosomaphis and Elatobium are two distinct genera. This view is perhaps more rational because in Elatobium the siphunculi are cylindrical or very weakly clavate, ultimate rostral segment is distinctly shorter than hind tarsus 2 and both apterae and alatae lack extensive dorsal abdominal pigmentation (Raychaudhuri, 1983). The nature of the siphunculi in Liosomaphis and Wahlgreniella is more or less similar but the former genus is easily separated from the latter by the antennae as long as or longer than the body and ultimate rostral segment always longer than hind tarsus. It will perhaps not be out of place to draw a morphological relationship between Liosomaphis and Amphorophora since both these genera have swollen siphunculi coupled with smooth to nearly smooth head. But Amphorophora can well be distinguished by the first antennal segment bearing spinules near the outer margin, segment III bearing secondary rhinaria and by the spinulose second tarsal segment.

Following are the species under the genus Liosomaphis Walker. Validity and synonymy of the species is based on Remaudière and Remaudière (1997).

atra Hille Ris Lambers, 1966
=neoempetri A.K. Ghosh, R.C. Basu and D.N. Raychaudhuri, 1971 (Wahlgreniella)
berberidis (Kaltenbach, 1843) (Aphis)
ESPECE-TYPE
=berberidis Fitch, 1851 (Aphis)?
=berberidis Narzikulov, 1957 (Rhopalalomyzus)?
evadens Rusanova, 1942 nomen nudum [G.R. p. 280 et 304]
himalayensis A.N. Basu, 1964
ornata Miyazaki, 1971
rhododendrophila G.-x. Zhang, Zhong and W.-y. Zhang, 1992
turanica Narzikulov, 1960

Key to the viviparous females of Liosomaphis Walker from India

1. Dorsum of the abdomen with dark pigmentation (Fig.1 I) and sclerotic pattern (Fig.1 H).....2

– Dorsum of the abdomen pale.....3
2. Processus terminalis 1.6–1.9 times as long as base of the last antennal segment. Dorsal pigmentation variable. Siphunculi (Fig.1 F) as long as head width across (including eyes).....**L. ornata Miyazaki**
– Processus terminalis 1.2–1.6 times as long as base of the last antennal segment. Dorsum usually with a complete shield. Siphunculus shorter than head width across eyes.....**L. atra Hille Ris Lambers**
3. ANT 0.52–0.73× body length, processus terminalis 1.6–2.1 times as long as base of the last antennal segment..... …….....**L. himalayensis Basu**
– ANT 0.4–0.5× body length, processus terminalis 0.8–1.4 times as long as base of the last antennal segment..... .....**L. berberidis Kaltenbach**

Description

Live aphid characters

Body oval to elongate, body yellowish green in younger specimens. Colour turns reddish-brown to dark brown as the aphid grows (Figs.1. A, B). Grown up aphid with paler thoracic segmental lines and a pale mid-dorsal line running from head to mid area of abdomen where it meets a dull central dorsal patch. Abdominal segments 1 to 6 dark brown but the segments beyond that become paler.

Characters of mounted female

The characters of mounted females are described in Fig. 1. Viviparous female is elongate to oval in shape (Fig. 1 I). Head smooth, pigmented, with dorsal setae 1/3-1/2 as long as middle width of 3rd antennal segment, with a pair of large, weak swellings mesially to eyes; antennal tubercle as high as or lower than median tubercle, with 2 setae apically (Fig.1 C). Antenna 6-segmented, about half as long as body; 3rd segment faintly imbricated, without rhinaria; processus terminalis 1.6-1.9 times as long as basal part of 6th (Fig.1 D). Thoracic tergites corrugated or papillated, with a brown colouration.

Rostrum reaching middle coxa; ultimate segment obtuse, 0.8-0.9 as long as 2nd segment of hind tarsus, about twice as long as wide, with 2 secondary setae (Fig.1 E) Femora smooth, sparsely imbricated at tip. Tibiae smooth, with setae at most as long as middle width of hind tibia. First tarsal chaetotaxy 3:3:3. Abdominal tergum sclerotized, corrugated or papillated (Fig.1 H); 1st-6th tergites pigmented, usually irregularly lightened in colour mesially; 7th and 8th tergites each with a dark broad band; 2nd-4th tergites each with 5-8 short pointed setae besides marginal ones, without marginal tubercles; 8th with 4 or 5 at most as long as middle width of 3rd antennal segment. Siphunculus markedly swollen, more strongly convex on inner side than on outer side, smooth, with a few rows of transverse striae at apex, as long as or longer than head width across eyes, 2-3 times as long as cauda; largest diameter 1.6-2.1 times as large as smallest diameter of basal cylindrical portion, 2.1-3.3 times as large as smallest diameter just below flange (Fig.1 F). Cauda finger-shaped, blunt at apex, without constrictions, with 5-8 setae (Fig.1 G). First detailed description of this species has been provided by Miyazaki (1971).

Measurements in mm

Body 1.89; antennal segments (1st-6th): 0.08, 0.07, 0.28, 0.15, 0.12, 0.13+0.20; ultimate rostral segment 0.11; hind femur 0.5; hind tibia 0.86; hind tarsus (2nd segment) 0.12; siphunculus 0.51; cauda 0.21.

Specimens examined

17 females, Naggar, Kullu, Himachal Pradesh, India, 6.v.2013 on Berberis lycium.

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A second species of the ant genus Romblonella from the Philippines (Hymenoptera: Formicidae)

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Abstract

Romblonella coryae sp. n. is described from Palawan Island. It is the second species known from the province of Palawan, Philippines. Romblonella coryae differs from R. opaca (F. Smith, 1861) (=R. grandinodis Wheeler 1935) in possessing the following characters: a longitudinally costulate first gastral tergite; a subrectangular head (with CI not exceeding 90); compound eyes located at midlength of head; a narrow median clypeus, only as wide as frontal lobe; and a strikingly bi-colored mesosoma. The worker caste of R. grandinodis is remeasured and rediagnosed and a lectotype and paralectotypes are designated. A key to the two species, a distribution map, and a short discussion are provided.

Keywords: Romblonella coryae, Formicidae, new species, Philippines, Palawan.

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Introduction

Wheeler (1935) erected the myrmicine genus Romblonella and described its type species R. grandinodis based on a small series of specimens from Romblon Island in the central Philippines. Unknown to him and, subsequently to M. R. Smith (1953, 1956), R. grandinodis had already been described in a different genus. Bolton (1976) found that Myrmica opaca F. Smith, 1861 from Sulawesi, Indonesia is the senior synonym of R. grandinodis. Thus, the type species of the genus is now known as Romblonella opaca (F. Smith, 1861) (Bolton et al., 2007).

Romblonella ants are characterized by a robust, hard and compact body, stout propodeal spines, massive petiole and postpetiole, and gaster formed largely by the first tergite (Wheeler, 1935).

Romblonella ants are found on islands ranging from the Philippines to Fiji and Australia (Sarnat and Economo, 2012; Shattuck et al., 2014). Each described species has a very limited range, is rarely collected, and is known from only a few collections (Smith, 1956; Taylor, 1991; Shattuck et al., 2014). For instance, Sarnat and Economo (2012) failed to collect R. liogaster (Santschi, 1928) in their extensive archipelago- wide survey of the Fiji Islands, its type locality. Only R. opaca is widespread, known from small collections (1-5 workers) from four islands in the Philippines and its type locality in Indonesia.

During a recent survey of the primary lowland forest of Cleopatra's Needle, Puerto Princesa City, Palawan Island, Philippines, we discovered a strikingly colored species of Romblonella. It has been 24 years since the description of R. heatwolei Taylor, 1991, the most recent addition to the genus. This current contribution brings the number of valid species to 9 (Bolton et al., 2007; Shattuck et al., 2014).

Materials and Methods

Measurements and Indices

Measurements (in millimetres)

EL Maximum eye length along maximum diameter.

GL Maximum length of gaster, from base of first gastral tergite to apex of gaster, measured in lateral view.

HFL Maximum length of hind femur in anterior view.

HL Maximum head length in full face view, measured from anterior-most point of clypeal margin to posterior-most point of head capsule.
HW Maximum head width in full face view.
ML Mesosomal length measured from anterior edge of the pronotum (excluding the collar) to posterior edge of propodeal lobe.
PW Maximum width of pronotum in dorsal view.
SL Length of scape, excluding basal neck and condyle.
TL The total outstretched length of ant from mandibular apex to gastral apex; when measured in profile the sum of mandibular length + head length + mesosomal length + lengths of waist segments + length of gaster.

Indices
CI Cephalic index: HW/HL x 100
EI Eye Index: EL/HW x 100
SI Scape index: SL/HW x 100

Collection Abbreviations (Brandao et al., 2000)
ANIC Australian National Insect Collection, Canberra, Australia.
BMNH Natural History Museum, London, UK.
CASC California Academy of Sciences, San Francisco, CA, USA.
DMGC Private Collection, David Emmanuel M. General.
MCZC Museum of Comparative Zoology, Harvard University, Cambridge, MA, USA.
NHMW Naturhistorisches Museum, Wien, Austria.
PACB Private Collection, Perry Archival C. Buenavente.
PNM National Museum of the Philippines, Manila, Philippines.
UPLB University of the Philippines Los Baños Museum of Natural History, Los Baños, Laguna, Philippines.

Specimens were examined and measured using a Wild M-5A stereomicroscope with ocular micrometer. Images were created using a Canon 7D digital camera attached to a Leica MZ16 stereomicroscope. Montage images were rendered using Helicon Focus 6. Images were edited with Adobe Photoshop CS6 Extended. The map was created in ArcMap 10.

All specimens were collected under PNM permit # CPD-A-CT-2014-02 dated January 28, 2014.

Taxonomy
Genus Romblonella Wheeler, 1935

Type species: Romblonella grandinodis Wheeler, 1935 (junior synonym of Myrmica opaca F. Smith, 1861), by original designation.

Key to Philippine Romblonella, based on the worker caste

1. In dorsal view, first gastral tergite (abdominal tergite IV) longitudinally costulate with interstitial punctulae. In full-face view, head longer than broad (CI 84-89); median clypeus narrow, only as wide as frontal lobe; mesosoma, in lateral view, distinctly dark orange and brown..... .....**R. coryae General and Buenavente**
- In dorsal view, first gastral tergite (abdominal tergite IV) punctulate but never longitudinally costulate. In full-face view, head subquadrate (CI =95); median clypeus wider than frontal lobe; mesosoma, in lateral view, entirely dark brown.....**R. opaca (F. Smith)**

Romblonella opaca (F. Smith, 1861)

Myrmica opaca F. Smith, 1861: 47 (w.). Indonesia: Sulawesi. Combination in Tetramorium: Emery, 1901: 567; Donisthorpe, 1932: 469. Combination in Romblonella: Bolton, 1976: 294.
Romblonella grandinodis Wheeler, 1935: 7 (w.). Philippines: Romblon. Junior synonym of M. opaca: Bolton, 1976: 294.

R. grandinodis syntype workers (MCZ Type 20977) [examined]; top worker specimen on double pin here designated as LECTOTYPE. The lower specimen and the collection (2 specimens) of the Smithsonian Institution are designated as PARALECTOTYPES (Ms. Eugenia Okonski kindly confirmed the existence of the last two specimens in the

Smithsonian Institution, by sending DEMG images of the specimens and labels.)
Labels: Romblon Is./ 5/6/24 / coll. L. Morato [not Marato, as in Wheeler, 1935 and subsequent publications]
Lectotype measurements: TL 5.13, HL 0.98, HW 0.93, CI 95, SL 0.55, SI 59, EL 0.19, EI 20, PW 0.65, ML 1.18, GL 1.08, HFL 0.75.

Re-diagnosis of worker

In full face view, posterior margin of head broadly concave; head subquadrate (CI = 95); sides of head gently converging anteriorly; eyes located laterally, slightly behind midlength of head; shallow antennal scrobe present; frontal carina long but about 1 eyelength short of posterior margin of head; antennal scapes short, exceeding posterior edge of eye by less than scape width of distal scape at distal margin; antennae with 12 segments and a 3-segmented club; mandibles triangular, with 6 robust teeth; palp formula 5:3; median clypeus with a median carina flanked by 2 pairs of lateral carinae; median clypeus wider than frontal lobe, posteriorly inserted between frontal lobes; anterior clypeal margin entire, without an isolated median seta; head reticulate with punctae in interstitial spaces; mandibles striate.

In lateral view, dorsal margin of mesosoma smoothly and slightly convex, without grooves or sutures; propodeal spines long and stout; petiole sessile, with anterodorsally directed angle over petiolar spiracle; petiole massive, larger and taller than postpetiole; anterior subpetiolar denticle present; spurs absent on meso- and metatibia.

In dorsal view, pronotum angulate; sides of promesonotum subparallel, propodeum noticeably narrower than promesonotum; propodeal spines divergent at bases but parallel in distal third of their length; mesosoma, petiole and postpetiole dorsally reticulate with interstitial punctulation; first gastral (= 4th abdominal) tergite punctulate.

Head and body with abundant short, blunt erect hairs about as long as distance between them; antennal scape with suberect hairs.
Body dark brown with lighter mandibles and antennae.

Other material examined: Philippines: Negros Island, Negros Oriental Province, "Camp, 1924/ Dumaguete, P.I./ coll. J.W. Chapman" [other labels: 1) (yellow) "Cotype/ Romblon, P.I./ L. Morato coll."; 2) (red) MCZ Co-Type [upside-down, no number]; 3) "Romblonella grandinodis Wh."] (double pin: bottom worker specimen headless and petiole and gaster re-glued to point) (UPLB); Albay Province, Rapu-rapu Island, 07.V.2003, leg. B. Nachor [image presented in Alpert et al., 2006]; Palawan Province, Tara Island, January 2000, leg. V. Samarita (PNM 9022).

Romblonella coryae General and Buenavente sp. n.

urn:lsid:zoobank.org:act:F98B881D-F9D2-42CD-A5C4-50F275B9D0D3

Worker measurements and diagnosis
Measurements (paratypes (n=8) in brackets): TL 4.60 [4.60–4.88], HL 0.93 [0.88–0.93], HW 0.80 [0.78–0.83], CI 86 [84–89], SL 0.58 [0.53–0.58], SI 72 [64–72], EL 0.19 [0.19–0.21], EI 23 [23–27], PW 0.60 [0.58–0.65], ML 1.08 [1.03–1.08], GL 1.03 [1.03–1.30], HFL 0.73 [0.68–0.75].

In full face view, posterior margin of head shallowly emarginate; head longer than wide (CI = 84-89); sides of head subparallel; eyes laterally located, at about midlength of head; shallow antennal scrobe present; frontal carinae long, almost reaching the posterior corners of head; antennal scapes short, exceeding posterior edge of eye by about the width of scape at distal margin; antennae with 12 segments and a 3-segmented club; mandibles triangular, with 6 robust teeth; palp formula 5:3; median clypeus with a median carina flanked by 3 pairs of lateral carinae; median clypeus about as wide as frontal lobe and posteriorly inserted between frontal lobes; anterior clypeal margin entire, without an isolated median seta; head rugo-reticulate with short cross-hatches that do not reach the adjacent rugae; punctae present in interstitial spaces; mandibles striate.

In lateral view, dorsal margin of mesosoma smoothly convex, without grooves or sutures; propodeal spines short and stout; petiole sessile, with anterodorsally directed angle over petiolar spiracle; petiole massive, larger and higher than postpetiole; anterior subpetiolar denticle present; spurs absent on meso- and metatibia.

In dorsal view, pronotum with marginate humeri; sides of promesonotum gently converging posteriorly to base of

propodeal spines, interrupted only by slight bulges at junction between pronotum and mesonotum and at the propodeal spiracle; stout propodeal spines slightly divergent at bases but parallel in distal third of their length; mesosoma, petiole and postpetiole dorsally reticulate with interstitial punctulae; first gastral tergite longitudinally costulate with interstitial punctulae; gastral sculpture disappears before distal edge of first gastral segment.

Head with evenly distributed short, blunt erect hairs that are shorter than distance between them; antennal scape with suberect hairs; short, blunt erect hairs sparsely distributed over rest of body.

Color: Head, antennal club, meso- and metapleura, coxae, legs except foretibiae, and gaster dark-brown; rest of mesosoma, petiole and postpetiole dark orange; mandibles, rest of antenna, fore- and midtibiae yellow.

Male and gyne unknown.

Holotype worker: PHILIPPINES: Palawan Island, Puerto Princesa City, Tanabag Village, Sitio Kalakwasan, Camp Palaka, 10°03'57" N, 118°58'23" E, 13-26.II.2014, 200 m above sea level, primary lowland rainforest, leg. D.E.M. General, P.A.C. Buenavente, A.M. Domingo and L.J.V. Rodriguez (PNM 9012, deposited at PNM).

Paratypes: 3 workers, same data as holotype; 2 workers from leaf litter collected at camp, same data as holotype; 3 workers from trail to camp, no coordinates recorded (PNM 9013-9020) (1 worker each to ANIC, BMNH, CASC, DMGC, MCZC, NHMW, PACB, UPLB).

Bionomics: Workers were opportunistically collected from low vegetation along the trail (estimated length = 15 km) from the road to camp and from the tarpaulin shelters at the camp, and from sifted leaf litter.

Etymology: This species is named in honor of our late former President, Corazon C. Aquino (known to all Filipinos by her nickname "Cory"), who led the country out of the dictatorship era. It is fitting that a genus named after a Philippine island has a species named after a modern Filipino hero.

Figures 1-4. Romblonella coryae sp.n. (holotype). 1. Lateral habitus; 2. Full-face view of head; 3. Dorsal view of body; 4. Labels.

Discussion

The biology of Romblonella ants is unknown. Collection notes for this species may provide a clue to its nesting preference. Four specimens were opportunistically collected from the tarpaulin covers sheltering our camp. Other specimens were collected from low vegetation along the trails and from the leaf litter between the buttresses of large forest trees. This implies that R. coryae may be arboreal and have simply been blown off the trees overhead. It may be necessary to apply arboreal sampling techniques to find a nest in the trees.

Previous to the discovery of R. coryae, only R. opaca was known from the Philippines (General and Alpert, 2012). The presence of two species in the Philippines implies that other species may remain undiscovered in other islands in the country. Figure 7 clearly shows the distribution of Romblonella ants in the Philippines and the spread of the five islands from which they were found.

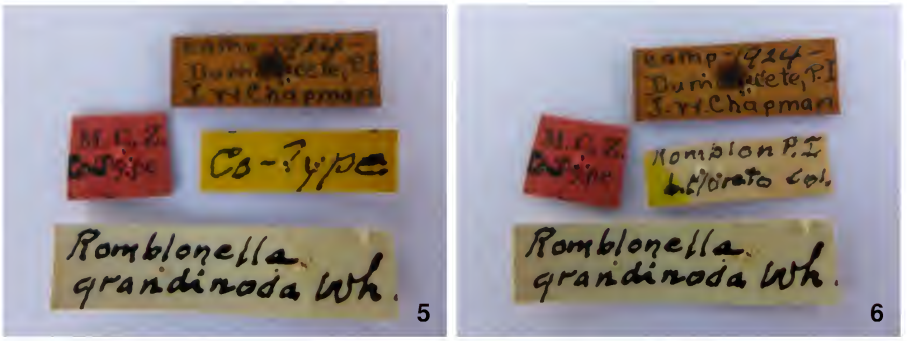
The UPLB material presents a couple of problems that might not be solvable. On a single pin with 2 mounted specimens, there are two legacy locality labels. The top tag, a typical label by Dr. J.W. Chapman, indicates "Camp, 1924/ Dumaguete, P.I./ coll. J.W. Chapman". "Camp" presumably refers to Dr. Chapman's favorite ant-collecting camp somewhere on the Dumaguete side of Mt. Cuernos de Negros, Negros Island. "P.I." is an abbreviation for Philippine Islands, the American colonial-era term for the Philippines, now disused. There is also a determination label which reads "Romblonella grandinoda Wh."[sic]. The problem arises from the other labels: "Cotype" on one side of yellow card and, on its flipside, a second locality label which reads "Romblon, P.I./ L. Morato coll."; and a printed red card which reads "MCZ Co-Type" with the handwritten characters "Co- "but no numbers and now pinned upside-down (see Figures 5 and 6). How is one to interpret this perplexing situation of one double-pin with two locality labels indicating two different islands (Figure 7)?

Wheeler (1935) wrote that the type series was composed of 4 workers. The four specimens in the type series are accounted for: two specimens are in MCZ and two are in the Smithsonian Institution (DEMG, unpublished notes, E. Okonski, pers. comm.) We speculate that there were originally 5 workers in the collection by L. Morato. Wheeler returned one specimen of what would become the type series to Chapman before Wheeler wrote the genus description. And that, when Wheeler wrote his 1935 paper, he forgot about the returned specimen as part of the type series, hence he mentioned only 4 workers in the type series and as a consequence, there is a MCZ Co-Type label without a number. This label is now pinned upside-down, probably to indicate that it is no longer considered a real co-type. As regards the second specimen on the pin, it is highly unlikely that Chapman was so short of insect pins that he combined 2 separate collections on a pin. It is more likely that storage space, e.g. insect drawers, was so limited that he combined the 2 collections on a single pin. We emphasize that this is simply speculation to explain the curious situation of 2 legacy locality labels on a single pin. In addition, there is still the intractable problem of which locality label goes with which specimen.

There are more than 7,100 islands in the Philippine archipelago, of which 1,400 islands belong to Palawan Province alone. It is interesting that Romblonella ants have not been recorded from the two largest islands, Luzon and Mindanao, although this may simply indicate sampling bias. Obviously, more islands and localities need to be surveyed to determine the actual distribution of these ants in the Philippines.

Acknowledgments

We thank the Batak tribesmen who served as our guides, porters and friends. We also thank Jonah van Beinen and Edgar Jose of The Centre for Sustainability for facilitating our collecting trip and introducing us to the



Figures 5 and 6. Labels of two specimens on a single pin, deposited at the Entomological Collection, University of the Philippines Los Baños Museum of Natural History (see text for discussion)

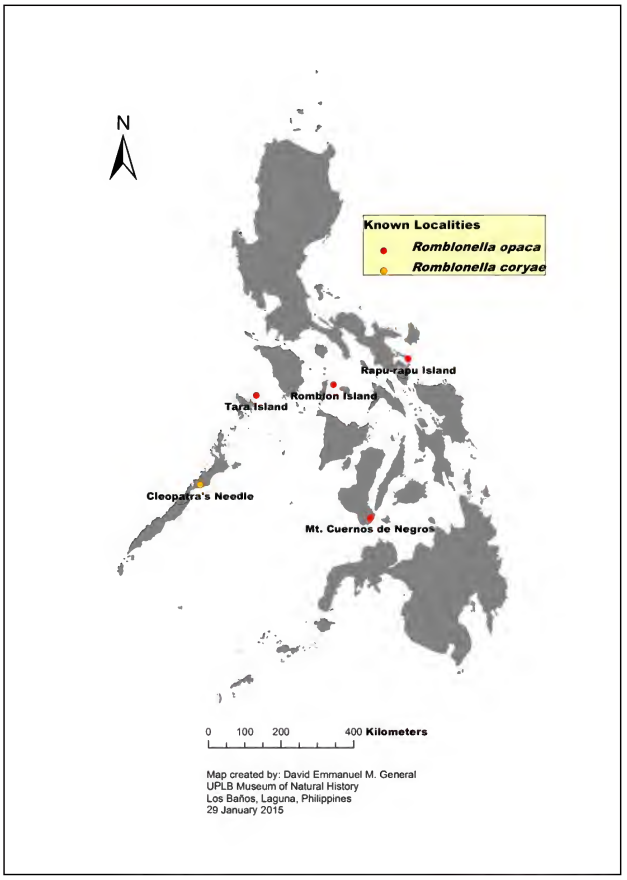


Figure 7. Known localities of Romblonella ants in the Philippines. Coordinates for Romblonella opaca localities were obtained from the Philippine Gazetteer (DIVA-GIS 2014)

gentle Batak. We also thank PNM for issuing the collecting and transport permits. We also thank Ted Schultz and Eugenia Okonski for verifying the presence of the Romblonella grandinodis co-types in the Smithsonian Institution and imaging them for us. We thank Lillian J. V. Rodriguez for imaging the labels of the UPLBMNH specimens. We also thank Philip S. Ward, Seiki Yamane, Robert W. Taylor, and Mostafa R. Sharaf for reviewing the manuscript and providing constructive comments that improved the paper. DEMG is extremely grateful to Gary Alpert and Mary Corrigan for graciously hosting his visit to Cambridge, MA for this study. He also thanks Gary for teaching DEMG how to use his new imaging system and to edit the images for publication. Thanks also to Jignasha Rana for her assistance in the MCZ Ant Room and to JJ Dida for the use of his computer to generate the map. Finally, DEMG is very grateful to the Harvard University Grant Committee for providing an Ernst Mayr Travel Grant for his visit to MCZ.

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Polleniopsis annamensis Kurahashi, 1972 (Diptera: Calliphoridae) a new record from India, with a revised key to the known Indian species

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Abstract

Polleniopsis annamensis Kurahashi, 1972 has been recorded for the first time from India (Western Ghats). A key to the four species of the genus Polleniopsis Townsend, 1917 recorded so far from India is provided.

Key words: Diptera, Calliphoridae, Calliphorinae, Polleniopsis, new record, Western Ghats, India.

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Introduction

Genus Polleniopsis Townsend, 1917 belongs to Onesia-group of tribe Calliphorini. It includes 43 species from East Asia, Australia and Oceania (Verves, 2005). Two species are earlier known from India: P. pilosa Townsend, 1917, P. kashmirensis Kurahashi and Okadome, 1976(Bharti, 2011) and an undescribed species Bharti and Verves,2015 (in press). The present species Polleniopsis annamensis Kurahashi, 1972 has been recorded from Nandi hills falling in the state of Karnataka. A key to the species of Indian Polleniopsis has been provided.

The ecology of these flies is poorly known. Majority of the species prefer mountainous regions and have been collected from up to an altitude of 4650m above mean sea level. The adults of few species (P. chosenensis, P. dandoensis, P. hokurikuensis, P. horii and P. mongolica) have been reported to frequent flowers of the mountains (Kurahashi, 1964; Kurahashi, 1972).

Materials and Methods

The fly was collected with an entomological net from an altitude of 1500m from Nandi Hills falling in the state of Karnataka. The material was examined under Nikon SMZ 1500 stereozoom microscope. Digital images were captured with the help of an MP evolution digital camera mounted on Nikon SMZ 1500 using Auto-Montage (Syncroscopy, Division of Synoptics, Ltd) software. The images were processed and cleaned with Adobe Photoshop CS5.

New Record

Polleniopsis annamensis Kurahashi, 1972
(Figs. 1, 2, & 3)

urn:lsid:zoobank.org:act:F62239D6-C4D6-4185-937C-EC4CE48E1DEB

Material Examined: INDIA: 1♂, Nandi Hills, Karnataka; 13°38" N, 77°70"E, altitude 1500m, 30.IX.2014, M. Bharti.

Distribution: India (Karnataka) (New record), China: Hainan Islands, Viet Nam

Remarks: The genus Polleniopsis is characterized by reduced number of presutural acr (0-2); well-developed facial carina; densely dusted body and lack of presutural intra alar bristles. The species P. annamensis closely resembles P. dalatensis Kurahashi,1972 but could be distinguished from the latter by the following combination of characters: facial carina well developed; post-jowls clothed with black hairs; sternopleurals 1+1; third antennal segment 3X as long as second.

Ecology: P. annamensis has been recorded from Nandi Hills falling in the state of Karnataka. The vegetation of the above mentioned place is typical of high hills. The evergreen forest patch on top of the hill is a

favoured wintering location for many migrant species of warblers, flycatchers and thrushes.

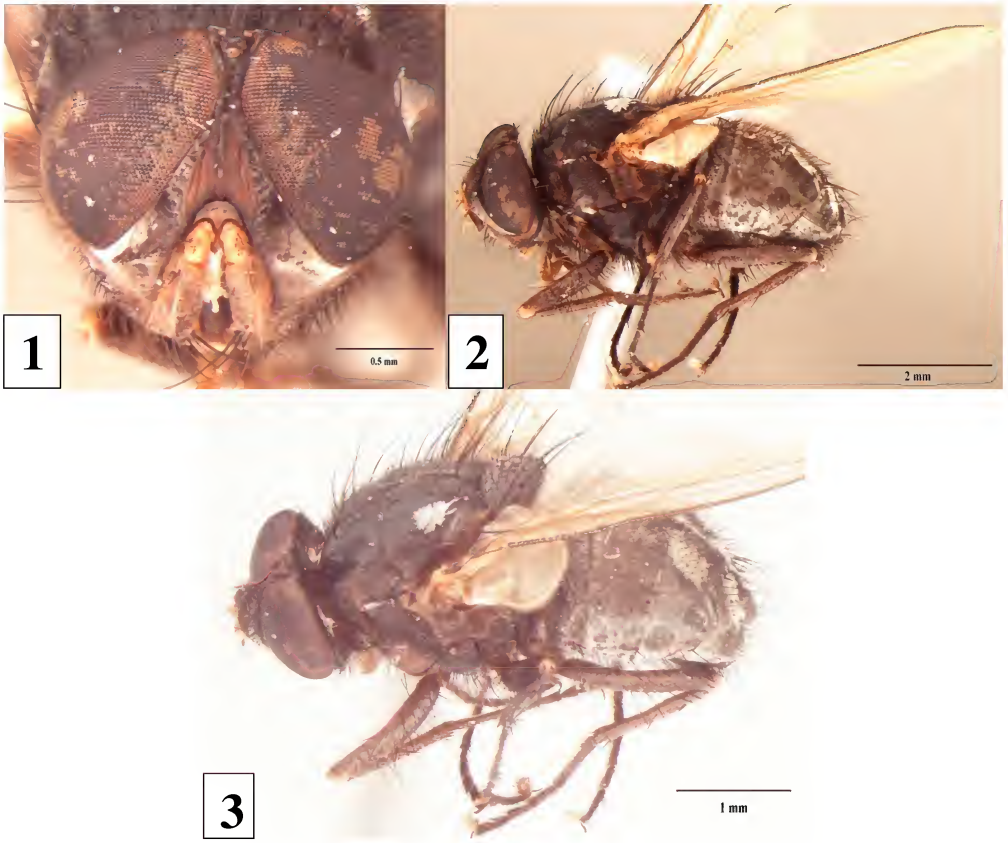

Fig.1-3: Polleniopsis annamensis, male, 1.head view; 2.profile view; 3. dorsal view

Key to the species of Polleniopsis from India

1. Abdomen entirely dark grey to black, silver-gray dusted…….....…….....2
- Abdomen metallic green or blue; legs entirely black 3
2. Scutellum blackish, covered with brownish grey dusting, femora and tibiae light brown; basicosta orange; acr 0+1. 6-7 mm……..**P. pilosa Townsend** India (Bihar, Meghalaya, West Bengal), Thailand (Chiang Mai, Kanchana Buri)
- Scutellum lead grey with grey dusting; legs entirely fuscous to black, at most tibiae and knees sometimes brownish; basicosta light brown; acr 1+2. 6.5 mm……**P. annamensis Kurahashi** (India, China, Viet Nam)
3. Abdomen entirely metallic green; basicosta orange; acr 1+3. 6.5-7.5 mm..... .**P. kashmirensis Kurahashi & Okadome** India (Jammu & Kashmir)
- 1+2nd and 3rd abdominal tergites dark blue, 4th and 5th ones light blue; basicosta black; acr 0+2. 10 mm ….**P. undescribed species** India(Arunachal Pradesh)

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Geographic origin and spread of cosmopolitan ants (Hymenoptera: Formicidae)

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Abstract

I have compiled a list of 42 "cosmopolitan" ant species, i.e., ants with multiple well-established populations in both the Old World and New World, spread through human commerce. Twenty of the 42 cosmopolitan ant species have established populations in all seven of the world's ant-inhabited biogeographic regions (i.e., all except the Antarctic): Afrotropic, Palearctic, Indomalay, Australasia, Oceania, Nearctic and Neotropic. Of the 42 cosmopolitan ant species, 35 (83%) are Old World natives and seven (17%) are New World natives. Cosmopolitan ant species are most often originally native to the Indomalay bioregion (17 species) and are least often native to the Nearctic bioregion (only one species). Only twelve cosmopolitan ants have become major ecological, agricultural, and/or household pest species: Anoplolepis gracilipes, Linepithema humile, Monomorium pharaonis, Nylanderia bourbonica, Paratrechina longicornis, Pheidole megacephala, Solenopsis geminata, Solenopsis invicta, Tapinoma melanocephalum, Technomyrmex difficilis, Trichomyrmex destructor, and Wasmannia auropunctata. The other 30 species are, at most, minor pests. Documenting the exotic spread of ant species within their own native hemisphere will be more complicated because it is often difficult to evaluate what geographic area constitutes the native range and what area, if any, constitutes the exotic range.

Key words: ants, exotic species, invasive species.

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Introduction

Numerous tramp ant species have been transported around the world, hidden in our plant products, packaging material, building supplies, and heavy machinery such as logging and military equipment. Some of these ant species have had great population explosions in areas they have invaded, causing serious ecological and economic problems. Other species have remained rare and/or inconspicuous and have had no discernable impact.

Forel (1911) compiled a list of ant species, spread by humans, which had achieved or were in the process of achieving broad cosmopolitan distributions in both the Old World and the New World (Table 1). Eight of these species are now global pests: Anoplolepis gracilipes (Smith, 1857), Linepithema humile (Mayr, 1868), Monomorium pharaonis (Linnaeus, 1758), Paratrechina longicornis (Latreille, 1802), Pheidole megacephala (Fabricius, 1793), Solenopsis geminata (Fabricius, 1804), Tapinoma melanocephalum (Fabricius, 1793), and Trichomyrmex destructor (Jerdon, 1851). Five others are widespread, but have not developed into major pests, with substantial ecological and/or economic impacts (see below): Cardiocondyla emeryi Forel, 1881, Monomorium floricola (Jerdon, 1851), Tetramorium bicarinatum (Nylander, 1846), Tetramorium lanuginosum Mayr, 1870, and Tetramorium simillimum (Smith, 1851), though in Forel's time, *Tetramorium "simillimum"* also encompassed a second distinct tramp species, Tetramorium caldarium (Roger, 1857). The last two species on Forel's list, Nylanderia vividula (Nylander, 1846) and Odontomachus haematodus (Linnaeus, 1758), may not be cosmopolitan tramps at all. Instead, in Forel's time these two names, each represented what are now recognized as several species with different regional ranges and whose taxonomic boundaries remain uncertain. After dropping N. vividula and O. haematodus, and adding T. caldarium to Forel's (1911) list, a striking trend

emerges: only two of these 14 cosmopolitan species are native to the New World: L. humile and S. geminata (Table 1).

Over the past century, many additional ant species, not on Forel's list, have achieved cosmopolitan distributions, with broad ranges in both the Old World and New World. In the present study, I evaluate the worldwide distribution of all ant species reported to have established populations outside their native hemisphere.

Methods

I compiled both published and unpublished site records for all ant species that have been reported as having exotic populations. I obtained unpublished site records from museum specimens in the collections of Archbold Biological Station, the Museum of Comparative Zoology, the Smithsonian Institution, and (for Forel's (1911) cosmopolitan species) the British Museum. In addition, I used online databases with collection information on specimens by Antweb (www.antweb.org), and the Global Biodiversity Information Facility (www.gbif.org). I also received unpublished collection information from numerous other researchers.

For analyzing the worldwide distributions of the ants, I categorized each site record as belonging to one of seven terrestrial biogeographic realms (following Olson et al., 2001; Old World = bioregions 1-5; New World = bioregions 6-7): 1) The Afrotropic bioregion (22.1 million km^2) includes sub-Saharan Africa, the southern and eastern coasts of the Arabian Peninsula, southern Iran, southwestern Pakistan, Madagascar, western Indian Ocean islands, Cape Verde, and southern mid-Atlantic islands. 2) The Palearctic bioregion (54.1 million km^2) includes Europe, northern Africa, Canary Islands, Madeira, northern and central Arabian Peninsula, and Asia north of the Himalayas, and the main islands of Japan. 3) The Indomalay bioregion (7.5 million km^2) includes southeastern Pakistan, the Indian subcontinent, Southeast Asia, southern China, Philippines, Taiwan, and Japan's Ryukyu Islands, and Indonesia west of Wallace's line. 4) The Australasia bioregion (7.6 million km^2) includes Australia, New Guinea, Indonesia east of Wallace's Line, Vanuatu, Solomon Islands, New Caledonia, and New Zealand. 5) The Oceania bioregion (1.0 million km^2) includes the Pacific islands of Fiji, Micronesia, and Polynesia (except New Zealand). 6) The Nearctic bioregion (22.9 million km^2) includes North America south to the Mexico highlands (except southern Florida), Bermuda, and Greenland. 7) The Neotropic bioregion (19.0 million km^2) includes South and Central America, south and central Mexican lowlands, Caribbean islands, southern Florida, and the Bahamas.

When an ant species occurs in both the Old World and the New World, it is almost always clear that one of these ranges is entirely exotic. Within a hemisphere, however, it is often much more difficult to evaluate what geographic area constitutes the native range and what area, in any, constitutes the exotic range. For this reason, when an ant has a fairly continuous distribution in its native hemisphere and I have no evidence to the contrary, I designated the entire continuous distribution as part of the native range, and listed the entire bioregion as native (Tables 1-4).

I defined a cosmopolitan ant as an ant species, with multiple well-established outdoor populations, outside their native hemisphere, spread through human commerce. Although in the tables I included indoor records for species with broad exotic ranges, I did not include species with records outside their native hemisphere known solely from indoors. I also have not included several ant taxa, with reports of spread outside their native hemisphere, whose worldwide status remains unclear due to taxonomic problems analogous to those of N. vividula and O. haematodus (see Introduction), and may represent multiple species, e.g., Brachymyrmex cordemoyi Forel, 1895, Brachymyrmex obscurior Forel, 1893, Camponotus herculeanus (Linnaeus, 1758), Lasius alienus (Foerster, 1850), Lasius flavus (Fabricius, 1782), Lasius niger (Linnaeus, 1758), Monomorium monomorium Bolton, 1987, Nylanderia vaga (Forel, 1901), Ochetellus glaber (Mayr, 1862), and Tetramorium caespitum (Linnaeus, 1758). I have also omitted several species whose extra-hemispheric records are all dubious, e.g., Pheidole anastasii cellarum

Forel, 1908 (= Pheidole bilimeki Mayr, 1870) (see Fischer and Fisher, 2013).

Results

My analyses distinguished 60 ant species with outdoor populations established outside their native range hemisphere (Tables 1-4). Of these, 42 species qualified as cosmopolitan, i.e., with multiple well-established outdoors populations outside their native hemisphere (Tables 1-3). Twenty of the 42 cosmopolitan ant species have established populations in all seven of the world's ant-inhabited biogeographic regions (i.e., all except the Antarctic, which ironically has no ants).

I subdivided the 28 cosmopolitan species that were not on Forel's (1911) list into two categories based on a fairly crude evaluation of their degree of geographic spread. Widespread cosmopolitan species (Table 2) have multiple well-established populations in at least five of the world's biogeographic regions: Cardiocondyla mauritanica Forel, 1890, Cardiocondyla minutior Forel, 1899, Cardiocondyla obscurior Wheeler, 1929, Cardiocondyla wroughtonii (Forel, 1890), Hypoponera opaciceps (Mayr, 1887), Hypoponera punctatissima (Roger, 1859), Nylanderia bourbonica (Forel, 1886), Plagiolepis alluaudi Emery, 1894, Pseudoponera stigma (Fabricius, 1804), Strumigenys emmae (Emery, 1890), Strumigenys membranifera Emery, 1869, Strumigenys rogeri Emery, 1890, Technomyrmex difficilis Forel, 1892, and Wasmannia auropunctata (Roger, 1863).

Incipient cosmopolitan species (Table 3) have multiple well-established populations in fewer than five bioregions: Brachyponera chinensis (Emery, 1895), Cardiocondyla venustula Wheeler, 1908, Cerapachys biroi Forel, 1907, Hypoponera eduardi (Forel, 1894), Hypoponera ragusai (Emery, 1894), Leptogenys maxillosa (Smith, 1858), Myrmica rubra (Linnaeus, 1758), Nylanderia flavipes (Smith, 1874), Pheidole teneriffana Forel, 1893, Solenopsis invicta Buren, 1972, Strumigenys hexamera (Brown, 1958), Technomyrmex vitiensis Mann, 1921, Tetramorium insolens (Smith, 1861), and Tetramorium lucayanum Wheeler, 1905.

In addition to 42 cosmopolitan ant species, I listed 20 ant species with only minor spread of outdoor populations outside their native hemisphere: Cardiocondyla tjibodana Karavaiev, 1935, Formica lugubris Zetterstedt, 1838, Monomorium salomonis (Linnaeus, 1758), Monomorium subopacum (Smith, 1858), Myrmica specioides Bondroit, 1918, Nylanderia steinheili (Forel, 1893), Pheidole fervens Smith, 1858, Pheidole moerens Wheeler, 1908, Pseudomyrmex gracilis (Fabricius, 1804), Solenopsis globularia (Smith, 1858), Strumigenys silvestrii Emery, 1906, Syllophopsis sechellensis (Emery, 1894), Tapinoma sessile (Say, 1836), Temnothorax longispinosus (Roger, 1863), Tetramorium pacificum Mayr, 1870, Tetramorium tonganum Mayr, 1870, Tetramorium tsushimae Emery, 1925, and Vollenhovia emeryi Wheeler, 1906 (Table 4). Some of these species appear to be spreading quickly, e.g., T. tsushimae (Reuther, 2009). Other species are known outside their native hemisphere from only a single outdoor site record: C. tjibodana (in Belize; Seifert, 2003), F. lugubris (in Quebec, Canada; Finnegan, 1975), M. subopacum (in Antigua; Wheeler, 1923), M. specioides (in Washington State; Jansen and Radchenko, 2009), N. steinheili (in Mauritius; Wheeler, 1922), P. fervens (in California; Martinez, 1996), Tapinoma sessile (in Hawaii; Buczkowski and Krushelnycky, 2012), T. longispinosus (in Spain; Espadaler and Collingwood, 2001), and T. tonganum (in Brazil; Fowler et al., 1994). In some cases, these single populations may be (or may have been) only temporary.

Discussion

My analyses identified 62 ant species with populations established outside their native range hemisphere. Based on their known world distribution, I classified 42 of these ant species as cosmopolitan, i.e., with multiple well-established outdoor populations outside their native hemisphere (Tables 1-3). Of these 42 cosmopolitan ant species, 35 (83%) are Old World natives and seven (17%) are New World natives. This pattern suggests that Old World species are more likely to be competitively dominant, possibly due to evolving in a more competitive environment. Overall, the highest

number of cosmopolitan ant species were originally native to the Indomalay bioregion (17 species; Tables 1-3) and the lowest number were native to the Nearctic bioregion (only one species; Table 2). Of the 20 ant species with only very minor spread of outdoor populations outside their native hemisphere (Table 4), thirteen (65%) are Old World natives and seven (35%) are New World natives.

Comparing the native ranges of Forel's (1911) cosmopolitans with those of the newer cosmopolitans suggests a shift in the main sources of cosmopolitans. For example, only one of Forel's (1911) 14 cosmopolitans (7%) is native to the Palearctic bioregion, despite the Palearctic having the greatest land area. In contrast, eight of the 28 new cosmopolitans (29%) are native to the Palearctic (Tables 1-3).

Of the 42 cosmopolitan ant species, I consider twelve to be major ecological, agricultural, and/or household pest species. Forel's (1911) list of 14 cosmopolitan ant species (Table 1) included eight of these major pest species (see Introduction). In contrast, my list of 28 additional cosmopolitan ant species (Tables 1 and 2) includes only four additional major pest species: Nylanderia bourbonica (Forel, 1886), Solenopsis invicta Buren, 1972, Technomyrmex difficilis Forel, 1892, and Wasmannia auropunctata (Roger, 1863). The other 24 new cosmopolitans are at most relatively minor pests. Thus, although the new list of cosmopolitan ants is much longer than that of Forel (1911), few of the additions have developed into major pests.

My classification of what constitutes a major pest, however, is not based on quantitative criteria, but instead is largely subjective, based on my experience with the different species and the papers I have read about their impact. All twelve of these major pest species commonly attain high local densities where they can have measurable ecological and/or economic impacts. There are certainly valid arguments for including other ant species on this list. For example, T. bicarinatum can sometimes be a serious agricultural pest and B. chinensis and M. rubra both have painful stings; all three of these species can attain high densities in some places.

In a related problem of categorization, Sarah Lowe, a staff member of the Invasive Species Specialist Group (ISSG) of the International Union for Conservation of Nature (IUCN), asked me in 1997 at a scientific meeting in Suva, Fiji, what I thought were the most harmful exotic ants, for inclusion on a list of 100 of the worst invasive species in the world. I was skeptical about the validity of comparing the relative impact of different ant species, much less comparing the impact of invasive ants with that of invasive mammals, fish, trees, etc. Some ants are enormous agricultural pests, others have a great impact on native species, while still others are important household pests. But Lowe assured me that this publication was a "publicity booklet," simply meant to call attention to exotic species and to IUCN's newly created Global Invasive Species Database. She emphasized this was not to be a list of the 100 worst, but instead 100 examples of harmful invasive species. I suggested six ant species based on my experience at the time: A. gracilipes, L. humile, P. megacephala, S. geminata, S. invicta, and W. auropunctata. In the publication, Lowe et al. (2000) dropped S. geminata because they did not want two species of the same genus. While I would certainly rank S. geminata ahead of A. gracilipes, Lowe et al. (2000) explicitly stated: "Absence from the list does not imply that a species poses a lesser threat." This distinction, however, seems to have been lost in the many dozens of papers that claim species x is one of the 100 worst exotic species, inappropriately citing Lowe et al. (2000) as if this paper were an authoritative primary source founded on definitive research, rather than being a collection of illustrative examples selected based on subjective impressions.

Another problem with categorization is exemplified in a paper by McGlynn (1999), which included a list of 147 ant species that have been “recorded outside their native habitat.” This paper has been a popular source reference regarding exotic ants, cited in the mistaken belief that it represented a comprehensive list of ant species with established exotic populations. McGlynn's (1999) list, however, included a great many ant species that have no known outdoors populations established in locales beyond their native range, e.g., numerous species that Nishida (1994, 2002) listed as intercepted by quarantine

on goods imported into Hawaii, but without any established populations (e.g., Camponotus exiguoguttatus Forel, 1886, Camponotus itoi Forel, 1912, Camponotus obscuripes Mayr, 1879, Crematogaster lineolata (Say, 1836), Formica subpolita Mayr, 1886, Lasius interjectus Mayr, 1866, Pheidole barbata Wheeler, 1908, Pheidole hyatti Emery, 1895, Pheidole noda Smith, 1874, Pheidole punctatissima Mayr, 1870, Carebara affinis (Jerdon, 1851), Polyrhachis argentea Mayr, 1862, Polyrhachis dives Smith, 1857, Polyrhachis femorata Smith, 1858, Ponera coarctata (Latreille, 1802), Prenolepis imparis (Say, 1836) and Prenolepis melanogaster Emery, 1893). In addition, some species on McGlynn's (1999) list have only exotic indoor populations (e.g., Camponotus atriceps (Smith, 1858), Dolichoderus thoracicus (Smith, 1860), Linepithema iniquum (Mayr, 1870), Carebara diversus (Jerdon, 1851) and Solenopsis texana Emery, 1895), and a few were based on site error (e.g., Anoplolepis custodiens (Smith, 1858), Dolichoderus quadripunctatus (Linnaeus, 1771), Gnamptogenys porcata (Emery, 1896), Odontomachus simillimus Smith, 1858 etc.) (Wetterer 2005, 2014c) or identification error (e.g., Cardiocondyla nuda (Mayr, 1866), N. vividula (Nylander, 1846), Pheidole variabilis Mayr, 1876, Brachyponera obscurans (Walker, 1859) etc.) (Seifert 2003). Finally, some species listed actually appear to be native throughout their known range (e.g., Hypoponera elliptica (Forel, 1900), Lasius turcicus Santschi, 1921) (Taylor, 1987;Seifert, 1992).

A complete list of all ant species that have ever been "recorded outside their native habitat" would be much greater than the 147 listed by McGlynn (1999), especially if the list included species simply intercepted in transit. For example, Suarez et al. (2005) listed 232 ant species intercepted by quarantine inspectors in the US. However, Suarez et al. (2005) found that only 28 of these "now occur as established nonnative species in the continental United States, and three species can be considered invasive." I believe that distinguishing these different categories is of vital importance.

Future research plans

I have authored or co-authored papers, reviewing, one species at a time, the known geographic distributions of most cosmopolitan ant species whose taxonomy are well established (Tables 1-3). I am working to review the rest, when the taxonomy can be properly ascertained and specimens with uncertain identities re-examined, often in collaboration with one or more taxonomic experts (e.g., T. simillimum with F. Hita-Garcia). My present list of cosmopolitan ant species is almost certainly incomplete. Taxonomic revisions will probably identify additional cosmopolitan ant species, e.g., one or more Brachymyrmex species appear to be widespread cosmopolitans. Unfortunately, the taxonomy of Brachymyrmex remains very confused.

I am also turning my attention to ant species reported to have exotic populations only within their own native hemisphere. Some of these species have large geographic gaps between their presumed native and exotic populations, e.g., Gnamptogenys triangularis (Mayr, 1887) (MacGown and Wetterer, 2012a) and Pheidole obscurithorax Naves, 1985 (Naves, 1985), so the limits of the known native and exotic ranges can be discerned. For many species, however, distinguishing where the native range ends and the exotic range begins is difficult, e.g., Brachyponera sennaarensis (Mayr, 1862) (Wetterer 2013a) and Strumigenys margaritae Forel, 1893 (MacGown and Wetterer, 2013).

In some cases, species that have been reported as exotic are actually native throughout their known range. Wittenborn and Jeschke (2011) wished to compare characteristics of native versus exotic ant species in North America, but appear to have misclassified numerous ant species as exotics, that are actually native to North America, such as Leptogenys manni (Wheeler, 1923), a species endemic to Florida (Trager and Johnson, 1988).

For example, Wittenborn and Jeschke (2011) considered Gnamptogenys hartmani (Wheeler, 1915), Labidus coecus (Latreille, 1802), Pachycondyla harpax (Fabricius, 1804), and Trachymyrmex jamaicensis (André, 1893) as exotic to North America, but all four have distributions in the southern US that are simply the northern end of continuous native ranges and give no indication that these species are exotic to

Table 1. Forel's (1911) fourteen cosmopolitan ant species. x-date = year first found outside native hemisphere. Bioregions: Af = Afrotropic, Pa = Palearctic, In = Indomalay, Au = Australasia, Oc = Oceania, Na = Nearctic, and Nt = Neotropic. Presumed native range in caps and bold and rest shows presumed exotic range

Species name	Worldwide range							x-date	Major exotic reference
Cardiocondyla emeryi	**AF**	Pa	In	Au	Oc	Na	Nt	1878	Wetterer, 2012d
Pheidole megacephala	**AF**	Pa	In	Au	Oc	Na	Nt	1858	Wetterer, 2012e
Tetramorium caldarium	**AF**	Pa	In	Au	Oc	Na	Nt	1908	Wetterer and Hita Garcia, 2015
Tetramorium simillimum	**AF**	Pa	In	Au	Oc	Na	Nt	1868	Bolton, 1980
Trichomyrmex destructor	Af	**PA**	In	Au	Oc	Na	Nt	1893	Wetterer, 2009b
Monomorium floricola	Af	Pa	**IN**	Au	Oc	Na	Nt	1863	Wetterer, 2010a
Monomorium pharaonis	Af	Pa	**IN**	Au	Oc	Na	Nt	1864	Wetterer, 2010c
Paratrechina longicornis	Af	Pa	**IN**	Au	Oc	Na	Nt	1859	Wetterer, 2008
Tapinoma melanocephalum	Af	Pa	**IN**	Au	Oc	Na	Nt	1793	Wetterer, 2009a
Tetramorium lanuginosum	Af	Pa	**IN**	Au	Oc	Na	Nt	1912	Wetterer, 2010b
Anoplolepis gracilipes	Af	---	**IN**	Au	Oc	Na	Nt	1859	Wetterer, 2005
Tetramorium bicarinatum	Af	Pa	**IN**	**AU**	Oc	Na	Nt	1850	Wetterer, 2009c
Linepithema humile	Af	Pa	In	Au	Oc	Na	**NT**	1858	Wetterer, et al., 2009
Solenopsis geminata	Af	Pa	In	Au	Oc	Na	**NT**	1851	Wetterer, 2011a

Table 2. Fourteen additional widespread cosmopolitan ant species with substantial geographic spread outside their native hemisphere. Symbols and abbreviations as in Table 1.

Species name	Worldwide range							x-date	Major exotic reference
Strumigenys membranifera	**AF**	Pa	In	Au	Oc	Na	Nt	1890	Wetterer, 2011b
Strumigenys rogeri	**AF**	Pa	In	Au	Oc	Na	Nt	1862	Wetterer, 2012a
Plagiolepis alluaudi	**AF**	Pa	In	Au	Oc	Na	Nt	1928	Wetterer, 2014a
Technomyrmex difficilis	**AF**	----	In	Au	Oc	Na	Nt	1986	Wetterer, 2013b
Hypoponera punctatissima	**AF**	**PA**	In	Au	Oc	Na	Nt	1892	Bolton and Fisher, 2011
Cardiocondyla mauritanica	Af	**PA**	In	Au	----	Na	Nt	1967	Wetterer, 2012f
Nylanderia bourbonica	Af	Pa	**IN**	Au	Oc	Na	Nt	1924	Deyrup et al., 2000
Cardiocondyla minutior	Af	----	**IN**	Au	Oc	Na	Nt	1924	Wetterer, 2014b
Cardiocondyla wroughtonii	Af	Pa	**IN**	Au	Oc	Na	Nt	1939	Seifert, 2003
Cardiocondyla obscurior	Af	Pa	**IN**	----	Oc	Na	Nt	1982	Seifert, 2003
Strumigenys emmae	Af	----	In	**AU**	Oc	----	Nt	1890	Wetterer, 2012c
Hypoponera opaciceps	----	Pa	In	Au	Oc	**NA**	Nt	1892	Wilson and Taylor, 1967
Pseudoponera stigma	----	----	In	Au	Oc	Na	**NT**	1858	Wetterer, 2012b
Wasmannia auropunctata	Af	Pa	----	Au	Oc	Na	**NT**	1893	Wetterer, 2013d

Table 3. Fourteen incipient cosmopolitan ant species with several well-established outdoor populations outside their native hemisphere. Symbols and abbreviations as in Table 1.

Species name	Worldwide range							x-date	Major exotic reference
Cardiocondyla venustula	**AF**	----	In	----	Oc	Na	Nt	1906	Seifert, 2003
Leptogenys maxillosa	**AF**	Pa	In	----	----	----	Nt	1861	Roger, 1861
Pheidole teneriffana	**AF**	**PA**	In	----	----	Na	Nt	1930	Wetterer, 2011e
Monomorium salomonis	Af	**PA**	----	----	----	----	Nt	1913	Wheeler and Mann, 1914
Myrmica rubra	----	**PA**	----	----	----	Na	----	1900	Wetterer and Radchenko, 2011
Hypoponera eduardi	Af	**PA**	----	Au	Oc	----	Nt	1914	Bolton and Fisher, 2011
Nylanderia flavipes	----	**PA**	**IN**	----	----	Na	----	1939	Wetterer, 2011c
Brachyponera chinensis	----	**PA**	**IN**	Au	----	Na	----	1932	Nelder et al., 2006
Tetramorium tsushimae	----	**PA**	**IN**	----	----	Na	----	1988	Reuther, 2009
Hypoponera ragusai	Af	Pa	**IN**	Au	Oc	----	Nt	1939	Bolton and Fisher, 2011
Cerapachys biroi	Af	----	**IN**	----	Oc	----	Nt	1930	Wetterer et al., 2012
Technomyrmex vitiensis	Af	Pa	**IN**	**AU**	**OC**	Na	Nt	1987	Bolton, 2007
Solenopsis invicta	----	----	In	Au	----	Na	**NT**	2001	Wetterer, 2013c
Solenopsis globularia	Af	----	----	----	Oc	----	**NT**	1958	Wetterer et al., 2007

Table 4. Twenty ant species with a small number of outdoor populations outside their native hemisphere. Symbols and abbreviations as in Table 1.

Species name	Worldwide range							x-date	Major exotic reference
Tetramorium lucayanum	**AF**	Pa	----	----	----	----	Nt	1904	Wetterer, 2011d
Monomorium subopacum	Af	**PA**	In	----	----	----	Nt	1920	Wheeler, 1923
Formica lugubris	----	**PA**	----	----	----	----	Nt	1973	Finnegan, 1975
Myrmica specioides	----	**PA**	----	----	----	Na	----	<2007	Jansen and Radchenko, 2009
Myrmica scabrinodis	----	**PA**	----	----	----	Na	----	2009	Clark et al., 2011
Vollenhovia emeryi	----	**PA**	**IN**	----	----	Na	----	1986	Wetterer et al., 2015
Strumigenys hexamera	----	**PA**	**IN**	----	----	Na	----	1987	MacGown and Wetterer, 2012b
Cardiocondyla tjibodana	----	**PA**	**IN**	**AU**	----	----	Nt	1997	Seifert, 2003
Pheidole fervens	----	**PA**	**IN**	**AU**	**OC**	Na	----	1995	Martinez, 1996
Tetramorium pacificum	Af	----	**IN**	**AU**	**OC**	Na	----	1950	Creighton, 1950
Tetramorium tonganum	----	Pa	**IN**	**AU**	**OC**	----	Nt	<1994	Fowler et al., 1994
Syllophopsis sechellensis	Af	----	**IN**	**AU**	Oc	----	Nt	2003	Wetterer, in prep.
Tetramorium insolens	Af	Pa	**IN**	**AU**	**OC**	Na	----	1979	Bolton, 1979
Temnothorax longispinosus	----	Pa	----	----	----	**NA**	----	1923	Espadaler and Collingwood, 2001
Tapinoma sessile	----	----	----	----	Oc	**NA**	----	2009	Buczkowski and Krushelnycky, 2012
Pseudomyrmex gracilis	----	Pa	----	----	Oc	**NA**	**NT**	1912	Wetterer, 2010d
Strumigenys silvestrii	----	Pa	In	----	----	Na	**NT**	2001	MacGown et al., 2013
Nylanderia steinheili	Af	----	----	----	----	----	**NT**	1908	Wheeler, 1922
Pheidole moerens	----	----	----	----	Oc	Na	**NT**	2000	Wilson, 2003
Cyphomyrmex minutus	Af	----	----	----	----	----	**NT**	2011	B. Fisher (pers. comm.)

North America (Wetterer 2014c, Wetterer and Snelling 2015).

I eventually plan to make a comprehensive analysis of all ant species with well-established exotic populations, including those that have not spread beyond their native hemisphere. This, however, will take much additional effort in compiling and evaluating specimen records.

More than 100 years ago, Forel (1911) compiled a list of cosmopolitan ants, calling attention to this important group of invasive species, spread around the world by human commerce. Forel (1911) identified most of what remain the dominant tramp ant species today. I hope that my present compilation will prove to be a useful extension of Forel's (1911) prescient work.

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Description of a new species of Alastor (Alastor) Lepeletier, 1841 (Hymenoptera: Vespidae: Eumeninae) from Telangana, India, with a key and a checklist of Oriental species

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Abstract

A new species, Alastor (Alastor) venkataramani sp. n. is described from Telangana, India, and compared with its most similar described species, A. punjabensis Dutt. A key to and checklist of the species of Alastor (Alastor) from Oriental region are provided.

Keywords: Alastor (Alastor), new species, key, checklist, Telangana, India.

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Introduction

Lepeletier (1841) described the genus Alastor for two species; the type species Alastor atropos Lepeletier, 1841, was designated by Ashmead (1902). This large genus is primarily Ethiopian and Palaearctic, with a few species in the Oriental Region (India and Sri Lanka). This genus is divided into four subgenera namely Alastor s. str., Alastorellus Giordani Soika, 1991, Megalastor Blüthgen, 1951, and Parastalor Blüthgen, 1939. Of these the subgenus Alastor is recorded from the Oriental Region with four species; one from India, one from Sri Lanka, and the other two species from both India and Sri Lanka (Bingham, 1897; Dutt, 1922; van der Vecht, 1981). In this paper a new species, namely Alastor (Alastor) venkataramani sp. n., is described from Telangana, India, and compared with its most similar described species, A. punjabensis Dutt. A key to and checklist of the species of Alastor (Alastor) from the Oriental region are also provided.

Material and Methods

The specimens were studied and photographed using a Leica stereo microscope with LAS software version 3.6.0. The holotype of the new species described here is deposited in the National Zoological Collections of the Zoological Survey of India, Kolkata(NZC).

Abbreviations used in the text: F = Antennal flagellomeres; H = Head; M = Mesosoma; OOL = Ocellocular distance; POL = Post ocellar distance; S = Metasomal sterna; T = Metasomal terga.

Results

Alastor (Alastor) venkataramani Kumar and Carpenter sp. n. (Figs. 1-12)

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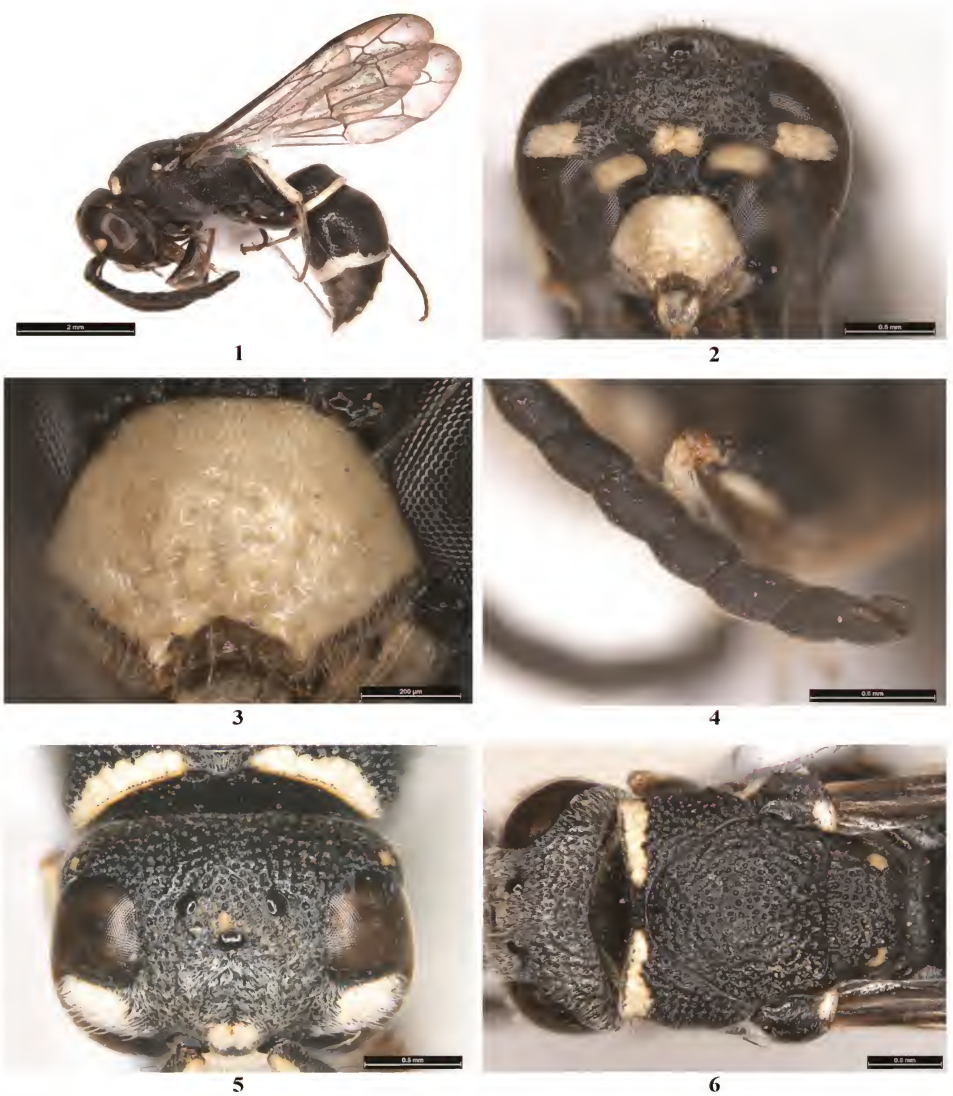
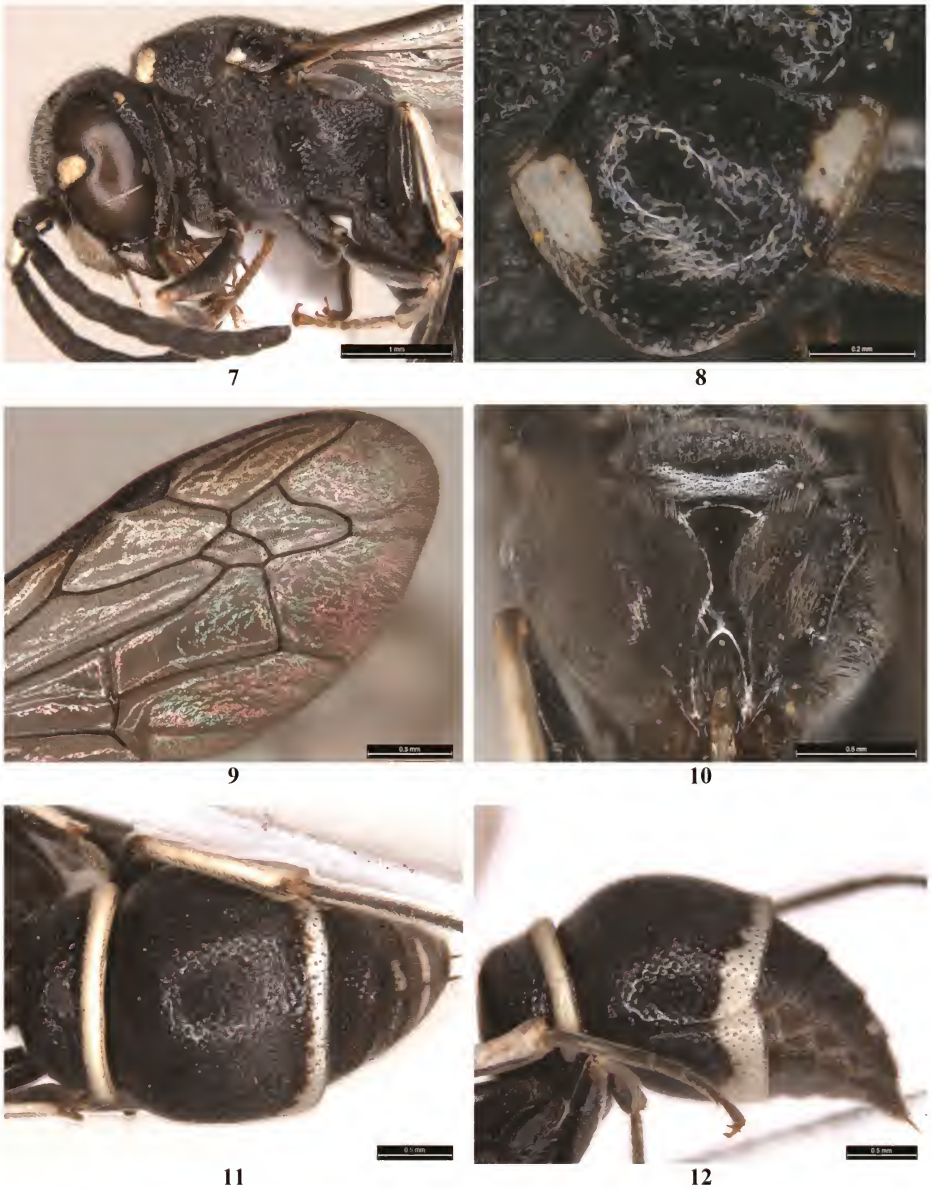
Description: Holotype male (Fig. 1): Body length (H+M+T1+T2) 5.5 mm; Forewing length 4.7 mm. Body black with whitish yellow markings as follows: a large mark on mandible; clypeus; a spot on lower front; ocular sinus; a very small spot on upper part of temple near eye margin; ventral side of scape; a medially interrupted transverse band on pronotum; anterior and posterior apices of tegula; two small spots on scutellum; a small spot on fore and mid femora in apical half; outer surface of all tibiae; a linear mark on outer surface of basitarsus of all legs; a regular band at apex of T1; an irregular band at apex of T2 expanded laterally; a similar but broader band on S2; traces of apical bands on

Plate I



Figs. 1–6. ***Alastor (Alastor) venkataramani*** sp. nov. Holotype male. (1). Body profile; (2). Head frontal view; (3). Clypeus; (4). Apical antennal articles; (5). Head dorsal view; (6). Head and mesosoma dorsal view.

Plate II



Figs. 7–12. ***Alastor (Alastor) venkataramani* sp. nov.** Holotype male. (7). Head and mesosoma lateral view; (8). Tegula; (9). Apical half of forewing; (10). Propodeum; (11). Metasoma dorsal view; (12). Metasoma lateral view.

T4 and T5 medially. All tarsal segments blackish brown except linear yellow marks on basitarsus of all legs. Wings slightly infumated; stigma blackish brown; veins brown. Body with rather sparsely to moderately dense fine silvery white pubescence, with some long hairs (length slightly longer than the diameter of anterior ocellus) on head and mesosoma.

Head: 1.16x as wide as long in front view (Fig. 2); clypeus (Fig. 3) convex, the apex with two acute teeth, the area in between them deeply incised, the distance between teeth 1.17x the distance between anterior tentorial pits, maximum width of clypeus 1.37x its length medially, with coarse punctures medially; frons, vertex and temple closely, strongly and uniformly punctured, diameter of punctures greater than the distance between punctures; POL 1.70x OOL (Fig. 5); diameter of anterior ocellus 0.77x as long as the distance between anterior ocellus and posterior ocelli; temple 0.62x as wide as eye in profile (measured through its ocular sinus) (Fig. 7); minimum interocular distance 1.53x as long as on vertex than at clypeus; occipital carina complete and narrowed ventrally at the margin of temple, almost absent on the margin of vertex. Antenna (Fig. 4) with scape 1.56x as long as F1; F1 1.10x as long as F2, 1.94x as long as wide; F2–F9 each broadened at middle and narrowed at both ends; apical antennal article finger-like, its apex not reaching the base of F9.

Mesosoma (Fig. 6): Anterior face of pronotum smooth with minute scattered punctures; pronotal carina strong except at middle depressed and absent, present on the lateral margin of pronotum; humeral angles of pronotum moderately projecting; posterior face and lateral sides of pronotum, mesoscutum and scutellum strongly and closely punctate, diameter of punctures greater than the distance between punctures; median length of mesoscutum 0.93x its maximum width; scutellum without distinct median longitudinal impression, its posterior apex smooth; metanotum bifaced, horizontal and 1/3 of vertical surfaces strongly punctured as that of scutellum; mesopleuron closely punctured except large area of epicnemium and posterior margin smooth; epicnemial carina not distinct; upper metapleuron with few strong punctures and strong transverse striae in upper half, lower metapleuron with a few weak punctures at lower half. Propodeum (Fig. 10) vertical, medially concave, smooth except lateral sides with punctures; sides of propodeum rounded in front of the apical tooth, which is distinctly curved upwards. Tegula (Fig. 8) with sparse but strong punctures; parategula absent; axillary fossa oval, not slit-like. Midtibia without spur. Forewing (Fig. 9) with pterostigma 1.89x prestigma, second submarginal cell petiolate, first and second recurrent veins both received in submarginal cell II.

Metasoma (Figs. 11 and 12): T1 without transverse carina but bifaced with smooth basal vertical half and punctured dorsal half, punctures of dorsal area of T1 weak but large, transverse yellow apical band of T1 smooth, maximum width of dorsal surface of T1 2.5x its median length in dorsal view; T1 0.84x as wide as T2; T2 0.91x as long as wide in dorsal view, with a narrow apical lamellae; T2 and S2 with distinct but weak and small punctures.
Female: Unknown.

Material examined: Holotype ♂, INDIA: Telangana, Adilabad district, Tarnam, 9.ix.2013, Coll. D. Prabhakaran and Party, NZC Regd. No. 16556/H3.

Distribution: India: Telangana.

Etymology: The species is named after Dr. K. Venkataraman, Director, Zoological Survey of India for his keen interest and encouragement in our studies.

Discussion

This new species comes close to Alastor (Alastor) punjabensis Dutt, 1922, described from India (Punjab), in having the apex of clypeus deeply incised (cf. the key in van der Vecht, 1981). But it distinctly differs from A. punjabensis in having: (1) Median area of clypeus densely punctured (in the latter clypeus sparsely punctured); (2) Scutellum densely punctured without distinct median longitudinal impression on apical half (in the latter scutellum sparsely punctured with median longitudinal impression on apical half); (3) Tegula punctured (in the latter tegula smooth and shining).

Key to Oriental species of Alastor (Alastor) Lepeletier, 1841

(Modified from van der Vecht, 1981)

1. Apex of clypeus deeply incised. ……2
– Apex of clypeus truncate or shallowly emarginate. ….....…....3
2. Clypeus slightly punctured; scutellum sparsely punctured with median longitudinal impression on apical half; tegula smooth and shining.....…….. .....**A. punjabensis Dutt**
– Median area of clypeus densely punctured (Fig. 3); scutellum densely punctured without distinct median longitudinal impression on apical half (Fig. 6); tegula punctured (Fig. 8)..... .....**A. venkataramani sp. n.**
3. Sides of propodeum angularly projecting in front of the apical tooth, which is short and not distinctly curved upwards (van der Vecht, 1981: fig. 40); humeral angles of pronotum strongly projecting (van der Vecht, 1981: fig. 39); ocular sinus black. ……..... **A.abditus van der Vecht**
– Sides of propodeum rounded in front of the apical tooth, which is distinctly curved upwards (van der Vecht, 1981: fig. 49); humeral angles of pronotum not or hardly projecting (van der Vecht, 1981: figs. 32, 48); ocular sinus with yellow spot. …….. 4
4. Antenna rather slender, in dorsal view 3rd article longer than 4th (van der Vecht, 1981: fig. 30); apex of clypeus shallowly emarginate (van der Vecht, 1981: fig. 29); dorsal surface of T2 on each side with oblique shallow impression, outline in profile beyond the basal constriction almost straight (van der Vecht, 1981: fig. 35); mandible with yellow spot; anterior margin of pronotum with interrupted band; T1 and T2 with narrow apical yellow band, T3 without yellow mark. (♀ unknown). …….. .....**A.variolosus Bingham**
– Antenna thicker, in dorsal view 3rd and 4th articles about equally long; apex of clypeus shallowly emarginate; dorsal surface of T2 with transverse impression, which clearly visible in profile (van der Vecht, 1981: fig. 50); mandible without yellow spot; anterior margin of pronotum with transverse spot near humeral angle; T1 and T2 with broad apical yellow band, T3 with abbreviated band...…….....**A.sulcatus van der Vecht**

Checklist of the species of Alastor (Alastor) Lepeletier, 1841, from the Oriental Region

1. Alastor (Alastor) abditus van der Vecht, 1981 — India (Tamil Nadu; Kerala) (Gusenleitner, 2006); Sri Lanka (near the town of Anuradhapura; Suriyawewa of Hambantota district; Hunuwilagama of Anuradhapura district; China Bay of Trincomalee district).
2. Alastor (Alastor) punjabensis Dutt, 1922 — India (Punjab).
3. Alastor (Alastor) sulcatus van der Vecht, 1981 — India (Pondicherry); Sri Lanka (Tissamaharama of Hambantota district; Padaviya archeological site of Anuradhapura district; China Bay of Trincomalee district).
4. Alastor (Alastor) variolosus Bingham, 1897 — Sri Lanka (Trincomalee).
5. Alastor (Alastor) venkataramani sp. n. — India (Telangana).

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Reassessment of the adult Stevenia signata (Mik, 1966) (Diptera: Rhinophoridae) from Turkey with comprehensive notes on its morphology, ecology and faunistic limits

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Abstract

Stevenia signata (Mik, 1866) has been recorded for the first time from South Turkey. Herein, the sexual forms of the species are described in detail with notes on their faunistic and ecological data.

Key words: Diptera, Rhinophoridae, Stevenia signata, morphology, faunistic, South Turkey.

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Introduction

Rhinophoridae is a small family of calypterate two-winged flies, comprising of 174 species from the world (Pohjoismäki and Kahanpää, 2014) out of which 89 species are known from the Palaearctic region (Cerretti et al., 2014; Cerretti and Pape, 2009; Crosskey, 1977; Herting, 1993; Pape, 1998; Verves, 2005a, b; Zeegers, 2011). The larvae of this group are internal parasitoids of woodlice whereas imagoes feed on the flowering plants (Bedding, 1973; Bürgis, 1991a, b; Bürgis, 1992a, b; Verves and Khrokalo, 2006; Wijnhoven, 2001).

Genus Stevenia Robineau-Desvoidy, 1830 consists of 25 species from Palaearctic, Oriental and Afrotropical regions (Cerretti et al., 2014; Cerretti and Pape, 2007; Crosskey, 1977; Herting, 1961, 1993; Peris and González-Mora, 2007; Zeegers, 2008). 6 species are known from several districts of Turkey (Koçak, 2014; Koçak and Kemal, 2015): S. angustifrons Villeneuve, 1912; S. atramentaria (Meigen, 1824); S. hertingi Kugler, 1978; S. kugleri Herting, 1961; S. pallidicornis (Loew, 1847); S. signata (Mik, 1866). The present species, S. signata has been recorded for the first time from South Turkey. The current paper deals with the detailed description of the sexual forms of the above mentioned species along with notes on its ecology and zoogeographic distribution.

Materials and Methods

The flies were collected with an entomological net at an altitude of about 100m a.s.l. All flies were examined and photographs were prepared using a stereomicroscope Leica M205C (Leica Microsystems, Wetzlar, Germany) with a Canon EOS 5D Mark II Body camera (Canon Inc., Tokyo, Japan). As the mount was essentially not flat, a series of photographs were taken at different focal depths. They were compiled into one sharp image, in accordance with the criterion of maximal power in the spatial high-frequency domain, with the software Helicon Focus Pro 5.3.14 X64 (Helicon Soft Ltd., Kharkiv, Ukraine).

All specimens are deposited in collection of Department of Ecological Monitoring, Institute for Evolutionary Ecology, National Academy of Sciences of Ukraine, Kyiv.

New Record

Stevenia signata (Mik, 1866)

(Figs 1-7)

Rhinophora signata Mik, 1866: 307 (description of ♂). Type locality: Italy: Mt Czavn nr Görz [= Gorizia] (by original designation).

Stevenia signata: Bürgis, 1991a: 295; Bürgis, 1992a: 50; Bürgis, 1992b: 100 (hosts); Cerretti and Pape, 2007: 37 (male

Figures: 1-7. Stevenia signata (Mik, 1866). 1-2. Male head in profile and frontal view; 3. Male left wing, dorsal view; 4-5. Male abdomen, dorsal view (two different specimens); 6. Male terminalia lateral view; 7. Female abdomen dorsal view.

abdomen dorsally and mid femur posteriorly figured), 39 (in key); Herting, 1961: 26 (short descriptions of ♂♀ and faunistic); Khitzova, 1981: 4 (faunistic; male cerci and surstyli dorsally figured); Koçak, 2014: 347; Koçak and Kemal, 2015: 342 (faunistic); Mihályi, 1980: 340 (faunistic); Peris and González-Mora, 2007: 53 (in key).

Stevenia femoralis (misidentification, not Stevenia femoralis Rondani, 1862): Stein, 1924: 188 (faunistic); Villeneuve, 1931: 66 (taxonomical notes).

Material Examined: TURKEY: 6 ♂♂ 4 ♀♀, Antalya Province, Side City, sand waste plot, 36°46′05″N, 31°23′24″E, 10-19.08.2011, Yu. Verves.

Distribution

Albania (Mihályi, 1980); Croatia (Bürgis, 1992a, b); Greece: mainland and Korfu I. (Herting, 1961); Italy: mainland (Mik, 1866); Russia: Astrakhan (Khitzova, 1981); Turkey: Antalya (Koçak, 2014; Koçak and Kemal, 2015).

Redescription of male and female:

Colour: Dark coloured. Head black, slightly silvery-grey dusted; frontal stripe matt black, at vertex with fine pruinescence; ocellar triangle black; antennae black, apex of pedicel light yellowish-brown; palpi yellowish-brown. Occiput shining black, slightly grey pollinated. Thorax entirely black, with fine grey pollination; three matt black longitudinal stripes are distinct before suture only; they separated by a pair silvery gray spots near fore margin of thorax; fore spiracles black, hind ones brownish-black. Legs black. Wings distinctly infuscated in fore part, especially along veins; basicosta and epaulette yellow, tegula black, veins brownish black. Colour of abdomen variable: from entirely shining-black (Fig. 5) to mainly brownish-yellow except black base of 1+2nd tergite, narrow longitudinal median stripes of 1+2nd and 3rd tergites, triangular median spot and fuscous hind border of 4th tergite and completely shining black 5th tergite (Fig. 7); sometimes 3rd tergite with brownish-yellow lateral spots in fore 0.5-0.8 (Fig. 4). All abdominal sternites shining black (♂) or dark brown to black (♀). Epandrium and female terminalia shining brownish-black.

Head (Figs 1, 2): Frons of both sexes at vertex 0.29-0.32x, at level of antennal base 0.38-0.40x of head-width, at its narrowest point about 0.6-0.8 times as wide as an eye in dorsal view. Frontal stripe almost parallel-sided, slightly widened anteriorly, at level of proclinate orb 0.9-1.3x as wide as one parafrontal, covered with very short microscopic setae, almost bare; second aristomere about 1.4-1.5x as long as wide. Parafacial, in profile, at level of antennal base 0.17-0.20x, genae 0.28-0.35x as high as compound eye; parafacial distinctly widened than postpedicel. Face with distinct narrow median longitudinal carina separating antennae. Vibrissal angle laterally distinctly placed in front of antertior margin of eye. Palpi short, at apex slightly clavate. Ocellar setae 1+2, strong and reclinate; vti very long and strong, vte absent; fr 6-11, middle long and strong; orb 1+1, slightly longer than fr; fronto-orbital plate and upper part of parafacial with microscopic setae; hind part of parafacial with a row of 2-3 elongate and several short setae along eye border. The upper part of facial ridge bare, its lower with several strong bristles, among them a pair of the longest angular vi present. Genae and occiput with erect black hairs; several pairs of black oral bristles well developed.

Thorax: Covered with short black hairs, bristles well developed; prosternum and proepisternum bare. Humeral callus with three strong setae; acr 1-3+1, only prescutellar pair distinctly developed; dc 2+3; ia 1+2; npl 2; kepst 2+1; propleuron bare. Scutellum with long, strong parallel apical and lateral bristles, short and fine basal setae; other setae hair-like. Subscutellum distinctly shortened than scutellum.

Wings (Fig. 3): Costal spine very long, about 2.0-2.5 times as long as crossvein r-m. Costal vein (C) covered with distinct numerous spines from base to the middle of 5th section (CS5); second costal portion (CS2) with short setulae ventrally. Base of R4+5 with 3-5 black dorsal and 1-2 ventral setulae; the strongest setulae equals to costal spine in length. Section of M between crossveins r-m and dm-cu distinctly shorter than the section between dm-cu and

right-angled bend of M. Petiole of cell r4+5 0.5-0.9 times as long as fast straight post-angular portion of M; dm-cu distinctly s-liked curved.

Legs: All male tibiae ventrally with short dense spine-liked setae. Almost straight elongate claws distinctly longer than tarsomere 5; pulvilli narrow and elongate. Fore leg: tibia with 3-4 anterodorsal and one posterodorsal setae. Mid leg: male femur with posteroventral ctenidium; tibia with 3-4 long anterodorsal and posterodorsal, one posterior, and one anteroventral setae. Hind leg: tibia with 3-4 long anterodorsal and posterodorsal, and one anteroventral setae.

Abdomen: 1+2nd and 3rd tergites with paired elongate median marginal bristles; 4th and 5th tergites with rows of strong marginals; discal setae absent.

Male postabdomen (Fig. 6): 6th and 7th tergites distinctly separated by deep junction; cercus straight, surstylus 2x as long as cercus, in profile slightly curved ventrally, with small apical hook. Paraphallus with a pair of well sclerotised dark ventro-lateral plates; small acrophallus directed dorsally, membranous.

Body length: 6.5-8.0 mm.

Ecology: The larvae are the internal parasites (parasitoids) of woodlice (Isopoda) Armadillidium frontirostre Budde-Lund (Bürgis, 1992b), A. vulgare (Latr.) (Bürgis, 1991a; Horstmann and Bürgis, 1991), Philoscia sp., Porcellio sp., Tracheoniscus sp. (Bürgis, 1991a, 1992a). The larvae of hymenopteran host Phygadeuon armadillidii Horstmann and Bürgis, 1991 (Hymenoptera: Ichneumonidae) are known as hyperparasites of pupae (Horstmann and Bürgis, 1991). Adult flies were collected at altitudes up to 4000 ft a. s. l. [about 1220 m] (Mihályi, 1980; Mik, 1866).

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Eucharitid wasp parasitoids in cocoons of the ponerine ant Diacamma scalpratum from Thailand

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Abstract

Different immature stages and adults of the new species Schizaspidia diacammae (Chalcidoidea: Eucharitidae) were found inside cocoons of Diacamma scalpratum (Formicidae: Ponerinae). Wasp larvae were feeding on ant pupae, while other host cocoons yielded five wasp pupae and both male and female adults. Parasitized cocoons are cut in a distinct manner by the wasps when they exit, and this feature can be used to assess the prevalence of parasitism. Dissection of the ovaries of one recently emerged physogastric female revealed thousands of eggs ready to be laid. These data are used to discuss the life history and reproductive strategy of this parasitoid wasp associated with Diacamma brood.

Keywords: Ponerinae, Eucharitidae, Schizaspidia diacammae, planidia, parasitism.

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Introduction

Ants are an insect group with very high biomass in most parts of the world, hence an abundant and stable potential food resource for other organisms, but surprisingly few parasitoids are known to attack ant brood. The life histories of the parasitoid wasps associated with ants are poorly understood (Pérez-Lachaud et al., 2012). Among 9 families of wasps parasitic on ants, only Eucharitidae (56 genera) specialize in attacking the immature stages of ants. Unlike most parasitoid wasps, eucharitid females deposit their eggs away from the host, in or on plant tissue (leaves and flower buds). The very active, minute, strongly sclerotized first-instar larvae ('planidia') are transported by foraging ants to their nest where they attach to ant larvae and development proceeds once the latter have spun a cocoon (Clausen, 1940; Heraty and Murray, 2013). The eucharitid parasitoids attacking Ponerinae, Ectatomminae, Myrmeciinae and Formicinae belong to a monophyletic derived lineage of Eucharitidae (Murray et al., 2013).

Diacamma belongs to the early branching ant subfamily Ponerinae, with all 20-30 species characterized by the presence of two minute appendages ('gemmae') on the mesosoma (thorax) of workers. The queen caste is absent and only one of the workers mates and reproduces (termed the 'gamergate') (e.g. André et al., 2001; Baratte et al., 2006; Cuvillier-Hot et al., 2002). Gemmae play a key role in the reproductive division of labour in Diacamma, and they are mutilated within hours of emergence (references above). Only the gamergate can retain her gemmae.

Within Ponerinae, Diacamma belongs to the Ponera genus group (consisting of Austroponera, Cryptopone, Ectomomyrmex, Emeryopone, Ponera and Pseudoponera; Schmidt and Shattuck, 2014). They are solitary hunters on a variety of small invertebrates. Diacamma scalpratum (F. Smith) has some of the largest workers in the genus, reaching 16-17mm in length. The species occurs in the hills of northern India, Sikkim, Assam, Myanmar and

northern Thailand (Bingham, 1903). Herein, we report on the first case of brood parasitism for this genus.

Materials and Methods

We excavated 4 complete colonies of D. scalpratum outside Thung Salaengluang NP (Phitsanulok province, northern Thailand, 16°34'32"N, 100°53'01"E) during September 2006. Ant colonies were plentiful and easy to find in a small patch of pine forest, and we selected nests that occurred away from the base of trees. Excavation continued to a depth of 90 cm in one colony. Several workers (including one gamergate) had a large mite on the dorsum of the mesosoma. Specimens of D. scalpratum workers examined in the British Museum (Natural History), London matched our samples, but proper taxonomic determination needs to be based on male genitalia (W.L. Brown Jr, pers. comm.), and a key is not yet available for Diacamma.

In the laboratory, colonies were maintained in plaster nests with a glass roof that allowed observations. Ants were fed with live mealworm pupae or small crickets. All workers were checked for the presence of gemmae, and half a dozen were dissected to assess ovarian activity and presence of sperm in the spermathecae. Cocoons were opened with forceps. Pupae were sexed whenever possible, and different developmental stages of a wasp parasite were collected.

Results

Four colonies of Diacamma scalpratum yielded 151±109 workers (mean number ±SD) and 69±38 cocoons (range 47-101). Only one worker had gemmae in each colony, and dissections confirmed that this was the gamergate (i.e. mated and egg-laying). All workers dissected had 16-20 ovarioles, and this number is diagnostic among closely related species (e.g. another species with large workers from Thailand has 8 ovarioles).

A proportion of field-collected cocoons eclosed in the laboratory and yielded workers. Other cocoons had been damaged during excavation and transport, and we cut them open to sex pupae. In addition to ant males and workers at various stages of pigmentation, we found different immature stages and two adults of the new species Schizaspidia diacammae (Chalcidoidea: Eucharitidae: Eucharitinae) (Heraty et al., 2015). Both second-instar and third-instar larvae were feeding on ant pupae (Fig. 1). One cocoon contained two wasp larvae attached to an ant pupa. Five wasp pupae and two adults (male and female) occurred singly in other host cocoons (Fig. 2). Adults (4.7–5.2mm long) are sexually dimorphic (Heraty et al., 2015). In total, we opened more than 50 cocoons of which 9 were parasitized, from 3 out of 4 ant colonies.

Diacamma ants routinely discard empty cocoons outside of the nest. In contrast to normal cocoons cut open midway by the ants, parasitised cocoons are cut at one extremity by exiting wasps, and could thus be recognized easily (Fig. 3); at least 3 of these were found. In the laboratory, one cocoon with a cut extremity was carried outside by the ants, and we observed a wasp walking out soon after. This female was strongly physogastric (Fig. 4a). Dissection revealed dozens of ovarioles with thousands of apparently mature (fully chorionated) eggs (Fig. 4b).

Discussion

This is the first record of eucharitid parasitoids in Diacamma ants. Adults and brood of Schizaspidia diacammae (Heraty et al., 2015) were found together in the same ant nests. Only rarely has more than one eucharitid species ever been found within a single ant colony (Pérez-Lachaud and Lachaud, pers. comm.), and we assume that wasp adults and immatures found in D. scalpratum nests all belong to the same species. Immature stages (eggs, first-instar exuvium, second and third-larval instars, pupa) of Schizaspidia diacammae are described in Heraty et al. (2015) and follow the general morphology of other eucharitids known to attack Ectatomminae and Ponerinae (Pérez-Lachaud et al., 2006).

The different developmental stages of S. diacammae extracted from Diacamma cocoons indicate that its life history is typical for Eucharitinae. Laboratory observations suggest that, in the field, Diacamma workers discard empty cocoons outside the nest, which may facilitate dispersal of the wasps from the nest. In

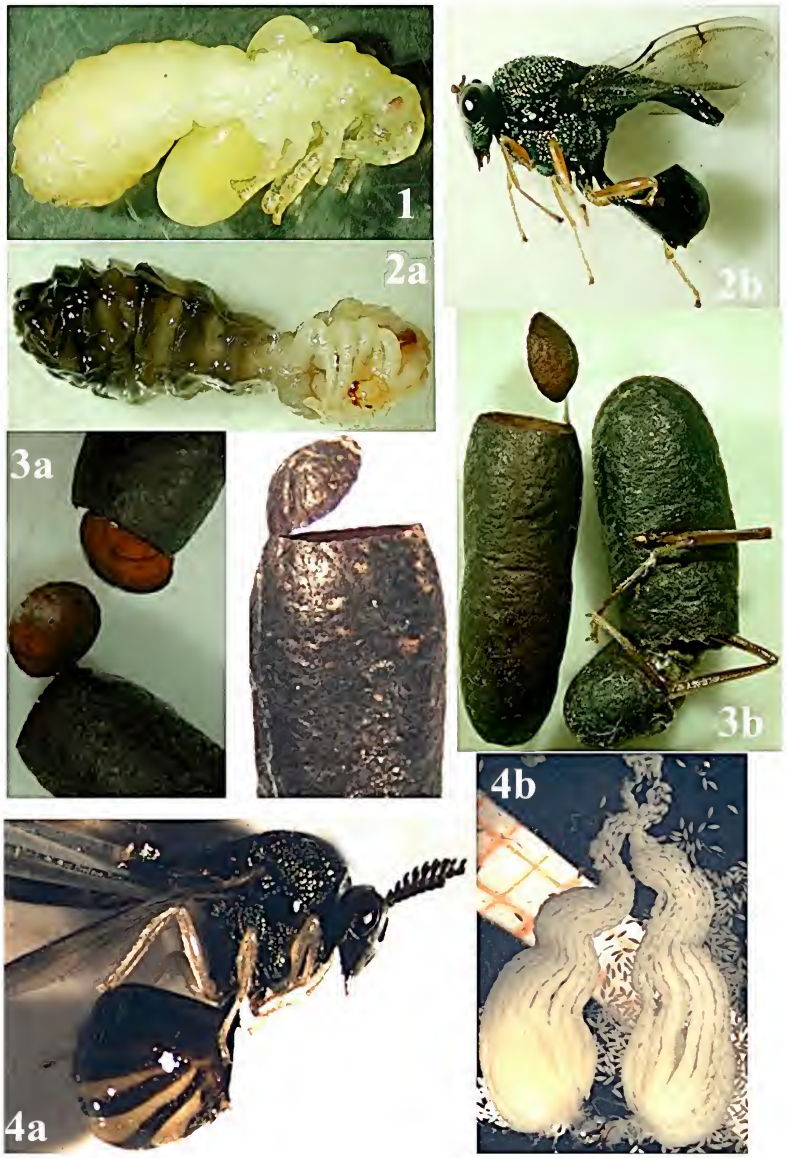

Fig. 1. Larva (second instar) of Schizaspidia diacammae feeding on the thorax of an ant pupa. The host cocoon has been removed. 2. Parasitized ant pupa (a) and adult male wasp (b) extracted from Diacamma cocoons. 3. Diacamma cocoons from which parasitoid wasps exited, showing the distinctive operculum cut at one extremity (a). In contrast, non-parasitized cocoons are cut midway by the ants (b, on right). 4. Physogastric female (a) of S. diacammae that exited from a Diacamma cocoon. Intersegmental membranes are exposed in dorsum of abdomen. Physogastry was confirmed by dissection (b) more than 10 ovarioles in each ovary, all packed with fully developed oocytes (scale: square = 1mm).

a similar case, a worker of Dinoponera lucida was observed to carry out a cocoon that was cut at one extremity (similar to Fig. 3), and a Kapala wasp exited from the cocoon in transit (Buys et al., 2010). This contrasts with reports that eucharitid adults exit cocoons inside the ant nests, and are then transported by workers (discussed in Buys et al., 2010).

Dissection of the gravid female of S. diacammae indicated that most eggs mature before dispersal (in contrast to oogenesis continuing throughout reproductive life). It would be advantageous for these parasitoids to complete oogenesis in the safety of the ant cocoon before dispersing outside the ant nest. At any rate, it is the parasitoids that cut cocoons open, and presumably they do this only once they are ready to disperse. This physogastric female seemed too heavy to fly, suggesting that she oviposits on a nearby host plant. We assume that males can disperse further, but it is not known where and when mating occurs. It is possible that female wasps lay their eggs on particular plant species. Unlike ectatommine ants, ponerine species are strict carnivores (Peeters, 1997), although Diacamma foragers may be attracted to sweet plant secretions. Torréns and Heraty (2012) reported that planidia have a propensity to jump, and they may attach to ant foragers walking under vegetation. A planidia of Schizaspidia nasua (attached to a larva of Odontomachus rixosus) is illustrated in Heraty et al. (2015).

Several Ponerinae have been recorded as hosts for Eucharitid (Eucharitinae) wasps (Pérez-Lachaud et al., 2006). The tribe Psilocharitini is linked with Hypoponera, while tribe Eucharitini is essentially parasitic on medium to large ponerines (Dinoponera, Neoponera, Odontomachus, Pachycondyla sensu stricto and Pseudoponera) and ectatommines (Ectatomma, Gnamptogenys, Typhlomyrmex and Rhytidoponera), but also myrmeciines (Myrmecia) and numerous formicines (Anoplolepis, Calomyrmex, Camponotus, Cataglyphis, Formica, Lasius and Polyrhachis). All verified host records for Eucharitinae involve ants that have a cocoon. There are no confirmed records with the naked pupae of myrmicine ants (Lachaud and Pérez- Lachaud, 2012). Another subfamily (Oraseminae) of Eucharitidae is restricted to myrmicine ants, mostly Pheidole, while Gollumiellinae attacks Nylanderia (a formicine that lost cocoons) (Murray et al., 2013). Thus, the ability to attack naked ant pupae appears ancestral within Eucharitidae, and the association with ant cocoons is derived (Heraty and Murray, 2013).

The genus Diacamma is conducive to the investigation of cocoon parasitoids because researchers open cocoons routinely: (i) newly emerged ant workers are quickly mutilated by nestmates, and workers with intact gemmae are more easily obtained from cocoons (e.g. pheromone or evo-devo studies); (ii) male genitalia are invaluable for taxonomy, and males are occasionally found in cocoons, after which they fly out. The first author of this paper has studied over 10 species of Diacamma throughout India, SE Asia and Australia, and has inspected thousands of cocoons, but this is the first discovery of a parasitoid. Five species of Diacamma are known from India (indicum, ceylonense, cyaneiventre, scalpratum, sculptum) (André et al., 2001; Cuvillier-Hot et al., 2002; Viginier et al., 2004), and future fieldwork may reveal more parasitoids.

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A new Anterhynchium species from Japan, with a key to the Northeast Asian species of the genus (Hymenoptera, Eumenidae)

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Abstract

A new species of the eumenid genus Anterhynchium is described from Japan. This new species, A. gibbifrons sp. n., was not noticed by Japanese hymenopterists until recently, but in these three years it has been found to be rather common in Fukui Prefecture, Honshu, Japan. A key to the Northeast Asian species of the genus Anterhynchium is presented, and discrimination of the four species is discussed.

Key words: Anterhynchium, new species, Japan, key to species, distribution.

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Introduction

The genus Anterhynchium Saussure, 1863 consists of three subgenera, i.e., Anterhynchium, Dirhynchium van der Vecht, 1963, and Epiodynerus Giordani Soika, 1958. The genus is distributed mainly in the Old World tropics (van der Vecht, 1963) and composed of 40 species world-wide (J. M. Carpenter, personal communication). All the forms found in Northeast Asia belong to the subgenus Dirhynchium (Yamane, 1990; Kim, 2014). Although Giordani Soika (1986) described a Japanese species, Anterhynchium kogimai, as belonging to Epiodynerus, this species actually belongs to another genus, Okinawepipona (Yamane, 1987). Up to now three species have been known for Anterhynchium in Northeast Asia, namely A. flavomarginatum (Smith, 1852), A. flavopunctatum (Smith, 1852), and A. melanopterum Yamane, 1981. A new species is herein described from Japan.

Materials and Methods

Collection data for the new species are given in 'Specimens examined'. For A. flavopunctatum one female identified by Dr. J. van der Vecht (Hangchow, China, 20.vii.1924, J. F. Illingworth leg.) and two males identified by Dr. A. Giordani Soika (Fukien, China, viii.1946, Tschung-Sen leg.) were examined.

The body length is given as a total length of the head, mesosoma, metasomal tergites 1 and 2, and roughly measured with naked eyes using an ordinary ruler. The head width and clypeal width (CW) and length (height) (CL) were measured with an ocular micrometer under a binocular microscope to the nearest second decimal. CW was measured excluding the 'wings of clypeus' sensu Richards (1962, pp. 10-11) or lateral lobe sensu Kim (2014, p. 32, Fig. 5C) (Fig. 14). CW/CL was calculated to show relative widths of the clypeus.

Collector names are abbreviated as follows: Hideyoshi Kurokawa (HK), Tadao Murota (TM), Tadashi Tano (TT).

Anterhynchium gibbifrons Yamane et Murota, sp. n.
(Figs. 1-4, 15, 18)

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Specimens examined. **Holotype**. ♀, Wada-chô, Sabae-shi, Fukui-ken, Honshu, Japan, 12.vii.2014, T. Murota (Entomological Collection, Hokkaido University, Japan).

Figs. 1-4. Anterhynchium gibbifrons sp. n. 1, 3. Female, Inzai-shi, Chiba-ken, Honshu; 2, 4. Male, Katsuyama-shi, Fukui Pref.

Paratypes. 3♀9♂, 12-17.vii.2014, same loc., T. Murota (Entomological Collection, Hokkaido University; Kyushu University Museum, Japan; Hyogo Prefectural Museum, Japan; Osaka City Museum of Natural History, Japan; J.-K. Kim Collection, Korea).

Non-type material. Chiba-ken, Honshu: 1♀, Matsumushi, Inzai-shi, 23.vi.2013, H. Suda (SY14001). Fukui-ken, Honshu: 3♂, Hamasaka, Awara-shi, 9.vii.2013, TM & HK; 11♂, same loc., 11.vii.2013, TM; 6♂, Kashizu, Echizen-chô, 18.vii.2014, TM; 4♂, Nozue, Echizen-chô, 17.vii.2014, TM; 5♂, Oimatsu, Echizen-chô, 18.vii.2014, TM; 3♂, Shimoyamanaka, Echizen-shi, 18.vii.2014, TM; 6♂, Tera, Echizen-chô, 17.vii.2014, TM; 2♂, same loc., 18.viii.2014, TM; 4♂, Tsuetate, Echizen-chô, 17.vii.2014, TM; 7♂, Uwado, Echizen-chô, 18.vii.2014, TM; 3♀, Asuwayama, Fuki-shi, 28.vii.2014, HK; 6♂, Futatsuya, Fukui-shi, 17-19.vii.2014, TM; 2♂, Ichinose chô, Fukui-shi, 20.vii.2014, TM; 1♂, Ichiôji chô, Fukui-shi, 19.vii.2014, HK; 2♀, Kamiikari-chô, Fukui-shi, 18.viii.2014, TM; 4♂, Kazao-chô, Fukui-shi, 19.vii.2014, TM; 11♂, Ohmori-chô, Fukui-shi, 17-18.vii.2014, TM & HK; 2♀2♂, same loc., 5.viii.2014, HK; 1♀8♂, Ohtani-chô, Fukui-shi, 20.vii.2014, TM; 1♀, same loc., 18.viii.2014, TM; 7♂, Sano, Fukui-shi, 16-19.vii.2014, HK; 6♂, Sanrihama, Fukui-shi, 11.vii.2013, TM; 1♂, same loc., 20.vii. 2013, HK; 2♂, Shimizubata-chô, Fukui-shi, 16.vii.2014, HK; 1♀4♂, same loc., 28.vii.2014, HK; 7♂, Shirakata-chô, Fukui-shi, 1.vii.2014, T. Murota; 3♀8♂, same loc., 8-12.vii.2014, T. Murota & H. Kurokawa; 1♀, same loc., 20.vii.2014, TM; 2♀, Higashino, Kitagô-chô, Katsuyama-shi, 10.viii.2014, TT; 12♂, Tani, Kitadani-chô, Katsuyama-shi, 20-21.vii.2013, TM & HK; 1♀1♂, Nigure, Ohno-shi, 26.vii.2014, TM; 6♂, Kitadani-chô, Katsuyama-

shi, 20.vii.2013, TM; 2♀, Mizuochi-chô, 22.vii. 2014, TM; 1♀, same loc., 28.vii.2014, TM; 1♀, 17.viii.2014, TM; 8♂, Wada-chô, Sabae-shi, 1.vii.2014, TM; 4♀19♂, same loc., 12-17.vii.2014, TM; 2♀, same loc., 3.viii.2014, TM; 1♀, same loc., 3.ix.2013, TM; 2♀, Yamagishi, Mikuni-chô, Sakai-shi, 9-12.vii.2014, TT; 10♂, Usogouchi Tsuruga-shi, 19.vii.2013, TM & HK; 1♀, same loc., 27.vii.2013, TT. Aichi-ken, Honshu: 1♂, Watagari-chô, Toyota-shi, 30.vi.2013, A. Kawazoe (5237-41-33). Ehime-ken, Shikoku: 1♀, Hoko-san, Hakata-jima, 25.vii.2010, S. Hashikoshi.

Diagnosis. Body including legs predominantly black with the following parts yellow: clypeus except in apical part, small median patch on lower frons, antennal scape below, medially interrupted line at anterodorsal margin of pronotum, metanotum, and regular apical bands on metasomal tergites 1 and 2 (♀). Mandible usually entirely black, sometimes slightly with reddish tinge (♀♂). Legs black with fore and mid-tibiae (often also hind tibia) yellow-marked. Clypeus almost as broad as long (CW/CL 1.02) (♀). Area between antennal insertions and ocelli strongly raised (♀). Cephalic foveae distinct, situated in a large transverse excavation (♀). With mesosoma in profile, erect hairs on mesonotum of uniform length, shorter than diameter of anterior ocellus (♀♂).

Female.

Measurements. Body length (head + mesosoma + metasomal tergites 1+2) (n=9): 14.5-17.0mm (mean(m)=15.9mm); head width (n=9): 4.23-4.93mm (m=4.65mm); clypeal width, CW (n=20): 1.84-2.24mm (m=2.07mm); clypeal length, CL (n=20): 0.90-0.98mm (m=0.94mm); CW/CL (n=20): 0.90-0.98mm (m=0.94mm).

Structure and sculpture. Clypeus slightly longer than broad; its anterior margin relatively broadly emarginate at middle, with blunt lateral teeth; clypeus entirely puncto-reticulate with a few irregular ridges running from its base toward apex. Frontal area surrounded by eyes, ocelli and antennal insertions distinctly raised. Cephalic foveal depression large, with anterior margin deeply emarginate; posterior margin very close to occipital margin. Antennal flagellum relatively long; seen from above, first to fourth flagellomeres longer than broad; others as long as or slightly shorter than broad. Mesoscutum puncto-reticulate; interpuncture ridges tending to run longitudinally; puncto-reticulation coarser on mesoscutellum than on mesoscutum. Dorsolateral part of propodeum anteriorly with distinct transverse rugae; lateral face of propodeum coarsely rugous, without isolated punctures. Punctures around basal area of dorsal face of metasomal tergite 1 much coarser than those in its posterior portion and those on tergite 2; lateral face of tergite 1 with very large punctures that are comparable to posterior ocellus in diameter.

Pilosity. Hairs on raised area of frons weakly curving, as long as or slightly longer than diameter of ocellus; those on vertex and temple suberect or appressed to surface. Clypeus with dense hairs that are slightly downward directed and much shorter than hairs on frons. Gena with very short hairs only. Venter of head with dense long erect hairs. With mesosoma in profile mesoscutum bearing very dense, relatively short hairs of equal length that are much shorter than those on frons; with mesosoma in dorsal view lateral face of pronotum and mesopleuron with short hairs that are at most as long as diameter of ocellus.

Colour pattern. Ground body colour black with the following parts yellowish (Figs. 1, 3): clypeus extensively (W-shaped black marking present in apical portion), triangular marking above frontal keel, minute spot on temple, underside of antennal scape, narrow band (often medially interrupted) on pronotum, parategula, narrow band (rarely interrupted medially) on metanotum (band absent in 25% of examined specimens), regular apical bands on metasomal tergites 1 and 2. Fore wing strongly infuscated; hind wing much paler.

Male.

Measurements. Body length (head + mesosoma + metasomal tergites 1+2) (n=20): 11.0-16.0mm (mean(m)=13.1mm); head width (n=20): 3.60-4.60mm (m=4.00mm); clypeal width, CW (n=70): 1.23-1.75mm (m=1.43mm); clypeal length, CL (n=70): 1.43-2.05mm (m=1.69mm); CW/CL (n=70): 0.81-0.89mm (m=0.84mm).

Structure, pilosity and colour pattern. Very similar to female, with ordinary sexual difference. Body size much more variable than

in female. Clypeus distinctly longer than broad. Frontal area in front of ocellar area more weakly raised than in female. Apical part of aedeagus broader than shaft that widens toward base, with apical margin entire or very indistinctly notched at middle. Erect hairs on frons longer than those of female; some of them longer than diameter of anterior ocellus. Erect hairs on mesoscutum of uniform length, much shorter than those on frons, much less than diameter of anterior ocellus. Colour pattern similar to that of female (Figs. 2, 4) with the following difference. Clypeus entirely yellow. Sinus of eye with short yellow line on lower margin. Yellow marking on metanotum more frequently lost or vestigial (in ca. 45% of examined specimens) than in female. Posterolateral corner of metasomal sternite 2 usually with yellow spot. Apical two to three flagellomeres often with yellowish to brownish areas. Upper faces of fore and mid-tibiae (also often of hind tibia) yellow; apical tarsomere often clear to dirty yellow.

Etymology. The specific epithet gibbifrons means 'inflated frons'.

Distribution. JAPAN. Honshu (Chiba Pref., Aichi Pref., Fukui Pref.) and Shikoku (Ehime Pref.).

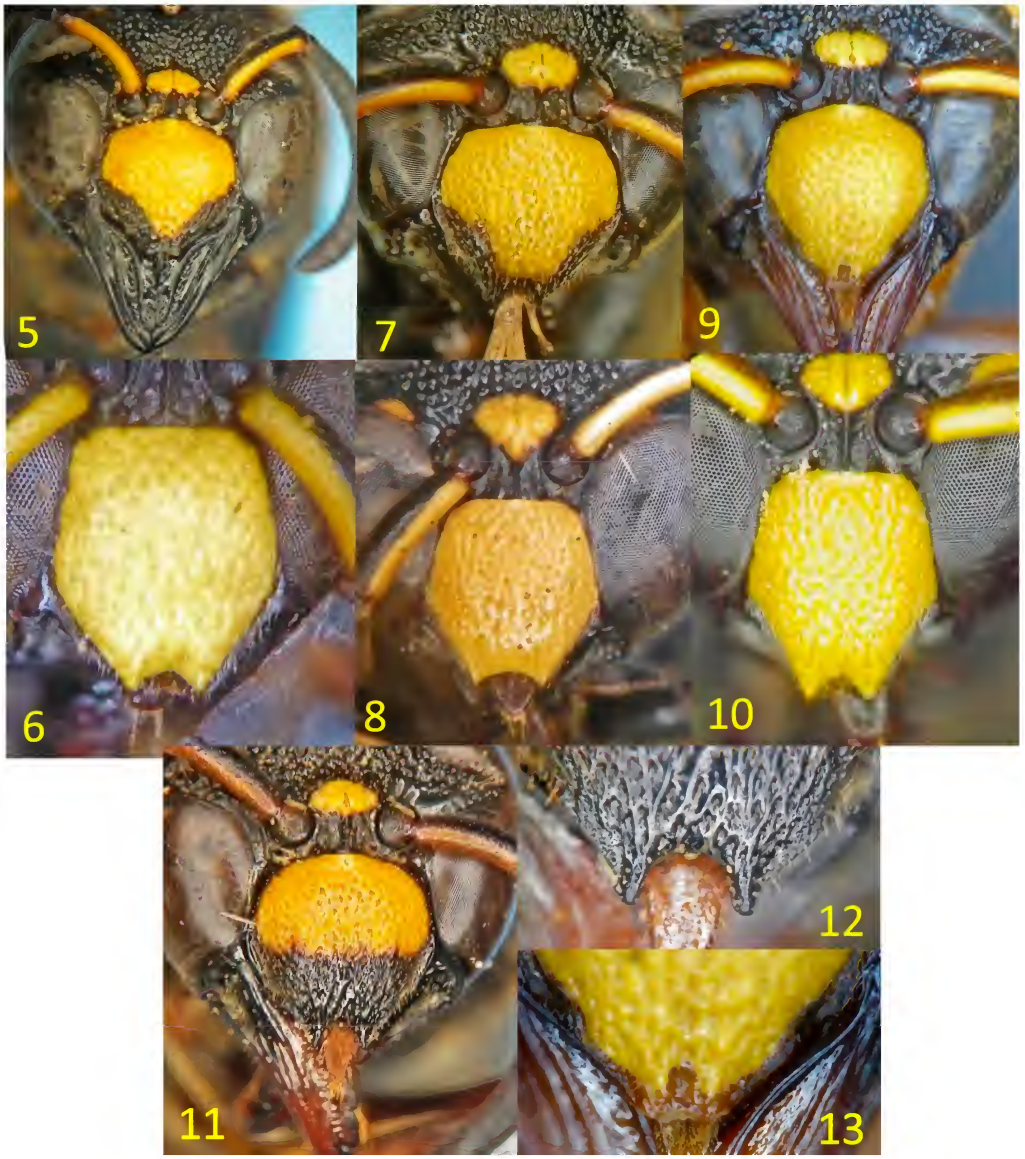
Taxonomic discussion

The shape of the clypeus is sometimes useful in separating the species (Figs. 3, 4, 5-13). In the female two conditions are observed in the anterior margin of the clypeus. In A. flavomarginatum, A. gibbifrons sp. n. and A. melanopterum the anterior margin is shallowly emarginated with lateral teeth that are apically round. In A. flavomarginatum the anterior margin is very shallowly emarginated, sometimes almost truncate and essentially without teeth, although the punctated yellow area is deeply incised (Figs. 9, 13). In the other species the emargination is slightly broader and deeper. On the other hand, A. flavopunctatum and A. inamurai (see below) have a deeply emarginated anterior margin and apically sharp lateral teeth (Figs. 7, 12). The male clypeus is less useful, but it is very broadly emarginated with sharp lateral teeth in A. flavopunctatum.

Anterhynchium gibbifrons sp. n. is very similar to the two previously known species from Japan, i.e., Anterhynchium flavomarginatum (Smith, 1852) and A. melanopterum Yamane, 1981. In colour pattern it is more similar to A. melanopterum than to the Japanese mainland subspecies micado (Kirsh, 1878) of A. flavomarginatum, but A. gibbifrons sp. n. and A. melanopterum are very different from each other in pilosity on the mesoscutum, structure of the vertex, and shape of the aedeagus (see 'Key to species'). The new species and A. flavomarginatum share the presence of a distinct depression for the cephalic foveae, and shorter and uniform erect hairs on the mesoscutum. However, A. flavomarginatum is very distinct in having the apical margin of the clypeus with a narrow and very shallow emargination, and distinctly broadened apical portion of the aedeagus.

The present new species is also similar to A. flavopunctatum (Smith, 1852) distributed in the Korean Peninsula and China (see van der Vecht 1963; Giordani Soika 1976; Kim and Yoon 1994; Kim 2014) in both structure and colour pattern. They are, according to the present knowledge, allopatric in distribution. In both the species, the vertex of the female has a distinct depression for cephalic foveae, and the punctures in the dorsobasal part of the metasomal tergite 1 are much larger than those on tergite 2. However, the new species differs from the latter in the following points: clypeus relatively longer (CW/CL 0.90-0.98 vs. 1.00 in ♀, 0.81-0.89 vs. 0.90-0.92 in ♂), with lateral teeth of anterior emargination rounded; frons more distinctly raised (♀); and occipital carina smoothly curved through the entire gena (♀) (in A. flavopunctatum a rather distinct angle present). The Korean subspecies koreanum Yamane, 1981 of A. flavomarginatum is similar in colour pattern to A. gibbifrons sp. n. However, in the former, the area in front of the ocellar region is not distinctly elevated (♀), and the dorsolateral faces of the propodeum have a pair of yellow markings (♀, ♂) (see also 'Key to species').

Anterhynchium gibbifrons sp. n. shares some character conditions with other related species as mentioned above, but is very unique in that the frontal area surrounded by eyes, ocelli and antennal insertions is distinctly raised in the



Figs. 5-13. Clypeus of Anterhynchium spp. 5, 6. A. melanopterum; 7, 8. A. flavopunctatum; 9, 10, 13. A. flavomarginatum; 11, 12. A. inamurai; 5, 7, 9, 11. Female; 6, 8, 10. Male; 12, 13. Apex of female clypeus.

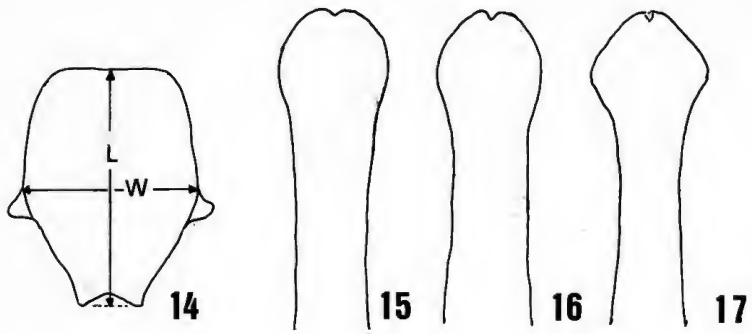


Fig. 14. Measuring points of clypeus (L: length, W: width). 15-17. Dorsal view of aedeagus. 15. Anterhynchium gibbifrons; 16. A. melanopterum; 17. A. flavomarginatum. (16 and 17 after Yamane, 1981)

female.

Distribution and Bionomics

Anterhynchium flavomarginatum is one of the most common tube-renting wasps in Japan (e.g. Iwata, 1938; Yamane, 1990). In many museum collections it can be found in large numbers. On the other hand the close relative A. melanopterum, easily recognized by its peculiar colour pattern, is relatively rare elsewhere in Japan and Korea (Yamane, 1990; Kim, 2014). The present new species A. gibbifrons should have attracted the eyes of wasp collectors due to the similarity in coloration to the rare species A. melanopterum. However, the oldest date for A. gibbifrons sp. n. material available is 25 July 2010 (collected on a small island in Setonaikai). During 2013 and 2014 a relatively large number of specimens were collected by several hymenopterists (see 'Specimens examined'). Although the known localities are concentrated to Fukui Prefecture, Honshu (Fig. 18), the real range should be much wider. Collection sites in Fukui Prefecture ranged from 5 to 573 m in altitude.

Anterhynchium flavomarginatum has one generation per year in Japan (Itino, 1986). Most specimens of A. gibbifrons sp. n. have been collected during July and August, although one male was collected in late June and one female in early September. However, females were seen even late September (29 Nov. 2013) by Mr. T. Tano in Tsuruga-shi. Although the flight season covers more than 3 months, at present this species is also considered to be univoltine. Most collected specimens were visiting flowers of Ampelopsis glandulosa (Wall.) Momiy. var. heterophylla (Thunb.) Momiy. or Cayratia japonica (Thunb.) Gagnep., both belonging to Vitaceae. These two vines commonly grow along the roadside of farmlands and forest edge, representing typical 'satoyama' plants, and are very important nectar sources for wasps and other insects. In most collection sites in Fukui Prefecture A. gibbifrons sp. n. occurs sympatrically with A. flavomarginatum. It is not clear that the remarkable size variation in the body size of A. gibbifrons is due to the competition for prey or nectar resources with the latter species.

Notes on Anterhynchium inamurai (Sonan, 1937) stat rev.

A. inamurai was originally described in the genus Rhynchium based on a single female specimen from Taiwan. Vecht (1963) synonymized it with A. flavopunctatum. However, A. inamurai is very distinct in having an exceptionally large body size: in the female, body length (head + mesosoma + metasomal tergites 1+2; BL) 19.5-20.0mm, head width (HW) 5.20-5.26mm, forewing length (WL) 18.5-

19.5mm (BL 14.0-14.5mm, HW ca. 4.33mm, WL ca. 16mm in A. flavopuntatum). Furthermore, the anterior excavation of the clypeus is broader and deeper as was illustrated by Sonan (1937, fig. 1). The anterior pronotal band is more developed than in A. flavopuntatum and not interrupted medially, and the mesoscutellum as well as metanotum is marked with orange. All this strongly suggests that this form is to be considered a good species.

We examined the following two specimens from Taiwan: ♀, Nantou Hsien, Kwantau Shi, 21-29.v.1973, So. Yamane leg.; ♀, Tao Yuan Hsien, Pa Lin, 14.viii.1987, K. Baba leg. (identified as A. flavomarginatum umenoi by Dr. J. Gusenleitner).

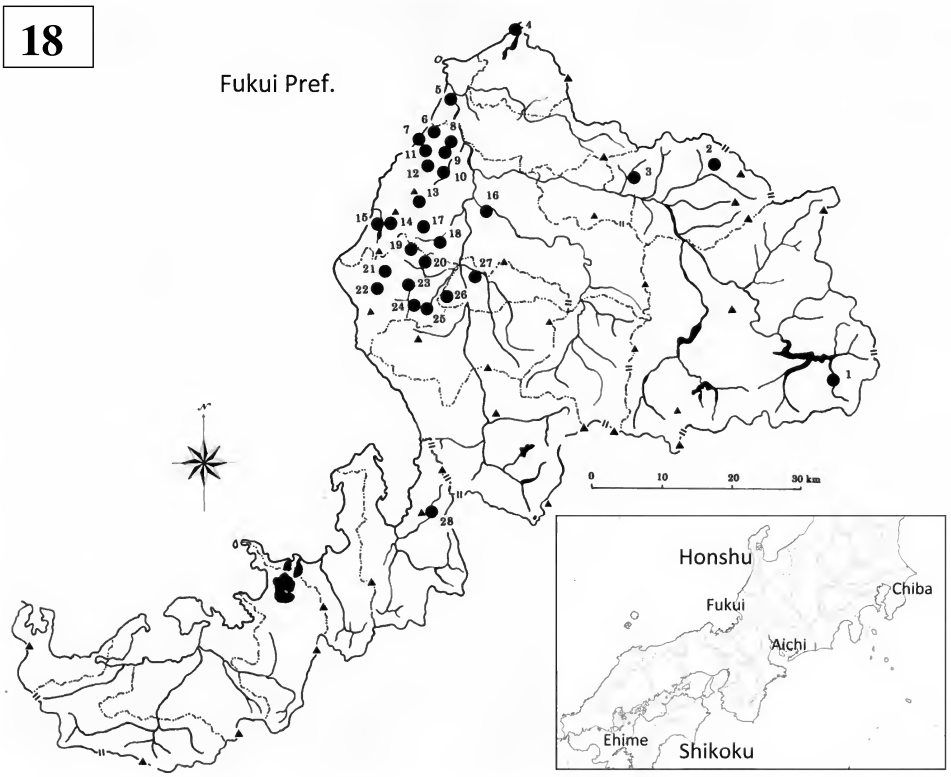


Fig. 18. Distribution of Anterhynchium gibbifrons in Fukui Prefecture, Honshu. 1: Ohno-shi; 2, 3: Katsuyama-shi; 4: Awara-shi; 5: Sakai-shi; 6-18: Fukui-shi; 19-25: Echizen-chô; 26, 27: Sabae-shi; 28: Tsuruga-shi.

Key to Northeast Asian Anterhynchium species

1. Standing hairs on mesonotum soft and long, slightly waving, similar in type to those on frons and vertex (♀, ♂). Depression bearing cephalic foveae obscure or essentially absent (♀). Clypeus broader than long, with CW/CL 1.07-1.09 (♀). Aedeagal shaft only slightly narrower than aedeagal tip (♂)..... .....**A. melanopterum Yamane** (China, Korean Peninsula, Japan)
- Standing hairs on mesonotum stiffer and shorter, of uniform length, distinctly different in type from those on frons and vertex (♀, ♂). Large depression present on vertex, bearing foveae (♀). Clypeus as long as or longer than broad, with CW/CL generally less than 1.0 (♀). Aedeagal shaft variable in shape (♂).....2
2. Punctures around dorsobasal part of metasomal tergite 1 as large as those in posterior half of tergite 1 and those on tergite 2 (♀, ♂). Female clypeus distinctly longer than broad (CW/CL: 0.89-0.95, generally less than 0.92); its apical emargination narrow and shallow. Pronotum (in addition to narrow anterior band), mesopleuron and mesoscutellum generally with yellow to orange maculae (in the specimens from the Nansei Islands in Japan yellow or orange maculae much more abundant). Apical part of aedeagus distinctly broadened, much broader than shaft (♂)..... .....**A. flavomarginatum (Smith)** (Southeast Asia, China, Korean Peninsula, Japan)
- Punctures around dorsobasal part of metasomal tergite 1 much larger than those in posterior half of tergite 1 and those on tergite 2 (♀, ♂). Female clypeus as long as broad or slightly longer than broad (CW/CL: 0.90-1.00, generally more than 0.93); its apex more broadly emarginate. Pronotum, mesopleuron and mesoscutellum generally without yellow to orange maculae (pronotum often with narrow anterior band). Apical portion of aedeagus slightly broader than shaft in A. gibbifrons sp. n. (this character is not known for A. flavopunctatum).....3
3. Frontal area surrounded by ocellar region, upper lobes of eyes and yellow frontal marking only weakly raised. Clypeus almost as broad as long in female (CW/CH: 1.00); its apex rather deeply emarginated with sharp lateral teeth; male clypeus moderately longer than broad (CW/CH: 0.90-0.92). Occipital carina with distinct bent on gena (♀).....**A. flavopunctatum (Smith)** (China, Korean Peninsula)
- Frontal area surrounded by ocellar region, upper lobes of eyes and yellow frontal marking strongly raised. Clypeus slightly longer than broad in female (CW/CH 0.90-0.98, mean=0.94); its apex relatively shallowly emarginate with blunt lateral teeth; male clypeus distinctly longer than broad (CW/CH ca. 0.81-0.89, mean=0.84). Occipital carina smoothly curved on gena (♀). .....**A. gibbifrons sp. n.** (Japan)

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The species of the ant genus Recurvidris Bolton, 1992 (Hymenoptera: Formicidae: Myrmicinae) in Thailand

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Abstract

The Thai ant species of the genus Recurvidris Bolton, 1992 is revised to include three species: Recurvidris browni Bolton, 1992, R. chanapaithooni sp. n., and R. recurvispinosa Forel, 1890. R. browni is newly recorded as found in Thailand and R. chanapaithooni is new to science. A key to the Thai species of the genus is presented, based on the worker caste. All species were collected from the forest floor.

Keywords: ant, Recurvidris, Thailand, new species, distribution, Myrmicinae.

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Introduction

Recurvidris Bolton, 1992 is a small myrmicine genus of the tribe Crematogastrini (Bolton, 2003; Ward et al., 2015). The genus was first described from India by Forel (1890) as Trigonogaster, with Trigonogaster recurvispinosus as the type species. Unfortunately, this name was preoccupied by a chalcid genus, Trigonogaster Guérin-Meneville, 1844 (Hymenoptera: Pteromalidae). Bolton (1992) proposed Recurvidris to replace Trigonogaster, treated seven species and classified them into two species groups (R. kemneri and R. recurvispinosa groups).

Until now, ten species have been described in the Recurvidris genus from the Oriental and Indo-Australian regions (Bolton, 1992; Xu and Zheng, 1995; Zhou, 2000; Zettel, 2008). Jaitrong and Nabhitabhata (2005) recorded only one species, Recurvidris recurvispinosa (Forel, 1890), from Thailand. Having further examined specimens of this genus from Thailand, three species were recognized; one of which is new to science and one is newly recorded as found in Thailand. In this paper the Recurvidris species from Thailand is revised, describing a new species and provides a key to species identification, based on the worker caste.

Materials and Methods

This study is mainly based on the materials deposited in the Ant Museum of Kasetsart University (Thailand) and The Natural History Museum of the National Science Museum, Thailand. Most morphological observations were made with an Olympus SZX12 stereoscope. Materials used in this study were compared with the images of paratypes of Recurvidris browni Bolton, 1992, R. pickburni Bolton, 1992, R. proles Bolton, 1992, and R. recurvispinosa (Forel, 1890), (Antweb, 2015).

Multi-focused montage images were produced using Axio Vision SE64 [IP-ExtendedFocusImage-10 (locked:1)*] from a series of source images taken by a Digital AxioCam ICc 5 camera attached to a ZEISS Discovery.V12 stereoscope. Worker measurements were made using an ocular micrometer, recorded to the nearest 0.01 mm.

The abbreviations used for the measurements and indices are as follows (edited from Bolton, 1987):

TL Total length. Roughly measured from the anterior margin of head to the tip of gaster in stretched specimens.

HL Head length. Length of head proper, excluding mandibles, measured in straight line from anterior clypeal margin to the mid-point of a line drawn across the posterior margin of head.

HW Head width. Maximum width of head, in full-face view measured behind eyes (excluding eyes).

CI Cephalic index. HW/HL × 100.

SL Scape length. Maximum straight line length of antennal scape excluding the basal constriction and condylar bulb.

SI Scape index. SL/HW × 100.

PW Pronotal width. Maximum width of pronotum in dorsal view.

ML Mesosoma length. Diagonal length of mesosoma in profile, from the point at which the pronotum meets the cervical shield to the posterior margin of metapleuron.

The abbreviations used for institutions are as follows:

AMK Ant Museum, Faculty of Forestry, Kasetsart University, Thailand.

BMNH The Natural History Museum, London, U.K.

MCZC Museum of Comparative Zoology, Cambridge, MA, U.S.A.

SKYC SKY Collection at Kitakyushu Museum of Natural History and Human History, Japan.

THNHM Natural History Museum of the National Science Museum, Thailand.

Systematics

Recurvidris Bolton, 1992

Recurvidris Bolton, 1992: 36, figs. 1-11. [Replacement name for Trigonogaster Forel, 1890: cix; junior homonym of Trigonogaster Guérin-Méneville, 1844: 1149 (Hymenoptera: Pteromalidae).]

Trigonogaster Forel, 1890: cviii. Type species: Trigonogaster recuvispinosus Forel, 1890: cix. [Junior homonym of Trigonogaster Guérin-Méneville, 1844: 1149 (Hymenoptera: Pteromalidae).]

Worker diagnosis: for a more extensive description of the worker caste of the genus, see Bolton (1992). Some of the important characteristics described by Bolton (1992) are reproduced here. Clypeus broad from front to back; antenna 11-segmented, with a conspicuous apical club of three segments; mandible with 4-5 teeth on masticatory margin; palp formula 4, 3; frontal carinae and antennal scrobes absent; propodeal spine present, curving upwards and forwards from its base; petiole pedunculate, with spiracle at about midlength of the peduncle, and node low and weakly conical in profile; first gastral segment in profile almost flat dorsally and strongly convex ventrally, with tergite strongly overlapping sternite laterally.

Key to Thai species, based on worker caste

1. Head entirely smooth and shiny; basal margin of mandible with a small tooth which is widely separated from basal (fourth) tooth (Fig. 2B); basal tooth acute apically; propodeal declivity lacking infradental lamella or ridge linking propodeal spine to metapleural lobe; dorsum of propodeum with 2 pairs of standing hairs (Fig. 2A).....**R. chanapaithooni sp. n.**

- Head largely sculptured (reticulate, reticulate-punctate to reticulate-granular); basal margin of mandible unarmed (Fig. 3B); basal (fourth or fifth) mandibular tooth blunt or bidenticulate apically; propodeal declivity with narrow infradental lamella or ridge linking propodeal spine to metapleural lobe; dorsum of propodeum without standing hairs (figs. 1A and 3A).....2

2. Masticatory margin of mandible with four teeth, basal tooth bidenticulate; much smaller species (HW 0.36-0.41 mm); head in full-face view narrow, rectangular and slightly longer than broad.....**R. recurvispinosa (Forel)**

- Masticatory margin of mandible with five teeth, basal tooth blunt or truncate at apex; larger species (HW 0.53-0.56 mm); head in full-face view round and almost as long as broad.....**R. browni Bolton**

Recurvidris browni Bolton, 1992
(Fig. 1)

Recurvidris browni Bolton, 1992: 43, figs. 1, 3. Holotype and 32 paratype workers from E. Malaysia, Sarawak, 4th Div. G. Mulu Nat. Pk., RGS Expd., Long Pala, lowl. Rainfor., forest floor, 5.x.1977, B. Bolton leg. (BMNH, MCZC).

Measurements: Non-type workers (n = 12): TL 2.50-2.60 mm, HW 0.53-0.56 mm, HL 0.56-0.58 mm, SL 0.50-0.51 mm, PW 0.28-0.30 mm, ML 0.73-0.76 mm, CI 91-97, SI 91-97.

Description: Head in full-face view round and almost as long as broad, with posterior margin strongly convex. Eye 0.12 mm in maximum diameter, with seven ommatidia along the longest axis. Antennal scape extending posteriorly, reaching posterolateral corner of head. Masticatory margin of mandible with five teeth, fifth (basal) tooth much larger than fourth, blunt or truncate apically; basal margin of mandible unarmed. Clypeus with indistinctly paired carinae. Mesosoma slender; promesonotum in profile weakly convex dorsally and sloping gradually to metanotal groove. Propodeum in profile with weakly convex dorsal outline; recurved propodeal spine long and narrow. Propodeal declivity with a fine but distinct infradental lamella or ridge linking propodeal spine to metapleural lobe. Peduncle of petiole relatively long, with its dorsal outline distinctly concave and ending posteriorly in sharp angle, its ventral outline weakly convex with long acute subpetiolar process.

Dorsum of head superficially reticulate, with some short fine longitudinal rugulae near mandibular base. Pronotum and mesonotum glassy smooth and shiny; mesopleuron, metapleuron, and propodeum finely reticulate; propodeal spines sculptured. Petiole and postpetiole finely punctate. Gaster smooth and shiny.

Head with relatively dense hairs that are very short; promesonotum sparsely with longer hairs (less than ten hairs); longest pronotal hairs 0.10-0.13 mm long; hairs absent from propodeal dorsum. Petiole with two short dorsal pairs of hairs. Postpetiole with three short dorsal pairs and one short ventrolateral pair of hairs. Body colour yellow.

Non-type material examined: S. Thailand, Nakhon Si Thammarat Prov., Thasala, 5°78′971″N / 99°53′378″E, 765 m alt., 17.iv.2007, W. Jaitrong leg., WJT07-TH675 (THNHM: THNHM-I2013-03922 THNHM-I2013-03923, THNHM-I2013-03924, THNHM-I2013-03925, THNHM-I2013-03926, THNHM-I2013-03927, THNHM-I2013-03929. AMK: THNHM-I2013-03928); S. Thailand, Narathiwat Prov., Wang Dist., 24.ii.2002, S. Hasin leg. (AMK).

Distribution: Indonesia (Kalimantan), Malaysia (Sarawak and W. Malaysia) (Bolton, 1992), and Thailand (new record, fig. 4).

Remarks: Recurvidris browni belongs to the R. recurvispinosa species group (sensu Bolton, 1992) that has the following characteristics: basal tooth on masticatory margin of mandible is enlarged and usually blunt, truncated or bidenticulate apically; basal margin of mandible unarmed; propodeal declivity with infradental lamella that links the spine to metapleural lobe. This species is closely related to Recurvidris williami Bolton, 1992, sharing the same form of mandibular dentition. R. browni is notably larger, has more slender propodeal spines. Furthermore, R. williami has a strongly reticulate-punctate sculpture, which is absent in R. browni. In Thailand R. browni is restricted to the primary evergreen rainforest in the south. The single colony from Nakhon Si Thammarat Province (WJT07-TH675) was collected by sifting leaf litter.

Recurvidris chanapaithooni Jaitrong and Wiwatwitaya, sp. n.
(Fig. 2)

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Types: Holotype (THNHM-I2015-00295) and three paratype workers (THNHM-I2015-00296, THNHM-I2015-00297, and THNHM-I2015-00298) from E. Thailand, Chanthaburi Prov., Soi Dao Dist., honey bait trap, 17.i.2008, W. Jaitrong leg., WJT170108-1 (THNHM).

Measurements: Holotype and paratypes (n = 4): TL 2.00-2.10 mm, HW 0.38-0.41 mm, HL 0.36-0.40 mm, SL 0.35-0.36 mm, PW 0.21-

0.25 mm, ML 0.53-0.54 mm, CI 104-105, SI 88-96.

Non-type (n = 7): TL 1.95-2.10 mm, HW 0.36-0.41 mm, HL 0.36-0.40 mm, SL 0.35-0.36 mm, PW 0.21-0.25 mm, ML 0.53-0.54 mm, CI 100-105, SI 88-96.

Description: (Holotype and paratypes). Head in full-face view round and almost as long as broad, with posterior margin convex. Eye 0.11-0.13 mm in maximum diameter, with eight ommatidia along longest axis. Antennal scape extending posteriorly slightly beyond posterolateral corner of head. Masticatory margin of mandible with four sharp teeth, fourth (basal) tooth larger than third; basal margin with a small tooth. Clypeus without paired carinae, its anterior margin convex. Promesonotum in profile strongly convex dorsally and sloping gradually to metanotal groove. Propodeum in profile with strongly convex dorsal outline; propodeal spines slender, divergent, and in caudal view very narrow. Propodeal declivity lacking infradental lamella or ridge linking propodeal spine to metapleural lobe. Peduncle of petiole relatively long, with its dorsal outline concave and ending posteriorly in right angle, its ventral outline convex with long acute subpetiolar process.

Head entirely smooth and shiny, lacking sculpture except some short longitudinal rugulae near mandiblular base. Mandible and antennal scape smooth and shiny. Promesonotum and lateral face of propodeum smooth and shiny; mesopleuron reticulate, partly smooth and shiny; propodeal dorsum and propodeal spine superficially reticulate. Petiole entirely reticulate; dorsum of postpetiole smooth and shiny. Gaster smooth and shiny.

Head with relatively dense short hairs; promesonotum with sparse longer hairs (11-13 hairs); longest pronotal hairs 0.10-0.13 mm long; propodeum dorsally with two pairs of short standing hairs in front of spiracles. Petiole with two dorsal pairs of long hairs. Postpetiole with two dorsal pairs of long hairs and one ventrolateral pair of short hairs. Body colour yellow.

Non-type material examined: E. Thailand, Chachoengsao Prov., Thathakiab Dist., 30.xii.2002, W. Jaitrong leg., WJT301202-1 (THNHM); S. Thailand, Trang Prov., Nayong Dist., Khao Chong B.G., 29.ix.2001, C. Bourmas leg. (AMK, SKYC, and THNHM).

Etymology: The specific name is dedicated to Mr. Sakorn Chanapaithoon, Vice President and Acting President of National Science Museum, Thailand who has supported W. Jaitrong in his myrmecological research in Thailand and Laos.

Distribution: Thailand (Chanthaburi, Chachoengsao and Trang Provinces) (fig. 4).

Remarks: Recurvidris chanapaithooni sp. n. is closely related to R. kemneri Bolton, 1992, R. nigrans Zettel, 2008, and R. proles Bolton, 1992 (all belonging to the R. kemneri group) in having 4 teeth on the masticatory margin of the mandible, sharp basal tooth, a small tooth on the basal margin. This species is most similar to R. kemneri, both sharing a small body size and clear yellow body colour. However, R. chanapaithooni is distinguished from R. kemneri by the presence of 2 pairs of hairs on propodeal dorsum (hairs absent in R. kemneri). It can be separated from R. proles by the following characteristics: body colour uniformly yellow in R. chanapaithooni (head brown, much darker than yellowish mesosoma in R. proles); petiole with a long acute anteroventral process in R. chanapaithooni (subpetiolar process- a small triangular tooth in R. proles); body much smaller (HW 0.38-0.41 mm in R. chanapaithooni; 0.66-0.68 mm in R. proles). R. chanapaithooni is easily separated from R. nigrans by the following characteristics: a much smaller body size (HW 0.38-0.41 mm in R. chanapaithooni; HW 0.61-0.65 mm in R. nigrans); clear yellow body colour in R. chanapaithooni (body colour dark in R. nigrans); tooth on basal margin of mandible smaller than fourth tooth in R. chanapaithooni (almost as large as fourth tooth in R. nigrans); promesonotum with 11-13 hairs in R. chanapaithooni (with nine hairs in A. nigrans); dorsum of propodeum superficially reticulate in R. chanapaithooni (smooth and shiny in R. nigrans); petiole finely reticulate in R. chanapaithooni (smooth and shiny in R. nigrans). The type series was collected using honey baits from the forest floor in a lowland evergreen forest in the areas noted above.

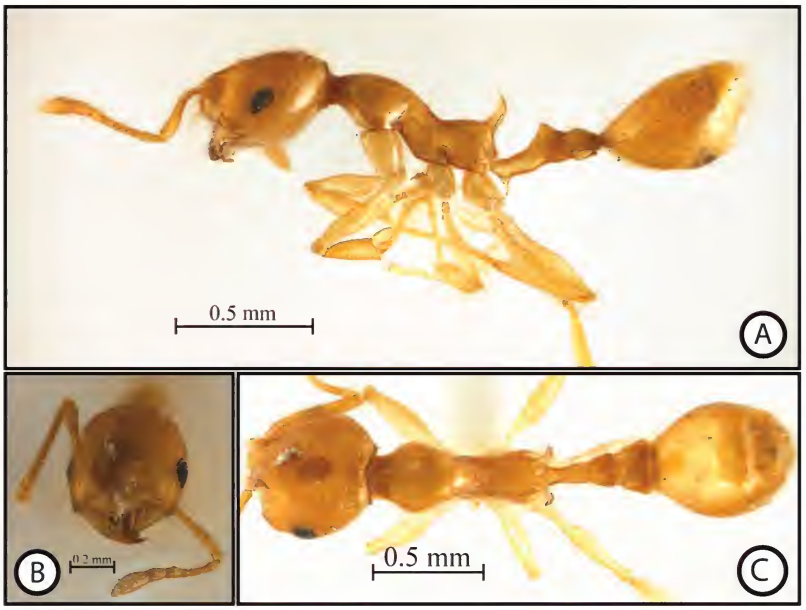

Fig. 1. Recurvidris browni (non-type worker from S. Thailand, THNHM-I2013-03924). A. Habitus in profile; B. Head in full-face view; C. Dorsal view of body.

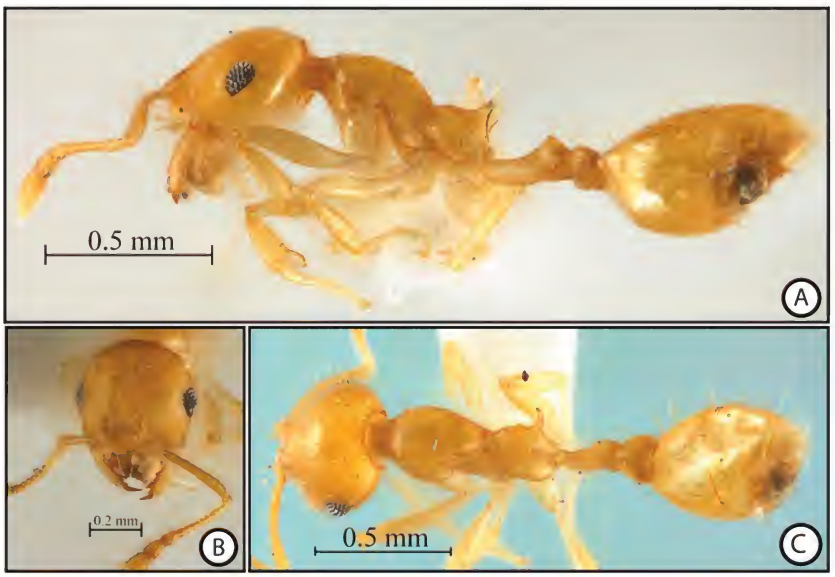

Fig. 2. Recurvidris chanapaithooni sp. n. (Holotype, THNHM-I2015-00295). A. Habitus in profile; B. Head in full-face view; C. Dorsal view of body.

Fig. 3. Recurvidris recurvispinosa (non-type worker from W. Thailand, THNHM-I2015-00299). A. Habitus in profile; B. Head in full-face view; C. Dorsal view of body.

Fig. 4. Distribution of Recurvidris browni, R. chanapaithooni and R. recurvispinosa in Thailand.

Recurvidris recurvispinosa (Forel, 1890) (Fig. 3)

Trigonogaster recurvispinosus Forel, 1890: cix, Syntype workers from India, Poona (R.C. Wroughton) (MHNG).

Trigonogaster recurvispinosa: Wheeler, 1927: 5 (description of male).

Recurvidris recurvispinosa: Bolton, 1992: 46; Bolton, 1995: 377; Jaitrong and Nabhitabhata, 2005: 41.

Measurements: Non-type workers (n = 15). TL 1.70-1.85 mm, HW 0.36-0.41 mm, HL 0.41-0.43 mm, SL 0.33-0.35 mm, PW 0.21-0.25 mm, ML 0.53-0.54 mm, CI 85-96, SI 87-95.

Description: Head in full-face view narrow, subreticular and slightly longer than broad, with posterior margin almost straight or feebly concave. Eye 0.10 mm in maximum diameter, with 6-7 ommatidia along longest axis. Antennal scape extending posteriorly slightly beyond posterolateral corner of head. Masticatory margin of mandible with four sharp teeth, fourth (basal) tooth enlarged and bidenticulate; basal margin of mandible unarmed. Propodeal spine stout, upcurved. Clypeus with distinct paired carinae, its anterior margin weakly convex. Mesosoma relatively slender; promesonotum in profile weakly convex dorsally and sloping gradually to metanotal groove. Propodeum in profile with feebly convex dorsal outline; recurved propodeal spine long and narrow. Propodeal declivity with infradental lamella or ridge linking propodeal spine to metapleural lobe. Peduncle of petiole relatively short, with its dorsal outline distinctly concave and ending posteriorly in blunt angle, its ventral outline convex. Subpetiolar process varying from a tooth to short spine.

Dorsa of head and mesosoma usually finely reticulate-punctate to reticulate-granular; on head the sculpture usually weaker on dorsum behind frontal lobes, and stronger posteriorly and laterally. Sculpture on pronotum much weaker than that on head. Petiole finely reticulate. Postpetiole superficially reticulate. Gaster smooth and shiny.

Head with relatively sparse short hairs; promesonotum with sparse short hairs (less than ten hairs); longest pronotal hairs 0.07 mm long. Hairs absent from propodeal dorsum. Petiole with two dorsal pairs of short hairs. Postpetiole with two dorsal pairs and one ventrolateral pair of short hairs. Body colour yellow to yellowish brown.

Non-type material examined: N. Thailand, Chiang Mai Prov., Muang Dist., Chiang Mai University, Teak Plantation, 29.viii.2014, W. Sangtow leg. (THNHM); same loc., Y. Onishi leg., 2.v.2013 (THNHM); Chiang Mai Prov., Doi Chang Khian, 21.vi.2013, Y. Onishi leg. (THNHM); W. Thailand, Tak Prov., Umphang Dist., Umphang W.S., Doi Huar Mod Forest Ranger Station, DDF, 27.i.2015, W. Jaitrong leg., TH15-WJT-248 (THNHM: THNHM-I2015-299, THNHM-I2015-300, THNHM-I2015-301, THNHM-I2015-302); W. Thailand, Kanchanaburi Prov., Thong Pha Phum Dist., 13.ii.2004, C. Bourmas leg. (AMK, THNHM); NE. Thailand, Nakhon Ratchasima Prov., Sakaerat Environmental Research Station, 17.vi.1998, D. Wiwatwitaya leg., THNHM-I2013-03931 (THNHM). NE. Thailand, Nakhon Ratchasima Prov., Pak Chong Dist., 5.xii.1998, W. Jaitrong leg. (AMK); E. Thailand, Chachoengsao Prov., Thathakiab Dist., 29.xii.2002, W. Jaitrong leg., THNHM-I2013-03934 (THNHM); same loc., 30.xii.2002, W. Jaitrong leg., WJT301202-2 (THNHM); same loc., Dry Evergreen Forest, 21.viii.2003, Sk. Yamane leg. (SKYC); E. Thailand, Chanthaburi Prov., Soi Dao Dist., 19.vii.1997, Sk. Yamane leg. (SKYC); same loc., 2.vi.2001, Sk. Yamane leg. (SKYC).

Distribution: India, Nepal, Myanmar, China, Hong Kong, Taiwan, Japan (Bolton, 1992; Xu and Zheng, 1995; Zhou, 2000; Terayama, 2009), and Thailand (Jaitrong and Nabhitabhata, 2005) (fig. 4).

Remarks: Recurvidris recurvispinosa is closely related to Recurvidris hebe Bolton, 1992 (Sulawesi) in having 4-dentate mandibles, and the basal mandibular tooth being enlarged and bidenticulate apically. R. recurvispinosa differs from R. hebe in the following points: in profile propodeal spine and petiolar peduncle relatively short and stout (relatively long and narrow in R. hebe); with head in full-face view, occipital corners round (more broadly round in R. hebe); mesosoma finely reticulate-punctate to reticulate-granular (superficially sculptured in R. hebe). All Thai specimens were collected from the forest floor.

The single colony from Tak Province (TH15-WJT-248) was collected by sifting leaf litter.

Table 1. List of the Recurvidris species and their distribution. Type localities are marked with *.

Species	Distribution
1. Recurvidris browni Bolton, 1992	Malay Peninsula (W. Malaysia and S. Thailand) and Borneo (Sarawak* and Kalimantan)
2. Recurvidris chanapaithooni sp. n.	Thailand*
3. Recurvidris glabriceps Zhou, 2000	China (Guangxi* and Hainan) and Vietnam
4. Recurvidris hebe Bolton, 1992	Sulawesi*
5. Recurvidris kemneri (Wheeler and Wheeler, 1954)	Borneo (Sarawak), and Java*
6. Recurvidris nigrans Zettel, 2008	Philippines (Negros*)
7. Recurvidris nuwa Xu and Zheng, 1995	China (Guizhou Province*)
8. Recurvidris pickburni Bolton, 1992	Sri Lanka*
9. Recurvidris proles Bolton, 1992	Sulawesi*
10. Recurvidris recurvispinosa (Forel, 1890)	India*, Nepal, Myanmar, China, Hong Kong, Taiwan, Japan and Thailand
11. Recurvidris williami Bolton, 1992	Flores Island* (Indonesia)

Discussion

Until now, eleven species of the genus Recurvidris have been known from Asia (Table 1). Among them, three species are found in Thailand and they belong to two species groups (sensu Bolton, 1992): R. kemneri group (R. chanapaithooni sp. n.) and R. recurvispinosa group (R. browni and R. recurvispinosa).

Mandibular dentition, the presence of hairs on propodeal dorsum and the presence of infradental lamellae or ridges that link the propodeal spines to the metapleural lobes were characters used by Bolton (1992) to distinguish the two species groups mentioned above. These morphological characters were confirmed and used by several authors who described new species of the genus after Bolton (1992) (Xu and Zheng, 1995; Zhou, 2000; Zettel, 2008). The present paper also follows the previous works. In the specimens used in the present paper, the number and shape of teeth on the mandible of the worker (4-5 on masticatory margin and 0-1 on basal margin) are constant within each species even in older specimens (aged individuals). We strongly recommend that whenever preparing dry specimens of the genus, the mandibles of several specimens from each colony must be opened. The peculiar shape of propodeal spine is an important character (curving upwards and forwards from its base) to separate the genus from the others and it seems to be constant in shape and length within each species. However, for Thai Recurvidris the shape, curve, and length of propodeal spine are not very useful to distinguish among the three species. The sculpturing, colour, and pilosity on the body are more important for species identification. The genus is clearly monomorphic in worker caste (at least in Thai species) with a small size variation occurring within species. Thus, body size can be separated into large and small species. In this paper body size has been used, separating R. browni from R. recurvidris (couplet 2) and R. chanapaithooni sp. n. from R. nigrans.

Recurvidris recurvispinosa is the most widely distributed member of the genus. It has been recorded from India, Nepal, to Southeast Asia including southern China, Hong Kong, Taiwan, and southernmost part of Japan (Bolton, 1992; Xu and Zheng, 1995; Zhou, 2000; Jaitrong and Nabhitabhata, 2005; Terayama, 2009). In Thailand, R. recurvispinosa can be found in the areas north of the Isthmus of Kra and in various types of forest such as dry evergreen forest, mixed deciduous forest and dry dipterocarp forest. Recurvidris browni was recorded from lowland rainforests in Sundaland (W. Malaysia, Sarawak and Kalimantan) (Bolton, 1992). In Thailand, this species is recorded for the first time in the lowland evergreen rainforest, south of the Isthmus of Kra. Recurvidris chanapaithooni sp. n. inhabits

primary forests and is sympatric with R. recurvispinosa in the eastern part and with R. browni in the southern part of Thailand (fig. 4).

Acknowledgements

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Three new species of Amblyaspis Förster (Hymenoptera: Platygastridae) from India along with a Key to Indian species

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Abstract

The genus Amblyaspis Förster, 1856 (Hymenoptera: Platygastridae) is represented by eight species in India. Three species of Amblyaspis: Amblyaspis hirsuta, Amblyaspis emarginata and Amblyaspis narendrani are hereby described as new to science. An identification key to the species of Amblyaspis in India is also included.

Keywords: Hymenoptera, Platygastridae, Amblyaspis, new species, India, key.

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Introduction

Amblyaspis Förster, 1856 (Hymenoptera: Platygastridae) comes under the subfamily Platygastrinae and are reported as the parasitoids on Contarinia pisi Winnertz on Pisum sativum L., Rhopalomyia californica Felt. etc. (Vlug, 1995). The genus is represented by 81 species all over the world, 18 species from the Oriental region (Cora and Johnson, 2015) and eight species in India (Mukerjee, 1978; Veenakumari et al., 2013; Veenakumari, et al., 2015). This genus is characterized by almost fused A9-A10 and by the dense hairs on the scutellum. Three species of Amblyaspis are hereby described as new to science. A key to Indian species of Amblyaspis is also included.

Materials and Methods

Standard morphological terminologies and abbreviations are after Masner and Huggert (1989). Description and imaging were carried out employing Leica M205A and Leica DFC-500 digital camera. All the specimens studied are deposited at Zoological Survey of India, Calicut.

Abbreviations

OOL= oculo ocellar line; LOL= lateral ocellar line; OD= ocellar diameter; POL= posterior ocellar line; A1-A10= antennal segments 1-10; IOS= interorbital space; T1-T6= metasomal tergites 1-6.

Amblyaspis Förster, 1856

Type species: Platygaster tritici Walker, 1856

Diagnosis

Head transverse, Antenna 10 segmented in both sexes; A9-A10 in female almost fused, separated only by a fine suture; scutellum flattened in dorsal view, somewhat pointed, hardly transverse, not separated from mesoscutum by a groove; scutellum with dense hairs; tarsi 5 segmented; metasoma six segmented; forewing with submarginal vein, not knobbed apically.

Key to Indian species of Amblyaspis Förster, 1856

1. Notauli present……...……2
- Notauli absent……....…..3
2. Scape 7 X as long as wide; clava 1.13 X A3-A6 combined……...……...…….....…**A.kurinji Veenakumari and Buhl**
- Scape 4.4 X as long as wide; clava subequal to preceding segments combined……..... .....**A. dalhousianus Mukerjee**
3. Fore wings emarginate medially (fig. 15) ……..……..**A. emarginata sp. n.**
- Fore wings rounded medially (fig. 7)…..…4

4. Mesoscutum smooth……..... .....**A. charvakae Veenakumari and Buhl**
- Mesoscutum finely reticulate (fig. 21)..…. 5
5. Occiput with six prominent longitudinal striae laterally.....…. .....**A. ashmeadi Veenakumari and Buhl**
- Occiput with faint striae or without striae…6
6. OOL equal to LOL…..**A. narendranii sp. n.**
- OOL not equal to LOL……7
7. OOL less than 1.5 X LOL……8
- OOL greater than 1.5 X LOL……...9
8. A10 elongate, 1.7 X as long as wide; metapleura with dense white setae…..... .....**A. hirsuta sp. n.**
- A10 not elongate, 1.08 X as long as wide; metapleura with sparse, posteriorly oriented yellow setae..…..... .....**A. khasiana Veenakumari and Buhl**
9. Occiput reticulate with sparse longitudinal striae in lower half; scape less than 5 X longer than wide……..…10
- Occiput reticulate without any longitudinal striae; scape more than 5.5 X longer than wide…….....…..…… …..**A. panhalensis Veenakumari and Buhl**
10. Head 1.7 X higher than long; A10 1.76 X as long as wide ……..…..…..……..... ……...…**A. fabrei Veenakumari and Buhl**
- Head 1.48 X higher than long; A10 1.43 X as long as wide.....……...….. .....**A. tipusultani Veenakumari and Buhl**

Amblyaspis hirsuta Anjana and Rajmohana, sp. n. (Figs. 1-8)

urn:lsid:zoobank.org:act:F6D13AA2-56EE-4437-BA79-5D95C182474E

Diagnosis: Length 1.13 mm; occiput strongly reticulate; hyperoccipital carina present; A3 equal to A4; notauli absent; scape 5.8 X as long as wide; mesoscutum finely reticulate.

Description

Female: Length 1.13 mm (holotype) (fig. 1); A1-A2 and legs yellowish brown; A3-A9 dark brown; tegula dark brown; microtrichia dark brown; last segment of tarsi slightly darkened.

Head from above 2.18 X as wide as long (fig. 2); 1.09 X as wide as mesosoma; head in front view 1.17 X as wide as high (fig. 4); occiput strongly reticulate and without any striae; frons finely reticulate; lower frons above toruli with several transverse striae; IOS 2.29 X eye height; eyes bare; OOL 2.8 X OD; OOL 1.89 X LOL; POL 2.4 X LOL; eye height (in dorsal) 3.2 X temples; hyperoccipital carina present, extending between orbits; scape 5.8 X as long as wide; A3 equal to A4; scape 1.36 X as long as A7-A10 combined; ratio of length and width of antennal segments A1-A10 being 0.29: 0.05; 0.07: 0.03; 0.05: 0.03; 0.05: 0.03; 0.04: 0.03; 0.04: 0.04; 0.06: 0.05; 0.05: 0.06; 0.05: 0.06; 0.06: 0.04 (fig. 3).

Mesosoma 1.39 X as long as wide (fig. 5); pronotum non angular, clearly visible from above; lateral sides of pronotum sparsely setose with fine reticulations; epomial carina present; mesoscutum 1.07 X as long as wide, finely reticulate with sparse setae; notauli absent (fig. 5); posterior margin of mesoscutum with median lobe, projecting above mesoscutellum; scutellum 1.07 X as long as wide; fully covered with white setae, mesopleuron smooth; metapleuron fully covered with white setae (fig. 6). Forewing 2.56 X as long as wide (fig. 7), with fine and dense microtrichia; marginal cilia on posterior margin of forewing 0.12 X width of wing.

Metasoma 1.96 X as long as wide (fig. 8); T1 with dense white setae laterally; T2 with white setae at basal region, rest smooth; T3-T6 with punctures at the apex; T5-T6 with a transverse row of white setae; length and width of T1-T6 being: 0.09: 0.10; 0.36: 0.29; 0.03: 0.24; 0.03: 0.19; 0.03: 0.11; 0.03: 0.06.

Specimen examined

Holotype: Female, India, Kerala, Anjuruli, Idukki (9.74^{0}N, 77.06^{0}E), 09.iv.2013, Coll. Rajmohana, on card, Reg. No. ZSI/WGRS/IR.INV.4636, paratype: female, India: Kerala, Anjuruli, Idukki (9.74^{0}N, 77.06^{0}E), 09.iv.2013, Coll. Rajmohana, on card, Reg. No.ZSI/WGRS/IR.INV.4637.

Etymology

This species is named 'hirsuta' as the metapleuron of the species is fully pilose.

Comments

Amblyaspis hirsuta sp. n. runs to A. vietnamensis in Buhl's key to Oriental species of Amblyaspis (2009), but differs in the following characters. OOL 1.89 X LOL in Amblyaspis hirsuta sp. n. whereas only 1.25 X in A. vietnamensis. Mesoscutum is with sparsely arranged short hairs in A. hirsuta sp. n. whereas, two broad stripes of short hairs along imaginary notauli is present in A. vietnamensis. Metapleuron is fully pilose in A. hirsuta sp. n. whereas sparsely pilose in A. vietnamensis.

A. hirsuta sp. n. is very similar to A. khasiana Veenakumari & Buhl, described from India but differs in the following. A10 in 1.7 X longer than wide in A. hirsuta sp. n. and 1.08 X in A. khasiana. Scape is 5.8 X longer than wide in A. hirsuta sp. n. whereas 5.3 X in A. khasiana. A. khasiana also has metasoma more than twice as long as wide, A8-A9 less transverse, and POL only 2.1 X LOL. Also body appendages distinctly brighter coloured in A. hirsuta sp. n. than in A. khasiana.

A. hirsuta sp. n. is compared with all known species of Amblyaspis from India. OOL is less than 1.5 X LOL in all the known species from India. In A. dalhousianus Mukerjee, OOL 1.45 X OD. In A. charvakae, A. fabrei, A. panhalensis, A. tipusultani mesoscutum is with setae arranged in an imaginary notaular line which is absent in Amblyaspis hirsuta sp. n. A. kurinjii is with deep notauli. Scape is 5.8 X as long as wide in Amblyaspis hirsuta sp. n. whereas, only 3.9 X as long as wide in A. ashmeadi.

Amblyaspis emarginata Anjana and Rajmohana, sp. n. (Figs. 9-16).

urn:lsid:zoobank.org:act:7403E0CB-2052-40A6-B89A-AC08043CA110

Diagnosis: Length 0.93 mm; head 1.86 X wider than long; scape 5.2 X as long as wide; wings emarginate medially; metapleuron setose only at the posterior corners.

Description:

Female: Length 0.93 mm (holotype) (fig. 9); scape yellowish brown; A2-A10 brown; legs including coxae yellow; tegula light brown; microtrichia dark brown; last segment of tarsi slightly darkened.

Head from above 1.86 X as wide as long (fig. 10); 1.18 X as wide as mesosoma; occiput finely reticulate with granulations and without any striae; head in front view 1.14 X as wide as high; frons reticulate (fig. 11); lower frons above toruli with several transverse striae; IOS 2.25 X eye height; eyes bare; OOL 3.45 X OD; OOL 1.4 X LOL; POL 1.96 X LOL; eye height (in dorsal) 3.5 X temples; hyperoccipital carina present extending between orbits; antennae covered with white setae (fig. 12); scape 5.2 X as long as wide, 1.24 X as long as claval segments combined; ratio of length and width of antennal segments A1-A10 being: 0.26: 0.05; 0.07: 0.04; 0.05: 0.04; 0.03: 0.03; 0.04: 0.03; 0.04: 0.04; 0.05: 0.05; 0.05: 0.06; 0.04: 0.05; 0.07: 0.04.

Mesosoma 1.45 X as long as wide (fig. 13); mesoscutum 1.09 X as long as wide, finely reticulate with sparse setae; notauli absent; pronotum non angular, clearly visible from above; lateral sides of pronotum with fine reticulations; mesopleuron smooth; metapleuron with sparse setae at the anterior margin and thick setae at posterior margin (fig. 14). Forewings emarginate medially; 2.18 X longer than wide, with fine and dense microtrichia; marginal fringe 0.15 X forewing width (fig. 15).

Metasoma 1.5 X as long as wide (fig. 16); T1 with white setae laterally; white setae on the base of T2, rest smooth; T3-T6 with a transverse row of setae; length and width of T1-T6 being: 0.05: 0.13; 0.26: 0.27; 0.03: 0.23; 0.02: 0.19; 0.02: 0.12; 0.03: 0.07.

Specimen examined

Holotype: Female, India, Kerala, Anjuruli, Idukki (9.74^{0}N, 77.06^{0}E), 09.iv.2013, Coll. Rajmohana, on card, Reg. No. ZSI/WGRS/IR.INV.4638.

Etymology

This species is named 'emarginata' as the forewing of the species is medially emarginate.

Comments

This species runs to A. cariniceps Buhl, 1997 in Buhl's key to Oriental species of Amblyaspis (2009). Even though, A. cariniceps also has OOL slightly shorter than LOL and metapleuron evenly covered by pilosity, A. emarginata sp. n. differs from A. cariniceps in having medially emarginate wing. This character makes this species unique from all other known species of Amblyaspis.

Plate 1. A. hirsuta sp. n. (1) Female (2) Head dorsal (3) Frons (4) Antenna (5) Mesosoma (6) Pleura (7) Metasoma (8) Wings.

Plate 2. A. emarginata sp. n. (9) Female (10) Head dorsal (11) Frons (12) Antenna (13) Pleura (14) Mesosoma (15) Metasoma (16) Wings.

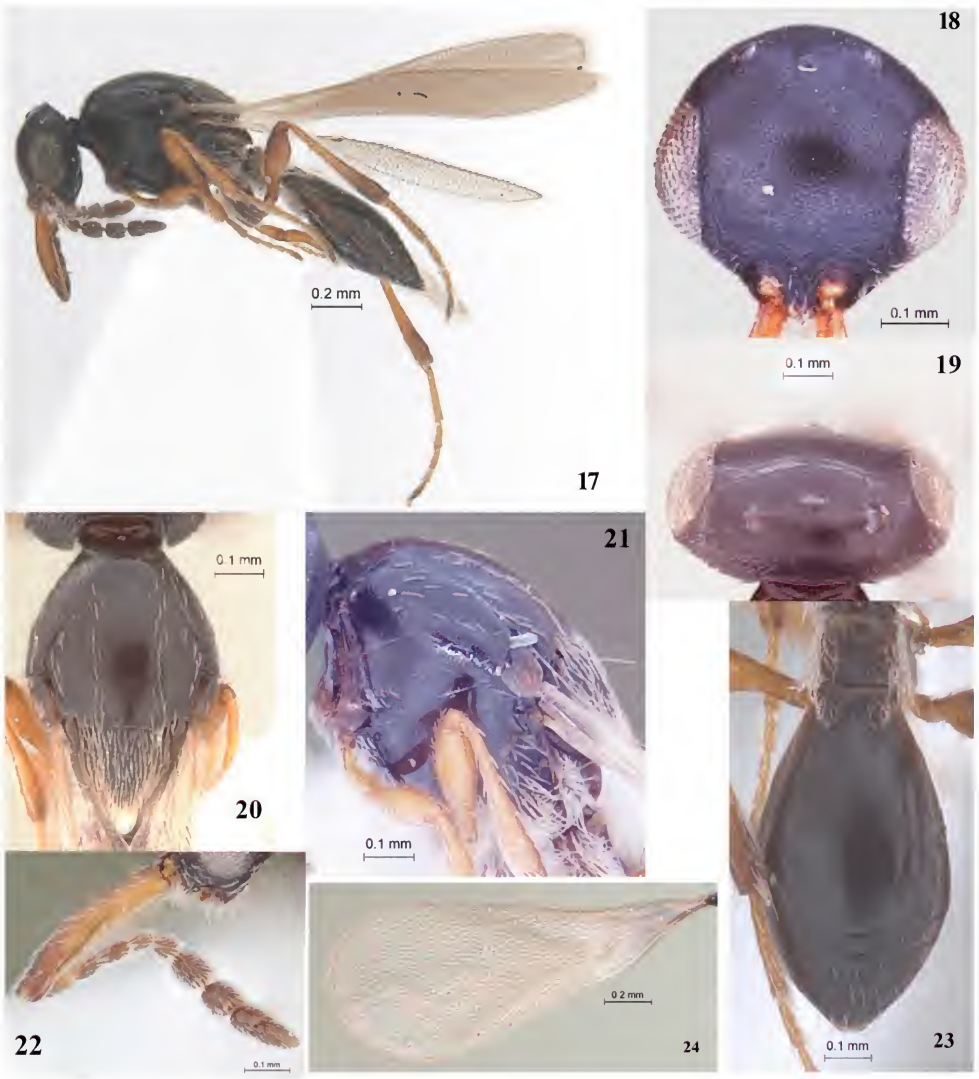

Plate 3. A. narendrani sp. n. (17) Female (18) Frons (19) Head dorsal (20) Mesosoma (21) Pleura (22) Antenna (23) Metasoma (24) Wings.

Amblyaspis narendrani Anjana and Rajmohana, sp. n. (Figs. 17-24).

urn:lsid:zoobank.org:act:B397FF6C-2391-4E05-B2B8-E088A4E59A64

Diagnosis: Length 1.72 mm; head 2 X as wide as long; scape 5.3 X as long as wide; occiput with longitudinal striae on the lateral ends; marginal fringe almost absent.

Description:

Female: Length 1.72 mm (holotype) (fig. 17); scape yellowish brown at the base and more darker towards the apex; A2-A10 brown; legs yellow; tegula black; microtrichia dark brown; last segment of tarsi slightly darkened.

Head from above 2 X as wide as long (fig. 18); occiput reticulate with longitudinal striae on the lateral ends; head in front view 1.04 X as wide as high (fig. 20); frons finely reticulate; lower frons above toruli with several transverse striae; IOS 1.36 X eye height; eyes bare; OOL 2.3 X OD; OOL equal to LOL; POL 2.4 X LOL; eye height (in dorsal) 2.3 X temples; hyperoccipital carina present extending between orbits; antennae fully covered with white setae; scape 5.3 X as long as wide; scape 1.25 X as long as claval segments combined; ratio of length and width of antennal segments A1-A10 being: 0.41: 0.08; 0.1: 0.03; 0.08: 0.04; 0.06: 0.04; 0.06: 0.04; 0.06: 0.05; 0.09: 0.08; 0.08: 0.08; 0.06: 0.07; 0.10: 0.06 (fig. 19).

Mesosoma 1.5 X as long as wide (fig. 21); mesoscutum 1.08 X as long as wide, finely reticulate; five setae present towards the lateral end of the mesoscutum; notauli absent, imaginary notaular line present; pronotum non angular, clearly visible from above; lateral sides of pronotum with fine reticulations with scattered white setae; scutellum fully covered with white setae; 1.2 X as long as wide; mesopleuron smooth; metapleuron with sparse setae at the anterior margin and thick setae at posterior margin (fig. 22). Forewings 2.4 X longer than wide, with fine and dense microtrichia (fig. 24).

Metasoma 1.5 X as long as wide (fig. 23); T1 with parallel median carinae with a depression in between; dense lateral setae present on T1; white setae on base of T2, rest smooth; T3-T6 with a transverse row of setae; length and width of T1-T6 being: 0.14: 0.18; 0.53: 0.4; 0.04: 0.35; 0.05: 0.3; 0.05: 0.24; 0.07: 0.13.

Specimen examined

Holotype: Female, India, Kerala, Manalar, Idukki (9.62^{0}N, 77.34^{0}E), 07.iv.2013, Coll. Abhilash Peter, on card, Reg. No. ZSI/WGRS/IR.INV.4639.

Etymology

This species is named 'narendrani' after the eminent taxonomist, late Prof. Dr. T. C. Narendran.

Comments

This species runs to A. cariniceps Buhl, 1997 in key to Oriental species of Amblyaspis (Buhl, 2009). A. cariniceps is much smaller compared to new species. Occiput is without longitudinal striae in A. cariniceps. Also, OOL is equal to LOL in new species whereas, LOL 1.2 X OOL in A. cariniceps. A. cariniceps also has metapleuron evenly covered by pilosity.

A. narendrani sp. n. is compared with all the Indian species of Amblyaspis. It differs from A. kurinjii Veenakumari and Buhl in not having notauli. New species having OOL equal to LOL differs from all other Indian species.

This species is very similar to Palearctic A. roboris (Walker, 1835), cf. Vlug (1985). Even though it runs to A. roboris (Buhl and Choi 2006; Buhl 1999), the frontal sculpture of A. narendrani sp. n. is a completely different (reticulate meshes) than on A. roboris (transversely granulate, uneven without meshes) sufficiently differentiate the two species. Also, LOL is not equal to OOL in A. roboris. Also, the longitudinal rows of setae on mesoscutum is more wider and more scattered in A. roboris than in A. narendrani sp. n.

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Morphology of third instar Boettcherisca highlandica Kurahashi & Tan, 2009 (Diptera: Sarcophagidae)

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Abstract

Five species of Boettcherisca have been recorded hitherto in Malaysia. The present study demonstrates that Boettcherisca highlandica Kurahashi & Tan could be playing a role in forensic investigations as the larvae were recovered from a rabbit carcass placed at Cameron Highlands, peninsular Malaysia. The third instar morphology of B. highlandica is described for the first time. The larval length ranged from 17-18 mm, with the anterior spiracle composed of 28-30 papillae arranged in two irregular rows. The posterior spiracle is large, heavily pigmented with dilated tails at the upper and lower end of peritreme. Morphological differences of third instar and adults between B. peregrina and B. highlandica are highlighted, and keys to differentiate species of Sarcophagidae of forensic importance are provided herein.

Keywords: Boettcherisca highlandica, Sarcophagidae, third instar, forensic entomology, Cameron Highlands, Malaysia.

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Introduction

The literature on the species richness and biogeography of Sarcophagidae in Malaysia is relatively scarce compared to other dipteran families such as Calliphoridae. Sugiyama et al. (1990) described six new species of sarcophagine flies from Malaysia and Singapore. Twelve years later, a taxonomic key of the oriental Sarcophagidae was provided by Kurahashi (2002), where several new species were added. In 2007, Kurahashi & Leh listed the species of Diptera collected in Sarawak, East Malaysia and documented 21 species of Sarcophagidae, along with a new species described, Parasarcophaga omari Kurahashi & Leh, 2007. It was then followed by Kurahashi & Tan (2009) who published a checklist on the sarcophagid flies from peninsular Malaysia which comprised two subfamilies, 20 genera and 45 species. Soon after that, a sarcophagid, Iranihindia martellata (Senior-White, 1924) was documented as a new locality record for peninsular Malaysia (Tan et al., 2010a).

The genus Boettcherisca was established by Rohdendorf in 1937 where he designated Myophora peregrina Robineau-Desvoidy, 1830 as type species. Lopes (1961) listed seven species in the checklist including B. septentrionalis Rohdendorf; B. formosensis Kiener & Lopes; B. nathani Lopes; B. javanica Lopes, B. peregrina (Robineau-Desvoidy); B. karnyi (Hardy) and B. atypica (Baranov). The phylogeny of Boettcherisca can be divided into two monophyletic and one paraphyletic group, namely peregrina-group, septentrionalis-group and karnyi-group, respectively. Based on the cladogram analysis and geographic distribution, the genus Boettcherisca is postulated to have a Sundaland origin (Kurahashi & Kano, 1984).

Recently, a total of 17 species of Boettcherisca were recorded by Xue et al. (2011), where most of the species are distributed in Oriental region. The oriental species include B. bengalensis Nandi; B. cabrerai Kano & Sugiyama; B. dumoga Sugiyama & Kurahashi; B. highlandica; B. javanica; B. koimani Kano & Shinonaga; B. krathonmai (Pape & Bänzinger); B. nepalensis Kano & Sugiyama; B. talomensis Magpayo & Kano; B. timorensis Kano & Shinonaga; and B. yuwanensis (Sugiyama). The remaining six species of Boettcherisca have a wider geographical distribution viz., B. formosensis (Palaearctic, Oriental); B. invaria (Walker)(= atypica Baranov) (Oriental, Australasian/Oceanian); B. karnyi (Oriental, Australasian/Oceanian); B. nathani (Palaearctic, Oriental); B. peregrina (Palaearctic, Oriental, Australasian/Oceanian, Madagascar); and B. septentrionalis (Palaearctic, Oriental).

In peninsular Malaysia, five species of Boettcherisca have been previously recorded. These included B. peregrina, B. karnyi, B. javanica, B. krathonmai and B. highlandica (Kurahashi & Tan, 2009). However, only four species were documented in Sarawak (Malaysian Borneo), with no record of B. highlandica (Kurahashi & Leh, 2007).

The biology of B. peregrina is relatively well documented. The adult flies of B. peregrina are extensively distributed in eusynantrophic as well as the semisynanthropic and asynanthropic zones on human feces especially in latrine areas (Feng et al., 1990). The adult flies feed on garbage, corpses, feces, flowers and fallen fruits (Xue et al., 2011). The larvae of B. peregrina can be found in a wide variety of decomposing organic matter such as carrion, dung, garbage and human feces (Hall & Bohart, 1948; Lopes, 1961), including dead giant African snail, Achatina fulica Bowdich (Senior-White et al., 1940, as Sarcophaga fuscicauda Boettcher). However, dead snails are probably not the primary breeding site for B. peregrina. According to Beaver (1986), B. peregrina rarely breeds on A. fulica in Thailand, and in three out of five experiments in Malaysia, but only in a single snail in each experiment. The numbers of individuals bred per snail varied from 1-6, and adults emerged from 15-19 days after exposure of the snails. Other than snails, the larvae have been recovered from dead common Indian toad (Bufo melanostictus) (Das & Dasgupta, 1986). Furthermore, adults have been reared from candle bush (Cassia alata L.)(= Senna alata (L.) Roxburgh) and on "rotting shells" (Lopes, 1961). The larvae can be parasites of sugarcane loopper, Mocis frugalis (Fabricius) (Mungomery, 1947), earthworms, and locust, Chortoicetes terminefera (Walker) and act as facultative predators of lepidopteran pupae of nymphalid and pyralid (Xue et al., 2011).

Previous studies demonstrated that B. peregrina breeds on human and animal remains (Sukontason et al., 2010) and served as an causative agent in various kind of myiases such as ophthalmomyiasis (Miura et al., 2005), otomyiasis (Chigusa, 1994), cutaneous myiasis (Kani et al., 1981), nasal myiasis (Kamimura & Arakawa, 1986), intestinal myiasis (Hasegawa et al., 1992), urogenital myiasis (Jiang, 2002), vaginal myiasis (Chigusa et al., 2005) and urethral myiasis (Sun & Ren, 1995). Furthermore, this species was reported in hospital-acquired myiasis (Uni et al., 2006). In forensic entomological studies, Early & Goff (1986) recovered the larvae of B. peregrina from the exposed carrion on the island of Oahu, Hawaiian islands, USA, as well as on human remains found indoor in Hawaii (Frost et al., 2010). Besides, the larvae of B. peregrina have been used in entomotoxicology studies to determine the effect of drug substances on its developmetal rates (Goff et al., 1989; Goff et al., 1991). From the medical perspectives, the adults have been incriminated as mechanical vectors of etiological pathogens of diseases acquired from decaying animal matter and human feces (Moribayashi et al., 2001). In molecular taxonomy, molecular profiles of forensically important flesh flies (which include Boettcherisca spp.) in Malaysia have been studied thoroughly by Tan et al. (2010b). Besides, the basic life cycle and life history of B. peregrina have been investigated by several researchers (Nishida et al., 1986; Majumder et al., 2012), including a study on its developmental rate under different temperature conditions (Wang et al., 2010).

However, there is little information on the biology and ecology of B. highlandica. The type localities of B. highlandica were Genting

Highlands and Cameron Highlands (both located in peninsular Malaysia) and this species appeared to be only present at high elevations (i.e., above 1,200 m a.s.l.) (Kurahashi & Tan, 2009). In this study, we had a chance to collect and examine several B. highlandica larvae obtained from a rabbit carcass placed in Cameron Highlands. The third instar morphology of B. highlandica is herein described for the first time.

Materials and Methods

A field experiment was conducted at Cameron Highlands (4.49N, 101.39E, 1,517.3 m a.s.l.). Observations and insect collections were carried out for 22 days continuously. A total of five rains were recorded during the study period, with mean temperature and relative humidity 23.56 ± 4.04^{0}C and 81.73 ± 21.56%, respectively. A rabbit (Oryctolagus cuniculus (L.)), weighted approximately 2.0 kg, was euthanized by phenobarbital overdose and then placed on the ground at the study site on Day 1 (initial day of study). The carcass was then surrounded by protective fence to prevent scavenging activities by vertebrates.

Three third instar larvae from a same maggot mass were collected beneath the carcass on the last day of observation (on day-22 postmortem). Two of them were preserved in 70% ethanol immediately on-site and the other one was allowed to grow by feeding it with beef liver and brought back to the laboratory for rearing purposes. The food source (i.e., beef liver) was added ad-libidum until the post-feeding stage, and then the larva was transferred to a dry container for pupation. The resulted adult fly was then killed by using Ether, pinned, and kept in the oven for desiccation over four days. The dried specimen was subsequently labeled and sent to the last author for species confirmation.

The preserved larvae were observed microscopically and the body length was measured by an ordinary ruler under a dissecting microscope (Olympus SZX10, Japan). Larval mouthparts, anterior spiracles, posterior spiracles and other morphological features were then prepared by using slide mounting methods modified from Lee et al. (2004) as follows: a transverse excision was made at eleventh body segment without separating it into two parts. The excised larvae were placed in 10% potassium hydroxide (KOH) for clearing purposes. Immersion in KOH was continued overnight. The next day, they were placed in 10% acetic acid for five minutes to neutralize the KOH solution. Forceps, pins and a half-shaved wooden stick were used to aid in removing the gut contents of the larvae. The larvae were placed through a series of ascending concentrations of alcohol solutions from 70%, 90%, 95% and absolute alcohol for 30 minutes each. They were then placed in xylene for 7 minutes and in clove oil for 30 minutes for colouring purpose. Lastly, the larvae were placed on glass slides and mounted with Canada Balsam and kept in an oven for drying process. The slides were labeled and observed under a compound stereoscope (Olympus BX53, Japan) with a standardized measurement bar according to the respective magnifications. The pinned adult specimen and larval slides were vouchered in the parasitology laboratory, Faculty of Medicine, Universiti Teknologi MARA.

Results and Discussions

The first sarcophagid that arrived on the rabbit carcass was Parasarcophaga albiceps (Meigen) and it was observed on the third day of decomposition. Two days later, a male and a female B. highlandica were noted on the carcass, indicating a temporal delay in this species to locate ephemeral resources (Table 1). Adults of B. highlandica were sighted continuously until day-13 of decomposition. On the other hand, Parasarcophaga taenionota (Wiedemann) was also recovered at the later stage of decomposition. However, neither P. albiceps nor P. taenionota larviposited on rabbit carcass, although the adults were seen frequently on the carrion. The complete faunal successional data on rabbit carcasses at Cameron Highlands can be retrieved from Silahuddin et al. (2015).

We did not record the date and time of the initial larviposition by B. highlandica on the rabbit carcass, the developmental time from egg to larva stage could not be precisely determined. However, the duration needed from initial pupation stage to emergence of adult was noted under a well-controlled laboratory condition with a recorded pupariation time. The pupa was

formed on 3.II.2012 and the adult emerged on 20.II.2012. Hence the pupal duration was 18 days (~432 hours) under the laboratory condition (23.7 ± 0.17°C, 83.6 ± 1.82% RH, 10 h light: 14 h dark cycle). This preliminary data was based on a single emerged adult, therefore the duration of pupal stage might vary as there was no replication. It is necessary to study the complete development duration of B. highlandica as this species could have ecological implications and forensic value especially in the ecoregion of montane forest.

Description: third instar B. highlandica (n=2). **General:** length from 17-18 mm, body slender-shaped, creamy white in color. **Cephalopharyngeal skeleton:** medium sized and heavily pigmented; basal piece of mouth hook rectangular, hook part short, stout and pointed downward slightly; ventral cornua approximately half length of dorsal cornua; anterodorsal processes inapparent. **Anterior papillae:** arranged in two rows with 28-30 papillae. **Body spination:** each segment covered by spinations; the spine is of single spike (unicuspid), long, slender, sharp pointed at tip and wholly pigmented. **Posterior spiracle:** large and heavily pigmented; peritreme incomplete with well developed inner slit projections; button absent; spiracular slits slender and long; distance between both posterior spiracles is approximately ¾ width of spiracle; tails of the upper and lower end of peritreme dilated (Figs. 1 and 2).

Comparative remarks. Since both B. peregrina and B. highlandica are carrion breeders, we provide taxonomic keys adapted from Sukontason et al. (2010) to distinguish these species and prevent misidentification in the future. We hereby highlighted some morphological differences between B. peregrina and B. highlandica larvae (Table 2) and adult stage (Table 3). The differences in their bionomics are also compared in Table 4.

We hope the information provided in this paper could be useful to forensic communities where B. highlandica could potentially serve as a forensic indicator in the determination of minimum postmortem interval and the location of body recovery.

Table 1. Succession of Sarcophagidae on a rabbit carcass placed at Cameron Highlands, Malaysia

Species recovered	Adult sighted on	Larval recovered from rabbit carcass
Parasarcophaga albiceps (Meigen)	Day 3	No
Boettcherisca highlandica Kurahashi & Tan	Day 5-13	Yes (larvae collected on Day-22 postmortem)
Parasarcophaga taenionota (Wiedemann)	Day 12-18	No

Table 2. Larval morphology between B. peregrina and B. highlandica

Key features of third instar larva	Boettcherisca peregrina (adapted from Sukontason et al., 2010)	Boettcherisca highlandica
Cephalopharyngeal skeleton	Apparent anterodorsal processes	Inapparent anterodorsal processes
Anterior spiracle	Arranged in two rows with 24-26 papillae	Arranged in two rows with 28-30 papillae
Body spination	The spine is of single spike, long, slender, sharp pointed at tip and wholly pigmented	The spine is of single spike, long, slender, sharp pointed at tip and wholly pigmented
Posterior spiracle	Tail of the upper end of peritreme is dilated	Tails of the upper and lower ends of peritreme are dilated
Length of third instar larva	17 mm	17-18 mm

Table 3. Adult morphology between B. peregrina and B. highlandica

Key features of the adult	Boettcherisca peregrina (adapted from Sukontason et al., 2010)	Boettcherisca highlandica (adapted from Kurahashi & Tan, 2009)
Body length	10-12 mm	10-12 mm
Gena	Black	Black
Third antenna segment	Black	Black
Palpus	Black or dark brown	Black
Postsutural ac	Present	Present
Abdomen	Grey pollinosity at least on tergite 4-5 in male	Golden yellow pollinosity at least on tergite 4-5
Epandrium	Brown, occasionally blackish or reddish	Reddish brown

Table 4. Natural history between B. peregrina and B. highlandica

Significant natural history	Boettcherisca peregrina	Boettcherisca highlandica
Medical and forensic importance	Both myiasis and forensic cases were reported from human and animal carcasses	No myiasis or forensic case yet to be reported from human corpses. The larvae were recovered from animal carcass at high elevation, indicating its potential role in decomposition at the highland
Habitat	Low land (highest altitude recorded was 1,300 m a.s.l.) (Xue et al., 2011) secondary forest, rural and urban settling, plantations	Highlands with montane forest more than 1,200 m above sea level
Geographical distribution	Peninsular Malaysia and Malaysian Borneo, Singapore, Bangladesh, Bhutan, Laos, Myanmar, Nepal, Vietnam, Cambodia, Thailand, Indonesia, Japan, China, Taiwan, North Korea, South Korea, India, Sri Lanka, Seychelles, New Guinea, New Britain, French Polynesia, Fiji, Samoa, Volcano Island, Bonin Island, Gilbert Island, Guam, Kiribati, Hawaii and Mariana Island, Papua New Guinea, Australia, New Zealand (Kurahashi & Kano, 1984; Xue et al., 2011)	Peninsular Malaysia (Genting Highlands and Cameron Highlands)
Ecology	Synantrophic and widely distributed. Larvae are necrophagous and adults are coprophilous (Heo et al., 2010)	Asynantrophic. Larvae breed on carrion at high elevation

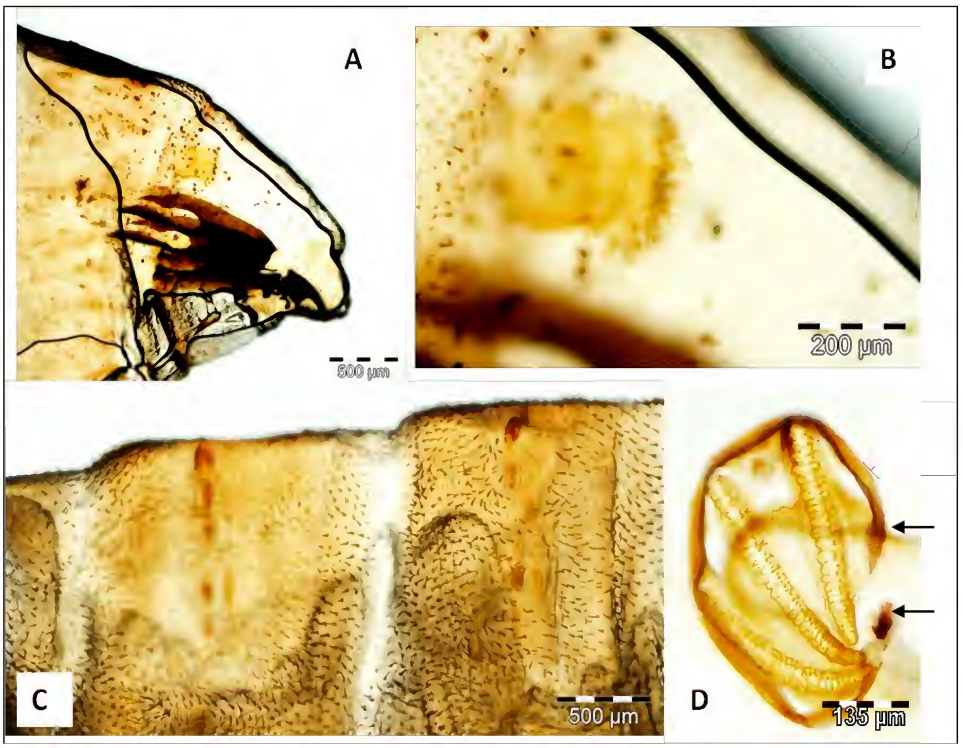

Fig. 1. Microscopic morphology of third instar B. highlandica. A, Cephalopharyngeal skeleton. B, Anterior spiracle with 28-30 papillae arranged in two irregular rows. C, Body spines with single spike (unicuspid). D, Left posterior spiracle. Peritreme with dilated upper and lower ends (arrows)

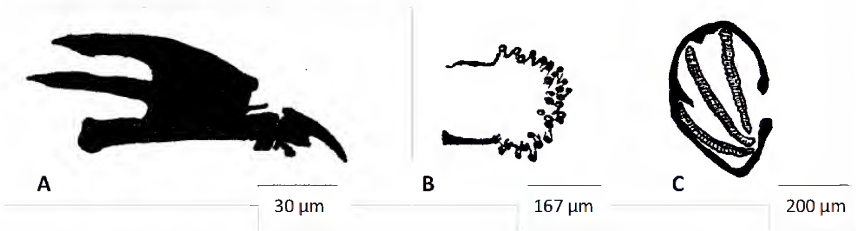

Fig. 2. Internal structures of third instar B. highlandica. A, Cephalopharyngeal skeleton. B, Anterior spiracle. C, Left posterior spiracle

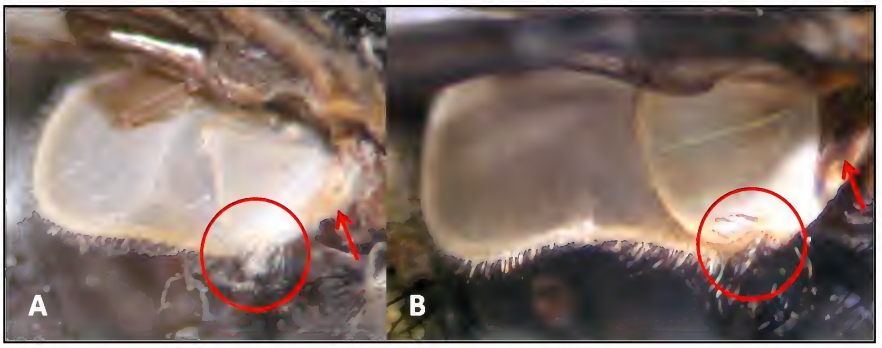

Fig. 3. Alar and thoracic squamae. A, Boettcherisca peregrina. B, Boettcherisca highlandica. Circle showed lower margin of alar squama. Arrow showed base of alar squama

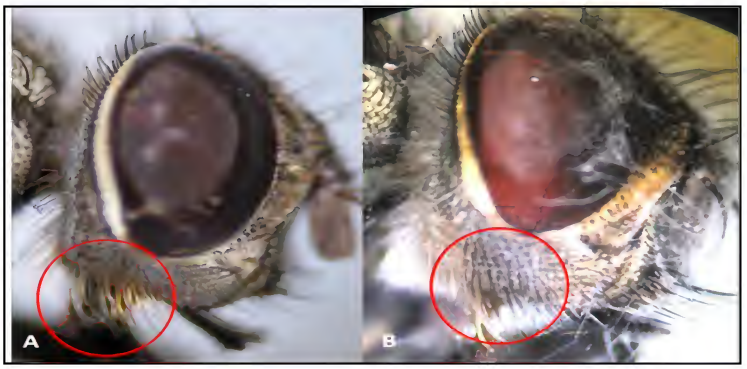

Fig. 4. Lateral view of head. A, Boettcherisca peregrina, gena clothed with yellowish hairs posteriorly. B, Boettcherisca highlandica, gena clothed with black hairs. Circle showed the gena region

Key to adult sarcophagids of forensic importance in Malaysia

1. Propleuron bare……..……..……2
- Proplueron hairy……..……..3
2. Third antennal segment largely orange; palpus wholly orange; epandrium orange; postsutural ac absent……..…….. ……..…**Liopygia ruficornis Fabricius**
- Third antennal segment largely fuscous black; palpus wholly dark brown; epandrium brown; postsutural ac present…..... ……**Parasarcophaga (Liosarcophaga) dux Thomson**
3. Inner lower margin of alar squama and outer lower margin of thoracic squama with tuft of pale white hairs (Fig. 3A); gena clothed with yellowish hairs posteriorly (Fig. 4A)……..... ……..**Boettcherisca peregrina (Robineau-Desvoidy)**
- Inner lower margin of alar squama and outer lower margin of thoracic squama with tuft of fuscous black hairs (Fig. 3B); gena clothed with black hairs (Fig.4B)……... .....**Boettcherisca highlandica Kurahashi & Tan**

Key to third instar of sarcophagids of forensic importance in Malaysia (parts of the key were adapted from Sukontason et al., 2010)

1. Marginal papillae of anterior spiracle arranged in one regular row, composed of 11-17 papillae……...2
- Marginal papillae of anterior spiracle arranged in two or more irregular rows; composed of 24-30 papillae.....….3
2. Anterior spiracle with 11-15 papillae; inter-slit projection distinct; lower end of peritreme located near base of middle or upper slit……...**Liopygia ruficornis Fabricius**
- Anterior spiracle with 14-17 papillae; inter-slit projection indistinct; lower end of peritreme located near base of lowest slit…**Parasarcophaga (Liosarcophaga) dux Thomson**
3. Anterior spiracle with 24-26 papillae; only tail of the upper end of peritreme dilated……**Boettcherisca peregrina (Robineau-Desvoidy)**
- Anterior spiracle with 28-30 papillae; tails of the upper and lower ends of peritreme dilated…..……**Boettcherisca highlandica Kurahashi & Tan**

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A practical guide to insect cell cultures: establishment and maintenance of primary cell cultures

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Abstract

In the last three decades several insect species have been studied in vitro using cell culture revealing fundamental information about their biology. However, numerous important species have never been investigated at a cellular level so that insect cell culture is still in an early stage of its potential development. In the present review, we summarized the main steps involved in the establishment of primary cell cultures to serve as a practical guide for current and future entomologists to leverage the power of cell cultures. This approach has the potential to generate valuable results and suggestions about insect metabolism, vectorial capacity and adaptation to different stresses and challenges. Although most published papers discussed immortalized cell lines, we focussed our review on primary cell culture since they can give precise data in different research fields.

Key words: primary cell culture, cell maintenance, in vitro analyses, immunocytes, insects.

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Introduction

The establishment of insect cell cultures played an important role in different research fields, such as insect pathology, toxicology, insecticide screening and activity assay (Lynn, 1999; Monti et al., 2014).

The main breakthrough occurred five decades ago when Grace and Gao established long-term cultures of insect cells (Grace, 1962; Vlak, 2007). Since then, more than 500 cell lines have been established from more than 100 insect species representing every economically important insect order (Hink, 1972, 1980; Hink and Hall, 1989; Lynn, 1996, 2002). These cell lines have been used in diverse research fields and for instance, plant and vertebrate pathologists isolated and maintained cells capable of replicating viruses transmitted by insects (for a variety of papers on vertebrate, invertebrate and plant viruses in insect cell cultures see Mitsuhashi, 1989).

Most published papers focus their attention on established cell lines. Actually, primary cell cultures can furnish pertinent results for several types of experiments, since they tend to retain in vivo characteristics to a greater extent than continuous cell lines. As a consequence, the development of a continuous cell line is not necessarily the only means of performing in vitro assays for entomologists. For instance, in order to perform RNA interference experiments it can be sufficient to maintain healthy cells only for few days. Primary cell lines could be therefore a potential source of material for entomologists to use in a wide range of studies.

Primary cell culture refers to the initial stage of the culture after cells are isolated from the tissue. At this stage, if the cells proliferate, they can be subcultured (i.e., passaged) by transferring them to a new flask/dish with fresh medium. If the cell culture continues to grow and it is passaged at least ten times, it is considered a continuously replicating cell line. As

these cultures are passaged, cells with the highest growth capacity predominate resulting in a degree of genotypic and phenotypic uniformity in the population. If a subpopulation of a cell line is positively selected from the culture by cloning or some other method, this cell line becomes a cell strain.

Few insect cell lines are commercially available from suppliers or culture collections (such as the American Type Culture Collection, ATCC, www.atcc.org), but most insect cell culturists make their lines freely available to other scientists for research purposes. If no cell lines exist for a specific application, it is up to the researchers to develop their own insect cell cultures.

In this paper, we provide a brief overview of how new primary cell cultures can be established from adult insects. In particular, we focussed our attention on immunocytes that are involved in the interaction with both pathogen and symbiotic bacteria making them useful in different research projects (Pandey and Tiwari, 2012; Monti et al., 2014).

Equipment

The isolation and maintenance of cell cultures can be performed in a small cell laboratory equipped with few instruments (Figure 1). Minimal equipment consists of a laminar flow hood (or biological safety cabinet) to manage cells and sterile reagents, an inverted phase contrast microscope to check cells with 10X (or 20X) and 40X phase contrast objectives, a mechanical pipetting device to dispense reagents, medium and cells, a refrigerated incubator to maintain cells at 24-28°C, glass bead sterilizers for forceps and a stereo microscope for insect dissection. In the absence of a sterilizer, autoclaving utensils or sterilizing them in low percent bleach and then in 70% ethanol also works well.

The presence of a room ultraviolet air purifier can greatly improve the quality of the work since UV light can penetrate cell walls of bacterium, fungi and mold resulting in their death and safer working conditions. However, UV light can also be dangerous for researchers, so that its safe use has to be carefully planned.

Selection of the proper medium

Insect cell culture media contain the same basal ingredients as mammalian cell culture media (carbohydrates, amino acids, and salts), but at concentrations adapted to insect cell metabolism (Lynn 2002, 2007). Furthermore, insect media are generally more acidic (ranging in pH from 6.2 to 6.9) and buffered with sodium phosphate so that a CO_2 incubator is not required for insect cell culture. A further difference is that the osmotic pressure is generally more than twice as high in insect cell culture media as the osmolarity typical of mammalian ones.

The most important point to consider in attempting to develop a new cell culture is the medium (Lynn, 1996, 2002). While perhaps the easiest way to do this is with a shotgun approach in which every commercially available medium is tried, a certain amount of thought can go into selecting the order in which these have been tried. Many commercial media, for instance, have been developed and sold for Lepidoptera (such as the EX-CELL 405 and the SF-900), whereas other commonly available media are for dipteran cell lines, such as Schneider's Drosophila medium. The main points you should consider in selecting a medium for insects other than Diptera and Lepidoptera are the pH, the osmolarity, the amount and ratio of inorganic salts. A never failing reference is still the paper published by Altman (1961) that gives information about the concentration of inorganic salts and amino acids and pH of haemolymph of many insects. Stating from Altman's paper, it is possible to compare values of these factors with the published formulation of commercial media to select the most appropriate for your insect and make the necessary modifications.

As reported in literature (Lynn, 1996, 1999), some media can be used in different species even if they do not belong to the same order. For instance, Grace's insect medium is popular for insect cell culture (Mather and Roberts, 1998) and it has been used with different dipteran

Figure 1. Minimal equipment needed consists of a table or bench that can be cleaned with ethanol solutions for the performance of dissections at the stereomicroscope (a), a laminar flow hood (or biological safety cabinet) to manage cells and sterile reagents (b), a refrigerated incubator to maintain cell flasks/dishes at 24-28°C (c, d, f) and an inverted phase contrast microscope to observe cells (with a 10X or 20X, and 40X phase contrast objectives) (e).

species, even though it was originally formulated for Lepidoptera (Grace, 1962). Schneider's Drosophila medium was originally developed for Drosophila cell culture, but it can be also used for other dipteran cell lines. EX-CELL 420 medium has been used for different Diptera and Lepidoptera and more recently for the red flour beetle, Tribolium castaneum (Goodman et al., 2012). Good results for Coleoptera have also been reported with the Schneider's medium (supplemented with 15% fetal bovine serum) and the Kimura's medium (Kimura, 1984) suggesting that Coleopteran cells can also be maintained and cultured in different media (Zhang et al., 2014).

In some cases, common cell media seem to be inadequate and specific media or combinations of commercial media have to be tested. For instance, the Kimura's medium (Kimura, 1984) has been developed for hemipteran species, but it resulted inadequate for the hemipteran Cacopsylla pyri, Cacopsylla melanoneura and Cacopsylla crataegi cell cultures, so that we recently compared the maintenance of psyllid cells in three media (EX-CELL ® 405, Sf-900™III and the psyllid Hert-Hunter medium) (Monti et al., 2014). The Sf-900™III medium did not support psyllid cells, which shrivelled and died in the first two days post culture. Better results were obtained with EX-CELL ® 405 medium, since cells remained viable for more than one month, though with a low growth rate. On the contrary, extremely positive results were obtained with HH70 medium, which kept cells alive for more than sixty days, according to Marutani-Hert et al. (2009). Interestingly, the HH70 medium is a combination of four media (Schneider's

medium, Sf900 III, Medium 199 and CMRL) supplemented with heat-inactivated fetal bovine serum (Marutani-Hert et al., 2009).

A comprehensive listing of most serum-free media currently available for insect cell culture has been compiled by Agathos (2007).

Preparation of hood and dishes/plates

At the beginning of an experimental trial to isolate insect cells, it could be useful to work with small medium volume so that, in place of the commonly used 12.5 or 25-cm^2 tissue culture flasks, it can be more suitable to start the work using 35mm x 10 mm polystyrene cell culture dishes (with 1 ml of medium) or use 24 well tissue culture plates (with 1 ml of medium in each well). Polystyrene cell culture dishes are less expensive, but they can be difficult to manage as they have to be sealed with Parafilm® to maintain sterility and to facilitate their examination with the inverted microscope.

We generally prefer to work with tissue culture plates since they can be managed more quickly, they do not need to be sealed and information can be written on their lids (such as number of dissected specimens, date, medium volume and type, etc.).

As described in detail by Lynn (2002, 2007), before starting to prepare plates, wipe down the working surface of the hood with 70% ethanol (keep a 100–200-ml squeeze bottle of ethanol next to the hood for this purpose) and sterilize the hood for at least 40 minute with the UV lamp. After sterilization, turn on the air movement of the hood for a few minutes before starting your work to ensure the moving air is clean. Then remove a bottle of fresh medium from the refrigerator and place it in the hood.

It is best to use single-use disposable serological pipets to manage media and cells. Pay attention that the major source of microbial contamination in cell cultures is not the laboratory room, but the laboratory workers, so that it is important to wash hands and wear clean gloves to minimize contamination.

One of the most practical advantages of working with insect cells is that many of the contaminants that vertebrate cell culturists have to contend with are not an issue with insects (Lynn, 2007). For example, mycoplasma (which is the major source of problem of cell workers) is adapted to grow at 37°C and the temperatures at which insect cells grow (typically 25-28°C) are not conducive to the effective growth of this organism (Lynn, 2007). In many cases, a further source of contamination is related to viruses due to contaminations of the bovine foetal serum, but generally they do not replicate in insect specific media.

Media supplements

In order to have the best growth conditions for cells, insect media are generally supplemented with 200 mM L-Glutamine solution.

If you plan to use the medium for primary cell cultures, it is essential to add antibiotics, in particular gentamicin (at a final concentration of 50 µg/ml) and penicillin/streptomycin (at a final concentration of 50U/ml and 50 µg/ml, respectively). Lastly, taking into account that the main problem with cell culture contamination is not related to bacteria but to mold, the antimycotic agents nystatin (100 U/ml) or amphotericin B (0.5 ug/ml) can also be added to the medium.

When reagents are ready to use, loosen the caps on the medium, carefully insert the pipet only as far as necessary to reach the fluid and draw the different reagents into the pipet and then transfer them into the bottle containing your medium for supplementation. Serological pipettes for cell culture and liquid management and the pipettor should be the only item in the hood. Do not use the hood as a storage area for buffers, tips, or other equipment since these will interfere with the airflow in the hood and can lead to contamination. When the supplemented medium is ready, pipette 1 ml of medium into a well in your plate (or in the Petri dish if you plan to use it) always being careful to work inside the sterilized hood.

Depending on incubation conditions, small volumes of the cell culture medium may evaporate quickly, especially during long-term experiments. In order to reduce this effect, pipette sterile water into wells near where you plan to put cells. If you plan to use dishes, they need to be placed in a tightly sealed plastic (or glass) container with a small beaker of sterile distilled water and the entire container placed within the incubator to discourage dehydration.

How to dissect insects

Two factors make primary tissue culture of insects particularly arduous (as reported in Lynn, 1996). The first is, their generally small size, whereas the second problem is that insects often live in dirty environments. The former problem can be overcome by setting up primary cultures in small volumes, whereas the latter issue can be dealt with by using antibiotics. It is generally not a good idea to use antibiotics in continuous cell lines (it could favour the occurrence of resistant bacteria), nevertheless they are beneficial in initiating a new primary cell culture.

The general procedures we currently use for isolating cells are shown in figure 2. We normally disinfect insects by submerging them for 1 minute in a series of dishes respectively containing 2 ml of 70% ethanol, 0.115% sodium hypochlorite, sterile water, 70% ethanol and sterile water before dissection. After drying on a filter paper for a couple of seconds, insects can be moved into a tissue culture dish or a multi-well plate (we generally prefer multi-well plates) where they can be dissected using sterilized forceps.

Different approaches can be used for the development of primary cell culture and may involve the use of adults, eggs or specific organs (Freshney, 1987; Lynn, 1996, 1999, 2001, 2002, 2007; Mitsuhashi, 2002). Embryos have been a common source of cells for the development of new cell lines since they can be frequently obtained in large quantities and the insect chorion is sufficiently impervious to simple disinfectants for effective decontamination. However, they are not always available and you may be interested in specific organs/cell types.

In order to isolate circulating immunocytes, it is sufficient to cut the insect body in half using forceps and shake the abdomen with a pair of sterile forceps favouring the release of immunocytes. Then, all the body fragments need to be removed using forceps and plates incubated at 24-28°C.

Observation of primary cell cultures with a microscope

After 16-24 hours (h), it is possible to start the observation of cells at the inverted microscope with a 10X (or 20X) phase contrast objective. The medium in the culture should be relatively clear (debris can be present due to dissection) and cells should be somewhat refractive under the microscope (Figures 3a, b).

A cloudy appearance, which makes it hard to see cells with the microscope, suggests a bacterial contamination; such cultures should be autoclaved and discarded (Figure 3a). A further unlucky event could be the presence of mould and also these plates should be autoclaved and discarded (Figure 3d).

As marvellously written by Lynn (1996): “Patience becomes the greatest virtues at this stage”, since little or no growth of cells can be seen for weeks. During this period, additional culture medium should be added to the cells. We generally add 0.2 ml of medium every 5-7 days to our 1 ml cell culture, whereas the observation of cell cultures and the evaluation of the cell growth are carried out daily using an inverted light microscope. After 7 days, 700 µl of spent culture medium is removed and replaced by fresh medium. We generally prefer to add room temperature medium, but several researchers add fresh medium at 4°C.

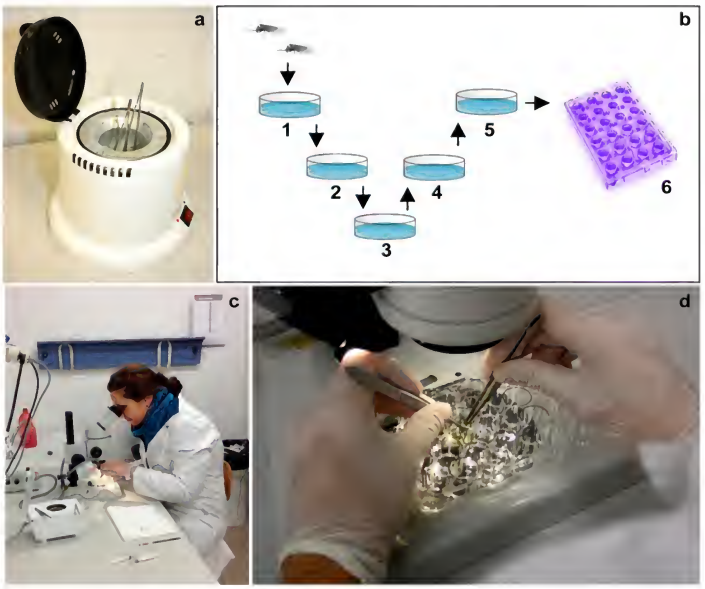

Figure 2. In order to have "clean" dissections it is important to use sterilized forceps to manage and dissect the insects (a). Before dissection, disinfect insects by submerging them for 1 minute in 75% ethanol (1), 0.115% sodium hypochlorite (2), sterile water (3), 75% ethanol (4) and sterile water (5), as summarized in panel b. After drying on a filter paper, insects are transferred into a multi-well plate and dissected using sterilized forceps (c-d).

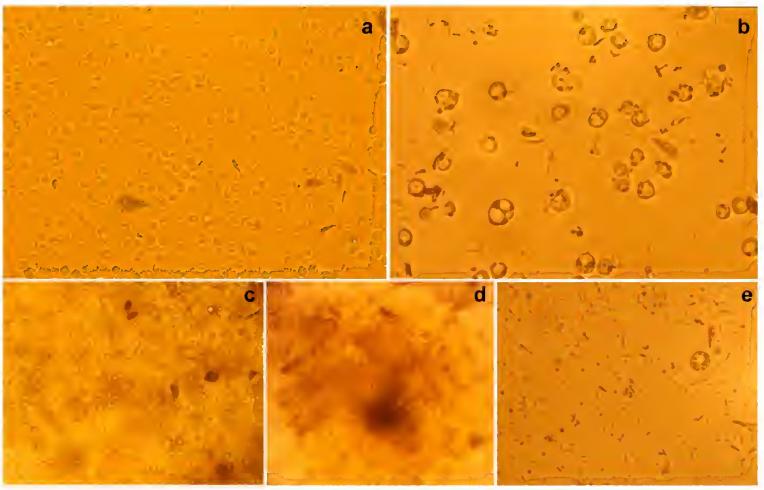

Figure 3. Observation of cells at the inverted microscope with a 20X (a, c) and 40X (b, e) phase contrast objective showing cell cultures in the absence of specific staining. A cloudy appearance is a direct evidence of bacterial contamination (c), whereas the presence of mold can be quickly evaluated (d).

Maintenance of primary cell cultures

Most primary cell cultures do not survive beyond 2 months, however this short period is sufficient for different type of analyses and work, such as the propagation of viruses in cultured cells and the study of immunocytes in mediating an immune response to different immunological challenges (for a review see Smagghe et al., 2009). The use of primary cell culture should be favoured for ex vivo analysis in respect to continuous cells, since stable cell lines assume morphological and physiological characteristics that can differ from the source of primary cultures. For instance established midgut cell lines bear little resemblance to midgut cells in vivo and their susceptibility to some stimuli (such as to toxins) is altered (Smagghe et al., 2009).

At 2–3 day intervals, it is necessary to examine the cell cultures using an inverted microscope and record what you observe/make in a record book. This information should include the date, the 'name' of the culture (cell line designation, dissection date, number of specimen dissected, etc.) and the type, amount and specific source of the used culture medium.

Even if it is true that microbial contamination is a relevant curse for cell culturists, well-maintained modern laminar flow hoods and proper aseptic techniques are sufficient to eliminate the need for some antibiotics and antimycotic reagents in the maintenance of the stock cultures. For this reason, after two weeks we start to use medium supplemented only with glutamine and penicillin/streptomycin, without any further use of gentamicin and antimycotic agents. Actually, to avoid the development of resistance to the antibiotic, all the antibiotics should be removed, but resistance occurs on rare occasions so that we use them and we immediately discard plates if a contamination does occur.

During the examination of cell cultures you can observe the number and the type of the cells that have been isolated during dissection, you can evaluate if their density increases week after week. In this regard, you may have to wait for more than a week before the observation of mitosis since cells must adapt to the new "environment".

A regular examination of the morphology of the cells in culture (i.e., their shape and appearance) is essential for successful cell culture experiments. In addition to confirm their healthy status, inspecting the cells by eye at a microscope each time they are handled will allow you to detect any sign of contamination early on and to contain it before it spreads to other cultures around the laboratory. Signs of deterioration of cells may include granularity around the nucleus, detachment of cells from the substrate and cytoplasmic vacuolation. Deterioration may be caused by a variety of reasons, including contamination of the culture, senescence of the cell line, the presence of toxic substances in the medium, or the need for a medium change.

Lastly, cell line cross-contamination is a serious concern because of the length of time it can go undetected so that it can be useful to avoid the simultaneous presence of different cell cultures in the hood.

Subculture of the cells

After an average of ten weeks (Goodman et al., 2001) you can start subculturing, also referred to as passaging, which consists of the removal of the spent medium, addition of fresh medium and the transfer of cells from the older vessel into a new vessel containing fresh growth medium. This procedure enables the further propagation of the cell line or cell strain. Indeed, when cells occupy all the available substrate or when cells in suspension cultures exceed the capacity of the medium to support further growth, cell proliferation is greatly reduced or ceases entirely. Additionally, to keep the culture at an optimal density for continued cell growth and to stimulate further proliferation, the culture needs to be supplied with fresh medium. If you observed an increase in the cell number you can decide to: i. increase the medium volume; ii. divide cells in two dishes.

It is important to pass your cells according to a strict schedule in order to

ensure a reproducible behaviour, which also allows you to monitor cell health on a consistent basis. Vary the seeding density of your cultures until you achieve consistent growth rate and yield appropriate for your cell type.

When cells are ready for passaging or you have to insert fresh medium, remove the spent medium with a sterile pipette and immediately insert fresh medium. If cells are not well attached to the dish, gently resuspend cells in the old medium, transfer them into a sterile tube and centrifuge the cell suspension at 100 × g for 3-5 minutes at room temperature. Discard the spent medium by pipetting it into a waste container and resuspend the cell pellet in fresh growth medium.

Optional : Determination of Cell Viability (for all cell types)

Several laboratories use trypan bleu staining to determine the number of viable cells (those not taking up the stain) (Lynn, 2002, 2007). We personally find this a time consuming step that does not greatly improve the probability of maintaining healthy cultures. We feel we can confidently recognize healthy cells just by examining them in the dishes with the inverted microscope. Beginners may want to use trypan blue staining until they gain confidence in their visual inspection of cells.

Immunocytes at work: an example of application of primary cell cultures

Immunocytes (frequently referred as hemocytes) play multiple functions in insect immunity, including nodule formation, phagocytosis, encapsulation and synthesis of antimicrobial peptides and other molecules, as recently revised by Pandey and Tiwari (2012). In view of these functions, primary cultures of immunocytes have been used for the characterization of the immune response in different insects, including the mosquitoes Anopheles gambiae and Aedes aegypti (e.g. Castillo et al., 2006), for studies of signalling mechanisms in immunity (Smagghe et al., 2009) and for the comprehension of the immunocyte migration at the infection site (Smagghe et al., 2009).

Recently, immunocytes have been shown to interact with symbionts and play a role in the vectorial capacity of insects (Mandrioli, 2009; Mandrioli et al., 2015), making their study an important challenge for the scientific community. There are therefore many scientific questions regarding the role of immunocytes in insect biology beyond their involvement in immunity, which also point towards the potential of future applications in entomological and biomedical fields.

Conclusions

The field of insect cell culture is facing a rapid expansion into different areas of biology, such as immunity, endocrinology, toxicology and biochemistry. We expect substantial advances to be made in the coming years in many different fields, including signal transduction, endocrinology, toxicology and in several other areas of insect cell biology. As reviewed by Smagghe et al. (2009), cell cultures will allow numerous advances in insect science that will become a part of the modern vanguard of agricultural science necessary for safer production of healthier foods to meet the demands of a rapidly increasing human population.

By paraphrasing Corrie S. Moreau (2014), in closing we hope that this practical guide may provide the foundation for future entomologists to leverage the power of cell cultures to address questions in insect metabolism, vectorial capacity and adaptation to different stresses and challenges.

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