Two new Oriental species of Paramanota Tuomikoski (Diptera: Mycetophilidae), with DNA sequence data

Heikki Hippa1, David Kaspřák2, Siti Rafhiah Haji Abd Kahar3 & Jan Ševčík2*

Abstract. Two new species of Paramanota (Diptera: Mycetophilidae: Manotinae), Paramanota rodzayi, new species, and P. trilobata, new species, are described from Brunei and Thailand, respectively. The new species, P. rodzayi, represents the first record of this genus from the island of Borneo. The barcode mitochondrial cytochrome oxidase subunit I (COI) sequence, partial 12S ribosomal RNA and partial nuclear 28S ribosomal RNA sequences of P. rodzayi are also provided, indicating its possible relationship to P. orientalis Tuomikoski, 1966.

Key words. Manotinae, Sciaroidea, fungus gnats, taxonomy, DNA barcoding, Brunei, Thailand.

INTRODUCTION

The family Mycetophilidae is one of the most speciose groups of Diptera. It is usually divided into six subfamilies (Rindal et al., 2009). The genus belongs to the monophyletic subfamily Manotinae (Hippa et al., 2005; Ševčík et al., 2013). The subfamily currently includes five genera: Manota Williston, 1896, Eumanota Edwards, 1933, Paramanota Tuomikoski, 1966, Promanota Tuomikoski, 1966, and the fossil genus Alavamanta Blagoderov & Arillo, 2002. The phylogenetic analyses based on both morphological (Hippa et al., 2005) and molecular (Ševčík et al., 2013) characters placed Paramanota as a sister group to the clade (Eumanota + Promanota).


The aim of this work is to describe two new species of Paramanota from Brunei and Thailand, respectively, to publish new records of some previously described species, and to provide DNA sequence data for one of the new species.

MATERIAL AND METHODS

The specimen from Brunei was collected in 2015 in a primary lowland rainforest at Kuala Belalong Field Studies Centre (KBFSC) in Ulu Temburong National Park (for the description of the locality and methods used see Ševčík et al., 2014). The new species from Thailand was found among the material collected within the project Thailand Inventory Group for Entomological Research (TIGER) in the Thailand national parks. Numerous other species of Manotinae have previously been described based on the material from that project (e.g., Hippa, 2010, 2011).

The material was preserved in ethanol. Abdomens of the specimens were detached and macerated in warm concentrations of potassium hydroxide (KOH). The hypopygium was also detached beyond segment 8. After washing in water and stepwise dehydration in alcohol, the parts of the abdomen were placed for a few seconds in clove oil (eugenol), after which they were mounted in “Euparal” between two pieces of cover glass, enabling the specimen to be studied from both sides under a compound microscope. These preparations are attached to regular glass slides by a couple of strips of adhesive tape across their edges and thus easily detachable when needed. Other parts of the body were not treated with potassium hydroxide, but after dehydration mounted as in “Euparal”.

The holotype of Paramanota rodzayi was treated with proteinase K (see below) and subsequently...
mounted in “Euparal” in the same way as the other specimens but without treatment in potassium hydroxide and eugenol.

Where possible the terminology follows Søli (1997). The most important structures on the male terminalia are indicated in Figs 1, 2 and 3. The aedeagi are difficult to see in the present mounts and the drawings are approximate. The wing length was measured from both humeral cross vein (and from the base) to wing apex.

Illustrations were made with the aid of a drawing tube attached to a Leitz Diaplan compound microscope.

The material is deposited in Queen Sirikit Botanic Garden, Chiang Mai (QSBG) and Universiti Brunei Darussalam, Brunei (UBDC).

**DNA extraction and amplification.** DNA was extracted using NucleoSpin Tissue Kit (MACHEVERY-NAGEL) following the manufacturer’s protocol. Individual fly specimen was rinsed in phosphate-buffered saline (PBS), placed in sterile Eppendorf tubes and incubated overnight at 56°C in lysis buffer with proteinase K. COI barcode fragment was amplified using primers LCO1490 and HCO2198 (Folmer et al., 1994), partial 12S and 28S ribosomal RNA genes were amplified using primers published in Cook et al. (2004) and Belshaw et al. (2001). Amplified products were purified using Gel/PCR DNA Fragments Extraction Kit (Geneaid). PCR products were sequenced by Macrogen Europe (Netherlands). Sequences were assembled, manually inspected, and primers trimmed, in SEQUENCER 5.0 (Gene Codes Corporation, Ann Arbor, MI), and deposited in GenBank and BOLD databases.

**Sequence alignment and analyses.** The sequences of COI, 12S and 28S were aligned using MAFFT version 7 (Katoh & Standley, 2013) on MAFFT server (http://mafft.cbrc.jp/alignment/server/) with default settings, checked based on amino-acid translation and yielded indel-free nucleotide alignment. We made three separate alignments for each gene and one concatenated alignment for all the genes and 8 taxa, including the new species *P. rodzayi*. We have not been able to extract DNA from the other new species, *P. trilobata*. Data matrix consists of 1592 characters: COI – 658 bp, 12S – 352 bp, 28S – 582 bp. The datasets were analysed using maximum likelihood method, with GTR + Γ model on CIPRES cluster using RAxML-HPC BlackBox 7.6.3 (Stamatakis, 2006), employing automatic bootstrapping on the partitioned dataset. Trees were rooted on the Palaeartic species *Manota unifurcata* Lundström, 1913. The other species used in the analysis were taken from the dataset by Ševčík et al. (2013).

**SPECIES DESCRIPTIONS**

*Paramanota rodzayi*, new species
(Figs. 1A–E)


**Female.** Unknown.

**Etymology.** This species is named after Rodzay Abdul Wahab (Universiti Brunei Darussalam and Institute for Biodiversity and Environmental Research in Brunei), who provided us field facilities to study Diptera in Brunei Darussalam and helped to arrange all the necessary permissions.

**Distribution.** Brunei. This species represents the first record of the genus *Paramanota* from the island of Borneo. Concerning the subfamily Manotineae, only several species of *Manota* and *Eumanota humeralis* Edwards, 1933 had previously been recorded from Borneo (cf. Edwards, 1933, Hippa & Ševčík, 2010, Ševčík et al., 2014).

**Description.** Male. **Colour.** Head yellowish, vertex and occiput darker brown, antennal flagellum becoming darker towards the apex; setae and other vestiture dark. Thorax yellowish, scutum, scutellum and the medial part of mediotergite brown, prothoracic pleura slightly darker than other pleural parts; setae dark. Legs yellowish, hind femur slightly infuscated ventrally near apex, the setae and other vestiture dark which makes the apical part of tibiae and all of tarsi seem dark under low magnification. Wing greyish brown; haltere yellowish with black knob. Abdomen grey brown, tergites and tergites concolorous, setae dark. **Head.** Similar to fig. 7a in Hippa et al. (2005) but the number of facial setae higher. Antennal flagellomere 4, Fig. 1E. The two basal palomeres of maxillary palp similar to fig. 7a in Hippa et al. (2005) but the setae longer and stronger, the two apical segments broken off from the specimen. The number of strong postocular setae 6–7, occiput with a group of 4 strong setae latero-ventrally. **Thorax** similar to Fig. 8a in Hippa et al. (2005). **Legs.** Front tibial organ not well visible in the specimen, apparently consisting of a transverse row of 3 setae. **Wing** similar to fig. 1F in Hippa (2010), stM and the basal part of M₁ and M₂ visible as shades. Wing length 1.7 (1.9) mm. **Hypopygium,** Figs. 1A–D: Gonocoxae ventrally separated by a membranous area, each side with a very large lobe which is posteriorly extending nearly as far as the gonostylius, with the posterior part simple, not divided into sub-lobes; the ventral surface of the lobe as well as the other ventral parts of gonocoxa evenly covered with strong setae, the dorsal surface of the lobe with a postero-mesial area of numerous megasetae subequal in size. Gonocoxa dorsally is simple with setae similar to those of the ventral side. Tergite 9 simple, with a few strong setae. Tergite 10 distinct, discernible as a separate sclerite, with a few lateral setae. Cercus simple. The hypoproct with three setae (one on the left side, two on the right side). Gonostylius with a dorsal lobe, a ventral lobe and a median lobe, each with difficultly observable largely membranous sub-lobes; the dorsal lobe in dorsal view subtriangular, with apical and mesial membranous part showing a striated or lamellar structure; the ventral lobe narrow, transverse, at anterior margin with a long comb-like row of narrow lamellae, the
Fig. 1. *Paramanota rodzayi*, new species (holotype). A, Hypopygium, dorsal view; B, Hypopygium, ventral view; C, Ventral and median lobe of gonostylus, anteroventral view; D, Aedeagus with associated structures, dorsal view; E, Antennal flagellomere 4, lateral view. Scale bar = 0.1 mm. cr = cercus, gs d = dorsal lobe of gonostylus, gs m = median lobe of gonostylus, gs v = ventral lobe of gonostylus, gx = gonoxa, gx l = ventral gonocoxal lobe, hp = hypoproct, tg 9 = tergite 9, tg 10 = tergite 10.
median lobe narrow, transverse, with an apical transverse comb-like row of narrow lamellae. Aedeagus subtriangular.

**Discussion. Possible relationships of the new species.** In the key to the species of *Paramanota* (Hippa, 2010), *P. rodzayi* fits couplet 1 including only *P. orientalis* because the ventral gonocoxal lobe is posteriorly simple, not divided into a more lateral and a more mesial sub-lobes. In this respect *P. rodzayi* is similar to *P. trilobata*, the other new species described in the present paper. Both these species are distinguished from *P. orientalis* by having all the megasetae on the dorsal side of the ventral gonocoxal lobe short, the longest ones at most one fourth the width of the lobe while in *P. orientalis* they are double of that length. *Paramanota rodzayi* is distinguished from all other known *Paramanota* by its very short antennal flagellomeres, being twice broader than long instead of being about as long as broad.

In the mount of the holotype, tergite 10 is well visible as a separate sclerite even if it is partly fused with tergite 9. In the other cases we have seen only the lateral part has been discernible (as in Fig. 2A) and have been described as posterolateral part of tergite 9.

**DNA sequences.** GenBank accession numbers for two mitochondrial (12S, COI) and one nuclear (28S) gene markers are presented in Table 1.

Table 1. GenBank accession numbers for the species included in the molecular analyses

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**Description.** Male. **Colour.** Head yellowish brown, vertex and antennal flagellum darker brown; setae and other vestiture dark. Thorax yellowish, scutum, scutellum and the medial part of mediotergite brown, anterior part of anepisternum infuscated; thoracic setae mostly worn out, the few existing ones dark, the short setae anteriorly on scutum seem pale. Legs yellowish, hind femur with an infuscated subapical patch ventrally, the setae and other vestiture dark which makes the apical part of tibiae and all of tarsi seem darker under low magnification. Wing yellowish brownish; haltere yellowish with dark brown knob. Abdomen brown, tergites and tergites concolorous, setae dark. **Head.** Similar to fig 7a in Hippa et al. (2005) but the number of facial setae higher. Antennal flagellomere 4, Fig. 2D. Maxillary palp similar to fig 7a in Hippa et al. (2005), ultimate palpmere 1.9 times longer than the penultimate one. The strong postocular setae are not countable in the specimen. **Thorax** similar to Fig. 8a in Hippa et al. (2005). **Legs.** Front tibial organ not well visible in the specimen, only one seta observable. **Wing** similar to fig 1D in Hippa (2010). Wing length 2.6 (3.0) mm. **Hypopygium.** Figs 2A–C: Gonocoxae ventrally to Fig. 8a in Hippa et al. (2005). Gonocoxa ventrally covered with strong setae, the dorsal surface of the lobe as well as the other ventral parts of gonocoxa evenly covered with strong setae, the dorsal surface of the lobe with a postero-mesial area of numerous megasetae enlarging in size towards the base, a few rather unmodified setae flanking on the lateral side the area with megaseta. Gonocoxa dorsally simple with the setae similar to those of the ventral side. Tergite 9 simple, with strong setae. Tergite 10 fused with tergite 9 and appearing as a small setose lobe at the posterolateral corner of the latter. Cercus simple. The hypoproct with one seta. Gonostylus with a dorsal lobe, a ventral lobe and a median lobe, each with difficultly observable largely membranous sub-lobes; the dorsal lobe in dorsal view elongated, nearly parallel sided, with membranous apical part showing a striated or lamellar structure; the ventral lobe narrow, oblique, at anterior margin with a long comb-like row of narrow lamellae, the median lobe narrow, oblique, without comb-like structures. Aedeagus elongate, with a broader basal part and narrower
Fig. 2. *Paramanota trilobata*, new species (holotype). A, Hypopygium, dorsal view; B, Hypopygium, ventral view; C, Outlines of aedeagus and associated structures, dorsal view; D, Antennal flagellomere 4, lateral view. Scale bar = 0.1 mm. cr = cercus, gs d = dorsal lobe of gonostylus, gs m = median lobe of gonostylus, gs v = ventral lobe of gonostylus, gx = gonocoxa, gx l = ventral gonocoxal lobe, hp = hypoproct, tg 9 = tergite 9, tg 10 = tergite 10.
Fig. 3. *Paramanota bifalx* Hippa, 2010 (Thailand). A, Right half of hypopygium, dorsal view; B, Median lobes and the ventral lobe of gonostylus, dorsal view. Scale bar = 0.1 mm. cm = comb-like row of pale lamellae, cr = cercus, gs d = dorsal lobe of gonostylus, fl = curved finger-like lobe, gs v = ventral lobe of gonostylus, gx = gonocoxa, gx l = ventral gonocoxal lobe, hl = hand-like lobe, hp = hypoproct, tg 9 = tergite 9, tg 10 = tergite 10.

Fig. 4. Molecular affiliation of *Paramanota rodzayi* based on Maximum likelihood analysis of the combined dataset (12S, 28S, and COI). The numbers below the branches indicate bootstrap support (BS) values above 75.
Discussion. In the key to the species of Paramanota (Hippa, 2010), P. trilobata fits couplet 1 including only P. orientalis because the ventral gonocoxal lobe is posteriorly simple, not divided into a more lateral and a more mesial sub-lobes. In this respect P. trilobata is similar to P. rodzayi, the other new species described in the present paper. Both these species are distinguished from P. orientalis by having all the megasetae on the dorsal side of the ventral gonocoxal lobe short, the longest ones at most one fourth the width of the lobe while in P. orientalis they are double of that length. Paramanota trilobata is distinguished from P. rodzayi by having the antennal flagellomeres longer, the flagellomere 4 being as long as broad instead of only half of that length, by having the dorsal aspect of the dorsal gonostylar lobe elongate subquadrangular, not subtriangular, and by lacking a transverse comb-like row of lamellae apically on the median lobe of the gonostylus. Also many other details in the hypopygium distinguish the two species (Figs. 1, 2).

MOLECULAR ANALYSIS

Maximum likelihood analysis based on the concatenated dataset (12S + 28S + COI) places P. rodzayi as sister taxon to P. orientalis with high bootstrap support (BS=83), see Fig. 4. P. peninsulacae is a sister taxon to P. rodzayi + P. orientalis and P. furcillata is sister to the entire latter clade, though both with low support (BS<75). The genus Paramanota forms a well-supported (BS=100) sister clade to Eumanota + Promanota, in concordance with previous phylogenetic hypotheses (Hippa et al., 2005, Ševčík et al., 2013).

Interestingly, the single-gene trees based on 12S and 28S, respectively, placed P. rodzayi as a sister species to P. furcillata (trees not shown), but with low support values (BS<75). In the tree based on COI barcode region only, P. rodzayi is grouped with P. orientalis, also with low support (BS<75).

NEW RECORDS

Paramanota bifalx Hippa, 2010
(Figs. 3A, B)

Material examined. 1 male (QSBG), THAILAND, Petchaburi, Kaeng Krachan NP, Panernthung/km27/, water pump, 12°49.151’N 99°22.483’E, 950 m, Malaise trap, 8–15 August 2008, Sirichai & Chusak leg. (T4350).

Discussion. The species was described from Thailand – the holotype from the same locality and three paratype males from Surat Thani, Khao Sok NP (Hippa, 2010).

Paramanota furcillata Hippa, 2010

Material examined. 1 male (QSBG), THAILAND, Petchaburi, Kaeng Krachan NP, Panernthung/km27/, water pump, 12°49.151’N 99°22.483’E, 950 m, Malaise trap, 8–15 August 2008, Sirichai & Chusak leg. (T4350).

Discussion. The species was described from Thailand – the holotype from the same locality and three paratype males from Surat Thani, Khao Sok NP (Hippa, 2010).

Paramanota orientalis Tuomikoski, 1966

Material examined. 1 male (QSBG), THAILAND, Nan, Doi Phu Kha NP, Office 13, 19°12.605’N 101°5.074’E, 1371 m, Malaise trap, 22–29 July 2007, Charoen & Nikom leg. (T3276).

Discussion. The species was described based on the holotype male only from Burma, Kambaiti (Tuomikoski, 1966). An additional male was recorded from Thailand, Nan Doi Phu Kha NP (Hippa, 2010).

Paramanota paxillosa Hippa, 2010

Material examined. 1 male (QSBG), THAILAND, Petchaburi, Kaeng Krachan NP, Panernthung/km27/, water pump, 12°49.151’N 99°22.483’E, 970 m, Malaise trap, 5–12 September 2008, Sirichai & Chusak leg. (T4377).

Discussion. The species was earlier known only by the holotype male from Thailand, Nachon Si Tammarat, Namtok Yong NP (Hippa, 2010).

ACKNOWLEDGEMENTS

This study was partly supported by the project CZ.1.07/2.2.00/28.0149 (“Innovation of ecological studies by complementary fusion of courses between Palacký University and University of Ostrava”) financed by the Structural Funds of the European Union and by the “National Feasibility Program I”, project LO1208, of Ministry of Education, Youth and Sports of the Czech Republic. We are grateful to the Institute for Biodiversity and Environmental Research, Universiti Brunei Darussalam, for allowing us to work in Kuala Belalong Field Studies Centre, and to the Biodiversity and Research Innovation Centre (BioRIC), Ministry of Industry and Primary Resources, for the issuance of our export permits. The type specimen of Paramanota trilobata was collected within the “Thailand Inventory Group for Entomological Research (TIGER)" project, funded by a U. S. National Science Foundation grant DEB-0542864 to Michael Sharkey, Lexington, Kentucky, and Brian Brown, Los Angeles, California. The project was supported by the National Research Council of Thailand.
and the Department of National Parks, Wildlife and Plant Conservation, Thailand, who gave permission for research and the collection of specimens. We also thank Peter Chandler (Melksham, Great Britain), Peter Kerr (Sacramento, USA) and one anonymous referee for their valuable suggestions and linguistic corrections.

LITERATURE CITED


