

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

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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 *Alavamanota* 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 species of *Paramanota* are relatively small, yellowish to brownish fungus gnats, about 2.5 mm long. They differ from the other manotines mainly by wing vein Sc ending in C, complete eye bridge and specific male terminalia. The genus currently includes the following eight extant species distributed exclusively in the Oriental region (see Papp, 2004, Hippa et al., 2005, Hippa, 2010): *P. awanensis* Hippa, Jaschhof & Vilkamaa, 2005, *P. bifalx* Hippa, 2010, *P. furcillata* Hippa, 2010, *P. orientalis* Tuomikoski, 1966, *P. paxillosa* Hippa, 2010, *P. peninsulae* Hippa, Jaschhof & Vilkamaa, 2005, *P. schachtii* Papp, 2004, and *P. sumatrana* Hippa, Jaschhof & Vilkamaa, 2005. In addition, one fossil

species, *P. grandaeva* Hippa, 2010, is known. The type species of the genus is *Paramanota orientalis* described by Tuomikoski (1966) from a single male from Burma and redescribed by Hippa (2010). Hippa (2010) also provided a key to species of *Paramanota*.

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 concentrated 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 they were in “Euparal”. The holotype of *Paramanota rodzayi* was treated with proteinase K (see below) and subsequently

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mounted in “Euparal” in the same way as the other specimens but without treatment in potassium hydroxide and eugenol.

Where possible the terminology follows Söli (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 (MACHEREY-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 SEQUENCHER 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-HPG BlackBox 7.6.3 (Stamatakis, 2006), employing automatic bootstrapping on the partitioned dataset. Trees were rooted on the Palearctic 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)

Type material. Holotype. Male (in UBDC). BRUNEI, Ulu Temburong N.P., Kuala Belalong Field Studies Centre

[primary lowland rainforest], Malaise trap ID3, 4°33’N 115°10’E, coll. D. Kaspřák & M. Mantič, 25 January–7 February 2015.

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 Manotinae, 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 palpomeres 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 gonostylus, 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). 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 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

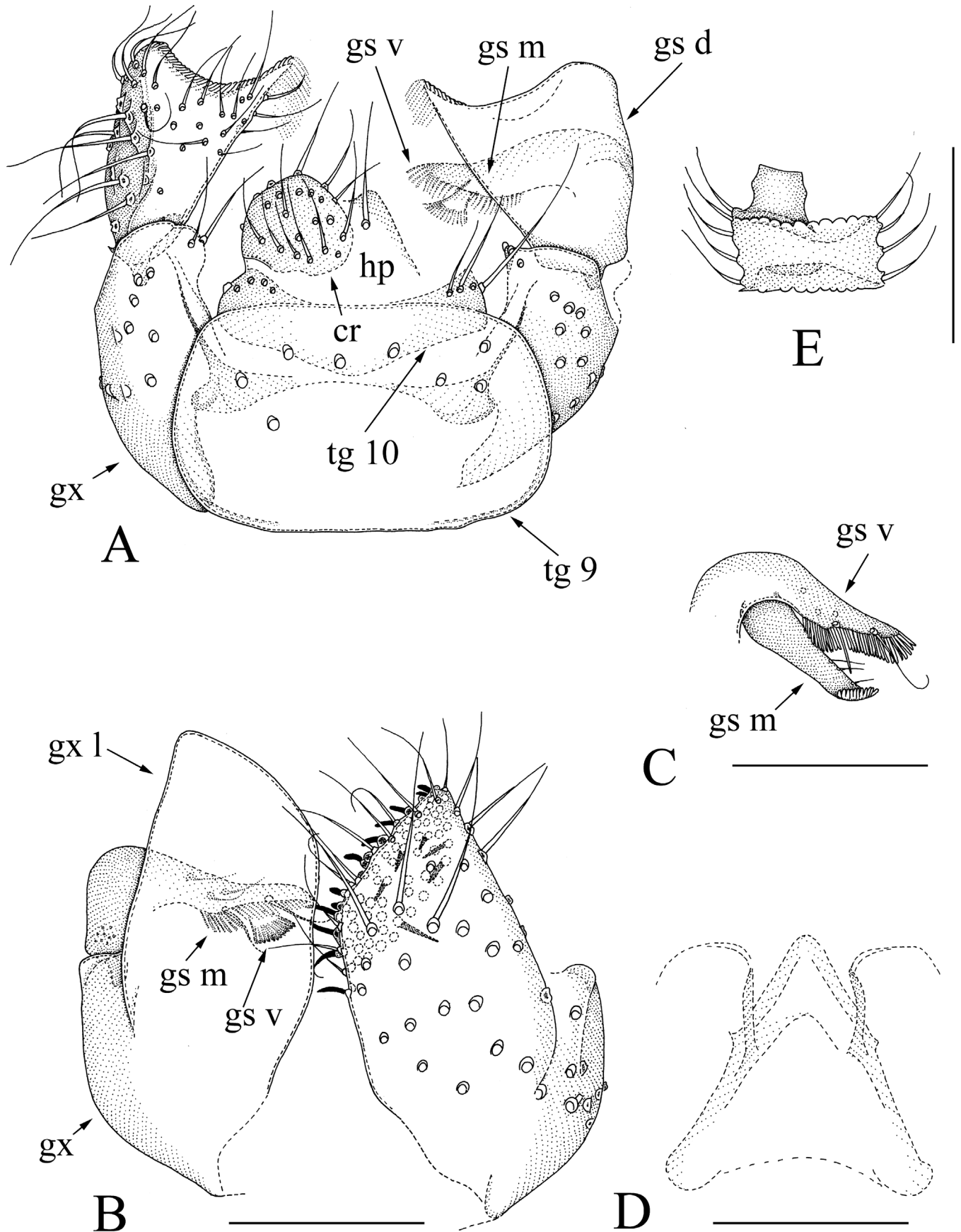


Fig. 1. *Paramanota rodzayi*, new species (holotype). A, Hypopygium, dorsal view; B, Hypopygium, ventral view; C, Ventral and median lobe of gonostylus, anteroventral view; D, Aedeagus with associated structures, dorsal view; E, Antennal flagellomere 4, lateral view. Scale bar = 0.1 mm. cr = cercus, gs d = dorsal lobe of gonostylus, gs m = median lobe of gonostylus, gs v = ventral lobe of gonostylus, gx = gonocoxa, gx l = ventral gonocoxal lobe, hp = hypoproct, tg 9 = tergite 9, tg 10 = tergite 10.

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

Taxa	GenBank accession numbers			BOLD process ID
	COI	12S	28S	
<i>Eumanota humeralis</i>	KC435643	KC435536	KC435608	GBDP14447-13
<i>Eumanota suthepensis</i>	KC435644	KC435537	KC435609	GBDP14446-13
<i>Manota maboakensis</i>	KC435647	KC435540	KC435612	GBDP14443-13
<i>Manota mazumbaiensis</i>	KC435648	KC435541	KC435613	GBDP14442-13
<i>Manota unifurcata</i>	KC435649	KC435542	KC435614	GBDP14441-13
<i>Paramanota furcillata</i>	KC435656	KC435550	KC435622	GBDP14434-13
<i>Paramanota orientalis</i>	KC435657	KC435551	KC435623	GBDP14433-13
<i>Paramanota peninsulæ</i>	KX713156	KX713154	KX713155	JSPAR003-16
<i>Paramanota rodzayi</i>	KU496908	KU496909	KU496910	JSPAR001-15
<i>Promanota malaisei</i>	KC435661	KC435557	KC435629	JSPAR002-15

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.

***Paramanota trilobata*, new species**
(Figs. 2A–D)

Type material. Holotype. Male (T4371 in QSBG). THAILAND, Petchaburi, Kaeng Krachan NP, Panernthung [km 27 water pump], 12°49.151'N 99°22.483'E, 970 m, Malaise trap, coll. Sirichai & Chusak, 12–19 September 2008.

Female. Unknown.

Etymology. The name is Latin, *trilobata*, three-lobed, referring to the three-lobed gonostylus.

Distribution. Thailand.

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 dark 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 palpomere 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 separated by a membranous area, each side with a very large lobe which is posteriorly extending as far as the gonostylus, 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 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 postero-lateral 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

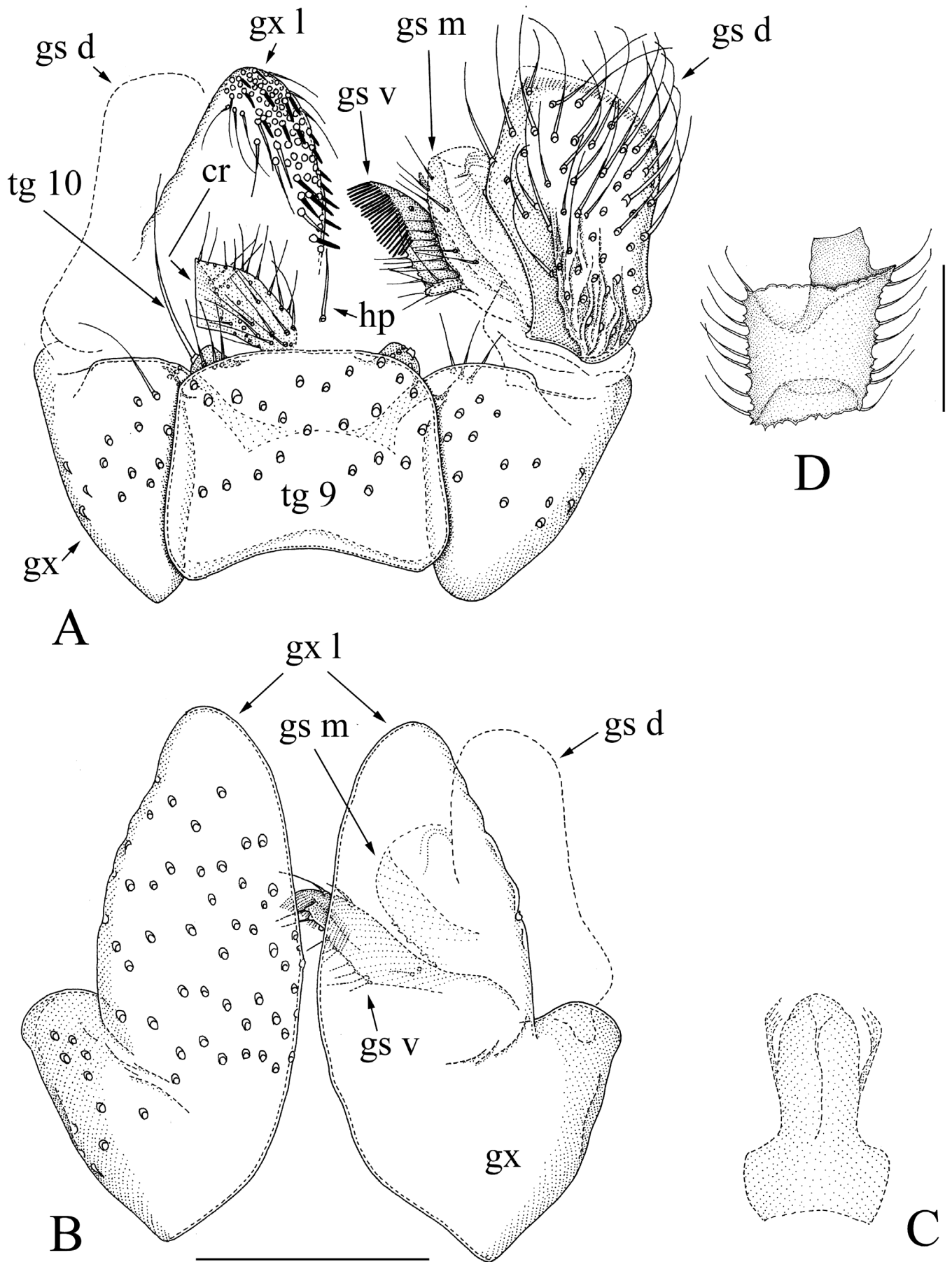


Fig. 2. *Paramanota trilobata*, new species (holotype). A, Hypopygium, dorsal view; B, Hypopygium, ventral view; C, Outlines of aedeagus and associated structures, dorsal view; D, Antennal flagellomere 4, lateral view. Scale bar = 0.1 mm. cr = cercus, gs d = dorsal lobe of gonostylus, gs m = median lobe 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.

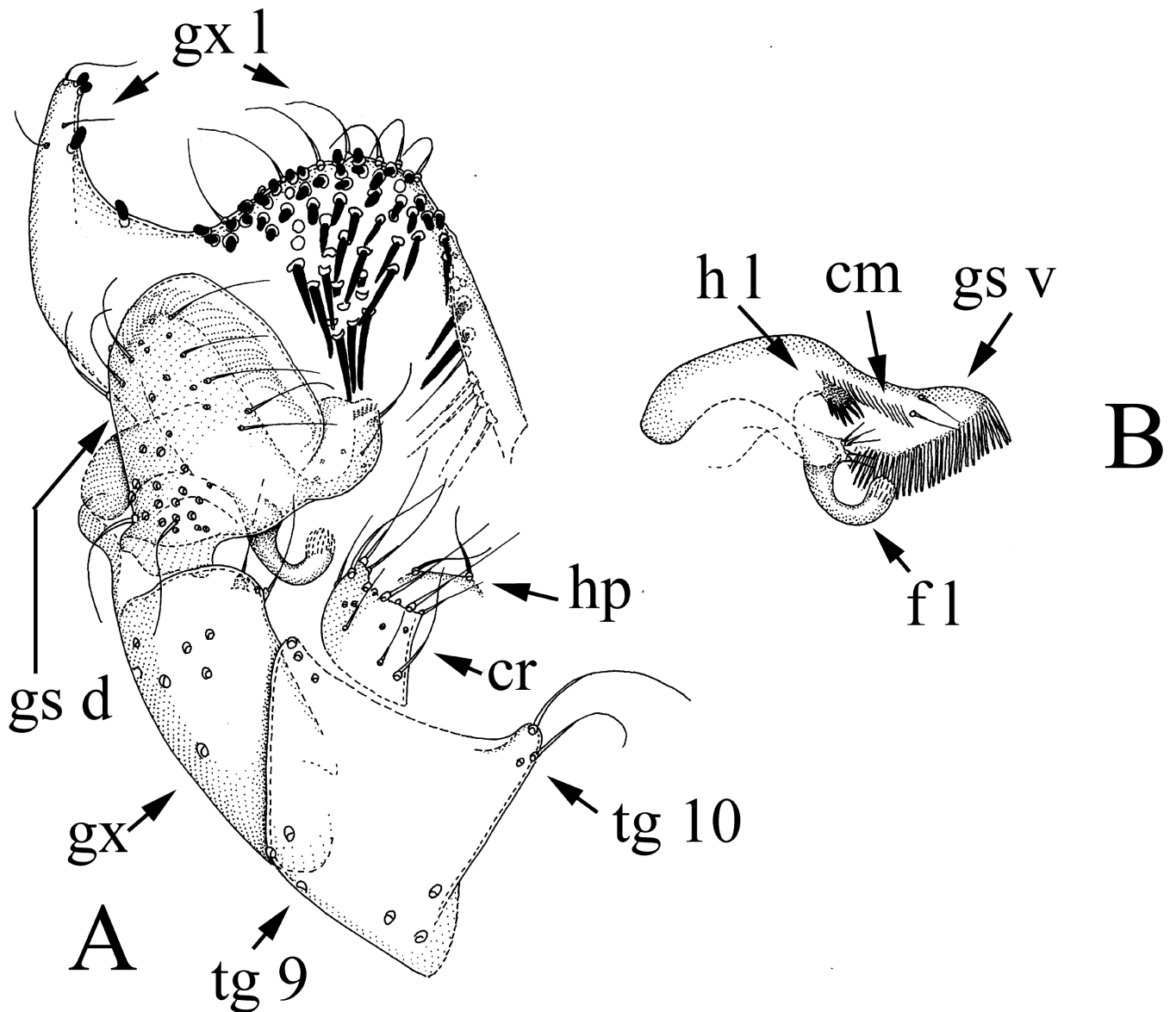


Fig. 3. *Paramanota bifalx* Hippa, 2010 (Thailand). A, Right half of hypopygium, dorsal view; B, Median lobes and the ventral lobe of gonostylus, dorsal view. Scale bar = 0.1 mm. cm = comb-like row of pale lamellae, cr = cercus, gs d = dorsal lobe of gonostylus, f l = curved finger-like lobe, gs v = ventral lobe of gonostylus, gx = gonocoxa, gx l = ventral gonocoxal lobe, h l = hand-like lobe, hp = hypoproct, tg 9 = tergite 9, tg 10 = tergite 10.

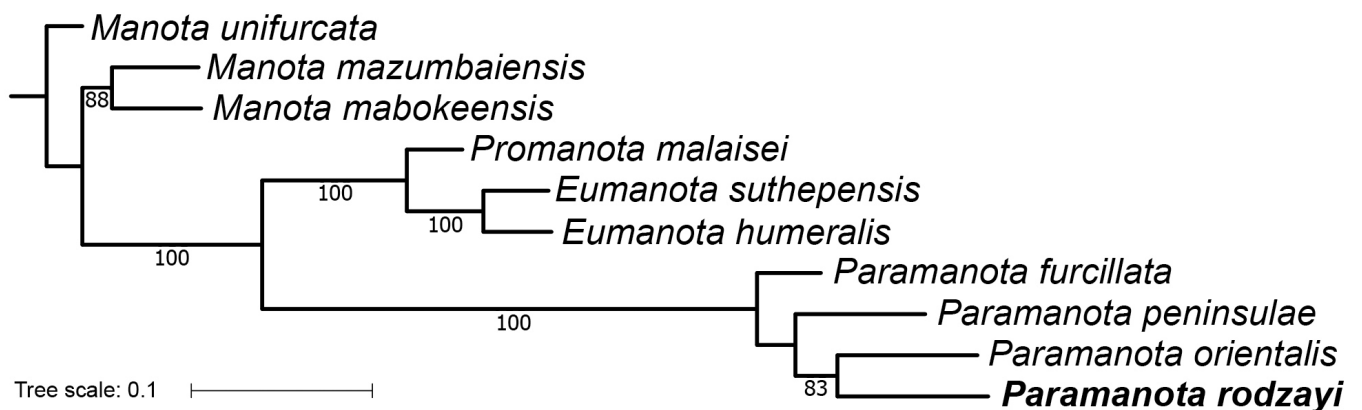


Fig. 4. Molecular affiliation of *Paramanota rodzayi* based on Maximum likelihood analysis of the combined dataset (12S, 28S, and COI). The numbers below the branches indicate bootstrap support (BS) values above 75.

almost parallel sided apical part, the details not discernible in the mount.

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. peninsulae* 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, Nakhon Si Thammarat, Namtok Yong NP, TV aerial, 8°14.262'N 99°48.289'E, 966 m, Malaise trap, 1–8 September 2008, Paiboon leg. (T3538).

Discussion. The species was earlier known only by the holotype male from the same locality in Thailand (Hippa, 2010). The present mount is tilted and some characters can be seen better than in the holotype. The curved finger-like lobe between the dorsal and ventral lobes of the gonostylus is well visible and it is visible that between this lobe and the ventral lobe of the gonostylus there is another small hand-like lobe with sclerotized lamellae as fingers. Further, on the dorsal side of the ventral lobe of the gonostylus there is a

comb-like row of fine lamellae. The curved finger-like lobe mentioned above is a good key character for *Paramanota bifalx*. A reminiscent but straight lobe is seen also in *P. paxillosa*.

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 Thammarat, Namtok Yong NP (Hippa, 2010).

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