Journal of Natural History
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tnah20

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Available online: 27 Feb 2012

To cite this article: Mitsuaki Sutou, Munetoshi Maruyama, Takashi Komatsu & Taisuke Kanao (2012): Discovery of a remarkable new species of black fungus gnat (Diptera, Sciaridae) from termite nests in Malaysia, Journal of Natural History, 46:15-16, 969-978

To link to this article: http://dx.doi.org/10.1080/00222933.2012.654478

Journal of Natural History
Vol. 46, Nos. 15–16, April 2012, 969–978

Discovery of a remarkable new species of black fungus gnat (Diptera, Sciaridae) from termite nests in Malaysia

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(*Received 30 June 2011; final version received 2 January 2012; printed 28 February 2012*)

A remarkable new species of black fungus gnat *Pnyxiopalpus roslii* sp. nov., was discovered from nests of the termite *Nasutitermes proatripennis* in Ulu Gombak, Malaysia, and is described based on the female morphology and DNA barcodes. This is the first record of a termitophilous black fungus gnat from South-east Asia. The new species is easily distinguished from its known congeners by the degeneration of all thoracic setae into tiny setulae. This species is described as a symbiont of the host termite based on field observations and the examination of specimens. The "wing stump" structure of the new species indicates that this species has wings after eclosion and later loses them, an adaptation to a cryptic life. A second still undescribed termitophilous species of *Pnyxiopalpus* was observed walking in a marching column of the termite *Longipeditermes longipes* in Ulu Gombak.

Keywords: Sciaridae; *Pnyxiopalpus*; termitophily; Malaysia; DNA barcode

Introduction

Due to the morphological and ecological diversity of social insects, their symbionts belong to various arthropod groups (Wheeler 1928; Kistner 1982). Among dipteran insects, flies of the suborder Brachycera are frequent symbionts of social insects, whereas mosquitoes, gnats, midges and flies of the suborder Nematocera are rarely symbionts (Kistner 1982). Within Nematocera, fungus gnats are members of the superfamily Sciaroidea, which is usually classified into 7–9 families, and contains ca. 7000 described species worldwide (Schumann et al. 1999). Currently, two types of fungus gnat symbionts are known: the myrmecophagous larvae of the keroplatid fungus gnats belonging to the family Keroplatidae (Aiello and Jolivet 1996; Matile 1996; Chandler and Matile 1998; Debout 2004) and the myrmecophilous and termitophilous inquilines of the black fungus gnats belonging to the family Sciaridae (Schmitz 1915; Schmitz and Mjöberg 1924; Bernard 1968 cited in Kistner 1982 and in Hölldobler and Wilson 1990; Evenhuis et al. 2007). Schmitz (1915) described the termitophilous sciarid fly *Termitosciara biarticulata* based on a female collected from the fungus garden of the termite *Odontotermes obesus* in Bombay, India. No reduction of the wings

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ISSN 0022-2933 print/ISSN 1464-5262 online

http://dx.doi.org/10.1080/00222933.2012.654478
http://www.tandfonline.com

was observed in this species. Menzel and Mohrig (2000) discussed the morphological similarity between the genera *Termitosciara* and *Cratyna* within sciarid flies. Schmitz and Mjöberg (1924) described a second termitophilous sciarid fly, *Austrosciara termitophila*, based on brachypterous females collected from the galleries of the termite *Glyptotermes trilineatus* in eastern Australia. No termitophilous fungus gnat has been recorded since these species were found.

The genus *Pnyxiopalpus* was established by Vilkamaa and Hippa (1999) with 16 new species; they included a phylogenetic analysis based on morphological characters. Vilkamaa and Hippa (2011) recently described an additional new species and presented a revised phylogeny. All known species of this genus have been recorded from South-east Asia, and their biology is unknown. We report the discovery of a termitophilous species of *Pnyxiopalpus* from Malaysia, and show its strong ecological and morphological specialization as a termitophile.

Materials and methods

Field collections were performed by MM and TKo in April 2007 and by TKa in November 2010 at the University of Malaya Field Studies Centre, Ulu Gombak, Malaysia. The new species was discovered when they carefully broke into a nest of the termite *Nasutitermes proatripennis* in rotten wood to collect termitophilous insects. Photos of live individuals in the nest were taken by TKo to record this symbiosis using a Panasonic Lumix FZ50 digital camera with a Raynox CM-3500 macro lens. The specimens were preserved in 80% ethanol. MM took micrographs of an example from various angles using a Zeiss LEO scanning electron microscope. Five specimens were transferred to 99% ethanol and were stored in a freezer (at –30°C) for subsequent molecular analysis. Seven specimens were mounted on microscope slides using xylol-based Canada balsam for later morphological description. The remaining six specimens were kept in 80% ethanol to facilitate morphological observation from various angles in future taxonomic studies. TKa collected one female of another species of sciarid fly that was walking in a marching column of the termite *Longipeditermes longipes* in December 2010 at the same site, and preserved it in 80% ethanol. The morphological terminology follows Vilkamaa and Hippa (1999). The type specimens were deposited in the Department of Zoology, National Museum of Nature and Science, Tsukuba, Japan (NSMT), the Kyushu University Museum, Fukuoka, Japan (KUM), and private collection of MS, Tokyo, Japan (PCMS).

The DNA barcode sequences of two individuals of the new species were determined using the universal primers for barcoding (Folmer et al. 1994). Total genomic DNA was extracted from all legs of each individual; the remainder of each body was kept as a voucher specimen. The temperature profile for the polymerase chain reaction (PCR) consisted of: 60 s at 94°C, 30 cycles of 30 s at 94°C, 30 s at 56°C, and 60 s at 72°C, and a final 60 s extension at 72°C. The PCR products were purified and sequenced in both directions on a CEQ8000 Genetic Analysis System. Trace files were saved in scf format. For both individuals, a 636 base pair partial fragment of the COI gene was identified as the DNA barcode. The sequences were deposited in the DNA Data Bank of Japan (DDBJ) and in the Barcode of Life Data System (BOLD) (Ratnasingham and Hebert 2007). Voucher specimens for the barcodes were deposited in NSMT as part of the paratypes.

Taxonomy

Pnyxiopalpus roslii Sutou and Maruyama sp. nov.
(Figures 1–3)

Materials studied

Holotype. female on a slide (Figures 1B, 3A–F), Malaysia, Selangor, Ulu Gombak, University of Malaya Field Studies Centre, 3°19′29″ N 101°45′11″ E, 260 m als., 7 April

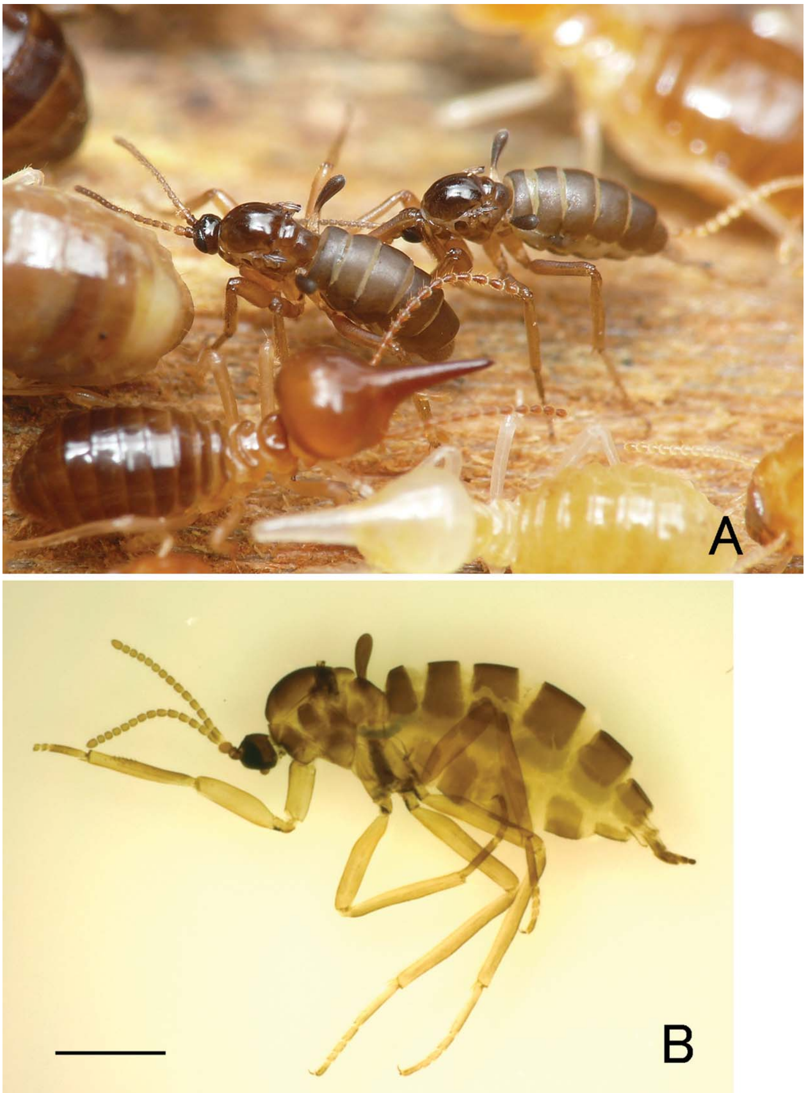

Figure 1. *Pnyxiopalpus roslii* sp. nov., habitus. (A) Alive individuals with host termites; (B) the holotype, left front leg was removed and mounted separately on the same microscope slide. Scale bar: 1 mm.

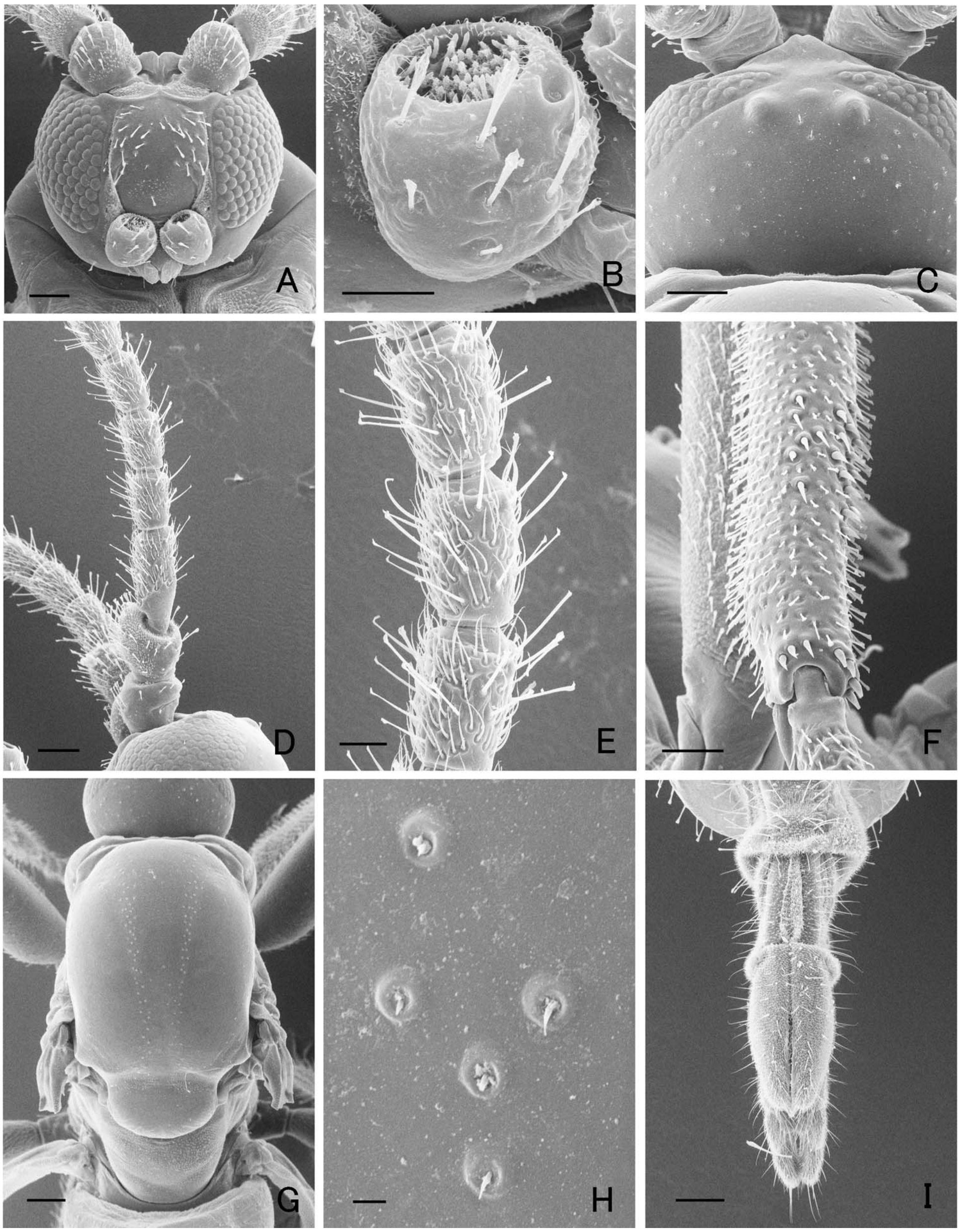

Figure 2. *Pnyxiopalpus roslii* sp. nov., scanning electron micrographs. (A) Head, frontal view; (B) right palpus; (C) head, dorsal view; (D) scape, pedicel and 1st–4th flagellomeres of antenna; (E) 5th–7th flagellomeres of antenna; (F) left front leg, anterior view; (G) thorax, dorsal view; (H) minute setae on anterior scutum; (I) ovipositor, ventral view. Scale bars: 60 μm for A, C, D, F and I; 30 μm for B and E; 100 μm for G; 4 μm for H.

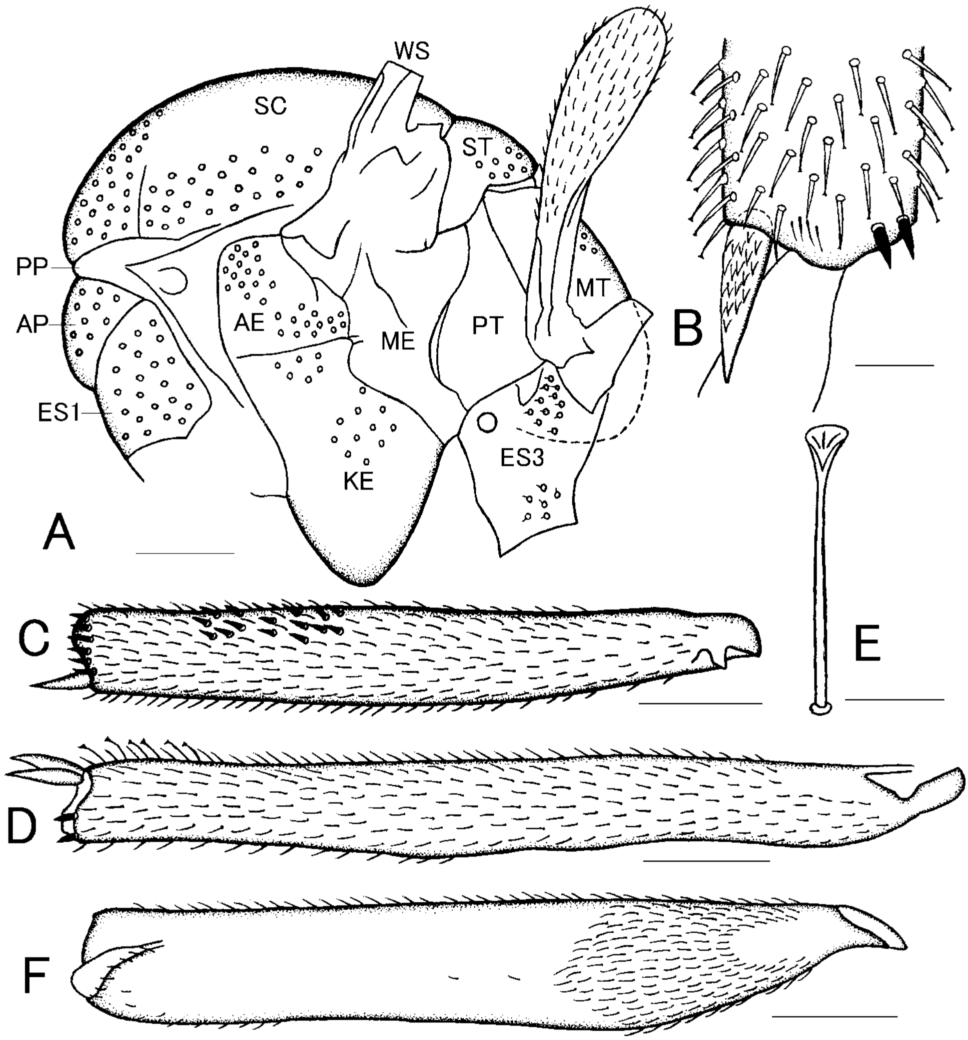

Figure 3. *Pnyxiopalpus roslii* sp. nov., thorax and legs of the holotype. (A) Thorax, lateral view; (B) inner apex of front tibia; (C) front tibia, outer lateral view; (D) hind tibia, outer lateral view; (E) proapical seta having scale-like expanded apex on hind tibia; (F) hind femur, inner lateral view. Scale bars: 0.2 mm for A, C, D and F; 0.05 mm for B, 0.02 mm for E. Abbreviations: AE anepisternum, AP anterior pronotum, ES1 episternum 1, ES3 episternum 3, KE katepisternum, ME mesothoracic epimeron, MT mediotergite, PP posterior pronotum, PT pleurotergite, SC scutum, ST scutellum, WS wing stump.

2007, M. Maruyama and T. Komatsu (NSMT-I-Dip-6783). *Paratypes.* Three females on slides and six in 80% ethanol, same data (KUM); two females on slides and two in 99% ethanol, same data (PCMS); two females in 99% ethanol as vouchers for DNA barcodes, same data (NSMT-I-Dip-6784 and 6785); one female on a slide and one female in 99% ethanol, same locality, 27 November 2010, T. Kanao (KUM).

Symbiotic host

Nasutitermes proatripennis (Ahmad, 1965)

Etymology

Dedicated to Dr Rosli Hashim who supported our fieldwork in Ulu Gombak.

Female

Head. Polished dark brown. Compound eyes blackish, each eye very sparsely with a few interfacetal setae. Eye bridge constricted medially (Figure 2C). Anterior ocellus smaller than posterior ones (Figure 2C). Anterior vertex prominent in lateral and dorsal view without setae (Figure 2C); clypeoprefrons with setae whose apex expanded apically (Figure 2A). Antenna consists of dark brownish scape and brownish pedicel and flagellomeres; proximal part of 1st flagellomere yellowish; 4th flagellomere about 1.8 times longer than wide with short neck (Figure 2D). Setae of antennae apically expanded (Figure 2E). Palpus one-segmented with 6–8 setae whose apex expanded apically, and with deep hollow in which many sensillae present (Figure 2B). Proboscis weakly developed (Figure 2A). *Thorax*. Polished dark brown. Thoracic chaetotaxy is as shown in Figure 3A: posterior pronotum, methothoracic epimeron and pleurotergite bare; scutum, scutellum, mediotergite, anterior pronotum, episternum 1, episternum 3, anepisternum and katepisternum with minute setae. Dorsal minute setae on scutum in two rows; minute setae on anepisternum and katepisternum weakly divided into two patches; short setae on episternum 3 clearly divided into two patches. All thoracic setae remarkably reduced: only sockets are visible at low magnification ($< \times 200$), but minute setae are observed at high magnification (Figure 2H); exceptionally short setae on episternum 3 visible at low magnification (Figure 3A). Scutellum weakly developed (Figure 3A). Wing stump normally truncate (Figures 2G, 3A). Halter long, knob covered by many short setae (Figure 3A). *Legs*. Brown. Front tibia with a patch of 8–20 dark brownish prolateral enlarged setae on distal two-fifth or on distal half, and with 4–7 dark brownish enlarged setae on outer apex (Figures 2F, 3C); inner apex of front tibia with two dark brownish enlarged setae and a tibial spur, and without differentiated patch of setae (Figure 3B). Front femur proximally with dense ventral setae. Middle tibia with 3–6 dark brownish enlarged setae and two spurs apically. Inner surface (outer surface depending on mounted angle) of middle femur with numerous setae proximally (similar to Figure 3F). Hind tibia with two dark brownish enlarged setae and two spurs apically, and posterior surface slightly undulating in lateral view (Figure 3D); proapical setae of hind tibia apically expanded forming scale-like apex (Figure 3E). Inner surface of hind femur with numerous setae proximally and nearly bare on middle and distal surface (Figure 3F). All tibial spurs covered by scale-like appressed setae (Figure 3B). All trochanters with dense ventral setae. *Abdomen*. Tergites and sternites dark brown. First–fifth tergites with minute setae similar to those on thoracic sclerites; sixth–ninth tergites and all sternites with short setae. Membranous parts of abdomen without setae but with microtrichia ventrally. Ovipositor bilobate ventrally with short setae (Figure 2I). *Body length.* 3.3–4.4 mm, from base of antenna to apex of abdomen.

Male

Unknown.

Remarks

Vilkamaa and Hippa (1999) described the following eight unique diagnostic characters of the genus *Pnyxiopalpus*: (1) front tibiae with enlarged conical setae; (2) tibial spurs covered by scale-like appressed setae; (3) tarsi with a medial ventral keel and a row of tightly placed setae; (4) setae of female femora and tibiae apically expanded; (5) thorax and femora lacking vestiture of microtrichia; (6) female abdomen divided into a broad preabdomen and a narrow postabdomen, the former consisting of the first six segments; (7) wing veins r-m, R and R1 ventrally setose; and (8) female mesothoracic pleura richly setose. This new species also has these characters, except for (7) not being studied due to the lack of wings. This species is easily distinguished from the known congeners by the degeneration of all thoracic setae (Figure 3A) and dense setosity on the inner proximal part of the middle and hind femora (Figure 3F). Condition of the character (4) of this species is remarkable as follows: apically expanded setae are observed in various parts of the body, coxae, trochanters, femora, tibiae and basitarsomeres of the legs (Figure 3E), clypeoprefrons (Figure 2A), palpi (Figure 2B), antennae (Figure 2E) and haltere. See the Discussion for the biology of this species.

DNA barcodes

The DNA barcode sequences of two females were identical. They were deposited in DDBJ under the accession numbers AB638762 and AB638763, and also in BOLD with barcode IDs JBOL053-11 and JBOL054-11.

Pnyxiopalpus sp.

Material studied

One female in 80% ethanol, same locality, 15 December 2010, T. Kanao (KUM).

Symbiotic host

Longipeditermes longipes (Haviland, 1898)

Remarks

This is an undescribed species of *Pnyxiopalpus* found in association with *Longipeditermes longipes*. The single female recorded here also has wing stumps, but the condition is very peculiar: the most membranous part of the wing, including the M and Cu veins, is lost, but the anterior basal veins, R, R1, Rs, bM, r-m, basal half of C, and proximal part of R5 remain. We preliminarily treat this species as *Pnyxiopalpus* sp. because only one female is currently available. The condition of the wing should be examined in multiple specimens of this species, preferably of an individual with fully developed wings. However, the locality and host record are worth reporting here.

Discussion

The biology of *Pnyxiopalpus* is unknown (Vilkamaa and Hippa 1999). However, our discovery of termitophilous species sheds light on the biology of this genus, which has the unique morphological characters described above. We think that the new species *P. roslii* is certainly a symbiont of the host termite for the following reasons. First, MM and TKo observed a mutual relationship between *P. roslii* and the termites in the field (Figure 1A). In 2007, *P. roslii* was found among gregarious individuals of *Nasutitermes proatripennis* inhabiting a standing piece of hard rotten wood, which measured about 30 cm in diameter and 200 cm in height. *P. roslii* and the termites touched each other closely, and the former was never attacked by the termites. Second, TKa found *P. roslii* again in a *Nasutitermes proatripennis* nest in 2010. This indicates that their mutual relationship continues over years. We note that *P. roslii* was found in April in 2007, but in November in 2010. The third reason is that *P. roslii* has wing stumps (Figures 2G, 3A), which are formed by shedding normal wings. Wing stumps have been observed in the termitophilous scuttle fly *Echidnophora dondroi* (Disney 1995), and it is found here for the first time in a nematoceran dipteran, *P. roslii*. All of the known apterous or brachypterous sciarid flies lack halters or have small rudiments, and their thorax is flat due to loss of the flight muscles. One such example is the Australian termitophilous sciarid fly *Austrosciara termitophila* (see Figure 1 of Schmitz and Mjöberg 1924). The well-developed scutum and long halters of *P. roslii* (Figure 3A) are nearly the same as those of the winged congeners (see figure 4 of Vilkamaa and Hippa 1999). This shows that this species has wings just after eclosion and that these are later shed, resulting in the formation of the wing stumps. The loss of the wings increases their mobility in the termite nest, and saves the energy required to maintain them. Therefore, we think that the wing stumps of *P. roslii* shows morphological specialization as a symbiont in a termite nest. *Pnyxiopalpus* sp. reported here also has wing stumps.

We described here the new species with DNA barcodes. As one of the benefits of DNA barcoding, a specimen can be identified at any life stage, using a variety of tissue types (Stoeckle 2003; Blaxter 2004; Savolainen et al. 2005; Waugh 2007). In arthropods, it has been used not only for assessing species diversity (Smith et al. 2005) but also for linking different life stages (Richard et al. 2010; Stur and Ekrem 2011; Sutou et al. 2011) and for associating conspecific males and females (Sheffield et al. 2009; Ekrem et al. 2010; Kurina et al. 2011). Therefore, the DNA barcodes of *P. roslii* provided here will be useful for correctly identifying the larvae, winged females, and males of this species. This will assist further studies of this interesting species.

Vilkamaa and Hippa (1999, p. 217) stated that "*Pnyxiopalpus* is exceptional in Sciaridae regarding the interspecific variation of female characters." This suggests that each species of this genus, especially the females, has a unique habit. Here, we show that females of at least two species of this genus are termitophile. The myrmecophilous sciarid *Vulagisciara myrmecophila* was sister to *Pnyxiopalpus* in the phylogenetic analysis of Vilkamaa and Hippa (2011). As well as seeking more examples of *Pnyxiopalpus* from the nests of social insects, our future endeavour will be to understand how termitophily evolved in this Oriental genus.

Acknowledgements

We thank Dr Rosli Hashim (University of Malaya) for his assistance in the field. MS thanks Dr Motomi Ito (University of Tokyo) for facilitating the molecular experiments. This paper was partially supported by a JSPS Postdoctoral Fellowship for Research Abroad and Grants-in-Aid for Scientific Research of JSPS (Young Scientists B: 22770085) to M. Maruyama.

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