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Freshwater trichomycetes from northern Thailand

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Abstract – Collections of black fly (Diptera: Simuliidae) larvae were made at previously unexplored lotic habitats in northern Thailand and their gut fungi identified. Eight trichomycete species were recovered, including one new species, *Harpella asiana*, and two possible new species: *Graminelloides cf. biconica* and *Harpella cf. tica*. *Paramoebidium* sp., *Pennella montana* Lichtwardt, *Stachylina nana* Lichtwardt and *Stipella vigilans* Léger & Gauthier are new Thailand records. The widely distributed species of gut fungus, *Harpella melusinae* Léger & Duboscq, that has previously been reported from Thailand, was also recovered. We have shown that trichomycetes are abundant in northern Thailand and undoubtedly many other Harpellales, beyond the taxa we report here, are present especially in unexplored regions of the country and in different hosts, habitats and times of the year. These findings correspond to 40% (6/15) of the trichomycete genera previously reported from tropical regions worldwide.

Amoebidiales / aquatic habitats / Harpellaceae / Kickxellomycotina / Legeriomycetaceae / macroinvertebrate

INTRODUCTION

The term "trichomycetes", previously known as Class Trichomycetes (Lichtwardt *et al*., 2001) belonging to Phylum Zygomycota, has recently been used to describe an ecological group of fungi and protists living in the guts of arthropods. Hibbett *et al*. (2007) recognized the polyphyletic nature of endobiotic microorganisms living in the guts of arthropods and this interpretation and terminology is used here.

Two orders of trichomycetes, Amoebidiales and Eccrinales, are now known to be related to Mesomycetozoan (protists), leaving the fungal group with two orders, Harpellales and Asellariales (Lichtwardt, 1996; Benny & O'Donnell, 2000; Lichtwardt *et al*., 2001; Cafaro, 2005; White *et al*., 2006a).

Although traditionally regarded as commensals, they are known to encompass the entire spectrum of symbiotic relationships, from commensalism to mutualism or parasitism, partly dependent on nutritional or environmental circumstances (Lichtwardt *et al*., 2003; Nelder *et al*., 2006). For example, *Smittium culisetae* may act as a commensal when the mosquito larvae (*Aedes agyptii*) are fed, but benefit the mosquito host when essential host nutrients are deprived. On

the other hand, another species of *Smittium, S. morbosum*, kills mosquito larvae (Horn & Lichtwardt, 1981; Lichtwardt *et al*., 2003), and the relationship is much narrower.

Whereas some members of Asellariales and Eccrinales are marine and terrestrial, nearly all of the Harpellales and Amoebidiales occur in freshwater. Harpellales comprise two families depending on the part of the gut where the endosymbionts are attached. Harpellaceae, characterized by their unbranched thalli and determinate growth occur in the peritrophic matrix (sometimes referred to as peritrophic membrane), and Legeriomycetaceae, characterized by branched thalli and undeterminate growth, occur in the hindguts. Within the Amoebidiales, species of *Paramoebidium* occur in the hindgut but species of Amoebidium are ectocommensals (Lichtwardt *et al*., 2001; 2003; Beard & Alder, 2002; Beard *et al*., 2003).

Hosts of Harpellales include black fly larvae, as well as larval mosquitoes, chironomid, soldier flies, coleoptera (family Scirtidae), and trichoptera and nymphs of mayflies and stoneflies, with one species from Isopoda (Lichtwardt *et al*., 1999; 2001; White, 1999; White *et al*., 2000; Strongman & White, 2008). Members of the Amoebidiales have been reported from many families of Crustacea, Insecta and Isopoda (Lichtwardt *et al*., 2001). Black fly larvae (Diptera: Simuliidae) are host to ten trichomycete genera, and have received the most attention as a trichomycete host because of their ease of collection, ubiquity, and status as one of the best-known taxonomic groups of aquatic insects at the species level (Adler *et al*., 2004, cited in Nelder *et al*., 2006). Interestingly, adult female black flies can carry and disperse certain species of Harpellales. *Gennistellospora homothallica*, *Harpella melusinae* and *Pennella simulii* have been reported as chlamydospores or cysts dissected from the ovaries of infected black flies (Moss & Descals, 1986; Labeyrie *et al*., 1996; White *et al*., 2006b).

Although trichomycetes can be found wherever the hosts occur, they are mostly reported from temperate regions. Only 15 genera (27%) and 39 described species (17%) have been reported from tropical regions (Misra, 2001). *Harpella melusinae* is the only previously known trichomycete species from Thailand (Takaoka & Adler, 1997).

We are carrying out studies on the biodiversity of fungi from various habitats in northern Thailand (e.g. insects: Aung *et al*., 2008; leaf litter: Duong *et al*., 2008; wood: Kodsueb *et al*., 2008a, b; monocotyledons: Pinruan *et al*., 2007; Thongkantha *et al*., 2008) which have previously been poorly studied. In the present study we investigated the trichomyctees occuring in black fly larvae in streams in northern Thailand.

MATERIALS AND METHODS

Collections were limited to lotic habitats (streams) in two provinces, Chiang Mai and Chiang Rai. Black fly larvae were collected randomly from the substrate, such as on the surfaces of trailing vegetation, sticks or rocks. Forceps were used to place hosts on moist filter paper in Petri dishes, for transport to the laboratory on ice. Whenever possible, 30 black flies larvae were collected from each site, with a total 270 larvae dissected. Each site was sampled once from April to November 2008. The collection dates and sites are listed in Table 1. Voucher slides are maintained in the herbarium of Mae Fah Luang University (MFLU).

Black fly larvae were dissected in a drop of water with the aid of a dissecting microscope. Gut fungi were then slide-mounted for identification using a compound microscope, following the procedure provided by Lichtwardt *et al.* (2001) (See Box 1 below). Wet mounts of gut fungi were photographed live

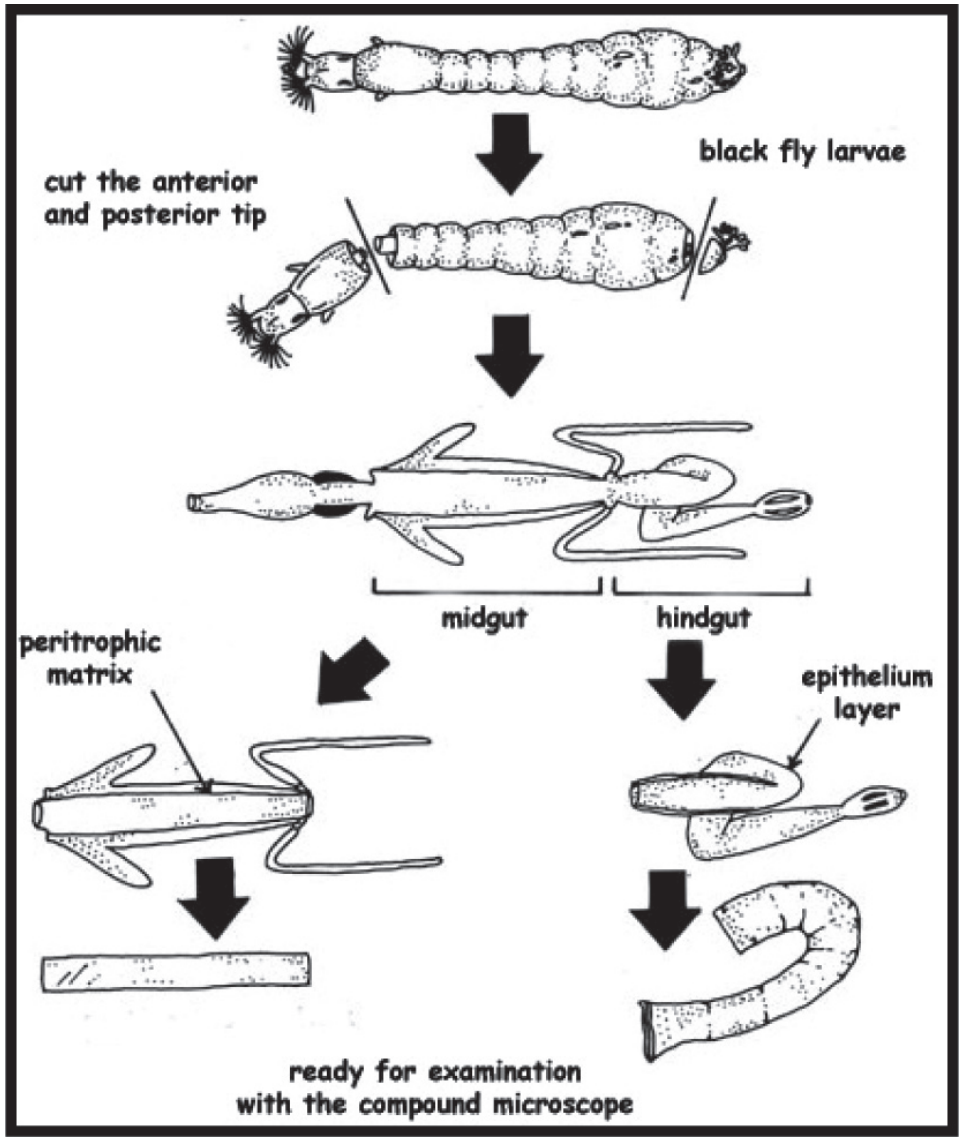
Table 1. Details of sites and dates of collection of black fly larvae.

Site[a]	*Date*	*Location*	*Latitude/ Longitude*	*Elevation (m)*	*Water Temperature (°C)*
1	05 May 08	Huai Khang Pla Waterfall (slow moving, unshaded, shallow rocky stream with mostly bamboo trees along the riverbanks)	N 20°05' E 099°46	512	24
2	19 July 08	Nang Lae Nai Waterfall (gravel bottom, fast flowing stream, muddy and unshaded)	N 20°02' E 099°49'	445	22
3	14 Nov 08	Pong Phrabat Waterfall (rocky, shallow, clear cold stream, located at Lam Nam Kok National Park)	N 20°00' E 099°48'	459	21
4	20 Oct 08	Huai Mae Sai Waterfall (rocky, clear cold stream, surrounded by diverse vegetation)	N 20°00' E 099°43'	522	21
5	21 Sept 08	Khun Khon Waterfall (rocky, clear cold stream, located at Lam Nam Kok National Park)	N 19°52' E 099°37'	671	19
6	14 April 08	New Waterfall (heavily shaded, cold and muddy stream)	N 19°52' E 098°41'	781	19
7	15 April 08	Fast flowing, cold stream located at Pong Dueat Hotspring National Park	N 16°06' E 099°43'	711	20
8	06 Aug 08	Slow flowing, silty and muddy stream located at Mae Sae village	N 19°14' E 098°39'	962	24
9	06 Aug 08	Mork Fah Waterfall (cold and clear stream, shaded by various vegetation)	N 20°02' E 099°52'	596	22

Note. [a] Site 1-5 are located in Chiang Rai Province. Site 6-9 are located in Chiang Mai Province.

(Nikon Coolpix 4500) or after infiltration of the specimens with lactophenol-cotton blue. Coverslips were sealed with clear fingernail polish to make the slides semi-permanent and deposited as vouchers at Mae Fah Luang University (MFLU) Herbarium. In some cases, the entire gut with gut fungi was placed in 2X CTAB buffer for ongoing molecular systematic studies. All the drawings were made from the semi-permanent slides using a Lucida Microscope.

Upon removal of the head, and optionally the last segment with the anus, the digestive tract may be removed from the host. Subsequently the midgut (left)



Box 1. Overview of dissection method (Lichtwardt *et al*., 2001).

and hindgut (right) are separately microdissected to extract the peritrophic matrix and the chitinous lining of the hindgut to check for microscopic gut fungi.

Species of gut fungi identified

Some of the following species appear to be new to science, but are not named here due to lack of the important characters in our collections.

Unidentified *Paramoebidium* **Fig 1**

Thalli straight to slightly curved, unbranched, non-septate, 335-375 × 7.5-10 µm. Attached to the hindguts of black fly larvae by a holdfast that are wider than the thallus. Amoeboid cells, 37.5-50 × 5-7.5 µm.

Material examined: Thailand, Chiang Mai Province, New Waterfall, in hindguts of black fly larvae, 04-10-2008, 20°C, M.P. Hapsari and P. Sysouphanthong, MFLU 08 1493; Chiang Rai Province, 20-10-2008, 19°C, *ibid*. MFLU 08 1519.

Notes: *Paramoebidium* sp. was found inhabiting the hindguts of black fly larvae and was common at most of the sites (Site 3, 4, 6, 7 and 9), but the specimens could not be identifed to species level based on the material collected. This is only the fourth tropical region where *Paramoebidium* spp. have been reported. The previous reports of the occurence of *Paramoebidium* spp. in tropical regions were in Australia, Costa Rica and Puerto Rico. Four species of

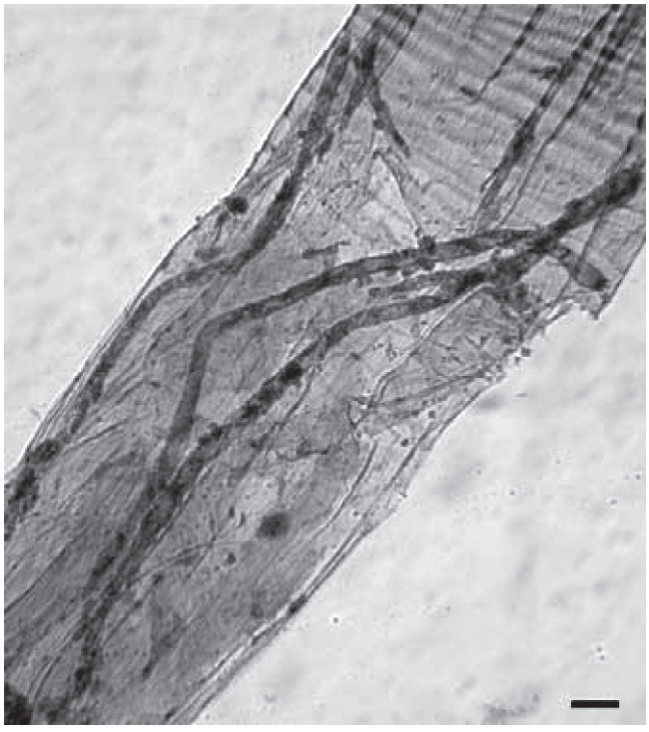


Fig 1. Several thalli *Paramoebidium* sp. attached to a black fly hindgut (Bar: 20 µm).

this genus have previously been reported from black fly larvae. *Paramoebidium* spp. also are considered cosmopolitan, but are likely to have been underestimated because of difficulties in identifying the species based on morphological criteria and the inability to culture *Paramoebidium* spp. (White *et al*., 2000).

***Graminelloides cf. biconica* Lichtwardt, 1997** **Fig 2**

Thalli unbranched, 62.5 µm long and 3-5.5 µm in diameter, attached to the hindgut cuticle of black fly larvae. The holdfasts are tapered and without mucilaginous secretion. The slightly curved thalli producing 7-12 trichospores, one from each generative cell. Trichospores are bluntly biconical (28-40 × 3-4 µm). Released trichospores were observed but the appendages were not clearly seen.

Material examined: Thailand, Chiang Rai Province, Nang Lae Nai Waterfall, in a hindgut of black fly larvae, 19-07-2008, 22°C, M.P. Hapsari and R.A. Kristanti, MFLU 08 1478.

Notes: One larvae of Simuliidae from Nang Lae Nai Waterfall (Site 2) harboured *Graminelloides cf. biconica* attached to its hindgut. Although no released trichospores were observed, we consider our collection to be *Graminelloides* as it has a tapered holdfast, one important character of this genus (Lichtwardt *et al*., 2001; Misra, 2001). Our collection resembles the monotypic species, *G. biconica*. It differs, however, in having shorter thalli (62.5 × 3-5.5 µm

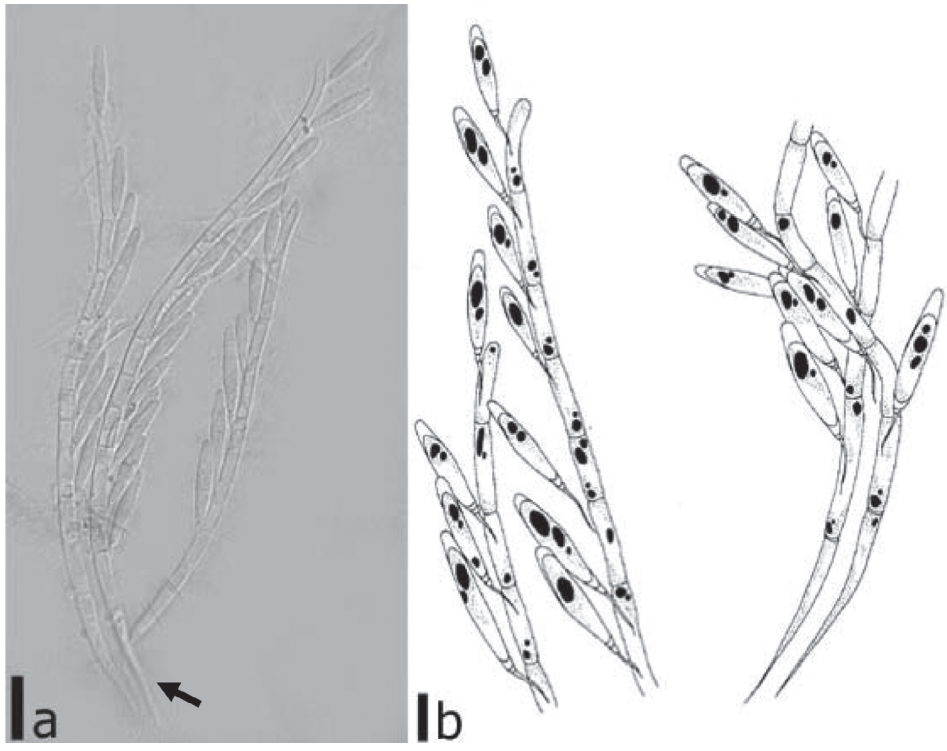


Fig 2. a & b. *Graminelloides cf. biconica* Lichtwardt. Note the tapered holdfast (arrowhead) and unicellular bluntly biconical trichospores (Bars: Fig a = 20 µm; Fig b = 10 µm).

vs. > 350 × 5-8 μm, *sensu* Lichtwardt *et al*., 2001) and in having larger trichospores (28-40 × 3-4 μm vs. 10-16 × 4.5 μm, *sensu* Lichtwardt *et al*., 2001). Since we only obtained one collection we consider it premature to introduce a new species to accommodate this taxon.

The monotypic genus, *Graminelloides*, was described from Costa Rica in black fly larvae and has never been reported elsewhere (Lichtwardt, 1997; Misra & Lichtwardt, 2000; Lichtwardt *et al*., 2001). Although specimens in our collections lacked released trichospores (released trichospores show the shape and size of the appendage(s) and are useful for generic identification (Lichtwardt, 1996)) we did observe a tapered base without mucilaginous (Fig. 2). This characteristic has never been reported from any other recently described trichomycete genera (Misra & Lichtwardt, 2000; Lichtwardt *et al*., 2001).

Harpella asiana **Hapsari, M.M. White & K.D. Hyde, sp. nov.** **Fig 3**

Thallus simplex, curvatiusculae (125-250 × 4-5 μm). Cellulae tenax processu acuto praedita tenuis secreta. Thallus formas cellulae genitales 2-4, unaquaque trichosporam curvatiusculae 50-75 × 4-5 μm. Quadro appendices helicte dispositae intra cellulam genitalem ante trichosporum liberotianem. Zygosporae nor inventae. Thalli in membrana peritrophica larvarum Simuliidarum (Dipterorum) affixa.

Thalli unbranched, slightly curved or straight, 125-250 μm long and 4-5 μm in diameter. Attached to the peritrophic matrix with a tapered secreted holdfast stucture. Thalli producing 2 to 4 generative cells, each producing one curved to tightly coiled trichospores (50-75 × 4-5 μm). Four helical appendages seen through the thallus prior to detachment. Attached to the peritrophic matrix of black fly larvae (Diptera: Simuliidae).

Etymology: In reference to its discovery in Asia.

Holotype: Thailand, Chiang Rai Province, Huai Mae Sai Waterfall, from the peritrophic matrix of black fly larvae, 20-10-2008, M.P. Hapsari and P. Sysouphanthong, microscope slide, MFLU 08 1518.

Other material examined: Thailand, Chiang Mai Province, New Waterfall, in peritrophic matrix of black fly larvae, 04-10-2008, 20°C, M.P. Hapsari and P. Sysouphanthong, MFLU 08 1503; *ibid*. MFLU 08 1505; *ibid*. MFLU 08 1506; *ibid*. MFLU 08 1507; Pong Dueat Hotspring stream, 03-11-2008, 22°C, *ibid*. MFLU 08 1480; Chiang Rai Province, Huai Mae Sai Waterfall, 20-10-2008, *ibid*. MFLU 08 1516; *ibid*. MFLU 08 1517.

Notes: Five species of *Harpella* have been described (Lichtwardt *et al*., 2001). Although the thalli and trichospores of *H. amazonica* are similar in width to *H. asiana*, these two species have different holdfast structures. The holdfast is rounded and without secreted material in *H. amazonica*, whereas it is tapered and with a small amount of secreted material in *H. asiana*. Our collection is different from *H. leptosa*, as that fungus has its diameter of trichospores generally narrower than its thalli, and its holdfast structure is without secreted materials. *Harpella asiana* can be distinguished from *H. meridianalis* by the dimension of the thalli and trichospores, which is larger in *H. meridianalis* and also by the different holdfast structure. *Harpella asiana* is different from *H. tica* in the dimension and shape of trichospores. Although the trichospores of *H. tica* are rarely curved (mostly sigmoid), thus similar to *H. asiana*, *H. tica* trichospores have a rounded base. Of these, *Harpella asiana* most closely resembles *H. melusinae*, a widespread species reported from all continents (except Antarctica) where Simuliidae occur.

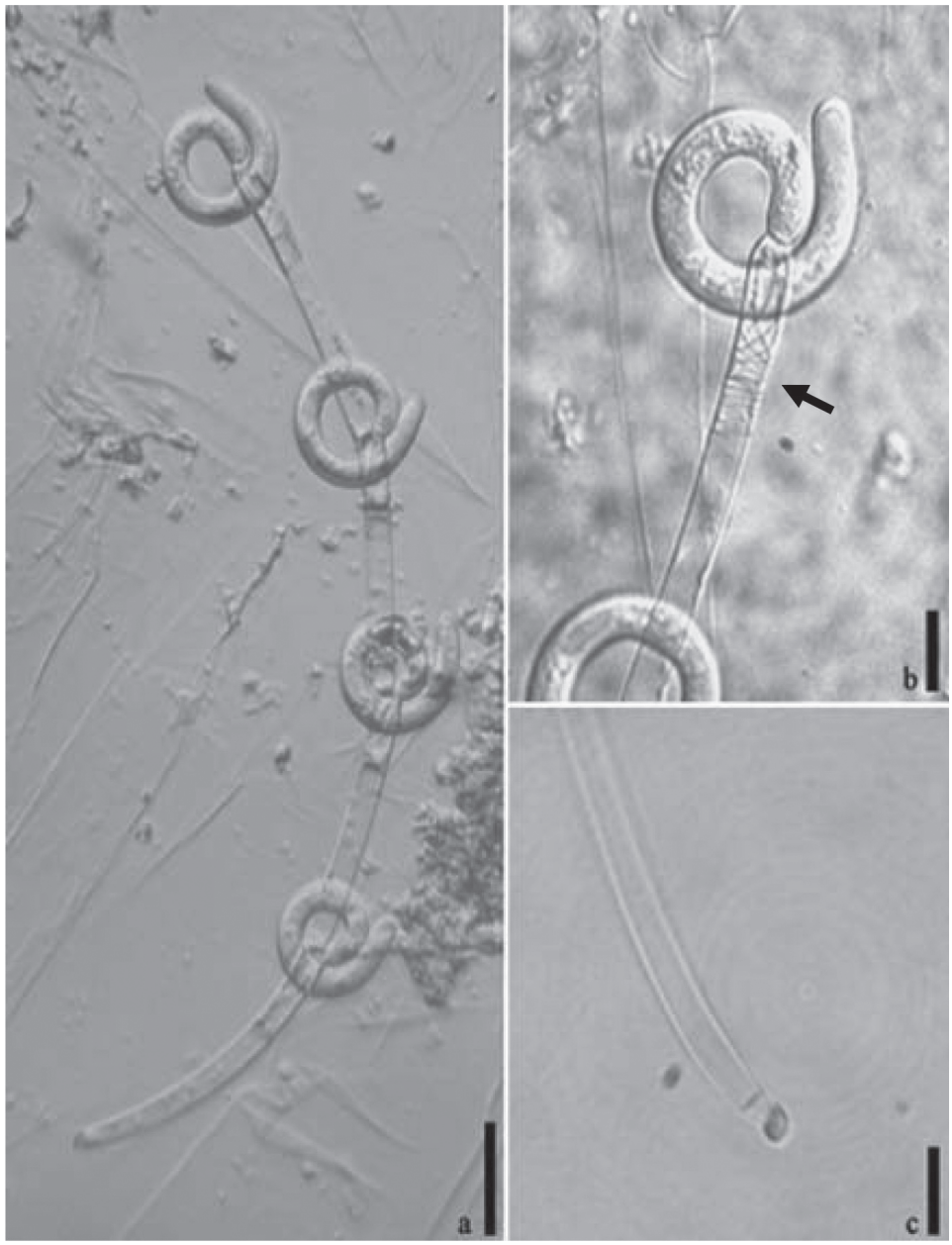


Fig 3. (a) *Harpella asiana*. a. *H. asiana* thallus bearing four coiled trichospores, attached to the peritrophic matrix of a black fly larvae; b. Attached trichospore with four helical appendages, seen through the thallus (arrow); c. Holdfast, abruptly tapering with small amount of secreted material. (Bars: Fig a = 25μm; Fig b & c = 10 μm).

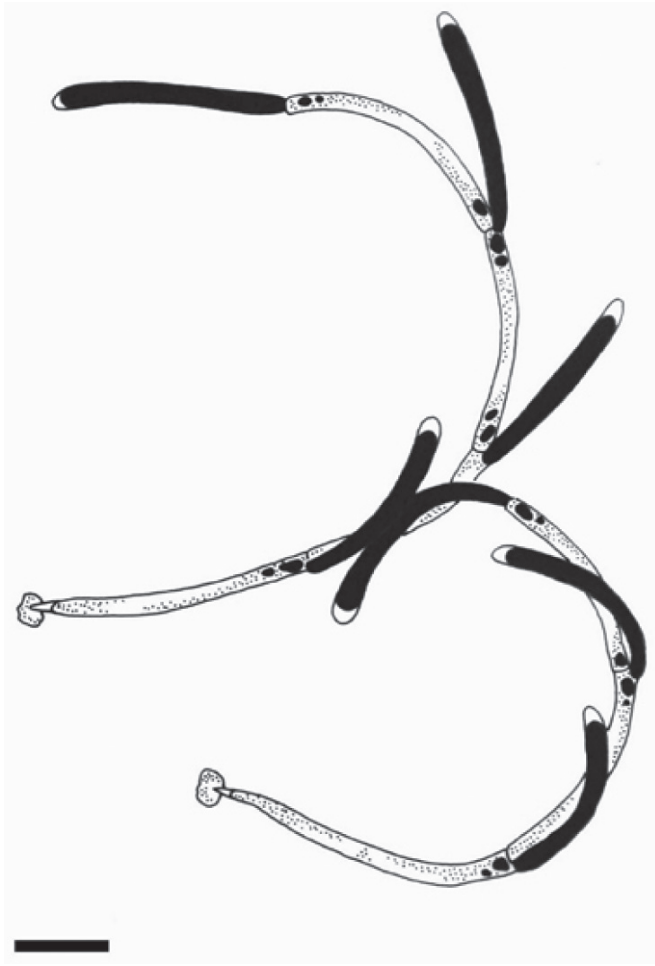


Fig 3. (b) *Harpella asiana* (Bar: 25 μm).

Several differences, however, are evident. *H. melusinae*, on the average, has larger trichospores (approximately 150 × 6-10 μm *sensu* Lichtwardt *et al*., 2001), and thalli are generally longer and wider (> 750 μm long by 6-10 μm diam *sensu* Lichtwardt *et al*., 2001). Although *H. melusinae* may rarely have as few as two trichospores, more commonly it produces 2-10 trichospores. Thalli of *H. asiana* most commonly produce only 2-4 trichospores. The new species commonly occurred on the same peritrophic matrix with *H. melusinae* and the differences of the two species are notable when they occurred along side each other as *H. melusinae* is about twice as large as compared to *H. asiana*.

***Harpella melusinae* Léger & Duboscq, 1929** **Fig 4**

Thalli slightly curved, unbranched, 135-250 μm long and 8-10 μm in diameter, attached to the peritrophic matrix of black fly larvae by a tapered secreted holdfast. Thalli producing 2-4 trichospores. Trichospores curved to

straight, 80-150 µm long and as wide as the thallus. Released trichospores were found but the appendages were not seen clearly.

Material examined: Thailand, Chiang Mai Province, Pong Dueat Hotspring stream, on peritrophic matrix of black fly larvae, 31-07-2008, 20°C, M.P. Hapsari and P. Sysouphanthong, MFLU 08 1508; *ibid*. MFLU 08 1504; *ibid*. MFLU 08 1509; *ibid*. MFLU 08 1510; New Waterfall, 19°C, *ibid*. MFLU 08 1511; *ibid*. MFLU 08 1512; *ibid*. MFLU 08 1513; Chiang Rai Province, Khun Khon Waterfall, 21-09-2008, 19°C, *ibid*. MFLU 08 1514; *ibid*. MFLU 08 1515; Huai Mae Sai Waterfall, 20-10-2008, *ibid*. MFLU 08 1517.

Notes: *Harpella melusinae* was common in our collections, and in most instances thalli were found conjugating. Conjugating thalli of *H. melusinae* have been reported to be common locally and temporally in host populations containing midgut-inhabiting Harpellales but zygospores rarely are found (Lichtwardt *et al*., 2001). Similar conjugations also have been reported from Japan (Lichtwardt *et al*., 1987). Although trichospores were no longer attached to the thalli while they were conjugating, and no zygospores were observed, we consider these conjugating thalli to be *H. melusinae* based on the tapered holdfast structure with secreted material that is broader in the area of attachment with the peritrophic matrix. Moreover, this species is the only *Harpella* species known to be capable of conjugating and producing zygospores (Lichtwardt *et al*., 2001). We found conjugation to be common at five sites, Site 3, 4, 5, 6 and 7. In some instances, we found *H. melusinae* thalli together with *Stachylina nana* (MFLU 08 1496, MFLU 08 1497 and MFLU 08 1498) attached to the same peritrophic matrix. In other cases, we found this species inhabiting the peritrophic matrix while members of Legeriomycetaceae (e.g. *Pennella*, *Stipella*) were inhabiting the hindguts. It is not uncommon to find one larvae to be infested by more than one species of gut fungi (Lichtwardt & Williams, 1999).

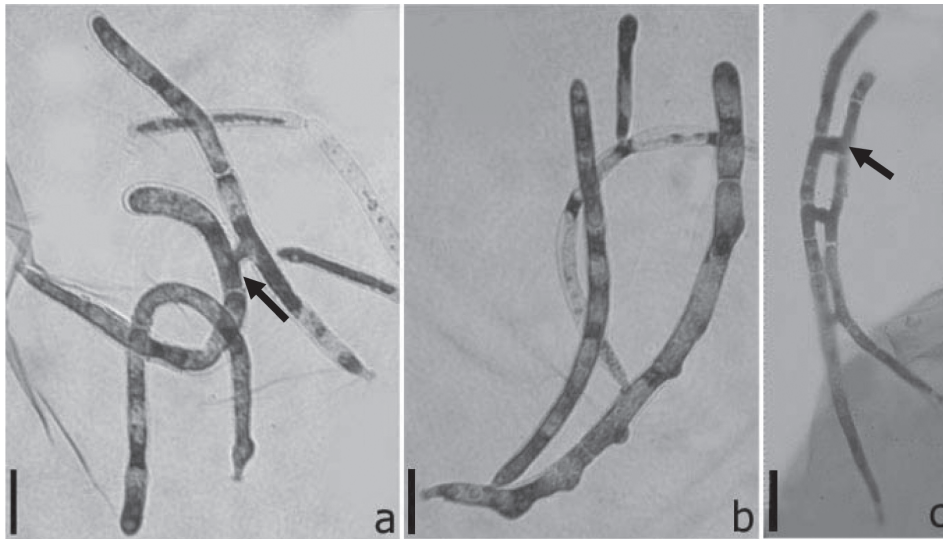


Fig 4. a-c. Conjugating thalli (arrows) of *Harpella melusinae* (Bars: 25 µm).

***Harpella cf. tica* Lichtwardt, 1997** **Fig 5**

Unbranched undulating thallus, 200-237.5 µm in length and 6 µm in diameter, attached to the peritrophic matrix of the host midgut by a tapered base, bearing 7-8 trichospores. Trichospores straight, long-cylindrical (62.5-87.5 × 3 µm), produced from a generative cell that is shorter than the trichospores (20-27.5 µm). No released spore were observed in our collection.

Material examined: Thailand, Chiang Mai Province, Pong Dueat Hotspring stream, 15-04-2008, 19°C, M.P. Hapsari and P. Sysouphanthong, MFLU 08 1477.

Notes: A single black fly larvae collected from Pong Dueat Hotspring stream (Site 7) had on its peritrophic matrix what appears to be *H. tica* but with several differences. The trichospores in our collection are straight to slightly curved, not sigmoid as mentioned in the original description of *H. tica*. Another difference is that the holdfasts in our collection are not rounded but tapered with shorter generative cells (20-27.5 µm vs. 40-60 µm *sensu* White *et al*., 2000). Therefore we consider our collection to represent a new species but it is not named here as there is only one collection.

Harpella tica, has been reported from the tropical Costa rica and Puerto Rico (White *et al*., 2000) from black fly larvae. Its occurence in Puerto Rico

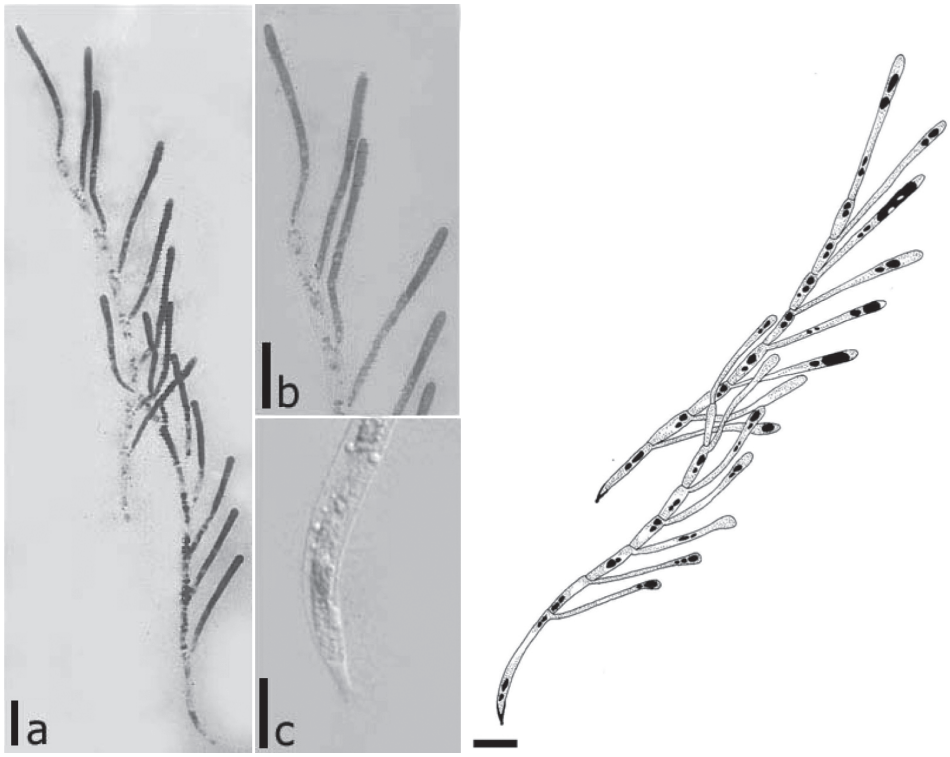


Fig 5. *Harpella cf. tica*. a. Two undulate thalli seen through the peritrophic matrix of black fly larvae; b. Trichospores attached to the generative cells; c. Tapered base (Bars: Fig a,b = 25 µm, Fig c = 10 µm, Drawing = 10 µm).

seemed to be restricted to the warmer streams (22-25°C) (White *et al*., 2000). Another study by Lichtwardt *et al*. (2000) in northwestern Argentina reported the occurence of *H. tica* from streams ranging from 17.0-24.5°C. In our streams the temperatures were also warmer (19-24°C), consistent with their observations of *H. tica* in warmer systems.

***Pennella montana* Lichtwardt, 1997** **Fig 6**

Mature thalli up to 1 mm long, main axis 9-12 µm in diameter producing numerous branches, 3.75-4 µm in diameter arising laterally. Basal cell bifurcated one or more times surrounded by a secreted mucilaginous cementing substance. Trichospores long ovoid, 37-56 × 3-6 µm. No released trichospores nor zygospores observed from our collections.

Material examined: Thailand, Chiang Mai Province, Pong Dueat Hotspring stream, on hindguts of black fly larvae, 16-04-2008, 21°C, M.P. Hapsari and P. Sysouphanthong, MFLU 08 1481; 03-09-2008, 22°C, *ibid*. MFLU 08 1483; *ibid*. MFLU 08 1484; Chiang Rai Province, Khun Khon Waterfall, 21-09-2008, 19°C, *ibid*. MFLU 08 1482.

Notes: *Pennella montana* has previously been reported from a tropical region in Costa Rica (Lichtwardt, 1997). This species of gut fungus is attached to the hindgut of simuliid larvae by a simple to dichotomously branched basal cell

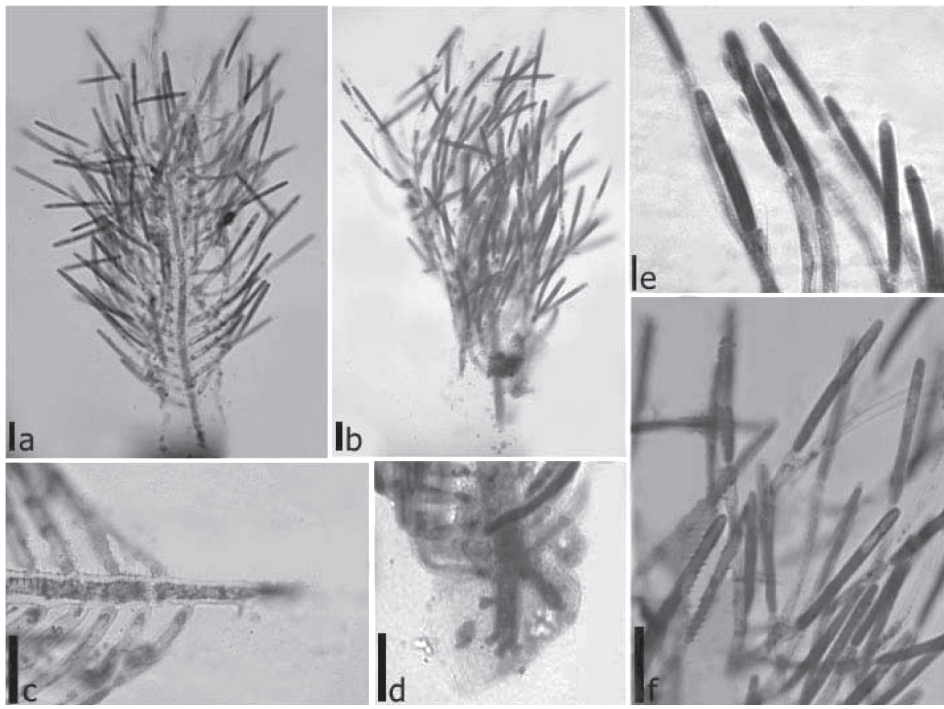


Fig 6. (a) *Pennella montana*. a,b. Main axis with numerous lateral branches producing trichospores; c,d. Simple and dichotomously bifurcated basal cell with mucilaginous secretion; e,f. Long-cylindrical trichospores (Bars: 25 µm).

covered by a mucilaginous substance, one important character of this genus. We found this fungus at three sites; Pong Dueat Hotspring stream (Site 7), Khun Khon Waterfall (Site 5) and Pong Phrabat Waterfall (Site 3), which all were located at higher altitude (671-781 m) compared to other sites where this fungus was absent, suggesting that it may be specific to certain environmental conditions. Though specimens in our collection lacked zygospores, this species is identifiable by its trichospore characters and dimensions.

The range of *P. montana* is now extended to Thailand. The previous report of this species in the tropics was in Costa Rica (Lichtwardt, 1997). We

Fig 6. (b) *Pennella montana* thallus reproducing asexually (Bar: 10 μm).

found specimens with both types of basal cell: simple and dichotomously bifurcated. Since it is not uncommon to find the immature (unbranched) thalli bearing trichospores, and to note extensive sporulation on fertile branchlets of the mature (branched) thalli, we consider *Pennella* to have considerable capacity to reproduce asexually. Conversely, the opposite is true of the morphologically similar genus, *Stipella* (see below).

Stachylina nana Lichtwardt, 1984 Fig 7

Thalli banana or boat-shaped, 40-62.5 × 5-8 μm, and narrowing at the apex and base (3 μm in diameter). Producing 2-4 generative cells, which are shorter than trichospores when having more than two trichospores. Trichospores ovoid, 16-32 × 4-8 μm, with one single appendage. Attached to the peritrophic matrix of black fly larvae by a small disk-like holdfast structure.

Material examined: Thailand, Chiang Rai Province, Huai Khang Pla Waterfall, on peritrophic matrix of black fly larvae (found side by side with *Harpella* spp.), 04-04-2008, 24°C, M.P. Hapsari and R.K. Wati, MFLU 08 1496; *ibid*. MFLU 08 1497; *ibid*. MFLU 08 1498.

Notes: The genus *Stachylina* was previously reported only from midges (Diptera: Chironomidae), until Labeyrie *et al*. (1996) reported this fungus from black fly larvae through their study at New York State. We found this gut fungus inhabiting the same peritrophic matrix with *Harpella* species in all of our collections. This species has been reported from tropical regions in Costa Rica from chironomid larvae (Lichtwardt, 1997), but has never been reported from tropical simuliid larvae. We found this fungus to be common at one site, Huai Khang Pla Waterfall (Site 1). This finding suggests that trichomycete taxa previously reported to be restricted to a certain family of host might not be as host-specific, pending studies of other hosts across broader geographic ranges. We also found immature thalli of *Stachylina* species. For these collections we consider them to be *Stachylina* sp. (Material examined: Thailand, Chiang Rai Province, Huai Khang Pla Waterfall, on peritrophic matrix of black fly larvae (together with *Harpella* sp.), 04-04-2008, 24°C, M.P. Hapsari and R.K. Wati, MFLU 08 1499; ibid. MFLU 08 1500; ibid. MFLU 08 1501; ibid. MFLU 08 1502).

Stipella vigilans Léger & Gauthier, 1932 Fig 8

Thalli 175-650 μm long, with 5-9 μm diameter of the main axis, producing branchlets about the same diameter as the trichospores. Attached to the hindguts of black fly larvae by a simple holdfast structure coated by a mucilaginous secretion. Small branches arise laterally from the basal cells. Trichospores straight cylindrical, 40-45 × 5 μm.

Material examined: Thailand, Chiang Rai Province, Khun Khon Waterfall, in hindguts of black fly larvae, 21-09-2008, 19°C, M.P. Hapsari and P. Sysouphanthong, MFLU 08 1485; *ibid*. MFLU 08 1492; Chiang Mai Province, Pong Dueat Hotspring stream, 06-07-2008, 23°C, *ibid*. MFLU 08 1486; *ibid*. 10-06-2008, 19°C, *ibid*. MFLU 08 1489; *ibid*. 1490; New Waterfall, 04-10-2008, 20°C, *ibid*. MFLU 08 1487; *ibid*. MFLU 08 1488.

Notes: Though our collections lacked released trichospores and zygospores, the ramified thalli and small number of trichospores produced distinguish this gut fungus from species of *Pennella*. The genus *Stipella* comprises two species, *S. latispora* and *S. vigilans* (Lichtwardt *et al*., 2001). *Stipella vigilans*

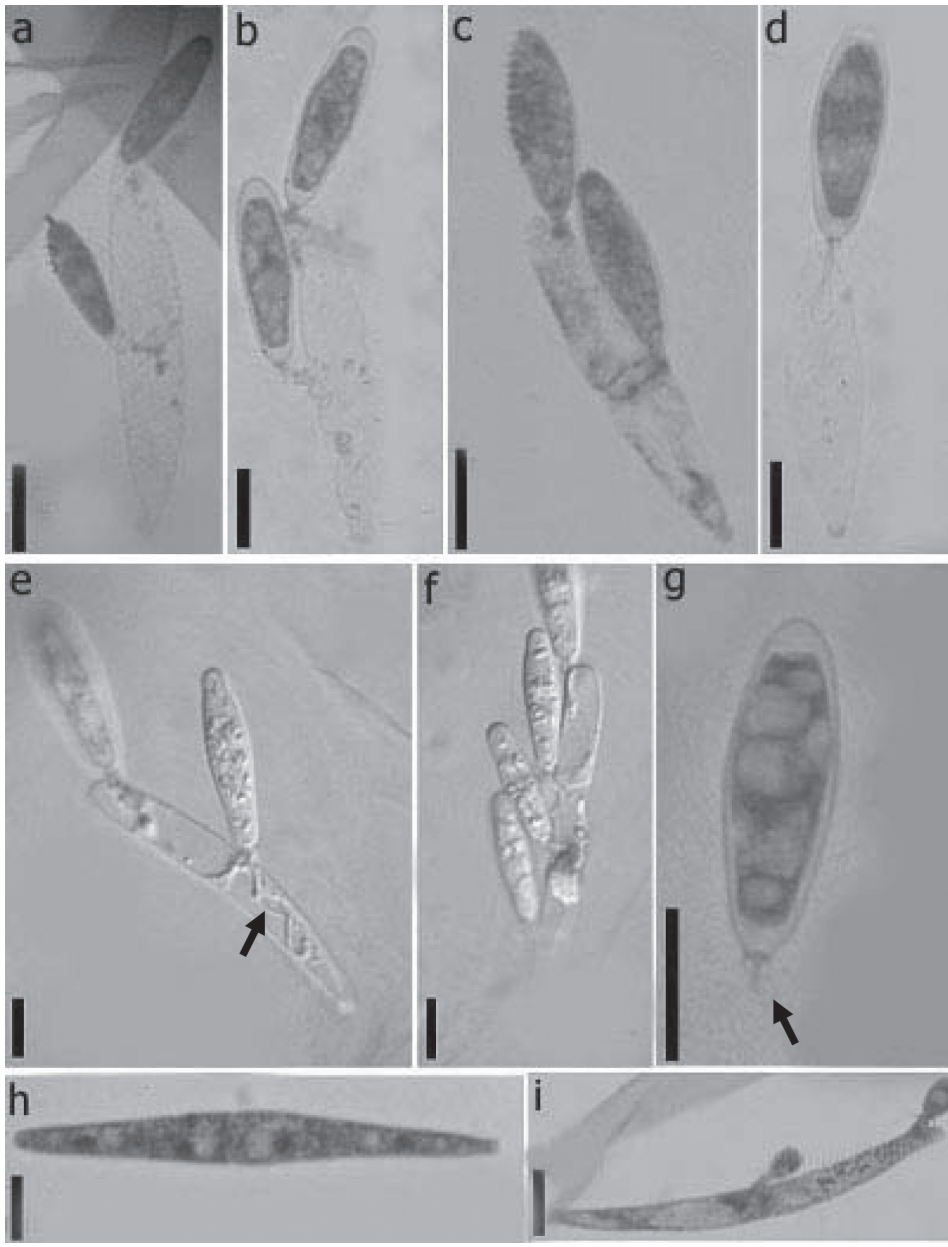


Fig 7. (a) *Stachylina nana*. a,b,c,d. Cymbiform thalli bearing trichospores (in lactophenol-cotton blue); e,f. Thalli in wet-mounts; g. Released ovoid trichospore showing one single appendage (arrow); h,i. Immature thalli (Bars: 10 μm).

is reported to have two types of holdfast: forked or simple, both with mucilaginous secretion. Moss (1970) found both types of holdfast: the first is a dichotomously branched holdfast and the second is a simple cellular, unbranched holdfast with either smooth or with spine-like non-cellular projections which appear to embed themselves in the cuticle. It is possible that these outgrowths aid in the attachment of the thallus. These microprojections are absent in the genus *Pennella*, thus it is also a useful character in differentiating both genera, as they have a mucilaginous secretion covering their holdfasts. Manier (1950) also noted both types of the holdfast, and in Manier (1963) she highlighted the differences in morphology that are possible. Although the online monograph (www.nhm.ku.edu/fungi~) did not mention these projections clearly in *S. vigilans*, from the papers combined, it is consistent enough on the thalli with simple bases. Our collections lack the spine-like projections, however, the smooth unbranched holdfast observed is consistent with the simple type of *S. vigilans* holdfast.

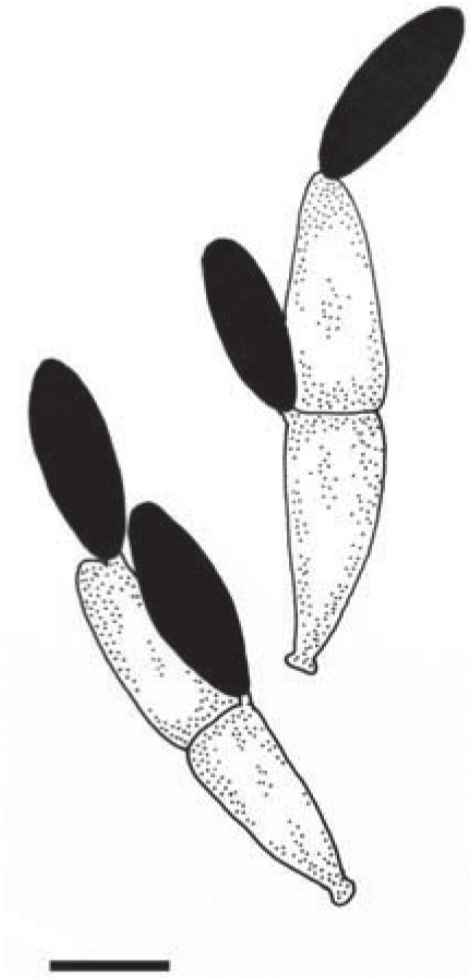


Fig 7. (b) *Stachylina nana* mature thalli with two attached trichospores (Bar: 10 μm).

We found this fungus to be common at three sites; Khun Khon Waterfall (Site 5), Pong Dueat Hotspring stream (Site 7) and New Waterfall (Site 6). In all instances, they were found inhabiting the high altitude (671-781 m) and lower temperature streams (19-22°C). These findings suggest that trichomycete taxa

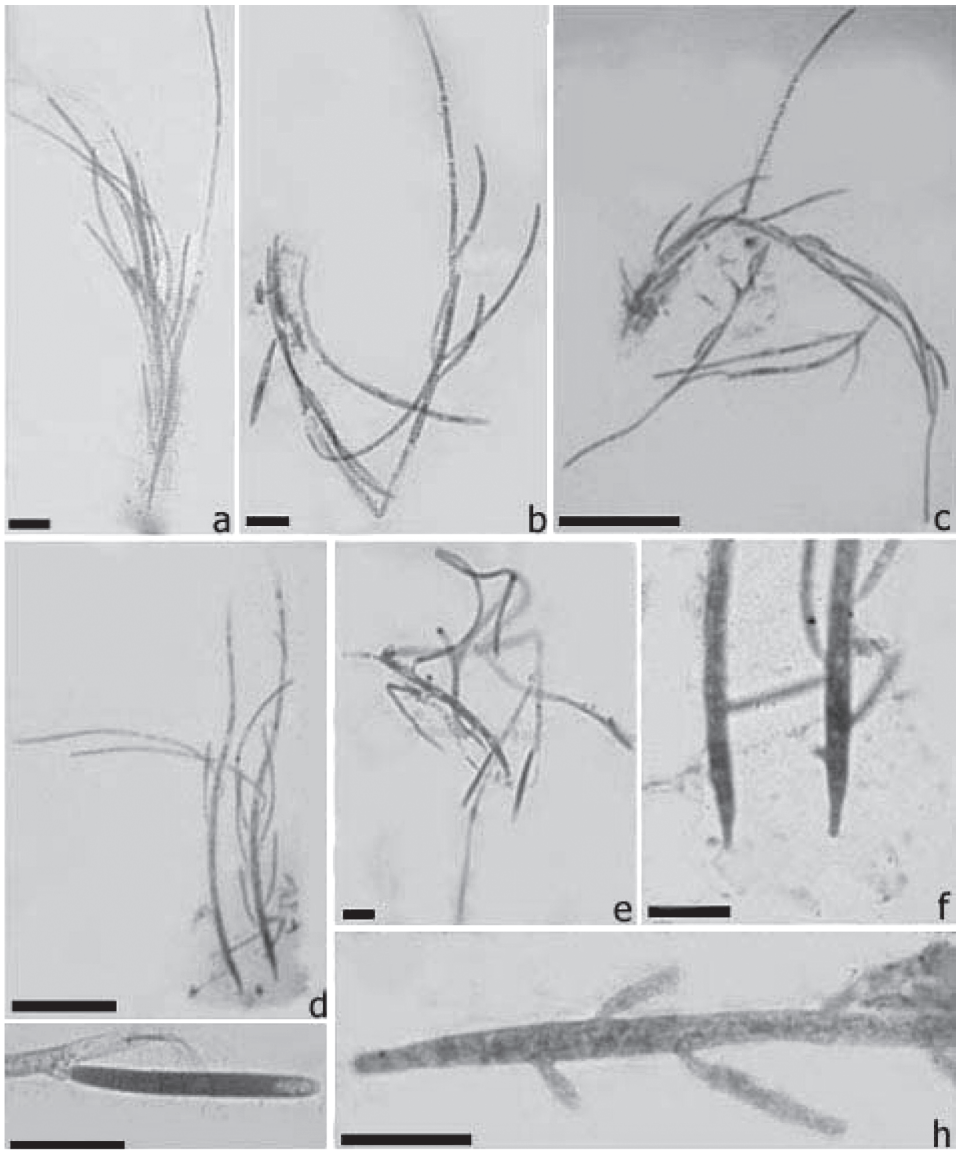


Fig 8. *Stipella vigilans* Léger & Gauthier. a,b,c,d. Ramified thalli dissected from hindguts; e. Thalli bearing trichospores; f,h. Simple base coated by a mucilaginous secretion; g. Long-cylindrical trichospore attached to the generative cell (Bars: Fig a,b,e = 10 μm; Fig c,d,f,g,h = 25 μm).

currently known only from the temperate regions can also be found in the tropical regions as the continued search for gut fungi is expanded globally. This genus has never been reported from the tropical regions. *S. vigilans* which was first discovered in France (Léger & Gauthier, 1932 cited in Lichtwardt *et al*., 2001), later in England (Moss, 1970), Spain (Valle & Santamaria, 2004; Valle, 2007), Armenia (Nelder *et al*., 2005) and now in Thailand, is considered to be rare.

DISCUSSION

Of the 38 genera of Harpellales, nine genera: *Barbatospora, Genistellospora, Graminelloides, Harpella, Pennella, Simuliomyces, Smittium, Stachylina and Stipella* are found typically in association with black fly larvae (Diptera: Simuliidae). Among the protistan members, both genera of Amoebidiales, *Amoebidium* and *Paramoebidium*, are known as simuliid-associated trichomycetes (Labeyrie, 1996; Lichtwardt *et al*., 2001; Misra & Lichtwardt, 2000).

Over a period eight months, we found six genera inhabiting the larval black fly; two genera of Harpellaceae, *Harpella* and *Stachylina*, were observed in midguts, and four genera of Legeriomycetaceae – *Graminelloides, Paramoebidium, Pennella,* and *Stipella* – in hindguts, for a total of eight species recorded. These findings suggest that trichomycetes are diverse; probably common in Thailand. Our data add new species or extend the host range or geographical distributions of previously known species.

The taxa identified and their occurrences are listed in Table 2 demonstrating that some species of gut endobionts were restricted to certain streams. For example, *Graminelloides cf. biconica*, *Harpella cf. tica* and *Stachylina*

Table 2. Species of trichomycetes collected from black fly larvae and their occurences at nine sites in northern Thailand

Taxon	*Collection site*								
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
Paramoebidium sp.			+	+		+	+		+
Graminelloides cf. biconica		+							
Harpella asiana	+	+	+ +	++	++	++	++	+	+
Harpella melusinae	+	+	++*	++*	++*	+*	++*	+	+
Harpella cf. tica							+		
Pennella montana			+		+		++		
Stachylina nana	+								
Stipella vigilans					+	+	+		

Note. + = present, ++ = abundant, * = conjugation of thalli.

nana were only found at Site 2 (22°C), Site 7 (20°C) and Site 1 (24°C), respectively. Stream temperatures ranged from 19 to 24°C. Collections made at different sites and months also did not reveal these three species, suggesting that other ecological factors may affect the occurrence of these fungi or the distributions of the black flies larvae. As mentioned by McCreadie and Adler (1998), canopy cover, conductivity, depth, discharge, dissolved oxygen, dominant streambed-particle size, pH, riparian vegetation, seston, velocity, water temperature and width are all useful predictors of the distribution of black fly larvae. These parameters, however, were not included in this preliminary study.

Harpella melusinae and *H. asiana* were found in all collection sites. They were more abundant in five collection sites (Site 3, 4, 5, 6, and 7) (See Table 2), which have lower water temperature than the other streams. All conjugating thalli of *H. melusinae* were also observed in black fly larvae collected from the lower water temperatures. This suggests that these fungi may prefer living on the peritrophic matrix of black fly larvae in the colder streams rather than in warmer streams. Lichtwardt *et al.* (2003) generalized that colder streams tend to be richer in trichomycete taxa compared to warmer streams.

This study focused on Harpellales inhabiting black fly larvae, with one new species, and others that may be forthcoming. Ranges are also extended for the known distribution of other species of the endobionts. With further studies, several new species species are likely from black flies, not to mention other immature aquatic insects, crustaceans, millipedes, or other kinds of terrestrial, freshwater or marine arthropods that are not included in this study. Further investigations will undoubtedly produce many new records and species of trichomycetes.

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