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Trichomycetes from China and the description of three new *Smittium* species

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Abstract: Trichomycetes were recovered from the guts of aquatic insect larvae collected from a stream in the Qinling Mountains in western China. These collections included *Smittium hecatei*, known only from Spain, as well as *Smittium simulii* and *Stachylina penetralis*, which appear to be more widely distributed. *Caudomyces japonicus*, previously recorded only from Japan, also is reported from crane fly larvae (*Antocha* sp.) from China. We describe three new species, *Smittium chinliense* from a tipulid host, as well as *Smittium naiadis* and *Smittium nodifixum*, both from chironomid larvae. A probable new species of *Gauthieromyces* was collected in mayfly nymphs and is illustrated but not described here.

Key words: aquatic insects, *Caudomyces*, *Gauthieromyces*, gut fungi, Harpellales

INTRODUCTION

Trichomycetes living in the guts of arthropods, mostly immature stages of insects in aquatic systems, are widely distributed. The best documented occurrences of these fungi are from the United States and western Europe (France, England and Spain) (Manier 1970, Moss et al 1975, Lichtwardt 1986, Santamaria 1997, Lichtwardt et al 2001, Valle and Santamaria 2004). Fewer published records exist for South America (Lichtwardt and Arenas 1996, Lichtwardt et al 2000), Central America (Lichtwardt 1994, 1997), Australia (Lichtwardt and Williams 1990) and New Zealand (Williams and Lichtwardt 1990). Although the Trichomycete survey to date has covered many widely separated continents, large areas with prime habitat and high arthropod diversity have yet to be examined in a systematic way (e.g. in Canada, Africa and China). The abundance and frequency of occurrence of Trichomycetes in hosts from areas that already have been surveyed suggest that sampling in new geographic areas will yield many undescribed species and may help better elucidate the ecology and biogeography of this fungal group.

Trichomycetes are poorly known from Asia. One publication reported species recovered in Japan (Lichtwardt et al 1987) and another describing parasites of blackflies from China noted the presence of a Trichomycete, *Harpella melusinae* Leger & Dubosq, in these hosts (Adler et al 1996). Chien and Hsieh (2001) reported Trichomycetes in the orders Eccrinales and Asellariales from aquatic and terrestrial crustaceans as well as millipedes in Taiwan, including two new species of eccrinids from crustacean hosts.

This paper reports Trichomycetes from aquatic insects collected in the Niu Bei Liang nature protection area, Qinling Mountains approximately 40 km from Xi'an, in western China. We describe three new species of Trichomycetes in the genus *Smittium* from these collections as well as six other taxa of trichomycetes.

MATERIAL AND METHODS

Insects were collected in a D-net by kick-sampling (Lichtwardt et al 2001) in a fast-flowing, shallow stream (Hao River) in the Niu Bei Liang nature protection area, western China (33°55.21N 108°56.49E) 26 and 27 Apr 2005. The stream had a sandy and pebbly bottom with little organic material and flowed through an area of large boulders. The stream yielded immature stages of mayflies (Ephemeroptera), stoneflies (Plecoptera), larval flies (Diptera) and beetles (Coleoptera). Samples of tipulid (Diptera) larvae also were hand-picked from rocks in the splash zone of a small waterfall near the sampling area.

The hindgut and peritrophic matrix (in the dipterans) were dissected out of the hosts and examined microscopically for the presence of trichomycetes and other gut inhabitants. Fungal identifications were made with the Lucid keys available at the University of Kansas Trichomycete Website (www.nhm.ku.edu/fungi/). Semipermanent slides, stained with lactophenol cotton blue were prepared as described in Lichtwardt et al (2001). Digital photomicrographs of the diagnostic features were taken at the Saint Mary's University Taxonomy Laboratory, Halifax, Nova Scotia, Canada. Voucher specimens of new species and *Caudomyces japonicus* Lichtw. Kobayasi & Indoh were deposited in the herbarium (SANU), College of Life Science, Shaanxi Normal University, Xi'an, 710062, Shaanxi, People's Republic of China. Other voucher specimens are in the collection of DBS, Saint Mary's University.

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RESULTS

The insects contained several species of Trichomycetes in the order Harpellales as well as protists in the genus *Paramoebidium*, which is currently classified within the Trichomycetes in the order Amoebidiales (Lichtwardt et al 2001). Harpellids recovered from midges (Diptera: Chironomidae) included *Smittium hecatei* L.G.Valle & Santam. and *Sm. simulii* Lichtw. from the hindgut. *Stachylina penetralis* Lichtw. was observed on the peritrophic matrix of these hosts. In addition two species of *Smittium* collected in chironomids had unique morphological features that warrant description as new species, *Sm. naiadis* (FIGS. 1–4) and *Sm. nodifixum* (FIGS. 5–8). Thalli of *Paramoebidium* spp. were common in the hindgut of these dipterans but insufficient characters were available to identify them to species.

The other dipterans commonly collected were two unidentified species of tipulids (*Antocha* spp.), one from wet rocks next to a waterfall and another from the stream. Although approximately 50 larvae from the waterfall site were dissected no fungi were present in the hindgut. Only three specimens of another *Antocha* sp. were collected from the stream. One of these had thalli of a *Smittium* sp. that did not match any described species so we describe a new species, *Sm. chinliense* (FIGS. 9–11), below. *Caudomyces japonicus* (FIGS. 12, 13) was collected in the hindguts of two tipulid larvae, including one larva that also had *Sm. chinliense*. This monotypic genus was known only from Japan where it was recorded from the same genus of tipulid host (Lichtwardt et al 1987).

Mayflies collected from Hao River had Trichomycetes in their hindguts from the same orders (Harpellales and Amoebidiales) as the chironomids. A *Gauthieromyces* sp. was recovered from *Baetiella ausobskyi* Braasch (Ephemeroptera: Baetidae). The genus *Gauthieromyces* and the type species, *G. microsporus* Lichtw., were described formally by Lichtwardt (1983) based on the original description and drawings from Gauthier (1960). No type material exists, but based on the drawings in the original species description the specimens from China belong to *Gauthieromyces*. Its distinctive minute, horseshoe-shaped trichospores could not be confused with any other described species. We collected this *Gauthieromyces* sp. from a baetid host, which is in the same family of insect that *G. microsporus* occurs (Gauthier 1960). J.K. Misra, (jitrachravi@sify.com pers comm) recently has discovered a *Gauthieromyces* sp. from India. Images from his collection compared with the specimen from China suggest they are the same fungus. Misra is describing a new species so we report the Chinese specimen simply as *Gauthieromyces* sp. and provide photomicrographs to show the features in this collection from China (FIGS. 14, 15). These baetids also had *Paramoebidium* spp. in their guts.

The guts of siphlonurid mayflies (Ephemeroptera: Siphlonuridae) contained *Paramoebidium* spp. Approximately 12 specimens of ephemerellid mayflies (Ephemerellidae) were dissected but no Trichomycetes were seen. One to three specimens each of nemourid stoneflies (Plecoptera: Nemouridae) and mayflies in the families Heptageniidae and Caenidae were dissected but the guts contained no fungi. The small number of these insects in the collections probably reflects their phenology and it may be that they would have Trichomycetes in the gut at a different time or stage in their development. Predacious beetle larvae were not dissected because predacious insects normally do not house Trichomycetes (Lichtwardt et al 2001).

TAXONOMY

Smittium naiadis Strongman et Shengquan Xu, sp. nov. FIGS. 1–4

Thallus diffusus, ramis primariis e fasciculo simplici cellularum basalium muco velatarum orientibus; ramificatio secundaria per longitudinem et in extremis ramorum principalium valde verticillata. Trichosporae cylindricae longae angustaeque (34–36 × 2.5–3.5 μm), cum collo conspicuo breve (3 μm) quod ut videtur 1–2 tumores plerumque habet. Trichosporae appendice singula tenui instructae. Zygosporae incognita. In proctodaeo larvarum Chironomidarum.

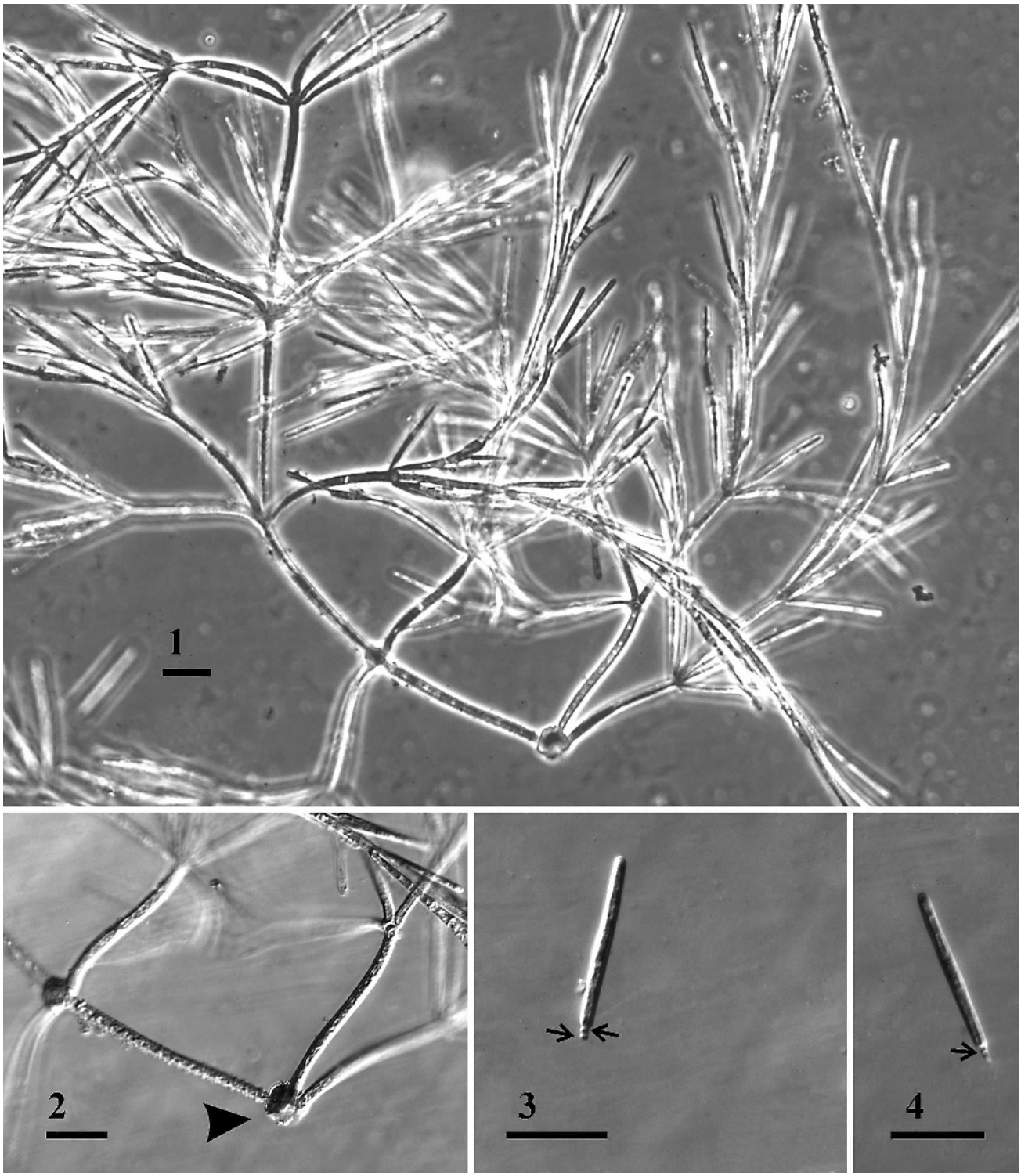
Thallus diffuse, primary branches arise from a simple cluster of basal cells covered with mucilage (FIGS. 1, 2), secondary branching strongly verticilliate along the length and at the end of the primary branches (FIG. 1). Trichospores cylindrical, long and narrow (34–36 × 2.5–3.5 μm) with a conspicuous, short (3 μm) collar that commonly appears to have 1 or 2 thickenings (FIGS. 3, 4). Trichospores with a single, thin appendage. Zygospores not found. In hindgut of bloodworms (Chironomidae).

Etymology. from the Latin *naias* meaning from a nymph, referring to the source being the immature (larval) stage of the host.

Specimens examined. CHINA. SHAANXI PROVINCE: Hao River, Niu Bei Liang nature protection area, 33°55.21N 108°56.49E. Slide CHI-2 prepared from the hindgut of a chironomid larva collected 27 Apr 2005 (HOLOTYPE SANU).

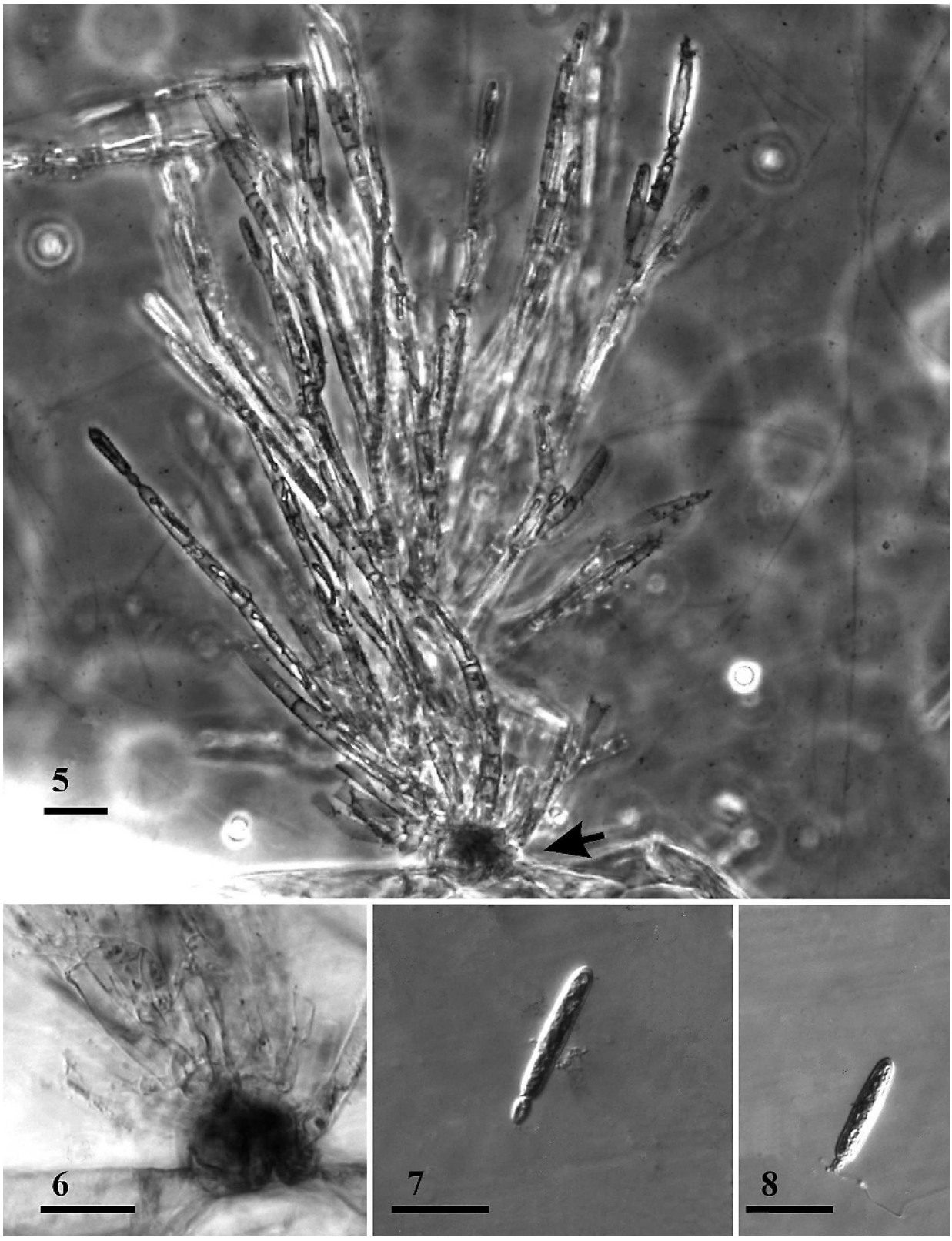
Note. This slide also contains thalli and trichospores of *Sm. nodifixum*.

Commentary. The trichospores in *Sm. naiadis* are long and narrow compared to most other species of *Smittium* (www.nhm.ku.edu/fungi/). The tricho-

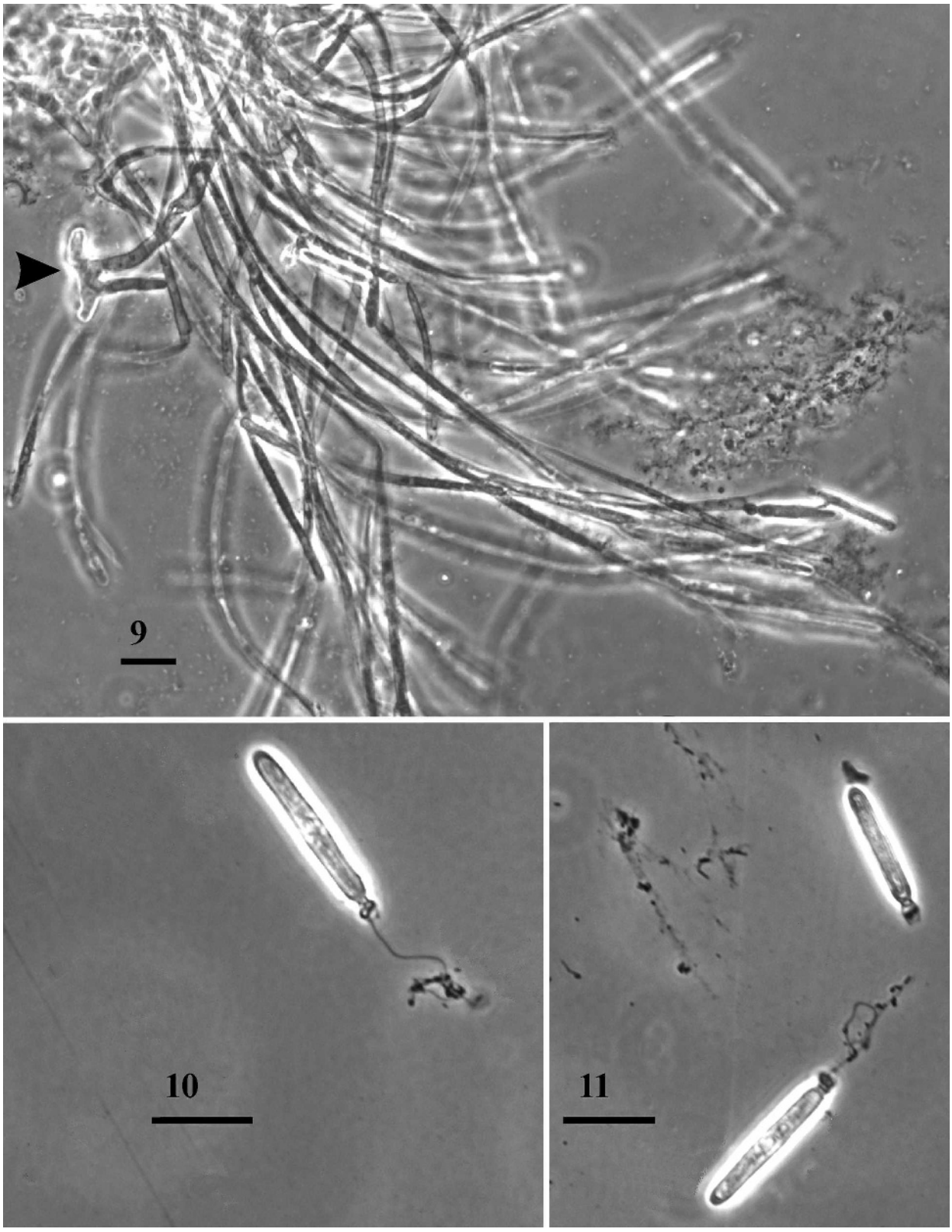


FIGS. 1–4. *Smittium naiadis*. 1. Thallus branching and holdfast characteristics. 2. Details of the holdfast (arrow). 3, 4. Trichospores. The arrows indicate apparent thickenings in collar. Bars = 20 µm.

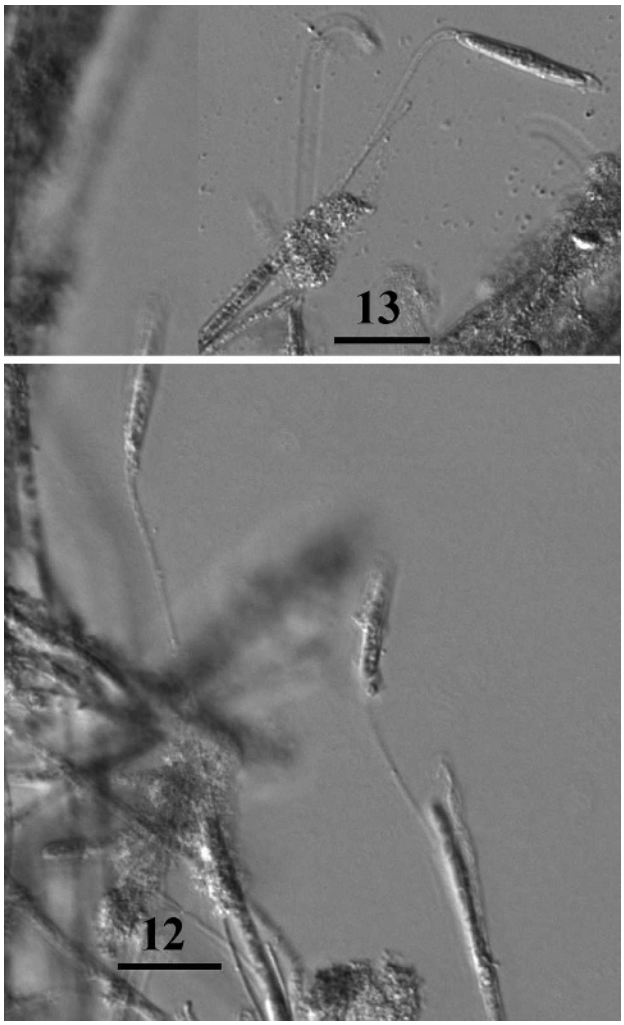
spore collar has distinctive thickenings and this feature seems to be consistent, therefore diagnostic. *Smittium gravimetallum* Lichtw. Ferrington & Hayford (Ferrington et al 2000) has narrow spores but in this species they are shorter on average and have a distinct swelling in the midregion, which is lacking in *Sm. naiadis*. Another species with long spores is *Sm. elongatum* Lichtw. but they are wider than in *Sm.*



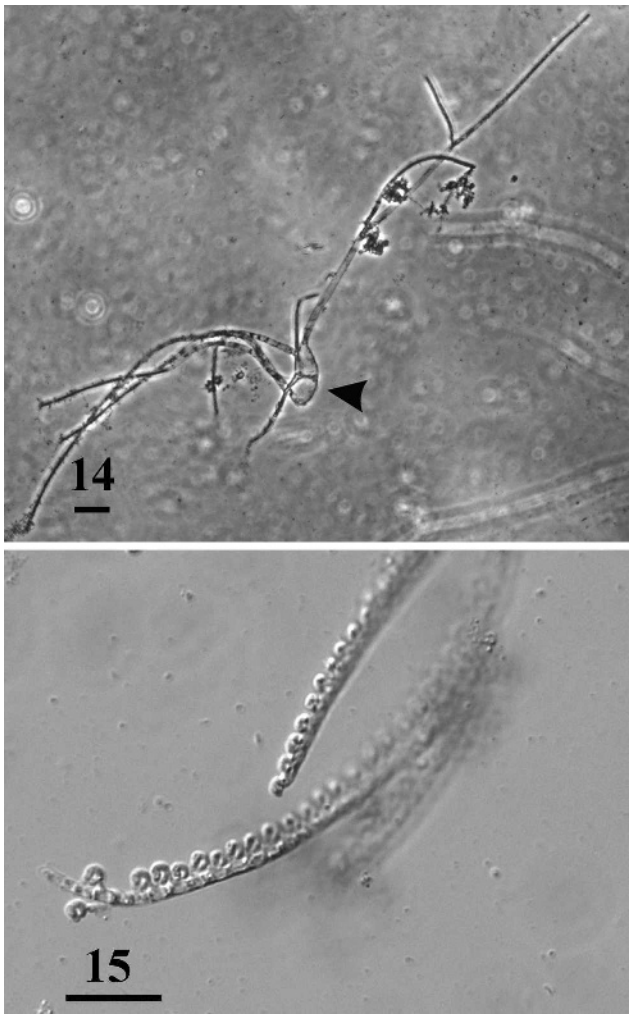
FIGS. 5–8. *Smittium nodifixum*. 5. Thallus arising from bulbous holdfast (arrow). 6. Details of knobby holdfast and individual branches. 7. Trichospore showing campanulate collar. 8. Trichospore with single appendage. Bars = 20 µm.



FIGS. 9–11. *Smittium chinliense*. 9. Thallus with foot-like holdfast (arrow). 10, 11. Released trichospores. Bars = 20 µm.



FIGS. 12–13. *Caudomyces japonicus*. 12. Trichospores with long collars attached to fertile branch. 13. A single trichospore with long collar typical of the species. Bars = 20 µm.



FIGS. 14–15. *Gauthieromyces* sp. 14. Immature thallus showing sparse branching from swollen basal cell at arrow. 15. Terminal fertile branch with many horseshoe-shaped trichospores. Bars = 20 µm.

naiadis, the holdfast in *Sm. elongatum* is inconspicuous (Lichtwardt 1972) whereas it is prominent and embedded in mucilage in this new species. There was no apparent coiling in the appendage as is described for *Sm. elongatum*. *Smittium angustum* M.C. Williams & Lichtw. has narrow spores but they are shorter and the thallus features differ greatly from *Sm. naiadis* (Lichtwardt and Williams 1992).

Smittium nodifixum Strongman et Shengquan Xu, sp. nov. FIGS. 5–8

Thallus compactus, ramis primariis e haptero conspicuo nodoso orientibus; ramificatio secundaria sparsa. Trichosporae subcylindricae, 26–29.5 × 5–6 µm, cum collo campanulato 5–6 µm longo. Zygosporae incognitae. In proctodaeo larvarum Chironomidarum.

Thallus compact, primary branches arising from a conspicuous knobby holdfast, secondary branching sparse (FIGS. 5, 6). Trichospores subcylindrical 26–29.5 × 5–6 µm with a campanulate collar 5–6 µm long (FIGS. 7, 8). Zygospores not found. In hindgut of bloodworms and other midge species (Chironomidae).

Etymology. from the Latin *nodos* and *fixum*, meaning fasten with a knot, in reference to the conspicuous, knobby holdfast in this species.

Specimens examined. CHINA. SHAANXI PROVINCE: Hao River, Niu Bei Liang nature protection area, 33°55.21N 108°56.49E. Slide CHI-4 prepared from the hindgut of a chironomid larva collected 27 Apr 2005 (HOLOTYPE SANU).

Commentary. The spore dimensions in *Sm. nodifixum* overlap with nine described species in this genus (www.nhm.ku.edu/fungi/). However, when

thallus features are considered along with spore shape and collar characteristics, the species can be separated. *Smittium acutum* Lichtw. & Grigg and *Sm. annulatum* Lichtw. share some features with this new species but the holdfast structures differ; *Sm. acutum* has a flared collar (Lichtwardt and Grigg 1998), compared to a campanulate collar in *Sm. nodifixum*, and the spores of *Sm. annulatum* are smaller on average (Lichtwardt 1997). *Smittium commune* Lichtw. & Grigg has variable spores (Lichtwardt and Grigg 1998) so the dimensions overlap substantially with *Sm. nodifixum*. However the spores in *Sm. commune* are ellipsoidal and shorter, on average, with a shorter collar so it can be differentiated from *Sm. nodifixum* on this basis. The new species resembles *Sm. cylindrosporum* Lichtw. & Arenas but trichospores in that species have a conspicuous bulge in the middle and the branching pattern in the thallus differs (Lichtwardt and Arenas 1996). There is no mention of a distinctive holdfast in the description of *Sm. cylindrosporum*, which is a conspicuous feature in *Sm. nodifixum*.

Smittium chinliense Strongman et Shengquan Xu, sp. nov. FIGS. 9–11

Thallus e haptero conspicuo pediformi oriens; ramificatio sparsa, trichosporis 3–4 in terminiis ramorum fertilium typice formantibus. Trichosporae cylindricae vel subcylindricae, 28.5–36 × 5–6 μm, cum collo tubulari 5–6 × 4 μm saepe sporam latitudine aequanti, appendice singula instructae. Zygosporae incognitae. In proctodaeo larvarum Tipulidarum.

Thallus arising from a conspicuous foot-like holdfast, branching sparse, typically with 2–4 trichospores forming at the tips of fertile branches (FIG. 9). Trichospores cylindrical to subcylindrical, 28.5–36 × 5–6 μm with a tubular collar 5–6 × 4 μm, often as wide as the spore, with a single appendage (FIGS. 10, 11). Zygospores not found. In hindgut of crane fly larvae (Tipulidae).

Etymology. from Qinling (pronounced chin-lee) Mountains of China where the collections were made.

Specimens examined. CHINA. SHAANXI PROVINCE: Hao River, Niu Bei Liang nature protection area, 33°55.21N 108°56.49E. Slide CHI-13 prepared from the hindgut of a tipulid (*Antocha* sp.) larva collected 27 Apr 2005 (HOLOTYPE SANU).

Note. This slide also contains thalli and trichospores of *Caudomyces japonicus*.

Commentary. This fungus does not resemble any of the three species known from tipulid larvae, *Sm. simulatum* Lichtw. & Arenas (Lichtwardt and Arenas 1996), *Sm. simulii* (Lichtwardt 1964) and *Sm. tipulidarum* M.C. Williams & Lichtw. (Williams and Lichtwardt 1987) (www.nhm.ku.edu/fungi/). The long, cylindrical trichospores of *Sm. chinliense* are similar in dimensions to *Sm. elongatum* and *Sm. cylindrosporum*, both from dipteran larvae (chironomids), but the collar in these species is narrower than the spore (Lichtwardt 1972, Lichtwardt and Arenas 1996) whereas the collar in *Sm. chinliense* is as wide as the spore. Also the thallus in both known species has verticilliate branching whereas *Sm. chinliense* does not.

DISCUSSION

This paper expands the known geographic range of several Trichomycete species to another continent, China, and describes three new species, *Sm. naiadis*, *Sm. nodifixum* and *Sm. chinliense*. *Smittium hecatei* is a recently described species from Spain (Valle and Santamaria 2004) and the Chinese collection fits the trichospore size ranges and characteristics for both spores α and spores β as described by these authors. We did not see the ''elephant leg'' basal cell that is typical for this species but the thallus characteristics match otherwise. *Smittium simulii* is a species that is widely distributed and common in chironomid larvae (Lichtwardt et al 2001) so it is not surprising that we collected it in these hosts from China as well. *Stachylina penetralis* has been reported from Japan as well as Europe (Lichtwardt et al 2001) suggesting it might have broad distribution.

Smittium is the largest, most widely distributed genus of Trichomycetes with more than 70 species described to date from a variety of insect hosts. Therefore typical morphological characteristics used to define species (e.g. spore dimensions) overlap greatly, which makes species identification challenging. This problem is further complicated by the occurrence of several dimorphic species that exhibit two distinct spore morphologies arising from the same thallus (Valle and Santamaria 2004, White and Lichtwardt 2004). Thallus characteristics such as the holdfast and a more standardized description of the branching patterns may help delineate taxa. More molecular studies like those described in Gottlieb and Lichtwardt (2001) will provide data on genetic relatedness that might help refine our interpretation of the significance of certain morphological characters and variation among species.

Caudomyces japonicus specimens recovered from *Antocha* sp. larvae in the Qinling Mountains matched the type species description well and are from the same genus of host (Lichtwardt et al 1987). This is an extension of the geographic range for this fungus and although we, like the authors on the original description, were unable to identify the host to species

it might be an indication that the host and fungus are widespread in Asia. The original description of *C. japonicus* included an illustration but this second collection from China supplements the illustration by providing photomicrographs (FIGS. 12, 13), which might help other collectors recognize the species and give us a better understanding of the morphological variation within the species.

The discovery of another species of *Gauthieromyces* is significant in that this monotypic genus has not been reported since its description from France in 1960 (Lichtwardt 1983). No type material exists for *G. microsporus* and subsequent attempts to collect the species at the type locality were unsuccessful (Lichtwardt 1983). The characteristics of the *Gauthieromyces* sp. from China differ from the type in that the swollen basal cell is shorter, tapers to the central axis more abruptly (FIG. 14) and the branching is more profuse with typically many spores (20+) per fertile branch (FIG. 15). The structure of the thallus is similar to species of *Graminella*, also known from baetid mayflies (www.nhm.ku.edu/fungi/). However the trichospores are diagnostic in that no other genus contains species with small, horseshoe-shaped spores. The spores in the Chinese collection are the same length around the curve (10–12[–14] µm) as the type and about 2 µm wide; no width is given in the original description (Lichtwardt 1983). The independent discovery of another species in both India and China provides reference material that will be useful when other species are found. The results from this limited survey of two habitats along a short stretch of one river suggest that China and other unexplored areas in Asia are rich in Trichomycetes and no doubt more extensive surveys will add many more new taxa.

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