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Diversity of trichomycetes in larval flies from aquatic habitats in Argentina

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Abstract: Trichomycetes, mainly from nonbiting midge (Chironomidae), mosquito (Culicidae) and black fly (Simuliidae) larvae (Insecta: Diptera), are reported from diverse freshwater environments principally from previously unexplored areas of Argentina. Four new species of Harpellales are described: *Austrosmittium patagonicum*, *A. lenticum*, *Smittium basiramosum* and *Legeriomyces lichtwardtii*. This is the first report of *Austrosmittium* spp. from the Americas and the first report of a *Legeriomyces* from South America. Two other species (*Simuliomyces* sp. and *Harpella* sp.) are described but not named. Fourteen previously described species were recovered, and their geographical distribution and host ranges have been extended for Argentina, with *Sm. phytotelmatum*, *Stachylina lentica*, *St. lotica and St. penetralis* being new records for the country.

Key words: Diptera, endosymbiotic fungi, Harpellales, Kickxellomycotina, macroinvertebrates

INTRODUCTION

Trichomycetes are cosmopolitan, symbiotic microorganisms inhabiting the digestive tract of nonpredaceous arthropods (Lichtwardt 1986), generally associated in a commensal relationship. Four orders (Amoebidiales, Asellariales, Eccrinales and Harpellales) were included in the former zygomycotan class Trichomycetes. Orders Amoebidiales and Eccrinales are now considered to be protists (Benny and O'Donnell 2000, Cafaro 2005, White et al. 2006a). The Asellariales and Harpellales were placed by Hibbett et al. (2007) within the subphylum Kickxellomycotina, with the suggestion that the term trichomycetes (lowercase t) be used to refer to them as an ecological group and not as a monophyletic or natural assemblage. We use that terminology herein.

More than 200 species of trichomycetes are described from around the world (Lichtwardt et al. 2001). What is known about the diversity of the trichomycetes from the Neotropics is scattered and relatively recent, with records from Costa Rica (Lichtwardt 1994, 1997), Chile (Lichtwardt and Arenas 1996), Brazil (Alencar et al. 2003), Argentina (López Lastra 1997; Lichtwardt et al. 1999, 2000; Cafaro 2000; López Lastra et al. 2005), Ecuador (Nelder et al. 2004), Mexico (Valle et al. 2008), Puerto Rico (White et al. 2000, Valle and Cafaro 2008) and Dominican Republic (Valle and Cafaro 2008).

In previous studies of trichomycetes from Argentina nine new species, as well as 16 previously known and four undetermined species, were cited. Those studies included the monotypic genus *Coleopteromyces*, which represents the only report of a Harpellales associated with the gut of a beetle larva. In addition two new species of Amoebidiales and one new species of Eccrinales were described. Herein we report the diversity of freshwater trichomycetes (with emphasis on Harpellales) from previously unexplored sites in six provinces in Argentina.

MATERIALS AND METHODS

Immature insects were collected in several freshwater environments from diverse sites in Argentina (Table I). The methods used were based on habitat characteristics. Insect larvae from streams and rivers were collected with limnologic D-nets or taken directly from the substrate. For lentic habitats we used a D-net or a 250 mL ladle. In the case of phytotelmata the impounded water containing insect larvae was extracted with a 3 mL plastic pipette. Insects were placed in collecting jars, and in the case of black flies, larvae and their substrates were placed in plastic bags or on moist filter paper lining disposable, sterile Petri dishes (White et al. 2006b) for transport to the laboratory in a cooler with ice and transferred to a refrigerator (4 C).

Insects were dissected with a Zeiss Stemi DV4 stereo microscope, and microscope slides were observed under an Olympus CH-30 microscope with phase contrast. Digital photographs were taken with an Olympus DP-71 or Sony DSC-P73 4MP camera. Semipermanent slides were prepared by infiltrating specimens with lactophenol-cotton blue (0.5% w/v) and sealing the cover slip with clear fingernail polish. Type and reference slides were deposited in the Spegazzini herbarium (LPSC).

Some of the thalli obtained from infected larvae were used as inoculum to isolate axenic cultures on a medium of a 1 : 1 mixture of dilute brain-heart infusion (1/10 BHI) and tryptone-glucose-salts (TG) with vitamins (BHIGTv) (White et al. 2006b) overlaid with sterile distilled water and

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TABLE I. Site codes and description, with GPS coordinates and dates of collection of trichomycetes in six provinces in Argentina

Site	Site description	Date	Longitude/latitude	Province
ARR-1	Rice field	*	34°59′20.1″S, 57°59′43.1″W	Buenos Aires
ARR-2	Ditches bordering Site ARR-1	22-XII-2004	35°59′20.2″S, 57°59′43.0″W	Buenos Aires
		01-II-2005		
		06-VII-2005		
BG-1	Salado River (river with variable concentrations of salts)	03-XI-2004	35°44′42.8″S, 58°31′31.1″W	Buenos Aires
BG-2	Small ponds next to Site BG-1	03-XI-2004	35°44′42.9″S, 58°31′31.0″W	Buenos Aires
BG-3	Ditches	03-XI-2004	35°45′43.9″S, 58°29′48.7″W	Buenos Aires
BG-4	*Eryngium* sp. (phytotelmata)	03-XI-2004	35°44′33.5″S, 58°28′59.6″W	Buenos Aires
BG-5	*Eryngium* sp. (phytotelmata)	03-XI-2004	35°30′54.0″S, 58°21′27.5″W	Buenos Aires
CEM	Flower vases at La Plata Cemetery	**	34°57′30.0″S, 57°57′48.0″W	Buenos Aires
CHA	Seco stream	08-XI-2004	38°10′39.0″S, 57°39′28.4″W	Buenos Aires
CHAC	Water reservoir	10-XI-2007	26°55′11.3″S, 61°37′42.7″W	Chaco
ChL-1	Temporary pond	20-VII-2005	35°00′41.3″S, 57°54′49.0″W	Buenos Aires
		28-VII-2005		
		08-VIII-2005		
		10-XI-2005		
		23-III-2006		
		30-X-2006		
		28-XII-2006		
		06-III-2007		
ChL-2	*Eryngium* sp. (phytotelmata)	20-VII-2005	35°00′41.3″S, 57°54′49.0″W	Buenos Aires
		28-VII-2005		
		23-III-2006		
		28-XII-2006		
ChL-3	Small artificial pool	28-XII-2006	35°00′41.3″S, 57°54′49.0″W	Buenos Aires
ENS-1	*Eryngium* sp. (phytotelmata)	16-V-2005	34°55′01.5″S, 57°44′33.3″W	Buenos Aires
LAN-1	Rucu Leufú stream	15-I-2005	39°43′13.6″S, 71°31′41.7″W	Neuquén
LAN-2	Small stream next to Site LAN-1	15-I-2005	39°43′15.6″S, 71°31′38.0″W	Neuquén
LAN-3	Raquitué stream	15-I-2005	39°45′39.3″S, 71°17′22.2″W	Neuquén
LAN-4	Unnamed stream	17-I-2005	39°42′51.7″S, 71°31′55.9″W	Neuquén
LAN-6	Unnamed stream	17-I-2005	39°45′06.9″S, 71°30′01.2″W	Neuquén
LF-1	Río Grande de Punilla	21-IX-2006	31°05′49.7″S, 64°30′04.5″W	Córdoba
LP-1	*Eryngium* sp. (phytotelmata)	14-XII-2004	35°00′17.2″S, 58°06′45.6″W	Buenos Aires
LP-4	Ditches	14-XII-2004	34°56′52.6″S, 58°05′40.5″W	Buenos Aires
LP-6	Ditches	25-I-2005	34°57′10.3″S, 58°02′34.3″W	Buenos Aires
LP-7	Ditches	04-II-2005	34°57′08.5″S, 58°02′35.2″W	Buenos Aires
LP-8	Temporary pond	04-II-2005	34°57′02.4″S, 58°57′30.3″W	Buenos Aires
LR-2	Cosquín river	20-II-2005	31°17′14.0″S, 64°27′38.4″W	Córdoba
LR-4	Anillaco stream	22-II-2005	28°47′45.2″S, 66°59′45.5″W	La Rioja
LR-5	Santa Vera Cruz stream	23-II-2005	28°40′37.6″S, 66°58′57.7″W	La Rioja
LR-8	Los Sauces River	24-II-2005	31°43′54.6″S, 64°54′04.6″W	Córdoba
LR-9	Mina Clavero River	24-II-2005	31°42′10.7″S, 64°53′44.9″W	Córdoba
LR-10	Unnamed stream	24-II-2005	31°37′35.8″S, 64°54′43.0″W	Córdoba
MP-1	Adelas Negras stream	13-VII-2005	37°37′56.5″S, 58°29′40.2″W	Buenos Aires
		23-X-2005		
		24-II-2006		
		03-V-2006		
MP-2	Unnamed stream	13-VII-2005	37°52′00.2″S, 58°02′21.6″W	Buenos Aires
MP-3	Unnamed stream	23-X-2005	37°10′33.5″S, 58°31′04.3″W	Buenos Aires
MP-4	Las Brusquitas stream	13-VII-2005	38°04′10.7″S, 57°50′16.7″W	Buenos Aires
		23-X-2005		
		24-II-2006		
		03-V-2006		

TABLE I. Continued

Site	Site description	Date	Longitude/latitude	Province
MP-6	De la Ballenera stream	13-VII-2005	38°09′40.2″S, 57°58′39.0″W	Buenos Aires
		23-X-2005		
		24-II-2006		
		03-V-2006		
MP-7	Tajamar stream	23-X-2005	37°51′41.2″S, 57°57′01.9″W	Buenos Aires
MP-8	Chocorí stream	23-X-2005	38°16′10.3″S, 58°09′23.0″W	Buenos Aires
MP-9	De la Tigra stream	23-X-2005	38°14′40.4″S, 58°06′18.0″W	Buenos Aires
PI-2	*Eryngium* sp. (phytotelmata)	25-VII-2006	35°14′06.1″S, 57°16′12.4″W	Buenos Aires
PI-3	Unnamed stream	20-VI-2006	35°11′13.1″S, 57°20′13.4″W	Buenos Aires
		25-VII-2006		
PI-6	Ditches bordering Site PI-5	25-VII-2006	34°57′57.2″S, 57°46′42.0″W	Buenos Aires
PI-8	Primera Estancia stream	25-VII-2006	35°08′46.9″S, 57°23′29.3″W	Buenos Aires
PL-1	*Eryngium cabrerae* Pontiroli (phytotelmata)	03-X-2005	34°51′53.0″S, 57°52′23.2″W	Buenos Aires
		10-II-2006		
		04-I-2007		
PL-2	Temporary pond	03-X-2005	34°47′05.5″S, 58°00′55.2″W	Buenos Aires
		10-II-2006		
		04-I-2007		
PL-3	Temporary pond	06-IX-2006	34°52′02.4″S, 57°57′30.3″W	Buenos Aires
PL-4	Temporary pond	03-X-2005	34°49′19.1″S, 57°58′07.4″W	Buenos Aires
		28-VI-2006		
PL-5	Temporary pond close to Site PL-1	10-II-2006	34°51′53.0″S, 57°52′23.2″W	Buenos Aires
R9-5	Temporary pond	03-III-2004	33°48′09.6″S, 59°37′04.2″W	Buenos Aires
SL-1	Larca stream	19-I-2007	32°38′10.3″S, 64°57′07.6″W	San Luis
SL-3	Cortadera stream	20-I-2007	32°30′31.6″S, 64°57′59.2″W	San Luis
SL-4	Papagayos stream	20-I-2007	32°40′38.9″S, 64°58′57.9″W	San Luis
SV-3	Unnamed stream	20-II-2006	38°07′30.0″S, 61°45′40.7″W	Buenos Aires
SV-4	Sauce Chico River	21-II-2006	38°02′01.2″S, 64°11′40.6″W	Buenos Aires
SV-6	El Loro stream	23-II-2006	38°03′52.1″S, 61°53′15.2″W	Buenos Aires
SV-7	Tres Arroyos stream	23-II-2006	38°23′03.5″S, 60°14′46.9″W	Buenos Aires
SV-8	Las Piedras stream	08-III-2007	38°04′45.7″S, 61°56′09.3″W	Buenos Aires
		11-VI-2006		
SW-1	Swimming pool	***	34°55′25.9″S, 57°55′36.2″W	Buenos Aires
SW-2	Swimming pool	29-XI-2004	34°53′12.6″S, 58°01′25.6″W	Buenos Aires
		20-VII-2005		

* Samples obtained biweekly Jan 2004–Jul 2005.
** Samples obtained Feb 2005–Mar 2006.
*** Samples obtained weekly Aug–Dec 2006.

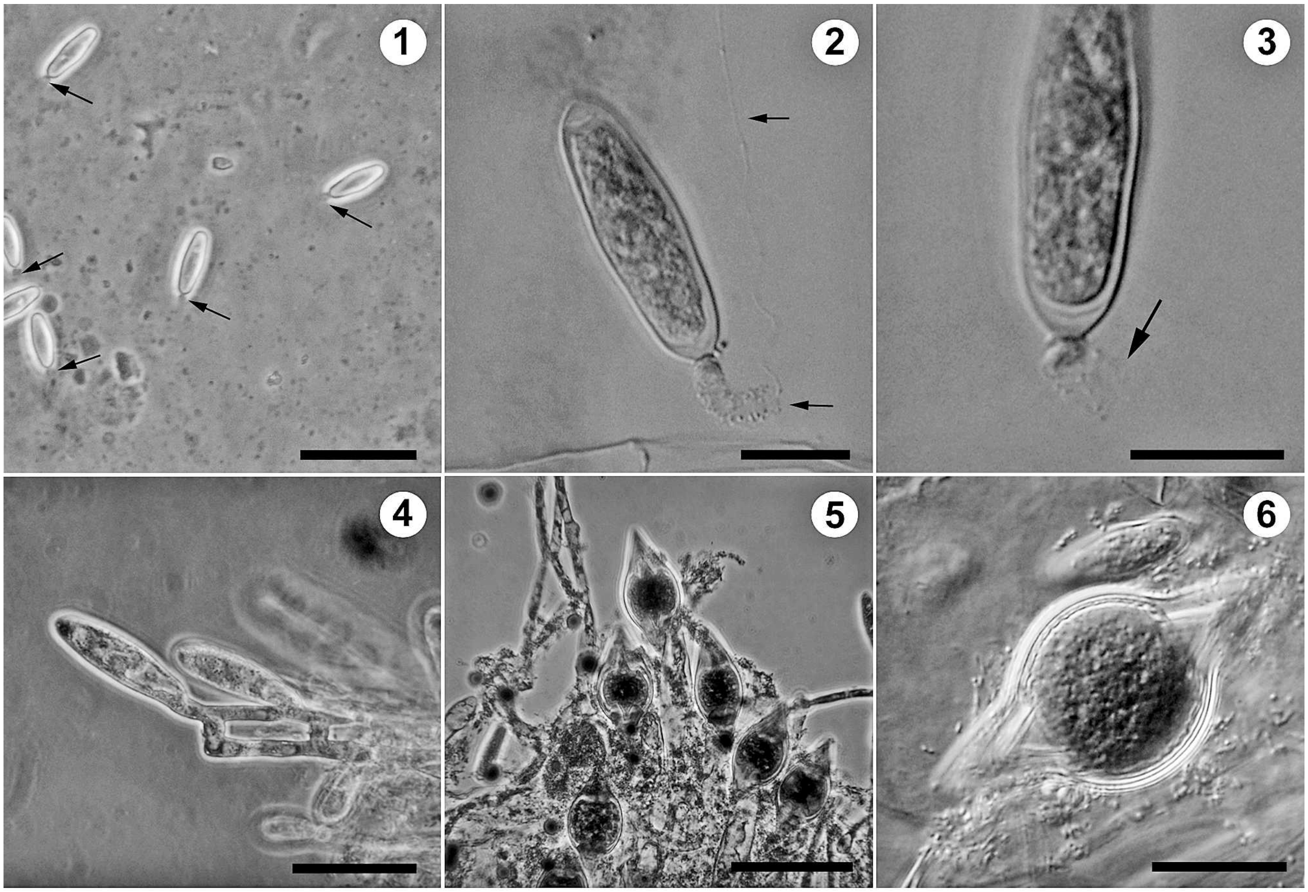
penicillin-streptomycin antibiotic solution (according to Lichtwardt et al. 2001). Thalli were preserved in 2× Cetyl trimethylammonium bromide buffer (CTAB) for future molecular studies. Insect hosts were preserved in 70% ethanol for later identification.

TAXONOMY

Austrosmittium patagonicum Siri sp.nov. FIGS. 1–6
MycoBank MB 514145

Thalli ramosi < 300 μm, structura basali ex cellulis in annulum circum appressorium ramificans dispositis. Trichosporae ellipsoidales (24–)28(–31) × 7 μm, appendice tenui, longa, collare 9–13 μm longo. Zygosporae biconicae (Type II), in medio distincte protuberantes (47–)54(–62) × (21–)24(–28) μm, collare obliquo praeditae; conjugationibus scalariformibus inter thallos orients. In proctodaeo larvarum Podonominatarum (Chironomidarum).

Branched thalli < 300 μm long, basal structure consisting of cells arranged in a ring around a holdfast from which branches arise. Ellipsoidal trichospores (24–)28(–31) × 7 μm, with a thin and long appendage, collar 9–13 μm long. Biconical zygospores (Type II) with a pronounced median bulge, (47–)54(–62) × (21–)24(–28) μm, and an oblique collar; zygosporophore 40 × 12 μm (n = 1)



FIGS. 1–6. *Austrosmittium patagonicum*. 1. Released trichospores before staining (arrows indicating the collar). 2. Appendage beginning to unfurl (arrows). 3. Base of trichospore (arrow indicating the collapsing collar). 4. Scalariform conjugations. 5. Thalli with zygospores. 6. Magnified, thick-walled zygospore. Bars: 1, 4, 5 = 50 μm; 2, 3 = 10 μm; 6 = 20 μm.

arising from scalariform conjugations between thalli. In hindgut of Podonominae (Chironomidae) larvae.

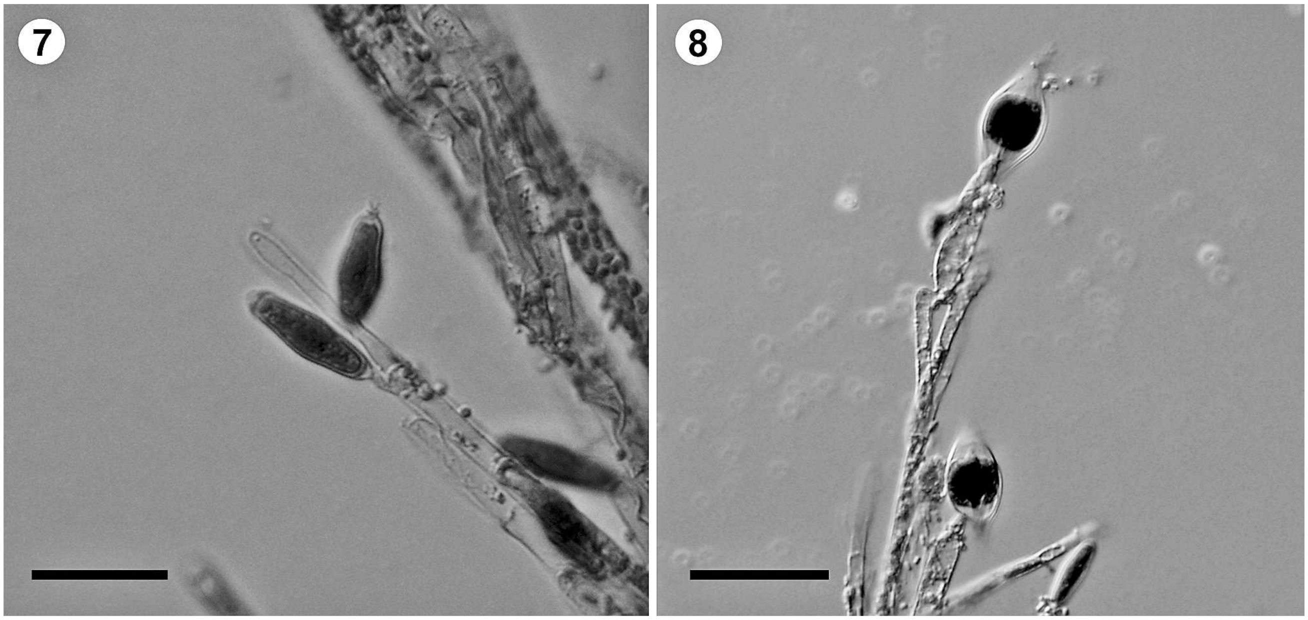
Etymology. patagonicum = from Patagonia (southern region of Argentina).

Specimens examined. ARGENTINA. NEUQUÉN: stream (unnamed) from Parque Nacional Lanín, 39°46′27.4″S, 71°40′56.8″W (Site LAN-4, TABLE I), 17-I-2005. Microscope slide LAN-4-2 (HOLOTYPE, LPSC 48159) showing thallus, trichospores and zygospores from hindgut of *Parochlus* sp. (Diptera: Chironomidae). ISOTYPES: from same collection and host species as the holotype, microscope slides LAN-4-1, LAN-4-5, LAN-4-6, LAN-4-8, LAN-4-10, LAN-4-11, LAN-4-12, LAN-4-13, LAN-4-14.

The main characters used to place this new species in genus *Austrosmittium* were the morphology of the trichospores and zygospores, as well as the type of host. Zygospores of *A. patagonicum* (FIGS. 5, 6) are larger in most cases than known zygospores from the other species of genus *Austrosmittium* (Lichtwardt and Williams 1990, Williams and Lichtwardt 1990).

Trichospores of *A. patagonicum* showed a short collar before staining (FIG. 1), although the infiltrated trichospores either appeared collarless or the collar was delicate and collapsed (FIG. 3). On release *A. patagonicum* trichospores reveal a long, thin coiled appendage, which extends after a few minutes (FIG. 2). Trichospores of other *Austosmittium* spp. have an evident long collar (Lichtwardt and Williams 1992a).

The size of *A. patagonicum* trichospores is similar to the larger trichospores of the dimorphic *A. biforme* Williams & Lichtwardt (Lichtwardt and Williams 1992a). As in *A. kiwiorum* Williams & Lichtwardt, there are scalariform conjugations (Williams and Lichtwardt 1990) from which zygospores are formed (FIG. 4). *Austrosmittium norinsulare* Lichtwardt and *A. aussiorum* Williams & Lichtwardt are the other species previously published, both with trichospores and zygospores of lesser size (Lichtwardt and Williams 1990, Williams and Lichtwardt 1990) than those of *A. patagonicum*. All previously published species of *Austrosmittium* were isolated from subfamily Orthocladiinae, the presence of *A. patagonicum* being the first report from subfamily Podonominae.



FIGS. 7–8. *Austrosmittium lenticum*. 7. Detached and attached trichospores. 8. Zygospores still attached to their zygosporophores. Bars = 20 μm.

Austrosmittium lenticum Siri sp. nov. FIGS. 7, 8
MycoBank MB 514146

Thalli minores quam 120 μm longi, axe ramificato principali effecto, trichosporis per ramum fertilem 2–4, secretione tenaci praediti. Trichosporae ovales vel ellipsoidales, (10–)12(–17) × 5–6 μm, collare 2–3 μm longo; appendiceque carente. Zygosporae biconicae (Type II), in medio protuberantes, (23–)26(–28) × 10–12 μm, collare obliquo 10–12 × 2–3 μm praeditae; zygosporophora circa 34–38 × 6–7 μm. In proctodaeo larvarum Orthocladiinarum (Dipterorum: Chironomidarum).

Thalli up to 120 μm long, with a ramified main axis, 2–4 trichospores per fertile branch and a secreted holdfast. Oval to ellipsoidal trichospores (10–)12 (–17) × 5–6 μm, collar 2–3 μm; appendage not apparent. Detached biconical zygospores (Type II) (23–)26(–28) × 10–12 μm, with a median bulge and an oblique collar 10–12 × 2–3 μm. Zygosporophores 34–38 × 6–7 μm. In hindgut of Orthocladiinae (Diptera: Chironomidae) larvae.

Etymology. lenticum = from a lentic environment.

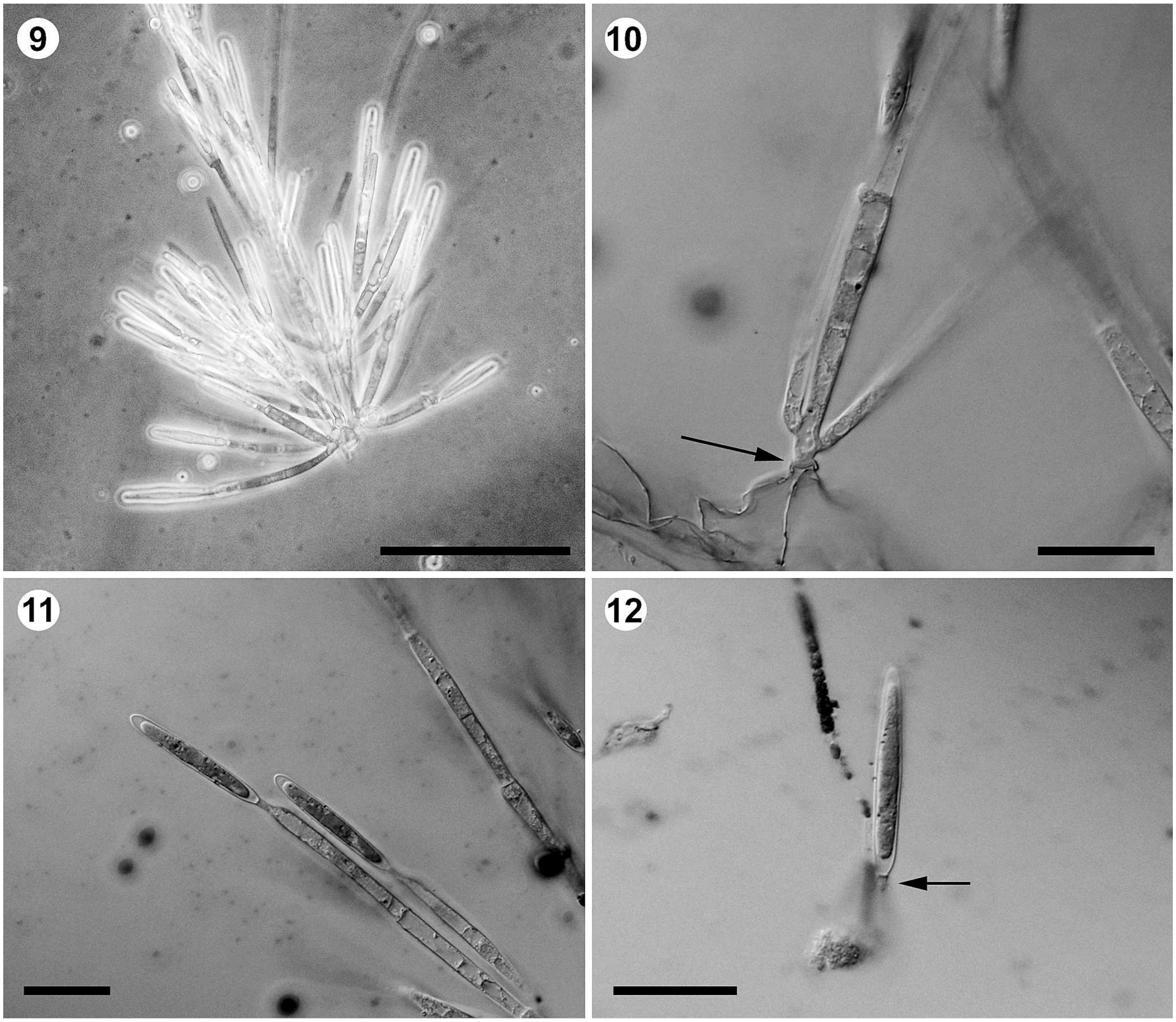
Specimens examined. ARGENTINA. BUENOS AIRES: ephemeral pond, in Arana (La Plata), 35°00′41.3″S, 57°54′49.0″W (Site ChL-1, TABLE I), 28-VII-2005. Microscope slide ChL-1-1 (HOLOTYPE, LPSC: 48160), which contains a mature thallus, trichospores and zygospores, from hindgut of *Corynoneura* sp. (Orthocladiinae: Chironomidae). Other vouchered specimens from the same site and host: ChL-1-2, ChL-1-4, ChL-1-5, ChL-1-7, ChL-1-8, ChL-1-12, ChL-1-15 on the same date as holotype; ChL-1-16, ChL-1-17, ChL-1-18, ChL-1-19, ChL-1-20, ChL-1-21, ChL-1-22 collected on 08-VIII-2005 and ChL-1-25, ChL-1-27, ChL-1-29 collected on 10-XI-2005.

Austrosmittium lenticum shows the Type II zygospore morphology of the genus (Moss et al. 1975) (FIG. 8). The location in the chironomid hindgut and trichospore morphology also support the generic placement of this new species. Nonetheless trichospores differ from the other *Austrosmittium* spp. because no appendage was observed and a collar was lacking (FIG. 7). The new species resembles *A. aussiorum* in trichospore size and length of zygospores (Williams and Lichtwardt 1990), however zygospore width is clearly greater in *A. lenticum* and its zygospores are more spherical in the mid-region. Trichospore sizes are similar to the smaller trichospores of *A. biforme*, although *A. lenticum* is not dimorphic like *A. biforme* (Lichtwardt and Williams 1992a).

Although trichospores of *A. norinsulare* (Williams and Lichtwardt 1990) have similar dimensions to *A. lenticum*, the zygospores of *A. norinsulare* are smaller than *A. lenticum*. *Austrosmittium lenticum* is the first species within this genus reported from an insect in a lentic environment.

Smittium basiramosum Siri sp. nov. FIGS. 9–12
MycoBank MB 514147

Thalli minores quam 350 μm longi, principe ad basem ex ea rami pauci orientes; ad hostem per appressorium discoideum affixum. Trichosporae subcylindricae, (30–) 33(–35) × 5–6 μm, collare 1–2 μm longo atque appendice unica praeditae. Zygosporae ignotae. In proctodaeo larvarum Chironomidarum affixum.



FIGS. 9–12. *Smittium basiramosum*. 9. Entire thallus showing the basal ramification. 10. Discoidal holdfast (arrow). 11. Attached trichospores. 12. Released trichospore with a short collar (arrow). Bars: 9 = 100 μm, 10–12 = 20 μm.

Thalli up to 350 μm long, ramified only at the base from which a few branches are produced; attached to the host hindgut by a disk-like holdfast. Subcylindrical trichospores, (30–)33(–35) × 5–6 μm, collar 1–2 μm long, bearing a single appendage. Zygospores unknown. In hindgut of Chironomidae larvae.

Etymology. basiramosum = branched at the base.

Specimens examined. ARGENTINA. CORDOBA: Río Grande de Punilla, at La Falda (Site LF, TABLE I), 31°05′49.7″S, 64°30′04.5″W, 21-IX-2006. Microscope slide LF-A-3 (HOLOTYPE, LPSC: 48161), with mature thalli, trichospores, and a distinguishable holdfast, from hindgut of *Polypedilum* sp. larvae (Diptera: Chironomidae). Other vouchered specimens collected from the same stream, date and host: LF-A-4, LF-A-5, LF-B-1, LF-B-2, LF-C-1, LF-C-2.

Smittium basiramosum is not distinguishable from other species of *Smittium* by a single character, but no other described *Smittium* species share all the characters of this new species. The holdfast of *Sm. basiramosum* is wide at the base, are disk-like (FIG. 10). The length range of the subcylindrical trichospores of *Sm. basiramosum* (FIGS. 11, 12) fit with *Sm. chinliense* Strongman & Xu (Strongman and Xu 2006), *Sm. mucronatum* Manier & Mathiez (Manier and Mathiez 1965, White and Lichtwardt 2004) and *Sm. naiadis* Strongman & Xu (Strongman and Xu 2006), as well as with the larger trichospores of *Sm. esteparum* Ferrington, Lichtwardt & López Lastra (Lichtwardt et al. 1999) and *Sm. hecatei* Valle & Santamaría (Valle and Santamaría 2004a). However these species differ mainly in their thallial branching. In addition trichospores of *Sm. naiadis* are narrower than *Sm. basiramosum*. *Smittium esteparum* and *Sm. hecatei* produce two size ranges of trichospores, and the collar is larger in *Sm. basiramosum* (FIG. 12). Concerning *Sm. mucronatum*, trichospores are wider overall, bearing a small nipple terminally, and the collars are larger than the new species.

The thallial branching in *Sm. basiramosum* (FIG. 9) resembles that of *Sm. angustum* Williams & Lichtwardt, where thalli grow in discrete radiations because branching occurs at the base (Lichtwardt and Williams 1992b), but the size of these species differs considerably.

Legeriomyces lichtwardtii Siri sp. nov. FIGS. 13–17
MycoBank MB 514148

Thalli ramosi, axe principali carentes, ca. 450 × 7–9 μm. Trichosporae obpyriformes (25–)28(–36) × 5–6 μm, collare carentes, appendices duas tenues longas gerentes, per ramum fertilemm, 2–4. In proctodaeo nympharum Ephemeropterorum per appressorium simplex affixus. Zygosporae ignotae.

Branched thalli without a main axis, about 450 × 7–9 μm. Obpyriform trichospores (25–)28(–36) × 5–6 μm, without a collar and bearing two thin and long appendages; 2–4 trichospores per fertile branch. Attached to the hindgut of Ephemeroptera nymphs by a simple holdfast. Zygospores unknown.

Etymology. lichtwardtii = named after R.W. Lichtwardt, who is considered the father of the trichomycetes (Cafaro 2003) and who has worked in this fungal group for more than 50 y.

Specimens examined. ARGENTINA. BUENOS AIRES: material collected from a stream at Site PI-3 (TABLE I), Punta Indio 35°11′13.1″S, 57°20′13.4″W, 25-VII-2006. Microscope slide PI-3-1a (HOLOTYPE, LPSC: 48162), obtained from the hindgut of nymphs of *Caenis* sp. (Ephemeroptera: Caenidae), showing a few mature thalli and released trichospores in which the appendages are clear. Other vouchered specimens from the same stream, date and host: PI-3-2, PI-3-3, PI-3-7, PI-3-8, and PI-3-10; PI-3-16, PI-3-17, PI-3-19, PI-3-21 from the same stream and host collected on 15-VIII-2006.

When *L. lichtwardtii* trichospores begin release, appendages are helical, but when trichospores are fully released the appendages are entangled (FIGS. 14–17). This species differs from the other *Legeriomyces* species principally in the dimensions of its trichospores. *Legeriomyces lichtwardtii* is similar to *L. rarus* Lichtwardt & Williams; however the trichospores of the latter species are smaller (Williams and Lichtwardt 1993, Valle and Santamaría 2004b) and the holdfasts in mature thalli are different. *Legeriomyces aenigmaticus* Lichtwardt & Williams, which was found in USA (Lichtwardt and Williams 1983), produces three types of trichospores with measurements of the middle size trichospores being the same as trichospores in the new species. However the thick trichospore appendages in *L. aenigmaticus* helps differentiate this species from the thin appendages of *L. lichtwardtii*. *Legeriomyces ramosus* Pouzar (ex *Genistella ramosus* Léger & Gauthier), which has the widest distribution in USA and Europe (White and Lichtwardt 2004), has larger trichospores than *L. lichtwardtii*. Trichospores of *L. dolabrae* Valle with two appendages differing in length and wider at proximal ends (Valle 2007) differs from *L. lichtwardtii*. Trichospores of *L. algonquinensis* Strongman & M.M. White, recently described from Canada (Strongman and White 2008), are considerably shorter than *L. lichtwardtii*.

In vitro growth of some specimens of *L. lichtwardtii* was attained initially, but all cultures were contaminated eventually, despite repeated transfer to new media with different antibiotics.

Harpella sp. FIGS. 18–20

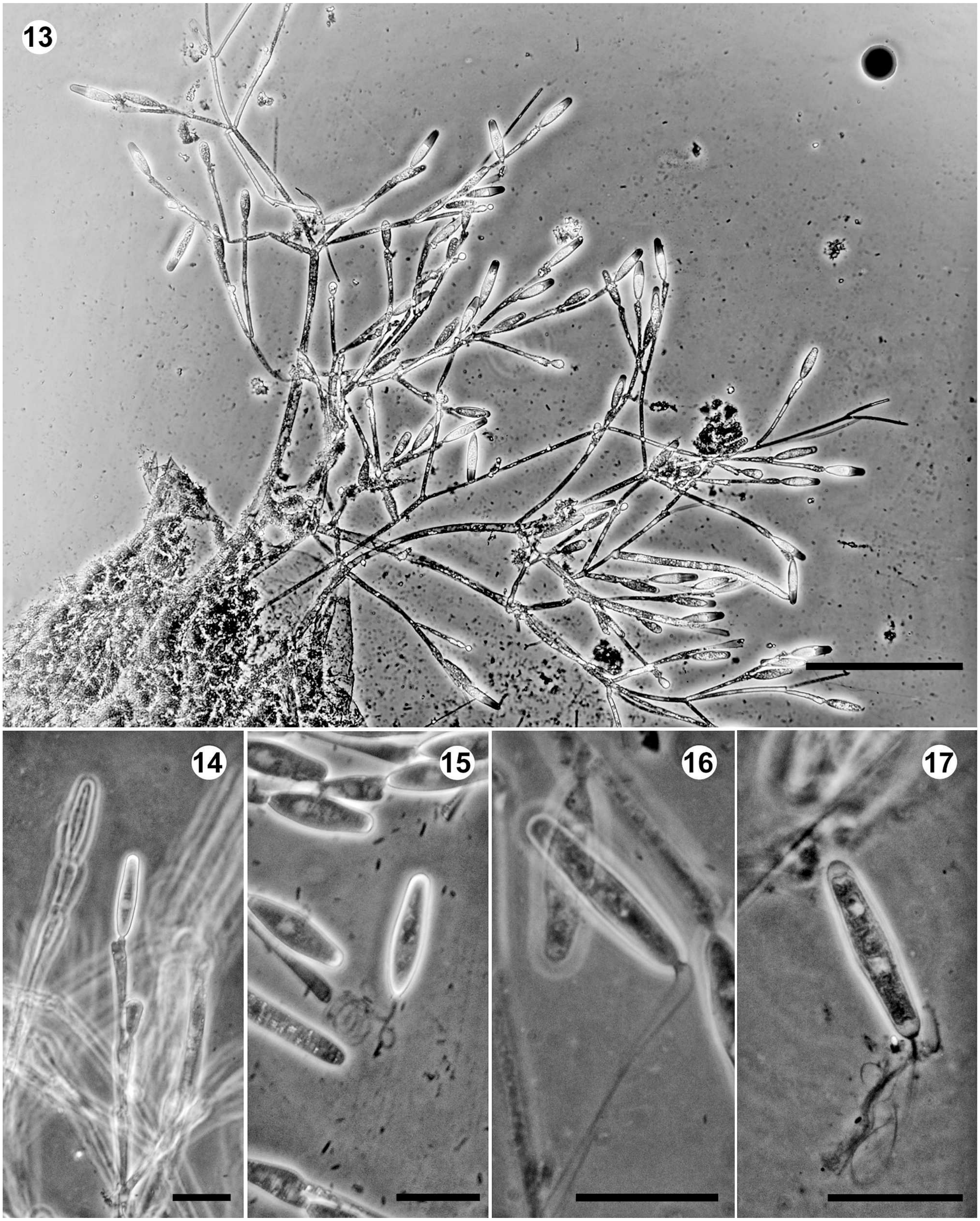
Thalli 400–700 μm long × 7–10 μm wide, partially or totally coiled, attached to simuliid larvae midgut (Site SL-3, TABLE I) by a subbasal holdfast. Zygospores and mature trichospores unknown. In hindgut of *Simulium wolffhuegeli* Enderlein (Diptera: Simuliidae) larvae.

The subbasal holdfast (FIG. 19), the transverse constrictions in the thallus (FIG. 18) and the totally or partially coiled thallus represent the main characters that differentiate *Harpella* sp. from others species in the genus. These features suggest that this might be a new *Harpella* species, but unfortunately we have not seen any mature development to allow a formal description. Additional collections of specimens with the full suite of morphological characters or molecular studies will be necessary to properly identify this species.

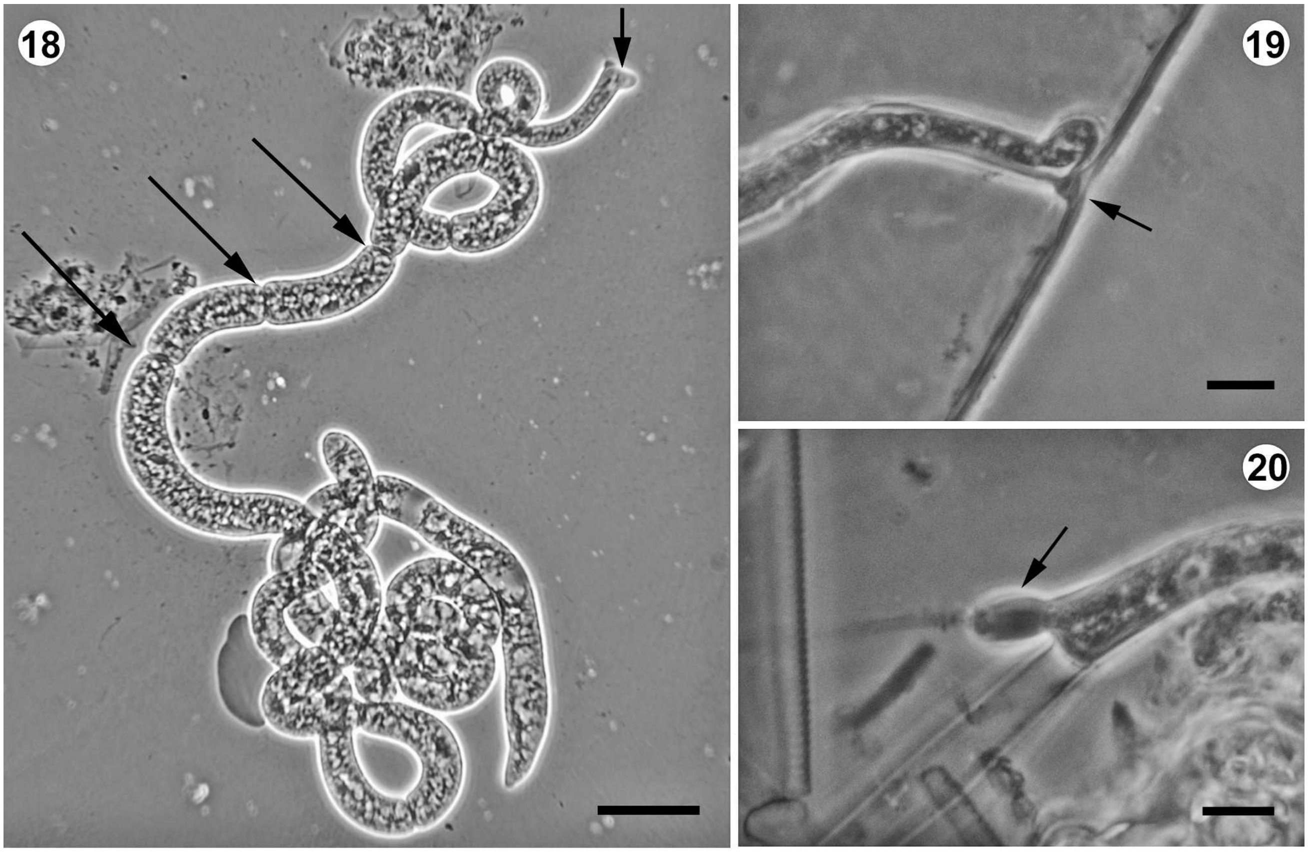
Simuliomyces sp.

Long ellipsoidal trichospores, (17–)19(–20) × 5 μm, bearing three thin appendages, longer than the trichospores body; without a collar. Biconical zygospores (Type I), 28 × 5 μm (n = 1), attached medially and perpendicularly to the zygosporophore; zygosporophore 15 × 6 μm. Branched thalli without a main axis, attached to the host by a spherical holdfast. In hindgut of *Simulium* (*Ectemnaspis*) *romanai* Wigodzinsky larvae (Diptera: Simuliidae) from Site LR-9 (TABLE I).

The ellipsoidal, collarless trichospores of *Simuliomyces* sp. (with three thin appendages) are similar to those of *Si. microsporus* Lichtwardt (with 2–4, typically two, thin appendages), but trichospores of *Si. microsporus* are longer (20–30 × 4–6 μm) (Lichtwardt 1972, White and Lichtwardt 2004). Also in both



FIGS. 13–17. *Legeriomyces lichtwardtii*. 13. Thallus overview. 14–17. Sequence of trichospore release, showing the two appendages. Bars: 13 = 100 μm, 14–17 = 20 μm.



FIGS. 18–20. *Harpella* sp. 18. Entire thallus attached by a subterminal holdfast (shorter arrow) and transverse constrictions (larger arrows). 19. Detail of the holdfast (arrow). 20. Immature trichospore (arrow). Bars: 18 = 50 μm, 19 = 20 μm, 20 = 10 μm.

species the tip of trichospores often appears thick-walled when trichospores are mounted in lactophenol-cotton blue.

Zygospores of *Simuliomyces* sp. are smaller than those of *Si. microsporus* (34–45 × 7–9 μm). Zygospores of *Si. microsporus* have the side of attachment flattened (Lichtwardt 1972) whereas our *Simuliomyces* sp. shows a different morphology. Thalli of *Si. microsporus* commonly attach to thalli of *Paramoebidium* sp. or other trichomycetes. We have seen thalli of *Simuliomyces* sp. attached only to the hindgut cuticle of the host. Both species are clearly different, and *Simuliomyces* sp. likely represents a new species; however the collected material is not sufficient to provide a complete description.

PREVIOUSLY DESCRIBED SPECIES

Genistellospora *homothallica* Lichtwardt

Trichospores [(24–)30(–41) × (8–)10(–13) μm] of this species might be confused with *Pennella* spp, although *G. homothallica* is easily recognizable by its prominent secreted holdfast, growth habit and Type III zygospores. Zygospores of *G. homothallica* [(77–)100(–113) × (15–)20(–22) μm] are unique among Harpellales, being formed without conjugations between thalli. This species has been found within the hindgut of Simuliidae larvae from streams in many countries including USA, Puerto Rico, England, Spain, Costa Rica, Chile and Argentina (White et al. 2000, Lichtwardt et al. 2001, López Lastra et al. 2005). In Argentina *G. homothallica* was found by Lichtwardt et al. (1999, 2000) and López Lastra et al. (2005) from several streams.

Some trichospore measurements from Site SV-6 were somewhat longer (43 μm) than other published measurements of this species. In all other cases our specimens fit the published measurements of *G. homothallica*. We found this species inhabiting the hindgut of black fly larvae from these hosts and sites: *Simulium* (*Psaroniocompsa*) *bonaerense* Coscarón & Wygodzinsky from MP-1 (13-VII, 23-X-2005, 24-II-2006), MP-2 (13-VII-2005), MP-3 (23-X-2005), MP-4 (13-VII, 23-X-2005, 24-II, 03-V-2006), MP-6 (13-VII-2005, 23-X, 24-II, 03-V-2006), MP-7 (23-X-2005), MP-8 (23-X-2005), MP-9 (23-X-2005); SV-3 (20-II-2006), SV-

4 (21-II-2006), SV-6 (23-II-2006), and *S. auripellitum* Enderlein from SV-8 (8-III, 11-VI-2007) (TABLE I).

Harpella meridianalis Lichtwardt & Arenas

The first report of *H. meridianalis* was by Lichtwardt and Arenas (1996) from southern Chile and later recorded in Argentina from Buenos Aires (Lichtwardt et al. 1999), Neuquén and Chubut (Lichtwardt et al. 2000) and Tierra del Fuego provinces (López Lastra et al. 2005). In this paper we extend the distribution of *H. meridianalis* La Rioja and Córdoba provinces at northwestern and central regions of Argentina respectively.

The curved to coiled trichospores of *H. meridianalis* easily distinguish this species from *H. tica* Lichtwardt, which is the other *Harpella* found in Argentina. *Harpella meridianalis* is similar to *H. leptosa* Lichtwardt & Moss (Moss and Lichtwardt 1980); however the trichospores of the former are shorter and narrower and the holdfast is wider. Conjugations were found between thalli on the material collected in Site CHA-1; however no zygospores were formed even after maintaining them within a humidity chamber for a few hours (Lichtwardt 1986, Lichtwardt and Williams 1988).

In the present study we found this species within the larval hindgut of: *Simulium* (*Psaroniocampsa*) *bonaerense* from CHA-1 (08-XI-2004); *S.* (*Trichodagmia*) *lahillei* Paterson & Shannon from LR-4 (22-IV-2005), *S.* (*Trichodagmia*) *huairayacus* Wygodzinsky from LR-5 (23-II-2005), *Simulium* sp. from LR-10 (24-II-2005); *Simulium* (*Psaroniocampsa*) *bonaerense* from MP-1 (13-VII, 23-X-2005, 24-II, 03-V-2006), MP-2 (13-VII-2005), MP-3 (23-X-2005), MP-4 (13-VII, 23-X-2005, 24-II, 03-V-2006), MP-6 (13-VII, 23-X-2005, 24-II, 03-V-2006), MP-7 (23-X-2005), MP-8 (23-X-2005), MP-9 (23-X-2005), SV-3 (20-II-2006), SV-4 (21-II-2006), SV-6 (23-II-2006), *S. auripellitum* from SV-8 (8-III, 11-VI-2007) and *Simulium wolffhuegeli* Enderlein from SL-3 (08-I-2007) (TABLE I).

Harpella tica Lichtwardt

Harpella tica was described by Lichtwardt (1997) from Costa Rica and later from Argentina (Lichtwardt et al. 2000, López Lastra et al. 2005) and Puerto Rico (White et al. 2000). In previous studies conducted in Argentina this species was described in the northwest and northeast. In the present study we found *H. tica* within the peritrophic matrix of the midgut of *Gigantodax* sp. larvae (Diptera: Simuliidae) collected from a stream in Parque Nacional Lanín (Site LAN-2-1). *Harpella tica* is identified easily by the sigmoid (or rarely curved to coiled) trichospores, while in other *Harpella* species the trichospores are almost always curved or coiled (rarely straight).

Pennella montana Lichtwardt

The base of thalli of *Pennella montana* is dichotomously bifurcated and the trichospores are long-ovoid [(39–)60(–72) × (8–10 µm)], bearing fine multiple appendages. We have not found zygospores of *P. montana* in our specimens. *Pennella montana* was found at Site LR-5 in *Simulium* (*Trichodagmia*) *huairayacus* larvae, LR-10 from *Simulium* sp. larvae, and SL-3 from *S. wolffhuegeli* larvae. *Pennella montana* was cited previously from Argentina in the northeast (Lichtwardt et al. 2000).

Simuliomyces microsporus Lichtwardt

Simuliomyces microsporus has similarities to genus *Smittium* and could be misidentified when their multiply appendaged trichospores or differently shaped zygospores (Type I) are not present. However *Si. microsporus* is easily distinguishable from *Smittium* spp. when trichospores are released because they are collarless and have 2–4 appendages. Also it is common to find thalli of *Si. microsporus* attached to *Paramoebidium* sp. thalli or other trichomycetes co-inhabiting the digestive tract of black fly larvae. Trichospores also are recognizable after mounting in lactophenol-cotton blue by the thick-walled appearance of the trichospore tips (Lichtwardt 1972).

Simuliomyces microsporus has been reported from several countries around the world, including Argentina (Lichtwardt et al. 1999, 2000). Size ranges for trichospores and zygospores, 20–30 × 4–6 µm and 34–45 × 7–9 µm respectively, in our specimens correspond to published measurements of this species. We have collected this species at sites LR-2 and LR-10 from *Simulium* sp. larvae and Site SV-8 from *S. auripellitum* larvae.

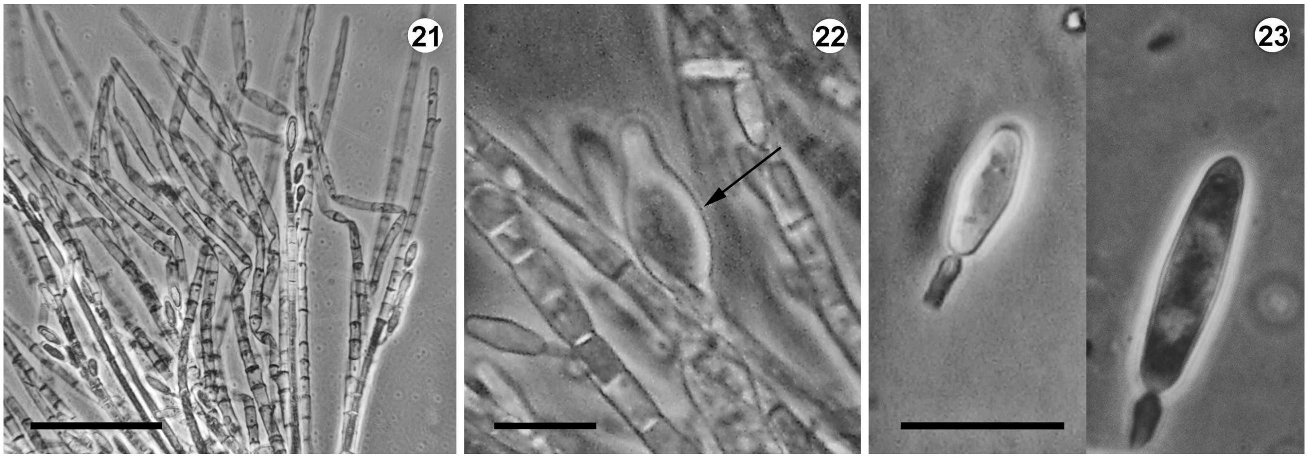
Smittium culicis Manier FIGS. 21–23

= *Orphella culicis* Tuzet & Manier 1947 *nom. nud.*

= *Rubetella culicis* Tuzet, Rioux & Manier 1961 *nom. nud.*

Smitium culicis has a cosmopolitan distribution and it is found principally infecting Culicidae larvae, but also Chironomidae, Simuliidae and Thaumaleidae larvae (Lichtwardt et al. 2001). This species is recognizable by the trichospores that are (15–)20 (–32) × (4–)6(–8) µm, with a median bulge and campanulate collar.

Specimens collected at sites PI-3 and PI-8 from simuliid and chironomid larvae were characteristic. In these cases we found trichospores of two sizes, 12–16 × 5 µm and 21–31 × 5–6 µm (FIG. 23), within the hindgut of both kinds of hosts (we have not



FIGS. 21–23. Unusual specimens of *Smittium culicis*. 21. Fertile branchlets with high number of generative cells (many already have released trichospores) and attached trichospores. 22. Slightly immature zygospore with rounded ends (arrow). 23. Shorter (left) and larger trichospore (right). Bars: 21 = 50 μm; 22, 23 = 20 μm.

considered this as dimorphism because we have not found both trichospore sizes within the same host at any time). In these specimens only a few biconical zygospores with rounded ends (about 46 × 10 μm, FIG. 22) were found from Site PI-8; however these zygospores might have been immature. The number of trichospores per fertile branch (< 20, FIG. 21) differs considerably from *Sm. culicis*. Similar to *Sm. culicisoides*, the simple holdfast of some specimens produced small projections that enlarge the adhesive area (Lichtwardt 1997). However our specimens differ from *Sm. culicisoides* by having narrower trichospores, a greater number of generative cells per fertile branch (1–4 in *Sm. culicisoides*) and by the simple holdfast. As with *L. lichtwardtii* collected from the same site of these rare specimens, in vitro growth was obtained initially but no axenic cultures ultimately were obtained. Additional material will be necessary to ascertain whether these specimens represent a new species.

We found *Sm. culicis* in mosquito larvae of *Aedes aegypti* L. from Site CEM (17-II, 23-III-2005); *Culex castroii* Casal & García from Site ChL-2 (15-XI-2005), LP-1 (14-XII-2004), PL-I (24-XI, 23-XII-2003, 09-XI-2004); PI-2 (25-VII-2006); *Cx. pipiens* Wiedeman from Site LP-6 (25-I-2005), CEM (17-III, 31-III, 27-IV-2005); ChL-1 (28-XII-2006); and *Ochlerotatus albifasciatus* Macquart on Site ARR-1 (26-I-2006); ARR-2 (06-VII-2005); PL-4 (28-VI-2006), PL-5 (10-II-2006), in chironomid larvae of *Chironomus* sp. from Site BG-3 (27-X-2004), ENS-1 (16-V-2005) and LF-1 (21-IX-2006). Also we found rare specimens from *Simulium delponteianum* (Wygodzinsky) and Orthocladiinae (Diptera: Chironomidae) larvae from sites PI-3 and PI-8 (25-VII-2006).

Axenic cultures of *Sm. culicis* were obtained from specimens of *Cx. pipiens* (accession number CEP-290, Site LP-6, 25-I-2005); *O. albifasciatus* (accession number CEP-291, Site ARR-2, 06-VII-2005); *Cx. pipiens* (accession number CEP-292, Site CEM, 23-III-2005) and Chironomidae (accession number CEP-293, Site BG-4, 03-XI-2004).

Smittium culisetae Lichtwardt

= *Smittium inopinatum* Manier 1969 (1970)

= *Rubetella inopinata* Manier, Rioux & Whisler 1961 *nom. nud.*

This is the other cosmopolitan harpellid species infecting mosquito larvae and less commonly infecting Chironomidae, Simuliidae, Ceratopogonidae and Tipulidae larvae (Lichtwardt et al. 2001). Because *Sm. culisetae* is an easily cultured species most of the physiological, biological and structural studies of Harpellales in vitro have been conducted with it. Despite the fact that *Sm. culisetae* is a common inhabitant of mosquito species around the world, zygospores have been found only once (Williams 1983).

Material obtained from the hindgut of *O. crinifer* from Site PL-2 has some differences with published data for this species. Thalli had a higher number of trichospores per fertile branch (< 20 in some cases) than published for *Sm. culisetae* (4–10). We found *Sm. culisetae* in mosquito larvae of *Ae. aegypti* from Site CEM (17-III-2005); *Cx. castroii* from Site BG-4 (03-XI-2004), BG-5 (03-XI-2004), PL-1 (06-XII-2004); *Cx. apicinus* Philippi from Site SW-1 (08-V, 15-VIII, 22-VIII, 23-X, 05-XII-2006), SW-2 (29-XI-2004, 20-VII-2005); *Cx. pipiens* from Site ChL-1 (28-XII-2006), CEM (27-IV-2005), LP-7 (04-II-2005); *O. albifasciatus* from sites ChL-1 (23-III-2006), ChL-3 (28-XII-2006); *Psorophora ferox* Humboldt from sites ChL-1 (23-III-

2006, 06-III-2007), PL-2 (03-X-2005, 10-II-2006, 04-I-2007), LP-8 (14-XII-2004); *P. cyanescense* Coquillet from Site PL-2 (04-I-2007).

Axenic cultures of *Sm. culisetae* were obtained from larvae of *O. crinifer* (accession number CEP-294, Site PL-2, 04-I-2007); *Cx. apicinus* (accession number CEP-295, Site SW-1, 08-V-2006); *O. albifasciatus* (accession number CEP-296, Site SW-1, 06-IX-2006) and *Ae. aegypti* (accession number CEP-297, Site CEP, 17-III-2005).

Smittium phytotelmatum Lichtwardt

The species previously was recorded only from Costa Rica (Lichtwardt 1994) in the hindgut of chironomid larvae living in phytotelmata and other semipermanent aquatic environments. We found *Sm. phytotelmatum* from the same microhabitats as in Costa Rica in *Polypedilum* sp. larvae from Site BG-5 (03-XI-2004), PL-I (03-X-2005, 10-II-2006, 04-I-2007), PL-2 (10-II-2006) and ChL-2 (20-VII-2005, 28-XII-2006). In addition *Cx. quinquefasciatus* Say (Diptera: Culicidae) larvae collected in a water reservoir from Site CHAC also were infected with *Sm. phytotelmatum*. Infections in these mosquito larvae represent the first report of *Sm. phytotelmatum* infecting culicid larvae. Axenic cultures of *Sm. phytotelmatum* were obtained from *Polypedilum* sp. larvae from sites BG-5, ChL-2 and PL-1 (accession numbers CEP-298, CEP-299 and CEP-300 respectively).

Smittium simulii Lichtwardt

Smittium simulii has a wide distribution, and it has been cited from USA, Japan, Australia, New Zealand and several European countries (Lichtwardt et al. 2001, White and Lichtwardt 2004). This species is associated with the hindgut of black fly and chironomid larvae and is attached to the host by a distinctive horseshoe-shape basal cell. Also it may rarely infect larva of mosquitoes and tipulids. In the present study we found *Sm. simulii* in larvae of *Simulium* sp. (sites LR-5 and LR-10), *Simulium* (*Ectemnaspis*) *romanai* (LR-9), *S. delponteianum* (LR-8) and *S. auripellitum* (Site SV-8). The published trichospore measurements of this species are (16–)23(–30) × (3–)5(–7) μm, and measurements of the Argentinean specimens collected in this study match those previously published.

Stachylina grandispora Lichtwardt

Stachylina grandispora might represent one of the most common harpellid species from chironomid larvae, and it has been found in several countries, including endemic species of midges from New Zealand, Australia and Hawaii (White et al. 2006b). Thalli and trichospore lengths of *St. grandispora* are variable, with trichospores 40–72 × 6–10 μm, but the large trichospores help to distinguish this species. Conjugations between thalli were found on some specimens, but no zygospores were formed. Specimens from Argentina were collected from *Chironomus* sp. from sites R9-5, BG-2 and ARR-1, and Orthocladiinae larvae from PI-6.

Stachylina lentica White & Lichtwardt

Before this study *St. lentica* was found inhabiting only the peritrophic matrix of chironomid larvae collected in a rocky bottom near shorelines of a lake in Norway (White and Lichtwardt 2004). We have found this species in Argentina (sites PL-1, ChL-2, ENS-1, TABLE I) from *Polypedilum* sp. and *Metriocnemus eryngiotelmatus* Donato & Paggi larvae living in the impounded water between the bracts of *Eryngium* spp. (phytotelmata).

The measurements of thalli (50–150 × 4–9 μm) and trichospores [(22–)35(–50) × (4–)5–6(–7) μm] of collected material in this study are the same as original measurements cited by White and Lichtwardt (2004). *Stachylina lentica* resembles *St. grandispora*; however the former species on average has shorter trichospores and thalli are usually shorter and narrower as well. In addition thalli of *St. lentica* produce 1 or 2 (rarely 4) trichospores, while *St. grandispora* produce commonly 4–16 (rarely fewer than 4).

Stachylina lotica Williams & Lichtwardt

Stachylina lotica was found attached to the peritrophic matrix that lines the midgut of *Chironomus* sp. (Diptera: Chironomidae) larvae sampled in an experimental rice field. Thalli (< 100 μm long) and trichospore measurements (24–32 × 8–10 μm), and the number of trichospores per thallus (< 8) coincides with published data (Williams and Lichtwardt 1984, Frost 1996). *Stachylina lotica* was present in most of biweekly collections Dec 2004–May 2005 from Site ARR-1 (TABLE I).

Stachylina platensis López Lastra, Lichtwardt & Ferrington.

Stachylina platensis was found in the midgut of *Chironomus* sp. from Site SW-1 and was cited once by Lichtwardt et al. (1999) from Argentina. The thalli of *St. platensis* are 6–10 μm diam, with four to more than 32 generative cells; trichospores ellipsoidal, 25–36 × 5–7.8 μm, with a short collar and one appendage that sometimes appears to split in two.

Stachylina penetralis Lichtwardt

Thalli (> 100 × 8–11 μm) bearing usually 2–8 trichospores per thallus. The trichospore on the terminal generative cell is produced one-third to one-half the distance from the cell apex. Trichospores 30–35 × 8–10 μm, with one long, narrowing appendage but no collar. Basal cell with a foot-like extension that penetrates through the peritrophic matrix. Within the peritrophic matrix of *Parametriocnemus* sp. (Chironomidae: Orthocladiinae) larvae from Site LF-1. Previous reports of this species were from France, Sweden, Japan and Spain within the peritrophic matrix of Diamesinae (Diptera: Chironomidae) larvae (Lichtwardt 1984, Valle 2007). This is the first report of *St. penetralis* from Argentina and from larvae of subfamily Orthocladiinae.

Amoebidiales

Amoebidium and *Paramoebidium* are the only two genera comprising the mesomycetozoan order Amoebidiales. *Amoebidium* spp. grow externally on the host, being the only ectocommensal of the trichomycetes.

Amoebidium parasiticum was found attached externally to the cuticle of *O. crinifer* (Diptera: Culicidae) larvae from Site PL-2. Thalli of *Paramoebidium* sp. were found within the hindgut of *Simulium* sp. larvae from sites LR-2, *Simulium* (*Ectemnaspis*) *romanai* from sites LR-9, SV-5 and SV-6, and *S. auripellitum* from SV-8.

DISCUSSION

As a result of this study four new species are described and 14 previously known species are reported, all from order Harpellales. Two undescribed species might be new, however material collected was not sufficient for a complete diagnosis. Also the mesomycetozoans *Paramoebidium* spp. and *A. parasiticum* (Amoebidiales) were recovered. These data increase our knowledge of Argentinean trichomycetes and reveal new hosts and distributions. Given the size of Argentina, there exists a wide range of biomes including a high diversity of aquatic environments, resulting in a potentially high diversity of trichomycetes. However most areas of Argentina still have not been explored.

Austrosmittium.—Before this study genus *Austrosmittium* was recorded only from Australia and New Zealand (Lichtwardt and Williams 1990, 1992b; Williams and Lichtwardt 1990). In the present study we report two new species of *Austrosmittium*, although White and Strongman (pers comm) have found other species of this genus in Canada and USA. Species of *Austrosmittium* found in Argentina were obtained from very different environments. *Austrosmittium patagonicum* was sampled in a high altitude cold stream from the southwest while *A. lenticum* was found in a temporary pond from the east-central part of the country. *Austrosmittium lenticum* is the only species of the genus collected in a lentic environment. Uncertainty surrounding this host's tolerance of lotic environments precludes any confident statement of *A. lenticum* being confined strictly to nonflowing systems.

Legeriomyces.—Species of *Legeriomyces* have been reported from USA, Australia, New Zealand, Spain, France, England, Norway and Sweden (Lichtwardt and Williams 1983, Williams and Lichtwardt 1993, White and Lichtwardt 2004, Valle and Santamaria 2004b, Valle 2007). *Legeriomyces lichtwardtii* represents the first report of *Legeriomyces* from the Neotropics.

Smittium.—This is the genus with the greatest number of species within the trichomycetes. More than 70 species have been described from the hindgut of various hosts (data published in the interactive lucid key available on the Internet at http://www.nhm.ku.edu/~fungi).

Studies with rDNA sequences have demonstrated that *Smittium* is not monophyletic, including at least five different lineages (Lichtwardt et al. 2001, Misra and Horn 2001, Gottlieb and Lichtwardt 2001, White 2002, White et al. 2006a).

Before this study 10 species of this genus were described from Argentina, therefore, considering the two new species and *Sm. phytotelmatum* (not previously described from Argentina), there are 13 species at present.

Smittium culicis and *Sm. culisetae* have been reported from practically all countries where trichomycetes have been studied. *Smittium simulii* is another cosmopolitan species of Harpellales, and it also has been found in various sites in Argentina. *Smittium phytotelmatum* has never been found before in Argentina, where it was collected from the hindgut of *Polypedilum* sp. sampled from phytotelmata and temporal ponds. Over the 2 y study of Siri et al. (2008), in which they investigated the prevalence of harpellid species from chironomid larvae, *Sm. phytotelmatum* and *St. lentica* were present throughout the year in *Polypedilum* sp. larvae. Also *Sm. phytotelmatum* was found in the hindgut of *Cx. quinquefasciatus* larvae, which is the first report of a culicid as a host of this species.

Pennella.—*Pennella montana* first was described by Lichtwardt (1997), who found this species growing in the hindgut of simuliid larvae from high altitude streams in Costa Rica. All specimens found in Argentina also were collected from high altitude streams, and there are no records of this species from low altitude streams. In this country the species has been collected in the western region, from the north to the central region.

Harpella.—*Harpella meridianalis* was reported exclusively from Chile and Argentina (Lichtwardt and Arenas 1996; Lichtwardt et al. 1999, 2000; López Lastra et al. 2005). *Harpella tica* is the other species found in Argentina (Lichtwardt et al. 2000) but also recorded from Cost Rica (Lichtwardt 1997) and Puerto Rico (White et al. 2000). Lichtwardt et al. (2000) have reported both *H. meridianalis* and *H. tica* growing in the midgut of the same host. We have not found these species growing within the same host, but we have found *H. meridianalis* and *Harpella* sp. growing within the same host.

Harpella amazonica is the fourth species known from South America, described from various streams in Brazil (Alencar et al. 2003). Trichospores of *H. amazonica* are similar to those of *H. meridianalis*, but trichospores in the former are shorter and narrower.

Stachylina.—This is a cosmopolitan genus, which is present principally within the peritrophic matrix of chironomid larvae, but there have been reports also of species associated with ceratopogonid, thaumaleid, psychodid and simuliid larvae. More than 30 species of *Stachylina* currently are described (Lichtwardt et al. 2001). *Stachylina* is also a polyphyletic genus (White et al. 2006a). To date no *Stachylina* species has been cultured. Four (*St. lentica*, *St. lotica*, *St. penetralis* and *St. grandispora*) of the five species of *Stachylina* found in the present study represent first reports from Argentina.

Genistellospora and Simuliomyces.—Both *G. homothallica* and *Si. microsporus* are common species of trichomycetes infecting the hindgut of simuliid larvae around the world. These species were collected in streams of Argentina, but in some samples they were found together. In Site SV-8 simuliid larvae were infected simultaneously with *G. homothallica*, *Si. microsporus*, *H. meridianalis* and *Paramoebidium* sp.

Amoebidiales.—*Amoebidium parasiticum* has a wide host range including many Crustacea and Insecta and has been found in numerous worldwide localities (White et al. 2004). This species is mainly found in lentic environments such as ditches or pools polluted with organic matter (Lichtwardt 1986). Specimens of *Paramoebidium* sp. were found inhabiting the hindgut of simuliid larvae from streams in Argentina.

Although the knowledge of trichomycetes diversity from Argentina has improved in the past few years, continued studies are needed to extend the knowledge of these gut fungi, especially in the unexplored or underexplored areas of the country.

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