

An updated checklist and literature review of Harpellales (Kickxellomycotina) and other endobionts associated with the guts of arthropods from Argentina

Lista actualizada y revisión de la literatura de Harpellales (Kickxellomycotina) y otros endobiontes asociados al tracto digestivo de artrópodos de Argentina

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ABSTRACT

The objective of this work is to further the knowledge of gut fungi, inhabiting the digestive tract of mandibulated arthropods, traditionally the Trichomycetes (currently Kickxellomycotina), in Argentina. The geographical distribution of these endobionts, especially in aquatic insects, but also other arthropods, is updated as well as host records and site information. Fungal species (Harpellales), which mostly colonize immature stages of aquatic insects, were identified in members of Coleoptera, Diptera, Ephemeroptera, Plecoptera, and Trichoptera from different biogeographical regions, from the far North in Jujuy province to the southernmost point in Tierra del Fuego. The overall goal of this review is to consolidate all the published and unpublished records from the country in order to summarize their geographical distribution.

Keywords: Diptera, Ephemeroptera, fungi, Kickxellomycotina, symbionts.

RESUMEN

El objetivo de este trabajo es reunir toda la información publicada hasta el momento referida a este grupo de hongos, tradicionalmente conocidos como Trichomycetes (actualmente Kickxellomycotina), de la Argentina. El objetivo fue realizar un estado de avance sobre la distribución geográfica de estos endobiontes, especialmente viviendo en insectos acuáticos, pero también en otros artrópodos, con la actualización de registros de hospedadores e información de los sitios de recolección. Las especies de Harpellales colonizan estados inmaduros de insectos acuáticos y fueron identificados en miembros de Coleoptera, Diptera, Ephemeroptera, Plecoptera, y Trichoptera, de diferentes regiones biogeográficas desde el extremo Norte en la provincia de Jujuy al extremo más al Sur en Tierra del Fuego. El objetivo principal de este trabajo es consolidar todos los registros del material publicado y no publicado de la Argentina para resumir su distribución geográfica y lista de especies citadas.

Palabras clave: Diptera, Ephemeroptera, hongos, Kickxellomycotina, simbiosis.

INTRODUCTION

Trichomycetes, traditionally, have been recognized as symbionts of a wide range of Arthropoda, usually recovered from the digestive tracts (guts) of aquatic insects, as well as crustaceans and millipedes (non-predaceous species). Most of them are considered to be commensals or mutualistic under certain conditions but can be pathogenic, or parasitic in adult females of hematophagous Diptera, such as black flies (Labeyrie *et al.* 1996). Trichomycetes were extensively studied by Professor Robert Lichtwardt, who published two books (Lichtwardt 1986, Misra & Lichtwardt 2000) and several chapters, reviews, and more than 200 articles on the taxonomy, diversity, and biology of this cryptic group (Lichtwardt 1986, 1994, 1996, Lichtwardt *et al.* 2001, Misra 2001, Strongman 2007, Strongman & White 2010, Wang *et al.* 2010, 2013, 2014).

Although the Trichomycetes were originally presented as a class of Fungi (Lichtwardt 1986), the four (original) orders are now known to be Fungi (Harpellales and Asellariales) and protists (Amoebidiales and Eccrinales) and “trichomycetes” is considered an ecological term for a polyphyletic group of gut dwelling microbes (Benny & O’Donnell 2000, Cafaro 2005, Reynolds *et al.* 2017, Wang *et al.* 2013, 2019). The term will be used in both the traditional and ecological sense for this summary, with some emphasis on the Harpellales, in part due to the focus of prior studies, but to be inclusive as a checklist, the non-fungal taxa will be included as well.

In the present work, the overall goal is to update and consolidate records of Harpellales (“gut fungi”), as well as Amoebidiales and Eccrinales found in insects reported in Argentina, as a synopsis and a species checklist. They are known to be distributed in 14 of the 23 provinces in the country, from the far North in Jujuy province to the southernmost point, Tierra del Fuego. Several of these records described new species and / or new host distribution records (Lichtwardt *et al.* 1999, 2000, López Lastra *et al.* 2003, 2005, Sosa Gómez *et al.* 2010, Siri & López Lastra 2010).

MATERIALS AND METHODS

Surveys of trichomycetes were carried out from the North in Jujuy to the south in Ushuaia, Tierra del Fuego, Argentina. The collection sites were aquatic environments such as streams, rivers, ponds, lakes, and lagoons, as well as tree holes. Material was collected and temperatures and relative humidity were recorded, and then the insects were kept on ice in coolers until arrival at the lab, where the guts were removed according to standard procedures described in Lichtwardt (1986).

Immature insects were collected mostly from freshwater sites in different regions of Argentina, and some of the insect orders sampled were Coleoptera, Diptera, Ephemeroptera, Plecoptera, and Trichoptera and other arthropods as Isoptera. Larvae from lotic habitats were collected with limnologic D-nets or taken directly from the substrate. In the case of lentic habitats, a D-net or a 250 mL ladle were used. For phytotelmata environments, accumulated water with insect larvae was extracted with a plastic pipette (Siri *et al.* 2008, Siri & López Lastra 2010). Insects were placed in collecting jars after sorting, except for blackfly larvae and their substrates, which were placed in plastic bags or on moist filter paper lining disposable, sterile (100 mm diameter) Petri dishes (White *et al.* 2006), transported in a cooler with ice to the laboratory where they were transferred to a refrigerator (4°C).

In general, fungal surveys were performed by dissecting insect larval guts with very thin sterilized forceps and needles under a binocular stereomicroscope, according to standard techniques (Lichtwardt 1986). Microscope slides of trichomycetes from insect guts were made and observed under a compound microscope with phase contrast or differential interference contrast illumination. Semi-permanent slides were prepared by staining with lactophenol-cotton blue (0.5% w/v), and coverslips were sealed with clear fingernail polish. Cultures of only a few species were made and these are deposited at the ARSEF Culture Collection (USA), and preserved material (microscope slides) are in the Farlow Herbarium (FH) (USA) and the La Plata Spegazzini Herbarium (LPSC) or in the culture collection at CEPAVE (La Plata, Buenos Aires, Argentina). Typically “*in vitro*” cultures are stored by freezing at -196 °C in liquid nitrogen, therefore, it was more feasible to deposit the specimens as microscopic preparations.

Few species of trichomycetes are culturable *in vitro*, and most of them are ones restricted to the hindgut of their hosts. Isolation and culture attempts were made according to the following procedure: first, the surface of the work area/bench or laminar flow hood was sterilized with 10% sodium hypochlorite and afterwards with 70% alcohol, and then a Bunsen burner flame was used to keep the bench clean. The insect guts containing fungi were transferred using a histological needle or fine forceps to several washes of 1 minute each in 5% sodium hypochlorite, then in sterile distilled water, then an antibiotic solution (chloramphenicol /250mg/50ml, gentamycine 25mg/50ml), and finally in sterile distilled water again; all in sterilized Petri dishes (35 mm diameter). Once rinsed, the thalli were removed from the gut and inoculated onto BHIA (Brain Heart Infusion Agar) medium diluted 1/10 or BHIA Tv with overlaying sterile water and 5 drops of an antibiotic solution (penicillin-streptomycin, 1150 mg/50 ml/528.5 mg/50 ml). The cultures were checked

every 24h and incubated for 7 days at 25°C. After checking purity and growth, they were transferred to culture tubes with BHIA medium and 3 ml of distilled water. Slant cultures were kept at 45° and slowly rotated every day to allow for oxygenation of thalli to promote growth. Separately, thalli were preserved in a cetyl-trimethylammonium bromide (2 % p/v CTAB DNA extraction buffer) 100 mM Tris-Cl (pH 8.0) 20 mM EDTA (pH 8.0) 1.4 M NaCl, 1% , PVP 40,000 (polyvinyl pyrrolidone) for molecular studies. Insect hosts were preserved in 70% ethanol for later identification. Several isolates from Argentinean hosts were preserved under ultra-low temperatures (-196°C) at the ARSEF Collection in Ithaca, N.Y., USA.

RESULTS

The most numerous species of trichomycetes (including all Argentinean collections) are from the Harpellales, now classified in the Subphylum Kickxellomycotina, Phylum Zoopagomycota (Spatafora *et al.* 2016, 2017). Species of Harpellales known to date in Argentina have been found in the provinces of Buenos Aires, Tucumán, Salta, Jujuy, Misiones, La Rioja, Chaco, Córdoba, San Luis, Río Negro, Neuquén, Chubut, and Tierra del Fuego in diverse insect species (Lichtwardt *et al.* 1999, 2000, López Lastra *et al.* 2003, 2005, Siri & López Lastra 2010). Species of Harpellales have been reported in 14 of the 23 provinces of Argentina (with several new species and an expanded species distribution range); they were found in several insect hosts from different biogeographic regions, from the northernmost to the southernmost points of the country (Fig. 1).

The fungal trichomycetes, as well as species of *Paramoebidium* (Amoebidiales) and *Palavascia* (Eccrinales), recorded to date are listed in Table 1.

DISCUSSION

Knowledge about the distribution and diversity of Trichomycetes fungal species in arthropods in Argentina is extensive and broad despite the large surface area of the country. Data from trichomycetes publications and reports compiled from the last 30 years record a total of 34 species, 15 of which were described as new species at the time (López Lastra 1997, 1999, Lichtwardt *et al.* 1999, 2000, López Lastra *et al.* 2003, 2005, Siri & López Lastra 2010).

Harpella species are non-branched and attached to the peritrophic matrix in the blackfly midgut of all larval instars. *Harpella meridionalis* is the predominant species present in Simuliidae in Argentina (Lichtwardt *et al.* 1999, 2000),

whereas *H. tica* is the other common midgut dweller in Simuliidae larvae. *Harpella tica* Lichtw. was reported in Costa Rica (Lichtwardt 1997), Puerto Rico (White *et al.* 2000), and northwestern Argentina (Lichtwardt *et al.* 2000), where the range extends to Misiones (Northeast of Argentina). *Harpella tica* was not found in Tierra del Fuego. *Harpella meridionalis* Lichtw. & Arenas, however, was present in Tierra del Fuego streams at most of the sites sampled and in several simuliid species; previous reports of *H. meridionalis* by Lichtwardt and Arenas (1996) from Southern Chile and by Lichtwardt *et al.* (1999) and from Patagonia, Argentina, were also from cooler waters.

Simulioomyces microsporus Lichtw., has been reported in Argentina as well as in other South American countries (Ecuador, Brazil, Chile) as several other species of Harpellales (Lichtwardt 1986, Lichtwardt & Williams 1990, Lichtwardt & Arenas 1996, Lichtwardt 1997, Lichtwardt *et al.* 1999, 2000, Alencar *et al.* 2003, López Lastra *et al.* 2005, Siri & López Lastra 2010). Compared to other Latin-American countries, the number of species and new species recorded from collections in Argentina have been more extensive and continuous over time.

Within the Harpellales, *Pennella* is a relatively common genus in Simuliidae hosts in the USA and Canada (Strongman 2007, Strongman & White 2010), but only two species have been found further South, globally, in Costa Rica: *P. simulii* Williams & Lichtw. and *P. montana* Lichtw., the latter being reported for Simuliidae in Northwestern Argentina (Lichtwardt *et al.* 2000). Chironomidae are frequent hosts of Harpellales with six genera known to inhabit their guts. The most common genera for these hosts are *Stachylinia* and *Smittium*; six *Smittium* spp. have been recorded from Chironomidae and Simuliidae in South America (Lichtwardt & Arenas 1996, Lichtwardt *et al.* 1999, 2000). It is important to note that the original name of *S. culisetae* Lichtwardt has been changed to *Zancudomyces culisetae* Yan Wang, Tretter, Lichtw. & White (Wang *et al.* 2013), and it is included in the Table 1 under this name.

Almost half of the trichomycetes recorded to date in Argentina (Table 1) were new species and/or new host records. Collection efforts have spanned the whole country producing new records, which suggests many more species may yet be discovered, especially considering that only one order (Harpellales) has been the focus of these studies. Only two of the four trichomycete orders are represented, so far, in the list if we include the protistan trichomycetes. Future surveys and studies in unexplored habitats, hosts and regions of the country, South America and the world, will only continue to increase our knowledge about gut fungi diversity and the role these endobionts play in the larger ecosystems.

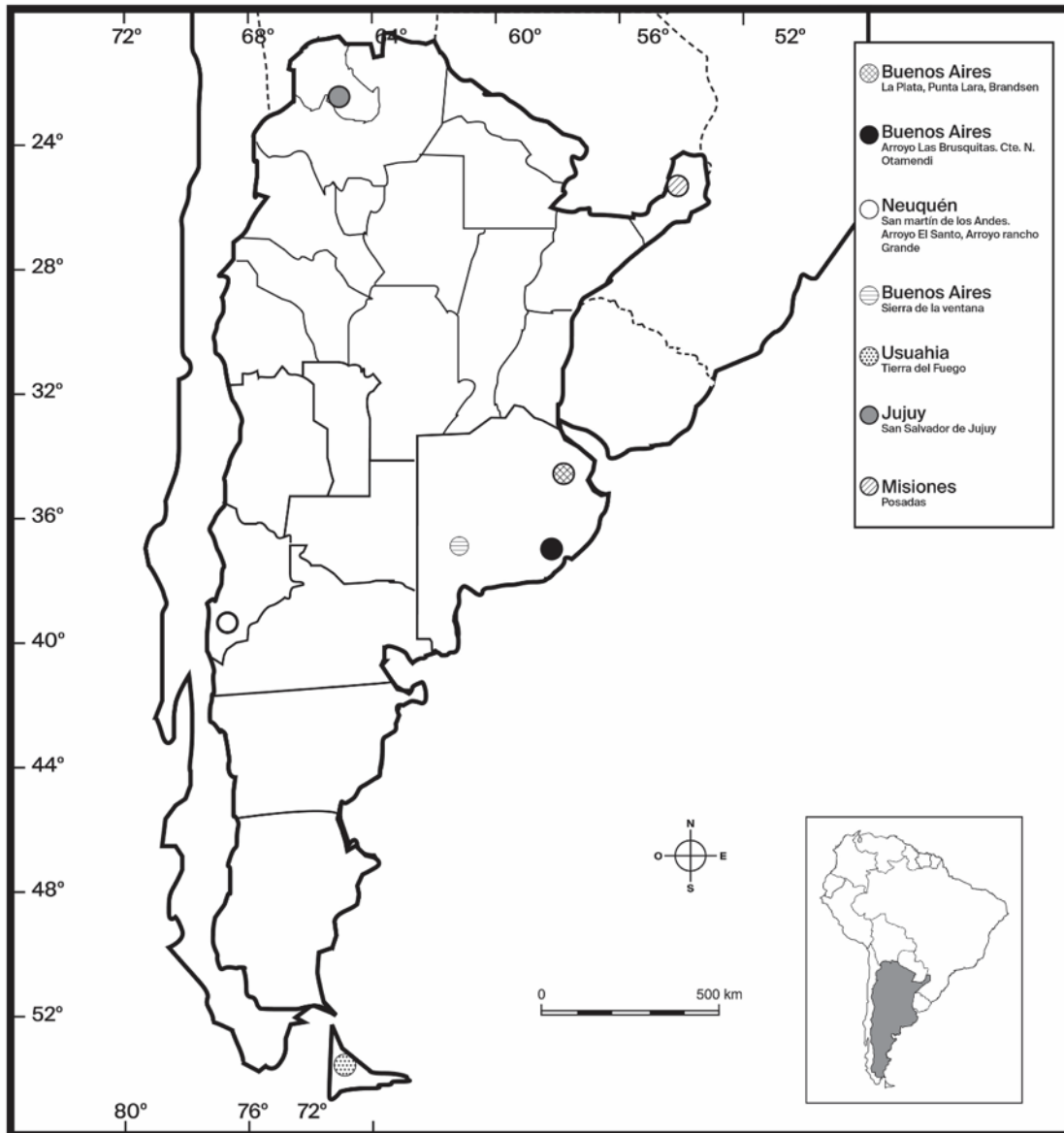


Figure 1. Known distribution of arthropod gut fungi (Kickxellomycotina) in Argentina. / Distribución de los hongos del intestino de artrópodos (Kickxellomycotina) de la Argentina.

Table 1. List of species of Harpellales and other “gut organisms” of arthropods from Argentina. / Lista de especies de Harpellales y otros organismos del intestino de artrópodos de la Argentina.

Gut Fungus / Order*	Site	Arthropod / Insect: Order/ Family	Host species / stage	Reference	Culture collection/ Holotype - Herbarium accession Number
<i>Autrosmittium patagonicum</i> Siri	Neuquen, Parque Nacional Lanín UN stream	Diptera: Chironomidae	<i>Parochilus</i> sp. larvae	Siri & López Lastra 2010	Holotype LPSC 48149 (slide) MB 514145
<i>A. lenticum</i> Siri	Buenos Aires, Arana Lentic environments: ponds	Diptera: Chironomidae	<i>Corynoneura</i> sp. larvae	Siri & López Lastra 2010	Holotype LPSC 48160 (slide) MB 514146
<i>Carouxella coemeteriensis</i> Lichtw., López Lastra & Ferrington	Buenos Aires province, La Plata ARG39-14	Diptera: Ceratopogonidae	<i>Dasyhelea necrophila</i> larvae	Lichtwardt et al. 1999	Holotype : microscope slide ARG 39-14 - FH
<i>Coleopteromyces amnicus</i> Ferrington, Lichtw. & López Lastra	Rio Negro province Arroyo La virgen ARG 16	Coleoptera: Scirtidae	Scirtidae larvae	Lichtwardt et al. 1999	Holotype ARG 16-3 FH
<i>Genistellospora homothallica</i> Lichtw.	Salta and Jujuy several sites	Diptera: Simuliidae	<i>Simulium bonaerense</i> and other spp. larvae	Lichtwardt et al. 2000 Lichtwardt et al. 1999	López Lastra et al. 2005 Siri & López Lastra 2010
	Buenos Aires several sites		<i>S. auripellitum</i> larvae		
	Misiones		<i>S. bonaerense</i> <i>S. auripellitum</i>		
<i>Glotzia</i> sp.	Tierra del Fuego	Ephemeroptera: Baetidae	Ephemeroptera NI nymphs	López Lastra et al. 2005	
<i>Harpella tica</i> Lichtw.	Tucuman, Salta and Jujuy several sites	Diptera: Simuliidae	Several species of <i>Simulium</i> Larvae	Lichtwardt et al. 2000	
	Misiones several sites	Diptera: Simuliidae	<i>Simulium auripellitum</i> larvae	López Lastra et al. 2005	
<i>H. meridionalis</i> Lichtw. & Arenas	Tucuman, Salta and Jujuy	Diptera: Simuliidae	Several species of <i>Simulium</i> Larvae	Lichtwardt et al. 2000	
	Tierra del Fuego	Diptera: Simuliidae	<i>Gigantodax</i> several spp., larvae	López Lastra et al. 2005	
	Córdoba, La Rioja several streams			Siri & López Lastra 2010	
<i>Legeriomyces lichtwardtii</i> Siri	Buenos Aires, Punta Indio	Ephemeroptera: Caenidae	<i>Caenis</i> sp. Nymphs	Siri & López Lastra 2010	Holotype LPSC 48162. MB 514148
<i>Paramoebidium argentinense</i> M.G. Mazzucch., López Lastra & Lichtw. / (Amoebidiales)	Jujuy province, “Yala river”	Ephemeroptera: Baetidae	<i>Baetodes</i> sp. Nymphs	Lichtwardt et al. 2000	Holotype: Ektachromes of the composite FIG. 8 FH

Gut Fungus / Order*	Site	Arthropod / Insect: Order/ Family	Host species / stage	Reference	Culture collection/ Holotype - Herbarium accession Number
<i>Paramoebidium digitoideum</i> Ferrington, Lichtw. & López Lastra / (Amoebidiales)	Río Negro province, Rio manso superior stream	Insecta: Notonemouridae	<i>Astronemoura</i> Nymph	Lichtwardt et al. 1999	Holotype ARG 25-5F-FH
<i>Palavascia patagonica</i> Cafaro / (Eccrinales)	Patagonia, Santa Cruz, Puerto Deseado Faro de Cabo Blanco	Isopoda: Sphaeromatidae	<i>Exosphaeroma studeri</i> Adults	Cafaro, 2000	Holotype Slide ARG-D4-C4b FH Plus, Paratype Ektachrome KU-330-22 FH
<i>Penella montana</i> Lichtw.	Tucuman, Salta and Jujuy provinces several sites Buenos Aires, La Rioja, San Luis several sites and streams	Diptera: Simuliidae	Several species of Simuliid larvae	Lichtwardt et al. 2000 Siri & López Lastra 2010	
<i>Penella simulii</i> M.C. Williams & Lichtw.	Tierra del Fuego	Diptera: Simuliidae	<i>G. antarcticus</i>	López Lastra et al. 2005	
<i>Plecopteromyces patagoniensis</i> Lichtw., Ferrington & López Lastra	Neuquen Province, small stream tributary of Traful River	Insecta: Plecoptera	<i>Limnoperla jaffueli</i> larvae	Lichtwardt et al. 1999	Holotype ARG 17-7 FH
<i>S. basiramosum</i> Siri	Cordoba, La Falda, Río Grande de Punilla	Diptera: Chironomidae	<i>Polypedilum</i> sp. larvae	Siri & López Lastra 2010	Holotype LPSC 48161 slide, MB 514147
<i>Smittium culisetae</i> Lichtw. <i>Zancudomyces culisetae</i> comb. nov. Y. Wang, Tretter, Lichtw. & M.M. White Basionym: <i>Smittium culisetae</i> Lichtw. 1964 Amer. J. Bot. 51:837.	Buenos Aires province Punta Lara, Ensenada, Misiones Buenos Aires; other sites	Diptera: Culicidae	<i>Aedes aegypti</i> <i>Culex dolosus</i> (larvae) <i>Ochlerotatus crinifer</i> <i>Aedes aegypti</i> larvae <i>Cx. castroi</i> , <i>Ae. aegypti</i> , <i>Cx pipines</i> , <i>Cx apicinus</i> , <i>Ps. ferox</i> larvae	López Lastra, (1997) López Lastra et al. 2005 Siri & López Lastra 2010	ARSEF 5203, 5430 ARSEF5200, LPS 500
<i>S. culicis</i> Lichtw.	Buenos Aires province Punta Lara, Ensenada, Buenos aires province; other sites	Diptera: Culicidae	<i>Cx. renatoi</i> larvae <i>Cx. castroi</i> , <i>Ae. aegypti</i> , Chironomids and simuliids larvae	López Lastra, (1997) Siri & López Lastra (2010)	ARSEF 5204, 5431, 5432 CEP 290, 291 and for Chironomidae CEP 293
<i>S. cellaspora</i> M.C. Williams	Tierra del Fuego	Diptera: Chironomidae	Chironomidae larvae	López Lastra et al. 2005	
<i>S. imitatum</i> Lichtw. & Arenas	Tierra del Fuego	Diptera: Simuliidae	<i>Gigantodax antarcticus</i> larvae	López Lastra et al. 2005	
<i>S. morbosum</i> var <i>rioplatensis</i> Sweeney / López Lastra	Buenos Aires Ensenada, Punta Lara, ponds	Diptera: Culicidae	<i>Culex renatoi</i> , <i>Cx. dolosus</i> , <i>Psorophora ferox</i> larvae	López Lastra 1999	

Gut Fungus / Order*	Site	Arthropod / Insect: Order/ Family	Host species / stage	Reference	Culture collection/ Holotype - Herbarium accession Number
<i>Smittium phytotelmatum</i> Lichtw.	Chaco, Buenos Aires several Phytotelmata	Diptera: Chironomidae	<i>Polipedylum</i> sp. larvae	Siri & López Lastra 2010 Siri et al. 2008	CEP 298, CEP 299, CEP 300 (slides)
<i>S. simulii</i> Lichtw.	Tucuman, Salta and Jujuy provinces several sites Buenos Aires, La Rioja several sites	Diptera: Simuliidae	<i>Simulium</i> sp. larvae Several Simuliidae species, larvae	Lichtwardt et al. 2000 Siri & López Lastra 2010	
<i>S. esteparum</i> Ferrington, Lichtw. & López Lastra	Chubut province Arroyo Rodeo	Diptera: Chironomidae	<i>Cricotopus</i> sp. Larvae	Lichtwardt et al. 1999	Holotype ARG D- 28 FHG
<i>S. tronadorium</i> Ferrington, Lichtw. & López Lastra	Rio Negro Province, unnamed stream tributary of Rio Manso	Diptera: Chironomidae	<i>Paraheptagya</i> sp. Larvae	Lichtwardt et al. 1999	Holotype F ARSEF 9030
<i>S. urbanum</i> López Lastra, M.G. Mazzucch. & Lichtw.	Tucuman province, stream at parque Guillermina	Diptera: Chironomidae	Chironomidae larvae (no identificado)	Lichtwardt et al. 2000	Holotype, mic. Slide ARG 59-1 FH
<i>Simuliomyces microsporus</i> Lichtw.	Tucuman, Salta and Jujuy provinces several sites Córdoba Mina Clavero UN stream	Diptera: Simuliidae	Several species of <i>Simulium</i> larvae Several species of <i>Simulium</i> larvae	Lichtwardt et al. 2000 Siri & López Lastra 2010	
<i>Stachylina grandispora</i> Lichtw.	Tucuman and Buenos Aires province several sites	Diptera: Chironomidae	<i>Chironomus</i> sp. larvae	Lichtwardt et al. 2000	
<i>Stachylina jujuyensis</i> M.G. Mazzucch., López Lastra & Lichtw.	Jujuy province, "La horqueta" stream	Diptera: Chironomidae	<i>Chironomus</i> sp. larvae	Lichtwardt et al. 2000	Holotype original Ektachrome slides deposited in FH
<i>Stachylina lentica</i> White & Lichtw.	Buenos Aires several water reservoirs in <i>Eryngium</i> spp. plants	Diptera: Chironomidae	<i>Polypedilum</i> sp. and <i>Metriocnemus eryngiotelmatus</i> larvae	Siri et al. 2008; Siri & López Lastra, 2010	
<i>S. lotica</i> M.C. Williams & Lichtw.	Buenos Aires <i>Eryngium</i> spp. plants	Diptera: Chironomidae	<i>Chironomus</i> sp. larvae	Siri & López Lastra 2010	
<i>Stachylina minima</i> M.C. Williams & Lichtw.	Tierra del Fuego	Diptera: Chironomidae	Chironomidae larvae	López Lastra et al. 2005	
<i>S. penetralis</i> Lichtw.	Cordoba, La Falda, Rio Grande de Punilla	Diptera: Chironomidae	<i>Parametriocnemus</i> sp. larvae	Siri & López Lastra 2010	
<i>Stachylina platensis</i> López Lastra, Lichtw. & Ferrington	Buenos Aires province Punta Lara, Ensenada	Diptera: Chironomidae	<i>Chironomus</i> sp. larvae	Lichtwardt et al. 1999	Holotype ARG 31-1- FH

*Most species listed are fungi and members of the Harpellales, but a few endobionts taxa are now recognized as protistan members. The two non-harpellids also include their order, for clarity.

FH: Farlow Herbarium, LPSC: acronym for Spegazzini Herbarium, MB: Mycobank access number, CEP: acronym for CEPAVE Culture Collection, ARSEF: Agricultural Research Service Entomopathogenic Fungi Culture collection.

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