

New records of chytridiaceous fungi (Chytridiomycota) from the Reserva Natural Selva Marginal Punta Lara (Argentina) with comments on some previously reported species

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The Chytridiomycota (chytrids) of Argentina are not well known from freshwater and terrestrial ecosystems. We collected samples of water and floating organic matter (vegetable debris) from a lotic environment and wet soil from four terrestrial habitats at the Reserva Natural Selva Marginal Punta Lara (Bs. As.) and added cellulosic, chitinic and keratinic baits to these samples. We also placed *Rosa* spp. fruits and corn leaves for colonization in the stream. We recorded 21 taxa. Four of them (*Chytrium hyalinus* var. *granulatus*, *Cylindrochytridium johnstonii*, *Cladochytrium replicatum* and *Septochytrium variabile*) are new records for Argentina and we describe and illustrate them here.

Chytrids (Chytridiomycota) are ubiquitous fungi that are found in soil and water bodies as saprobes or parasites of macrophytes, algae, amphibians, invertebrates and other fungi. Chytrid saprobes play a major role in freshwater ecosystems as decomposers of plant and animal substrates. They are characterized by the presence of zoospores with a single, posteriorly inserted, smooth flagellum (Müller et al. 2004). According to Hibbett et al. 2007, the phylum contains two classes with four orders: Monoblepharidiomycetes (Monoblepharidiales) and Chytridiomycetes (Chytridiales, Rhizophydiales and Spizellomycetales). The orders Blastocladales and Neocallimastigales, which previously were classified within the Chytridiomycota, have each been raised to the rank of phylum (James et al. 2006, Hibbett et al. 2007). Most of the diversity of the Chytridiomycota is in the Chytridiales. Members of this order were first described in the 1850s, and according to the 'Dictionary of the Fungi' (Kirk et al. 2001) the order contains four families (Chytridiaceae, Cladochytriaceae, Endochytriaceae and Synchytriaceae). However, these families do not necessarily represent monophyletic groups and not all of the diversity within the order is contained within these families (James et al. 2006, Letcher et al. 2006). Although the concept of the Chytridiales continues to change, it currently contains about 80 genera and more than 600 species.

Studies of the fungal biota of Chytridiomycota in Argentina are scanty (Steciow 1998, Steciow et al. 2006). Recently, Marano et al. (2006) isolated *Rhizophlyctis rosea* (Spizellomycetales) for the first time in Argentina and Marano et al. (2007) noted five species of *Nowakowskiella* (Cladochytriaceae, Chytridiomycota) in aquatic environments of the Buenos Aires province.

During a survey of chytridiaceous fungi carried out in the stream Las Cañas and four terrestrial habitats at the Reserva Natural Punta Lara (Partido de Ensenada, Buenos Aires, Argentina), we found some species belonging to the Chytridiomycetes and Monoblepharidomycetes. Four of them are new records for Argentina, and we here explain and illustrate their morphology in gross culture.

Material and methods

The Reserva Natural Punta Lara is located on the riverine side of the Río de la Plata on the northeast of Ensenada and Berazategui districts, Buenos Aires, Argentina (Cabrera 1960). The area is ca 6000 ha (Martínez et al. 2006) consisting of basins and levees (50–500 m width) with alluvial to gley humic soils (Cappannini and Mauriño 1966), high organic carbon content and low dissolved oxygen availability (Martínez et al. 2006). Four types of terrestrial habitats with different soil features and vegetation can be recognized: (i) floodplain marsh, (ii) grassland, (iii) dry forest, (iv) marginal forest and (v) coast.

Las Cañas stream is a small lotic environment that belongs to the Río de la Plata. Las Cañas is surrounded by a riparian forest, locally known as 'selva marginal' (marginal forest).

We collected samples of fresh water and floating organic matter (vegetable debris) in the stream seasonally during 2006–2007 and also placed corn leaves and *Rosa* spp. fruits in plastic mesh bags tied to the shore for 10-day periods (in situ samples). In December 2007 we collected three subsamples of 300–400 g of soil from each terrestrial habitat

(floodplain marsh, grassland, dry forest, marginal forest and coast) by random point sampling. We followed the baiting techniques of Sparrow (1960) and Stevens (1974) to find chytridiaceous fungi. We divided each water sample in two sub-samples of 30 ml and placed them in separate Petri dishes with corn leaves and snake skin. The in situ material was processed by direct observation and then baited with the same substrate. We employed sterilized pollen grains of *Cedrus* sp. to get some of the species into unifungal cultures. Soil sub-samples from each habitat were mixed and five grams was flooded with sterile deionized water. We incubated baited samples at room temperature (18–20°C) up to 42 days and examined them every two days. Observations were made with an Olympus BX 40 microscope equipped with phase contrast optics.

We brought *Chytriomycetes hyalinus* var. *granulatus* in to unifungal cultures on pollen grains of *Cedrus* sp. We isolated and maintained species in pure culture (Johnson 1973) on corn-meal agar medium or Emerson's YpSs/10 with antibiotics (5 g l⁻¹ of streptomycin sulfate and 2.5 g l⁻¹ of chloranphenicol) as described in Fuller and Jaworski (1987). Stock cultures were maintained as fixed slides and as photographs of developmental stages.

We identified species by comparison with drawings and descriptions in Sparrow (1960), Karling (1977), Rocha and Pires-Zottarelli (2002) and Letcher and Powell (2002).

Results

We recorded a total of 21 taxa. The species composition and some additional information about their ecology are shown in Table 1. Four of them are noted for the first time in Argentina (*).

Chytridiomycota

Chytridiaceae

Chytriomycetes hyalinus var. *granulatus* Karling (1967, p. 120). (Fig. 1A)

Thallus epibiotic, monocentric. Zoosporangium hyaline, spherical or subspherical, (20 –) 25–61 (–87) µm in diameter, sessile, sporangial wall smooth. Apophysis

Table 1. Chytridiaceous fungi from the Reserva Natural Selva Marginal Punta Lara, with additional information about the type of sample and nutrition, substrate employed and months of isolation. Abbreviations are as follows: w = water, is = in situ, om = organic matter, s = soil samples, sa = saprotrophic, p = parasitic, 1–12 = months of isolation, * = new record for Argentina.

Taxa	Type of sample	Nutrition	Substrate	Months of isolation
CHYTRIDIOMYCOTA				
CHYTRIDIOMYCETES				
Chytridiales				
Chytridiaceae				
<i>Chytridium</i> sp. Braun	w	pa	zoosporangia of <i>Allomyces arbuscula</i>	6
<i>Chytriomycetes hyalinus</i> Karling	w	sa	corn leaves	10
<i>Chytriomycetes hyalinus</i> var. <i>granulatus</i> Karling*	w	sa	snake skin	6, 10
<i>Cylindrochytridium johnstonii</i> Karling*	is, s	sa	corn leaves	2, 10, 12
Cladochytriaceae				
<i>Cladochytrium replicatum</i> Karling*	is, s	sa	corn leaves	2, 6, 9, 10, 12
<i>Macrochytrium botrydioides</i> Minden	is	sa	<i>Rosa</i> spp. fruits	5
<i>Nowakowskiella elegans</i> (Nowak.) Schroeter	om, w, s	sa	corn leaves	3, 6, 10, 12
<i>Nowakowskiella hemisphaerospora</i> Shanor	w, s	sa	corn leaves	3, 10, 12
<i>Nowakowskiella multisporea</i> Karling	om	sa	corn leaves	10
<i>Nowakowskiella profusa</i> Karling	s	sa	corn leaves	12
<i>Nowakowskiella ramosa</i> Butler	om	sa	corn leaves	2, 6, 10
Endochytriaceae				
<i>Catenochytridium</i> sp.	om, s	sa	corn leaves	9, 10, 12
Rhizophydiales				
Rhizophydiaceae				
<i>Rhizophyidium carpophyllum</i> (Zopf) Fischer	w	pa	oogonia of <i>Achlya</i> sp. and <i>Dictyuchus monosporus</i>	6
<i>Rhizophyidium keratinophilum</i> Karling	s	sa	snake skin, human hair	12
<i>Septochytrium variabile</i> Berdan*	w, is	sa	corn leaves	6, 12
Spizellomycetales				
Spizellomycetaceae				
<i>Rhizophlyctis rosea</i> (de Bary & Woronin) Fischer	w, om, s	sa	corn leaves, snake skin, fish scales	3, 8, 12
<i>Olpidium</i> sp.	om	pa	eggs of Gastrotricha (Chaetonotidae)	6
MONOBLEPHARIDOMYCETES				
Monoblepharidales				
Gonapodyaceae				
<i>Gonapodya polymorpha</i> Thaxter	is	sa	<i>Rosa</i> spp. fruits	10
<i>Gonapodya prolifera</i> (Cornu) Fischer	is	sa	<i>Rosa</i> spp. fruits	5, 10
Monoblepharidaceae				
<i>Monoblepharis polymorpha</i> Cornu	om	sa	<i>Ligustrum lucidum</i> leaves	9
<i>Monoblepharis hypogyna</i> Perrot	om	sa	<i>Ligustrum lucidum</i> leaves	10

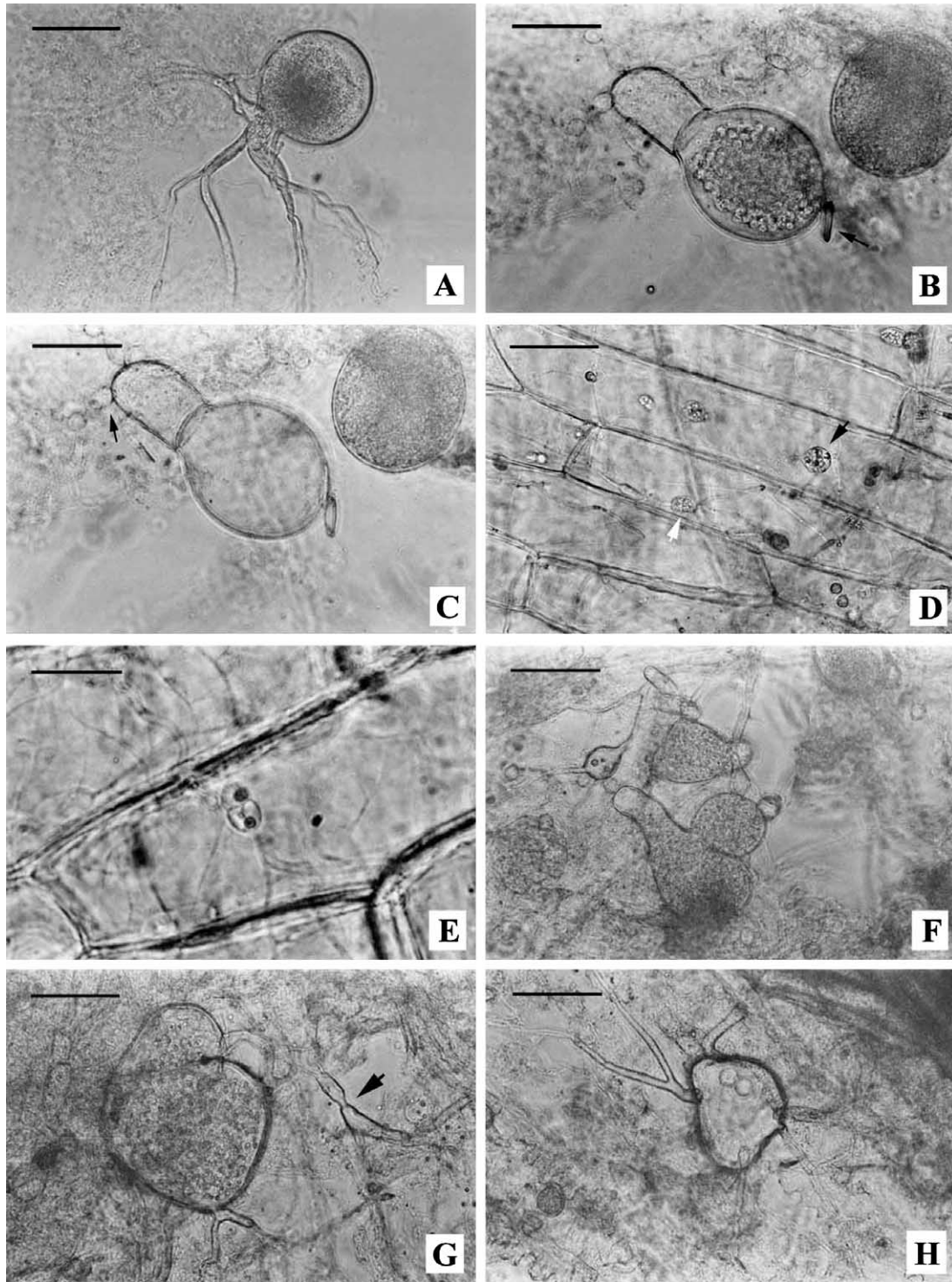


Figure 1. (A) thallus of *Chytrium hyalinus* var. *granulatus* with spherical zoosporangium and branched rhizoids, growing in cornmeal agar, (B)–(C) *Cylandrochytridium johnstonii*, (B) thallus with operculate (arrow) zoosporangium and a conspicuous basal portion, (C) detail of the empty zoosporangium after zoospores discharge, with a rhizoidal system bearing catenulate enlargements (arrow), (D)–(E) *Cladochytrium replicatum*, (D) polycentric thallus growing on onion skin, with zoosporangia (black arrow) and characteristic turbinate cells (white arrow), (E) detail of a turbinate cell, (F)–(H) *Septochytrium variabile*, (F) aspect of the polycentric thallus on corn leaves, (G) detail of a zoosporangium with septate rhizoids (arrow), (H) empty zoosporangium after zoospore discharge. Scale bars = 50 μm .

commonly absent, spherical when present. Operculum persistent, apical, 20 μm in diameter, discharge pore single; zoospore discharge as a mass, involving in a vesicle outside the sporangium before dispersal. Rhizoids intramatrical,

well developed, branched and coarse, extended for distances up to 522 μm (in corn-meal agar). Zoospores spherical, hyaline, 2–5 μm in diameter, with several refractive lipid globules. Resting spores not observed.

Ecology

Rare, on snake skin (specimens examined); frequent on cellulosic substrates, but also common on snake skin and shrimp exoskeleton (Karling 1967), whereas *C. hyalinus* prefers chitinic substrates (Letcher and Powell 2002).

Distribution

Argentina (specimens examined), Oceania, New Zealand.

Specimens examined

Argentina. Buenos Aires. Partido de Ensenada: Las Cañas stream (Reserva Natural Selva Marginal Punta Lara), 28 Jun 2006 and 18 Oct 2006, saprotrophic, from water baited with snake skin; leg. Marano, Melo & Saparrat. Marano (LPS 47771).

Notes

This species is uncommon at this stream. It was first isolated from water samples on keratinic substrata (snake skin). It was cultivated in corn-meal agar from colonized pollen grains (*Cedrus* sp.), where it develops as small white colonies. Our observations are in agreement with those made by Letcher and Powell (2002) who noted that this species differs from *C. hyalinus* in having bigger zoosporangia, that usually were non-apophysate and in the presence of several refractive globules in the zoospore (instead of the single refractive globule present in *C. hyalinus*).

Discussion

Our isolate is strikingly similar to the variety as summarized by Letcher and Powell (2002), except that our sporangia in pure culture attained a greater size (between 12–120 µm).

Cylindrochytridium johnstonii Karling (1941, p. 383). (Fig. 1B–C)

Thallus exo-endogenous in gross culture, monocentric, 92–170 (–338) µm long, excluding rhizoids. Zoosporangium hyaline, smooth, with a thin wall, usually tubular, cylindrical or clavate, (57 –) 72–105 (–120) × (32 –) 57–80 (–88) µm, occasionally oval, pyriform and sessile; basal portion (27 –) 32–120 (–213) µm long, with the same shape and diameter of the zoosporangium, but sometimes apophysis-like. Operculum oval, elliptical, 15–23 (–25) µm width, persistent on the discharge pore or moving away. Zoospores discharge as a mass (87–110 µm maximum diameter). Rhizoidal system extending from the sporangium in one or more directions, with irregular enlargements, frequently catenulate. Zoospores spherical, 5–7.5 µm in diameter, with

large refractive globule, commonly with ameoboidal shape when not swimming. Resting spores not observed.

Ecology

Frequently growing on corn leaves (specimens examined); saprophytic on organic materials, onion skin and grass leaves and on damp soil (Whiffen 1941, Karling 1942).

Distribution

Argentina (specimens examined), Brazil, Japan, Mexico, United States of America.

Specimens examined

Argentina. Buenos Aires. Partido de Ensenada: Las Cañas stream (Reserva Natural Selva Marginal Punta Lara), 18 Oct 2006, from corn leaves placed in the stream; floodplain marsh (Reserva Natural Selva Marginal Punta Lara), 7 Nov 2007, from soil baited with corn leaves; leg. Marano, Melo & Saparrat. Marano (LPS 47775).

Notes

This species is characterized by its clavate zoosporangium and an extensive rhizoidal system with catenulate enlargements.

Discussion

In Karling's description (1941) the zoosporangia attained a greater size (30–800 × 12–25 µm), whereas Pires-Zottarelli and Milanez (1993) and Rocha and Pires-Zottarelli (2002) describe sporangia of smaller size (29–60 × 11–25 µm and 62–100 × 20–30 µm, respectively) than our isolations.

Cladochytriaceae

Cladochytrium replicatum Karling (1931, p. 538). (Fig. 1D–E)

Taxonomic synonym: *Cladochytrium nowakowskii* Sparrow (1931, p. 619).

Thallus polycentric in gross culture, eucarpic, intramatrical. Zoosporangia terminal, pyriform or oval, rarely spherical, 10–26 µm in diameter, with a single discharge tube of variable length. Operculum and apophysis absent. Rhizoidal system delicate, branched, with spindle-shaped hyphal swellings and fusiform turbinate cells at regular intervals. Zoospores spherical, 5 µm in diameter, with a single orange lipid globule, discharged in a vesicle and remaining motionless for a short time, then escaping rapidly with active movement. Resting spores spherical or oval, 10–21 µm in

diameter, with an eccentric golden-brown globule and a hyaline smooth wall.

Ecology

Cladochytrium replicatum is a ubiquitous saprotroph, very common on corn leaves placed into the stream, or from water samples baited with the same substrate (specimens examined). Frequently found on submerged parts of phanerogams such as *Elodea canadensis*, *Eriocaulon septangulare*, *Myriophyllum verticillatum*, *Allium cepa*, *Typha* sp. leaves, roots of *Panicum variegatum* and also on other vegetable debris, decomposed animals and parasitic in *Cladophora glomerata*, *Spirogyra crassa*, *Oedogonium* spp. and *Coleochaete* sp. (Karling 1931, Sparrow 1960). As a weak parasite it could infect a wide variety of host tissues, including fungi, water ferns and flowering plants (Karling 1935). It is widespread in lakes and ponds and common in soil from which it may be trapped on cellulosic substrata (Karling 1977).

Distribution

Widespread.

Specimens examined

Argentina. Buenos Aires. Partido de Ensenada: Las Cañas stream (Reserva Natural Selva Marginal Punta Lara), 28 Jun 2006 and 18 Oct 2006 from corn leaves placed into the stream and from water samples; 5 Sep 2007, 16 Sep 2007, 10 Oct 2007, 12 Nov 2007, from submerged *Ligustrum lucidum* leaves baited with corn leaves; 10 Oct 2007, from submerged *Pouteria salicifolia* leaves baited with corn leaves; floodplain marsh (Reserva Natural Selva Marginal Punta Lara), 7 Dec 2007, from soil baited with corn leaves; leg. Marano, Melo & Saparrat. Marano (LPS 47774).

Notes

This species is frequently found growing at Las Cañas in association with *Nowakowskiella elegans*, *Septochytrium variable*, *C. johnstonii* and occasionally with *R. rosea* on corn leaves. It was easily distinguished by the presence of turbinate cells in its rhizomycelium and the characteristic golden-orange refractive globule in the zoospores. It was found also in temporary water bodies from the El Pescado floodplain, Partido de La Plata (Arellano, pers. comm.). Production of resting spores was not common in the material obtained from Las Cañas stream, so our observations of resting spores were made on the strains from the temporary water bodies from El Pescado floodplain. We cultivated this species on onion skin, filter paper on the surface of YpSs/10 agar and YpSs/10 agar. In accordance with Karling (1935), we found it difficult to isolate into pure culture.

Discussion

Sparrow (1960) described zoosporangia of smaller size (up to 18 μm) and slightly bigger zoospores (4–7.3 μm in diameter). Pires-Zottarelli (1990, 1999) also described zoosporangia of smaller size (9–17 and 14–22 μm). Chen et al. (2000) described sporangia of 10–32.5 μm in diameter whereas our isolates were up to 26 μm in diameter. Resting spore characteristics were as described in Sparrow (1960), but were larger than those described previously (15 μm) by this author (Sparrow 1931). Our experience agreed with that of Karling (1935) who found that the length of time before cellulosic substrata became infected varied from 4 to 20 days.

Septochytrium variable Berdan (1939, p. 461). (Fig. 1F–H)

Thallus polycentric on agar medium, intramatrical, with additional zoosporangia of smaller size (secondary zoosporangia). Zoosporangia spherical, (25–) 35–82 (–102) μm in diameter, oval or pyriform, (36–) 61–112 (–180) (35–) 46–67 (–88) μm , sometimes bearing a long discharge tube (neck); brownish wall, 2–6 μm thick. Opercula 10–21 μm in diameter, refractive; discharge pore 10–15 (–20) μm in diameter. Rhizoidal system extensive, septate and branched. Rhizoids up to 368 μm long and 5–21 μm in diameter that grow from 2 or 3 insertion points from the zoosporangium. Discharge vesicle present, in which zoospores complete differentiation, and then swim away. Zoospores spherical, with a single refractive globule. Resting spore not observed.

Ecology

Frequently found on corn leaves placed in the stream or from water samples baited with the same substrate (specimens examined); saprophytic on grass, rye, oats and corn leaves and *Narcissus* sp. stems from soil samples (Karling 1941, Sparrow 1960); on onion skin and cellophane from water and soil samples (Rocha and Zottarelli 2002); from water and soil samples (Schoenlein-Crusius et al. 2006).

Distribution

Widespread.

Specimens examined

Argentina. Buenos Aires. Partido de Ensenada: Las Cañas stream (Reserva Natural Selva Marginal Punta Lara), 28 Jun 2006 and 18 Oct 2006, from corn leaves placed into the stream and from water samples baited with the same substrate; leg. Marano, Melo & Saparrat. Marano (LPS 47776).

Notes

Stock cultures were maintained as fixed slides and as photographs of developmental stages.

Discussion

The characters that we observed were similar to those noted for this species by Pires-Zottarelli and Milanez (1993). Nevertheless, according to Rocha and Pires-Zottarelli (2002) the sporangia of *S. variabile* are much smaller (spherical ones 25–30 µm in diameter; ovals up to 50 µm long and 37 µm in diameter). Later, Pires-Zottarelli and Rocha (2007) described spherical zoosporangia of greater size (37–95 µm in diameter) that are in agreement with our observations. In contrast, Sparrow (1960) mentioned pyriform sporangia up to 220 µm. He also described this species as having a thicker rhizomycelium and with up to 12 insertion points of the rhizoids in the sporangia. Pires-Zottarelli (1999) described spherical sporangia of 46–86 µm in diameter and oval ones that ranged from 79–102 × 56–89 µm.

Conclusions

Until now, 26 species of chytridiaceous fungi have been noted in Argentina (Malacalza 1968, Lopez and Mac Carthy 1985, Steciow 1993, 1998, 1999, Lopez Lastra and García 1997, Steciow and Arambarri 2000, Steciow and Elíades 2001, Steciow et al. 2001a, 2001b, 2006, Steciow and Elíades 2002, Herrera et al. 2005, Marano et al. 2006, 2007) and recently, 13 other species were described as new ones (Steciow and Marano 2006, Letcher et al. 2008a, 2008b). One hundred and twenty nine species of chytridiaceous fungi have been recorded for Brazil (Milanez et al. 2007) and while 20 of them are shared by both countries, *Blastocladiella tenuis* Kanouse, *Chytridium olla* Braun, *Coelomyces iliensis* var. *indus* Dubitskij et al., *Olpidium entophyllum* (Braun) Rabenhorst, *Rhizophyidium subangulosum* (Braun) Rabenhorst and *Woronina glomerata* (Cornu) Fischer are exclusive to Argentina.

In this contribution, we recorded 21 chytridiaceous taxa, 18 saprotrophs and 3 parasites. Four of them belong to the Monoblepharidomycetes and 17 to Chytridiomycetes. Twelve of them were Chytridiales, with four taxa belonging to Chytridiaceae, seven to Cladochytriaceae and only one to Endochytriaceae. *Chytriomycetes hyalinus* var. *granulatus* is a new record for South America. The genera *Cladochytrium* Nowakowski, *Chytriomycetes* Karling, *Cylindrochytridium* Karling and *Septochytrium* Berdan are new records for Argentina.

The greatest number of taxa was recovered in June, October and December. Almost all the saprophytic taxa listed in this paper (with the exception of *Chytriomycetes hyalinus* var. *granulatus* and *Rhizophyidium keratinophyllum*) were isolated on cellulosic materials and are typical members of the cellulose-decomposing microbiota of aquatic systems.

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