Sydowia

An International Journal of Mycology

Volume 64 (1) Issued Juni 30 2012

Verlag Ferdinand Berger, Horn/Austria

Southernmost occurrence of two species of *Monoblepharis* (Monoblepharidomycetes, Chytridiomycota) in America

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Steciow M. M. & Marano A. V. (2012) Southernmost occurrence of two species of *Monoblepharis* (Monoblepharidomycetes, Chytridiomycota) in America. – Sydowia 64 (1): 119–127.

Two species of *Monoblepharis* (Monoblepharidomycetes, Chytridiomycota): *M. hypogyna* and *M. polymorpha*, were isolated from litter samples (mainly floating leaves and twigs) of two aquatic environments in Argentina. These findings represent the first record of both species in South America and the southernmost occurrence of the genus in America. It is also the first record of a member of the Monoblepharidaceae in Argentina.

Keywords: *Monoblepharis hypogyna, M. polymorpha,* Monoblepharidaceae, Argentina

The Monoblepharidales is a small order of Chytridiomycota (Fungi) which currently contains five known genera: *Gonapodya, Monoblepharella, Monoblepharis, Harpochytridium* and *Oedogoniomyces* (Hibbett *et al*. 2007, Kirk *et al*. 2008). This order was previously placed in the Class Chytridiomycetes and accomodated two families: Monobleparidaceae and Gonapodyaceae (Sparrow 1960, Karling 1977, Dayal & Kiran 1988) but has been recently reclassified by James *et al*. (2007) and elevated to the level of class (Cl. Monoblepharidomycetes). It currently includes four families: Gonapodyaceae, Harpochytriaceae, Monoblepharidaceae and Oedogoniomycetaceae, with five genera and 26 species (Kirk *et al*. 2008).

Members of this order are characterized by their unique mode of sexual reproduction (oogamous), which involves the fertilization of a non-flagellated female gamete (oosphere) by a flagellated, motile, male gamete, forming a zygote within the oogonium after fertilization, which becomes a thick-walled oospore (Sparrow 1960).

All genera within this order are saprobic and mainly decomposers of vegetable debris (Perrott 1955, 1960, Sparrow 1960). Some of them, such as *Monoblepharella mexicana* Shanor, have been more frequently collected in tropical and subtropical soils from warm regions of the Western Hemisphere (Shanor 1942, Karling 1977). Other members such as *Gonapodya* and *Monoblepharis* are commonly associated with cool and permanent fresh water

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habitats (Perrott 1955, Sparrow 1960). While species of *Gonopodya* usually colonize submersed fruits (such as apple and rose fruits) and form pustules on their surface together with *Blastocladia, Rhipidium* and *Sapromyces* (Sparrow 1960, Steciow *et al*. 2001 a), species of *Monoblepharis* generally occur on entirely submersed and corticated twigs of broad-leaved and coniferous trees and on various kinds of substrata such as insect cadavers, submersed fruits, lichens and fungi (Sparrow 1960, Karling 1977, Whisler 1987). *Monoblepharis* is associated with relatively quiet pools, free from silt and with a low content of decomposed organic matter since the fungus apparently invades the twigs through their lenticels (Perrott 1955, Sparrow 1960). Species of *Gonapodya*, on the contrary, have been recovered in aquatic and soil habitats with high content of organic matter and polluted with hydrocarbons (Steciow & Arambarri 2000, Steciow *et al*. 2001 a, b).

During a biodiversity survey of zoosporic fungi and Oomycetes in different aquatic environments in Argentina, we found two species belonging to the genus *Monoblepharis: M. hypogyna* Perrott and *M. polymorpha* Cornu. While other members of the order (family Gonapodyaceae), i.e. *Monoblepharella mexicana, Gonapodya polymorpha* Thaxter and *G. prolifera* (Cornu) Fischer have been previously recorded in Argentina from different aquatic and soil habitats (Steciow & Arambarri 2000, Steciow *et al*. 2001 a), no members of the family Monoblepharidaceae have been reported until now. The aim of this paper is to describe two species of *Monoblepharis* from freshwater sites in Argentina.

Materials and methods

Study sites

Sampling procedures were carried out at two freshwater habitats with different environmental characteristics and climate conditions:

(i) Las Cañas stream is located in the "Selva Marginal Punta Lara" Natural Reserve (34° 47′ 58.5″ S, 57° 57′ 19.3″ W; 34° 47′ 29.3″ S, 57° 59′ 49.2″ W) in Ensenada and Berazategui districts, Buenos Aires Province (Marano *et al*. 2008, 2011) and belongs to the Oriental Pampean District of the Pampean Province, Chaquenian Domain (Cabrera & Dawnson 1944). The stream is a 600 m long lotic system and runs through a riverine marginal forest of native species, *Blephalocalix tweedii* (Hook. & Arn.) Berg., *Ocotea acutifolia* (Nees.) Mez., and *Pouteria salicifolia* (Spreng.) Radlk., which represent 82 % of the biomass with the remaining 18 % belonging to the exotic *Ligustrum lucidum* (Cabrera & Dawson 1944, Cabrera 1960, Dascanio *et al.* 1994). This stream is an affluent of Río de La Plata river and thus experiences diurnal fluctuations in its water level related to the river tides. The water temperature ranged from 9 °C in winter to 24 °C in summer and the pH varies according to seasons from neutral to slightly alcaline (Marano *et al*. 2008).

(ii) Fantasma pond (41° 05′ 33″ S, 71° 27′ 00″ W) is a temporary wetland of approximately 10 000 m^2 that is dry in summer and can be frozen during winter months. It is located in a deciduous forest that belongs to the Deciduous Forest District of the Subantarctic Province, Subantartic Domain, in the proximity of San Carlos de Bariloche city, Río Negro Province. The riverine and aquatic vegetation is mainly composed of *Juncus procerus* E. Mey., *Carex aematorrhyncha* E. Desv., and *Potentilla anserina* L. The water temperature in this pond varies according to seasons, reaching 0 °C during winter, approximately 6 °C in early spring and 24 °C in summer. Its pH tends to neutral and the conductivity is low (Jara & Perotti 2009, Chaparro 2009).

Sampling procedures

Samples of floating dead leaves and twigs were collected in plastic bags in September and October at Las Cañas stream and in June 2008 at Fantasma pond. In addition, leaf bags of *Ligustrum lucidum* were submersed at Las Cañas stream in August 2007 for up to 90 days and collected periodically (Marano *et al*. 2011). In the laboratory, samples were placed in Petri dishes with sterile destilled water and sesame (*Sesamum indicum* L.) seeds and corn (*Zea mays* L.) leaves used as baits. Dishes were incubated at room temperature (± 20 °C) for 4–42 days. Sampled leaves, twigs and baits were examined periodically under the microscope (Olympus BX 40 microscope).

Results

Both species of *Monoblepharis* did not colonise any of the additional baits (i.e. sesame seeds and corn leaves) in gross culture and appeared as a pearly-gray mycelium that only grew at the margin of leaves. Since their hyphae grew more slowly than the associated species of zoosporic fungi and Oomycetes we were unable to isolate them into pure culture. The material is preserved as permanent slides in herbarium of the Instituto the Botánica Spegazzini (LPS).

Description of species

Monoblepharis hypogyna Perrott, Trans. Brit. Mycol. Soc. 38: 272. 1955.

Basionym. – *Monoblepharis sphaerica* Cornu, emend. Woronin, Mem. Acad. Sci. St. Péters. Phys. Math. 16: 1–24. 1904.

Mycelium scanty or well developed, characterized by highly vacuolated hyphae, cylindrical, branched or unbranched; hyphae thin, 3–5 µm in diam. at the base (Fig. 1). – Zoosporangia not observed. – Oogonia narrowly pyriform, 25–31 × 10–22 µm, occurring singly and terminally or occasionally in a series alternating with antheridia, or in fascicles at a hyphal tip; rarely intercalary (Figs. 2, 4). – Antheridia narrowly cylindrical, hypogynous, opening by a slightly exserted tube just beneath the oogonial crosswall (Fig. 3). An antheridium is present at the base of each oogonium, except in the case of some series of oogonia and antheridia, where the hypha gives rise to two oogonia without the alternation of an antheridium. – Antheridium containing inside 4–7 amoeboid uniflagellate antherozoids. – Oospore single, spherical, 15–31 µm in diam., exogenous, thick-walled, golden brown, covered with light yellow, transparent bullations at maturity.

Ecology. – Saprobic on submersed leaves of *Ligustrum lucidum* and aquatic plants (material examined), on submersed twigs of *Quercus robur* L. (Perrott 1955), and twigs of an unspecified tree species (Barnes & Melville 1932; Sparrow 1933, 1936; Beneke 1948).

Distribution. – Argentina (material examined), Finland, Germany, Latvia, United Kingdom, United States of America.

Material examined. – ARGENTINA, Buenos Aires, Ensenada district: Las Cañas stream ("Selva Marginal Punta Lara" Natural Reserve), 10 Oct 2007, on submersed leaves of *Ligustrum lucidum*; *leg. & det.* A. V. Marano (LPS 48237); Río Negro, Bariloche city: Fantasma pond, Jun 2008, from leaves of grasses; *leg.* M. G. Perotti, *det.* A.V. Marano s.n. (this isolate has not been preserved in herbarium).

Notes. – The oospore diameter is in agreement with the description in Sparrow's monography (1960).

Monoblepharis hypogyna was found growing on leaves of *Ligustrum lucidum* collected after 42 days of submersion in Las Cañas stream. At Fantasma pond it was very abundant on grass leaves. This species was unable to grow on additional substrates such as corn leaves.

Monoblepharis polymorpha Cornu, Bull. Soc. Bot. France 18: 59. 1871.

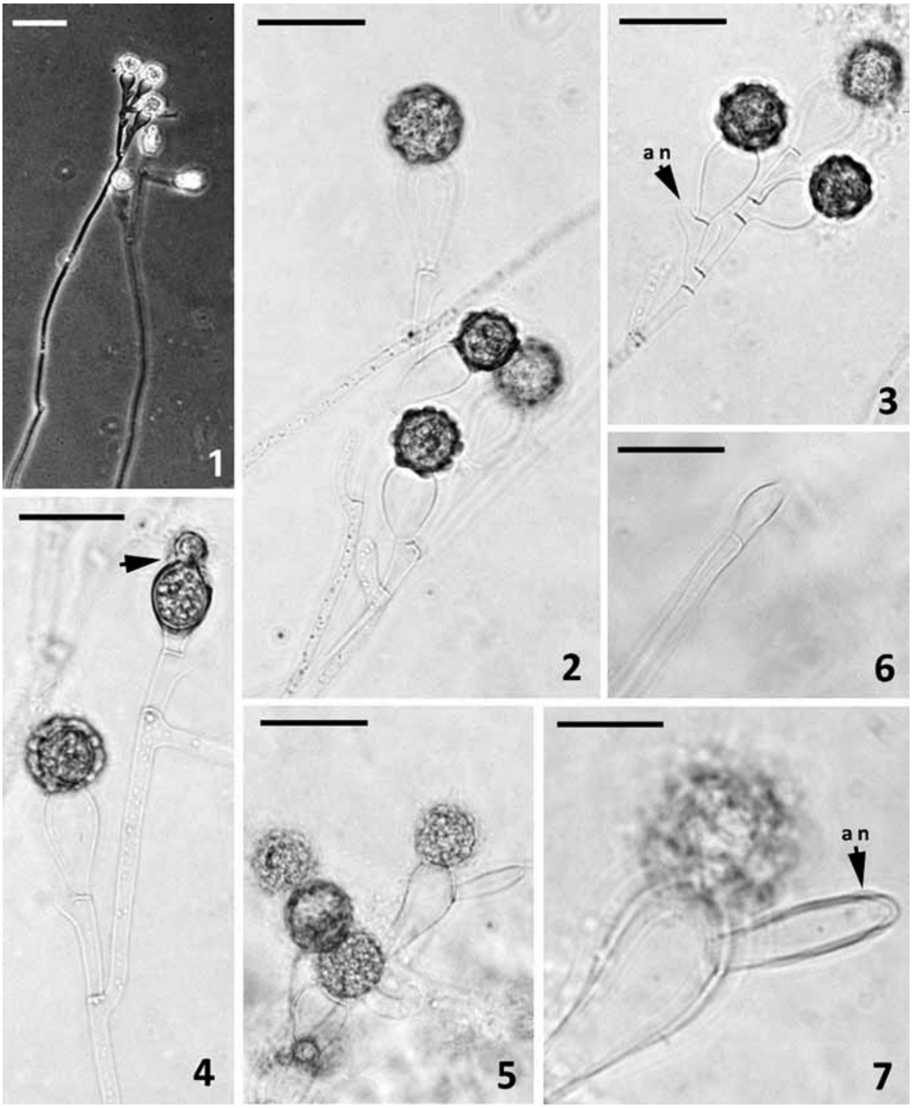
Basionym. – *Monoblepharis brachyandra* Lagerheim, Bih. Kgl. Svensk. Vetensk. Ak. Handl. 25: 37. 1900.

Mycelium filamentous, well-developed, characterized by highly vacuolated hyphae, cylindrical, branched or unbranched; hyphae thin, with a stout basal portion. – Zoosporangia cylindrical, 20 × 10 µm, occurring terminally single or solitary (several sporangia on one branched hypha but not directly fasciculate), or occasionally in clusters sympodially arranged; discharge pore single, apical (Fig. 6). – Oogonia somewhat variable in shape, broadly to narrowly pyriform in young material, and cylindrical in older thalli, (10) 20–30 (40) × (7) 12–18 (20) µm (Fig. 5). – Antheridia cylindrical, narrow, (7) 12–18 × 5-8 µm, epigynous or alternating with oogonia (Fig. 7). – Oospore spherical, exogenous, (10) 17–22 (28) µm in diam. (including ornamentations), with thick brown-orange wall ornamentated with undulations or bullations. – Oospheres not observed.

Ecology. – Saprobic on submersed leaves of *Ligustrum lucidum* (material examined), twigs, animal debris, fruits of *Betula* spp., *Quercus robur*, *Fraxinus excelsior* L., *Pyrus pashia* Buch. & Ham. (Perrott 1955, Sparrow 1960).

Distribution. – Argentina (material examined), China, Denmark, Finland, France, Latvia, Sweden, Switzerland, United Kingdom, United States of America.

Material examined. – ARGENTINA, Buenos Aires, Ensenada district: Las Cañas stream ("Selva Marginal Punta Lara" Natural Reserve), 16 Sept 2007, on submersed leaves of *Ligustrum lucidum*; *leg.* & *det.* A.V. Marano (LPS 48235).



Figs. 1–4. – *Monoblepharis hypogyna*. **1.** General aspect of the mycelial thallus. **2.** General aspect of the thallus bearing oogonia with mature oospores and antheridia. **3.** Detail of the oogonia with mature exogenous oospores and hypogynous antheridia (an). **4.** Detail of the oogonia with an immature oospore migrating outside the oogonium (arrow). **Figs. 5–7.** – *Monoblepharis polymorpha*. General aspect of the thallus bearing oogonia with oospores and antheridia. **6.** Detail of a zoosporangium. **7.** Detail of an oogonium with a mature exogenous oospore and epigynous antheridium (an). Bars: **1.** 60 µm. **2–6.** 40 µm. **7.** 20 µm.

N o t e s . – *Monoblepharis polymorpha* differs from *M. hypogyna* in the antheridia disposition, which alternate with the oogonia or are widely exserted in *M. polymorpha*, whereas in *M. hypogyna* they are hypogynous or only slightly exserted.

The size of the oospores is in agreement with the description in Sparrow (1960), while the diameter of the mycelium (12–15 μm) and the size of zoosporangia (130–234 × 10–13 μm) are smaller and the oogonia (20–28 × 5–7 μm) and antheridia (10–35 × 5–10 μm) are slightly bigger.

This species was found on leaves of *Ligustrum lucidum* collected after 21 days of submersion in Las Cañas stream. It was unable to grow on additional substrates.

Discussion

Monoblepharis hypogyna and *M. polymorpha* were found growing on leaves collected from freshwater habitats with different environmental characteristics and subjected to different climate conditions. It is also interesting to note that both species were recovered from shallow freshwater habitats that are subjected to fluctuating water levels as previously reported for *Monoblepharis* (Perrott 1955) and other members of the order, i.e. *Monoblepharella mexicana* (Shanor 1942), which can be related to the production of thick-walled dormant oospores that are able to resist drought conditions (Perrott 1955).

Most references (Perrott 1955, Sparrow 1960, Fuller & Jaworski 1987, Whisler 1987) indicated that entirely submersed corticated twigs of certain deciduous species such as *Betula* spp., *Quercus* spp., and *Fraxinus* spp. and of coniferous trees appeared to be the most favourable substrates for obtaining *Monoblepharis* in gross culture in the Northern Hemisphere. However, in our study both species were observed growing directly on leaves. It is important to note that even though Las Cañas stream has been sampled over a period of four years, different types of samples (water, floating vegetable debris) have been collected and substrates of various types (corn leaves, fish scales, insect exoskeletons, snake skin) have been placed *in situ* for colonization, we have never found these species before. The potential role of both species in the decomposition of leaves should be further investigated, particularly because the ecology of *Monoblepharis* species is not very well known.

Most species of *Monoblepharis* are commonly found in cold freshwater habitats and may grow vigorously in temperatures as low as 3 °C. Our sampled material rarely showed visible growth when it is was first brought in the laboratory as reported by Perrott (1955). Hudson (1980) reported vigorous growth in the laboratory after two to four weeks of collection when samples were taken in spring and fall, whereas, when samples were collected during summer and winter months, growth was delayed for one to four months under laboratory conditions. This could be related to the fact that some of the species appeared to have a seasonal periodicity of growth in natural habitats (Perrott 1960). The oospores germinate in early spring and produce sex organs and oospores shortly after germination, and these spring-formed oospores remain dormant until late fall when they germinate. Thus, in one year they might have two periods of germination and growth, one in spring and another in fall with dormant periods in summer and winter (Karling 1977). In agreement with the observations of Perrott (1960), both species were found during the spring months at Las Cañas stream.

Both species developed sexual and asexual structures when incubated at room temperature (± 20 °C). Temperature conditions appear to play a significant role on the growth of mycelia and development of sexual and asexual structures (Perrott 1955, Sparrow 1960). For example, if the sampled material is placed in sterile distilled water and incubated at 8–15 °C for 3–7 days, the mycelia of *Monoblepharis* will develop in abundance and produce only sporangia but if it is placed at room temperatute (± 21 °C), sexual reproduction can be observed (Sparrow 1960). As previously reported by Perrott (1955), the fungus was found growing in freshwater habitats with "neutrally alkaline" conditions. On the other hand, light seems to have little effect on the growth of this genus as reported by Perrott (1955), since in our study both species were found growing in freshwater habitats with different conditions of light.

It is evident that many aspects on the ecology and distribution of members of the Monoblepharidales and particularly of the genus *Monoblepharis*, are poorly known and need further investigation. This is the first report of the Monoblepharidaceae in Argentina. Up to now, the only species of the family Monoblepharidaceae found in Souh America is *M. regignens* Lagerh. that was obtained from water samples of various sites in São Paulo and Rio de Janeiro, Brazil (Milanez *et al.* 2007). Our findings therefore are the first record of *M. hypogyna* and *M. polymorpha* in South America and represent the southernmost occurrence of this genus in America.

Acknowledgements

We are indebted to Dr. Gabriela Perotti for providing the samples from Fantasma pond and to Dr. José I. de Souza (Instituto de Botânica, São Paulo, Brazil), who kindly helped in the preparation of the figures. We thank CONICET (project PIP 1422) and Universidad Nacional de La Plata (project N 566) for financial support.

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(Manuscript accepted 8 Mar 2012; Corresponding Editor: I. Krisai-Greilhuber)