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A new species of *Saprolegnia* (Saprolegniales, Straminipila), from a polluted Argentine channel

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Abstract *Saprolegnia variabilis* sp. nov. is described from litter (floating twigs, leaves, and roots) in a artificial polluted channel, near a petroleum refinery, in Buenos Aires Province, Argentina. The species is illustrated and compared with other species of the genus. It differs from *S. australis* in having mature subcentric oospores; the oogonia and the oospores are larger and more variable in shape and size, and the number of oospores is greater. The oogonial stalks are also variable in length and the zoosporangia are very much longer.

Keywords Straminipila; *Saprolegnia*; Argentina; systematics

INTRODUCTION

During a survey of zoosporic organisms occurring in hydrocarbon-polluted water and organic matter floating in streams and channels, near the YPF-Repsol Petroleum Refinery, Partido de Ensenada, Buenos Aires Province (Argentina), we found a species which belongs to the genus *Saprolegnia* but with distinctive features, thereby separating it from other species in the genus: it is here named *S. variabilis* Steciow et Elíades. It belongs to the Kingdom Straminipila, Phylum Heterokonta, Class Peronosporomycetes (Alexopoulos et al. 1996; Dick 2001).

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Very little is known about Argentinian water moulds since the literature contains few references, principally about polluted habitats of Buenos Aires Province (Steciow 1988, 1993a,b, 1997). Previous contributions have been made (Steciow et al. 2001a,b) about zoosporic organisms isolated from the above habitat.

MATERIAL AND METHODS

The methods described by Coker (1923), Sparrow (1960), and Seymour (1970) were used. Samples of brown decaying twigs and leaves and wood of the local dominant vegetation, collected from an artificial channel, were brought to the laboratory in separate sterile polyethylene bags. This channel is located at Partido de Ensenada, Buenos Aires Province, Argentina. It receives the effluents of the refinery and of coke production, with principally aliphatic (3.642 μg l^{-1}) and aromatic (4.34 μg l^{-1}) hydrocarbons found in water samples (established by gas chromatography).

The samples were placed in water culture in sterilised Petri dishes containing several halves of hemp seeds (*Cannabis sativa*), for incubation at room temperature (15–20°C). After growth of the fungus on the seeds a single hypha or spore was isolated and transferred to cornmeal agar medium. After 3–4 days, a block of agar from the edge of each colony was cut off and placed in sterilised Petri dishes containing distilled water, and halves of hemp seeds were added in order to obtain new colonies. Measurements and observations were made on those colonies. Some colonies were incubated at 5, 10, and 25°C to observe the possible effect of temperature on the variation of sexual structures. Mean diameters of fungus colonies, diameters of oogonia, numbers of oospores per oogonium, and diameters of oospores were calculated from 50 counts of each of three replicates. The total percentage of type of antheridial branches was calculated from all these replicates.

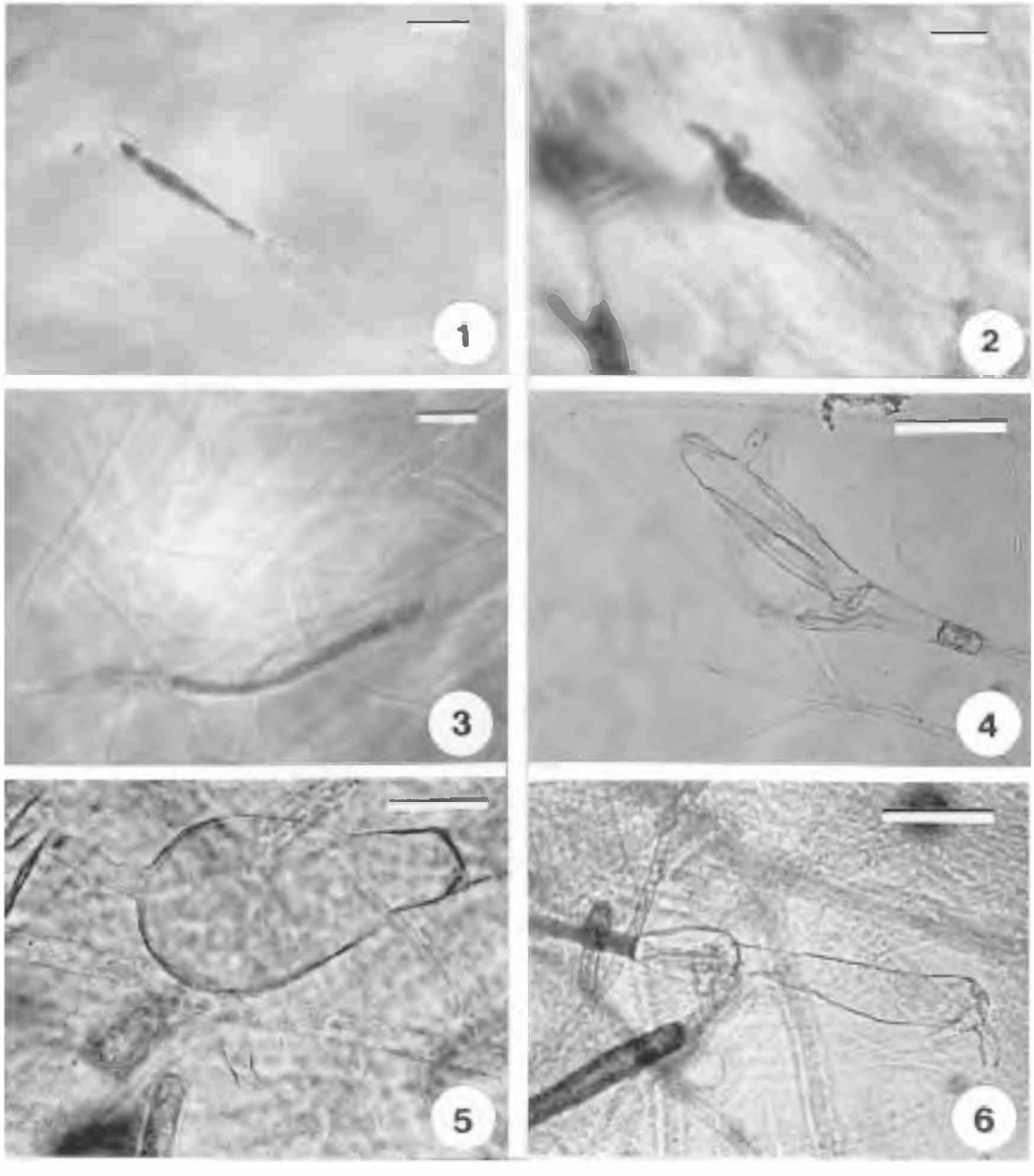
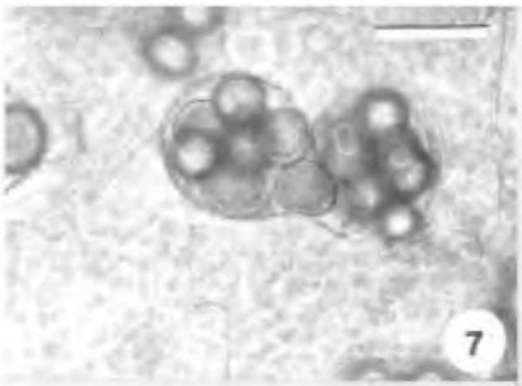


Fig. 1–6 *Saprolegnia variabilis*. **Fig. 1** Detail of zoosporangium renewal by internal proliferation, with development of a new one inside. **Fig. 2** Zoosporangium with a long papilla with zoospores inside. **Fig. 3** Filiform zoosporangium with zoospores. **Fig. 4–6** Gemmae functioning as zoosporangia with some papillae for discharge. Scale bars: Fig. 1–3 = 100 µm; Fig. 4, 6 = 50 µm; Fig. 5 = 10 µm.

Fig. 7–14 *Saprolegnia variabilis*. **Fig. 7** Mature pyriform oogonium with detail of subcentric oospores of different size. **Fig. 8** Obovate oogonium. **Fig. 9** Spherical, pyriform, and fusiform oogonia. **Fig. 10** Catenulate oogonia. **Fig. 11** Oval oogonium. **Fig. 12–13** Intercalary and doliform oogonia with numerous oospores. **Fig. 14** Clavate oogonium. Scale bars = 50 µm. ➤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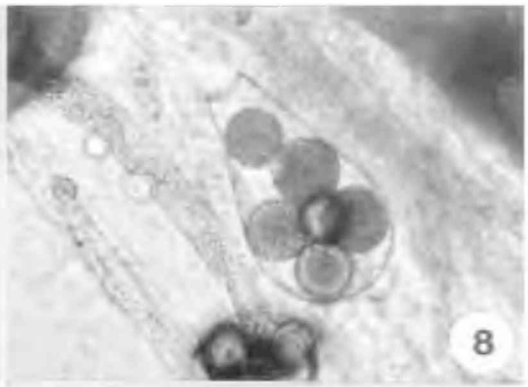
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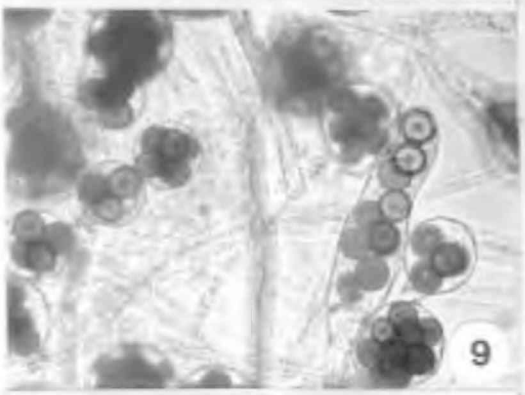
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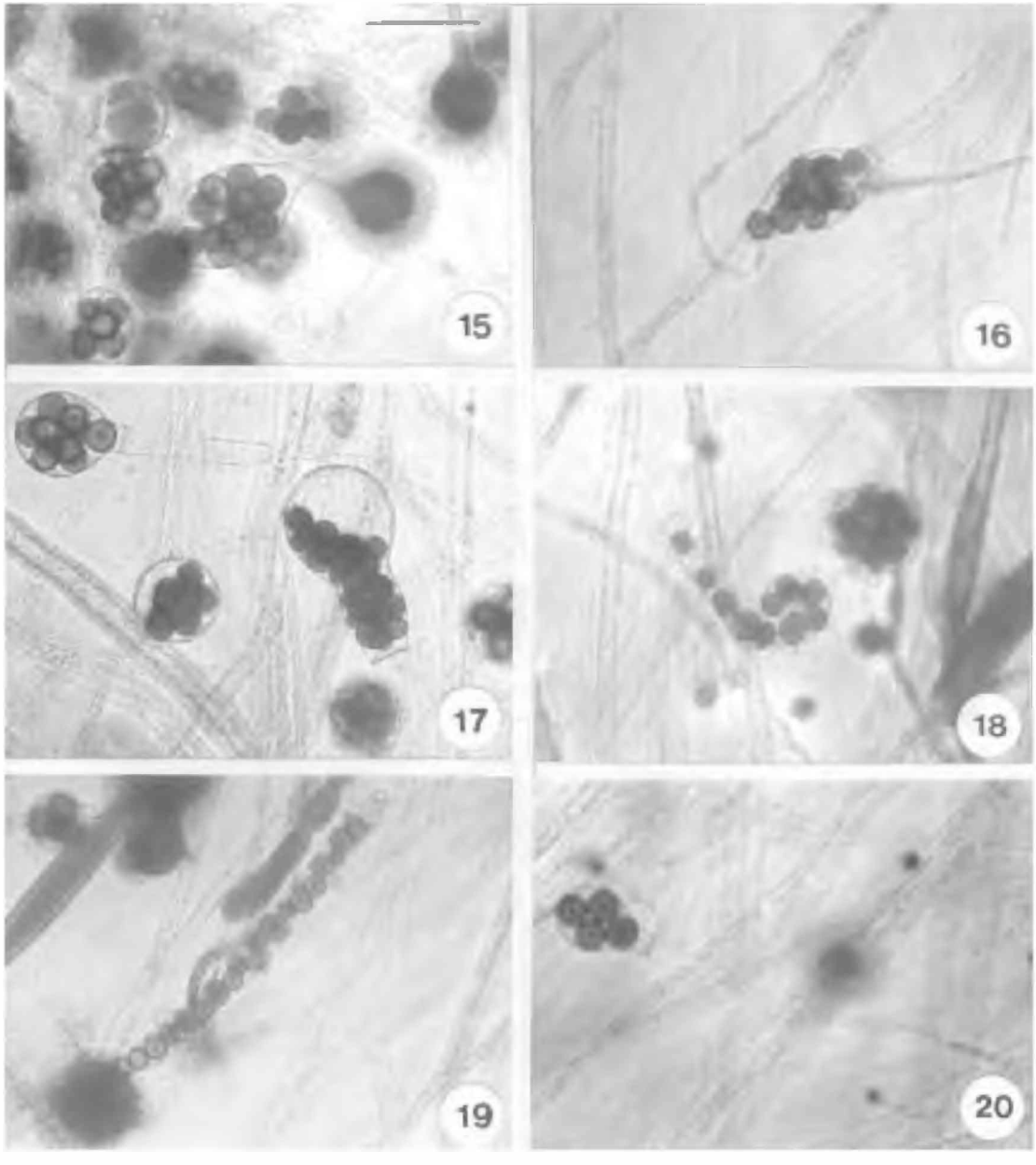
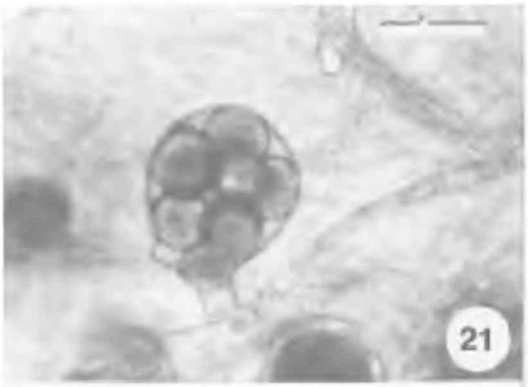


Fig. 15–20 *Saprolegnia variabilis*. **Fig. 15–16** Naviculate oogonia. **Fig. 17–18** Pyriform and irregular oogonia. **Fig. 19** Filiform oogonium. **Fig. 20** Long oogonial stalk tapering towards the end. Scale bars = 50 µm.

Fig. 21–28 *Saprolegnia variabilis*. **Fig. 21** Pyriform oogonium with a short lateral projection. **Fig. 22–26** Oogonia with spherical and ellipsoid subcentric oospores, variable in number and size. **Fig. 27** Diclinous antheridial branches. **Fig. 28** Oogonium with monoclinous antheridial branch. Scale bars: Fig. 21–26 = 50 µm; Fig. 27–28 = 100 µm.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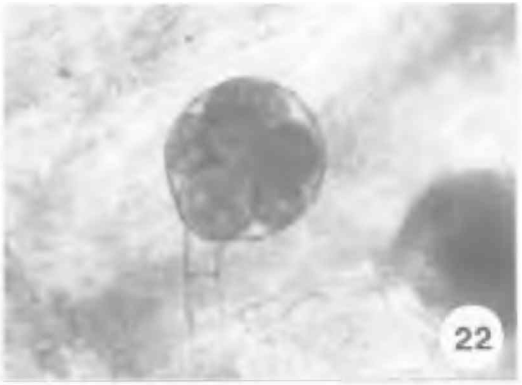
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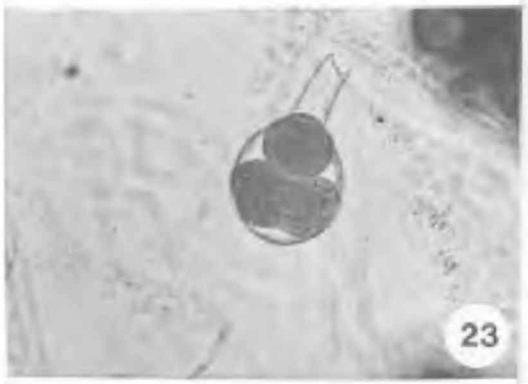
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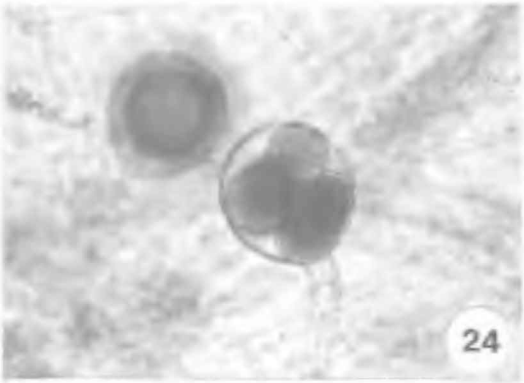
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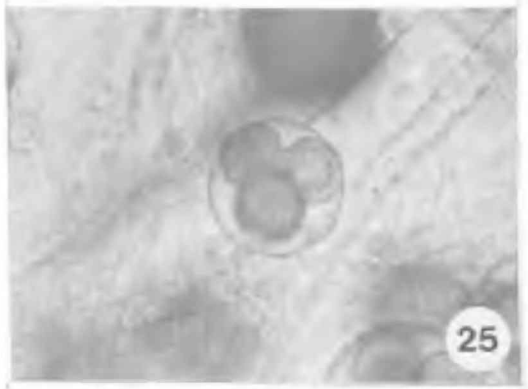
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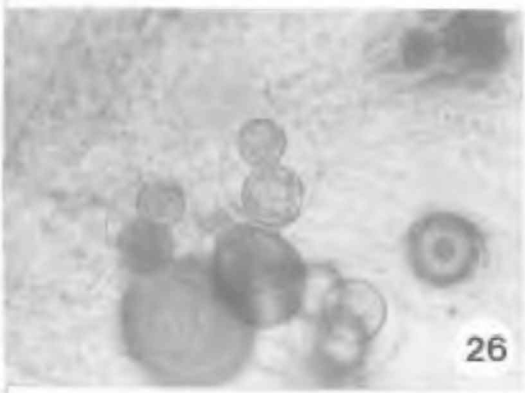
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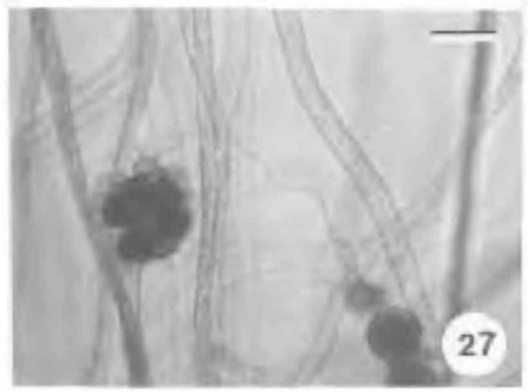
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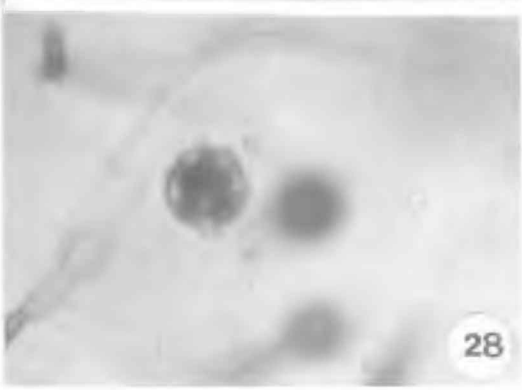
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The type specimen is deposited in the mycological herbarium of Spegazzini Institute (LPS) and its culture collection.

SPECIES DESCRIPTION

Saprolegnia variabilis Steciow et Elíades, sp. nov.
Fig. 1–28

Mycelium densum, cultura in seminibus *Cannabis sativae* 1.5–3 cm diam. Hyphae ramosa, pleraque 25–56 µm late diam. ad basim. Sporangia in culturis juvenilibus, filiformia, fusiformia vel clavata, (127–)137–407(485) µm larga et 20–49 µm lata, renovata per proliferationem internam. Ejecto sporarum pro genus typica, zoospori incystatis globosi 10–12 µm. Gemmae frequentis. Oogonia copiosa, pyriformia, sphaerica, cylindrica, obovate, naviculate vel apiculata, (39)51–92(153) µm diam. Paries oogonia laevis, ramulus lateralibus provenientia, 200–1000 µm. Oospori (1)3–18(60) per oogonium, subcentrici, (9)15–30(56) µm diam. Ramulus antheridiales, ramosus, diclina (84% ± 9), monoclina (9% ± 7) et androgina (7% ± 3).

Mycelium extensive, denser near substratum, 2-week-old hemp seed colony, 1.5–3 cm diam.; principal hyphae stout, sparingly branched, 25–56 µm diam. at the base. Gemmae abundant, spherical, pyriform or irregular, single or often catenulate, functioning as zoosporangia with papilla for discharge. Zoosporangia filiform, fusiform, clavate or irregular; (127)137–407(485) × 20–49 µm; short or long, often with one or two papillae, usually terminal, renewal by internal proliferation. Zoospore discharge saprolegnoid. Encysted spores globose, 10–12 µm diam. Oogonia very abundant, terminal or lateral, often intercalary; variable in shape; pyriform, spherical, obovate, cylindrical, or often naviculate or with short lateral projections when terminal, or sometimes doliform or filiform when intercalary; (39–)51–92(153) µm. Oogonial wall smooth; pitted or pitted only under point of attachment of antheridial cell. Oogonial stalks usually slender, frequently short to very long, tapering toward the end; straight or frequently bent; 25–650 µm long, sometimes branched and helicoidal in old cultures. Oospheres maturing. Oospores subcentric, type I, filling or not filling the oogonium; spherical or ellipsoid; (1)3–18(60) in number; variable in size, (9)15–30(56) µm diam. Antheridia present, or very rarely absent. Antheridial branches slender, principally diclinous (84% ± 9), occasionally monoclinous (9% ± 7), rarely androgynous (7% ± 3), branched. Antheridial cells simple or branched; attached by projections or laterally appressed. Fertilisation tube not observed.

HOLOTYPUS: Argentina, Buenos Aires Province, Ensenada, polluted artificial channel, on floating litter; April 2001, leg. M. Steciow, LPS N° 45649; culture collection N° 697.

ETYMOLOGY: referring to the variability in oogonial shape, oospore shape, oospore size, and length of oogonial stalks of this species.

DISCUSSION

Saprolegnia variabilis is closely related to *S. australis* Elliott (Elliott 1968; Seymour 1970). Both species have smooth oogonia, principally diclinous and also monoclinous and androgynous antheridial branches, and subcentric oospores. However, *S. australis* has immature subcentric oospores (type I or II), and the mycelial hyphae are more slender (30 µm diam. at the base).

In *S. variabilis* the oogonial stalk is very variable in length, slender, and tapering toward the end (up to 650 µm, 4 times the diameter of the oogonia), whereas in the original description for *S. australis*, the author failed to document the length of the

Table 1 Means and ranges of morphological measurements of *Saprolegnia variabilis* grown at 3 temperatures for 20 days.

Temp.°C	Colony diam. (cm)	Oogonia diam. (µm)	Oospores diam. (µm)	Oospores per oogonium	Length of oogonial stalk
5	1.5–4.0	(40)51–77(87)	(10)15–25(41)	3–15(60)	(25)51–102(600)
10	2.0–4.0	(39)53–92(111)	(9)15–30(44)	(1)3–18(21)	(51)121–300(650)
25	2.0–3.5	(40)51–81(153)	(10)15–30(56)	(1)3–17(25)	(25)76–127(420)
Overall mean ± SD	2.5±0.4	67.1±6.0	25.4±4.6	9.2±2.7	174±8.8

subtending hyphae ("two to many times the diameter of the oogonia in length"). Examination of the type slide has confirmed that this species is distinct in this respect.

In *S. variabilis*, the zoosporangia are longer (up to 485 µm), 10 times larger than in *S. australis*. They are cylindrical, filiform, fusiform, clavate or irregular whereas in *S. australis* they are filiform or clavate. Both species have saprolegnoid internal proliferation. The oospores are subcentric, spherical and ellipsoid, type I, and (1)3–18(60) per oogonium and are of variable and larger size, (9)15–30(56) µm, in *S. variabilis*, whereas the oospores are immature, type I or II, and the number and size of oospores are smaller, 6–12(30) and 22–24(27), respectively, in *S. australis*.

In the original description of *S. australis*, the oogonia showed variation in shape, pyriform to obovate, when the water culture was kept at 20 or 25°C. In *S. variabilis* the oogonia also change shape at those temperatures; they are principally pyriform or spherical, doliform or filiform when intercalary, and larger in size at 25°C. The same increase in the size of the oospores appears with higher temperatures; however, the great number of oospores was developed at 5°C. Measurements of *S. variabilis* colonies, oogonia diameters, oospore diameters, oospores per oogonium, and length of oogonial stalks were made at three different temperatures (Table 1). We were unable to make the same measurements on *S. australis* for comparison because the type culture was no longer available (S. Lorraine pers. comm.).

Saprolegnia longicaulis from other freshwater environments in Argentina (Steciow 2001) is characterised by having a longer oogonial stalk (up to 1300 µm long) and subcentric oospores of type I but fewer in number (up to 26 oospores per oogonium), but it differs from the new species in having only diclinous and monoclinous antheridial branches, and smaller oospores (up to 34 µm diam.) which are constant in shape and size inside the oogonium.The zoosporangia are longer, up to 700 µm.

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