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Saprolegnia milnae (Saprolegniales, Straminipila), a new species from an Argentine river (Tierra del Fuego Province, Argentina)

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Abstract *Saprolegnia milnae* is described from litter (floating dead twigs, leaves, and roots) in the Milna River, Ushuaia Department, Tierra del Fuego Province (Argentina). The new species is illustrated and compared with other species of the genus. Distinguishing characteristics of *S. milnae* are the production of smooth oogonia and predominantly contorted androgynous antheridial branches, and also monoclinous and diclinous ones. The oogonial stalks are predominantly bent, curved or once coiled; oospores are subcentric, (1–)3–23(–40) per oogonium, and are variable in size, reaching a diameter of up to 60 µm.

Keywords Stramenopiles; Oomycota; *Saprolegnia*; Argentina; systematics; new species

INTRODUCTION

During a study of zoosporic organisms occurring in water and floating organic matter in the Milna River, Ushuaia Department, Tierra del Fuego Province (Argentina), a species was recovered belonging to the genus *Saprolegnia* but with distinctive features that separate it from other species in the genus: the new species is herein described as *S. milnae* Steciow. It belongs to the Kingdom Straminipila, Phylum Heterokonta, Class Peronosporomycetes (Alexopoulos et al. 1996; Dick 2001).

This is the third in a series of contributions to the knowledge of zoosporic organisms from Tierra del Fuego Province, the southernmost province of Argentina and of South America (Steciow 2001a,b).

MATERIAL AND METHODS

The methods described by Coker (1923), Sparrow (1960), and Seymour (1970) were used to isolate zoosporic organisms. Samples of brown decaying twigs, leaves, and wood of the local dominant vegetation collected from the Milna River were brought to the laboratory in separate sterile polythylene bags. These samples were placed and distributed in water culture in sterilised Petri dishes containing several halves of hemp seeds (*Cannabis sativa*) and incubated at room temperature (15–20°C). After growth of the fungus on the seeds was observed, a single hypha or spore was isolated and transferred to a 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 variations of sexual structures. Diameters of fungus colonies, diameters of oogonia, number of oospores per oogonia, and diameters of oospores were calculated from 50 counts of each of 3 replicates. Measurements and observations were made using an Olympus BX 40 microscope (Olympus Optical Co., Ltd, Tokyo, Japan) equipped with phase contrast optics.The total percentage of type of antheridial branches was calculated from all of these replicates.

The type specimen is deposited in the mycological herbarium of the Spegazzini Institute (LPS) and its culture collection.

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TAXONOMY

Saprolegnia milnae Steciow, sp. nov.

Fig. 1–26

Mycelium densum, cultura in seminibus *Cannabis sativae* 2–4.5 cm diam. Hyphae ramosa, pleraque 18–70 µm late diam. ad basim. Sporangia in culturis juvenilibus, cylindrica, filiform vel clavata, 127–582 µm larga et 19–46 µm lata, renovata per proliferationem internam. Ejecto sporarum pro genus typica, zoospori incystatis globosi 10–15 µm. Gemmae parcus. Oogonia copiosa, pyriformia vel sphaerica, rarissimo apiculata, (33)51–98(145) µm diam. Paries oogonia laevis, ramulus lateralibus provenientia, 100–900 µm. Oospori (1)3–23(40) per oogonium, subcentrici, (14)18–30(60) µm diam. Ramulus antheridiales, ramosus, plerumque origine androgyna (44% ± 2) et diclina (35% ± 10) sed interdum monoclina (21% ± 5).

Mycelium extensive, denser near substratum, two-week-old hemp seed colony, 2–4.5 cm diam.; principal hyphae stout, sparingly branched, 18–70 µm diam. at the base, with secondary branches, short, irregular and contorted. Gemmae scanty, spherical, pyriform or irregular, simple or catenulate. Zoosporangia cylindrical, filiform, clavate or irregular; 127–582 × 19–46 µm; short or long, often with one or two papillae, usually terminal, renewal usually by internal proliferation or proliferating sympodially. Zoospore discharge saprolegnoid. Encysted spores globose, 10–15 µm diam. Oogonia very abundant, terminal or lateral, rarely intercalary; pyriform, spherical or obovate, infrequently doliform or apiculate; (33)51–98(145) µm; immature oogonia frequently proliferating. Oogonial wall smooth, very rarely with one papilla; pitted, or pitted only under point of attachment of antheridial cell. Oogonial stalks frequently stout and variable in length, tapering toward the end; sinuous, bent, curved or once coiled, very rarely slender and straight; 100–900 µm long. Oospheres maturing. Oospores subcentric, type I, filling the oogonium; spherical or ellipsoid; (1)3–23(40) in number; (14)18–30(60) µm diam. Antheridia present, or very rarely absent. Antheridial branches principally androgynous (44% ± 2) and diclinous (35% ± 10), occasionally monoclinous (21% ± 5), branched. Antheridial cells simple or branched; attached by projections or laterally appressed. Fertilisation tube not observed.

HOLOTYPE: Argentina, Tierra del Fuego, Ushuaia, Milna River, on floating litter; Feb 2000, *M. M. Steciow*, LPS no. 45646; culture collection no. 739.

ETYMOLOGY: Referring to the freshwater site of collection, the Milna River, where this new species was found.

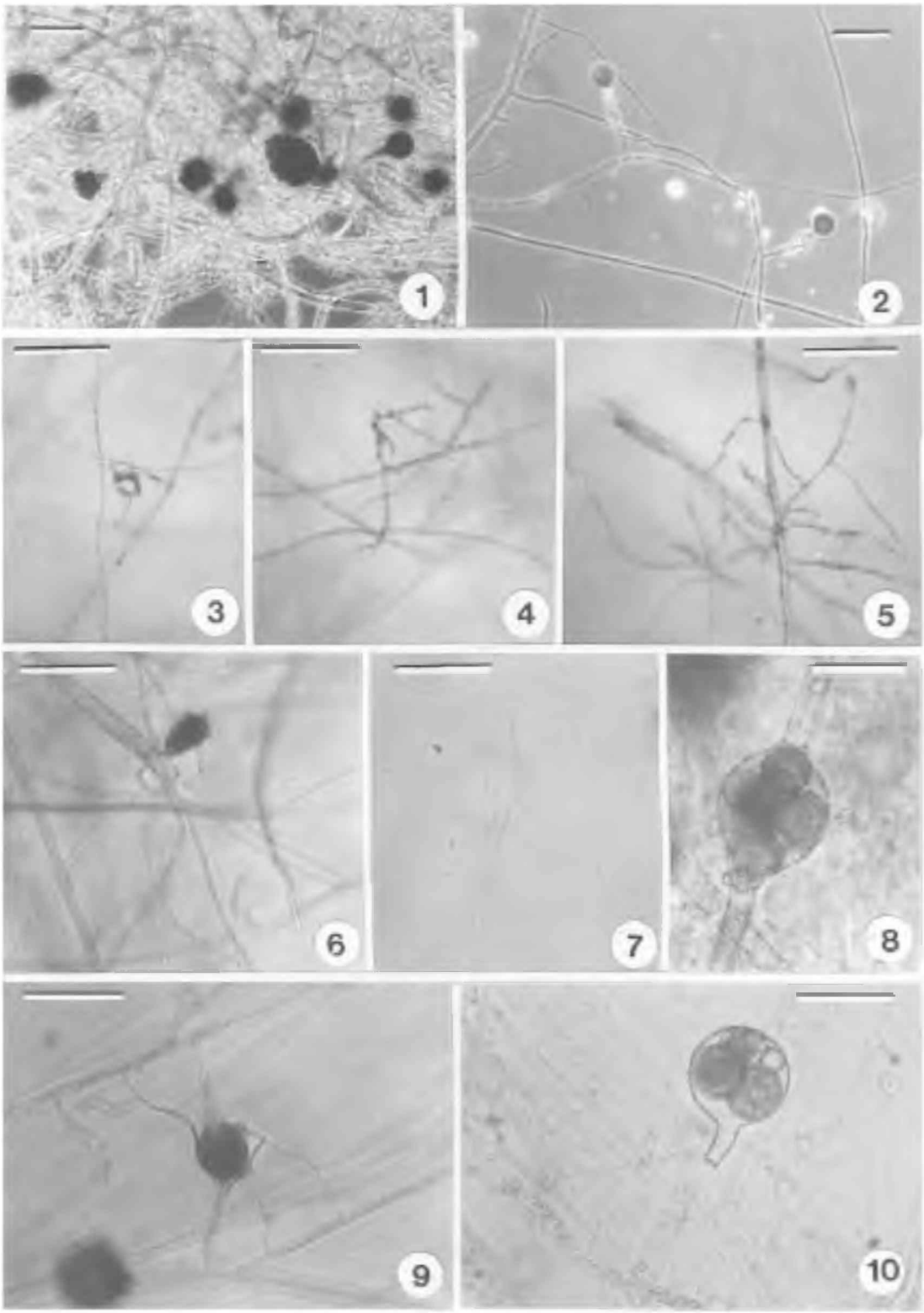
NOTES: It is important to note that the type of oospore, antheridial branches, and the measurements of oogonia are very constant features of this species. There was little variation in type and size of zoosporangia in different temperatures; they are cylindrical, filiform, clavate or irregular, and reached a mean range in length of 127–582 µm. The shape of oogonia remained constant, mainly pyriform, spherical or doliform, sometimes apiculate (smooth, rarely with one papilla).

Table 1 Ranges of morphological measurements of *Saprolegnia milnae* grown at 3 temperatures for 20 days.

Temp °C	Colony diam. (cm)	Oogonia diam. (µm)	Oospores diam. (µm)	Oospores per oogonium	Zoosporangia
5	2–3	(50) 76–86 (108)	15–35 (60)	1–20 (35)	204–434 × 35–41
10	2–4.5	(33) 48–84	(15) 23–26 (40)	(1) 10–25	260–450 × 30–43
25	3–4.5	(35) 51–97 (102)	15–29	3–27 (40)	127–460 × 20–46

Fig. 1–10 *Saprolegnia milnae*. **Fig. 1** Mycelium with immature and some mature oogonia with oospores. **Fig. 2–5** Mycelium with distinctive secondary hyphae that are profusely branched, contorted and twisted. **Fig. 6–7** Zoosporangia. **Fig. 8** Intercalary oogonium with one papilla. **Fig. 9** Detail of diclinous and monoclinous antheridial branches on immature oogonium. **Fig. 10** Oogonium with an androgynous antheridial branch and oospores of different sizes. Scale bars: Fig. 1–2 = 100 µm; Fig. 3–10 = 50 µm.

Fig. 11–18 (over page) *Saprolegnia milnae*. **Fig. 11–12** Detail of once coiled and bent oogonial stalk with androgynous antheridial branches. **Fig. 13** Sinuous oogonial stalk with distinctive contorted androgynous antheridial branches. **Fig. 14** Lateral oogonium with a long once-coiled stalk. **Fig. 15** Terminal oogonium with a long bent stalk. **Fig. 16** Aspect of subcentric oospores within a smooth and pyriform oogonium. **Fig. 17–18** Two oogonia on curved oogonial stalk. Scale bars = 50 µm.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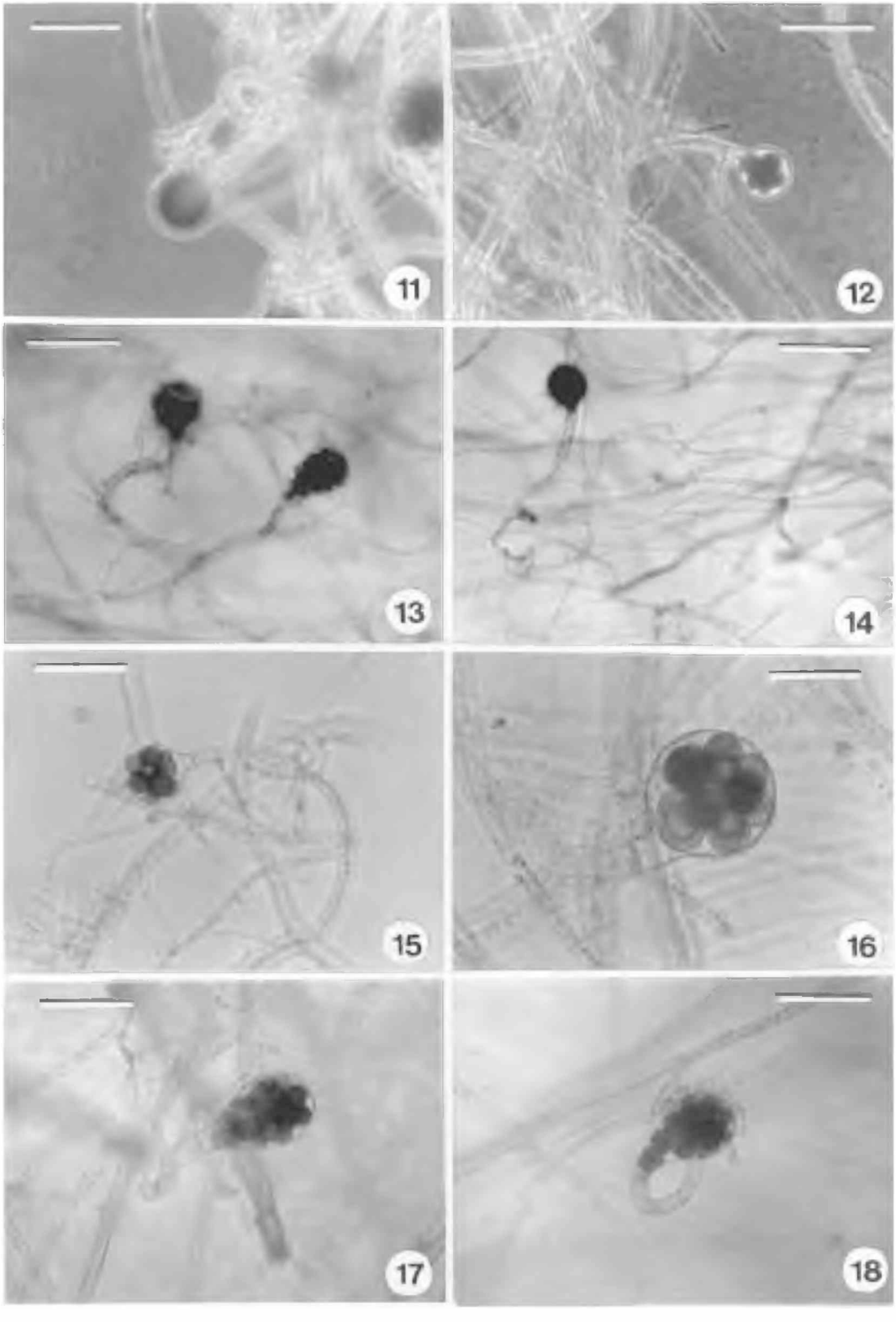
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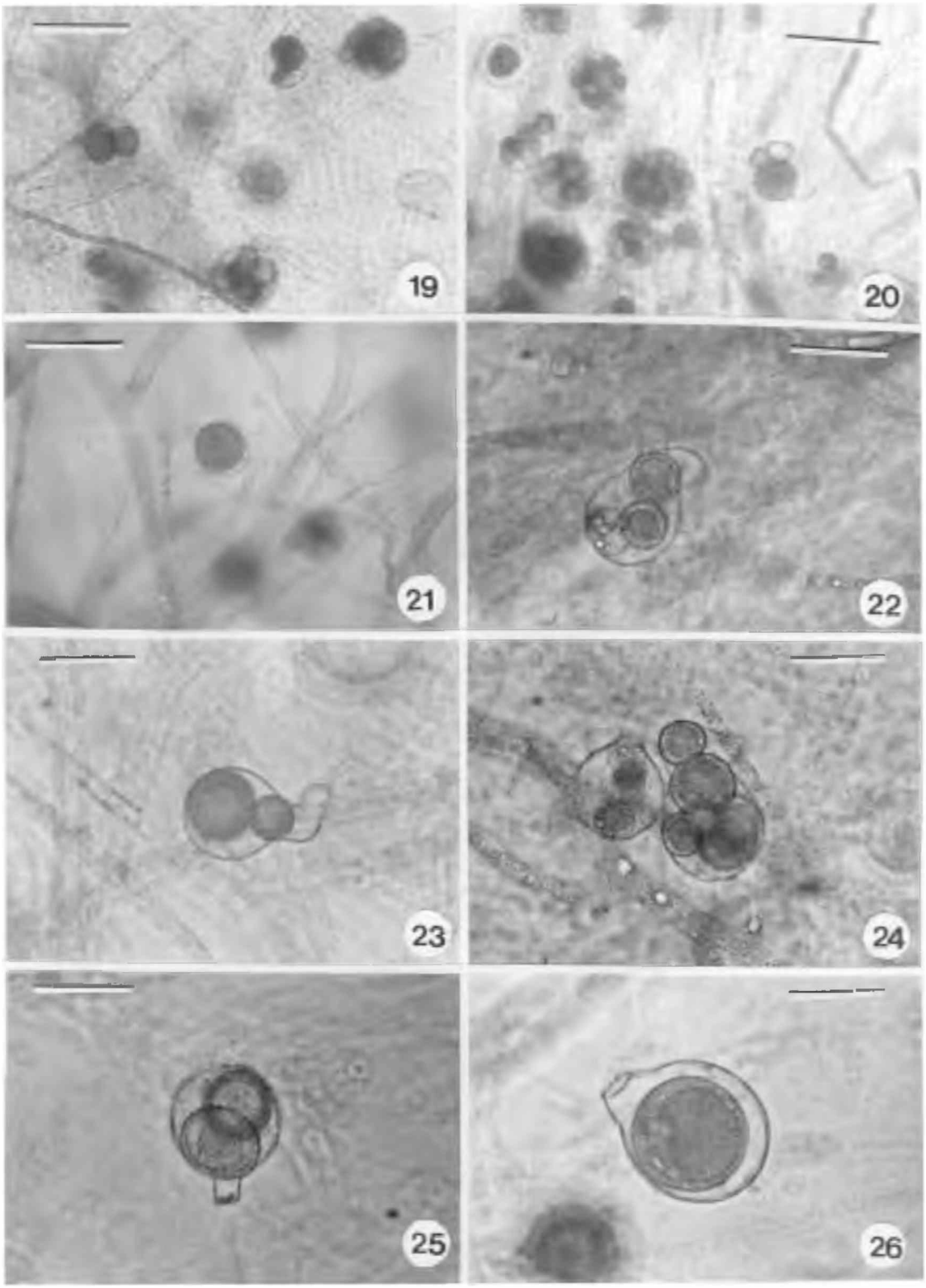
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The initial growth rate was faster at higher temperature (25°C), but after 20–30 days there was little difference in mean diameter of colonies from the other two temperature regimes (Table 1). At 25°C, the colonies develop a higher number of slender secondary hyphae, giving the colonies a denser aspect than they reached at lower temperature (5°C).

Oogonial production was unaffected by the different temperatures; all colonies developed oogonia, but mature oospores were less abundant at lower temperatures and the number of oogonia and the oospores were proportionally lower as the temperature decreased. However, the oogonial stalk reached a greater length (400–900 µm) and the oospores were particularly larger (40–60 µm) at 5°C.

DISCUSSION

Saprolegnia milnae Steciow appears to be related to *S. furcata* Maurizio (Seymour 1970). Both species have smooth oogonia and predominantly androgynous contorted antheridial branches, but also monoclinous and androgynous branches and a predominantly bent or coiled oogonial stalk. However, *S. milnae* has only subcentric oospores, larger (up to 60 µm) and often reaching different sizes inside the oogonium (mainly at lower temperatures, 5°C), whereas *S. furcata* has principally centric oospores, rarely subcentric, the oospores are smaller, (19.4–)20–22(–45) µm, and the oogonial stalk is often coiled up to 6 times (Seymour 1970).

In the Argentine species, the oogonia are larger, (35–)51–98(–146) µm diam. (sometimes apiculate and pitted), with (1–)3–23(–40) oospores per oogonium and the oogonial stalk never is so coiled (up to once coiled) as appears in *S. furcata*, whereas in the latter species, the oogonium is never apiculate, is unpitted or very rarely pitted, and the oogonia are smaller (33–)43–48(–80.6) µm diam. and fewer inside the oogonium ((1–)4–9(–25) in number).

Saprolegnia milnae appears to resemble *S. glomerata* (Tiesenhausen) Lund in having slender hyphae, these usually delicate and with lateral branches, generally short, irregular or contorted, giving a twiggy appearance and gemmae are not abundant (Seymour 1970). However, the latter has only (1–)6–9(–25) centric, spherical oospores, and they are smaller ((10–)23–26(–25) µm diam.). The zoosporangia are also different because they often have a bulbous base in *S. glomerata*, a feature absent in the Argentine species.

Saprolegnia australis Elliott and *S. longicaulis* Steciow, with subcentric oospores, also differ from *S. milnae* in having predominantly diclinous antheridial branches, and scanty monoclinous ones that are never contorted; the oogonial stalks are mainly straight (Elliott 1968). *Saprolegnia longicaulis* never has androgynous antheridial branches and the oogonial stalk is much longer (102–1300 µm) than in this new Argentine species (Steciow 2001c).

On the other hand, *S. litoralis* Coker also has predominantly androgynous antheridial branches on principally terminal oogonia, but it has centric oospores and fewer oospores ((1–)2–6(–15) per oogonium (Seymour 1970)).

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◀ **Fig. 19–26** (previous page) *Saprolegnia milnae*. **Fig. 19–20** Mycelium with oogonia containing variable numbers of oospores, with larger oospores at 5°C. **Fig. 21** Detail of oogonia with a larger oospore at 5°C. **Fig. 22–23** Two lateral apiculate oogonia. **Fig. 24–26** Aspect of oogonia with subcentric oospores, spherical or ellipsoid ones. Scale bars = 50 µm.

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