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A. robusta sp. nov., a new species of *Achlya* (Saprolegniales, Straminipila) from a polluted Argentine channel

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Abstract

Achlya robusta sp. nov. was found on litter (floating twigs, leaves, and roots) in an artificial polluted channel, near a petroleum refinery, in Buenos Aires province, Argentina. The species is described, illustrated and compared with other species of the genus. *A. robusta* produces mainly smooth and papillate, tuberculate or bullate oogonia and monoclinous antheridial branches. It develops spherical and subglobose oogonia, with the oogonial wall yellowish and containing mainly immature oospheres. The oospores are eccentric and ranging from (1) 4–17 (30) per oogonium.

Key words: *Achlya* – new species – Oomycota – pollution – Straminipila

Introduction

During a survey of zoosporic organisms occurring in water polluted with hydrocarbons and organic matter floating in streams and channels, near the YPF-Repsol Petroleum Refinery, Partido de Ensenada, Buenos Aires Province (Argentina), we found a novel species which belongs to the genus *Achlya*; it is here named *Achlya robusta* Steciow.

The Oomycota were historically classified as fungi, but ultrastructural, biochemical and molecular sequence analyses strongly indicate that they are in a lineage that includes some algal groupes, belong to the monophyletic Kingdom Straminipila, Phylum Heterokonta, Class Peronosporomycetes (Alexopoulos *et al.* 1996; Dick 2001).

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

Material and methods

The methods for collection and isolation described by Coker (1923), Johnson (1956) and Sparrow (1960) were used. Samples of brown decaying twigs, leaves and wood of the local dominant vegetation were collected from an artificial channel and brought to the laboratory in separate sterile polythylene bags. The channel is located at Partido de Ensenada, Buenos Aires Province, Argentina. It receives the effluents of the refinery, and of PROSUL industry, a coke producing company. Aliphatic (15.390 µg/l) and aromatic (3.327 µg/l) hydrocarbons were found in the water samples, estimated by gas chromatography.

The samples were placed and distributed in water culture in sterilised Petri dishes containing several halves of hemp seeds (*Cannabis sativa*), and were incubated 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. Hemp seed halves were added in order to obtain sister colonies. Measurements and observations were made on those colonies. Some colonies were incubated at 5, 10, and 25°C to observe possible effect of temperature on the variations of sexual struc-

tures. Diameters of fungus colonies and oogonia, the number of oospores per oogonium, and diameters of oospores were calculated from 50 counts of each of 3 replicates. The total percentage of type of antheridial branches was calculated from all these replicates.

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

Results

Species Description

Achlya robusta Steciow and Elíades, sp. nov. (Figs. 1–20).

Mycelium densum, cultura in seminibus *Cannabis sativae* 2–5 cm diam. Hyphae ramosa, pleraque 45–158 µm late diam. ad basim. Sporangia in culturis juvenilibus, fusiformia, filiformia, cylindrica, vel clavata, (135) 229–500 (945) µm larga et 19–72 µm lata. Ejecto sporarum pro genus typica, zoospori incystatis globosi 5–10 (15) µm. Gemmae frequentis. Oogonia copiosa, sphaerica, subglobosa et pyriformia, (29) 66–117 (148) µm diam. Paries oogonia foveatus, laevis vel papillatus, ramulus lateralibus provenientia, 200–1000 µm. Oospori (1) 4–17 (30) per oogonium, eccentrici, (10) 16–40 (48) µm diam. Ramulus antheridiales, ramosus, monoclina (49%), diclina (30%) et androgina (21%).

Mycelium extensive, denser near substratum, two hy week-old hemp seed colony, 2–5 cm diam.; principal hyphae stout, sparingly branched, 45–158 µm diam. at the base. Gemmae abundant, cylindrical, pyriform or irregular, single or often catenulate, functioning as zoosporangia. Zoosporangia fusiform, filiform, cylindrical or claviform; (135) 229–500 (970) X 19–72 µm; usually terminal, renewed usually by distinctive basipetalous or cymose succession. Zoospore discharge achlyoid. Encysted spores globose, 5–10 (15) µm diam. Oogonia very abundant, lateral or terminal; spherical or subglobose, rarely pyriform; (29) 66–117 (148) µm. Oogonial wall smooth or with ornamentation, papillate, tuberculate or bullate; pitted or pitted only under attachment point of antheridial cell; yellowish at maturity. Inner wall surface irregular, refringent and of varying thickness. Oogonial stalks usually slender, frequently short, straight or frequently bent, rarely curved; 15 to 200 µm long, sometimes branched. Oospheres often not maturing. Oospores eccentric, not filling the oogonium; spherical or ellipsoid; (1) 4–17 (30) in number; (10) 16–40 (48) µm diam. Antheridia always present. Antheridial branches slender, principally monoclinous (49%), occasionally diclinous (30%), rarely androgynous (21%), 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; 12-Oct-00, leg. M. Steciow, LPS N° 45704; culture collection N° 737.

Etimology: The name refers to the big size and the vigorous aspect of the colonia, the diameter of the basal hyphae and to the oogonial and oospores size.

The shape of oogonia, type of oospore (with many immature oospheres) and of antheridial branches are very constant features in *A. robusta*. The type and size of zoosporangia, in different conditions of temperature did not show mainly variations; they are fusiform, filiform, cylindrical or claviform and reach a length from 230 µm to 750 µm. The oogonia are constant in shape; they are mainly spherical or subglobose, rarely pyriform, often ornamentated, mainly papillate, tuberculate or bullate.

Measurements of colonies, oogonia diameters, oospores diameters, oospores per oogonium, length of

Table 1. Ranges of morphological measurements of *Achlya robusta* grown at 3 temperatures for 30 days

Temp. °C	Colony diam. (cm)	Oogonia diam. (µm)	Oospores diam. (µm)	Oospores per oogonium	Length of oogonial stalk	Zoosporangia
5	1.5–2.5	(50) 58–106 (120)	14–24 (35)	(1) 5–12	30–40	(261) 300–500 (945) X 29–68
15	2.0–5.0	(29) 41–95 (140)	(10) 16–36 (48)	(2) 4–17 (30)	(15) 24–48 (102)	(135) 257–750 (970) X 14–46
25	2.0–4.0	(51) 66–117 (148)	20–44	(1) 4–15 (20)	(20) 51–76 (200)	(219) 229–500 (810) X 20–72

Fig. 1–8. *Achlya robusta.* **1.** Detail of filiform zoosporangium with a characteristic discharge achlyoid; spore cluster persistent **2–3.** Zoosporangium renewed in distinctive basipetalous succession **4.** Two characteristic monoclinous antheridial branches and one androgynous in water culture 6.7. Detail of smooth oogonia with immature oospheres 8. Oogonium with detail of two eccentric oospores. Scale bars, Fig. 1–5 = 100 µm; 6–8 = 50 µm

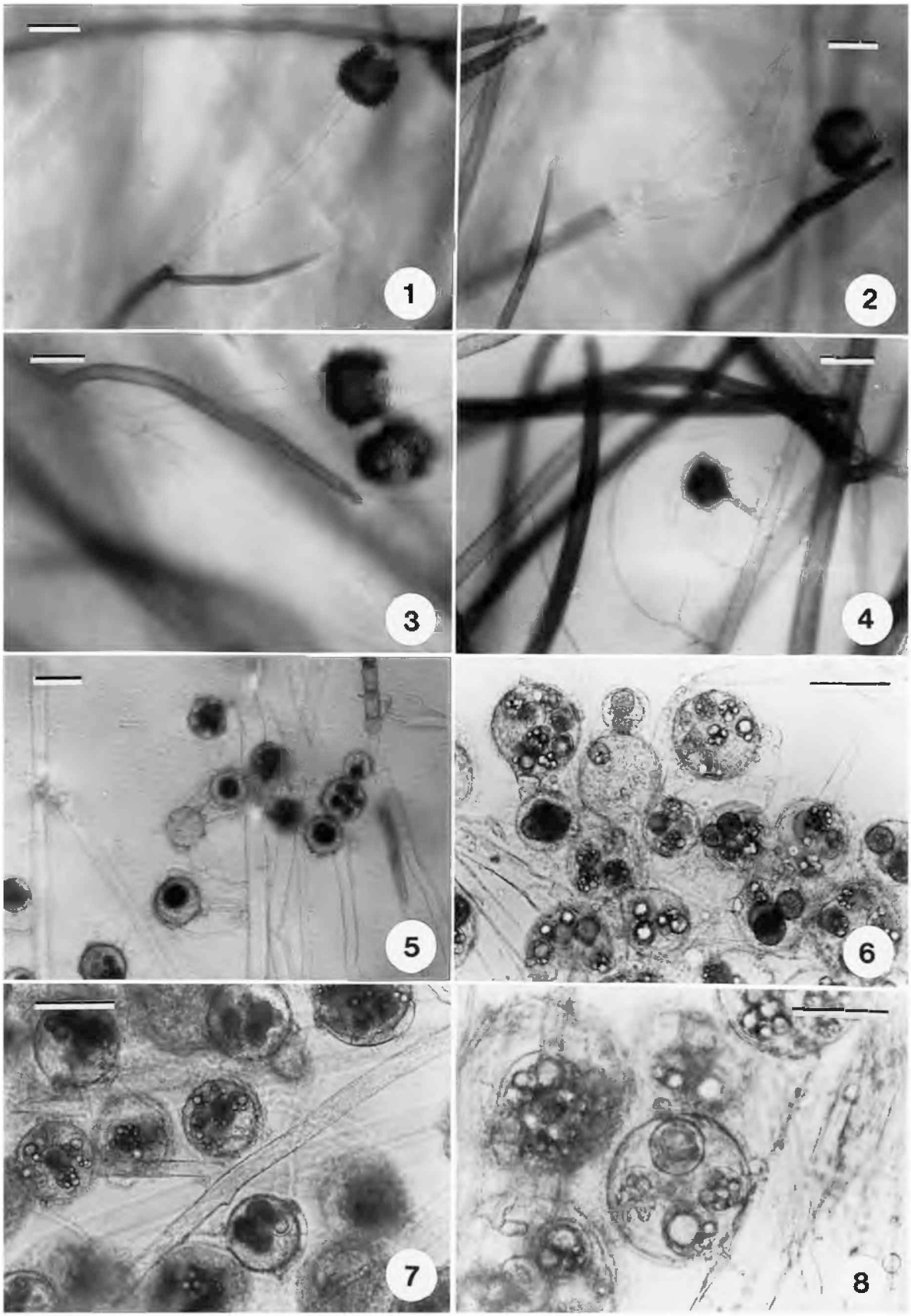

Fig. 1–8.

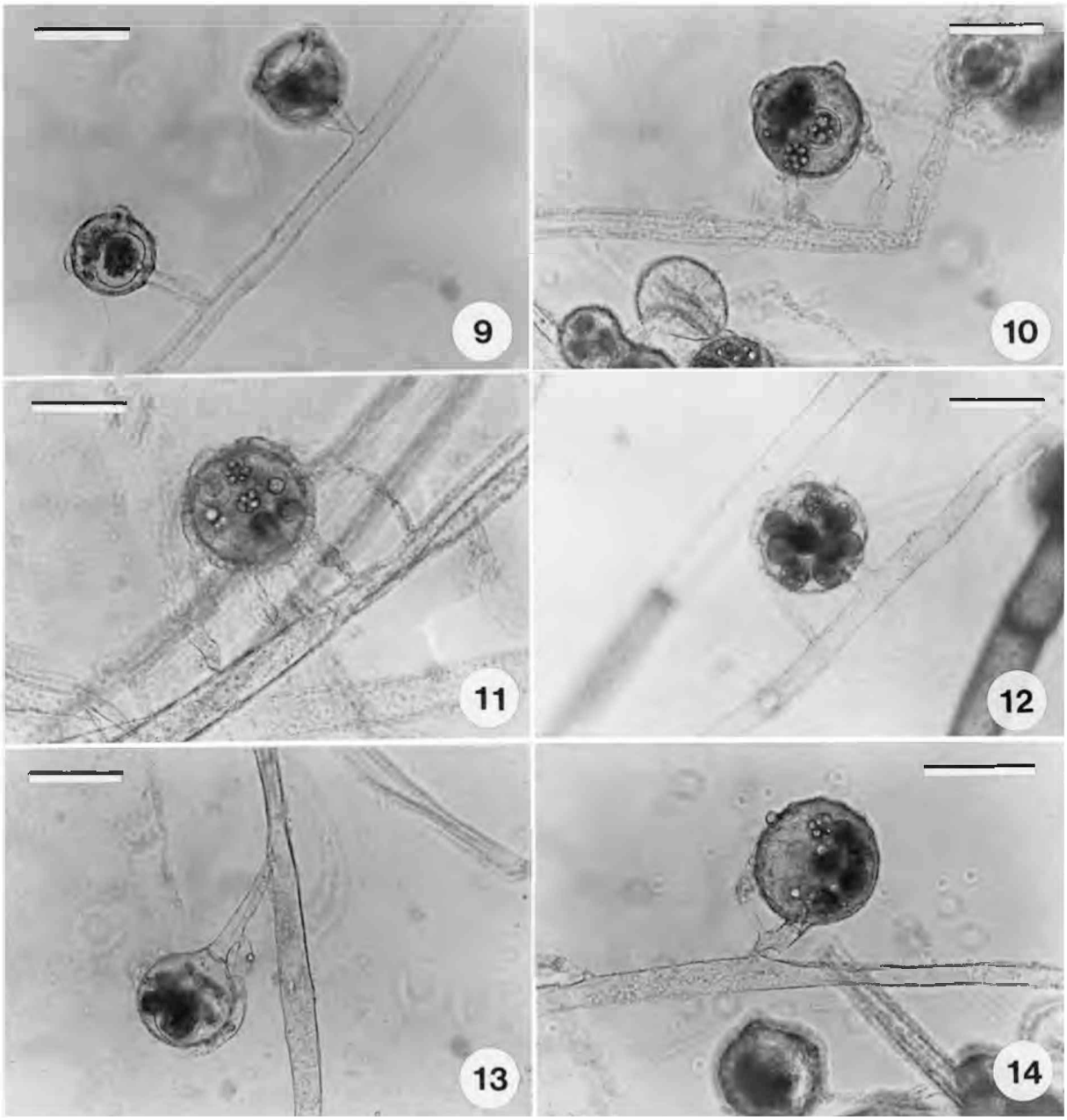

Fig. 9–14. *Achlya robusta.* **9–12.** Spherical or subglobose oogonia with distinctive monoclinous antheridial branches with immature oospheres inside, developed on slender and short stalk **13–14.** Androgynous antheridial branches in oogonia developed on bent oogonial stalk. Scale bars, Fig. 9–14 = 50 µm

oogonial stalks and size of zoosporangia were taken at three different temperatures (Table 1). In *A. robusta* the initial growth rate was faster at 25 °C. But after 25–30 days, the mean diameter of colonies at 15 °C, was not significantly different. Cultures incubated at 25 °C showed little further linear growth, while cultures at 15 °C continued to grow to form larger colonies with a great number of zoosporangia that reached a greater size (Table 1).

Oogonial production was affected by temperature. All develop oogonia, but mature oospores are less abundant at 5 °C, and the number of oogonia is lower at this temperature. The size of oogonia and oospores increases at higher temperatures.

The number of zoosporangia is higher in cultures at 15 °C than at 25 °C. At all temperatures, gemmae are more abundant with increasing age of the culture. After 10–15 days, they were very scanty in all of them.

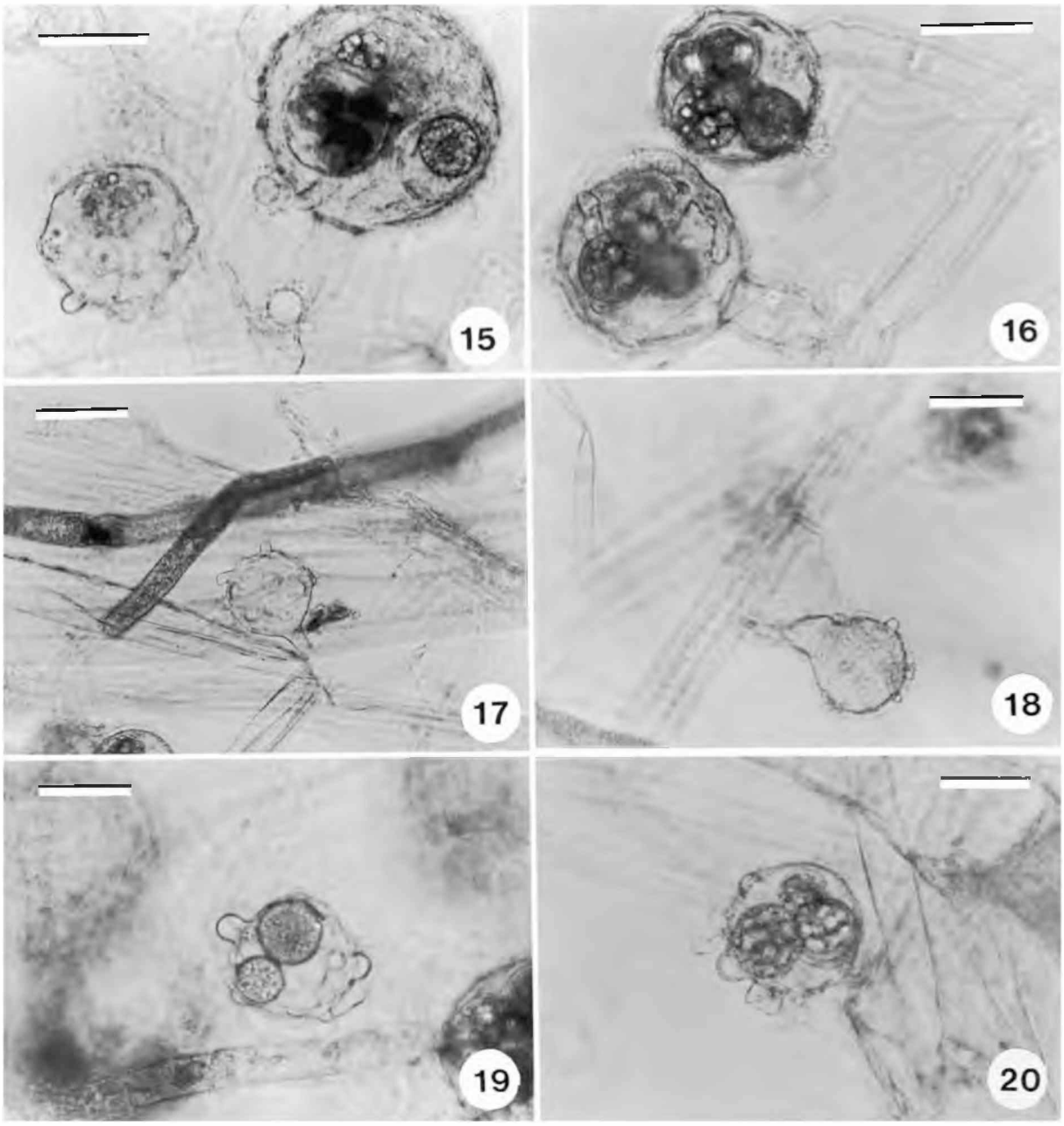

Fig. 15–20. *Achlya robusta.* **15–16.** Detail of ornamentated oogonia with the oogonial wall pitted and irregular inner surface **17–18.** Tuberculate and papillate oogonia with aborted oospheres **19–20.** detail of papillate and bullate oogonia. Scale bars, Fig. 15–20 = 50 µm

Discussion

Achlya robusta Steciow and Elíades (2002) has affinities to *A. americana* Humphrey (Johnson 1956; Johnson and Seymour 1974), due to the preponderance of simple or sparingly branched monoclinous antheridia of near origin, and eccentric oospores. *A. americana* is different from the Argentine species because of the scarcity of other types of antheridial branches (rarely diclinous, some isolates lack androgynous antheridial branches), the smoth-walled oogonia, spherical or pyriform with numerous conspicuous pitts, containing (1) 6–14 (38) oospores filling the oogonial cavity, and the oospores smaller: (15) 20–25 (47) µm diam. (Coker 1923; Coker and Mathews 1937; Johnson 1956). In *A. robusta*, the oogonia are distinctive spherical or subglobose, ornamentated, and with often immature or aborted oospheres. This latter features relates this new Argentine species

with *A. conspicua* Coker, which has spherical, oval or pyriform oogonia containing nearly mature oospheres, but usually with monoclinous or androgynous antheridial branches, rarely with diclinous ones, inside always smooth-walled oogonia. However *A. conspicua* has smaller oogonia and oospores than *A. robusta*, which reach (45) 65–80 (120) µm diam. and (19) 24–26 (35) µm respectively, and develops 1–40 oospores per oogonia.

A. cambrica Trow has also smooth or ornamentated oogonia with sparse papillate ornamentations but develops mature oospheres inside oogonia of different shapes and positions (terminal, lateral or intercalary). The zoosporangia are also smaller, reaching 400 µm (Johnson 1956).

Achlya debaryana Humphrey differs from *A. robusta* in having mature eccentric oospores of smaller size: (13) 22–26 (34) µm in iregular walled oogonia, but develops mainly monoclinous antheridial branches (90%) and diclinous ones (10%) in small proportion (Jonhson 1974).

Recently a new species was described from the southernmost province from Argentina, *Achlya fuegiana* Steciow, with papillate oogonia but with centric-subcentric oospores and principally androgynous antheridial branches (Steciow, 2001).

Little is known about the role in decomposition and community development of saprotrophic Oomycetes, their ubiquity and their large number may outline their ecological importance (Cooke and Rayner 1984). This contribution sheds some light on a particular environmental condition, in which species of Oomycetes are able to survive due to the fact that these organisms may be an indigenous or probably immigrant species of aquatic origin, and spend all or a part of its life cycle in stagnant water or in submerged muds (Dick 1971; Park 1972). Oil entering a freshwater and soil ecosystem has two major effects on the indigenous microorganisms. It is toxic to some of them, whereas other microorganisms can use oil as a source of carbon and energy. For this reason *A. robusta* may belong to a microbial community with species diversity that is enriched in hydrocarbons-using organisms.

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