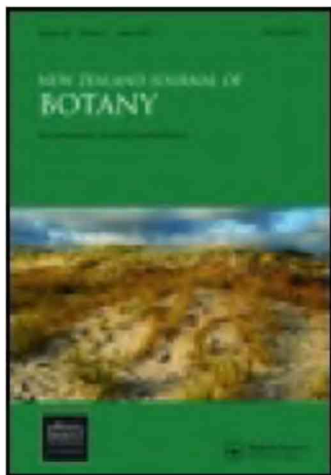


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A new freshwater species of *Achlya* from Tierra del Fuego Province, Argentina

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Abstract *Achlya ambispora* sp. nov. occurring on litter (floating dead leaves and twigs) in Las Cotorras stream, Tierra del Fuego Province, Argentina, is described and illustrated and compared with similar species. The species produces principally androgynous and monoclinal, rarely declinal antheridial branches; oogonial wall and oospores are distinctly yellowish at maturity and differ from other species in having also 1–6(–22) centric-subcentric oospores, in oogonia sometimes apiculate, that are frequently intercalary or proliferate in chains.

Keywords Chromista; *Achlya*; new species; Argentina; systematics

INTRODUCTION

During a survey of Chromistan organisms on floating dead leaves and twigs in streams of Tierra del Fuego Province, Argentina, a new species belonging to Oomycota was found. It is unique in producing androgynous, monoclinal, and declinal antheridial branches and 1–6(–22) centric-subcentric oospores.

It is here described as *Achlya ambispora* sp. nov. and illustrated.

This is the first contribution to the knowledge of the water moulds of Tierra del Fuego, the southernmost Province of Argentina. Very little is known about the Argentinian water moulds since the literature contains few references, principally about

habitats of Buenos Aires Province (Beroqui de Martínez 1970; Steciow 1988, 1993a, 1993b, 1998).

MATERIAL AND METHODS

The method described by Johnson (1956, 1974) and Sparrow (1960) was used. Isolations were made from water samples, containing small dead twigs, decaying leaves, etc., brought into the laboratory and distributed in sterilised Petri dishes containing several halves of hemp seeds (*Cannabis sativa*). After growth of the fungus on the seeds a single hypha or spore was isolated and transferred to a weak medium (cornmeal agar). After 2–3 days a block of agar at the edge of the colony was cut off and placed in another sterilised Petri dish containing distilled water, with half a hemp seed on the agar block in order to obtain a new colony. Measurements and observations were made on that colony.

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

To study morphological variations caused by temperature effects, age of culture, and different sources of water, the cultures were examined after incubation at 10°C, 15°C, 25°C, and 31°C for 10, 15, and 30 days.

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 (in distilled water and sterilised pond water). The total percentage of type of antheridial branches and type of oospores was calculated from all these replicates.

SPECIES DESCRIPTION

Achlya ambispora Steciow, sp. nov. Fig. 1–22
Mycelium densum, cultura in seminibus *Cannabis sativae*, 1–3 cm diam. Hyphae ramosae, pleraque 24–106 µm late in base. Sporangia copiosa in

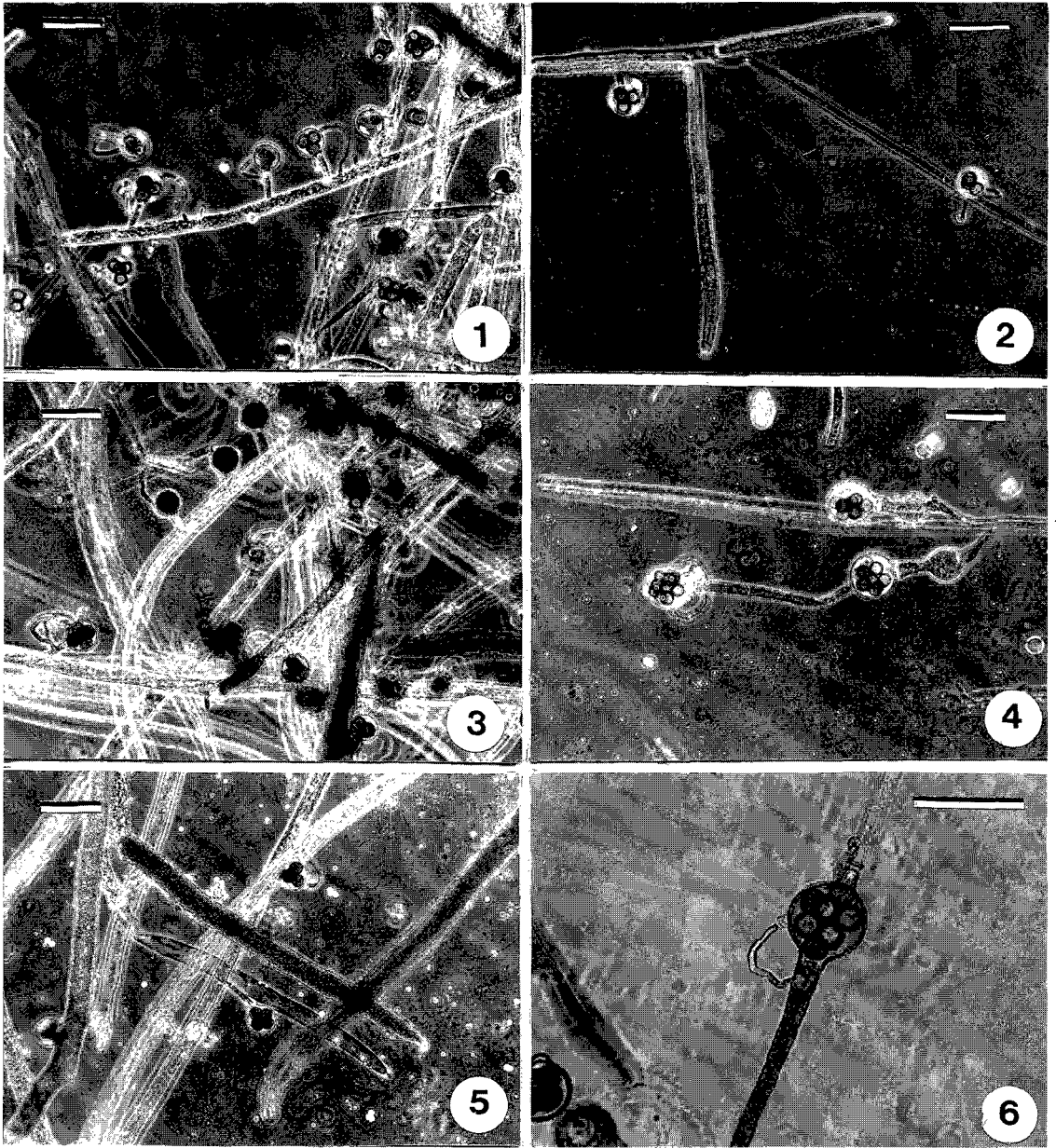
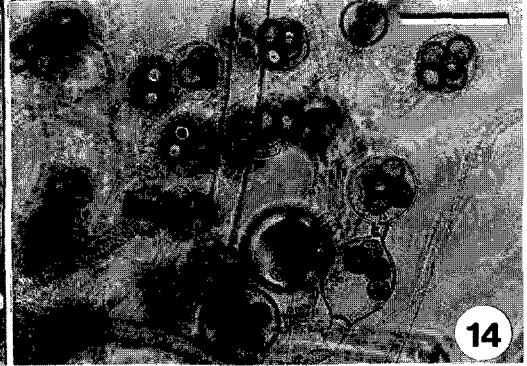
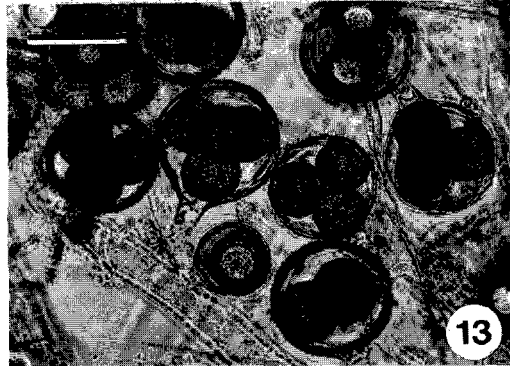
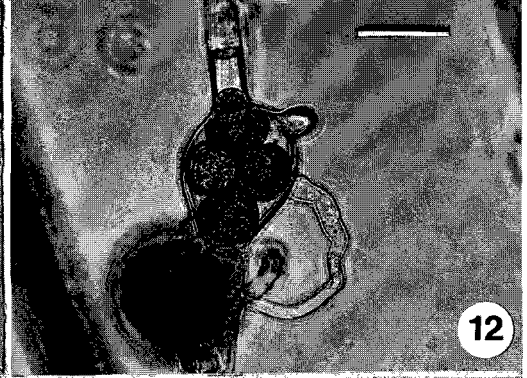
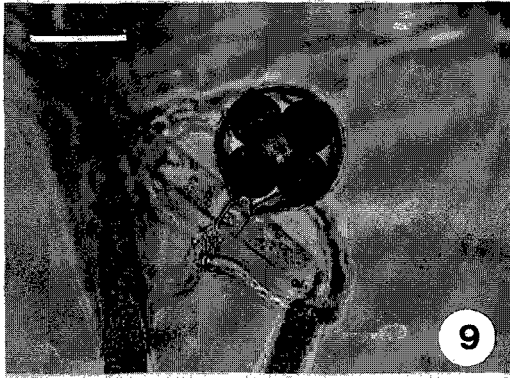
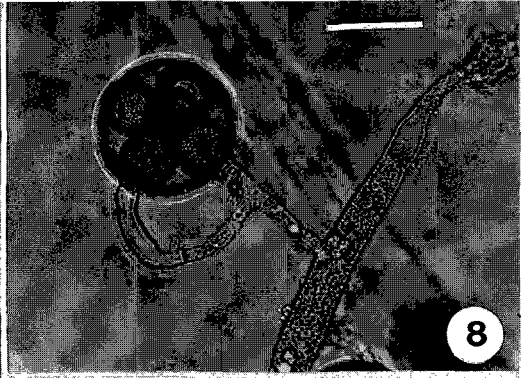
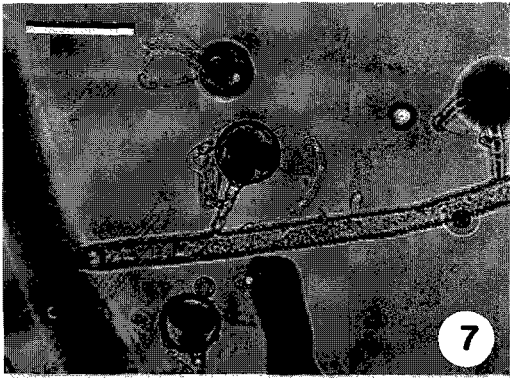
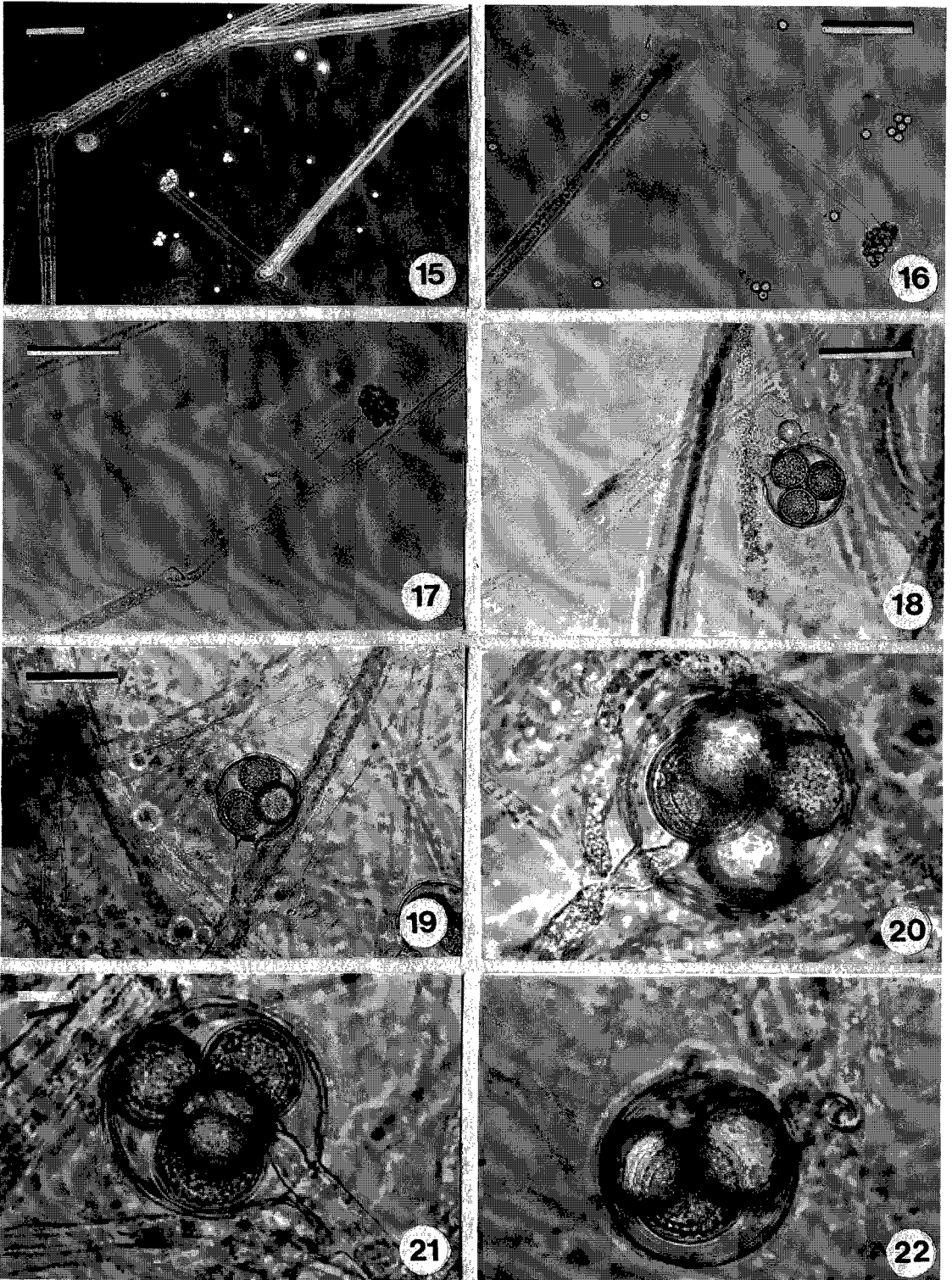


Fig. 1–6 *Achlya ambispora*. **Fig. 1** Mycelium with oogonia and monoclinous and androgynous antheridial branches, PCM. **Fig. 2** Terminal and intercalary gemmae, PCM. **Fig. 3** Oogonia with declinous and androgynous antheridial branches, PCM. **Fig. 4** Oogonia proliferating in chains, PCM. **Fig. 5** Zoosporangia, PCM. **Fig. 6** Intercalary oogonia with monoclinous antheridial branch. Scale bars: Fig. 1–5 = 100 μm ; Fig. 6 = 50 μm .

Fig. 7–14 *Achlya ambispora*. **Fig. 7** Detail of androgynous and monoclinous antheridial branches. **Fig. 8–10** Androgynous antheridial branches; antheridia apically appressed. Centric-subcentric oospores. **Fig. 11** Lateral apiculate oogonia with declinous antheridial branch. **Fig. 12** Intercalar apiculate oogonia with monoclinous antheridial branch. **Fig. 13–14** Oogonia with 1–several centric-subcentric oospores inside. Scale bars: Fig. 7, 14 = 50 μm ; Fig. 8–13 = 10 μm .





culturis juvenilibus, filiform vel naviculata, 250–650(–995) μm longa et 15–44 μm lata, sympodia vel basipeta. Ejuncto sporarum pro genus typica, spori globosi 10–12 μm . Gemmae frequentis. Oogonia copiosa, sphaerica, pyriform, doliiform, rarissimo apiculata, (30–)48–80(–110) μm diam ramulus lateralibus, intercalariibus vel terminalibus provenientia, 12–114 μm diam. Paries oogoni sine projectionibus, oospori 1–6(–22) per oogonium, centrici (80%) ad subcentrici (20%), (16–)20–27(–29) μm diam. Ramulus antheridialis, ramosus, plerumque origine androgyna (60%) sed interdum monoclina (30%) et diclina (10%).

HOLOTYPE: In foliis et ramis dejectis non determinatis, Arroyo Las Cotorras, Departamento de Ushuaia, Provincia de Tierra del Fuego, Argentina; *M. Steciow*, 5 Dec 1997, LPS no. 45528; culture collection no. 619 (Spegazzini Institute).

Mycelium limited or extensive, denser near periphery of the colony; 2-week-old hemp seed colony, 1–3 cm diam., principal hyphae branched, slender to stout, 24–106 μm at the base. Gemmae abundant in old colonies, filiform or irregular, terminal or intercalary, single or in chains, germinating by a slender hypha or functioning as zoosporangia. Zoosporangia moderately abundant in young colonies, filiform or naviculate, 250–650 (–995) $\mu\text{m} \times$ 15–44 μm (taken at the widest point), proliferating sympodially, cymosely or in basipetalous succession. Zoospore discharge achlyoid (from primary and secondary zoosporangia); spore cluster persistent or not at exit pore, remaining as an irregular clump at the tip of the sporangium. Encysted spores globose, 10–12 μm diam. Oogonia very abundant, lateral, occasionally terminal or intercalary, spherical, pyriform or doliiform, sometimes apiculate, (30–)48–80(–110) μm

diam. Oogonial wall smooth, without projections, yellowish at maturity, pitted only at the point of attachment of antheridial cells; inner surface occasionally irregular. Oogonial stalks usually stout, straight, rarely bent, 12–114 μm diam. ($\frac{1}{2}$ –2 times the diameter of the oogonium in length). Antheridial branches always present. Antheridial cells simple, apically appressed. Fertilisation tubes not observed. Oospores centric (80%) or subcentric (20%), always maturing, not filling the oogonium, sphaerical, or ellipsoid; 1–6(–22) in number; (16–)20–27(–28) μm . Antheridial branches 60% androgynous, 30% monoclinal; 10% diclinal, frequently branched.

ETYMOLOGY: From the Latin, *ambi* meaning both, *sporum* meaning spore; referring to the dimorphic nature of the oospores (sexual resting spores).

SPECIMEN EXAMINED: ARGENTINA, Tierra del Fuego Province, Ushuaia Department, Las Cotorras stream, on unidentified dead leaves and branches; *M. M. Steciow*, 5 Dec 1997, LPS 45528, culture collection no. 619.

MORPHOLOGY: It is important to note that the types of oospores and antheridial branches and the measurements of oogonia and oospores are very constant features of this species. There was little variation in type and size of zoosporangia in different temperature and water conditions; they are filiform and reached a mean range of length of 350–600 μm . The shape of oogonia remained constant, mainly spherical, rarely pyriform, sometimes apiculate.

This species did not grow at 31°C, which is related to the original habitat conditions where this species was found (5°C). The initial growth rate was faster at 25°C (in sterilised pond water and distilled water), but after 10 days there was little difference in mean diameter of colonies from the other two

Table 1 Ranges of morphological measurements of *Achyla ambispora* grown at 3 temperatures for 10 days.

Temp. °C	Colony diam. (cm)	Oogonia diam. (μm)	Oospores diam. (μm)	Oospores per oogonium
5	3	38–51	18–23	(1) 2–3 (7)
10	1.5–3	(38) 49–52	20–26	(1) 2–5
25	2.3	46–51 (59)	23–26	(1) 2–4 (5)

Fig. 15–22 *Achyla ambispora*. **Fig. 15–17** Detail of filiform zoosporangia with characteristic discharge achlyoid; spore cluster persistent, remaining as an irregular clump. **Fig. 18** Oogonium with centric oospores. **Fig. 19–22** Details of oogonia with subcentric oospores. Scale bars: Fig. 15 = 100 μm ; Fig. 16–19 = 50 μm ; Fig. 20–22 = 10 μm .

temperature regimes (Table 1). Cultures incubated at 25°C showed little further linear growth, while cultures at lower temperatures continued to grow to form larger colonies. At 5°C the colonies develop slender secondary hyphae, giving the colonies a denser aspect than they reached at higher temperatures (10 and 25°C).

Oogonial production was unaffected by the different temperatures; all developed oogonia, but mature oospores were less abundant at lower temperatures (principally at 5 and 10°C after 10–15 days) and the number of oogonia was lower at these temperatures. However, the number of zoosporangia at 5 and 10°C was higher than in cultures kept at 25°C.

At these temperatures and water types, production of gemmae was more abundant in older cultures. At 10–15 days, they were very scanty.

DISCUSSION

Achlya ambispora has close affinities to *A. racemosa* (Coker 1923; Johnson 1956). Both species have predominantly androgynous antheridial branches and some declinous, a smooth oogonial wall, and the oospores are yellowish at maturity.

However, *A. ambispora* also has characteristic monoclinal branches and not exigenous ones, as develops in *A. racemosa*. In the Argentine species, the oogonia are sometimes apiculate and the oospores are centric-subcentric and 1–6(22) per oogonium, whereas in *A. racemosa* the oogonia are never apiculate and the oospores are only centric, 1–10 per oogonium (Johnson 1956, 1974).

This new species also differs from *A. apiculata*, which has mainly monoclinal antheridial branches and occasionally androgynous and declinous ones, and the oospores are all subcentric within oogonia often apiculate, occasionally spherical or pyriform (rarely oval or irregular); the oospores are larger, (20–)35–40(–48) µm, and the oogonia stalks are bent, curved, or once-coiled (rarely straight as in *A. ambispora* (Johnson 1956).

A. ambispora appears to resemble *A. colorata*, but the latter has papillate oogonia with larger oospores and frequently immature oospheres, and androgynous, exigenous, and declinous antheridial branches (Johnson 1956, 1973, 1974).

According to Johnson (1956) and Seymour (1970), the sexual features are important to characterise a particular species of Saprolegniaceae. Oospores of similar structure have been found in most species of *Achlya* (Johnson 1956) except

A. oblongata and *A. treleaseana*. However, it is very common to see two types of oospores in several *Saprolegnia* species; *S. furcata*, *S. ferax*, *S. declina*, and *S. uliginosa* have centric oospores, rarely subcentric ones, and *S. terrestris* has subcentric oospores, rarely centric ones (Seymour 1970).

This species did not show variability in types of antheridial branches, types of oospores, size and shape of oogonia, and number of oospores per oogonium, nor in frequency of these features at different conditions.

These features provide strong support for erecting the new species, and particularly the development of two types of oospores at maturity.

Oospores were observed leaving some hyphae from an old culture in a new water Petri dish (at 20°C) and germinating 4–5 days later. Both centric and subcentric oospores germinated within the oogonia, which had a broken wall. Of a total of 50 oospores observed, only 20 had germinated, previously fertilised, after 5 days.

Following the classification of Johnson (1956), *A. ambispora* would belong to the subgenus *Centroachlya*.

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