RESEARCH LETTER

Achlya spiralis, a new aquatic oomycete with bent oogonial stalks, isolated from the Burgundian region of France

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Abstract

Achlya spiralis sp. nov. was isolated from water samples collected in the river Tille in the Burgundian region of France. The new oomycete is described, illustrated and compared with related species of the genus *Achlya*. It is characterized by the presence of smooth-walled oogonia that are usually borne on bent or twisted oogonial stalks; mainly monoclinous, androgynous and diclinous antheridial branches and eccentric oospores which generally do not mature or mature after a long period of time. The internal transcribed spacer (ITS) region of its rRNA is comprised of 671 bases. The taxonomic description of this new species, its comparison with related oomycetes and the sequence of the ITS region of its rRNA are discussed here.

Introduction

The biota of aquatic habitats includes water molds that can be isolated easily from submerged and floating organic matter in well-aerated waters. Most of these 'water molds' belong to *Oomycota* that were historically classified as fungi (Barr, 1992), but ultrastructural, biochemical and molecular sequence analyses strongly indicate that they are closer to algae than to fungi and hence have been classified within 'Stramenopiles' (Patterson, 1989). Stramenopiles is one of the eukaryotic Kingdoms, which includes water molds and brown algae. The position of the oomycetes as a unique lineage of stramenopile eukaryotes, unrelated to true fungi but closely related to heterokont (brown) algae, has been well established using molecular phylogenies that are based on rRNA sequences (Kumar & Rzhetsky, 1996). Surprisingly, mycologists of the mid-1800s had already classified these organisms within the algal groups and oomycetes like *Saprolegnia*, *Achlya* and *Pythium* were given a status equal to that of the algal family of *Oedogoniaceae*. Hence, we are back to the 'blemished early history' of *Achlya* in that a member of this genus was described as *Vaucheria aquatica* (Johnson *et al.*, 2002). The term 'alga' was frequently used to describe the oomycetes in general. Undoubtedly, the advent of molecular tools has come a long way to prove that these organisms have evolved from algae by losing their chloroplasts and thus becoming 'saprophytes' or 'parasites'.

The taxonomy of the water molds is known to be difficult, and the study of oomycetes in general has not received much attention in France. The much-studied genus is *Plasmopara viticola* because it is the causal agent of the 'downy mildew' of the grapevine. This is easily understandable because France is one of the biggest wine-producing countries. The first author, ever since his arrival in France in 1990, has written a series of papers on the genus *Pythium* and, during a survey of zoosporic organisms in the Burgundian region, isolated some members of *Saprolegniaceae*. A new species *Saprolegnia multispora* was described from the river Tille (Paul & Steciow, 2004). A previously uncharacterized water mold occurring in the same flowing freshwater river in the Burgundian region of France is being described here as *Achlya spiralis* sp. nov.

Materials and methods

Oomycetes isolation

Achlya spiralis (type strain F-1409) was isolated four times (F-1409, F-1409.1, F-1409.2 and F-1409.3) at different locations from the river Tille flowing through the village of

Saint Julien (latitude: 47.398349° N, longitude: 5.135851° E) near the city of Dijon. Omycete isolation was performed by baiting techniques described by Johnson (1956), Sparrow (1960) and Paul & Steciow (2004). Samples of brown decaying twigs and leaves from the river were placed in sterile capped bottles and brought to the laboratory. The samples were placed aseptically in sterile Petri dishes containing several boiled hemp seed halves (*Cannabis sativa*) floating on the surface of sterile water. This was then incubated at room temperature (15–20 °C). After 3 days, the hemp seed baits were examined for their colonization by oomycetes. Some hyphal filaments were detached from the baits with the help of sterile needles and transferred to another sterile Petri dish having sterile water and boiled hemp seed halves. Repeated cultures enabled us to obtain a bacteria-free colony on the hemp seeds. The oomycete mycelium was also inoculated on solid media like potato carrot agar (PCA), potato dextrose agar (PDA) and corn meal agar (CMA). Colonies in water and on solid media were examined microscopically, and all the measurements of the asexual and asexual structures were taken on a regular basis.

DNA extraction

The oomycetes were grown in potato dextrose broth. DNA was extracted from the oomycete mycelium using the method described earlier (Paul *et al.*, 2006, 2008) in which DNA was purified from mycelia with the use of the DNA-Easy Plant Mini kit (Qiagen, Basel, Switzerland), according to the manufacturer's specifications. Quality was checked by visualization under UV light following electrophoretic separation with a molecular mass standard (HindIII/EcoRI DNA Marker, Biofinex, Praroman, Switzerland) in 1% agarose (Biofinex) gel in 1 × TBE, subjected to 100 V for 1 h and stained with ethidium bromide (0.5 mg mL^{-1}). Concentrations were assayed in an S2100 Diode Array spectrophotometer (WPA Biowave, Cambridge, UK).

Internal transcribed spacer (ITS) amplifications of the oomycete were carried out using previously described universal primers ITS4 and ITS6 that target conserved regions in the 18S and 28S rRNA genes (White *et al.*, 1990; Cooke *et al.*, 2000). Amplifications were carried out in a Master Gradient thermocycler (Eppendorf, Hamburg, Germany) according to the following amplification program: an initial denaturation step of 95 °C for 2 min, followed by 30 cycles including denaturation for 20 s at 95 °C, annealing for 25 s at 55 °C and extension for 50 s at 72 °C. Amplification was terminated by a final extension step of 10 min at 72 °C (Cooke *et al.*, 2000). DNA sequencing was performed by Genome Express (Paris), and the DNA sequence has been deposited in Genbank.

Results and discussion

Taxonomy

Achlya spiralis Paul, B. & Steciow, M. sp. nov. (Figs 1–6).

Mycelium densum, cultura in seminibus Cannabis sativae L. circa 1,4–3 cm diam. Hyphae ramosa, pleraque 19–72 (−121) μm late ad basim. Sporangia copiosa in culturis juvenilibus filiformia, fusiformia vel clavata, 145–750 (−970) μm longa et (19-) 29–50 μm lata, sympodia, basipetala vel cymosa. Ejecto sporarum pro genus typica, spori globosi 6–12 (−14) μm. Gemmae frequentis. Oogonia copiosa,

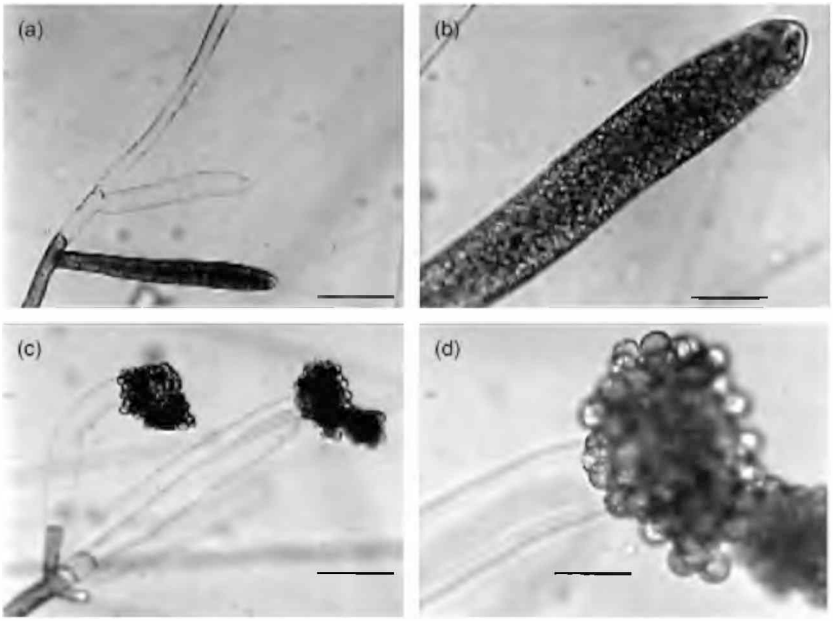

Fig. 1. *Achlya spiralis*. (a) Basipetalous zoosporangial renewal. (b) Filiform zoosporangium. (c) Cymose zoosporangial renewal. (d) Achlyoid zoospore discharge. (a, c) Scale bar = 50 μm; (b, d) scale bar = 20 μm.

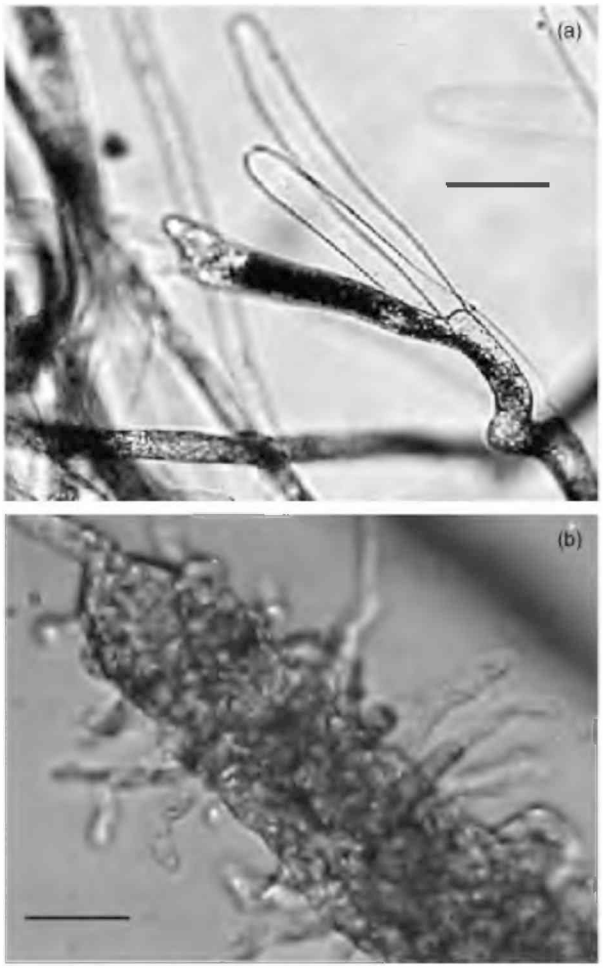

Fig. 2. (a) Sympodial zoosporangial renewal. (b) Aplanoid zoospore discharge. (a) Scale bar = 25 μm; (b) scale bar = 15 μm.

sphaerica, subglobosa, pyriformia vel doliiformia, (25-) 43–78 (−102) μm diam. Paries oogoni laevis; ramulus lateralibus vel terminalibus provenientia, 48–300 μm. Oospori (1) 2–8 (−18) per oogonium, eccentrici, (24-) 30–36 (−41) μm diam. Ramulus antheridialis, ramosus, plerumque monoclina, sed interdum androgina et diclina.

Morphological characteristics

Mycelium of *A. spiralis* is moderately extensive, denser near the substratum and a 2-week-old hemp seed colony measures up to 1.4–3 cm in diameter. Principal hyphae are branched, slender to stout, 19–72 (exceptionally up to 121) μm at the base and are profusely branched into secondary hyphae near the tips. Gemmae are sparsely produced, and are cylindrical, fusiform, pyriform, simple or catenulate. Zoosporangia are moderately abundant in young colonies, and are filiform, fusiform, to clavate, straight and tapering towards the end and frequently furnished with one to several lateral discharge pores or tubes in addition to the terminal orifice, straight or curved at the tips (Figs 1a–d and 2a). The zoosporangia measure 145–750 (exceptionally up to 970) μm × (19-) 29–50 μm and are renewed sympodially (Fig. 2a), or at times in basipetalous (Fig. 1a) or cymose succession (Fig. 1c). Zoospore discharge is mostly achlyoid (Fig. 1c and d), sometimes aplanoid (Fig. 2b) and the spore clusters are not persistent at the exit pore. Encysted spores are globose, 6–12 (rarely up to 14) μm in diameter. Oogonia are formed abundantly and are lateral, occasionally terminal or intercalary, spherical, subglobose, pyriform, oval, irregular, or dolioform, rarely proliferating. These usually measure between 25 and 78 (exceptionally up to 102) μm in diameter. Oogonial walls are slender, smooth,

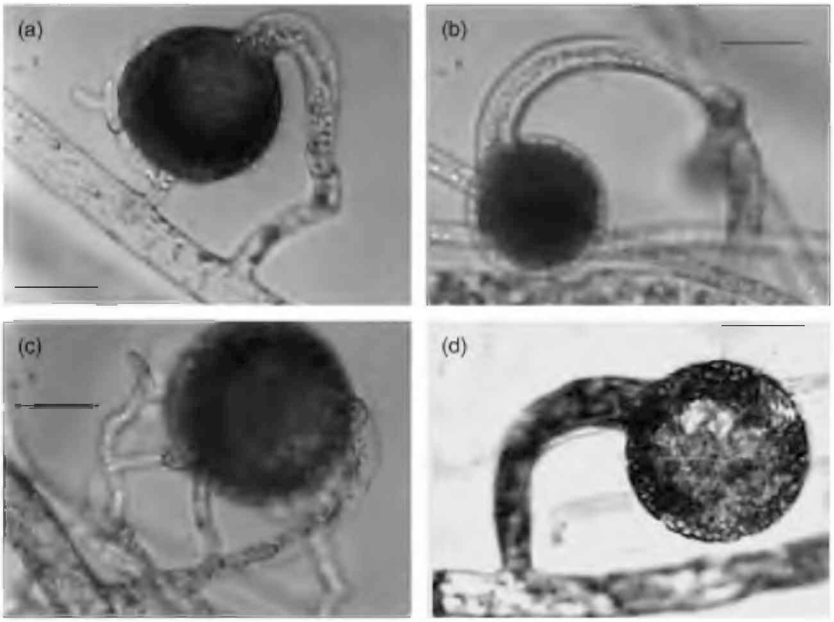

Fig. 3. (a–d) Smooth-walled oogonia on curved oogonial stalks with monoclinous, androgynous and diclinous antheridial branches. (a–d) Scale bar = 20 μm.

pitted or pitted only under the attachment point of the antheridial cell. The oogonia are generally borne on bent, curved (Figs 3a–d and 4) or even coiled stalks (Fig. 5c). Sometimes the oogonial stalks are straight. The length of the stalks can vary from 48 to 300 μm or longer when coiled. Antheridia always present. Antheridial branches are slender, principally monoclinous, sometimes androgynous (Figs 3a, b and 4) or diclinous (Fig. 5d), frequently branched, at times twisted or coiled. Some nonfunctional 'antheridial' branches also emerge towards the oogonia (Fig. 4). Antheridial cells are simple and laterally appressed (Fig. 5a). Fertilization tubes arise as peg-like projections from long laterally applied antheridial cells. Oospheres are frequently aborting or maturing after a long period of incubation. Oospores are eccentric (Fig. 6a–d), filling or not filling the oogonium, spherical (Fig. 4a, b and d), ellipsoid or irregular (Fig. 4c), usually 2–8 in number (exceptionally 1 and up to 18). The oospores usually measure between 19 and 30 (exceptionally 12–36) μm in diameter.

Fig. 4. Aspect of mycelium with characteristic oogonia on straight, bent or curved oogonial stalks and monoclinous, androgynous or diclinous antheridial branches. Scale bar = 50 μm.

Molecular characteristics

The ITS region of *A. spiralis* is composed of 671 bases (Genbank accession no. AY676020). The ITS1, 5.8S and ITS2 are 175, 159 and 337 bp in length, respectively. The sequence of the ITS and the flanking regions (18S gene partial sequence, ITS1 complete sequence, 5.8S gene complete sequence, ITS2 complete sequence and 28S gene partial sequence) are given in supplementary Table S1.

HOLOTYPE. F-1409 (type culture) isolated from the river Tille is maintained at the culture collection of the corresponding author, the 'Université de Bourgogne' in Dijon, France. This is also being deposited at CBS culture collection in Baarn, Holland. *Etymology.* The name *A. spiralis* refers to the characteristic bent or spirally coiled disposition of the oogonial stalks.

Achlya spiralis is a spectacular oomycete with bent and sometimes twisted oogonial stalks. It has affinities to *Achlya papillosa* Humphrey, previously named as *Achlya spiracaulis* Johnson and now placed in synonymy under the name *Newbya recurva* (Cornu) M.W. Dick & Mark A. Spencer (Index Fungorum, 2004). Both species have curved, recurved, loosely or tightly coiled oogonial stalks. However, *A. papillosa* differs from this new species in having centric oospores that mature and are usually 1–44 (usually 4–12) in number (Johnson *et al.*, 2002). There are fewer oospores per

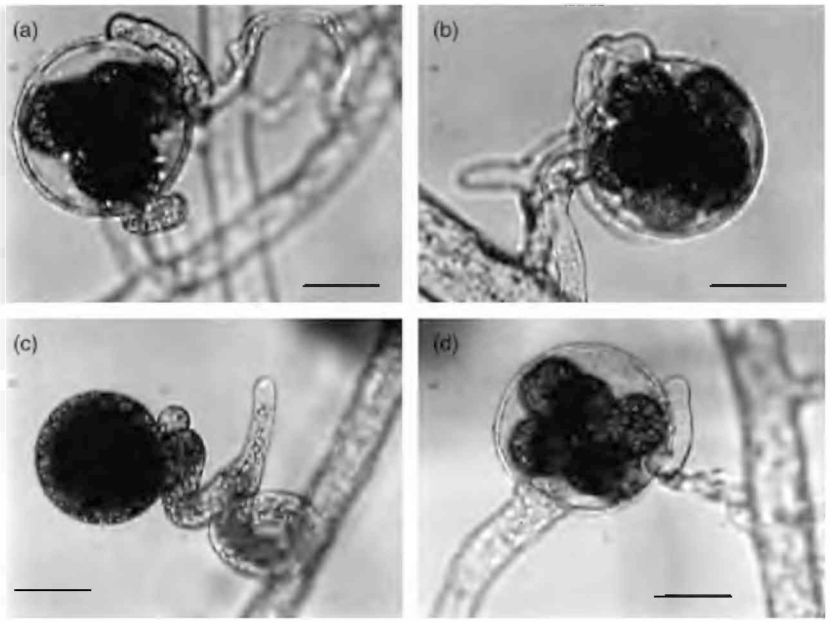

Fig. 5. (a, b) Smooth oogonia with monoclinous antheridial branches. (c) Oogonium on a typical coiled oogonial stalk. (d) Diclinous antheridial branch on pitted oogonium. (a–d) Scale bar = 15 μm.

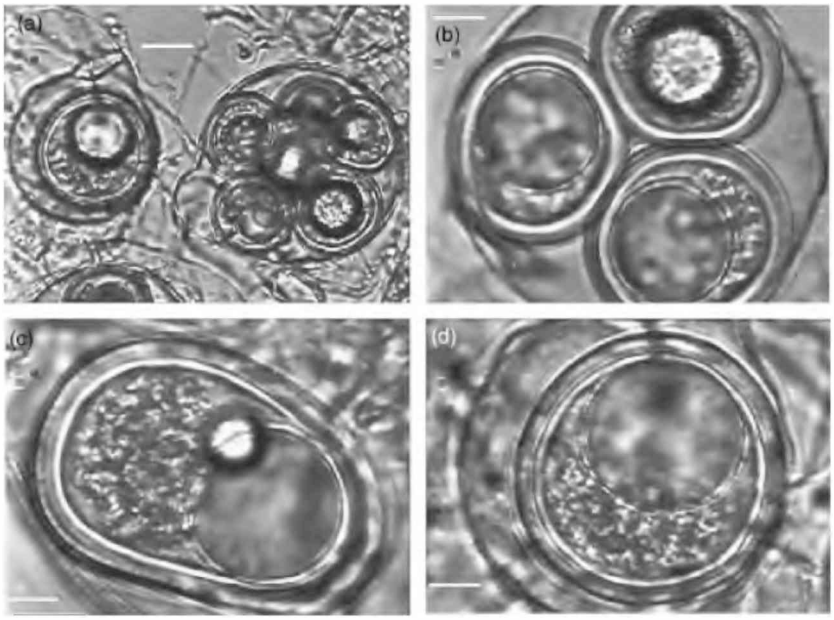

Fig. 6. (a, b) Oogonia containing one to several eccentric oospores. (c) Oval oospore. (d) Spherical oospore. (a) Scale bar = 10 μm; (b–d) scale bar = 5 μm.

oogonium in *A. spiralis* (usually 2–8), which often abort or do not reach maturity, and are eccentric. Sporangial characters are also different in these two species as the zoosporangia of *A. spiralis* are frequently furnished with one to several lateral discharge pores or tubes in addition to the terminal orifice, similar to the ones seen in the case of *Achlya caroliniana* Coker and *Achlya klebsiana* Pieters (now considered to be a synonym of *Achlya debaryana* Humphrey by Johnson *et al.*, 2002). This feature is absent in *A. papillosa*. *Achlya curvicollis* Beroqui and *Achlya brasiliensis* A.I. Milanez (Dick, 2001; Index Fungorum, 2004) are considered to be synonymous of *Achlya apiculata* by Johnson *et al.* (2002), and were then renamed by Spencer *et al.* (2002) as *Newbya curvicollis* (Beroqui) Mark A. Spencer and *Newbya brasiliensis* (A.I. Milanez) Mark A. Spencer; both these species have coiled, spiral or curved oogonial stalk and often have apiculate oogonia, but they possess subcentric or centric oospores. *Achlya spiralis* does not possess any apiculate oogonia.

Achlya spiralis also resembles *Achlya orion* Coker & Couch, which has oogonia borne in bent or strongly recurved, or twisted sharply or loosely coiled oogonial stalks. However, the oospores in this species are larger when compared with those of *A. spiralis* and the length of the oogonial stalks can be up to 12 times the diameter of the oogonia. This feature is completely lacking in our isolate. *Achlya spiralis* also differs from *Achlya robusta* Steciow & Elíades, which has mainly smooth or papillate, tuberculate or bullate oogonia; the oogonial stalks are slender, frequently short, straight, bent, rarely curved, but never coiled, and the antheridial branches are mainly monoclinous, occasionally diclinous, rarely androgynous (Steciow & Elíades, 2002).

The morphological characters of *A. spiralis* and related species are summarized in supplementary Table S2.

A BLAST query with the 671 bases of the ITS sequence of *A. spiralis* gives close affinities with *Achlya glomerata* (Genbank AF218149), *Achlya aquatica* (Genbank AF218150), *Achlya flagellata* (Genbank AF218143), *Achlya heterosexualis* (Genbank AF18165), *Achlya* sp. Strain S3 (Genbank AY666086) and *Achlya conspicua* (Genbank AF218144). However, all these species are morphologically different, and molecular evidence is not contradictory to this. The ALIGN matches with ITS sequences of all these species with those of *A. spiralis* show that the ITS size and the sequence homologies are quite different. The closest relative is *A. glomerata* with 99.5% similarity with *A. spiralis*. However, this is an entirely different species having ornamented oogonia, and the ITS region of its rRNA is smaller (668 bases). Other closely related species are *A. aquatica* (98.4% similarity, ITS size 664 bases), *A. flagellata* (97.5% similarity, ITS size 668 bases) *A. heterosexualis* (97% similarity, ITS size 670 bases) and *A. conspicua* (97% similarity, ITS size 668 bases). None of these have the ITS region composed of 671 bases and none of these have the characteristic combination of bent oogonial stalks and eccentric oospores. The very close similarities in the sequences of the ITS of many species of *Achlya* among themselves and with *A. spiralis* are not very surprising for the group of oomycetes. It has been reported that species can differ with as low as one base difference (Timothy *et al.*, 2003; Paul & Bala, 2008). The differences between the sequences and also the size of the ITS region,

coupled with unique morphological characteristics, justify the creation of this new taxon.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Sequence of the ITS and flanking regions of the rRNA of *A. spiralis*

Table S2. Comparison of morphological characteristics of *A. spiralis* and related species

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1574-6968.2008.01183.x (This link will take you to the article abstract).

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