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SHORT COMMUNICATION

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Saprolegnia semihypogyna sp. nov., a saprolegniaceous oomycete isolated from soil in Japan

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Abstract *Saprolegnia semihypogyna*, a saprolegniaceous oomycete (Saprolegniales, Oomycetes), is described based on two strains isolated from soil samples collected in Japan. It is characterized by having a new combination of sexual characters in the genus *Saprolegnia*, namely, producing mostly monosporous oogonia with subeccentric oospores and semihypogynous or androgynous antheridial branches. The species is morphologically similar to *Scoliolegnia subeccentrica*, although the oogonial wall of the former is smooth and that of the latter is densely ornamented. Morphological comparison of the two species indicates that the generic status of the genus *Scoliolegnia* is questionable.

Key words New species · Oomycetes · Peronosporomycetes · Saprolegniales · Taxonomy

In the traditional classification of the family Saprolegniaceae (Saprolegniales, Oomycetes), the generic definition was mainly based on the asexual characters, especially the method of zoospore discharge (de Bary 1888; Coker 1923; Coker and Matthews 1937; Sparrow 1960), whereas the species within a genus were mostly delimited by the characters of sexual organs (Johnson 1956; Scott 1961; Seymour 1970). Although this principle has been followed in most cases, the genus *Scoliolegnia* M.W. Dick is exceptional in that it was erected based on the combination of characters of vegetative hyphae and asexual and sexual reproduction by Dick (1969a). However, the asexual character of the genus is not clearly distinguishable from that of the genus *Saprolegnia* Nees, and it is considered that the genera are closely related (Powell and Blackwell 1998; Riethmüller et al. 1999).

During a floristic study of water molds in Japan, we obtained an undescribed species of the genus *Saprolegnia* that shared some taxonomically important characters with *Scoliolegnia* (*Sc.*) *subeccentrica* M.W. Dick. In this article, we describe and illustrate the new species, and discuss the validity of the genus *Scoliolegnia* as an independent genus by comparing its major characteristics with those of *Sc. subeccentrica*.

The oomycete was isolated from a soil sample by the baiting technique described by Seymour (1970). Autoclaved hemp seed halves (Seymour and Fuller 1987) were used as bait. To obtain a single species culture, a hyphal tuft was taken from the gross culture, washed several times with sterilized tap water, and plated onto cornmeal agar (CMA; Nissui, Tokyo, Japan). To prepare a zoospore suspension, a hemp seed was place on the edge of the colony, incubated for 36 h at 20°C, and the infected hemp seed was transferred to a petri dish with sterile distilled water. A single-spore culture was established by capturing an encysted zoospore from the zoospore suspension with a micropipette. The spore was inoculated on the surface of a CMA plate and incubated at 20°C. An agar block (5 × 5 mm) was cut from the edge of the growing colony and inoculated into a new plate. By repeating this procedure, a bacteria-free culture was established. The following species description was based on this bacteria-free culture grown on hemp seeds in sterilized distilled water according to the method of Seymour (1970), except for the incubation temperature, 15°C instead of 18°C.

Saprolegnia semihypogyna S. Inaba et Tokumasu, sp. nov. Fig. 1–6

Hyphae vegetativae delicatae, modice ramosae, 16–60 μm diam. Gemmae, interdum praesentes, sphaericae, pyrifomes vel irregulares, laterales vel terminales. Zoosporangia abundantia, elongata, cylindrica vel clavata, 125–660 × 34–70 μm. Zoosporangia secundaria ad zoosporangia prima intus proliferantia vel subter vel e latere sympodialiter formata. Zoosporae primariae saprolegnoides dimissae, pyriformes, biflagellatae, incystatae; cystosporae 12–14 μm diam. Zoosporae secundariae e zoospora incystata emergentes, reniformes. Oogonia abundantia, lateralia vel terminalia, raro intercalaria, sphaerica vel

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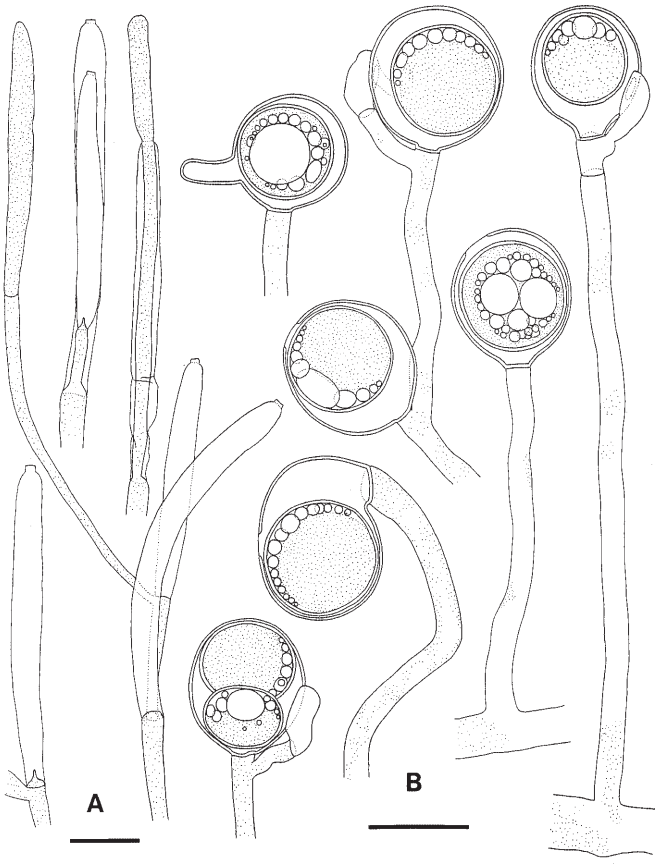


Fig. 1. Morphological characteristics of *Saprolegnia semihypogyna*. **A** Zoosporangia. **B** Oogonia with or without antheridium: the oospores are subeccentric. *Bars* **A** 100 μm; **B** 25 μm

pyriformia, (25–)30–45(–55) μm diam. Paries oogonii foveatus vel non foveatus, laevis vel sparsim papillatus. Stipites oogonii longi, recti vel curvati, interdum ramosi. Oosporae subeccentricae, sphaericae, (18–)25–33(–43) μm diam, in oogoniis singulares vel raro duae. Rami antheridii semihypogyni vel infra basim oogonii exorientes. Cellulae antheridiorum simplices, clavatae, ad apicem vel ad latus oogonio adhaerentes. Tubi fecundationis adsunt.

Holotypus: TNS-F-3688.

Hyphae slender, delicate, moderately branched, 16–60 μm in diameter at base. Gemmae, when present, spherical, pyriform, or irregular, terminal or lateral. Zoosporangia moderately abundant, elongate, cylindrical, clavate, occasionally pyriform in small ones, occasionally curved, terminal or lateral, 125–660 × 34–70 μm, renewed internally or sympodially. Primary zoospore discharge saprolegnoid, pyriform, biflagellate, encysted zoospores 12–14 μm in diameter. Secondary zoospore emerged from encysted primary zoospore, reniform. Oogonia abundant, lateral, terminal, rarely intercalary, occasionally clustered on a hypha, spherical, pyriform or obovate, doliform when intercalary, (25–)30–45(–55) μm in diameter. Oogonial wall pitted, unpitted, or pitted only under point of attachment of antheridial cells, smooth, occasionally with one or a few papillate projections. Oogonial stalks long, straight or curved, occasionally branched, then bearing oogonia cymosely. Oospheres usually maturing. Oospores subeccentric, spherical or subspherical, one, rarely two in number, (18–)25–33(–43) μm in diameter, oospore germination not observed. Antheridial branches semihypogynous, androgynous; when androgynous, usually arising from just beneath the basal walls of the oogonia, lacking on some oogonia. Antheridial cells simple, laterally or apically appressed, fertilization tubes observed.

Holotype: TNS-F-3688. Microscope slide of hempseed culture isolated from soil of grassland in Shirahama, Shimoda-shi, Shizuoka Prefecture, Japan, collected by Y. Kurihara, isolated by S. Inaba, 3 April 1999, preserved in herbarium of National Science Museum, Tokyo. Living cultures are deposited in the Centraalbureau voor Schimmelcultures (CBS 109568) in the Netherlands, Institute for Fermentation (IFO 33252) in Japan.

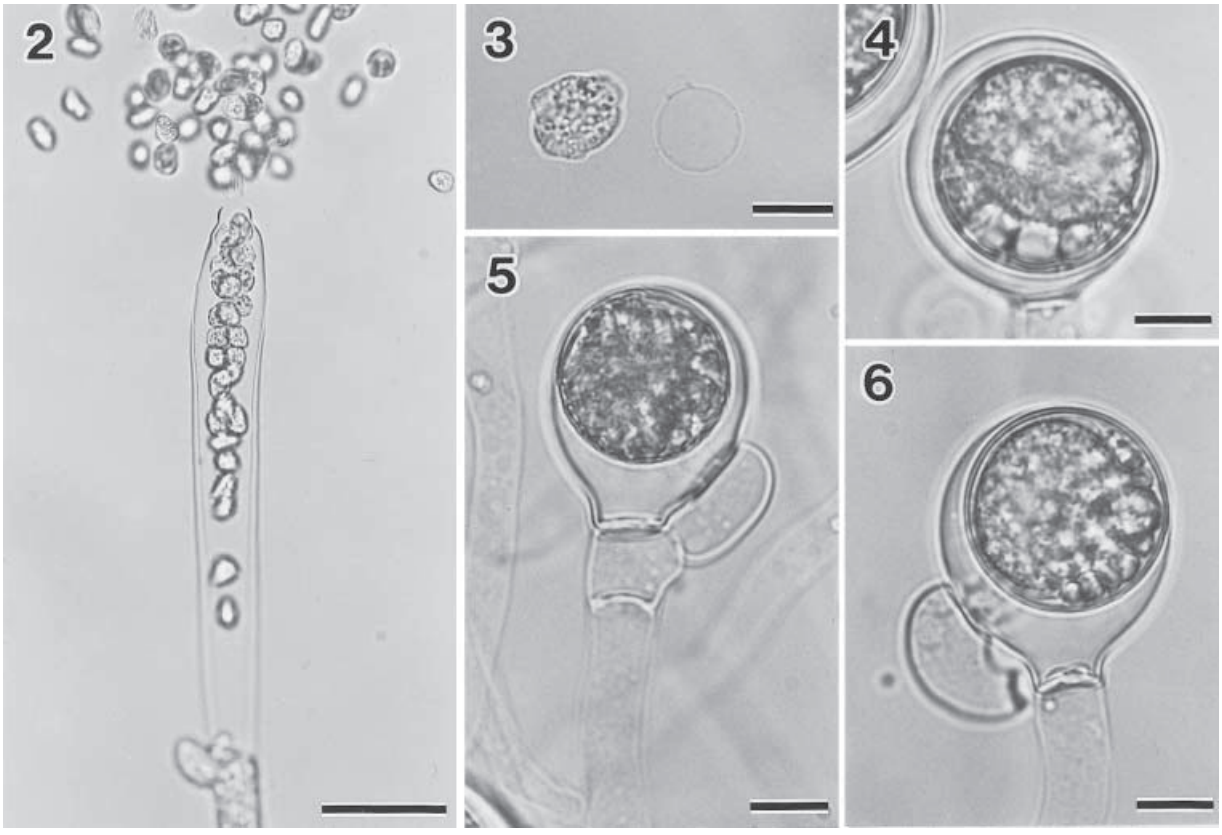
Other isolate examined: IA1379, Seni River, Suzaka-shi, Nagano Prefecture, from wet soil of bank, collected and isolated by S. Inaba, 3 May 1999.

Etymology: from the Latin *semi* = half, *hypogyna* = situated below base of oogonia; referring to the semihypogynous type antheridium.

Notes. The primary zoospores of the new species emerged directly through the exit pore of the sporangium (saprolegnoid; Fig. 2), and the encysted primary zoospores released the secondary zoospores (dimorphic; Fig. 3). In Saprolegniaceae, three genera have the saprolegnoid zoospore discharge and the dimorphic zoospore: *Isoachlya* Kauffman, *Saprolegnia*, and *Scoliolegnia* (Dick 2001). The genus *Isoachlya* differs in renewing zoosporangia by cymose branching (Kauffman 1921), whereas in the other two genera zoosporangia are mainly renewed by internal proliferation. The new species is not a member of *Isoachlya* because the zoosporangia are renewed internally or sympodially. The new species produced oogonia with smooth and pitted walls, unlike those of *Scoliolegnia*, which have more or less numerous papillae. From this evidence, we concluded that the new species is a member of the genus *Saprolegnia*.

In the genus *Saprolegnia*, there is no reported species having the combination of subeccentric oospores and semihypogynous antheridia. Indeed, there is no reported species with semihypogynous antheridia, like those typically observed in the *Pythiopsis cymosa* de Bary (Dick 1969b). On the other hand, subeccentric oospores are sometimes formed in the oogonia of both *Saprolegnia eccentrica* (Coker) R.L. Seym. (as *Isoachlya eccentrica* Coker) and *Saprolegnia unispora* (Coker & Couch) R.L. Seym. (Dick 1960, 1990), although Seymour (1970) indicated that the typical oospores are eccentric in the former species and subcentric or centric in the latter. Both species, however, can be easily distinguished from the present species by the lack of antheridial branches. Thus, we concluded that the present species is a new species of *Saprolegnia*.

In gross morphology, the present species is similar to *Scoliolegnia subeccentrica* in the size of oogonia and oospores, the oospore number per oogonium, and the type of mature oospore (Table 1). The only major difference



Figs. 2–6. *Saprolegnia semihypogyna*. **2** Emergence of primary zoospores from zoosporangium. **3** Secondary zoospore (*left*) and empty primary cyst (*right*). **4** Oogonium including single subeccentric oospore. **5** Oogonium with semihypogynous antheridium. **6** Oogonium with androgynous antheridial branch. *Bars* **2** 50 μm; **3–6** 10 μm

Table 1. Comparison of morphological characters of *Saprolegnia semihypogyna* and *Scoliolegnia subeccentrica*

Character	*Saprolegnia semihypogyna*	*Scoliolegnia subeccentrica*[a]
Behavior of zoospores	Dimorphic	Dimorphic
Zoosporangial renewal	Internal or sympodial	Basipetal or internal or lateral
Diameter of oogonia	(25–)30–45(–55) μm	(30–)39–57(–76) μm
Ornamentation of oogonial wall	Smooth, sometime with a few papillae	With numerous papillae
Pitting	Pitted	Unpitted
Oospore number per oogonium	One, rarely two	Nearly always one
Type of droplet distribution in oospore	Subeccentric	Subeccentric
Diameter of oospores	(18–)25–33(–43) μm	(19–)28–37(–49) μm
Type of antheridium	Androgynous or semihypogynous, sometimes absent	More often absent, androgynous or semihypogynous

[a] Modified from Dick (1969a)

between the two species is in the ornamentation of the oogonial wall, namely, smooth and pitted in the former and papillate and unpitted in the latter (see Table 1). On the other hand, the subeccentric type oospore is commonly formed in *Scoliolegnia blelhamensis* M.W. Dick and *Sc. subeccentrica*, but only occasionally in a few *Saprolegnia* species. These facts suggest that *S. semihypogyna* is an intermediate form between the two genera.

In other genera of the family Saprolegniaceae, the ornamentation of oogonial wall is usually regarded as a species-defining character. For example, *Achlya colorata* Pringsh. is primarily distinguished from *Achlya racemosa* Hildebr. on the basis of its dense papillate oogonial walls, although those species closely resemble each other in the other characters (Johnson 1956). According to this concept, it may be controversial to assign *S. semihypogyna* and *Sc. subeccentrica* to different genera because the asexual characters of both species are almost identical. Recently, some investigators have called into question the independence of the genus *Scoliolegnia*. Powell and Blackwell (1998) indicated its close relationship to *Saprolegnia* based on the phenetic analysis of genera of Saprolegniaceae. Similarly, Riethmüller et al. (1999) analyzed the phylogeny of the saprolegniaceous genera using 28S rDNA sequences and found that *Scoliolegnia asterophora* (de Bary) M.W. Dick (formerly *Saprolegnia asterophora* de Bary) distributed in a

cluster with species of the genus *Saprolegnia*. From this result, they suggested that it was of doubtful validity to separate the genus *Scoliolegnia* from the genus *Saprolegnia*. Dick (2001) also suggested that the generic status of *Scoliolegnia* is debatable. To bring this problem to a conclusion, the molecular data of the new species will be valuable as the species is intermediate in morphology between the two genera.

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