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Atkinsiella parasitica sp. nov. isolated from a rotifer, *Brachionus plicatilis*

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A new species of *Atkinsiella* (Lagenidiales, Oomycetes), *Atkinsiella parasitica* is described and illustrated. It was isolated from the eggs and bodies of a rotifer, *Brachionus plicatilis*, with fungal infection in 1992. The rotifer was reared in a hatchery as the first food supply for seed production of crustaceans and fishes. The fungus is characterized by producing monoplanetic, lateral biflagellate zoospores, and infrequently branched discharge tubes. Optimum temperature for the fungus was 25°C. The fungus was considered an obligate marine fungus, because its growth was observed only on PYGS medium including seawater.

Key Words——*Atkinsiella parasitica*; fungal infection; monoplanetic; Oomycetes; rotifer.

Introduction

Three species belonging to *Atkinsiella*, Lagenidiales, Oomycetes have hitherto been reported, and these were observed in aquatic animals and the environment. *Atkinsiella dubia* (Atkins) Vishniac was first isolated from the eggs and larvae of *Pinnotheres pisum* Pennant and *Gonoplax angulata* Pennant (Atkins, 1954), and later from eight different genera of green, red and brown algae including *Chordaria* sp. and *Cladophora* sp. (Fuller et al., 1964) and from the eggs of various crabs belonging to the genera *Paguristes, Hyas, Oregonia, Pugettia, Chorilia, Scyra, Chionoectes* and *Cancer* (Sparrow and Gotelli, 1969; Sparrow, 1973). *A. dubia* experimently infected the eggs of *Typton spongicola* de Costa, *Crangon vulgaris* Fabricius, *Macropodia* sp. and other species of crabs (Atkins, 1954). *A. entomophaga* Martin has been reported in the eggs of various midges in Virginia, including *Endochironomus nigricans* Johannsen, *Chironomus decorus* Johannsen (=*C. attenuatus*), *Glyptotendipes lobiferus* Say (Martin, 1977; 1981; 1984; 1991). *A. hamanaensis* Bian & Egusa is known as a parasite of the eggs and larvae of the mangrove crab, *Scylla serrata* Forsskål (Bian and Egusa, 1980). An unidentified species of *Atkinsiella* also has been isolated from zoea of the mud crab, *Scylla serrata* in Japan (Hamasaki and Hatai, 1993). This genus was characterized by a saccate lobed thallus, vacuolate stages at early zoosporogenesis and diplanetic biflagellate zoospores.

In 1992, a fungal infection occurred in the eggs and bodies of a rotifer, *Brachionus plicatilis* Müller, artificially reared in a hatchery at Chiba Prefectural Tokyo Bay Sea Farming Center, in Chiba Prefecture, Japan. The fungus could be classified as a member of the genus *Atkinsiella* (Nakamura et al., 1994). It was different from the three previously reported species of *Atkinsiella* in its morphological characteristics, such as monoplanetic biflagellate zoospores, infrequently branched discharge tubes, dimensions of discharge tubes, zoospores and encysted spores, and its mineral requirement.

In this paper, the fungus isolated from the rotifer is discribed and illustrated as a new species of the genus *Atkinsiella.*

Materials and Methods

Identification The fungus NJM 9231 was isolated from a rotifer, *Brachionus plicatilis* in 1992 (Fig. 1). The present isolate was incubated in PYGS broth (Nakamura et al., 1994) at 25°C for three days for morphological observation. To observe zoospore discharge, the mycelia were washed twice with sterilized artificial seawater (Aqua-Ocean®, Japan Pet Drugs, Tokyo), then placed in a Petri dish (8.25 cm diam) containing about 30 ml of sterilized artificial seawater and incubated at 25°C. For observation of zoospore liberation, a thallus undergoing zoospore formation was put onto a slide and covered with a coverslip. The morphology of zoospores was observed in a drop of zoospore suspension with a drop of formalin under a microscope. Encysted zoospores were put into PYGS broth and incubated at 25°C to observe germination. The fungus was identified according to Karling (1981), Atkins (1954), Sparrow (1973), Fuller et al. (1964), Martin (1977), and Bian and Egusa (1980).

Effect of temperature on growth The present isolate was inoculated into PYGS broth and incubated at 25°C for three days to produce mycelia. The mycelia were washed twice with about 30 ml of the sterilized artificial seawater in a Petri dish, transferred into another 30 ml of sterilized artificial seawater in a Petri dish and incubated at 25°C for 24 h to induce zoospores. The mycelia were removed by filtration with gauze to make a zoospore sus-

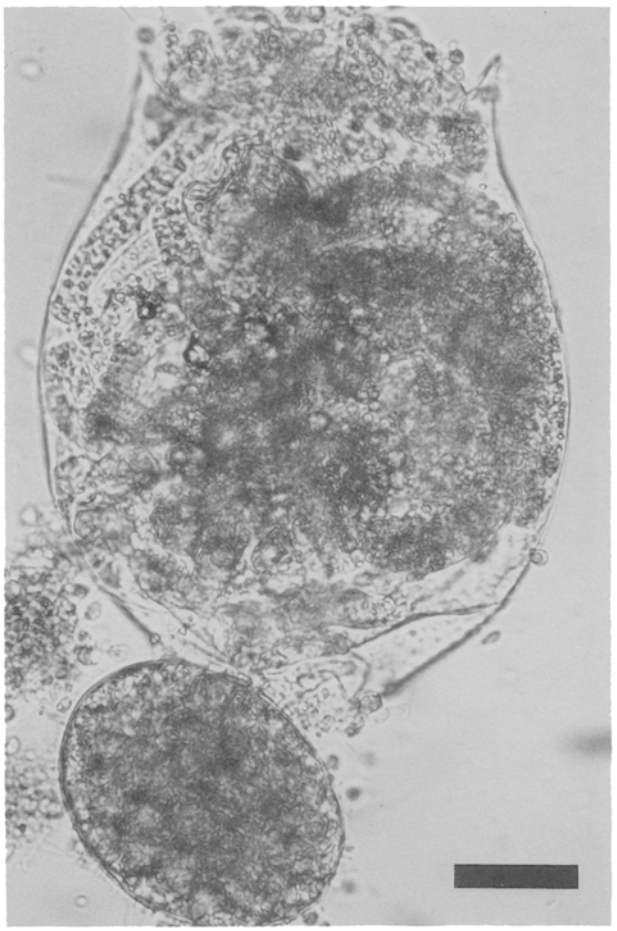

Fig. 1. A rotifer, *Brachionus plicatilis*, with fungal infection. (Scale: 50 μm.)

pension. A sterilized paper disc (8 mm diam) was put onto PYGS agar, then 0.05 ml of zoospore suspension was inoculated on the disc. Zoospore suspension was adjusted to 2.5×10^4 spores/ml. Each medium was prepared with 25 ml of PYGS agar. Five different temperatures (10, 15, 20, 25 and 30°C) were used to examine mycelial growth on the PYGS agar. The growth rate was determined at 3, 5, 7, 14, 21 and 28 days after inoculation by measuring the colony diameter at two points with vernier callipers.

Mineral requirement for growth To examine the mineral requirement of the fungus, PYG agar was prepared in the normal way but with distilled water replacing artificial seawater, and with three different concentrations of NaCl or KCl (0, 1.0, 2.5 and 5.0%). PYGS agar was prepared as a control medium. Portions of 30 ml of each medium were placed in Petri dishes, then inoculated with zoospore suspension at a level of 1.0×10^3 spores at the center. Colony appearence on each medium was checked after incubation for 30 days at 25°C. Zoospore suspension was prepared as described above.

Results

Taxonomy After cultivation on PYGS agar at 25°C for 20 days, the isolate NJM 9231 appeared as a yellowish to creamy moist colony with folds, heaped at the center, and attained a diameter of 2-3 cm (Fig. 2). The yellow coloration of the colony surface was decreased by subculture. The colony on the solid medium was easily removed with tweezers, because the fungus grew on the surface of the medium and did not penetrate deeply. In PYGS broth, vegetative hyphae appeared as spherical colonies of 0.7-3.0 mm diam after incubation for three days at 25°C. During zoosporogenesis, large vacuoles appeared in subthalli, and then a mass of protoplasm

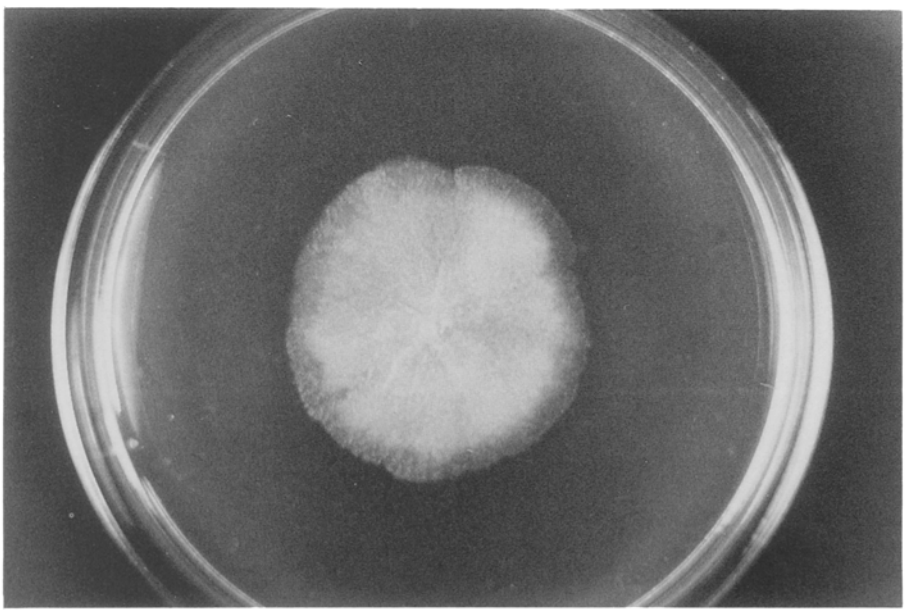

Fig. 2. Yellowish moist colony incubated on PYGS agar plate at 25°C for 20 days.

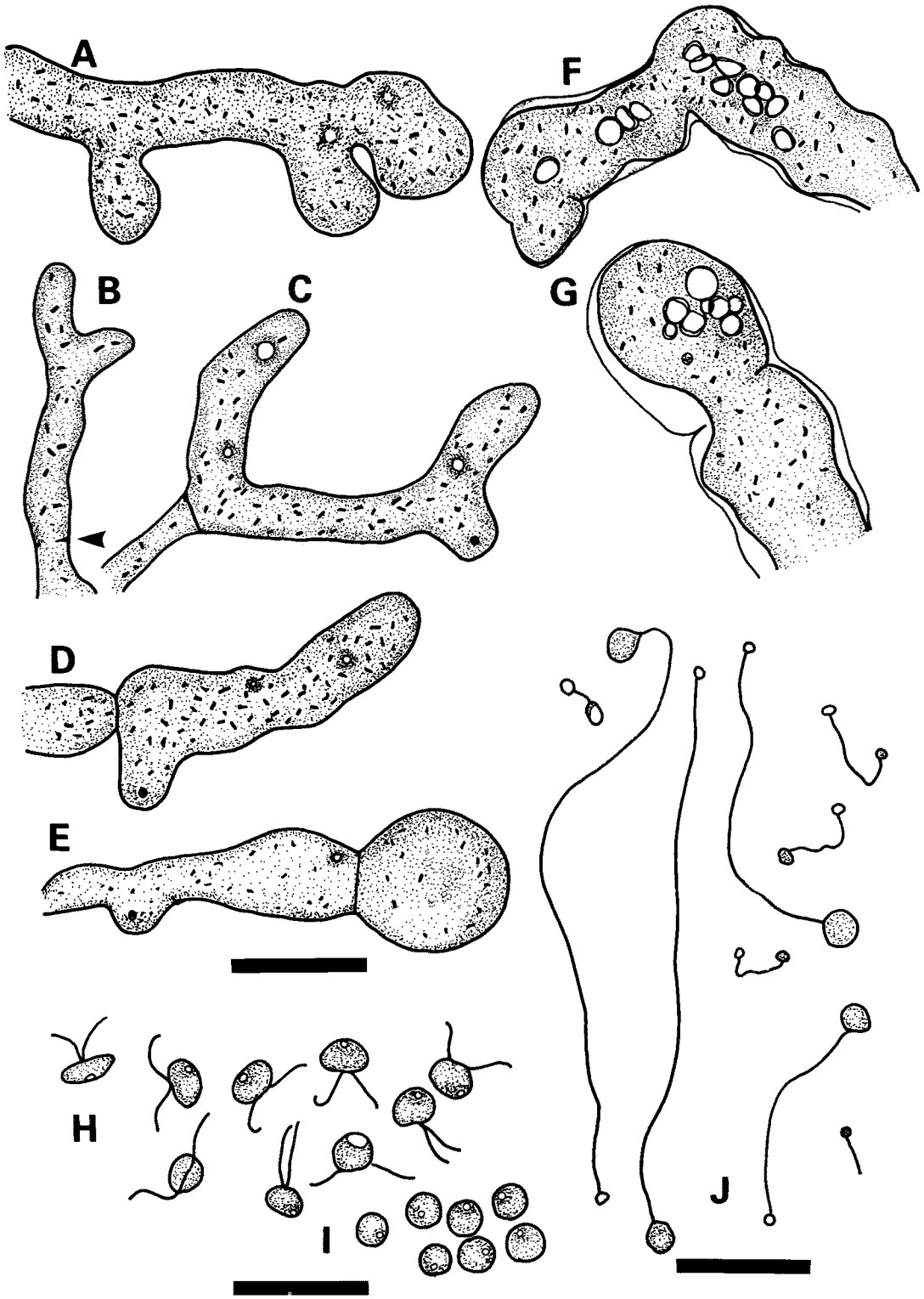

Fig. 3. *Atkinsiella parasitica* sp. nov. isolated from the rotifer *Brachionus plicatilis*. (Scales: A-G=50 μm; H-I=20 μm; J=50 μm.) A. Thallus with numerous shiny granules. B. Septum appeared in thallus (arrow head). C-D. Subthallus separated with a septum. E. Swollen hyphal tip. F-G. Gemmae. H. Zoospores, laterally biflagellate. I. Encysted zoospores. J. Germination.

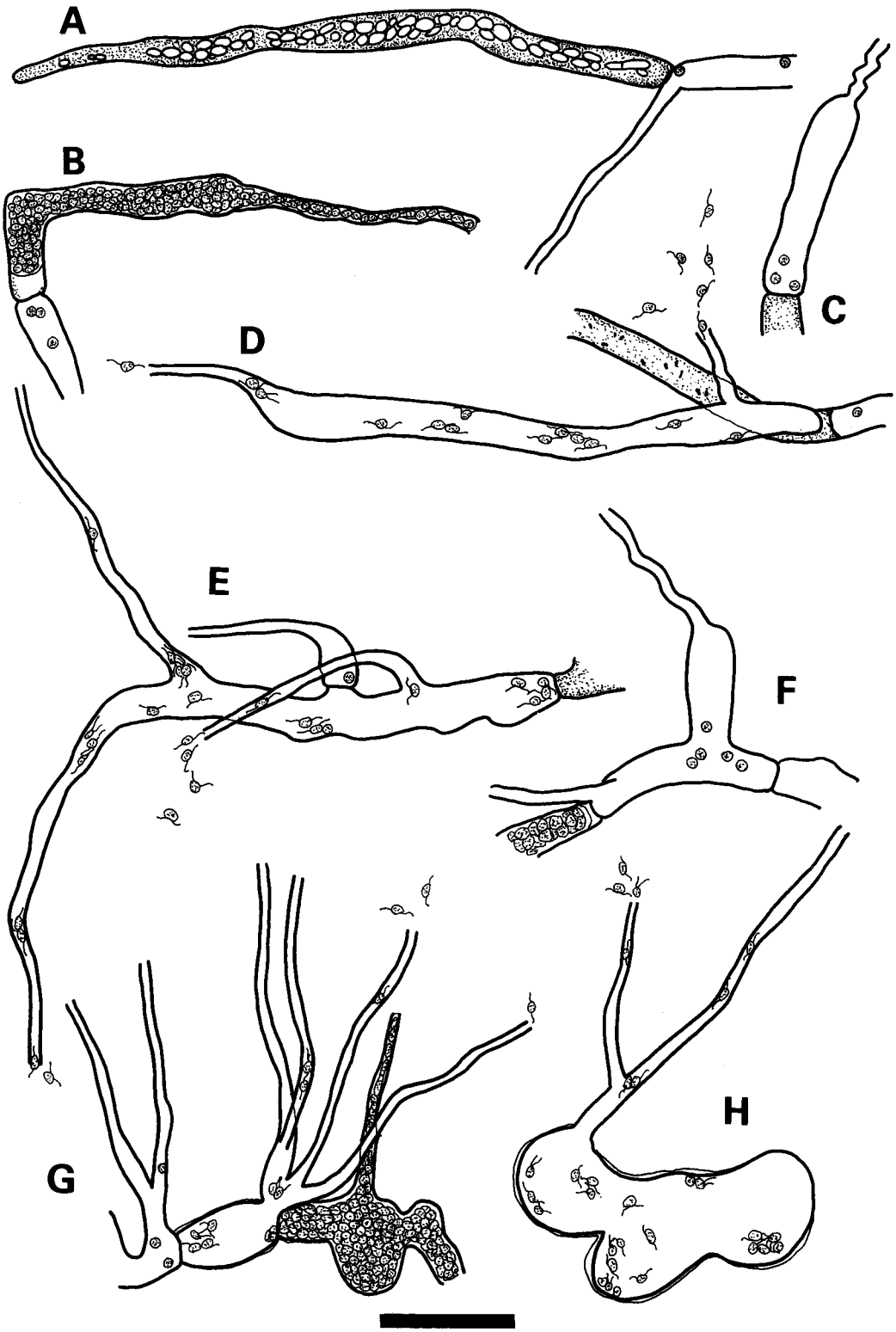

Fig. 4. Zoosporogenesis of *Atkinsiella parasitica* sp. nov. (Scale: A–H=50 μm.) A. Numerous large vacuoles appeared at an early stage of zoosporogenesis, and later discharge tubes developed. B. Zoospore formation in a zoosporangium and a discharge tube at the final stage of zooaporogenesis. C–F. One to three discharge tubes formed from a zoosporangium. G–H. Branched discharge tubes.

separated into initial zoospores. The present isolate has been placed in the culture collection of Nippon Veterinary and Animal Science University as NJM 9231. Its morphological characteristics are as follows.

Atkinsiella parasitica Nakamura et Hatai, sp. nov.
Figs. 3, 4

Thallus endobioticus holocarpicus, crassus, ramosus, in maturitate septatus et divisus in subthallos. Subthalli cylindrici, lobati vel iregulares. Zoosporangium subthallum conforme, e latere vel apice tubulos emittentes singulos vel nonnullos formans. Tubulus attenuatus vel aequaans in diametro, raro ramosus, 6-14×20-780 μm. Zoosporae pyriformes, monoplaneticae, lateraliter biflagellatae, 4.8-7.4×4.0-5.6 μm. Cystosporae globosae vel subglobosae, 5.5 (4.8-6.0) μm in diametro. Cystospora in fibrarum, 8-250 μm longarum germinans. Reproductio sexualis ignota. Mari incolens.

Holotypus: NJM 9231, colonia exsiccata e cultura ex corpis *Brachioni plicatilis* Müller, Chiba Pref. in Japonia, 24 Apr. 1992, a K. Nakamura isolata et ea collectione culturae in Universitate Veterinarii et Scientificae Animalis Nipponensis (NJM) conservata.

Thalli endobiotic, holocarpic, partly eucarpic in age or at lower temperatures, stout, branched, non-septate, saccate-lobed, 15-50 μm diam, swollen hyphal tips up to 110 μm diam. In PYGS broth at 25°C, the thallus at first was non-septate, generally vacuolate, with numerous shiny granules, septate in age dividing into subthalli. Gemmae present, saccate-lobed, thick-walled, with shiny globules, 40-200 μm diam, developing zoosporangia in seawater. Subthalli cylindrical, saccate, irregular, tuberculate, very variable in size and shape. Zoosporangia of same size and same shape as subthalli, extending one to several simple or infrequently branched discharge tubes. Discharge tubes straight, wavy or coiled, usually with a broad cone-shaped base, tapering or equal diameter, 6-14×20-780 μm, formed laterally or terminally from a zoosporangium. Zoospore production occurred within the zoosporangium and discharge tubes, 18-21 h at 25°C after vegetative hyphae were transferred into sterilized seawater. In the course of zoospore formation, flagellae appeared around the mass of protoplasm, and then protoplasm divided into initial zoospores. This behavior occurred first in the zoosporangium, then in the discharge tubes. The sequential zoospore production of subthalli separated from a single thallus with septa was observed. Proliferation was not observed. Zoospores pyriform, oblong, slipper-shaped, spherical, monoplanetic, laterally biflagellate, isokont, 4.8-7.4×4.0-5.6 μm, 6.0×4.6 μm on average. Zoospores were discharged within 30 min after beginning to move in the zoosporangium and discharge tubes, by rupture of the orifice of discharge tubes. In zoosporangia with several discharge tubes, zoospores were generally released first from one of the discharge tubes, then from others; but sometimes other discharge tubes did not open, because the movement of zoospores at their orifices was too weak. When discharge failed, zoospores swam within the zoosporangium, encysting and germinating in situ. The period of swimming after discharge was about 10 min. Encysted zoospores spherical to subglobose, 4.8-6.0 μm diam, 5.5 μm on average. Germination was observed about 2 h after spore encysted at 25°C with a hair-like filament measuring 8-250 μm length. Sexual reproduction was not observed. Parasitic in the eggs and bodies of the rotifer *Brachionus plicatilis* Müller.

Effect of temperature on growth The optimum growth temperature of isolate NJM 9231 was 25°C (Table 1). It showed good growth at 20-30°C, slight growth at 15°C, but did not grow at 10°C. When a plate incubated at 10°C was transferred to 25°C, 28 days after inoculation, fungal growth was not observed.

Mineral requirement for growth Isolate NJM 9231 grew on PYGS medium but not on PYG media prepared with any concentration of NaCl or KCl (Table 2). It was therefore judged as an obligate marine fungus, because its growth was observed only on medium including seawater.

Discussion

The present isolate could be placed in the genus *Atkinsiella* on the basis of the following characteristics: parasite in aquatic animals; holocarpic and endobiotic; zoosporangia of same size and same shape as subthalli and forming one to several discharge tubes; zoospores with lateral biflagella; germination with a hair-like filament. The genus *Atkinsiella* includes three species, *A dubia, A. entomophaga* and *A. hamanaensis*, but the fungus isolated from the rotifer differs in some characteristics from these three species.

The present isolate appeared as a yellowish to creamy, moist colony on PYGS agar. In *A. hamanaensis* the colony was creamy white, then gray to light brown with pigmentation (Bian and Egusa, 1980). Pigmentation was also observed in *A. entomophaga* (Martin, 1977), but not in *A. dubia* and the present isolate.

Rhizoid-like structures growing from hyphae with a septum was observed in *A. dubia* (Sparrow and Gotelli, 1969; Sparrow, 1973). However, there were no rhizoid-like structures in the present isolate, or in the other two strains.

Table 1. Effect of temperature on growth of *Atkinsiella parasitica* NJM 9231 on PYGS agar.

Temperature (°C)	Colony radius (mm)					
	Days after inoculation					
	3	5	7	14	21	28
10	−*	−	−	−	−	−
15	−	−	−	+**	+	3.2
20	+	+	3.5	5.8	7.5	9.3
25	+	+	3.5	7.2	10.1	12.7
30	+	+	3.3	5.7	7.6	11.6

*−: No growth.
**+: Little growth (difficult to measure).

Table 2. Effect of NaCl and KCl on growth of *Atkinsiella parasitica* NJM 9231 at 25°C.

Medium	NJM 9231**
PYGS agar*	+
PYG agar+1.0% NaCl	−
PYG agar+2.5% NaCl	−
PYG agar+5.0% NaCl	−
PYG agar+1.0% KCl	−
PYG agar+2.5% KCl	−
PYG agar+5.0% KCl	−
PYG agar	−

* PYGS agar consisted of 0.125% peptone, 0.125% yeast extract, 0.3% glucose and 1.2% agar in seawater. PYG agar was PYGS agar containing distilled water instead of seawater.

**+: Mycelial growth was observed 30 days after inoculation.
−: No mycelial growth observed.

Discharge tubes of *A. entomophaga* were up to 3.7 mm length, the longest among the species of *Atkinsiella*, and branched (Martin, 1977). In the present isolate, the discharge tubes also branched at the base of the sporangium, but was shorter than those of *A. entomophaga*. The dimensions of the discharge tubes in *A. hamanaensis* were 5-15×40-1150 μm (Bian and Egusa, 1980), closely similar to those of the present isolate (6-14×20-780 μm). In *A. dubia*, the length was reported as 9-10 μm and 50-400 μm by Sparrow (1973) and Atkins (1954), respectively.

At 25°C, zoospore production of the present isolate occurred 18-21 h after the mycelia were transferred into sterilized seawater. Zoospore production of *A. dubia* (Fuller et al., 1964) and *A. hamanaensis* (Bian and Egusa, 1980) was observed after about 12 h at 23°C and after 2-3 days at 25°C, respectively. The sequential zoospore production of parts of a single thallus was present in *A. dubia*, without presence of subthalli (Sparrow, 1973). In the present fungus, all parts of each subthallus transformed into zoosporangia at the same time, while the sequential zoospore production of each subthallus in a single thallus was observed. With the exception of subthalli presence, the behavior was similar to that of *A. dubia*. Proliferation occurred in *A. dubia* (Atkins, 1954), but was never observed in this fungus or the other two species of *Atkinsiella*.

Zoospores of the present isolate showed monoplanetism, but the other three species of *Atkinsiella* showed diplanetism (Atkins, 1954; Fuller et al., 1964; Sparrow, 1973; Martin, 1977; Bian and Egusa, 1980). Martin (1977) transferred Eurychasmaceae including *Atkinsiella* from Saprolegniales to Lagenidiales, because the pattern of zoosporogenesis and the type of diplanetism in Eurychasmaceae were similar to those in Lagenidiales. The swimming phase of primary zoospores was suppressed in *Atkinsiella* in place of the presence of diplanetism. The difference in planetism between this fungus and the other species was not thought to be of enough importance to distinguish this fungus as a separate genus, because primary zoospores of the present isolate were thought to have the most suppressed swimming phase with one motile. The genus *Lagenidium* belonging to Lagenidiales also includes both monoplanetic and diplanetic fungi (Karling, 1981). Secondary zoospores of *A. dubia* emerged from spores cysted within the zoosporangium (Sparrow, 1973). Contrary to *A. dubia*, primary zoospores were released outside the zoosporangium in *A. entomophaga* and *A. hamanaensis* (Martin, 1977; Bian and Egusa, 1980). Thus, various kinds of swimming phase were recognized in the genus *Atkinsiella*.

The dimensions of the zoospores in this fungus were 6.0×4.6 μm, and appeared similar to those of *A. dubia*, 6-8×4-6 μm (Sparrow, 1973) and *A. hamanaensis*, 6.3×4.5 μm (Bian and Egusa, 1980). Those of *A. dubia* (Atkins, 1954) and *A. entomophaga* (Martin, 1977) were 10 μm and 11.6×6.9 μm, respectively, easy to distinguish from the present isolate.

Encysted zoospores of the fungus germinated after about 2 h at 25°C, forming a hair-like filament and spherical hypha. However, the germination of *A. dubia* was observed after 24 h at 23°C, with or without a slender germ tube (Sparrow, 1973). In *A. hamanaensis*, encysted zoospores germinated with a filament in seawater and without a filament on agar plates (Bian and Egusa, 1980). Filaments of the isolate NJM 9231 were 8-250 μm length, similar to the 10-270 μm of *A. hamanaensis* (Bian and Egusa, 1980). Only in *A. entomophaga*, was a filament with swellings observed (Martin, 1977). No swellings of filament appeared in pure cultures of the present isolate or the others.

The optimum temperature of the present isolate was 25°C, while that of *A. hamanaensis* was 29-32°C (Bian and Egusa, 1980), higher than the present isolate. *A. dubia* and *A. entomophaga* were not researched.

The present species was considered an obligate marine fungus, because its growth was observed only on PYGS medium including seawater. *A. dubia* and *A. hamanaensis* could grow on media with NaCl replacing seawater (Aronson and Fuller, 1969; Bian and Egusa, 1980). *A. entomophaga*, which was isolated from the eggs of freshwater insects, could grow in PYG medium (Difco) and released zoospores in sterilized water (Martin, 1977).

On the basis of its morphological and physiological characteristics, we concluded that the fungus isolated from the rotifer should be considered as a new species of the genus *Atkinsiella*.

Comparison of the descriptions of *A. dubia* by Atkins (1954) and Sparrow (1973), reveals several apparent differences between them in the characteristics of the species. First, the rhizoid-like structures were observed by Sparrow, but not by Atkins. Second, the dimensions of the discharge tubes were 50-400 μm length and 10-30 μm diam according to Atkins, and 9-10 μm length and 4-6 μm diam according to Sparrow. Third, the dimensions of the zoospores were reported as 10 μm by Atkins and 6-8×4-6 μm by Sparrow. Fourth, proliferation was

observed only by Atkins, while the sequential zoospore production of a single thallus was observed only by Sparrow. We doubt whether the isolate observed by Sparrow should have been classified as *A. dubia*.

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