Mycoscience 36: 431–438, 1995

Atkinsiella dubia and its related species

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Accepted for publication 20 October 1995

***Atkinsiella dubia*, isolated from the mantle of abalone (*Haliotis sieboldii*), is described and illustrated as a new record from Japan. The fungus was also obtained from the gills of swimming crab (*Portunus trituberculatus*). Six other species of the genus *Atkinsiella* have hitherto been reported from various aquatic animals. The fungus is distinguished from the other six species by the morphology of its mycelia and the process of zoospore production. The most distinctive feature is that zoospores in the first motile stage of *A. dubia* encyst in zoosporangia, unlike the other species. We therefore propose *Halocrusticida* gen. nov. (Lagenidiales, Haliphthoraceae) for the other six species of *Atkinsiella*. A key to species of the genus *Halocrusticida* is provided.**

Key Words——*Atkinsiella dubia*; *Halocrusticida*; Japan; marine fungus; taxonomy.

Atkinsiella dubia (Atkins) Vishniac was originally isolated from the eggs of a pea crab, *Pinnotheres pisum* Pennant, in England by Atkins (1954), and assigned to the genus *Plectospira*. Atkins observed the same species on the eggs of *Gonoplax rhomboides* Pennant, and succeeded in experimentally infecting the eggs of some species of crustaceans. The morphology of the fungal parasite on the crabs' eggs was studied at that time. Later, Vishniac (1958) established a new family, Haliphthoraceae (Saprolegniales), for holocarpic biflagellate filamentous fungi, including *Haliphthoros milfordensis* Vishniac and Atkins' fungus, which was renamed *A. dubia*, although she did not actually observe *A. dubia*. Its morphology and development in pure culture were followed by Fuller et al. (1964) and Sparrow (1973) from marine algae and the eggs of various crabs, respectively.

During the survey of the fungi belonging to Lagenidiales on marine animals without clinical signs, an interesting fungus was isolated from the mantle of abalone, *Haliotis sieboldii* Reeve, at Chiba Prefectural Fisheries Experimental Station, Chiba Prefecture, Japan. The same species was also obtained from the gills of swimming crab, *Portunus trituberculatus* Miers, at Chiba Prefectural Tokyo Bay Sea Farming Center. The fungus was characterized by crystalline, tuberculate and moist colonies, dimorphic and diplanetic zoospores, and zoospores which remained in the zoosporangia during the first motile stage, and identified as *A. dubia*, new to Japan. To date, six other species of *Atkinsiella* have been reported from various aquatic animals (Martin, 1977; Bian and Egusa, 1980; Nakamura and Hatai, 1994, 1995; Kitancharoen et al., 1994; Kitancharoen and Hatai, 1995). They differ from *A. dubia* in regard to the morphology of the mycelia, the process of zoospore production and the behavior of zoospores in the first motile stage. We herein propose *Halocrusticida* gen. nov. (Lagenidiales, Haliphthoraceae) for the other six species of *Atkinsiella*. A key to the six species of *Halocrusticida* is provided.

Description

Atkinsiella dubia (Atkins) Vishniac, J. Mar. Biol. Ass. U.K. **33**: 731. 1954. Figs. 1–4

Colonies on PYGS agar attaining a diameter of about 30 mm in 1 month at 25°C, crystalline, tuberculate, and moist; moderately heaped at the center. Mycelia in the broth non-septate, radially branched, stout, swollen up to 250 μm in diam, with clusters of shiny spherical granules, without oil droplets and vacuoles. Granular clusters evenly distributed inside mycelia, generally consisting of several decades of granules. Mycelia in seawater developing narrow branches (discharge tubes), followed by zoospore production. Gemmae absent. Zoospores in the first motile stage produced after about 35 h at 20–25°C. Protoplasmic masses due to gathering of granular clusters on zoosporogenesis, supported at the center of zoosporangia by several protoplasmic threads; differentiated into loose networks of zoospores, then into free individual zoospores in the first motile stage. Zoosporangia the same in size and shape as the mycelia, with several discharge tubes extending from each zoosporangium. Zoospores in the first motile stage swimming dully and encysting within zoosporangia and discharge tubes, biflagellate, subglobose to globose, 8–11 μm in size. Zoospores in the second motile stage releasing one by one from encysted zoospores within zoosporangia and discharge tubes, swimming freely for a long time; laterally biflagellate, pyriform, slipper-shaped, isokont, 4–7 $\times$ 8–12 μm. Zoospores dimorphic, diplanetic. Encysted spores globose to subglobose, 7–9 μm in diam. Discharge tubes unbranched or occasionally bran-

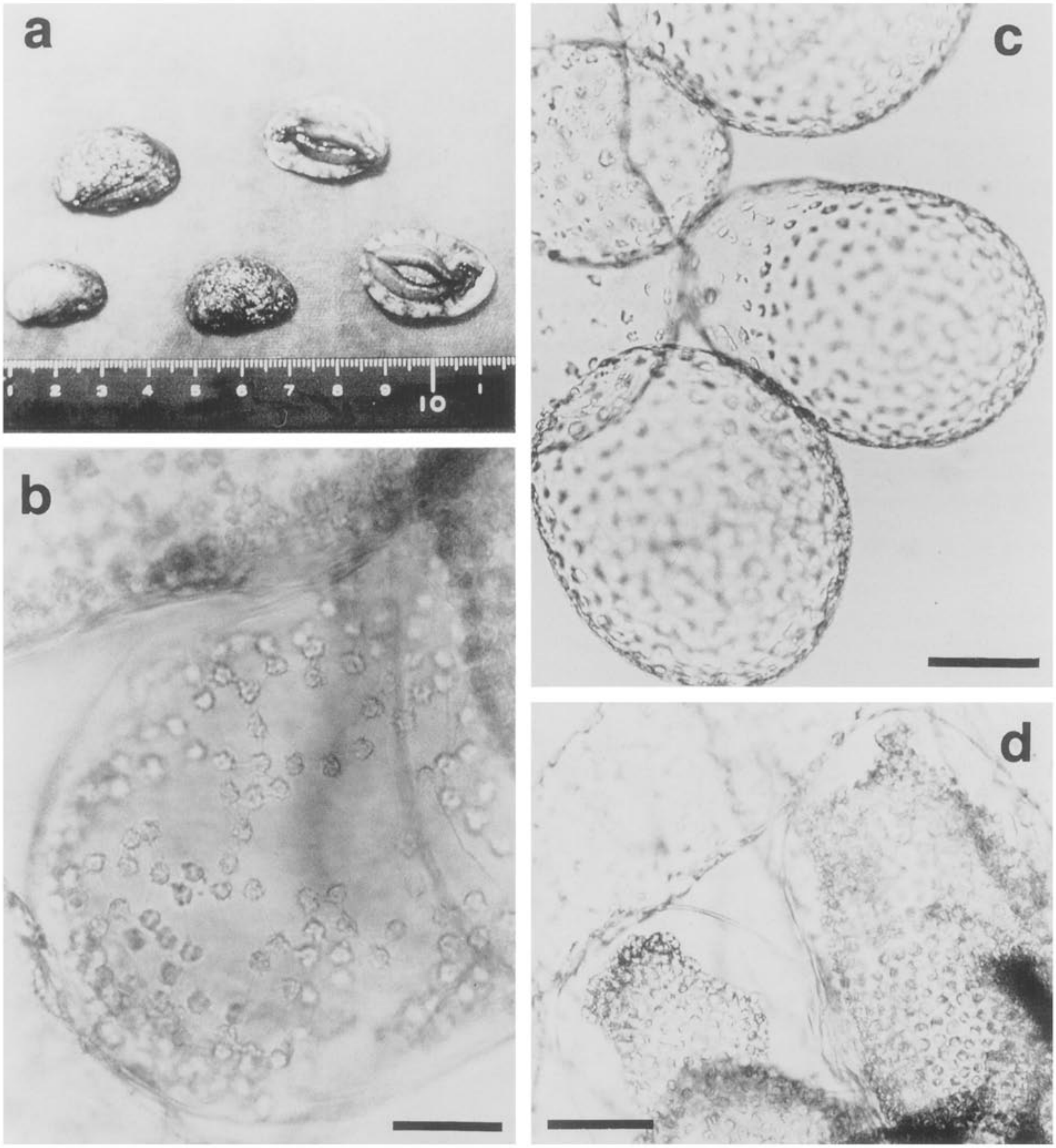

Fig. 1. a. *Atkinsiella dubia* was obtained from the mantle of abalone, *Haliotis sieboldii*, which was the same size as the abalones in the figure. b-d. Mycelia of *A. dubia* NJM 9455. b. A mycelium in PYGS broth. Granular clusters evenly distributed inside mycelium. c. Networks of granular clusters during zoosporogenesis in seawater. d. Gathering of granular clusters. Scales: b, d=100 μm; c=50 μm.

ched, straight or tapering with flared openings, rarely with a central swelling, 8-50 μm in width, 8-470 μm in length. Germination with a slender long tube. No sexual reproduction observed.

Growth temperatures: 10-25°C, optimum 25°C (Fig. 5).

Mineral requirements: growth with seawater and 2.5% (w/v) NaCl (Table 1).

Specimen examined: NJM 9455, isolated from the mantle of abalone, *Haliotis sieboldii*, obtained from Chiba Pref., Japan, 16 Dec. 1994; NJM 9531, isolated from the gills of adult swimming crab, *Portunus trituberculatus*, obtained from Chiba Pref., Japan, 17 Feb. 1995. Both fungi are deposited at Nippon Veterinary and Animal Science University.

Notes: Three morphological studies of *A. dubia* have hitherto been reported from marine crustaceans and algae (Atkins, 1954; Fuller et al., 1964; Sparrow, 1973).

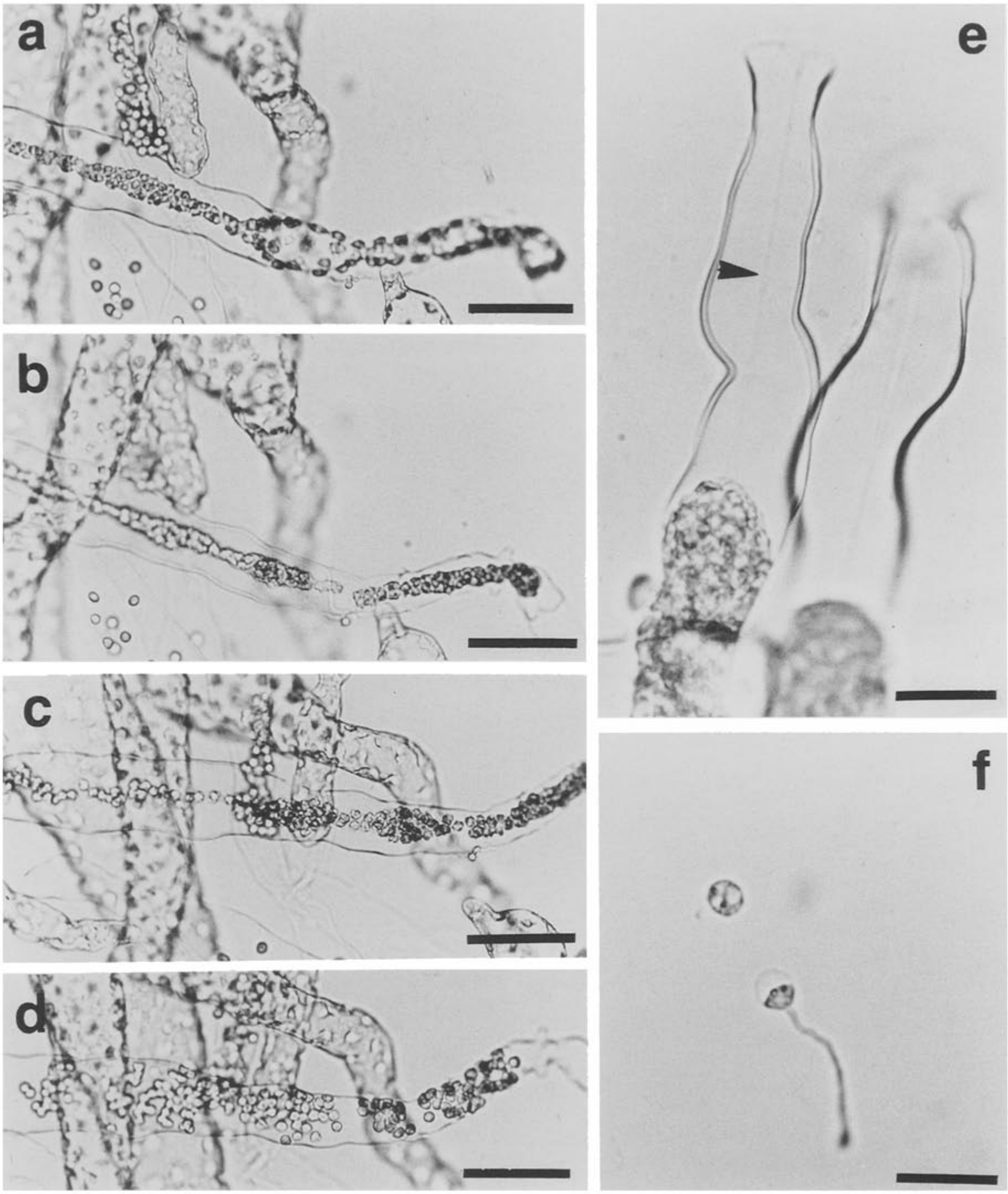

Fig. 2. Zoosporogenesis of *Atkinsiella dubia* NJM 9455.
a. Gathering of granular clusters. b. A protoplasmic mass inside zoosporangium. c. Initial zoospores. d. Encysted zoospores after the first motile stage. e. Discharge tubes with flared openings. Protoplasmic masses were supported by protoplasmic threads (arrowhead). f. Encysted zoospores and germination. Scales: a–d = 100 μm; e, f = 25 μm.

The present fungus differs from the previous reports in the proliferation of zoosporangia and the presence of rhizoids, as described by Atkins (1954) and Sparrow (1973), respectively (Table 2). However, except for these disagreements, the present isolate closely resembles Atkins' description morphologically. Therefore, the present isolate is identified as *A. dubia*, new to Japan.

The other six species of *Atkinsiella* were reported from various aquatic animals (Martin, 1977; Bian and Egusa, 1980; Nakamura and Hatai, 1994, 1995; Kitancharoen et al., 1994; Kitancharoen and Hatai, 1995). The sources of the six species are listed in Table 3. Mycelia contained granular clusters without oil droplets

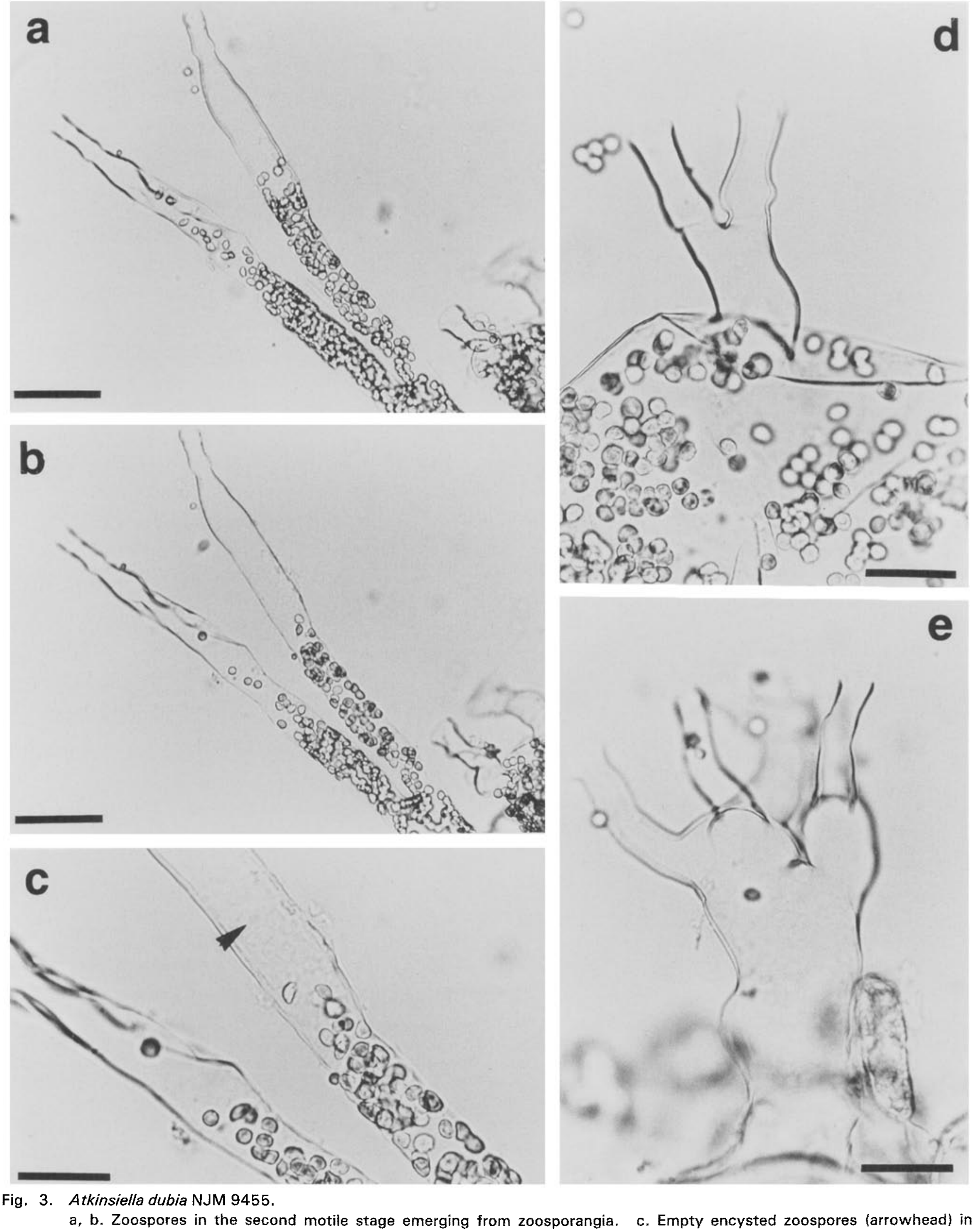

Fig. 3. *Atkinsiella dubia* NJM 9455.
a, b. Zoospores in the second motile stage emerging from zoosporangia. c. Empty encysted zoospores (arrowhead) in zoosporangia. d, e. Branched discharge tubes. Scales: a, b = 100 μm; c–e = 50 μm.

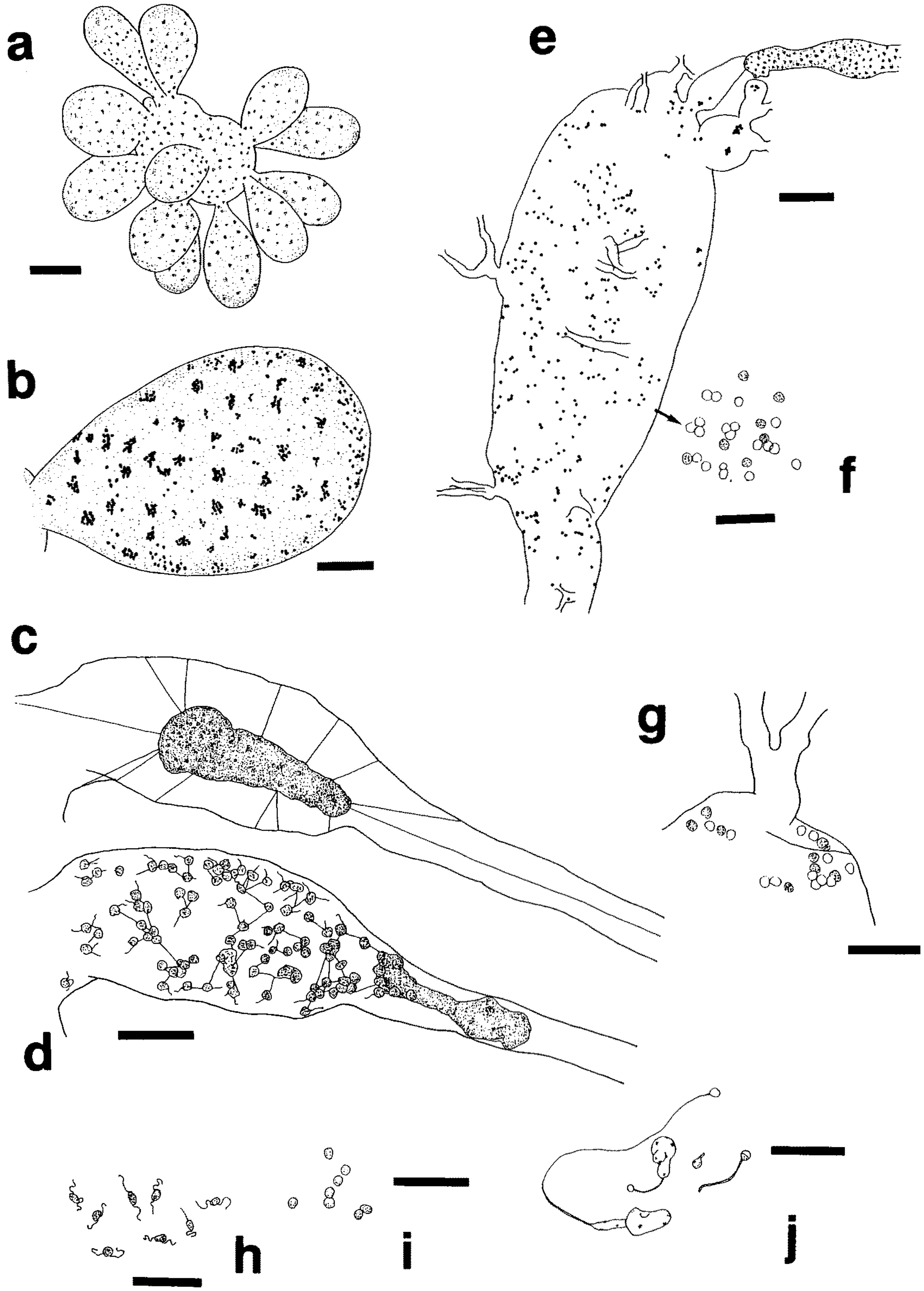

Fig. 4. *Atkinsiella dubia* NJM 9455.
a, b. Mycelium with granular clusters. c. A protoplasmic mass supported by several protoplasmic threads. d. Loose networks of zoospores. These differentiated into free individual zoospores in the first motile stage. e. A zoosporangium with branched discharge tubes. f. Empty encysted zoospores, and encysted zoospores with protoplasm from which zoospores in the second motile stage will emerge. g. A branched discharge tube with flared openings. h. Zoospores in the second motile stage. i. Encysted zoospores after the second motile stage. j. Germination. Scales: a, e = 150 μm; b = 70 μm; c, d, g–j = 50 μm; f = 40 μm.

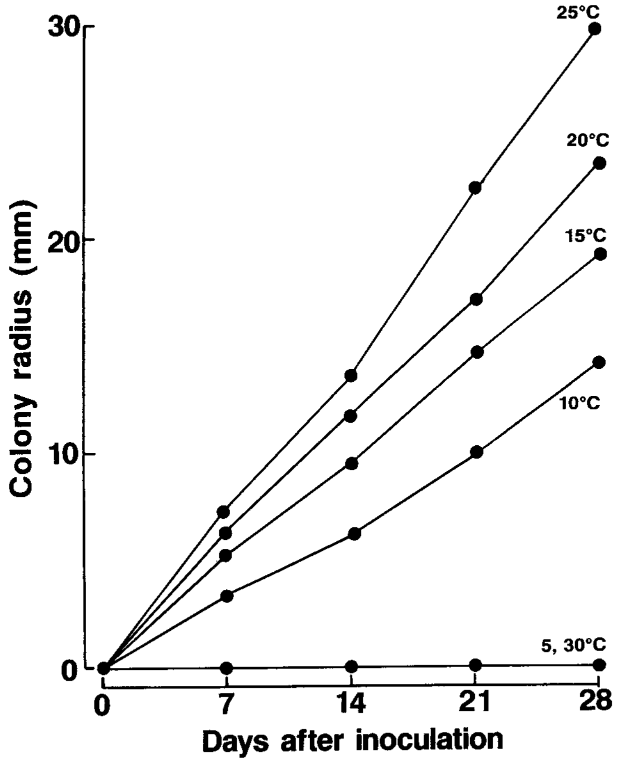

Fig. 5. Effect of temperature on growth of *Atkinsiella dubia* NJM 9455 isolated from the mantle of abalone.

Table 1. Effects of NaCl and KCl on growth of *Atkinsiella dubia* NJM 9455 at 25°C.

Medium*	NJM 9455**
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 1.25 g of peptone, 1.25 g of yeast extract, 3.0 g of glucose and 12 g of agar in 1 L of seawater. PYG agar contained distilled water instead of seawater in PYGS agar.
** NJM 9455: inoculated with a small colony on each medium.
*** Mycelial growth (+: growth, −: no growth) was checked after 1 month of incubation.

and vacuoles on *A. dubia*, but many vacuoles and numerous shiny granules were found on the others (Martin, 1977; Bian and Egusa, 1980; Nakamura and Hatai, 1994, 1995; Kitancharoen et al., 1994; Kitancharoen and Hatai, 1995). Central protoplasmic masses supported by several protoplasmic threads in the process of zoospore production were observed on *A. dubia*, but not on the others. The most apparent difference between *A. dubia* and the other six species of *Atkinsiella* was the behavior of zoospores in the first motile stage. Zoospores encysted within zoosporangia and discharge tubes following the first motile stage in *A. dubia*, while zoospores in the first motile stage were released from zoosporangia in the other six species (Martin, 1977; Bian and Egusa, 1980; Nakamura and Hatai, 1994, 1995; Kitancharoen et al., 1994; Kitancharoen and Hatai, 1995). We therefore propose *Halocrusticida* gen. nov. (Lagenidiales, Haliphthoraceae) for the other six species of *Atkinsiella*. A key to the species of *Halocrusticida* is provided.

Table 2. Comparison of morphological characteristics of the present strain (NJM 9455) with the previous descriptions of *Atkinsiella dubia*.

	Present strain (NJM 9455)	Description by		
		Atkins (1954)	Fuller et al. (1964)	Sparrow (1973)
Rhizoids	absent	—*	—	present
Discharge tubes	8–50 μm (width) 8–470 μm (length)	10 μm (width) 50–400 μm (length)	short tubes	4–6 μm (width) 9–10 μm (length)
Zoospore production**	about 35 h	—	about 12 h	—
Proliferation	not observed	observed	—	—
Zoospores***	f. 8–11 μm s. 4–7×8–12 μm	f. 10 μm s. 11–12 μm	f. 6×8.2 μm s. 4.8×8.7 μm	4–6×6–8 μm
Encysted zoospores	7–9 μm	f. 7–8 μm s. 6–7 μm	f. 7.4 μm s. 6.8 μm	7–9 μm
Germination	with a slender germ tube	—	with a small tube, about 1.7 μm in diam	with a long slender germ tube
Gemmae	absent	present	—	absent
Sexual reproduction	absent	absent	absent	absent

* —: not described.
** Zoospore production: time lapse between transferring hyphae to seawater and beginning of zoospore production.
*** f. or s.: zoospores in the first or second motile stage.

Table 3. Six species reported previously as *Atkinsiella*.

Species	References	Hosts	Locality
A. entomophaga	Martin (1977)	Insect eggs (Chironominae and Tanypodinae)	U.S.A.
A. hamanaensis	Bian and Egusa (1980)	Eggs and larvae of mangrove crab (*Scylla serrata*)	Japan
A. parasitica	Nakamura and Hatai (1994)	Rotifer (*Brachionus plicatilis*)	Japan
A. awabi	Kitancharoen et al. (1994)	Awabi (*Haliotis sieboldii*)	Japan
A. okinawaensis	Nakamura and Hatai (1995)	Zoea of the crab (*Portunus pelagicus*)	Japan
A. panulirata	Kitancharoen and Hatai (1995)	Philozoma of spiny lobster (*Panulirus japonicus*)	Japan

Halocrusticida Nakamura et Hatai, gen. nov.

Thallus endobioticus, holocarpicus, crassus, ramosus. Zoosporangium thallum conforme, tubulos emittentes singulos vel nonnullos formans. Zoosporae priores plerumque liberantes e zoosporangio. Zoosporae monoplaneticae aut diplaneticae, isokontae, lateraliter biflagellatae. Germinatio zoosporae cum tubulo tenui subiens. Repoductio sexualis ignota.

Species typica: *Halocrusticida entomophaga* (Martin) Nakamura et Hatai, comb. nov.

Basionym: *Atkinsiella entomophaga* Martin, Amer. J. Bot. **64**: 767. 1977.

Etymology: *halo*=sea (Greek), *crusta*=crustaceans (Latin), and *cida*=destroyer, murderer (Latin).

Thallus endobiotic, holocarpic, stout, branched. Zoosporangia the same in size and shape as thalli. Discharge tubes 1 to several per sporangium. Zoospores in the first motile stage emerge from the zoosporangia. Zoospores monoplanetic or diplanetic, isokont, laterally biflagellate. Germination with a slender germ tube. Sexual reproduction absent. Parasitic on aquatic animals, especially marine crustaceans.

Type species: *Halocrusticida entomophaga* (Martin) Nakamura et Hatai, comb. nov.

Basionym: *Atkinsiella entomophaga* Martin, Amer. J. Bot. **64**: 767. 1977.

Formal proposals for the new combinations for the other species of *Halocrusticida* are also given as follows.

Halocrusticida hamanaensis (Bian et Egusa) Nakamura et Hatai, comb. nov.

Basionym: *Atkinsiella hamanaensis* Bian et Egusa, J. Fish Dis. **3**: 379, 1980.

Halocrusticida parasitica (Nakamura et Hatai) Nakamura et Hatai, comb. nov.

Basionym: *Atkinsiella parasitica* Nakamura et Hatai, Mycoscience **35**: 387, 1994.

Halocrusticida awabi (Kitancharoen, Nakamura, Wada et Hatai) Nakamura et Hatai, comb. nov.

Basionym: *Atkinsiella awabi* Kitancharoen, Nakamura, Wada et Hatai, Mycoscience **35**: 267, 1994.

Halocrusticida okinawaensis (Nakamura et Hatai) Nakamura et Hatai, comb. nov.

Basionym: *Atkinsiella okinawaensis* Nakamura et Hatai, Mycoscience **36**: 89, 1995.

Halocrusticida panulirata (Kitancharoen et Hatai) Nakamura et Hatai, comb. nov.

Basionym: *Atkinsiella panulirata* Kitancharoen et Hatai, Mycoscience **36**: 100, 1995.

Key to species of *Halocrusticida*

1 Colonies filamentous, less than 2 tubes produced from each sporangium ········ *H. awabi*
1 Colonies lobed, bulbous ········ 2
2 Encysted spores more than 9 μm, parasitic on insect eggs ········ *H. entomophaga*
2 Encysted spores less than 9 μm, parasitic on crustaceans ········ 3
3 Branched discharge tubes present ········ 4
3 Branched discharge tubes absent ········ 5
4 Zoospores generally formed two or more deep in the discharge tubes ········ *H. okinawaensis*
4 Zoospores generally formed in a single row in the discharge tubes ········ *H. parasitica*
5 Pigmentation from gray to light brown, optimum temperature for growth 30-32°C ········ *H. hamanaensis*
5 No pigmentation, optimum temperature for growth 25°C ········ *H. panulirata*

Fungal infections belonging to Lagenidiales have been a serious problem in Japanese marine culture. Among the fungi, *Lagenidium callinectes* Couch and *Haliphthoros milfordensis* Vishniac have been recognized as the most serious pathogens toward marine animals (Sparks, 1985), also reported in Japan (Hatai, 1982; Hatai et al., 1992; Nakamura and Hatai, 1995). Usually fungal diseases occurred on the egg and larval stages of Decapoda (Crustacea). Holocarpic fungi previously isolated from marine crustaceans in Japan may be identified by the following key.

Key to the genera of the holocarpic fungi from marine crustaceans in Japan

1 Colonies filamentous, mycelioid ············ 2
1 Colonies lobed, bulbous ············ 3
 2 Vesicles produced on the orifices of the discharge tubes ············ *Lagenidium*
 2 Vesicles not produced ············ 4
3 Zoospores encysted in the zoosporangia following the first motile stage ············ *Atkinsiella*
3 Zoospores in the first motile stages released from the zoosporangia ············ *Halocrusticida* gen. nov.
 4 Zoosporangia disarticulating from thalli ············ *Sirolpidium*
 4 Zoosporangia fragmenting from thalli ············ *Haliphthoros*

Acknowledgements——We wish to thank Chiba Prefectural Fisheries Experimental Station and Chiba Prefectural Tokyo Bay Sea Farming Center for sampling specimens. We sincerely thank Dr. Ken Katumoto for recommending the scientific name *Halocrusticida*.

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