

The Peculiar Marine Phycomycete *Atkinsiella dubia* from Crab Eggs

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Summary. *Atkinsiella dubia* (Atkins) Vishniac has been found doubtfully parasitic in eggs of various crabs in the vicinity of Friday Harbor Laboratories.

Its morphology and development are followed in pure culture on nutrient media. The peculiarity of the sequential transformation of the intricate lobed thallus contents into a zoosporangium and of the similar development of the zoospores themselves is pointed out. The thallus itself is sometimes holo-sometimes encarpic. Resemblances of *Atkinsiella* to *Eurychasma*, a parasite of marine algae are pointed out.

Atkins in 1954 published her observations on a puzzling saprolegniaceous fungus with dimorphic (“diplanetic”) zoospores found by her parasitic in eggs of *Pinnotheres pisum* and *Gonoplax rhomboides* from the Plymouth (England) Laboratory. Eggs of crustaceans belonging to five other genera were infected when placed in contact with them. All eggs became slightly enlarged after infection. The organism, according to her was definitely parasitic and elements of the fungus invaded eggs containing embryos with the heart beating. The true nature of the thallus was greatly obscured by being within the confines of the host but it was evident from specimens dissected from the eggs that it was a complex of swollen, lobulate elements. Sometimes spherical gemmae were formed but in no instances were cross walls found. Although Atkins placed her fungus tentatively in *Plectospira*, she rightly pointed out that in contrast to this genus there were no purely vegetative hyphae. In the egg parasite the whole thallus became converted into a single sporangium (i.e., it was holocarpic). Features of its reproduction essentially like those about to be described did point to its being saprolegniaceous but not closely allied to *Plectospira*. Vishniac (1958) in describing a new holocarpic mycelial biflagellate fungus from egg cases of the oyster drill (*Urosalpinx cinerea*), *Haliphthorus milfordensis*, placed her own and Atkins’ fungus which she named *Atkinsiella dubia* in a new family, the Haliphthoraceae, erected for holocarpic biflagellate mycelial saprolegniaceous fungi. She, herself, however, did not study material of *Atkinsiella*.

Fuller *et al.* (1964) reported isolating *Atkinsiella* on antibiotic solid culture media from bits of the marine algae *Chordaria* and *Cladophora* in summer months near Newport, Rhode Island. It seemed probable that the propagules of the fungus were adherent to the algal surfaces. On solid media the thallus was saccate lobed and without purely vegetative elements for it was converted as a whole into a single sporangium when placed under appropriate conditions. As Miss Atkins showed, the zoospores were dimorphic and Fuller *et al.* found the primary ones to be anteriorly biflagellate as in *Saprolegnia*. These encysted and from the cysts laterally biflagellate or secondary zoospores emerged. The wall of *Atkinsiella* has been well-studied by Aronson and Fuller (1969) who found it to be like that of other Oomycetes and composed primarily of B 1—3 and B 1—6 linked glucans with lesser amounts of cellulose.

In the Pacific Northwest in the vicinity of the Friday Harbor Laboratories, eggs of crabs belonging to species of *Hyas*, *Oregonea*, *Pugettia*, *Chorilia*, *Skyra*, *Chianectes* and *Cancer* have all been found to harbour the fungus but never in epidemic proportions, at least in the summer of 1972. The occasional infected eggs in a "sponge" were readily distinguishable by their opaque and shaggy appearance compared with the clear and smooth uninfected one (Fig. 15). Furthermore, at the onset of nonsexual reproduction numerous broad tubular efferent structures often of bizarre shapes which emerged from the eggs were unmistakable evidence of the presence of *Atkinsiella* (Figs. 14, 16—17). No other fungus was found in infected eggs of these crabs.

When drops of seawater containing zoospores from infected eggs were spread out on the antibiotic agar surface abundant growth was obtained which could be transferred to media lacking antibiotics. Here, after a week a glistening pearly-gray lobed, non-mycelial colony developed.

In initiating thallus development, the encysted zoospore usually forms after 24 h a long slender germ tube from the tip of which a new thallus is produced (Figs. 2 and 3). Often such a tube is lacking and the developing thallus appears as a bud on the cyst which it soon outstrips in size (Fig. 4). Even at a very immature stage the new thallus has formed the large central vacuole so characteristic a feature of the saprolegniaceous vegetative cell (Figs. 2—4). After 48 h the thallus may be distinctly hyphal and somewhat dichotomously branched (Figs. 5 and 6), or irregular because of the formation of swellings. At 72 h the hyphal nature is lost as the swellings become more pronounced and there is imparted to the young plant a three-dimensional character (Fig. 7). This becomes more pronounced at 120 h and eventuates at 5 days in plants composed of a complex of lobes and swollen branches (Fig. 8). Such plants represent the mature vegetative stage and it is these which are sometimes released or can be teased out of very disintegrated eggs.

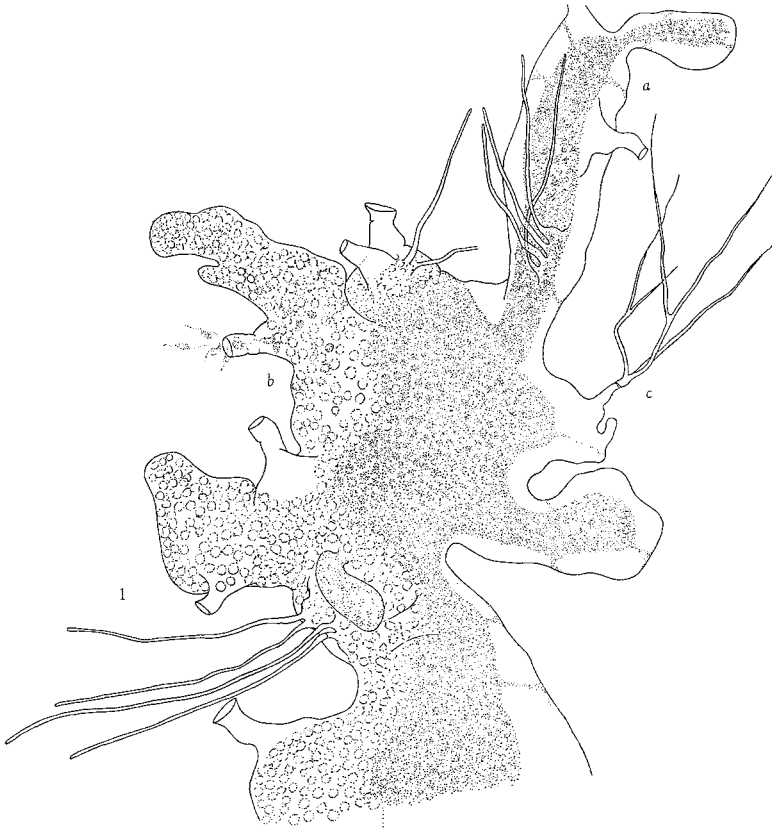
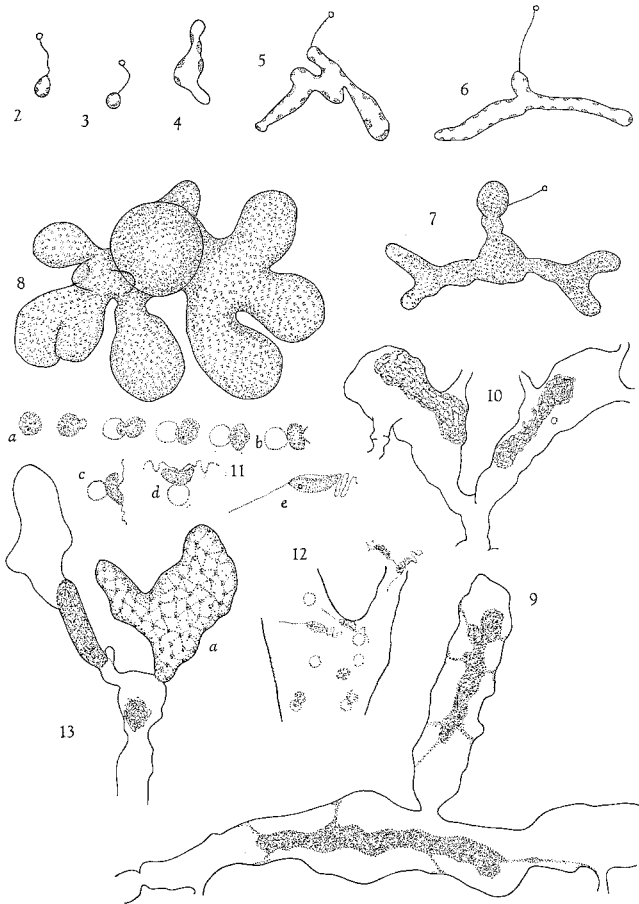


Fig. 1. Portion of thallus of *Atkinsiella* grown in nutrient broth and washed several days in seawater. *a* Cytoplasm is collapsing preparatory to primary zoospore formation. Discharge tube open. *b* Secondary zoospores are quitting cysts of primary zoospores and escaping through broad open discharge tube. *c* Rhizoids cut off by cross wall are seen. Throughout, contents of thallus in various stages of development, with numerous scattered cysts of primary zoospores. Approximately $\times 170$

Nonsexual reproduction can be induced by subjecting thalli grown in liquid media to sudden starvation by washing them in sterile seawater, as long ago pointed out by Klebs for other Phycomycetes. In many thalli subjected to this regime another, rather unexpected event occurs namely, rhizoids frequently cut off by well-defined cross walls are formed. Such a thallus as shown in Fig. 1 can therefore, hardly be called holocarpic.

Reproductive activity is signalled by the formation of numerous tubular outgrowths from infected eggs which were called "efferent



Figs. 2—13. Development of *Atkinsiella dubia* at 18° C. Figs. 2—4. 20 h plants on solid media, Figs. 2 and 3 showing zoospore cyst, long germ tube and young thallus. In Fig. 4 lower expansion original cyst with remainder of thallus budded from it. No germ tube. Figs. 5 and 6. 48 h plants. All young plants show strong and early development of central vacuole. Fig. 7. Five-day plant with cyst and tube still visible. Fig. 8. Five-day plant at room temperature showing strong 3-dimensional lobulate character of thallus. Fig. 9. Portion of a thallus showing collapsing of cytoplasm into center of cell; the first stage in primary zoospore formation. Fig. 10. Two lobes of thallus with open discharge tubes. The cytoplasm mass is dividing into primary zoospores. Fig. 11 a—e. A series of freehand drawings showing from a to d germination of primary zoospore cyst and stages in formation of secondary zoospore; e an interpretation of the secondary zoospore. Fig. 12. Portion of a thallus showing secondary zoospores emerging from cysts and escaping through broad discharge tube. Fig. 13. Portion of a thallus showing one lobe (a) in vegetative stage whereas others are forming primary zoospores. Figs. 2—7 and 12 $\times 300$. Figs. 8—10 and 13

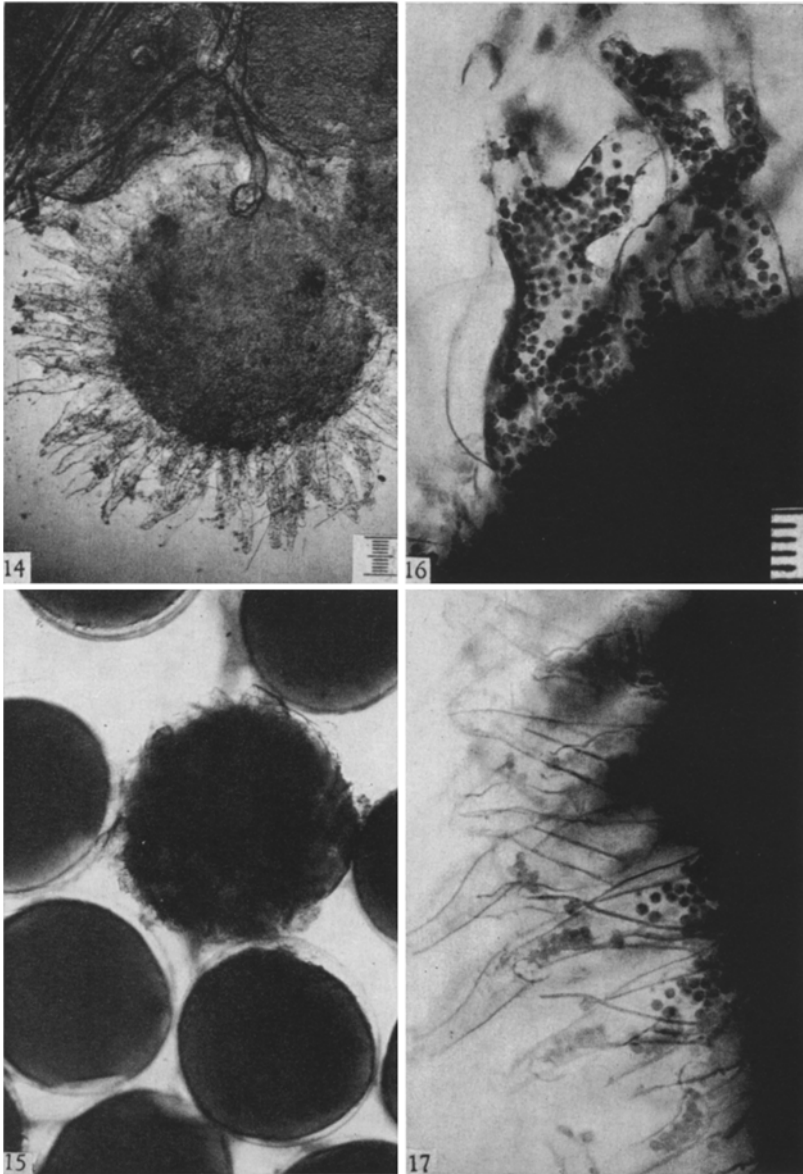


Fig. 14. "Efferent hyphae" (discharge tubes) amerging from heavily infected egg. Fig. 15. Single infected "fuzzy" egg in cluster of normal ones. Figs. 16 and 17. Discharge tubes with encysted primary zoospores emerging from eggs. Absolute scales (10 μ least division) for Figs. 14 and 15 on Fig. 14; for Figs. 16 and 17 on Fig. 16. Fig. 15 courtesy of Dr. Waaland

hyphae" by Atkins (1954) (Fig. 14). These were often broad and irregular and were the future discharge tubes for the zoospores. Meanwhile, the contents which are disposed reticulately around the enormous central vacuole (Fig. 13a) undergo changes associated with zoospore formation. The first of these is the loss of the net-like appearance and assumption of a granular aspect. There then follows a collapsing of the contents, save for delicate strands connected with the inner thallus wall, into the center of the cell (Figs. 1a and 9). Fairly quickly the mass is now cleaved out apparently basipetally into squirming bodies which often as they oscillate move apart from the expanding mass and are seen to bear flagella (Fig. 10). Just how these flagella are borne is not clear to me even under phase microscopy but according to Fuller *et al.* (1964) they are anteriorly attached to the pyriform body of the spore as in the "primary" spore of *Saprolegnia*. At this time, too, the tips of the numerous discharge tubes open. Such spores continue to writhe and oscillate within a very limited but expanding area for about an hour when they encyst. They are 6–8 μm long by 4–6 μm wide. The cysts are 7–9 μm in diameter. Atkins (1954) has reported a few primary swimmers may leave the sporangium. At intervals the cysts germinate. In the process (Fig. 11) a papilla forms on the cyst wall and then it dissolves and the contents rather rapidly (3–5 min) emerge (Fig. 11a and b). Outside after 3 to 5 min the spore mass begins to rock and stubby flagella appear which steadily elongate and start to wave (Fig. 11b and c). During the next 20 min the spore body assumes its mature shape, which is rather narrow, and bears two oppositely directed nearly equal flagella arising from a shallow groove on one surface (Fig. 11e). Such "secondary" zoospores swim in a lively fashion inside the sporangium until they find their way out the nearest broad orifice of the discharge tube (Figs. 1, 16 and 12). They are 9–10 μm long by 4–6 μm wide and tapered at their apices. The scattered empty, extremely thin-walled cysts are left behind as dimly-seen objects which could easily be overlooked (Fig. 1). The disproportionately wide discharge tubes and cysts are conspicuous features of infected eggs (Figs. 16 and 17). There is evidence that the zoospores may undergo repeated emergence in the medium if they do not reach a substratum.

No evidences for sexual reproduction or formation of resting structures of any kind were found.

Discussion

Several aspects of *Atkinsiella* are worthy of further consideration. The capacity of the Friday Harbor material to invade healthy crab eggs has not, owing to time limitations, been tested. Inasmuch as the fungus was only found in sponges of eggs removed from the crab and stored for

some weeks in a refrigerator, it was probable that fungus propagules present in the egg mass, or those from field-infected eggs, attacked weakened or moribund hosts. A study of Atkins' paper (1954) reveals that here, too, the fungus only appeared under laboratory conditions. That *Atkinsiella* can be a virulent parasite of healthy eggs to my mind remains to be proved.

Morphologically, the fungus presents several points of interest. Thalli grown in pure culture in nutrient broth and suddenly starved do occasionally produce rhizoids which are cut off by distinct cross-walls from the thallus. This feature which has not as yet been observed in material dissected from eggs, renders a holocarpic fungus eucarpic, and hence has a bearing on its relationship to other saprolegnians. How much weight should be accorded this character, not as yet known from material from nature, becomes a question.

In the establishment of the thallus from the encysted zoospore, the quick appearance of the large central vacuole—a well-known saprolegniaceous character—was noteworthy.

Non-sexual reproduction presented several unusual aspects. The first of these was the sequential transformation of parts of a single thallus into zoospore production. Thus, a glance at Fig. 1 will show parts of the contents of this thallus to be 1. in the vegetative stage, 2. collapsing towards the center preparatory to dividing into primary zoospores, 3. primary encysted zoospores from which secondary zoospores are leaving the stove-pipe-like open discharge tubes. Such development results in zoospore production being extended over a relatively long period of time and probably is the reason, rather than numerous thalli from multiple infections, why it has been observed a single infected egg can produce zoospores for nearly a week. Such a sequential development in a single thallus has also been noted by me in another marine Phycomycete *Haliphthorus*.

The collapsing of the cytoplasm into the center of the cell and the basipetalous cleavage of zoospores are also unusual features. Formation of the disproportionately broad discharge tubes and their precocious opening for escape of the small zoospores and encystment of the latter in the sporangium are all features found in the unusual saprolegnian parasite of the brown seaweeds *Striaria* and *Ectocarpus* (*Eurychasma dicksonii*) (Sparrow, 1934). Despite the supposed lack of rhizoids in *Eurychasma*, its resemblances in other ways to *Atkinsiella* suggests to me a close relationship of the two.

The supposed absence of sexuality and resting structures in *Atkinsiella* as in other marine Phycomycetes I suspect may be due to some sort of as yet hidden sexuality and alternation of generations, as in the seaweeds.

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