A FUNGUS PARASITE IN OVA OF THE BARNACLE CHTHAMALUS FRAGILIS DENTICULATA

T. W. JOHNSON, JR.

Department of Botany, Duke University, Durham, North Carolina

Species of fungi in marine animals are apparently not numerous, although some are very destructive parasites. Outstanding among the latter are Ichthyosporidium hoferi (Plehn and Mulso, 1911; Sproston, 1944) in herring, salmon, and flounder, and Dermocystidium marinum (Mackin et al., 1950; Ray and Chandler, 1955) in oysters. Among other reports of marine zoophagous fungi worthy of mention are: Cycloptericola marina in Cyclopterus lumpus (Apstein, 1910); Leptolegnia marina in the pea crab, Pinotheres pisum (Atkins, 1929, 1954a); a saprolegnaceous and a pythiaceous fungus in the calcareous shells of molluscs (Bornet and Flahault, 1889); Spongiophaga sp. in sponges (Galtsoff, 1940); three species of Nephromyces in the ductless kidneys of various ascidians (Giard, 1888); a pink yeast (Torula) in oysters (Hunter, 1920); boring fungi in various shell-forming animals (Kölliker, 1860); two species of Thalassomyces in the decapod Pasiphaea (Niezabitowski, 1913); a marine Laboulbenia on Aepus robini (Picard, 1908); an Ascomycete, Didymella conchae (Bonar. 1936) in mollusc shells (a marine lichen according to Santesson, 1939); Sirolpidium zoophthorun in lamellibranch larvae (Vishniac, 1955), and unnamed marine eccrinids in Panopeus herbstii and Emerita talpoida (Wolf and Wolf, 1947). A very few species of fungi occur in the ova of marine invertebrates: Lagenidium callinectes (Couch, 1942) in the blue crab, Callinectes sapidus; Plectospira dubia and Pythium thalassum (Atkins, 1954b, 1955) in the pea crab, and an unnamed fungus suggestive of Sirolpidium zoophthorun and Plectospira dubia in the oyster drill, Urosalpinx cinerea (Ganaros, 1957). Two fungi have been described from barnacles. The Ascomycete Pharcidia marina (Santesson, 1939, places this organism in the lichen genus Arthropypenia) occurs on the shells of Balanus balanoides (Bommer, 1891); a second Ascomycete, Didymella balani (also renamed as a lichen) develops on the test of Chthamalus stellatus (Hariot, 1887). A new species of Phycomycete, Lagenidium chthamalophilum, parasitic in the ova of Chthamalus fragilis var. denticulata, is described in this paper.

Lagenidium chthamalophilum sp. nov.

Hyphae crassae, contortae vel irregulares, ramosae; intra- et extramatricales, vacuolis et guttulis multls, pallide flavae usque ad hyalinis; plerumque 10–18 μ in diam. Sporangium ex septatione hyphae formatum, tubulo singulo apice dilato in vesicam sphaericam. Sporae reniformes, a lateri biflagellatae, in vesica formantur et vesica deliquescente emittuntur. Oögonia rara; lateralia vel terminalia vel intercalaria sed semper in hyphis intramatricalibus; globosa vel subglobosa vel elongato-irregularia; 19–47 μ diam. Oösporae singulae, vel raro binae; sphaericae

205

Hyphae stout, contorted or irregular, branched; filling the ova and emergent from them; generally reticulately vacuolate, with numerous minute cytoplasmic oil bodies, infrequently with very diffuse cytoplasm containing oil bodies; pallid golden-yellow to hyaline; occasionally constricted at points of penetration through the egg membrane; variable in diameter, generally 10–18 μ; occasionally producing globose, lateral swellings up to 39 μ in diameter. Sporangia formed by segmentation of intramatrical hyphae, very rarely produced on extramatrical hyphae; variable in length, diameter coincident with that of hypha; each producing one stout emergent discharge tube expanded apically into a spherical vesicle. Planospores reniform, laterally biflagellate, 8.5–10.2 × 6.8–8.5 μ; cleaved from sporangial protoplasm within the vesicle; aplerotic; discharged upon rapid deliquescence of vesicle. Oögonia rare: lateral, terminal, or intercalary on intramatrical hyphae; globose, subglobose, or slightly elongate-irregular; 19–47 μ in diameter. Oöspores 1, rarely 2; spherical, blunt-conic, or nearly ellipsoid; containing a single centric or eccentric mass of small oil globules; 18–27 × 16–23 μ, spherical ones 21–25 μ in diameter; germination unknown. Antheridia not observed.

Parasitic in ova of Chthamalus fragilis denticulata, Beaufort Inlet, North Carolina, June 17, 1957 (TYPE), leg. J. D. Costlow.

Chthamalus fragilis denticulata, common in the Beaufort, North Carolina region, is a small, pallid-brown to grayish-white, sessile barnacle attaching to pilings, rocks, sea walls, to other barnacles, and to the stems and leaves of Spartina alterniflora. The animal occurs only on the uppermost portions of pilings, for example, near the high water line. These barnacles, among the last to be covered by water at incoming tide, are submerged for the short slack high water period, and are the first to be exposed on ebb tide. Chthamalus fragilis denticulata occurs with, in fact often attaches to, a second equally abundant barnacle, Balanus amphitrite. The lamellae (egg cases) of C. fragilis denticulata are usually paired and lie free within the mantle. The larval planktomic stage, the nauplius, develops and is liberated within the parent barnacle. Uninfected eggs (in mass) change color as the embryo develops: bright orange-yellow, pallid yellow, pale cream. Infected lamellae, however, are often pallid gray or grayish-green.

Lagenidium chthamalophilum may develop in the ova of Chthamalus fragilis denticulata at any time between late gastrulation and emergence of the nauplii. Released nauplii are apparently not infected, nor are there any somatic tissues of the parent animal invaded by the fungus. Ova showing three or more appendage buds are generally most often infected (Fig. 6). Early stage embryos (one or more appendage buds) in entire egg masses may be destroyed, leaving only a cluster of egg membranes filled with fungus mycelium (Figs. 1, 2). On the other hand, if lamellae with more mature or differentiated embryos within the egg mass become infected, some embryos escape invasion by the fungus and develop into

Figures 1–9. Lagenidium chthamalophilum. 1, 2, hyphae within ova membrane; 3, early infection stage showing branching of hypha; 4, emergent hyphae with a sporangial and oögonial initial; 5, coiled extramatrical hypha; 6, infection by two spores of an embryo with three appendage buds; 7, vacuolate extramatrical hypha; 8, guttulate extramatrical hypha; 9, intramatrical hyphae showing constrictions and two sporangial initials.
Figures 10-27.
the planktonic stage. Infection visible in one or two peripheral ova in a lamella spreads rapidly through the entire cluster so that within two days (continual submersion in raw sea water) all embryos are invaded.

Inoculation is brought about by laterally biflagellate planonts. After a 10–15 minute period of active swimming (in 50 ml. of raw, aerated sea water, at 25° C.) the spores settle on an ovum (Fig. 18a) without rounding up. Within three minutes after attaching to the egg membrane, the spore protoplast penetrates the membrane (Fig. 18b), enlarges rapidly into a foot-like hyphal rudiment (Fig. 18c), and grows along the embryo. Within 25 minutes after inoculation, infection has been established and the young hypha is developed (Fig. 6). Whether hyphae actually penetrate the embryo is not known; the dense, opaque embryonic host cells prevent direct observation, and a suitable technique for fixing and sectioning infected eggs has not been developed.

The vegetative hyphae of Lagenidium chthamalophilum are very characteristic. Early in the incubation period, the hyphae become highly vacuolate, and generally maintain a reticulate vacuolation throughout development. Hyphae are characteristically “foamy” in appearance, suggestive of those of Monoblepharis. Emergent hyphae, similarly, are usually extremely vacuolate (Figs. 4, 7), but occasionally have very diffuse, strand-like cytoplasm (Fig. 8). In either case, the many minute refractive oil bodies in the cytoplasm give it a readily discernible golden-yellow cast. Emergent hyphae (other than the sporangial discharge tubes and vesicles) are not often observed in the lamellae immediately after dissection from the animal. Such hyphae form in abundance, and sporangial discharge occurs frequently, however, when infected lamellae are placed in sterile sea water and incubated for 12–18 hours at room temperature. Hyphae emerging from an ovum penetrate the membrane either with or without constriction (Figs. 2, 10). The extramatrical hyphae are generally stout and somewhat contorted, freely branched, and of a diameter coincident with that of the intramatrical hyphae. Occasionally, the emergent hyphae are very slender and coiled (Fig. 5), as in Pythium thalassium (Atkins, 1955). Neither the intra- nor extramatrical hyphae are septate except where reproductive cells are delimited.

Sporangia are formed by segmentation of the intramatrical hyphae almost exclusively. During formation of the delimiting septa, that portion of the hypha destined to become a sporangium accumulates protoplasm, and often has a few large vacuoles (Fig. 12). These vacuoles disappear prior to movement of the protoplasm into the apical vesicle. A stout discharge or exit tube (Fig. 11) develops from the cylindrical sporangium, penetrates the egg membrane (without constriction) and elongates. The apex of the exit tube enlarges to form, at first, a subglobose or slightly irregular swelling (Figs. 12, 20) containing very diffuse, vacuolated cytoplasm (Fig. 12). The bulbous discharge tube apex subsequently becomes perfectly spherical; it is completely formed before the sporangial content flows into the tube. The vesicle wall is cellulose as is the basal sporangium and vegetative hypha wall.

Figures 10–27. Lagenidium chthamalophilum. 10, infection in a pre-emergence embryo with eye spot: 11–17, stages in formation of vesicle and spores, and spore discharge (see text); 18, germination and penetration of spore protoplast; 19, planonts; 20, sporangial discharge tube and immature vesicle; 21, immature oögonium; 22–27, mature oögonia; Fig. 19, scale a, others, scale b.
Sporogenesis begins with movement of the sporangial protoplasm through the discharge tube and into the vesicle. Occasionally, the protoplasmic stream separates partially, leaving one or more fusiform masses connected to the main complement by a slender strand (Fig. 13). The sporangial content and the cytoplasm of the tube aggregate into a slightly irregular mass centrally located in the vesicle (Fig. 14). Spores are cleaved out in the protoplasmic mass, appearing first as polygonal units, then as definite spherical or reniform cells (Figs. 15, 16). In no instances observed did the spores fill the vesicle.

Spore discharge is initiated with a slow “shimmering” motion of the vesicular spore mass. The movement then becomes undulating and increases in rapidity until the spores are moving rapidly over and around one another in the center of the vesicle. For one or two minutes the spores are moving extremely rapidly. If such spores are killed with osmic acid fumes and stained with acid fuchsin or gentian violet, short, stubby flagella are visible on the peripheral spores. The vesicle deliquesces (Fig. 16) within 30 seconds, leaving the rapidly moving spores hanging together momentarily. One or two peripheral spores dart away, and subsequently, within a few seconds the spore mass breaks up to liberate rapidly but evenly swimming spores. The entire process, from migration of the undifferentiated protoplasm to spore discharge, is completed within 20 minutes. in raw, aerated sea water at 25–27° C.

Sexual reproductive cells are rarely produced in vivo. Short lateral branches with enlarged apices mature into oögonia containing a single oöspore (Fig. 27), but oögonia may also develop as intercalary (Figs. 22, 26) or terminal (Figs. 23, 25) hyphal segments. Intercalary or terminal oögonia often contain two oöspores. Whether antheridia are produced by L. chthamalophilum is not known; hypogynous antheridial cells, certainly, are not in evidence. In a few instances, short hyphae were observed near oögonia and in contact with them (Figs. 21, 27), but no antheridial cells were evident. These hyphal branches may be nonfunctional antheridial branches; if so, they are of monoclonous and androgynous origin (Johnson, 1955, pp. 14, 15). Extrametrical hyphae do not produce sex cells.

Attempts to culture Lagenidium chthamalophilum were successful. Spores germinated well on aged sea water agar, and on sea water agar fortified with 0.1% glucose and 0.05% yeast extract. Subsequent growth was very sparse, though extensive, and neither sexual nor asexual cells developed in culture. Very slender, sparingly branched, contorted, vacuolate hyphae are produced on the agar media.

The parasite can, in my opinion, be assigned equally well to Pythium (Middleton, 1943) or Lagenidium (see Sparrow, 1943) as these genera are presently understood and circumscribed. In both genera, planont maturation occurs, generally, in an evanescent vesicle produced at the apex of a sporangium or sporangial discharge tube. In neither genus, however, is the vesicle pre-formed. This fact alone, were it to be considered significant at the generic level, would exclude the barnacle parasite from both genera. On the other hand, the evidence is stronger in favor of assigning the fungus to Lagenidium than to Pythium. The lagenidioseous features of the parasite are: the “foamy,” granular cytoplasmic content of the stout, branched hyphae; sporangial delimitation by hyphal segmentation, and oöspore formation by contraction of hyphal segment content. The nonseptate nature of the hyphae, of course, suggests Pythium rather than Lagenidium, although members of the latter genus having pythiaceous mycelium are known (Sparrow,
1943). The fungus in barnacle ova, while suggestive vegetatively of the Aphragmium type of Pythium, has a simplified sexual apparatus, that is, no well defined antheridium, and no periplasm in the oögonium. In the final analysis, assignment of the fungus to Lagenidium turns, I believe, on simplicity of the sexual structures.

Two marine species of Lagenidium are known. Lagenidium sp. (Johnson, 1957) produces sporangia formed by hyphal segmentation just as does L. chthamalophilum, but the hyphae of the former are not stout and vacuolate, and the vesicle is not pre-formed. These two features also separate L. chthamalophilum from L. callinectes (Couch, 1942), parasitic in ova of Callinectes sapidus. Furthermore, L. callinectes has a persistent vesicle, the barnacle parasite does not. Lagenidium chthamalophilum differs in several significant respects from all other known species in the genus. The irregular, contorted, stout hyphae (with lateral lobulations) of L. entophytum (Pringsheim) Zopf suggests L. chthamalophilum, but other features separate the two immediately. Lagenidium closterii deWildeman produces an extramatrical sporangial discharge tube, as in L. chthamalophilum; the hyphae of the former are more delicate and the discharge tube is bulbous at the base. The pythiaceous hyphae of L. marchalianum deWildeman are very slender and markedly constricted; these differ significantly from the stout, vacuolate hyphae of L. chthamalophilum. The only other myceloid member of Lagenidium suggestive of the barnacle parasite is L. giganteum (Couch, 1935). Couch’s species, however, has segmented mycelium, and lacks a pre-formed vesicle.

Vegetatively, Lagenidium chthamalophilum resembles Plectospora dubia (Atkins, 1954b), particularly in the stout, irregularly branched and swollen hyphae. In other characteristics, notably those of sporogenesis and discharge, these two fungi are obviously dissimilar. Pythium thalassium, parasitic in Pinnotheces pism ova (Atkins, 1955), produces very stout hyphae resembling those of L. chthamalophilum, but the Pythium has two major characteristics distinguishing it from the barnacle parasite: the sporangia of P. thalassium are filamentous and proliferate internally, and the hyphae are not highly vacuolate.

The geographical distribution of Lagenidium chthamalophilum is not known, inasmuch as only host barnacles in the immediate vicinity of the Duke Marine Laboratory have been examined. Forty-four collections (totalling 1284 individuals) of Chthamalus fragilis denticulata were made within a five-mile radius of the Laboratory, including two series of collections on the outer banks of the Inlet region. The number of egg-bearing parent barnacles, and the number of infected lamellae in any one collection varied considerably. A sample series of ten collections, showing infection incidence, is given in Table I (each animal with paired lamellae). Occasionally, barnacles were collected in which only one egg mass was found. Twenty-one per cent of such masses were infected. It should be noted that some infected lamellae may have been overlooked, especially if they had been inoculated shortly before the animals were collected. For example, in eight dissections out of twenty-nine, visually uninjected ova masses showed infection after three days in storage in sterile, filtered sea water. Thirty-four per cent of all examined C. fragilis denticulata lamellae (1016 individual cases) were infected. Percentages of infection are based on hosts collected from pilings and mooring stakes. The same species of barnacle occurring on Spartina alterniflora showed some infection, but only infrequently. Only 3 of 86 barnacles (with one or two lamellae) from Spartina were invaded by the Lagenidium.
A replicated experiment was performed to determine whether *Lagenidium chthamalophilum* is actively parasitic in ova of *Chthamalus*, or is an invader of moribund eggs. Egg masses were dissected from the barnacles with sterilized needles, examined immediately with a dissecting microscope, and separated into two lots, infected and uninfected. As each lamella was removed, it was placed in a drop of sterile sea water to eliminate inadvertent inoculation in handling infected and uninfected eggs. The obviously parasitized egg masses were easily detected with the dissecting microscope. Infected lamellae were placed in small Petri plates, covered with sterile sea water, and incubated overnight. This short period of incubation induced sporulation. The uninfected lamellae were kept in pairs, as they were dissected from the parent animal, and incubated for 12–24 hours in drops of sterile sea water on slides in damp chambers. The masses were then examined; if no infection was visible, one lamella of the pair was placed in the dish with the sporulating fungus, the other lamella (from the same animal) in a separate Petri plate of sterile sea water, and utilized as the control. No specific stages of embryo development were selected for the tests. The plates were incubated at room temperature, and examined at 1, 3, and 5 days. Some plates containing a parasitized and nonparasitized egg mass were discarded when the control lamella (one of the uninfected pair) showed signs of the fungus.

No infection was visible in the "uninfected" egg cases by the end of 24 hours. At 72 hours, however, all naturally uninfected lamellae had been invaded by *Lagenidium chthamalophilum*. Two controls showed infection, and three lamellae in the dishes with the fungus had matured into nauplii; these plates were discarded. In sea water, in the laboratory, visible infection develops between 24 and 72 hours; inoculation, presumably, may occur during the first 24-hour period. Under natural conditions, inoculation must occur (since the "inoculum" is a motile spore) during the short periods (twice in approximately 24 hours) that the opercula of the barnacle are open while the animal is submerged at high tides. This period of time varies roughly—during the neap tide periods, at least—between 45 and 90 minutes, diurnally, for those individuals highest on the substratum. It is true that an exposed, closed animal retains sufficient water within the mantle to enable the

### Table 1

*Incidence and percentage infection of Chthamalus fragilis denticulata lamellae by Lagenidium chthamalophilum*

<table>
<thead>
<tr>
<th>No. animals in sample</th>
<th>No. animals with ova</th>
<th>No. of lamellae</th>
<th>No. infected lamellae</th>
<th>Percentage infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>41</td>
<td>14</td>
<td>28</td>
<td>8</td>
<td>28.5</td>
</tr>
<tr>
<td>34</td>
<td>31</td>
<td>62</td>
<td>42</td>
<td>67.7</td>
</tr>
<tr>
<td>15</td>
<td>15</td>
<td>30</td>
<td>28</td>
<td>93.3</td>
</tr>
<tr>
<td>13</td>
<td>12</td>
<td>24</td>
<td>16</td>
<td>66.6</td>
</tr>
<tr>
<td>43</td>
<td>37</td>
<td>74</td>
<td>54</td>
<td>72.9</td>
</tr>
<tr>
<td>22</td>
<td>3</td>
<td>6</td>
<td>6</td>
<td>100.0</td>
</tr>
<tr>
<td>11</td>
<td>6</td>
<td>12</td>
<td>2</td>
<td>16.6</td>
</tr>
<tr>
<td>41</td>
<td>40</td>
<td>80</td>
<td>62</td>
<td>77.5</td>
</tr>
<tr>
<td>65</td>
<td>49</td>
<td>98</td>
<td>84</td>
<td>85.6</td>
</tr>
<tr>
<td>23</td>
<td>8</td>
<td>16</td>
<td>2</td>
<td>12.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. of lamellae</th>
<th>No. infected lamellae</th>
<th>Percentage infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>28</td>
<td>8</td>
<td>28.5</td>
</tr>
<tr>
<td>62</td>
<td>42</td>
<td>67.7</td>
</tr>
<tr>
<td>30</td>
<td>28</td>
<td>93.3</td>
</tr>
<tr>
<td>24</td>
<td>16</td>
<td>66.6</td>
</tr>
<tr>
<td>74</td>
<td>54</td>
<td>72.9</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>100.0</td>
</tr>
<tr>
<td>12</td>
<td>2</td>
<td>16.6</td>
</tr>
<tr>
<td>80</td>
<td>62</td>
<td>77.5</td>
</tr>
<tr>
<td>98</td>
<td>84</td>
<td>85.6</td>
</tr>
<tr>
<td>16</td>
<td>2</td>
<td>12.5</td>
</tr>
</tbody>
</table>
planonts of the fungus to swim about and presumably cause infection. This seems a less likely time for infection to occur, however, since tests show that heavily fouled, oxygen-depleted sea water has a retarding effect on spore discharge and movement.

The close natural association of Chthamalus fragilis denticulata and Balanus amphitrite prompted a replicated series of artificial inoculations using infected lamellae from C. fragilis denticulata and uninfected masses from B. amphitrite. Lamellae were dissected and incubated in the manner described previously. Forty-three attempts at inducing infection in B. amphitrite ova were made. No infection in B. amphitrite eggs was evident at the end of 21 days, although the infected ova of C. fragilis denticulata were completely destroyed at the end of the three-week incubation period. While some lamellae of B. amphitrite were actually inoculated with planonts of the Lagenidium (visual inspection of individual ova), these spores did not germinate, or, if they germinated, did not penetrate the egg membrane. Many B. amphitrite egg masses were examined from animals collected in the same localities as C. fragilis denticulata, but none was infected. Dr. J. D. Costlow has never observed infection in the lamellae of B. amphitrite, although he has used ova from this species in extensive studies on larval development. These observations, in view of the proximity of B. amphitrite and C. fragilis denticulata in natural habitats, suggest the hypothesis that the ova of the former are, if not immune, certainly highly resistant to L. chthamalophilum.

The importance of Lagenidium chthamalophilum in reducing Chthamalus fragilis denticulata populations cannot be judged from this preliminary investigation. Certain further studies on the fungus and its host, however, may be of significance in elucidating the effect of the fungus. Significant among these studies are: distribution and severity of infection; conditions favorable to establishment and spread of infection; the period, in the reproductive cycle of the host, at which the animal is most susceptible; any fluctuations (and causes thereof) in percentage of infection, and a search for other suspects.

The support of the National Science Foundation, through Grant G-2324, is gratefully acknowledged. I am indebted to Dr. J. D. Costlow, Duke University Marine Laboratory, for technical guidance in the zoological aspects, and to several of my colleagues for opinions and criticisms of the mycological portions of the investigation. Mr. Thomas M. Simkins, Jr., Duke University Library, very kindly prepared the Latin diagnosis.

Summary

1. Lagenidium chthamalophilum is described as a parasite of the ova of Chthamalus fragilis denticulata. The pathogen is characterized by the formation of a vesicle before sporangial protoplasm migration, and by highly vacuolate, stout vegetative hyphae. In these features, L. chthamalophilum differs from the usual interpretation of members of the genus. The fungus is compared with other Phycomycetes known to parasitize crustacean ova.

2. Artificial inoculation experiments show L. chthamalophilum to be specific for C. fragilis denticulata. The associated barnacle, Balanus amphitrite, is resistant to the fungus.
LITERATURE CITED


