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The Role of Marine Fungi in the Penetration of Calcareous Substances

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SYNOPSIS. All publications dealing with fungi described from marine calcareous substrates are discussed in this survey of the literature. Original investigations of fungi from lime tubes of teredinids are included. Wood-inhabiting marine fungi, especially in warm waters, grow within calcareous linings of teredinid burrows. Fruiting structures of the Ascomycetes, *Halosphaeria quadricornuta* and *Remispora salina*, and the Deuteromycete, *Periconia prolifica*, form cavities in the tube linings, making them brittle and soft. Representatives of *Cirrenalia* and *Humicola* sporulate on the surface of calcareous linings. The fungus, *Pharcidia balani*, sometimes referred to as a lichen, decomposes shells of barnacles and molluscs. Another Ascomycete, *Lulworthia kniepii*, parasitizes calcareous algae, probably living on the middle lamellae, not on the calcified cell walls.

The first record of fungus-like structures in calcareous matter of marine animals dates back more than 100 years. Since that time, hyphae and reproductive organs of marine fungi have been found in shells of many Balanidae and Mollusca, as well as some calcified algae. These penetrating organisms are members of the higher fungi (Ascomycetes and Deuteromycetes) as well as the lower fungi (Phycomycetes). In the following survey of the literature, publications are discussed which deal with fungi described from calcareous substrates in the sea. Original investigations of fungi occurring in lime tubes of Teredinidae are also reported.

PENETRATION OF CALCAREOUS ANIMAL PRODUCTS BY MARINE FUNGI

Only those fungi are considered which actually grow in the interior of calcareous substrates. There are others, especially arenicolous representatives of the genus *Corollospora* Werderm., which fruit frequently on fragments of calcareous animal products in marine beaches (Kohlmeyer, 1966); however, these species develop only on the surface of such substrates and do not penetrate them.

Fungi in Shells of Molluscs

"Shells" sensu stricto. Several of the earlier reports on so-called fungal growth in bivalve shells are obscure concerning the penetrating organisms. Today, it is impossible, in most cases, to clarify whether the boring organisms described were fungi or algae. Kölliker (1860*a*, *b*) found what he called "unicellular fungi" in hard tissues of molluscs, balanids, and other animal groups. Parasites observed by Kölliker are today considered algae by most investigators (v. Pia, 1937). Fungoid sporangia with filamentous processes were found by Stirrup (1872) in shells of Mollusca. Bornet and Flahault (1889) described the fungus, *Ostracoblabe implexa*, which later (Bornet, 1891) was believed to be the fungal part in the lichen, *Verrucaria consequens* Nyl. The identity of another fungus, *Lythopythium gangliiforme*, described by Bornet and Flahault (1889) remained uncertain as well (Bornet, 1891).

New interest arose in recent years in microorganisms that inhabit calcareous substrates when Porter and Zebrowski (1937) and Zebrowski (1936) reported on

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lime-loving Phycomycetes as well as other fungi related to Myxomycetes. These authors observed the fungi in calcareous sands from Australia, China, Africa, Texas, North Carolina, and the West Indies. These fungi penetrate the calcareous substance and produce a network of hyphae with sporangia. Fragments of molluscan shells were the most common substrates; however shells of Foraminifera, Ostracoda, and spicules of calcareous sponges were also found to be attacked. None of the microorganisms described by Porter and Zebrowski (1937) and Zebrowski (1936) was cultured in the laboratory. Thus, it is not possible to judge the penetrative mechanisms of these organisms or whether they are of recent or fossil origin.

Johnson and Anderson (1962) found hyphae and chlamydospores of a fungus that showed affinities to *Endogone* (Phycomycetes) in a cast-off shell of *Anomia simplex* d'Orbigny collected in North Carolina. No information on the penetrative mechanism of this organism was available.

Korringa (1951) reported a shell-disease in the oyster (*Ostrea edulis* L.) in Dutch waters. An unidentified fungus was thought to be the causal agent of the disease. At first, tiny specks of a chalky white color appeared on the shell's inner surface, followed by green and brown rubber-like spots and warts, caused by an abnormal intense secretion of conchyolin. Fungal hyphae were found branching abundantly in the shell's interior. No description of the microorganism nor of its activity in the shell were furnished by Korringa. Alderman and Jones (1967) finally cultured and described the fungus causing the shell-disease in Britain as a biflagellate Phycomycete, a member of Saprolegniales. How the fungal zoospores penetrated the shell and whether the branching mycelium decomposed the calcareous substance or lived on the protein matrix remains unresolved.

The most recent note on the occurrence of fungal hyphae and empty fruiting bodies within shell fragments was given by Höhnk (1967) who observed these unidentified fungi on the Rumanian coast of the Black Sea.

In many of the publications mentioned above, the identity and taxonomic position of the penetrating organisms have remained uncertain and the mechanism of penetration into the calcareous substrate has not been considered.

Calcareous linings of teredinid tubes (original investigations). Hyphae of wood-inhabiting, higher, marine fungi are present in the outer layers of wood subjected to the marine environment. Because of their high O_2-requirement, hyphal penetration is rarely deeper than 500 μ. Wood-boring animals, shipworms and gribbles, penetrate deep into the substrate, and it is through their tunnels that fungi are able to invade the interior of the wood. After death of teredinids, calcareous linings of their tubes undergo a deterioration that appears to be caused by fungi. Fungal hyphae develop in the wood surrounding the calcareous tubes. They finally grow on the lime surface and enter it, and the once whitish tubes turn brownish by the action of the fungi. The calcareous material crumbles and may separate into thin layers. After treatment of old tubes with decalcifying solutions (*e.g.*, hydrochloric acid) a network of brown hyphae and a thin cuticula (conchyolin?) remain.

Those fungi found on and in the calcareous linings of teredinid tubes are Ascomycetes and Deuteromycetes. Fruiting bodies of marine Ascomycetes, especially the warm-water species, *Halosphaeria quadricornuta* Cribb et Cribb, and *Remispora salina* (Meyers) Kohlm. (Kohlmeyer, 1968), often develop within the calcareous substrate and can be seen as dark shadows in the tube linings (Fig. 1). Broken tubes exhibit perithecia that have grown inside the calcareous matter forming cavities in this substrate (Figs. 2a-c). The necks of the perithecia pierce the material around the fruiting body and reach the surface of the tube (Fig. 2b). Ascomycete ascomata can be found predominantly in the thickened posterior ends of the tubes. Within these calcareous tubes,

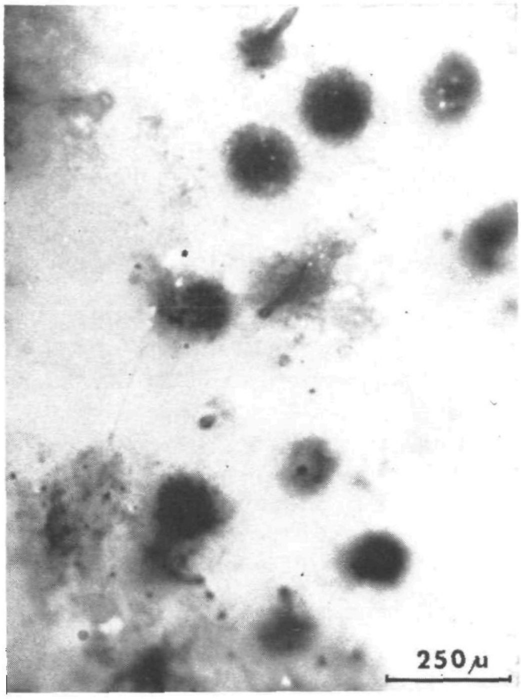

FIG. 1. Black perithecia of *Remispora salina* embedded in the calcareous lining of a teredinid burrow; tips of necks reach the surface of the tube (Liberia, Herb. J. K. 1888).

the diameter of perithecia is often smaller than that of the perithecia which have developed in wood. The relative hardness of the substrates probably influences the size of the fruiting bodies. Perithecia developing within bends of the tube where the calcareous substance is not as hard as in the posterior end, separate the substrate into thin lamellae, pushing them apart.

Ascomycete fruiting bodies from cold and warm waters were found in wood tissues under the calcareous linings of empty teredinid tubes. While the perithecia were embedded in the wood, their necks had pierced the calcareous material, penetrating with their tips into the cavity of the tube. Thus, ascospores were able to be transported with water currents away from the orifice of the neck, even if the perithecium was completely covered by the teredinid tube.

Marine Deuteromycetes often develop on the surface of empty teredinid tubes. Again, fungi from warm waters are more frequently found on these substrates than species collected in temperate zones. *Cirrenalia pygmea* Kohlm. and *Humicola alopallonella* Meyers et Moore (Fig. 3) belong to species sporulating on the surface of the calcareous linings while the Hyphomycete, *Periconia prolifica* Anast., from Hawaii fruits within the substance of

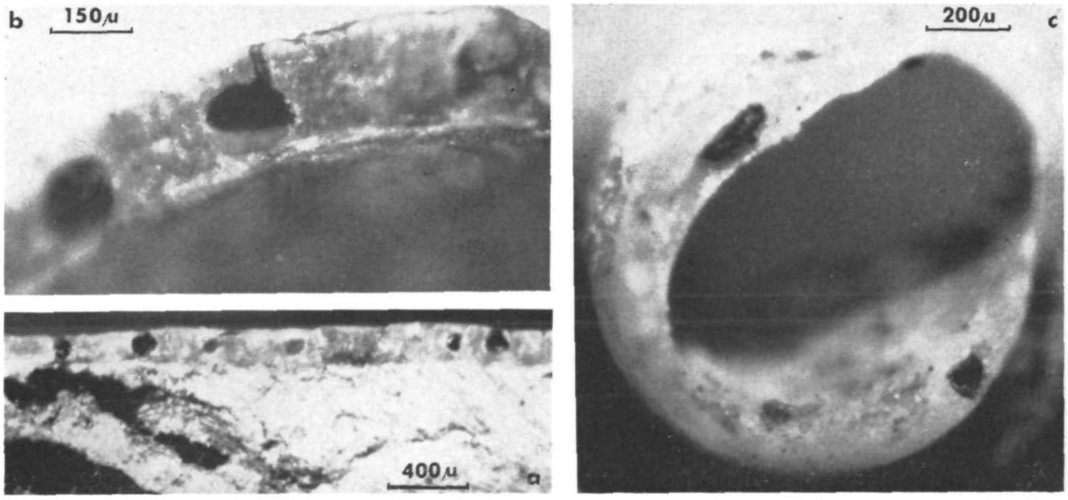

FIG. 2. Black perithecia of marine Ascomycetes within the calcareous linings of teredinid burrows, exposed by breaking of the tubes. a = *Halosphaeria quadricornuta* (Liberia, Herb. J.K. 1812). b = *Remispora salina,* neck reaches the outer surface of the tube (Liberia, Herb. J.K. 1888). c = *R. salina,* perithecia in the posterior end of a tube (Liberia, Herb. J.K. 1888).

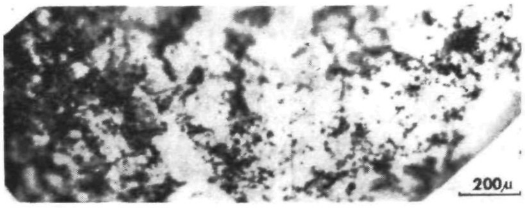

FIG. 3. Conidia and hyphae of *Humicola alopallonella* on the inner surface of calcareous lining of an empty teredinid burrow (Liberia, Herb. J.K. 1812).

the tubes (Fig. 4). This last fungus causes pustules within the tubes which finally break open. In early stages of development, the pustules appear as small umbos on the inner wall of the calcareous linings. These umbos contain brownish hyphae and chains of immature, hyaline, one-celled conidia. At a later stage, these pustules are filled with brown conidia and open at the top like craters (Figs. 4a, b). Apparently, the fungus is developing within the calcareous substrate and causes its inflation.

The teredinid tube containing the fungus was empty when the investigation was performed. Therefore, it was not known whether the Deuteromycete developed in the calcareous layer before or after the animal died. The firm structure of the lining around the pustules suggested that the fungus was growing while the animal was still alive and that the umbos formed were reactions of the mollusc in encysting the foreign organism. Fungal hyphae must have penetrated the calcareous substrate, probably entering from the wood side, and, finally, sporulating within the lime layer.

At this time, the chemistry of the breakdown of teredinid tubules by marine fungi cannot be explained. Even the chemical composition of the calcareous linings had not been examined until 1968. Using X-ray diffraction, Mr. John L. Culliney of the Duke University Marine Laboratory (pers. comm.) determined that the tube material is magnesium calcite. My own observations indicate that marine Ascomycetes and Deuteromycetes are able to grow and reproduce within the calcareous material, forming cavities in the substrate and making it brittle and, finally, soft. It is possible that the organic matrix (conchyolin?) of the tubes serves as a source of nitrogen for the fungi. Dissolution of calcium carbonate crystals or amorphous $CaCO_3$ by these marine fungi has not been demonstrated.

Isolates of *Halosphaeria quadricornuta* are cultured in our Institute on cellulosic substrates and fragments of shipworm tubes. Small colonies develop on cellulose foil in yeast-extract and sea-water and some hyphae also cover calcareous particles. However, because growth of this fungus is slow and sparse in pure culture and no fructification occurs, it has not been possible to demonstrate actual penetration of the $CaCO_3$ substrate in these preliminary experiments. Hopefully, experiments with pure cultures of other marine fungi and ultra-microscopic investigations will

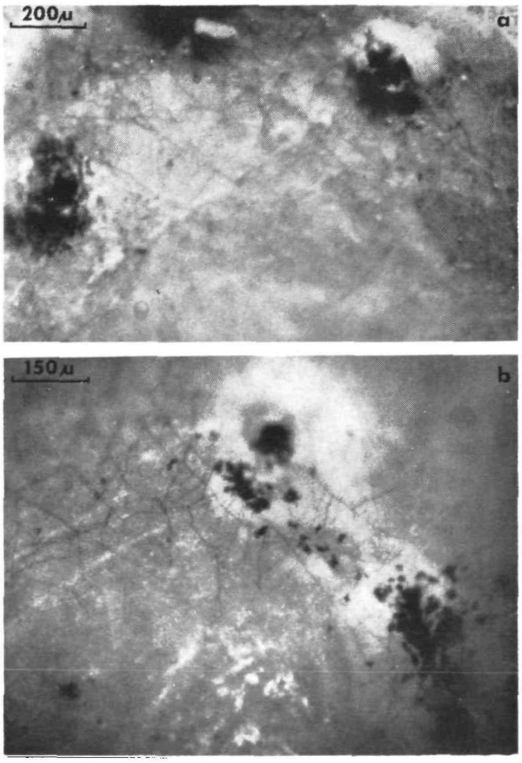

FIG. 4a,b. Crater-like pustules caused by *Periconia prolifica* in the calcareous linings of teredinid tubes, opening towards the inside; hyphae and conidia on the surface (Hawaii, Herb. J.K. 2527).

give clues to the mechanism and chemistry of penetration and destruction of teredinid tubes by fungi.

Fungi in Shells of Balanids

The fungus, *Pharcidia balani* (Winter) Bauch, occurs in calcareous shells of intertidal marine animals throughout the world. Sometimes this organism is associated with microscopic algae and, hence, has been described as a lichen, *Arthopyrenia sublitoralis* (Leight.) Arnold. In this discussion, this organism is treated as a fungus because a symbiotic relationship with algae is not always evident (Kohlmeyer, 1967).

Growth of this fungus in shells of barnacles, as well as in those of intertidal molluscs (Santesson, 1939) is apparent as a roughening of the surface that finally appears spongy and pitted (Bonar, 1936). A branched network of hyphae develops throughout the shell while the animal matrix becomes fragile and granular. The calcareous material of the tubes is disintegrated by the fungus, which eventually develops perithecia and pycnidia. These blackish fruiting bodies are found half-embedded in plates and the operculum of balanids (Fig. 5). As in the case of teredinid tubes, the mechanism and chemistry of deterioration of the calcareous substrate of barnacles are unknown.

PENETRATION OF CALCAREOUS PLANTS (ALGAE) BY MARINE FUNGI

Finally, a case of parasitism will be discussed in which a fungus penetrates highly calcified red algae. The Ascomycete, *Lulworthia kniepii* Kohlm., parasitizes calcareous algae of the genera *Lithophyllum* and *Pseudolithophyllum* (Bauch, 1936; Kohlmeyer, 1963, 1967). I found the same fungus recently in Hawaiian collections of *Porolithon*. The parasite develops a dark mycelium within the host while the surrounding medium becomes discolored. Immersed fruiting bodies with protruding necks grow among the hyphae (Kohlmeyer, 1963). Bauch (1936) described at

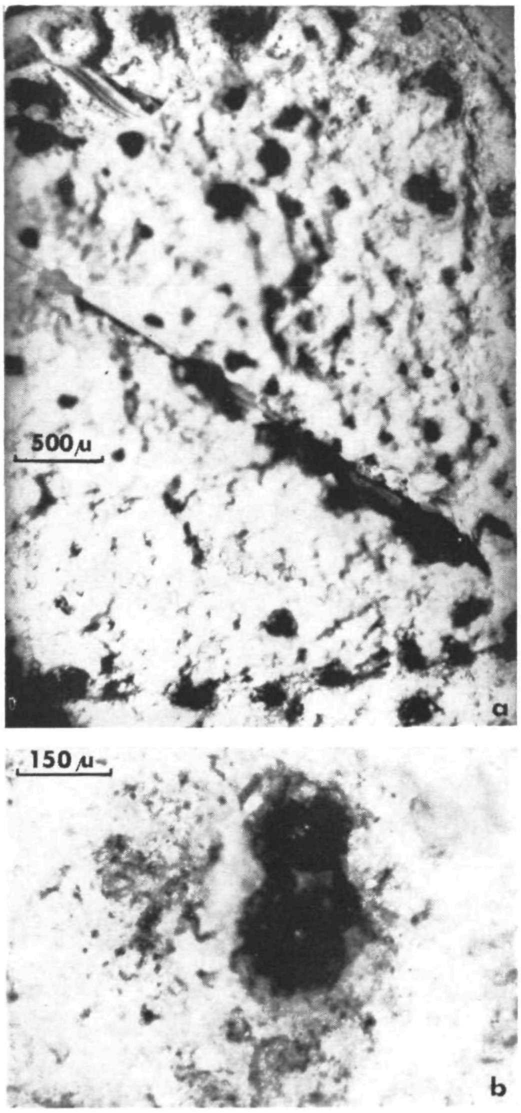

FIG. 5. Black fruiting bodies of *Pharcidia balani* partly immersed in the operculum of *Balanus* sp. a = pitted calcareous surface with perithecia (large) and pycnidia (small). b = two adjoining perithecia with central ostiola (Massachusetts, Herb. J.K. 2601).

length the growth of the fungus within the algal thallus. Hyphae develop in the middle lamellae of the plant, destroying the protoplasm of neighboring cells without penetrating them. Apparently, the calcified, cellulosic, cell walls are not attacked, and thus the hard structure of the alga is not affected by the parasite. Bauch concluded that the calcareous deposit was not dissolved by the fungus. Submicroscopic examinations should help to substantiate this proposition.

Summarizing, we can confirm that many cases of fungal attacks of marine calcareous substrates have been reported in the literature and found in our own investigations. However, information on the mode of penetration and decomposition of $CaCO_3$ by fungal hyphae is completely lacking. It is hoped that this survey will stimulate research along these lines.

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