LAGENIDIUM CALLINECTES COUCH IN BARNACLE OVA

By T. W. Johnson, Jr. and Rupert R. Bonner, Jr.

Reprinted from the Journal of the Elisha Mitchell Scientific Society
Vol. 76, No. 1, May, 1960
Printed in U.S.A.

Reprinted from the JOURNAL OF THE ELISHA MITCHELL SCIENTIFIC SOCIETY
Vol. 76, No. 1, May, 1960
Printed in U.S.A.

LAGENIDIUM CALLINECTES COUCH IN BARNACLE OVA

BY T. W. JOHNSON, JR.[1] AND RUPERT R. BONNER, JR.[2]

In 1942, Couch described *Lagenidium callinectes* from ova of the blue crab, *Callinectes sapidus* Rathbun. Subsequently, Rogers-Talbert (1948) published an account of the fungus in "sponges" (egg masses) from blue crab in the Chesapeake Bay. She gave particular attention to ecological factors. Through the courtesy of Dr. John D. Costlow of the Duke University Marine Laboratory, we were initially provided with infected lamellae of the barnacle *Chelonibia patula* Ranzani. The fungus in these egg masses, and in ones subsequently collected, was examined in various developmental stages, and identified as *L. callinectes*. This paper reports our observations.

Chelonibia patula is a large, white or gray-white barnacle that occurs commonly on the carapace of *Callinectes sapidus* (particularly the old females), and on *Limulus polyphemus* L. (King or Horseshoe crab), among other marine animals. *Chelonibia* lamellae are large, usually paired, and white; as the embryos develop, the eggs undergo color change.

Lamellae were dissected from the animals and placed in raw (as opposed to "aged") sea water. For examination, the material was mounted on ordinary slides or in depression slides. Cross-inoculation and infection tests were carried out as follows: 25 ml of raw sea water were put in each of 10 Petri plates. A small portion of blue crab "sponge" (about 25 ova) was put in each dish, and one infected barnacle lamella added. These cultures were incubated for 48 hours at 25°C, the barnacle eggs removed, placed in separate dishes, and all plates then re-incubated for one week. On the ninth day the crab eggs were examined for infection. Three control plates, consisting only of the crab ova in sea water, were also incubated for the 9-day period. Ova for controls were taken from the same "sponge" used in the cross-inoculation plates.

Characteristics of *Lagenidium callinectes* in barnacle ova differ in some respects from those of the same species as recorded by Couch (1942). The most obvious difference, perhaps, is the preponderance of extramatrical hyphae in the fungus from barnacles. According to Couch, *L. callinectes* mycelium develops entirely within the ovum membrane of the blue crab, but sporulates extramatrically by producing sporangial discharge tubes. The extramatrical hyphae of the fungus in *Chelonibia* are 8–14 μ in diameter, and usually profusely branched; the longest hypha measured was 1030 μ. Internally, the extramatrical hyphae are either reticulately vacuolate or have very diffuse cytoplasm containing numerous refractive oil globules. The emergent hyphae are without septa, but the intramatrical ones are sparingly septate. Commonly, the emergent hyphal apices are bulbous; as far as is known, these expansions do not develop into sporangia. Intercalary globose swellings also develop in the extramatrical filaments. Hyphae within the ovum, however, are very similar to those described by Couch: stout, sparingly septate, irregular, and filling the ovum.

Sporangium and discharge vesicle formation in the fungus from *Chelonibia* are similar, but not identical, to the stages described and illustrated by Couch (1942, pl. 19, figs. 12–14) for the crab-egg fungus. Two features are dissimilar. The first of these is development of the sporangial vesicle at the apex of the long, emergent hyphae. The second difference is in the nature of the vesicle in its early formation. As the vesicle enlarges (at the tip of the sporangial discharge tube), its wall does not seem to be gelatinous. Rather, it is consistent with, and of the same thickness as, the hyphal wall. The vesicle, therefore, is merely an expansion of the discharge tube apex, and is not secreted internally from the tube.

Sporangia are produced through intramatrical hyphal segmentation. Consequently, some of the emergent hyphae (those ultimately producing a vesicle) are the sporangial discharge tubes. In *Lagenidium callinectes* from blue crabs, these discharge tubes are short; in the same species from *Chelonibia* ova, the tubes are very long. Figures 10 and 11 accompanying Couch's paper (1942, pl. 19), applying to the fungus in crab eggs, also illustrate adequately the features of

[1] Department of Botany, Duke University, Durham, North Carolina.

[2] Bureau of Commercial Fisheries, Beaufort, North Carolina.

discharge tube penetration through the barnacle ovum membrane. However, the discharge tube base, as it enters into and emerges from the membrane (barnacle), is not markedly thickened as Couch figures it (1942, pl. 19, figs. 12, 13) for *L. callinectes* in blue crab.

Spore emergence in the *Lagenidium* from *Chelonibia* ova is precisely like that described for *L. callinectes*. The planonts are cleaved within the vesicle and escape through a lateral or terminal break in the persisting vesicular wall. Planonts are the same size as those described by Couch.

As in the *Lagenidium* from blue crab ova, the fungus in barnacles produces resting cells (18–36 μ in diameter), but these are apparently uncommon. Of the few examined, all but one were intercalary in the hyphae; the exception was terminal. Two such resting "spores" were observed during germination. The oil deposits migrate centrally in the resting cell, and gradually disappear. As the oil bodies disintegrate (?) the resting cell wall becomes thin, and the entire body enlarges slightly. At one point on the wall, an evagination develops which elongates to become a hypha with the characteristics of the intramatrical filaments. Because the hyphae from the two germinating resting cells became intermingled with other intramatrical filaments, it was impossible to follow further developmental stages.

Seven of the ten cross-inoculation and infection cultures were positive; that is, the *Callinectes* ova were infected by the fungus growing in *Chelonibia* lamellae. The three negative cultures probably resulted from failure of the fungus in barnacle eggs to sporulate, hence inoculation could not occur. None of the blue crab ova in the controls was infected. Only one sporangium that developed in experimentally-infected *Callinectes* ova produced a discharge tube. This tube was short and thick-walled at the entrance and exit through the ovum membrane, just as Couch (1942) figured it for the fungus in blue crab. Sporulation had occurred, since the discharge tube apex was expanded into an empty, partially deliquesced vesicle. Other than this single discharge tube, there were no extramatrical hyphae coming from the *Callinectes* ova, although the eggs were filled with mycelium. The successful cross-infection confirms, we believe, the morphological evidence that the fungus in barnacle lamellae is the same species as that in the *Callinectes* "sponges."

Both Couch (1942) and Sparrow (1939) recognized that the generic limits of *Lagenidium*, could well be revised. Including, for example, *L. chthamalophilum* (Johnson, 1958) with its preformed, evanescent vesicle, markedly changes certain concepts with respect to the genus. *Lagenidium callinectes* from *Chelonibia* has one characteristic, certainly, that is not a part of the genus as it is now understood. This feature is the profuse extramatrical hyphal system. In assimilative growth, at least, *L. callinectes* approaches certain myceloid members of the Pythiaceae and Peronosporales. There is increasing evidence, we believe, that if the generic limits of *Lagenidium* are not extended, the coarse mycelial forms such as *L. callinectes* and *L. chthamalophilum* cannot in all likelihood be retained in the genus.

One point in connection with *Lagenidium callinectes* should be emphasized. In keys to members of *Lagenidium* there is at least an indication that most species are host specific. *Lagenidium callinectes*, certainly, is not. However, it is not surprising to find the fungus from blue crab ova invading the eggs of the barnacle, or vice versa. The close proximity of the two suscepts is adequate to account for the cross-transfer.

SUMMARY

Lagenidium callinectes Couch is reported from lamellae of *Chelonibia patula*, a barnacle developing on the carapace of *Callinectes sapidus*. Extramatrical hyphae are very abundant in the fungus as it occurs in *Chelonibia*, in contrast to the exclusively intramatrical hyphae of the same species in *Callinectes* ova. The fungus can infect blue crab eggs, under laboratory conditions, in nonsterile, raw sea water.

ACKNOWLEDGMENTS

We are indebted to Drs. J. N. Couch and C. G. Bookhout for their critical review of the manuscript. Facilities provided by the Duke University Marine Laboratory are gratefully acknowledged.

LITERATURE CITED

COUCH, J. N. 1942. A new fungus on crab eggs. Jour. Elisha Mitchell Sci. Soc. **58:** 158–162.

JOHNSON, T. W., JR. 1958. A fungus parasite in ova of the barnacle *Chthamalus fragilis denticulata*. Biol. Bull. **114:** 205–214.

ROGERS-TALBERT, ROSALIE. 1948. The fungus *Lagenidium callinectes* Couch (1942) on eggs of the blue crab in Chesapeake Bay. Biol. Bull. **95:** 214–228.

SPARROW, F. K. 1939. A new species of *Lagenidium* parasitic on rotifer eggs. Mycologia, **31:** 527–532.