

## MYCAUREOLA DILSEAE, A MARINE BASIDIOMYCETE PARASITE OF THE RED ALGA, DILSEA CARNOSA

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*Mycaureola dilseae*, originally described as a pyrenomycetous ascomycete, is shown to be a basidiomycete with sigmoid, sessile basidiospores. The fungus is a parasite of the marine, subtidal red alga, *Dilsea carnosa*, on which it produces circular necrotic lesions. Dolipore septa have been observed in the fungal hyphae which penetrate and destroy the cortical cells of the algal host. A revised description is given for *M. dilseae*, the first aquatic basidiomycete to be described as an algal parasite.

Fungal diseases of marine macroalgae have been the subject of few recent reports (Kohlmeyer & Demoulin, 1981; Kohlmeyer & Kohlmeyer, 1975; Kazama & Fuller, 1979; Schatz, 1983; Thompson, 1981; van der Meer & Pueschel, 1985). Algal pathology is a subject that has attracted little attention (Andrews, 1976), but as our commercial use of algae expands, interest in fungal diseases of algae is likely to increase. The *Pythium* disease of *Porphyra* is the best studied fungal disease of an economically important alga (Kazama, 1979). Among the Ascomycotina there are more than 30 species known to be parasites of marine algae. However, most of these have only minor effects on the host, such as gall formation, and none has been reported to be epidemic (Kohlmeyer & Kohlmeyer, 1979). Epidemic infections may occur that have been unreported. One of us (WFF) has observed that infection of the red alga *Grateloupia filicina* (Lamouroux) C. Agh. by the marine ascomycete *Chadefaudia gymnogongri* (J. Feldmann) Kohlm. may occur in as high as 80% of the plants in a particular population.

*Mycaureola dilseae* Maire & Chemin was described originally as a pyrenomycete (Maire & Chemin, 1922). It was shown to be a parasite of *Dilsea carnosa* (Schmidel) O. Kuntze (syn. *D. edulis* Stackh.), a relatively common subtidal, foliose member of the Cryptonemiales (Rhodophyta) which extends from Arctic Russia to Portugal (Irvine, 1983). To our knowledge, this is the only described fungal parasite of *D. carnosa*, and it has not been reported in the literature since its original description from Roscoff (northern France), apart from a few more localities in Brittany listed by Feldmann (1954). J. Kohlmeyer observed the

holotype material from the Institut de Botanique, Montpellier (MPU), and concluded that it contained only immature reproductive structures, and thus designated both the genus and species as nomina dubia (Kohlmeyer & Kohlmeyer, 1979).

During September and October 1985 we made several collections of *D. carnosa* with circular, necrotic lesions, identical to those described by Chemin (1921) and Maire & Chemin (1922). In this paper we report that the fungus responsible for these lesions is a basidiomycete, with small, ostiolate, puffball-like basidiocarps formed on the surface of the lesions on the blades of the algal host. Previously, only three basidiocarp-forming basidiomycetes have been described from the marine environment (Kohlmeyer & Kohlmeyer, 1979). *Nia vibrissa* Moore & Meyers, *Digitatispora marina* Doguet and *Halocyphina villosa* Kohlm. & Kohlm. are all lignicolous, and usually produce their basidiocarps on exposed or intertidal substrates. *M. dilseae* differs from these three in its parasitic mode of nutrition and in the production of basidiocarps on the subtidal host alga.

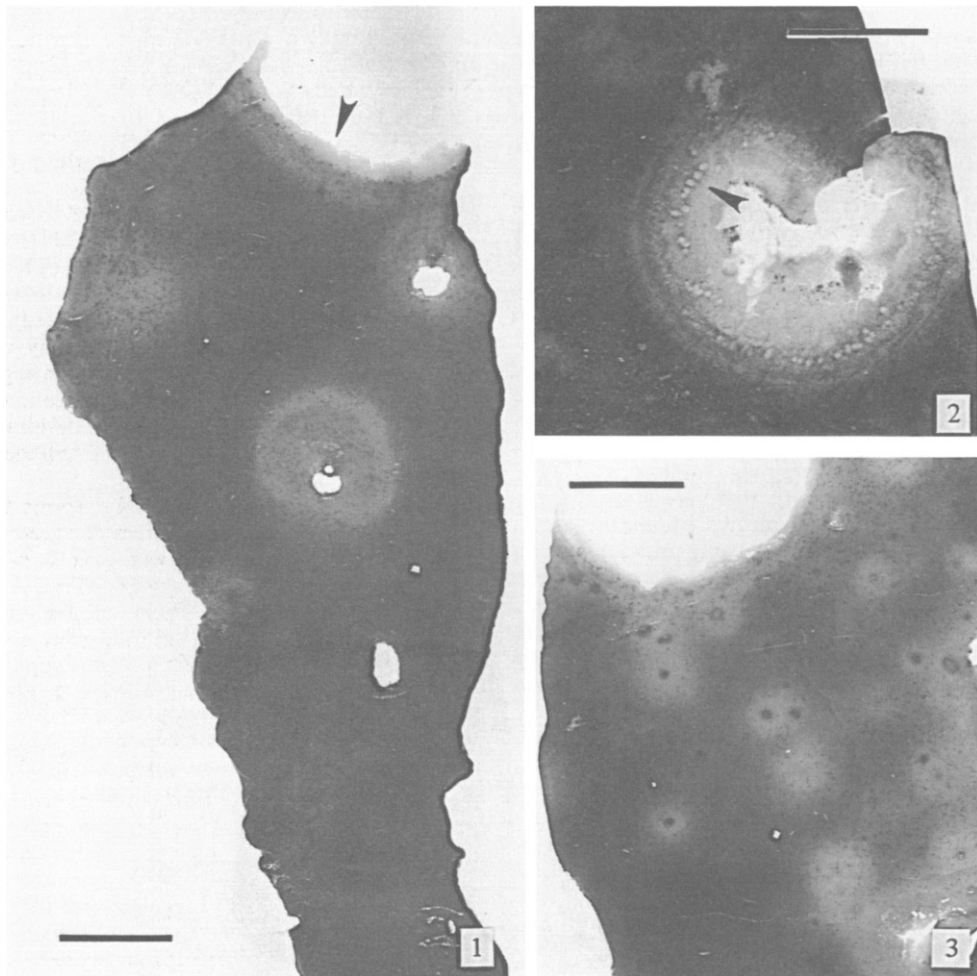
### MATERIALS AND METHODS

Attached plants of *Dilsea carnosa* infected with *Mycaureola dilseae* were collected from shallow sublittoral water at Swanage Pier, Dorset, U.K., on 20 Sept. 1985; at Gifford Bay on 16 Oct. 1985 and at L'Archirondel on 17 Oct. 1985, both on Jersey, Channel Islands; and off the Nothe, Weymouth, Dorset, on 24 Oct. 1985. Over 200 specimens of *D. carnosa* in the British Museum (Natural History) herbarium (BM) from the British Isles as well as from Denmark, the Netherlands and France were

examined to determine the presence of the fungus in previous years.

Freezing microtome sections or squash preparations were made of fresh material and observed with a Leitz Dialux 20 equipped with interference contrast optics. Material processed for electron microscopy was fixed for 2 h at room temperature in a 0.05 M sodium cacodylate-buffered mixture, pH 7.7, of 0.8% glutaraldehyde, 3.0% paraformaldehyde and 0.5% acrolein (final concentrations). This fixative contained 0.01%  $\text{CaCl}_2$  and 2.75% sucrose to match the osmolarity of sea water. After

fixation, the material was rinsed three times in buffer, post-fixed for 2 h in 1.0% osmium tetroxide and rinsed again in buffer. Material further prepared for SEM was frozen in supercooled Freon, fractured in liquid nitrogen with a cold razor blade, thawed, dehydrated through acetone, critical-point dried, sputter coated with gold and observed with a JEOL T20 SEM. For TEM, after post-fixation, material was dehydrated through alcohol, embedded in Spurr's resin, sectioned on a LKB Ultratome III, stained, and observed with a JEOL 100S TEM.



Figs 1-3. *Dilsea carnosa* with lesions caused by *Mycaureola dilseae*. Bar lines = 10 mm. Fig. 1. Small blade of *D. carnosa* with circular, zonate, necrotic lesions and a marginal arc of infected algal tissue (arrow). Note elongate perforation caused by grazing invertebrates. The small, dark spots visible in the lesions are thalli of the endophytic alga, *Chlorochytrium*. Fig. 2. Circular lesion with basidiocarps (arrow) in the central whitish zone near the border of the greenish ring. The centre of the lesion is torn away. Fig. 3. Portion of an algal blade with young, small greenish lesions. Smallest dark spots are thalli of *Chlorochytrium*.

## RESULTS

The necrotic lesions caused by *M. dilseae* in the blades of *D. carnosus* are easily recognized in the field because of their distinctive colour pattern. They are circular in outline as a result of the radial growth of the fungal hyphae within the algal blade. They reach a maximum diameter of about 20 mm. The central part of the lesion is whitish where all the algal photosynthetic pigments have broken down. This area is surrounded by a discrete zone of greenish tissue, typically about 2–4 mm wide, where the phycobilin pigments of the alga have been degraded. The greenish ring gives way to the reddish tissue of the uninfected algal blade (Figs 1, 2). The whitish tissue of the lesions is fragile and is usually torn or perforated. Basidiocarps number from a few to several dozen per lesion and develop on either or both surfaces of the algal blade. They appear as small white protuberances in clusters or arcs on the whitish tissue of the lesion and are located within about 1 mm of the greenish zone (Fig. 2). The young infection stages are greenish patches about 2 mm diam (Fig. 3). When these lesions were sectioned, fungal hyphae were observed only in the very centre of the greenish algal tissue. As the infections increase by hyphal growth into the healthy algal cells, the greenish ring expands and demarcates the region of actively growing hyphae (Fig. 4). Some of these circular lesions increase in size and fuse with other lesions to result in the loss of a portion of the algal blade by breakage. This is suggested by the frequent observation of arcs at the edge of the algal blade (Fig. 1) bordered by the characteristic greenish zone and containing active hyphae when viewed microscopically. Basidiocarps are also observed at times on these marginal infections.

Type material of *M. dilseae* from MPU was found to be identical with our collections.

None of the more than 200 herbarium specimens from BM exhibited convincing signs of the lesions typical of *M. dilseae*.

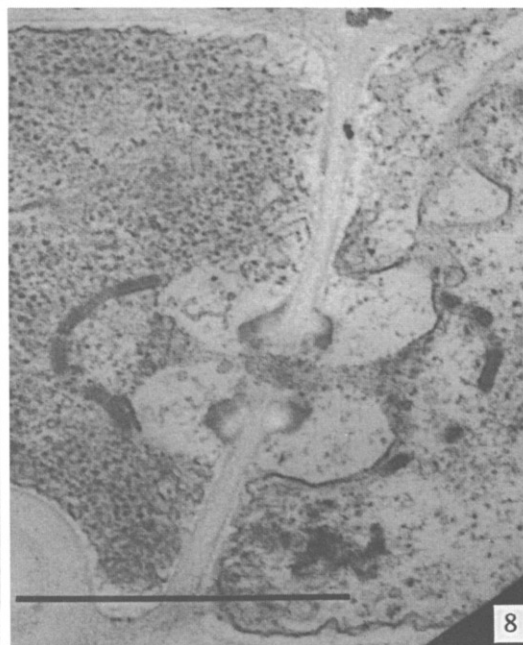
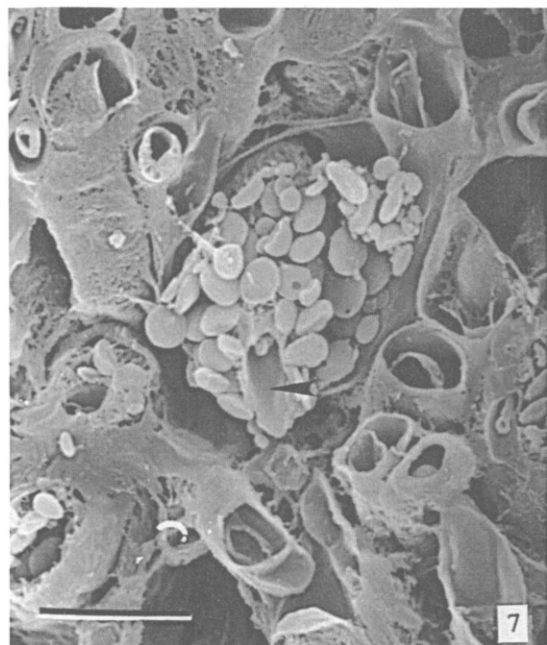
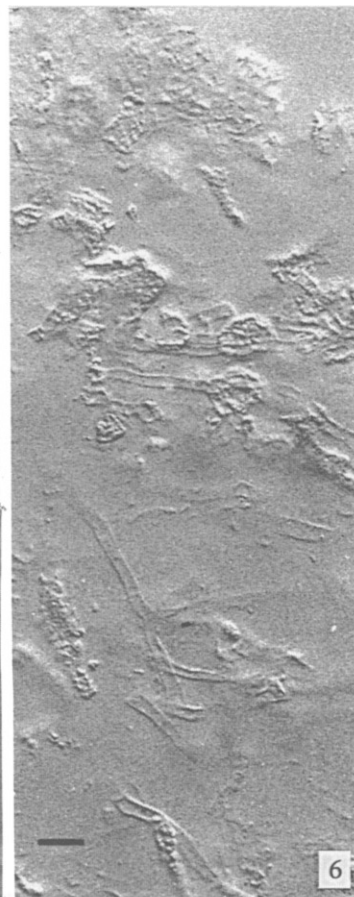
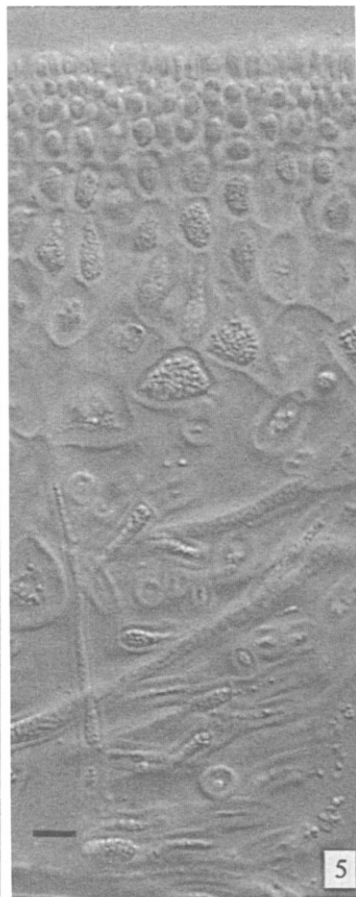
*D. carnosus* blades frequently have other perforations (Fig. 1) which are caused by grazing of various invertebrates, especially molluscs. These perforations differ from the fungal lesions in their irregularly elongate shape and lack of any intermediate greenish zone. Normal reddish tissue of the pigmented cortex of the alga is present right up to the margin and lines the rim of these perforations. This appearance suggests an active repair mechanism by the alga in response to the mechanical injury caused by grazing. Fungal hyphae have not been observed associated with these grazed perforations. A short account of the normal anatomy of *D. carnosus* is given by Bert (1981).

A microscopic comparison of the different regions of the zonate lesion caused by *M. dilseae* reveals that the active hyphae, which average 2–3  $\mu\text{m}$  diam, are present in the middle of the greenish zone (Fig. 4). No hypha is observed in the more distal portion of this zone, nor in any of the adjacent normal tissue of the alga (Fig. 5). Thus the initial loss of the red phycobilin pigments precedes the actual growth of the hyphae. Compared to the perforations caused by grazing, there appears to be no wound repair by the alga of the fungal lesions. Medullary algal filaments are present (Fig. 5), but these are easily distinguished from the hyphae by their wider diameter (7–10  $\mu\text{m}$ ), thick walls, and cytoplasm packed with floridean starch granules. Hyphae of the fungus grow in the mucilaginous intercellular matrix of the alga. The hyphae penetrate virtually every cortical cell (Fig. 4), causing the collapse of the algal cytoplasm and resulting in a marked physical disruption of the algal cells. The SEM clearly reveals the presence of fungal hyphae inside algal cells (Fig. 7). In the central whitish zone, little coherent algal structure remains (Fig. 6). The fragments of algal tissue appear to be held together by a network of hyphae. But even the hyphae are generally void of cytoplasm, presumably having depleted their nutritional base. It is only in the vicinity of the basidiocarps, typically located near the margin of the central white zone, that there is a more compact tissue. This tissue which supports the basidiocarps is composed of a denser growth of hyphae, but contains algal cells as well (Fig. 9).

Numerous endophytic unicells belonging to the chlorochytrium stage of the filamentous green alga, *Spongomorpha* (Lewin, 1984), were present in some of the infected *D. carnosus* plants. These algae, which were nearly invisible in the uninfected parts of the *D. carnosus* blades, appeared as green spots in the lesions (Figs 1, 3). The endophytes apparently were unaffected by the *M. dilseae* hyphae, a conclusion supported by microscopic observation. This suggests that the endophytes might possess antifungal properties.

Hyphae of *M. dilseae* have dolipore septa with perforate parenthosomes (Fig. 8) that are typical of the basidiomycetes. Clamp connexions were not observed.

The basidiocarps are white, hemispherical to ellipsoidal, sessile structures 300–500  $\mu\text{m}$  diam (Figs 9, 10), with an apical ostiole about 30  $\mu\text{m}$  diam (Figs 10, 11), frequently with a short, broad papilla. The ostiole is surrounded by short appendages up to 25  $\mu\text{m}$  long composed of relatively thin-walled hyphal branch tips (Fig. 11), but otherwise the basidiocarp surface is smooth. The wall of the basidiocarp is composed of



thick-walled, compact hyphae (Fig. 12). It is as much as  $50\ \mu\text{m}$  thick near the base but becomes thinner towards the apex. Within the basidiocarp is a basal hymenium extending part way up the sides of the central cavity (Fig. 9). The numerous elongated spores are massed in the central cavity (Fig. 13), and may exude through the ostiole when pressure is applied to the wall of the basidiocarp. The natural mechanism of spore release was not observed.

The basidiospores are unusual in their sigmoid shape (Fig. 14). They are one-celled and have an average size of  $2.8\ \mu\text{m}$  in width by  $115\ \mu\text{m}$  in length. Some of the spores have an end segment that is void of cytoplasm (Fig. 15). The spores are hyaline and smooth-walled. The basidia are cylindrical to subclavate (Figs 16, 17), and average  $50\ \mu\text{m}$  in length by no more than  $5.0\ \mu\text{m}$  in width at the tip. Cystidia were not observed. New basidia arise as branches from the base of existing basidia (Fig. 16). One to four spores are produced on each basidium. The basidiospores are sessile, without sterigmata (Fig. 17); they develop blastogenously (Fig. 18) and enlarge synchronously (Figs 17, 18).

Since *M. dilseae* was originally described as an ascomycete, a revised description is offered which incorporates our observations.

*MYCAUREOLA DILSEAE* Maire & Chemin, C. R. Acad. Sci., Paris 175: 319 (1922).

*Basidiocarps* white, solitary to clustered, 0.3–0.5 mm diam, hemispherical to subellipsoidal, ostiolate, with a single central cavity. *Peridium* smooth, 50 mm thick at base. *Hymenium* basal, without cystidia. Hyphae lacking clamp connexions. *Basidia* cylindrical to subclavate,  $50\ \mu\text{m} \times 3.5\text{--}5.0\ \mu\text{m}$ , aseptate, without sterigmata, one- to four-spored. *Basidiospores* elongated, sigmoidal,  $2.5\text{--}3 \times 105\text{--}118\ \mu\text{m}$ , smooth, hyaline, one-celled.

Material deposited as IMI 298343, BM and MPU.

#### DISCUSSION

Our observations on the interactions between *Mycaureola dilseae* and its host alga confirm those

of Chemin (1921). He accurately described the lesions on *D. carnosa* and the action of the fungus on the host tissue, including the suggestion that the fungus appeared to secrete a diffusible toxin that caused the loss of the phycobilin pigments ahead of the growth of the fungus. Maire & Chemin (1922) however, misinterpreted the structure of the sporocarp of the fungus as the perithecium of an ascomycete. The present demonstration of dolipore septa and basidia reveals the true nature of *M. dilseae* as a basidiomycete.

Given the size and the distinct appearance of the lesions formed by *M. dilseae*, it is difficult to understand why the fungus has been so little reported. The lack of any infected plants in the extensive collection of *D. carnosa* in BM suggests that the fungus may be sporadic in its appearance and not as common every year as it was in 1985. Perhaps future collectors of *D. carnosa* will look out for the presence of this fungus. Although not an obvious threat to the natural populations of this common red alga, *M. dilseae* is distinctly pathogenic to its host. If interest in the uses of *Dilsea* were to be translated into mariculture of this alga, then the pathogen might become a significant pest, but Levring, Hoppe & Schmidt (1969) report limited use of *Dilsea* at present.

Similarities exist between this disease and the disease of another foliose red alga, *Porphyra*, caused by *Pythium marinum* Sparrow. Like *M. dilseae*, *P. marinum* grows intracellularly (Kazama & Fuller, 1979) and produces circular necrotic lesions in the blade of the algal host (Fuller, Lewis & Cook, 1966). However, *P. marinum* in *Porphyra perforata* J. Aghard does not appear to produce a diffusible toxin, as there is no alteration to the host cells ahead of the hyphae of the parasite (Kazama & Fuller, 1979).

*M. dilseae* appears to be the product of a long evolutionary history in the marine environment resulting in, among other things, its host specificity. The sigmoid basidiospores that are not actively discharged are clearly adapted to the aquatic environment. Sigmoid spore morphology has evolved by convergence in diverse groups of

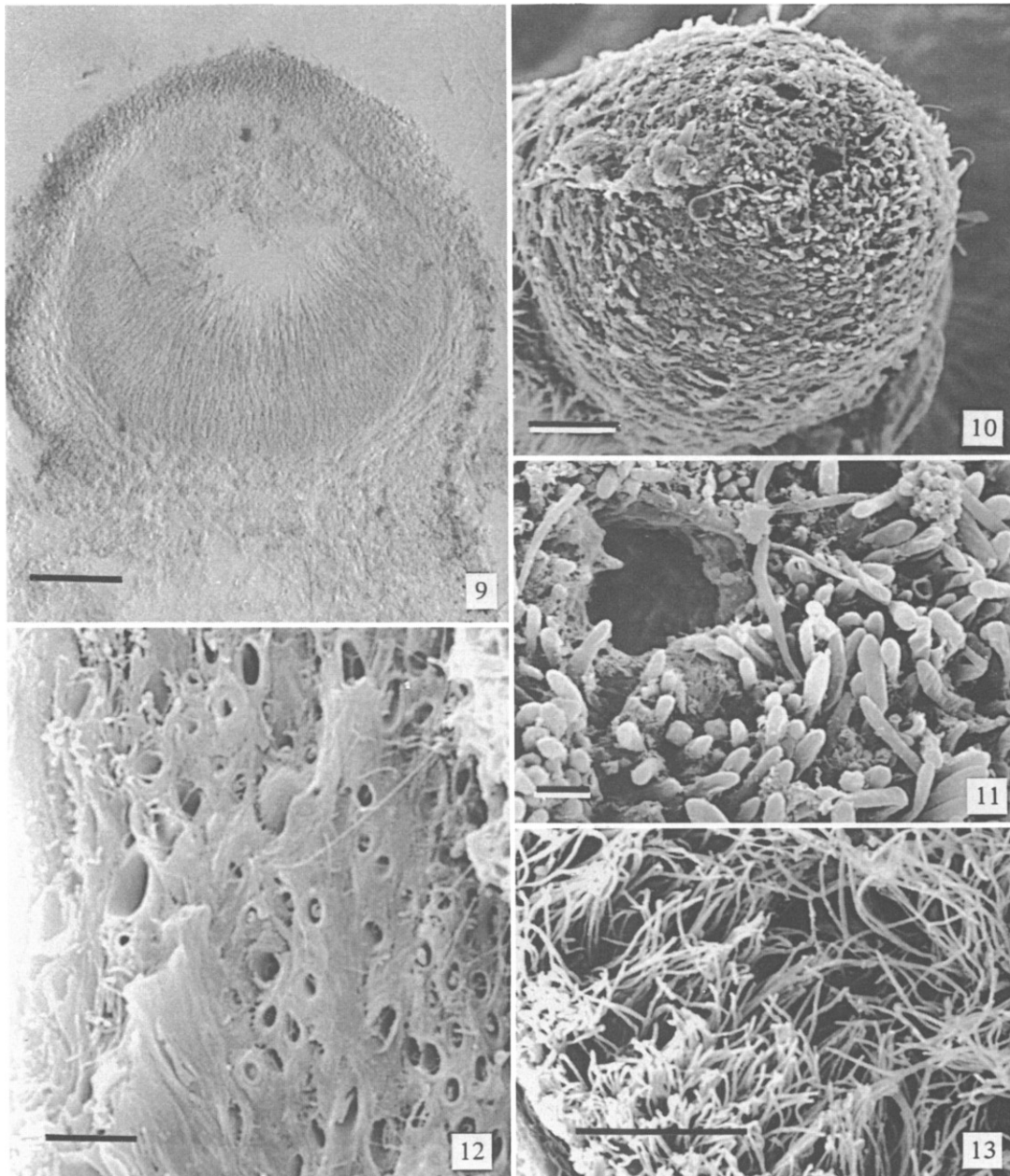
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Figs 4–6. Sections through infected and uninfected portions of the blade of *D. carnosa*. Bar lines =  $10\ \mu\text{m}$ .

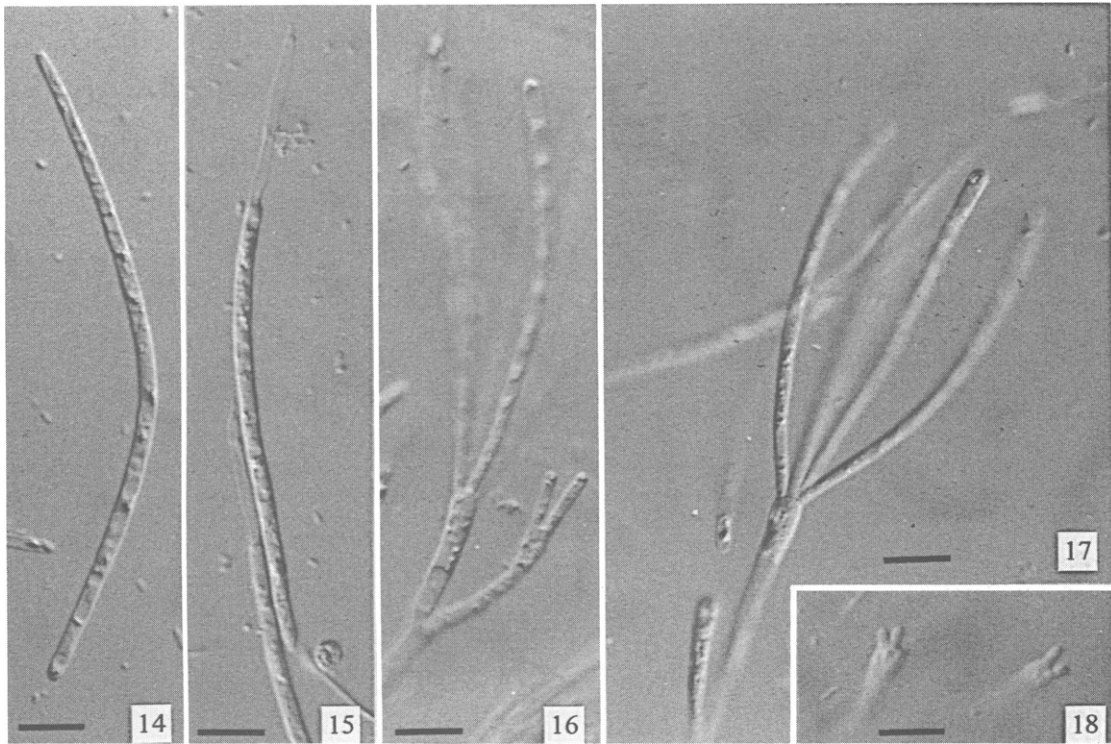
Fig. 4. Mid region of the greenish zone of a lesion with actively growing hyphae of *M. dilseae*. Note penetration of hyphae into algal cells and the collapse of the cytoplasm of infected algal cells. Fig. 5. Uninfected algal tissue. Note the thick cell walls of the medullary filaments and the cytoplasm packed with floridean starch granules. Fig. 6. Central whitish zone of a lesion. Only remnants of the algal tissue remain among the fungal hyphae.

Fig. 7. SEM of algal cells in the compact tissue beneath a basidiocarp. Hyphae are within the cell wall and have penetrated the cells (arrow). Small ovoid structures are floridean starch granules. Bar line =  $10\ \mu\text{m}$ .

Fig. 8. TEM of dolipore septum of *M. dilseae*. Bar line =  $1\ \mu\text{m}$ .



Figs 9–13. Basidiocarp of *Mycaureola dilseae*. Fig. 9. Non-median longitudinal section through a basidiocarp showing the basal hymenium and the central cavity. Interference contrast microscopy. Bar line = 100  $\mu\text{m}$ . Fig. 10. Apical view of basidiocarp. SEM. Bar line = 100  $\mu\text{m}$ . Fig. 11. Higher magnification of Fig. 10 showing ostiole and surrounding hairs. Bar line = 10  $\mu\text{m}$ . Fig. 12. Basidiocarp wall composed of thick-walled hyphae. Interior is to the right. Bar line = 10  $\mu\text{m}$ . Fig. 13. Transverse fracture across basidiocarp revealing spores within the central cavity. Bar line = 100  $\mu\text{m}$ .



Figs 14–18. Basidia and basidiospores of *Mycaureola dilseae*. Interference contrast microscopy. Bar lines = 10  $\mu\text{m}$ . Fig. 14. Sigmoid basidiospore. Fig. 15. Basidiospore with empty end segment. Fig. 16. Basidium with developing basidiospores. Fig. 17. Basidium with nearly mature basidiospores. Fig. 18. Tips of two basidia with initial blastogenous growth of basidiospores.

aquatic Ascomycotina and Deuteromycotina (Webster & Davey, 1984). *M. dilseae* now extends that convergence to include the Basidiomycotina.

Among the few known aquatic basidiomycetes (Jones, 1982), *M. dilseae* is the first parasitic, algicolous species. In addition, the formation and maturation of basidiocarps on a submerged substrate typical for *M. dilseae* is rare for other aquatic basidiomycetes. The lignicolous marine basidiomycetes usually form basidiocarps on intertidal or moist wood (Kohlmeyer & Kohlmeyer, 1979). Among the fresh-water basidiomycetes, the aquatic gasteromycete, *Lymnoperdon incarnatum* Escobar, McCabe & Harpel, produces basidiocarps which develop and mature at the surface of the water (Escobar, McCabe & Harpel, 1976), and the several fresh-water hymenomycetes appear to form only conidia in the submerged, aquatic environment (Webster & Descals, 1979). The number, small size and rapid production of basidiocarps by *M. dilseae* may be an adaptation to the relatively short life and fragile nature of the algal substrate on which they

are produced. In fact, the biology and structure of this fungus more closely resembles that of an ascomycete or deuteromycete than a basidiomycete.

Based on its morphology and mode of nutrition it is difficult to determine clearly any close relatives of *Mycaureola*. Even at higher taxonomic levels it defies placement; however, from the angiocarpic nature of basidiocarps and the sessile basidiospores on holobasidia, affiliation with the Gasteromycetes seems logical if not completely comfortable.

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