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Fungal Parasites of the Marine Green Algae, *Cladophora* and *Rhizoclonium*

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Abstract

Diseases caused by three fungal parasites in the green algal genus *Cladophora* from the beaches of Goa and Lakshadweep islands in Arabian sea in India are described. These are caused by *Sirolopidium bryopsisidis*, *Olpidium rostriferum* and *Labyrinthula* sp. Algal species in the genus *Rhizoclonium* was also heavily parasitized by *Labyrinthula* sp. A species of *Coenomyces* was found to be associated with *Cladophora repens* and *Rhizoclonium*.

The maximum occurrence of these fungi was found to be between July and September which is also the monsoon period in the western coast of India. Optimum salinity for maximum infection to occur under laboratory conditions was found to be between 10 and 15‰.

Except *Labyrinthula*, the other fungi could not be cultured on artificial media. The species of *Labyrinthula* required cholesterol (0.001%) for growth on artificial medium.

Most of these parasites were host specific and they could not be cross inoculated on other algae or even other species of *Cladophora*.

Introduction

Recent review articles have focused attention on the importance of research on pathology of marine algae (Andrews 1976, 1979). Fungi are very important disease-causing agents in marine algae and several fungal diseases have been reported (Aleem 1950, Fuller *et al.* 1966, Sparrow 1969, Kohlmeyer 1973, 1979, Kohlmeyer and Demoulin 1981, Van der Meer and Pueschel 1985). All groups of algae are parasitized by both higher and lower fungi. However, apart from studies on the physiology, biochemistry and ultrastructure of fungal diseases in algae, which are of great relevance to algal pathology, extensive surveys for fungal pathogens on algae have not been made. This is particularly so in the tropics. The author has recently reported a few diseases and associations between algae and fungi (C. Raghukumar 1986a and b, in press). Algal diseases appear to be a common feature of the marine algal ecosystem. This paper reports on naturally occurring phycomycete parasites of the green alga *Cladophora* from India.

Materials and Methods

Monthly samples of *Cladophora* species were collected in 1984 from the following sites in Goa, India (15°27'–15°38'N and 73°42'–73°50'E) (Fig. 1)

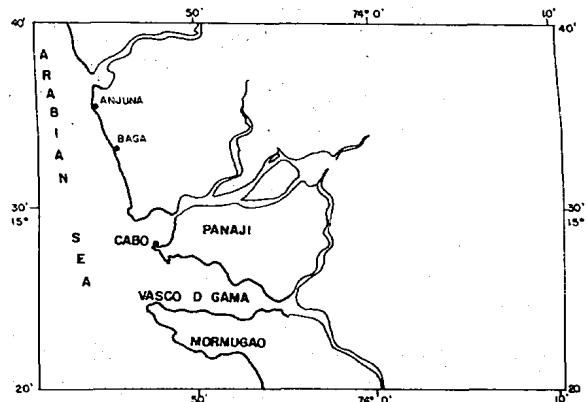


Fig. 1. Sites of sampling in Goa.

1. Anjuna
2. Baga, at the mouth of the Baga estuary
3. Opposite Cabo Raj Niwas, Dona Paula

Species of *Cladophora* were also collected from intertidal zones of coral reef lagoons on some of the Lakshadweep Islands in the Arabian sea and near the west coast of India between 10°30'–11°N and 72°–73°E.

The algae were brought to the laboratory immediately after collection and kept in streptomycin-penicillin solution for 24 h (1 ml of stock solution to 20 ml sterile filtered seawater; the stock solution consisted of 1 g of streptomycin sulphate and 1 g of penicillin [400 000 units] in 200 ml of filtered seawater) followed by a thorough wash with sterile seawater. The washed algae were separated into small clumps and incubated in sterile seawater in Petri dishes. The seawater was replaced every two days for one week and thereafter every fifth day for up to one month. The algae were screened thoroughly at weekly intervals for the presence of fungal pathogens. The percentage of infection was calculated by counting the number of infected cells per one hundred cells at random.

To investigate the influence of salinity on infection, the algal filaments were incubated in sterile seawater of different salinities immediately after collection and the percentage infection was calculated after 10–15 days.

Filaments of *Cladophora* infected with *Labyrinthula* were incubated in streptomycin-penicillin solution for 24 h, washed thoroughly with sterile seawater and plated on thraustochytrid isolation medium I (S. Raghukumar 1986), to which cholesterol (dissolved in ethanol) to a final concentration of 0.001% was added. The fungus isolated was maintained on pine pollen seawater medium (Sparrow 1960) and was used to inoculate healthy algae.

Sporulation of the infecting fungi in algae was observed by incubating the infected material in a moist chamber for 24 h. Healthy *Cladophora* cultures were maintained in Erdschreiber medium (Schreiber 1927). The cultures were maintained in diffuse day-light near a window and aerated continuously using an aerator pump.

Results

Description of the diseases observed

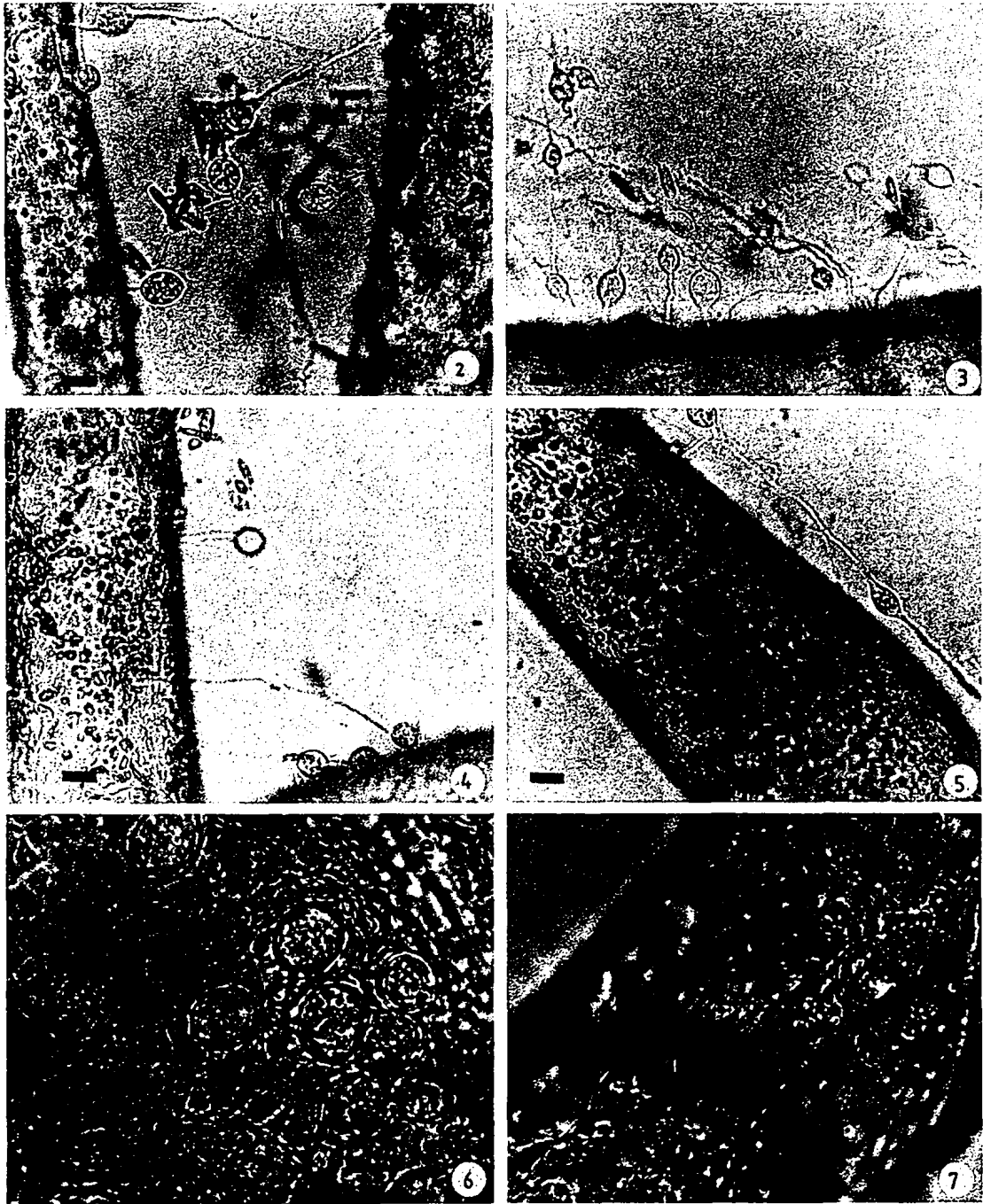
A polycentric chytrid associated with Cladophora repens (J. Agårdh) Harvey. A polycentric chytrid was regularly found to occur in *Cladophora repens*. The thallus was epi- and endobiotic, eucarpic consisting

of a filamentous, branched, segmented loose hypha-like complex of tubes bearing terminal and intercalary turbinate swellings (Figs 2, 7). Sessile sporangia-like structures were observed directly inside the host cells (Fig. 6) and these were inoperculate, judging from the empty intact sporangia (Fig. 3). The intercalary turbinate swellings, 15–17 µm in size might be sporangia with a long (65–70 µm) discharge tube. No zoospore liberation could be observed, although these sporangia-like structures were occasionally found to be empty of their contents (Fig. 3) and sometimes they were turgid with contents (Fig. 2). Based on the above characters, the fungus has been tentatively identified as a species of *Coenomyces*.

The alga appeared healthy and no morphologically visible symptoms of fungal infection were seen. After longer incubation in seawater, fungal sporangia were produced in abundance and they were always found as groups emerging out of algal filaments (Figs 3, 4, 5). When the algal filaments were bleached and dead, the fungus also appeared to die with it.

A species of *Rhizoclonium* collected from Agatti Island of Lakshadweep group of islands also harboured this fungus.

Terminal browning of Cladophora filaments. Cladophora frascatii Collins et Harvey was collected from rocks at the mouth of the Baga estuary. These rocks are submerged during high tide and partially exposed during low tides. However, they are constantly splashed by seawater during low tides. *C. frascatii* is a highly branched filamentous green alga with short tapering terminals. These terminal cells were often found to be golden brown in colour under a dissection microscope at 60x magnification. Golden brown patches alternated with hyaline patches (Figs 8, 9). On closer examination these hyaline patches were found to be fungal sporangia with a discharge tube penetrating the algal wall. About 5–7 sporangia were found in a row in a single cell. Adjacent sporangia produced discharge tubes on alternate sides, thus forming a regular pattern. Occasionally, the discharge tubes were bifurcated (Fig. 14). In the early stages, a single large cell occupied the entire algal cell. Later, this fungal cell became lobed and dissected into separate segments and each segment became a distinct sporangium (Figs 10, 11, 12). The host chloroplast was destroyed at this stage and the remnants appeared as golden brown accumulations at the sides of the fungal sporangia. The infected algal filaments were distinctly hypertrophied. When a cluster of filaments was viewed under the stereoscopic microscope, the narrow terminal cells appeared brown and swollen.

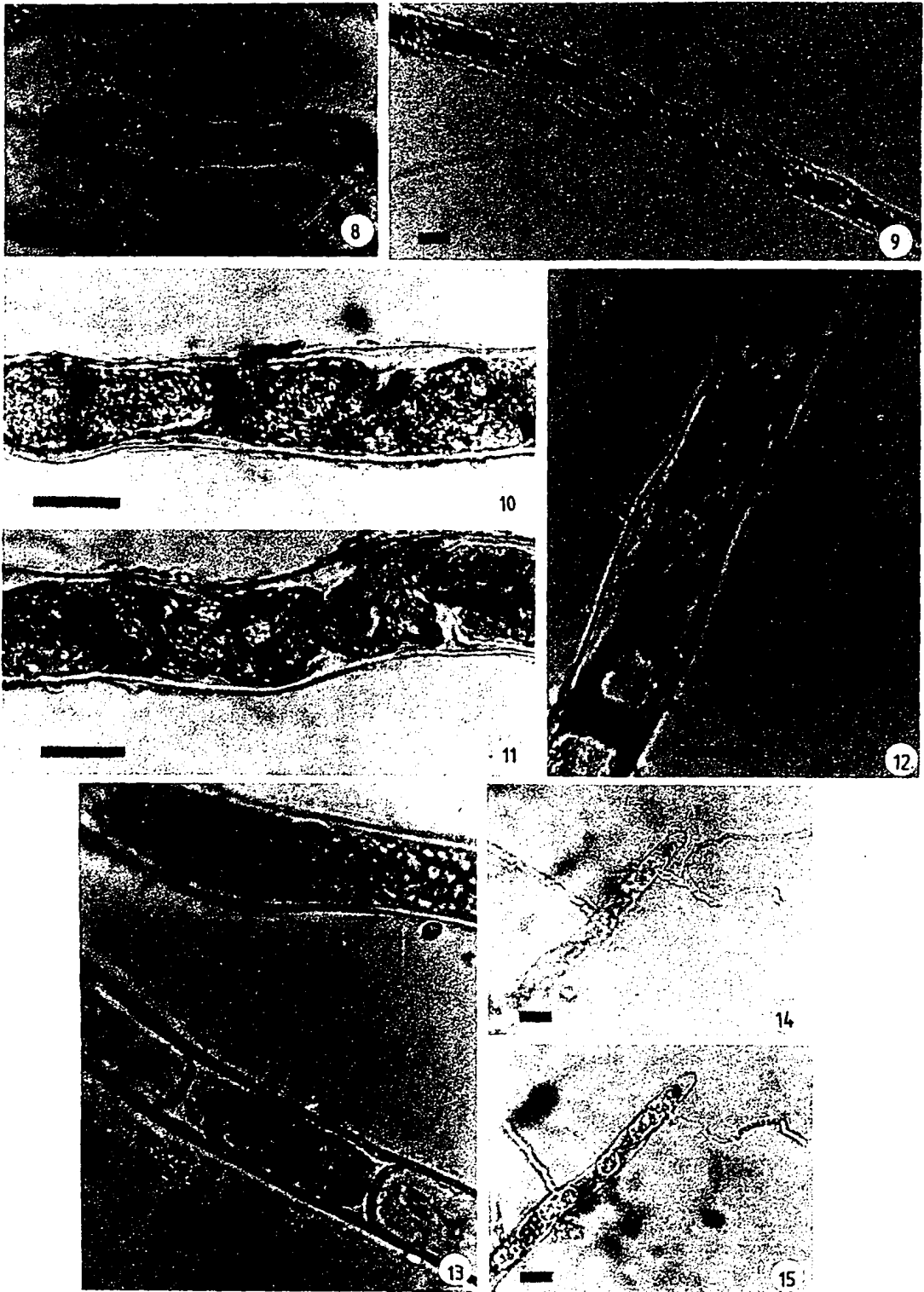


Figs 2–7. *Coenomyces* sp. in *Cladophora repens* (Scale bars = 20 μ m)

Fig. 2. Fungal cells with hypha-like tubes. Fig. 3. Group of fungal cells emerging out of the host filament. Fig. 4. Turgid cells, probably sporangia with granular content – growing towards another host filament. Fig. 5. Fungal cells with hypha-like filaments growing along the host filament. Fig. 6. Sporangia (?) inside the host cell. Fig. 7. Hypha-like filament with intercalary swellings growing over the host wall.

Zoospores were formed inside the sporangium and were liberated through the discharge tube. Sporangia were 15–25 μ m in height and 10–15 μ m in diameter, colourless and with thin smooth walls. The sporangial wall was appressed to the host cell wall (Fig. 13).

The cylindrical discharge tube was 2.5 μ m wide and 10–12 μ m in length and extended well beyond the algal wall. Zoospores were 2–4 μ m in length with two flagella. Based on these characters the fungus has been identified as *Sirolopidium bryopsidis* (de Bruyne)



Figs 8–15. *Sirolpidium bryopsidis* in *Cladophora frascatii* (Scale bars = 20 μ m)
 Fig. 8. Terminal cell of *C. repens* having fungal sporangia. Fig. 9. Filament with empty sporangia alternating with brown coloured content of the host cell. Fig. 10. Young sporangia demarcated inside the host. Fig. 11. Further lobed fungal sporangia.
 Fig. 12. Empty sporangia with discharge tube piercing the host wall. Fig. 13. Discharge tube constricted at the point of emergence.
 Fig. 14. Bifurcated discharge tube. Fig. 15. Abnormally long discharge tube. (Figs 14–15: samples were incubated in total darkness for 5 days).

H. E. Petersen. When the infected material was kept in total darkness, abnormally long discharge tubes were produced by the fungal sporangia (Figs 14, 15).

Browning disease of *Cladophora*. *Cladophora frascatii* collected from opposite Cabo Raj Niwas, when viewed under a stereoscopic microscope, showed browning of most of the cells (Fig. 16). The chloroplasts of the infected cells turned golden brown and accumulated around the fungal sporangia (Fig. 17). Cells of the alga harboured spherical to ovoid sporangia, 14–16 μm in diameter with thin smooth walls. Ripe sporangia had densely granulated contents. Eight to fifteen sporangia were found in a single host cell. (Figs 18, 19.) All of them produced discharge tubes simultaneously, which were single, 25–30 μm in length, strongly constricted when passing through the host wall and extended to various distances outside the host wall (Figs 20, 22–25).

Zoospores were spherical, about 3–4 μm in diameter with a single flagellum. Based on the above characters this fungus has been identified as *Olpidium rostriferum* Tokunaga.

Labyrinthula infection of *Cladophora*. Another virulent pathogen of *Cladophora frascatii* Collins et Harvey, *C. repens* (J. Agardh) Harvey and *Rhizoclonium* was *Labyrinthula* sp. It was found to occur in healthy green cells where the fungus caused extensive damage and discolouration of chloroplasts (Figs 26–30). The algal cells were filled with spindle shaped cells moving on ectoplasmic nets. During the late stages of infection when the host cells were dead, the fungus appeared to move out of the host cells.

Heavy infections of *C. frascatii* and *Rhizoclonium* sp. were noted in the samples collected from Agatii and Kalpeni Islands in the Lakshadweep Islands.

Ecology. Maximum occurrence of the fungus *Coenomyces* in *Cladophora repens* collected from Goa was recorded in August (Fig. 31). The fungus was not observed during March–June and only once again during November and December. Terminal browning of *C. frascatii* (collected from Goa) by *Sirolopidium bryopsidis* and browning disease of *C. frascatii* (also collected from Goa) by *Olpidium rostriferum* also appeared to occur maximally in August.

Optimum salinity for maximum infection of *C. repens* and *C. frascatii* by their respective pathogens was between 10‰ and 15‰ (Fig. 32), at 28 °C under laboratory conditions.

Figure 33 indicates the distribution of four parasites in relation to different species of *Cladophora*. Besides *C. repens*, *Coenomyces* was found to be associated

also with *Rhizoclonium* which was collected from Kalpeni island. *Sirolopidium bryopsidis* was found to occur only in *C. frascatii* collected from Baga. Similarly *Olpidium rostriferum* was found infecting *C. frascatii* collected only from opposite Cabo Raj Niwas. *C. frascatii* collected from Agatti island was only once found to be infected with *O. rostriferum*. *Labyrinthula* infection was found to occur naturally in *C. repens* and *Rhizoclonium* and in *C. frascatii* under laboratory conditions.

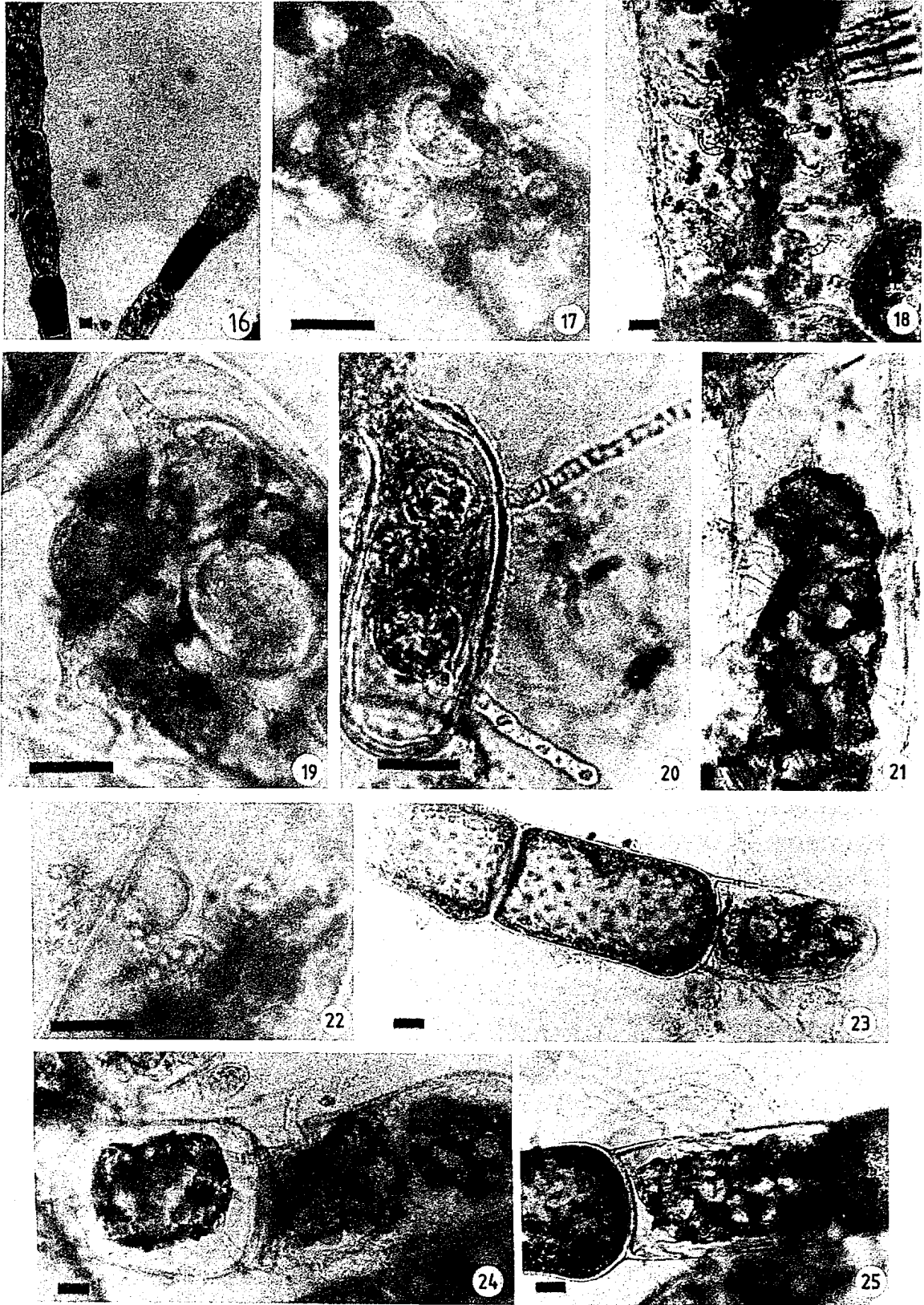
Cross inoculation between different species of *Cladophora* and *Rhizoclonium* with these fungi under laboratory conditions, was not successful. *Labyrinthula* alone could infect both species of *Cladophora* by incubating with infected cultures.

Coenomyces, *S. bryopsidis* and *O. rostriferum* could not be cultured on cellophane, corn meal agar, peptone maltose agar or peptone glucose agar or gelatine hydrolysate (Vishniac 1955, Fuller *et al.* 1964). Extracts of healthy living cells or healthy killed cells when incorporated into the media did not help in culturing these fungi. The above mentioned media were also prepared with the salinity adjusted to 10–30‰ but were not found to be useful in culturing the fungi.

Labyrinthula sp. alone could be cultured on Thraustochytrid medium but only in the presence of cholesterol. Once isolated on this medium, bacteria-free culture was maintained on pine pollen seawater medium and also on agar slopes.

Discussion

Each fungus produced distinctive disease symptoms such as terminal browning of cells by *S. bryopsidis* and browning of all cells and total destruction of filaments as in *O. rostriferum* and *Labyrinthula* infection. *Olpidium* infection was marked by the presence of projecting discharge tubes from the algal filaments. *Labyrinthula* species have been implicated in diseases of marine plants (see Rasmussen 1977). However, it has not been observed to produce such distinct disease symptoms on any host as in the present case. Even in *Zostera*, where it has been suspected to decimate whole populations, no proof has been obtained by means of disease symptoms (Young 1938). However, in the present case, *Labyrinthula* is apparently capable of decimating its hosts, namely *Rhizoclonium* and *Cladophora*. Similarly, *O. rostriferum* caused total destruction of *C. frascatii* within a span of one week in the laboratory. Ultrastructural studies on these host-parasites would throw more light on



Figs 16–25. *Olpidium rostriferum* in *C. frascatii* (Scale bars = 20 μ m)

Fig. 16. An infected branch. Dark coloured cell is the healthy one, the remaining ones are filled with fungal sporangia. Fig. 17. Young sporangia inside the host cell. Fig. 18. Sporangia with discharge tubes. Fig. 19. Sporangia with discharge tubes. Fig. 20. Abnormally long discharge tubes. Fig. 21. The chloroplast of the host cell has shrunk. All the sporangia have produced discharge tubes. Fig. 22. A sporangium liberating its zoospores. Fig. 23. An infected terminal cell with 8–10 sporangia. Fig. 24. Late stage of infection where almost all the cells are infected. Fig. 25. Abnormally long discharge tubes produced in a culture, incubated in total darkness for 5 days.

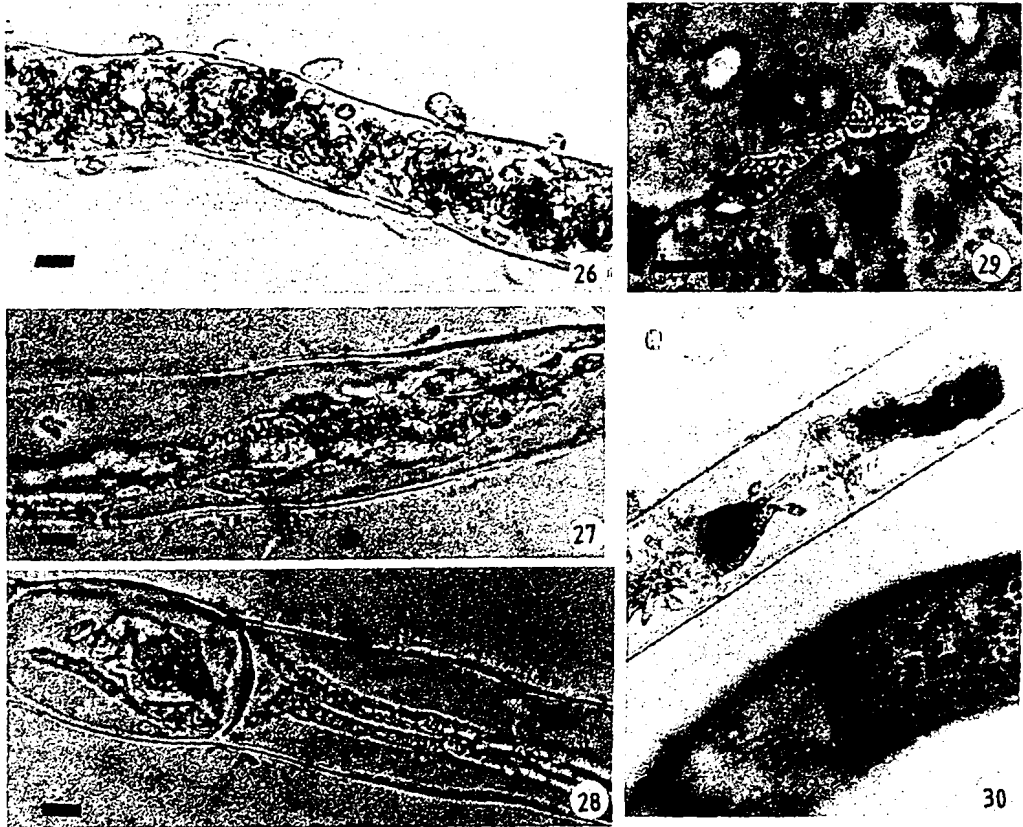
the detailed aspects of disease. Although *Coenomyces* species was found inside the host cells, it did not cause any morphological damage to the (host) cells. Therefore, this relationship is referred to as an association in this paper. Similar associations involving marine fungi and algae are reported by TeStrake and Aldrich (1984). However, in their report, the fungi did not penetrate algal walls.

During these studies it was observed that maximum growth of all species of *Cladophora* was during the months of monsoon, namely June–September, when the salinity of estuarine water fluctuates between 5‰ and 15‰. During this period, luxuriant growth of *Cladophora* was observed at all the sampling sites. During December–May, very scanty growth of *Cladophora* was observed in these regions. Maximum infection by fungal parasites was also noted during the peak period of algal growth. *Cladophora* prefers a reduced salinity and so do its parasites. During monsoon, when there is an influx of rainwater, the salinity drops and the terrestrial run off, bringing

an influx of phosphates and nitrates, promotes the growth of *Cladophora* (see also Gordon *et al.* 1981). During summer (March–May) the salinity of these waters is higher and therefore growth of *Cladophora* may also be limited. Also in the laboratory it was found that infection was best at lower salinities of about 15‰.

During the period when *Cladophora* was absent, the parasites were not detected in any alternate hosts. It is an intriguing question where and how the parasites survive in the absence of their host. *Olpidium rostriferum* and *Labyrinthula* sp. which caused 60–70% infection after incubation in the laboratory could be responsible for wiping out *Cladophora* under natural conditions.

As part of the survey programme, all the algae including brown and red algae were screened for the presence of fungal pathogens, but *Cladophora* appeared to be the most favourable for fungal parasites. Other green algae like *Ulva*, *Monostroma*, *Enteromorpha* and *Caulerpa* which existed with *Cladophora* did not



Figs 26–30. *Labyrinthula* infection (Scale bars = 20 μ m)

Fig. 26. Spindle-shaped cells with ectoplasmic net on the host wall. Fig. 27. The host chloroplast is completely destroyed and the cell is filled with fungal cells. Fig. 28. *Labyrinthula* cells in *Rhizoclonium*. Fig. 29. *Labyrinthula* cells in *Cladophora*. Fig. 30. Healthy and infected *Rhizoclonium* filament. The dark coloured content in the infected filament (upper) is the shrunken host chloroplast.

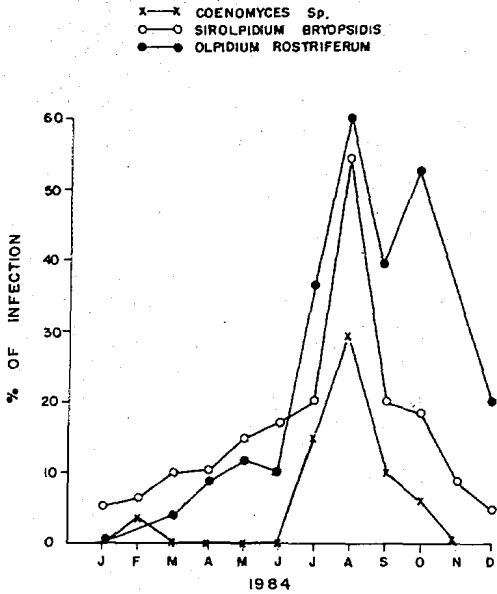


Fig. 31. Disease incidence recorded from sites of sampling in Goa during 1984.

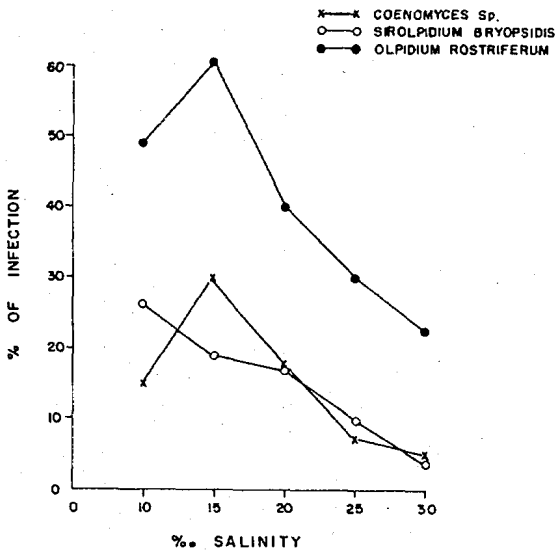


Fig. 32. Influence of salinity on infection of different spp. of *Cladophora* by their respective parasites under laboratory conditions, at 28°C.

harbour these fungal parasites. Cross inoculation with these parasites was also not successful in infecting these algae. These parasites are obviously host specific. Except for *Labyrinthula* the others are even species specific. They did not infect other species of *Cladophora* except the original host species on which they were found in the field.

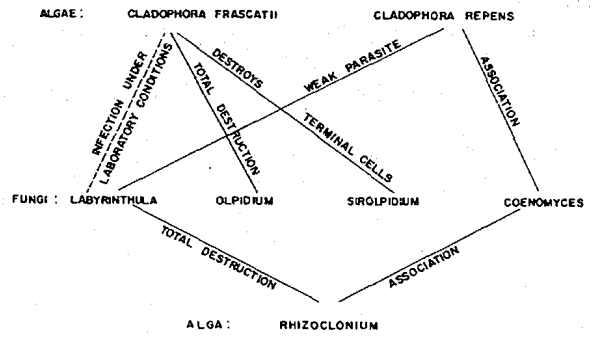


Fig. 33. Schematic representation of relationship between different host algae and their parasites.

Surprisingly all the phycomycetous parasites were of an obligate nature and except for *Labyrinthula* none of them could be successfully cultured on artificial media. Vishniac (1955) has reported culturing *Sirolpidium zoophthorum* Vishniac on a gelatine hydrolysate medium. However *Sirolpidium* from *Cladophora* could not be cultured employing the same medium. *Labyrinthula* could be isolated from the infected alga only in the presence of cholesterol. For growth on pine pollen also it required cholesterol enriched seawater. This is common with many species of *Labyrinthula*. Nutritional requirements of other parasites are to be screened in detail in order to bring them in culture.

Numerous workers (see Gordon *et al.* 1981), have reported the growth of *Cladophora* to nuisance proportions in estuaries and beaches and a search for a fungus as a biological control was carried out by some of them (Redhead and Wright 1978, Bott and Rogenmuser 1980). Naturally occurring algal parasites like *O. rostriferum* and *Labyrinthula* which cause total destruction of *Cladophora* and *Rhizoclonium* respectively, in a short time and which are being reported for the first time from India could be used in biological control of these algae.

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