

## *Chytridium polysiphoniae*—A Fungal Pathogen on the Red Alga *Centroceras clavulatum* (C. Agardh) Montagne, from Goa

CHANDRALATA RAGHUKUMAR

National Institute of Oceanography, Dona Paùla, Goa 403 004

Received 26 February 1985; revised received 7 October 1985

*Centroceras clavulatum* collected from rocky shores was found to harbour an epiphytic fungus identified as *Chytridium polysiphoniae*. Maximum infection of the host alga occurred during June-July 1984. Under laboratory conditions optimum infection occurred at  $25 \times 10^{-3}$  salinity and  $30^{\circ}\text{C}$  temperature. The infection was restricted to one locality (Anjuna), although the host alga was found at other sites in Goa. *C. polysiphoniae* could not infect killed host alga and it could not be cultured on artificial media, suggesting its obligate parasitic nature. The fungus and its occurrence on *C. clavulatum* are reported for the first time from India.

The number of fungi reported as potential algal parasites<sup>1-8</sup> shows that algae including phytoplankton could be as susceptible to fungal infection as terrestrial plants. During regular screening of coastal intertidal algae and phytoplankton for the presence of fungal pathogens, *Centroceras clavulatum*, one of the common red algae, was found to be generally infested with a fungal parasite. This alga was observed to occur abundantly throughout the year along the coast of Goa. It grows attached to rocks in the intertidal regions which were either partially submerged or received splashes of seawater even during the low tide. It was also found growing abundantly in the rock-pools. When this alga dominated one rock, it excluded growth of other algae on the same rock. This alga, whenever collected from Anjuna beach was found to harbour a chytridiaceous fungus growing as an epiphyte. Identification, developmental stages and culture characteristics of this fungus are reported in this communication.

### Materials and Methods

Monthly samples of *C. clavulatum* were collected during Jan.-Dec. 1984 from Anjuna, Baga and Dias beach and from the mouth of Mandovi estuary in Goa. The alga was washed thoroughly with sterile seawater after collection. Small bits (2 cm long) were then incubated in sterile seawater in Petri dishes and the seawater replaced every 2 d for 1 week and thereafter every 5 d up to 1 month. The number of fungal sporangia were counted next day after collection and 1 week later. The number of fungal sporangia for every 25 cells of the algal filament were counted to calculate the disease index. For isolating the fungus, small segments of the algal filament, containing fungal sporangia, were cut under the dissection-microscope

and washed in antibiotic solution (streptomycin -  $0.01 \text{ g.ml}^{-1}$ ; penicillin -  $2000 \text{ units.ml}^{-1}$ ). After washing thoroughly with sterile seawater, the segments were inoculated into seawater pine pollen medium<sup>9</sup> and modified Vishniac's medium<sup>10</sup> containing agar.

### Results

Filaments of *C. clavulatum* sometimes immediately after collection and often 3-4 days after collection showed epiphytic sessile sporangia (Fig. 1). The sporangia were urn shaped or subpyriform and ranged from 10-20  $\mu\text{m}$  diam. The sporangia had fairly stout wall and were found to rest with their broad base on the substratum. Spherical zoospores (2.5-4  $\mu\text{m}$ ) with a single long posterior flagellum escaped from the sporangium on dehiscence of an apical convex operculum (3-7  $\mu\text{m}$  diam.). Resting spores were not observed. Fungal sporangia were found occasionally growing on the setae of the alga also. This operculate, monocentric, epibiotic fungus was identified as *Chytridium polysiphoniae* Cohn. (family-Chytridiaceae, series-Operculatae, class-Phycomycetes)<sup>9</sup>.

The fungus could not be cultured on pine pollen nor on the modified Vishniac's medium. It did not grow on dead filaments of the host alga.

*Ecology of infection*—Maximum infection of alga occurred during June-July after which percentage of infection declined drastically (Fig. 2). The disease index in June-July represents the number of sporangia found immediately after collection, whereas the data during other months show the number of sporangia appearing 4 d after collection. When healthy alga was incubated with infected bits of alga at different salinities in the laboratory,  $25 \times 10^{-3}$  salinity was optimum for heavy

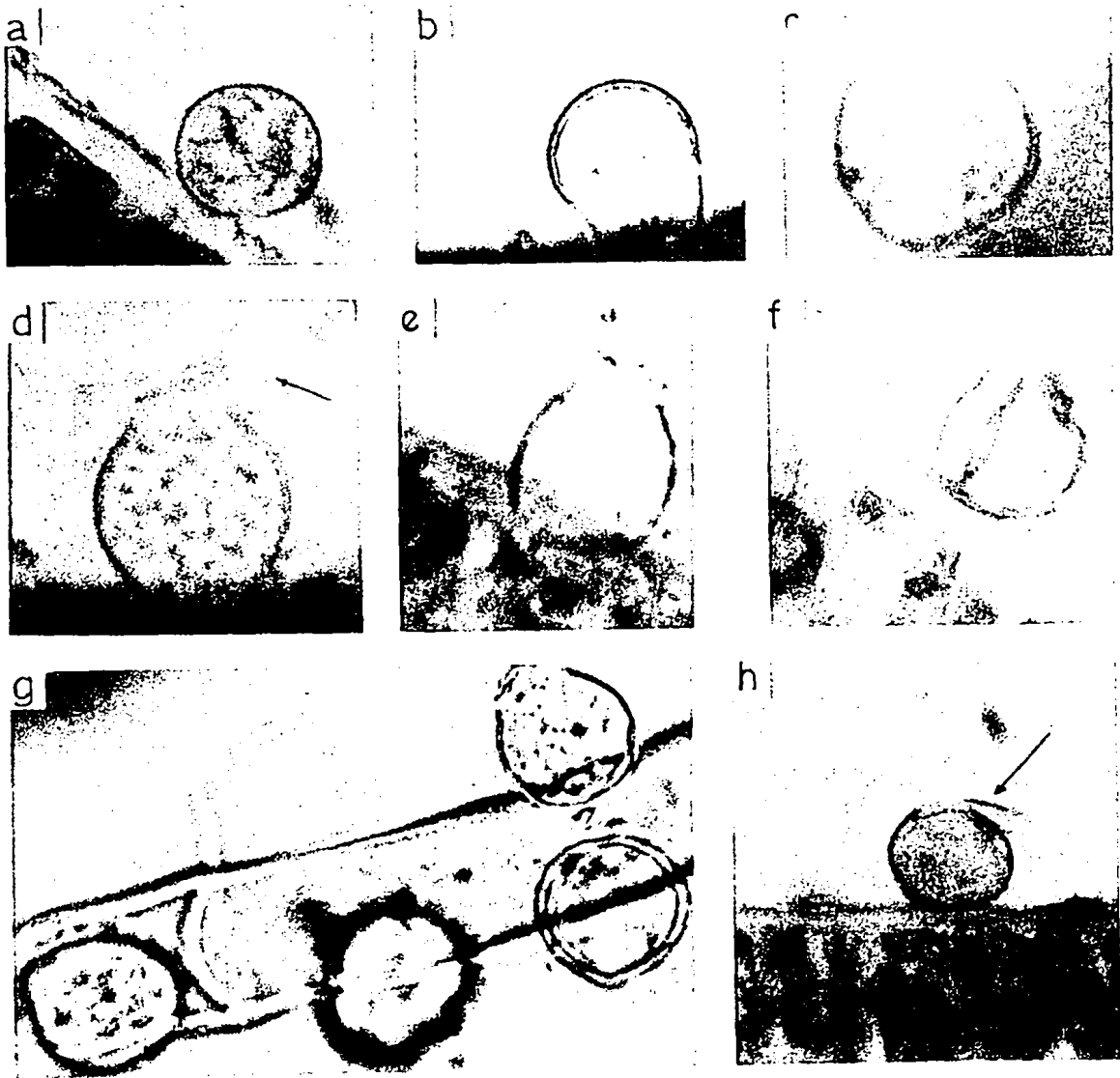


Fig. 1 - Different developmental stages of *Chytridium polysiphoniae* sporangia.  $\times 1300$  [a - spherical sporangium on algal wall. b - obpyriform sporangium on algal wall. c - ripe sporangium with its content cleaved into zoospores (arrow indicates operculum). d - sporangium with its operculum raised (arrow indicates *in situ* germination of zoospores). e - sporangium with operculum lifted and the zoospores escaping. f - empty sporangium with shrivelled walls. g - sporangia at different stages of development on the seta of the alga and h - operculum (arrow) is flipped back, the sporangium is empty.  $\times 1100$ ]

infection (Fig. 3). Under laboratory conditions better infection was achieved at the room temperature ( $30^{\circ}\text{C}$ ) than at  $24^{\circ}\text{C}$ . Incubation in light or dark did not have significant influence on the percentage of infection. Whenever the alga was heavily colonized by epiphytic diatoms chytrid infection was very low or absent.

The fungus was found to restrict itself to *C. clavulatum* alone and was not found on *Ceramium* sp., another red filamentous alga usually found in the vicinity of the infected host. Under laboratory conditions also it failed to infect *Ceramium*.

The samples collected from Anjuna were always found to harbour this fungus and samples of the same alga collected from Dias beach, Baga or from Mandovi

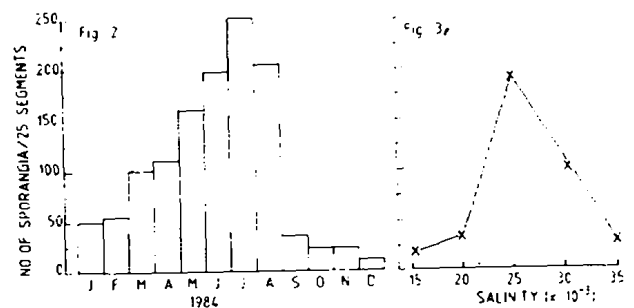


Fig. 2 - Variation in disease index during 1984

Fig. 3 - Influence of salinity on infection under laboratory conditions

near Cabo were never infected with this fungus. However, under laboratory conditions, all the samples could be infected by incubating with the infected *C. clavulatum*. *C. clavulatum* collected from Agathi atoll (Lakshadweep islands) was also found to be infected with *Chytridium polysiphoniae*.

### Discussion

The epiphytic fungus, *Chytridium polysiphoniae* on the red alga *C. clavulatum* is the first report from India. Sparrow's<sup>9</sup> description of *Chytridium polysiphoniae* resembles very much the fungal species reported here, but *C. clavulatum* as the host of this species has not been reported earlier.

The reason for restricted occurrence of the fungus to only one locality (Anjuna) although the same host alga is available at several sites is not understood. Comparison of physical and nutritional parameters of different sampling sites and the nutritional requirements of the fungus may provide a clue.

Since this fungus is epiphytic and draws nourishment from the host alga through rhizoids it does not cause any morphological changes in the alga. However, changes of biochemical nature are yet to be looked into. Alternatively, it might be commensalistic type of relationship where the fungus benefits and the host alga is unaffected.

Whenever the alga was found to be heavily colonized by epiphytic diatoms, chytrid infection was very low or totally absent. Probably the zoospores of the fungus do not find a place to settle down on the host wall, hence no subsequent infection took place. Alternatively, the extracellular metabolites of the diatoms might have an adverse effect on the successful settlement of zoospores and their further growth.

The failure of the fungus to grow either on the killed host filaments, or on synthetic media or on pine pollen seawater medium suggests obligate parasitic nature of the fungus. Besides, its inability to grow on other related algal genera, shows its host specificity. Phycomycetous parasites of phytoplankton and algae appear to be extremely difficult to culture<sup>11</sup>. They are reported in such cases to be maintained in dual cultures<sup>12-14</sup>, wherein cultures of healthy and infected algae are maintained separately.

### Acknowledgement

The author thanks the Director for facilities and Dr T S S Rao and Dr D Chandramohan for encouragement. Thanks are also due to Dr S Raghukumar for helpful discussions and to Mr S B Kamble for field and laboratory assistance.

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