

## A Report on the Occurrence of Thraustochytrid species in Indian Waters

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### ABSTRACT

Thraustochytrids were isolated from 52 samples of 14 substrata from the coastal waters of Goa, Tamil Nadu and Lakshadweep islands and the Arabian Sea. A total of 9 species are reported in this paper. These are: *Corallochytrium linacisporum* Raghukumar, *Labyrinthuloides minuta* (Watson and Raper) Perkins, *L. yorkensis* Perkins, *Thraustochytrium aggregatum* Ulken, *T. motivum* Goldstein, *T. multirudimentale* Goldstein, *T. striatum* Schneider, *Schizochytrium mangrovei* Raghukumar and *Ulkenia visurgensis* (Ulken) Gaertner. The substrata examined were decaying mangrove leaves, living algae, estuarine, and offshore water samples, phytoplankton samples, gill tissues of green mussels, hydroid polyps, detritus samples, scrapings of oyster shell, coral polyps and coral mucus. *Ulkenia visurgensis* was the most common species isolated, occurring in 57% of the substrata.

### INTRODUCTION

While fungi are known to play a very important role in the terrestrial ecosystem, their function in the sea is poorly understood. The fungi-like marine protists, the thraustochytrids (Moss, 1986) are extremely common in the sea. These are unicellular, eukaryotic, osmotrophic microorganisms. Most of the reports on the occurrence of these organisms, of which about 40 species are known (Moss, 1986) have been from temperate waters. These investigations have thrown light not only on the species occurring in temperate waters but have also revealed that they occur in a wide range of substrata. Water and sediments harbour them (Bremer, 1976; Raghukumar and Gaertner, 1980 and Jacobsen, 1984) and they are associated with micro and macroalgae (Sparrow, 1968; Gaertner, 1979; Miller and Jones, 1983 and C. Raghukumar, 1986) and invertebrates (Jones and Alderman, 1974; Hoehnke and Ulken, 1979; Wagner-Merner *et al.* 1980; Richter, 1985 and Alderman and Polglase, 1986). Apparently, these organisms have a definite role in the marine processes, probably similar to that of

fungi in the terrestrial ecosystem. Reports of thraustochytrids from tropical waters are relatively rare (Ulken, 1984; 1986; Raghukumar, 1987 and 1988) and there is a paucity of information on the species inhabiting tropical waters and the substrata harbouring them. Such information is a necessary first step for assessing their importance in the marine ecosystem.

During ecological studies of thraustochytrids in Indian waters, many thraustochytrids were isolated from various substrata. These isolates were identified and some results of these studies are presented here.

### MATERIALS AND METHODS

The thraustochytrids were isolated either by the pine pollen baiting technique of Gaertner (1968) or by plating the substrata on a nutrient medium. Larger substrata such as algae were gently rinsed in sterile seawater prior to isolation to ensure that only cells adhering and growing on the substrata were isolated. For the baiting technique, 5 ml of sterile seawater was taken in 5 cm Petri dishes and small bits of the substratum were placed in it. A small amount

of pine pollen, sterilised in an oven at 100 C for 48 h, was sprinkled on the water. Thraustochytrids grew on the pollen in about 4-5 days. For isolating thraustochytrids from seawater, various dilutions of the water were taken in Petri dishes and baited with pine pollen as above. Such cultures also harboured bacteria. To make cultures bacteria-free, they were grown in seawater/pine-pollen containing 5 mg streptomycin and 2000 units penicillin ml<sup>-1</sup>.

In the second method, bits of the substratum were placed on a solid medium (Raghukumar, 1986) containing streptomycin and penicillin in concentrations given above. Bacteria-free colonies of thraustochytrids formed around the bits. These were subsequently subcultured onto pine pollen/seawater on which all the cultures were maintained. Observations on the morphology and development were made in paraffin chambers (Bahnweg and Sparrow, 1972) and continuous flow chambers (Raghukumar, 1987).

*In situ* examinations of algal bits were carried out in continuous flow chambers. Algal bits were placed in the chambers with pine pollen as the substrate. The medium was seawater containing antibiotics as above. Thraustochytrids grew under these conditions and could be identified up to the species level. The developmental patterns of all the species studied corresponded to the authentic descriptions (Goldstein, 1963 a, b; Ulken, 1965; Schneider, 1967; Perkins, 1973, 1974; Raghukumar, 1988).

## RESULTS AND DISCUSSION

A list of the substrata and the thraustochytrid species which were isolated from them is presented in Table 1. A total of 9 species are reported in this paper, isolated from 52 samples of 14 different substrata. A list of the species is presented in Table 2.

Mostly only single species were isolated when the substrata were placed in petri dishes and baited or plated. However, in some cases, when the substratum was mounted directly in continuous flow chambers along with pine pollen, the presence of two or more species could be detected. Thus, filaments of the blue-green alga, *Lyngbya* sp., collected from Anjuna beach, Goa, first revealed the presence of *Labyrinthuloides yorkensis* Perkins after 24 h in the continuous flow chamber. By 48 h, sporangia of *Ulkenia visurgensis* (Ulken) Gaertner were detected along with the spindle cells and ectoplasmic net system of *Labyrinthuloides minuta* (Watson and Raper) Perkins. All these species completed their development in the continuous flow chamber, thus facilitating identification. Another association between *Labyrinthuloides yorkensis* and *Ulkenia visurgensis* was observed when bits of the green alga *Caulerpa* sp. were mounted directly in continuous flow chamber and observed (Table 1).

Thus, apparently, consortia of thraustochytrid species could be present in natural substrata.

*Ulkenia visurgensis* was the most common species isolated, occurring in 57% of the substrata examined (Table 2). It was present in the algae *Lyngbya* sp. and *Caulerpa* sp., phytoplankton samples in the Arabian Sea, gill tissue of the green mussel, polyps of an obeliinid hydroid, water samples from the Arabian Sea, scrapings of the shells of rock oyster and in coral mucus. This species, therefore, appears to have a wide substrate range. Ulken (1984) has also found this species to be common in tropical muds. The occurrence of *U. visurgensis* in hydroid polyps has been reported in detail elsewhere (Raghukumar, 1988). The isolation of this species from the gill tissue of the green mussel might be related to the filter feeding

TABLE I  
List of thraustochytrid species and their substrata

Substratum	Date and Locality (No. of samples)	Species
1. Decaying mangrove leaves (a) <i>Rhizophora mucronata</i> Lam.	Pitchavaram mangroves, Tamil Nadu, June 1982 (1) Orda, Goa, December 1982 (1) Orda, Goa, April 1986 (4)	<i>Thraustochytrium striatum</i> Schneider <i>T. striatum</i> Schneider <i>T. motivum</i> Goldstein <i>T. aggregatum</i> Ulken <i>Schizochytrium mangrovei</i> Raghukumar
(b) <i>Avicennia officinalis</i> L.	Orda, Goa, May 1987 (1)	<i>S. mangrovei</i> Raghukumar <i>Thraustochytrium multirudimentale</i> Goldstein
2. Blue-green alga, <i>Lyngbya</i> sp.	Anjuna beach, Goa, June 1986 (1)	<i>Labyrinthuloides yorkensis</i> Perkins <i>L. minuta</i> (Watson and Raper) Perkins <i>Ulkenia visurgensis</i> (Ulken) Gaertner
3. Brown-alga, <i>Sargassum</i> sp.	Anjuna beach, Goa, June 1987 (3)	<i>Labyrinthuloides minuta</i> (Watson and Raper) Perkins
4. Green-alga <i>Caulerpa</i> sp.	Mandapam, Tamil Nadu, March 1986 (1)	<i>L. yorkensis</i> Perkins <i>Ulkenia visurgensis</i> (Ulken) Gaertner
5. Red-alga <i>Laurencia</i> sp.	Mandapam, Tamil Nadu, April 1986 (1)	<i>Thraustochytrium motivum</i> Goldstein
6. Estuarine water sample	Zuari estuary, Goa, Dec. 1982 (1)	<i>T. striatum</i> Schneider
7. Phytoplankton samples	Central Arabian Sea, Sagar Kanya Cruise 1, July 1983 (2)	<i>Ulkenia visurgensis</i> (Ulken) Gaertner
8. Gill tissue of the green mussel	Arambol beach, Goa, October 1986 (1)	<i>U. visurgensis</i> (Ulken) Gaertner
9. Polyps of an obeliinid hydroid	Baga beach, Goa, March 1986 (5)	<i>U. visurgensis</i> (Ulken) Gaertner
10. Offshore water samples	Arabian Sea, Sagar Kanya Cruise 1, July 1983 (3)	<i>U. visurgensis</i> (Ulken) Gaertner
11. Detrital particles from an intertidal pool	Anjuna beach, Goa, April 1986 (2)	<i>Schizochytrium mangrovei</i> Raghukumar
12. Shells of the rock oyster	Anjuna beach, Goa, November 1986 (1)	<i>Ulkenia visurgensis</i> (Ulken) Gaertner
13. Corals ( <i>Acropora</i> spp.)	Lakshadweep islands (21)	<i>Corallochytrium imacisporum</i> Raghukumar
14. Mucus from the stag-horn coral	Lakshadweep islands, Minicoy (3)	<i>C. limacisporum</i> Raghukumar <i>Ulkenia visurgensis</i> (Ulken) Gaertner <i>Labyrinthuloides yorkensis</i> Perkins

TABLE 2  
Percentage of substrata harboring the various species of thraustochytrids

Species	Percentage
1. <i>Corallochytrium limacisporum</i> Raghukumar	14.3
2. <i>Labyrinthuloides minuta</i> (Watson and Raper) Perkins	14.3
3. <i>L. yorkensis</i> Perkins	21.4
4. <i>Schizochytrium mangrovei</i> Raghukumar	14.3
5. <i>Thraustochytrium aggregatum</i> Ulken	7.1
6. <i>T. motivum</i> Goldstein	14.3
7. <i>T. multirudimentale</i> Goldstein	7.1
8. <i>T. striatum</i> Schneider	14.3
9. <i>Ulkenia visurgensis</i> (Ulken) Gaertner	57.1

habits of the animal, resulting in the concentration of cells of this thraustochytrid from water in the gill.

*Labyrinthuloides yorkensis* was isolated from the algae *Lyngbya* sp. and *Caulerpa* sp. and from the mucus of the staghorn coral in the Lakshadweep islands. This species has also been reported by the author from Central Arabian Sea (Raghukumar, 1986) and therefore appears to be quite common. It had a percentage occurrence on 21.4 substrata examined (Table 2). The results also suggest that some species have a narrow substrate range (Tables 1 and 2). *Corallochytrium limacisporum* Raghukumar was isolated only from the coral reef lagoon of Lakshadweep islands, in coral polyps, mucus and lagoon waters. This species is very common in the lagoon waters of Lakshadweep islands (Raghukumar, 1987a), but was not isolated from any substratum outside it. *Thraustochytrium aggregatum* Ulken and *T. multirudimentale* Goldstein occurred in only 7.1% of the substrata examined and were isolated only once from decaying leaves of the mangrove, *Rhizophora mucronata* Lam.

Only qualitative aspects are presented in this paper. Quantification of thraustochytrids on the various substrata is needed in future to study their ecology. Raghukumar (1988) demonstrated *Ulkenia visurgensis* in great numbers in an obeliid hydroid, using immunofluorescence and presented some data on their numbers in the waters of the Arabian Sea (Raghukumar, 1986). These aspects will have to be studied in greater detail in future, using techniques which are available for such quantitative studies (Gaertner, 1968; Raghukumar, 1986).

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