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SCHIZOCHYTRIUM MANGROVEI SP. NOV., A THRAUSTOCHYTRID FROM MANGROVES IN INDIA

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Schizochytrium mangrovei sp. nov. on decaying mangrove leaves is described from Goa, India. In sea water/pine pollen cultures, the encysted zoospore divides by repeated binary divisions to form 4, 6, 8 or 12 cells. Instead of forming sporangia as in the other species of *Schizochytrium*, these cells directly become zoospores. In nutrient liquid media, the developmental stages resemble those of *Ulkenia*. A key to the species of *Schizochytrium* is given.

While investigating the thraustochytrid flora of the seas around India, a species was isolated from decaying mangrove leaves, collected from the mangrove vegetation at Orda, Goa (lat. 15° 25′N; long. 73° 40′E). The leaves were brought to the laboratory and small bits were placed in 5 cm Petri dishes to which 5 ml of sterile sea water and sterile pine pollen were added. The thraustochytrid grew on to the pine pollen in about a week. Four isolates were obtained, all from the same site. One of the isolates was obtained from decaying leaves of *Rhizophora mucronata* leaves during April 1986. The others were obtained from decaying leaves of *Avicennia officinalis* during March 1987.

The isolates were made bacteria-free by subculturing them on to sea water/pine pollen, containing 500 μg streptomycin and 1000 units of penicillin/ml seawater. The morphology and development of the organism were studied on pine pollen in sea water and also on nutrient liquid media in a continuous flow chamber (Raghukumar, 1987*a*). The nutrient medium was composed of glucose, 0·25 %, peptone, 0·125 %, yeast extract, 0·05 %, vitamin mix according to Vishniac (1956) and sea water, 100 ml.

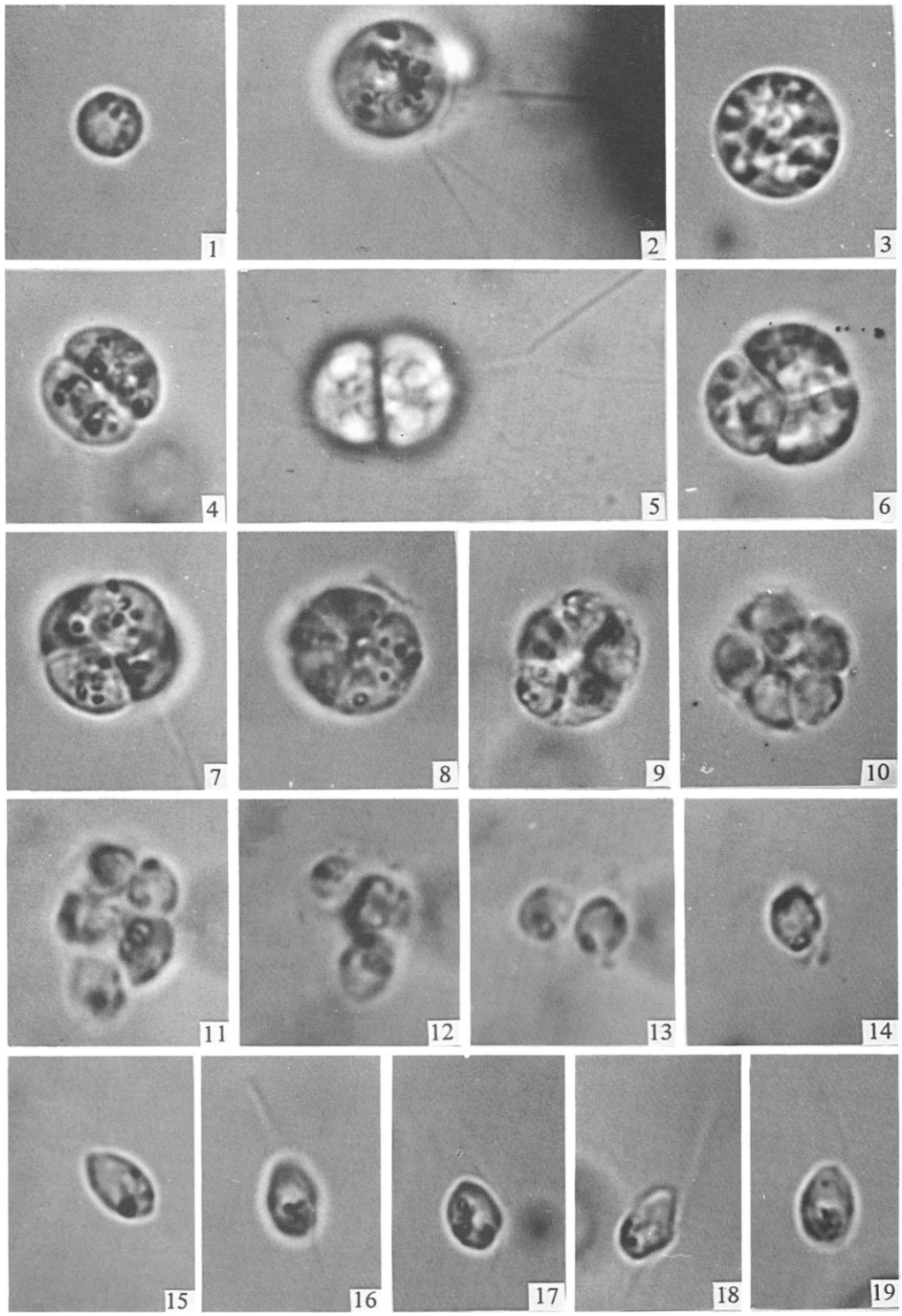
Schizochytrium mangrovei Raghu-kumar sp. nov.

In aque marinum/pinus pollen culturae, sporangia globosa initio 3·7–4·4 μm diam; maturitatae 6·5–9·2 μm diam, monoaxialum disciplinum reticulum ectoplasmaticum formant. Sporangium dividitur per continua bipartita amplificatione; producit 2, 4, 6, 8 vel 12 zoosporae. Maturitatae paries totaliter cedit. Zoosporae ellipticae sunt, 4·1–4·8 × 2·6–3·3 μm, laterialiter biflagellatae, flagellum afore longioris, flagellum posticos brevior.

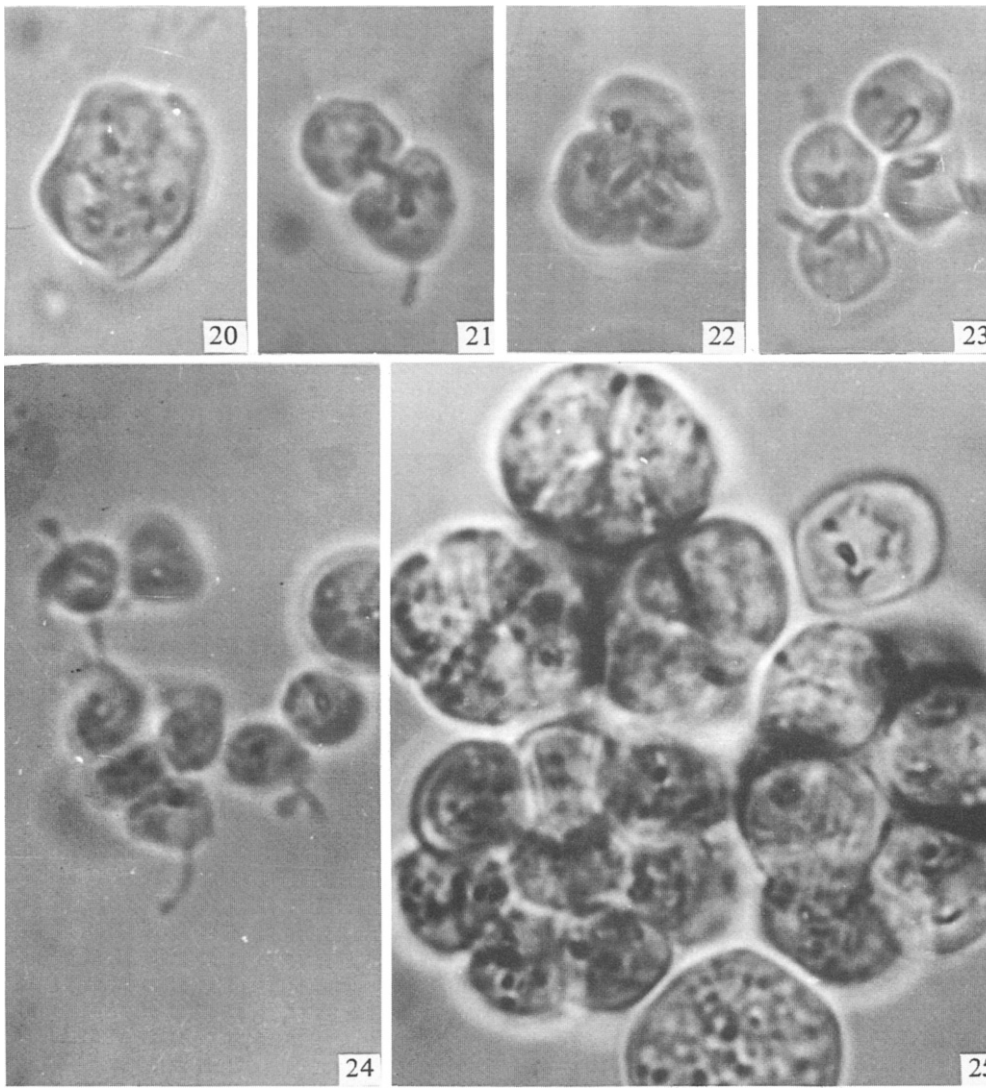
Isolata e foliis emortuis *Rhizophorae mucronatae*, Orda, Goa, India, Holotypus figs 1–19 et descriptio.

In sea water/pine pollen cultures, the organism developed epibiotically on pine pollen, interbiotically with only the ectoplasmic net elements touching the pine pollen or attached to the surface of the culture vessel. The young cell formed by encystment of the zoospore was globose and measured 3·7–4·4 μm diam (Fig. 1). The mature cell, namely at a stage prior to division, which was formed nearly 24 h after encystment of the zoospore, was globose or subglobose and measured 6·3–9·2 μm diam (Figs 2, 3). Ectoplasmic net elements were formed from a single site on the cell (Fig. 2). The mature cell divided by a binary division to form a diad (Fig. 4). Each cell of the diad could produce ectoplasmic net elements (Fig. 5). Another binary division led to the formation of a tetrad (Figs 6, 7). All the cells of the tetrad divided in two, or only two or three of the tetrad divided, resulting in 6, 7 or 8 cells in a cluster (Figs 8–10). Often further division resulted in 12 cells. Throughout, the individual cells resulting from the divisions did not increase in size and the diameter of the cell cluster remained more or less the same as the original mature thallus. The number of cells in the cluster was difficult to discern during early stages. Gradually, however, the cells rounded up and became more distinct (Fig. 10). At this stage, which is just prior to liberation of the zoospores, the cell wall gradually began to disintegrate. One after the other, each individual cell developed two flagella and swam away (Figs 11–14). The escape of the zoospores was successive. The zoospores (Figs 15–19) were elliptical in shape, and in lateral view possessed a slight depression at the anterior end (Fig. 18). They measured 4·1–4·8 × 2·6–3·3 μm, with a length-to-width ratio of 1·4:1 to 1·9:1. The long anterior flagellum and the shorter posterior flagellum were laterally attached.

Living cultures of *S. mangrovei* are deposited at the NIOCC (National Institute of Oceanography Culture Collection), under Nos. 90, 97 and 98.



Figs 1–19. *Schizochytrium mangrovei*, NIOCC 90. Figs 1–14. Development in sea water/pine pollen cultures (× 2800). Figs 15–19. Live zoospores (× 2800).



Figs 20–25. *Schizochytrium mangrovei*, NIOCC 90 in nutrient liquid medium. Figs 20–24. *Ulkenia* mode of development (×2800). Fig. 25. In situ growth of cells in a cluster (×2800).

Repeated binary divisions characterize the present species when grown in sea water/pine pollen. Of the eight genera known among the thraustochytrids (Olive, 1975; Moss, 1986; Raghu-kumar, 1987*b*), such development is seen in three genera, namely *Schizochytrium* Goldstein & Belsky (1964), *Labyrinthuloides* Perkins (1973) and *Corallochytrium* Raghu-kumar (1987*b*). Of these, only *Schizochytrium* produces zoospores, and the species described here resembles it in most features. The distinguishing characters of these genera are presented in Table 1.

However, this species differs from the other three species of *Schizochytrium* described (Goldstein & Belsky, 1964; Gaertner, 1981; Raghu-kumar, 1987*c*). In these species, the cell formed by the encysted zoospore grows and divides by repeated binary divisions to form a cluster of cells, each of which develops into a zoosporangium, producing 2, 8 or more zoospores, which escape from the cell wall of the zoosporangium to become free. In the present species also, the encysted zoospore grows and divides by repeated binary divisions. However, the resulting cells do not form

Table 1. *Distinguishing taxonomic features of thraustochytrid genera showing repeated binary divisions during development*

Characters	*Schizochytrium*	*Labyrinthuloides*	*Corallochytrium*
Ectoplasmic net system	Present; monoaxial	Present; radiate	Not present
Division of cell formed by encysted zoospore	Repeatedly binary	Repeatedly binary or by progressive cleavage	Repeatedly binary
Cluster of cells formed by binary divisions	Irregular with up to 100 cells, or globose with up to 12 cells	Globose cluster of up to 64 cells	Globose to sub-globose cluster with up to 32 cells
Nature of spores	Biflagellate zoospores	Non-flagellate, with ectoplasmic net system	Non-flagellate, limaciform spores

zoosporangia. On the other hand, each of the cells resulting from the division becomes a zoospore and escapes.

In nutrient liquid medium, the development differed from that in sea water/pine pollen in two ways. (1) When mature, the globose cells, resulting from the encysted zoospores, measuring 5·0–12·0 μm diam, become amoeboid and irregular in shape due to the loss of the thin wall (Fig. 20). Very soon, they twist and cleave exactly into 2, 4, 8, 16 or rarely 32 zoospores (Figs 21–24), accompanied by a constant change in the outline of the entire mass, indicating the absence of confinement in the parent cell wall. Small cells cleaved into only two cells. Larger ones first cleaved into four cells, with or without further divisions, leading to a greater number of zoospores. (2) Rarely, the cell wall of the mature thallus did not disappear. The contents of the globose thallus divided into 4, 8, 16 or rarely 32 cells. These did not develop into zoospores, but grew in situ, later resulting in large clusters of cells (Fig. 25).

Variations of developmental characters of thraustochytrid species on media other than sea water/pine pollen have been well documented (Booth & Miller, 1968; Kazama *et al.*, 1975). As Kazama *et al.* (1975) pointed out, studies of such variation would be of significance in understanding the taxonomic relationships of thraustochytrid species. In the present study, on liquid nutrient medium, the organism resembles the genus *Ulkenia* Gaertner in the disintegration of the cell wall and cleavage of the amoeboid protoplasmic mass into zoospores. However, all species of *Ulkenia* known have been described based on characters in sea water/pine pollen cultures (Gaertner, 1977). In sea water/pine pollen, the present species differs considerably from *Ulkenia*. In our opinion, a reasonable taxonomy of the thraustochytrids can be achieved by using a standard medium such as sea water/pine pollen or an equivalent medium, while other media will give an insight into the variability and relationships of the taxa.

KEY TO KNOWN *SCHIZOCHYTRIUM* SPECIES

1. Encysted zoospore growing and dividing by repeated binary divisions to form a cluster of cells, each of which becomes a zoosporangium 2

1. Encysted zoospore growing and dividing by repeated binary divisions to form a cluster of cells each of which becomes a zoospore. . . . . *S. mangrovei* Raghu-kumar

2. Zoosporangia 15–25 μm diam; zoosporangia forming 16–64 zoospores . *S. aggretatum* Goldstein & Belsky

2. Zoosporangia less than 14 μm diam, each zoosporangium forming 2 or 8 zoospores 3

3. Zoosporangia forming 8 zoospores each . . . . . *S. octosporum* Raghu-kumar

3. Zoosporangia forming 2 zoospores each . . . . . *S. minutum* Gaertner

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