

Detection of the thraustochytrid protist *Ulkenia visurgensis* in a hydroid, using immunofluorescence

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Abstract

An immunofluorescence technique was employed to detect the thraustochytrid protist *Ulkenia visurgensis* (Ulken) Gaertner, originally isolated from an algal bed, in natural samples in Goa, India. Collections were made monthly from September 1985 to November 1986. By treating the samples with antiserum prepared against this organism and conjugated with FITC stain, the protist was regularly found to occur in association with a hydroid. Several cells of the organism were observed in the coelenteron and the hydranth of the hydroid. The thraustochytrid could be repeatedly isolated in cultures from the hydroid. The cells of the protist differed from those in culture in that they lacked ectoplasmic net elements.

Introduction

The fungi-like marine protists, the thraustochytrids, occur abundantly in seawater and sediments (Bremer 1976, Gaertner and Raghu-kumar 1980, Raghu-kumar and Gaertner 1980, Jacobsen 1983) and on many vegetative substrates (Sparrow 1969). However, till now, they have only been recorded by using indirect culture techniques. In situ observations to determine the substrate and niches on which they occur are few and inconclusive. Such observations are essential to understand the role of these interesting organisms in nature. Sparrow (1936) reported them on moribund algae. Gaertner (1979) and C. Raghu-kumar (1986) reported them on living diatoms from the Rosfjord, Norway and the Arabian Sea. Riemann and Schrage (1983) reported a mass occurrence of thraustochytrid-like cells in Antarctic soils. The major reason for our scanty knowledge on this aspect may be the fact that cells of these organisms cannot be identified in natural samples, since they fall within the size range of many other protists (5 to 20 μm). It is also possible that

they occur in morphological forms different from those observed in laboratory cultures or grow on or in unexpected niches. I have hence resorted to the immunofluorescence technique to detect the thraustochytrid *Ulkenia visurgensis* in situ. The immunofluorescence (IF) or the fluorescence antibody technique (FAT) is particularly suitable for identifying species or even strains of individual microorganisms in nature and can be of immense value in ecological studies (Bohloul and Schmidt 1980).

Materials and methods

Ulkenia visurgensis (Ulken) Gaertner was originally isolated from algal beds of *Cladophora* sp. from the beaches of Baga at Goa, India (Lat. 15°33'N; long. 73°44'E). A few algal filaments were baited with sterile pine pollen in sterilised seawater, fortified with 0.1% streptomycin and 2 000 units of penicillin/ml, following the technique of Gaertner (1968). The bacteria-free culture of the thraustochytrid was subsequently maintained on pine pollen in seawater. The protist was identified by observing its developmental morphology in continuous flow chambers (Raghu-kumar 1987). The culture has been deposited in the National Institute of Oceanography Culture Collection under the No. NIOCC 83. The methodology for IF technique was according to Preece (1971 a, b), Cruickshank et al. (1975), Smibert and Krieg (1981) and Koti Reddy and Ananthanarayanan (1984).

The organism was grown in stationary cultures in liquid Thraustochytrid Medium I (Raghu-kumar 1985). The cells were harvested after one week's growth and suspended in physiological saline. The protein concentration was estimated according to a simplified method of Lowry (Peterson 1977), and adjusted to 600 $\mu\text{g/ml}$. 0.25 ml of this antigen was injected into White New Zealand rabbits on the first day. Dosage was increased by 0.25 ml every day after this till the 8th day and 2.0 ml antigen was injected on Days 8, 9, 10 and 11. Fifteen days after this schedule, a

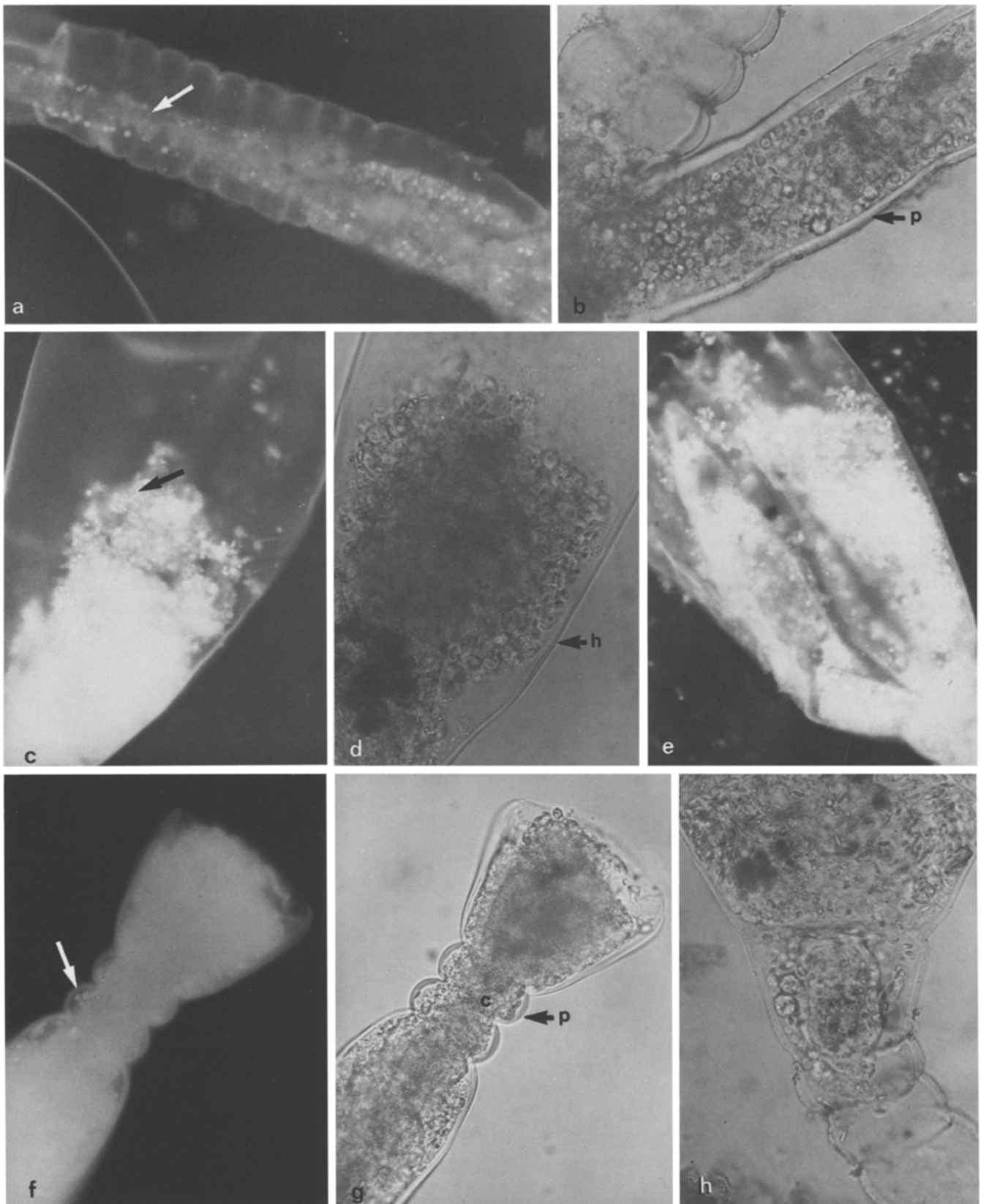


Fig. 1. *Ulkenia visurgensis*. (a) Fluorescent antibody (FA) stained preparations of the coelenteron revealing fluorescing cells of *Ulkenia visurgensis*. $\times 250$; (b) light micrograph of the coelenteron with globose cells resembling the thraustochytrid. $\times 400$; (c) fluorescence micrograph of the hydranth containing cells of *U. visurgensis* (arrow). $\times 200$; (d) light micrograph of a hydranth containing globose cells resembling the thraustochytrid. $\times 250$; (e) presence of fluorescing thraustochytrid cells (arrow) in the hydranth with retracted tentacles. $\times 250$; (f) young polyp of the hydroid stained with FA and showing bright fluorescence due to presence of *U. visurgensis* (arrow). $\times 250$; (g) light micrograph of f. $\times 250$; (h) cells resembling the thraustochytrid between the perisarc and the coelenteron. $\times 400$; c: coelenteron; h: hydranth; p: perisarc

booster dose of 2.0 ml antigen was injected for four more days. Seven days later, the antiserum was obtained and titer values were estimated. A value of 1/1280 was obtained. Gamma globulin was separated and purified following standard methods and was conjugated with Fluorescein Isothiocyanate (Merck, Darmstadt). The conjugate was stored at -20°C .

Material was regularly collected every month from the Baga Beach, starting from September 1985, stained with the FITC conjugate (Fluorescent Antibody or FA) and observed. A drop of a suitable dilution of the conjugate was placed in a cavity slide and bits of the material (algal filaments and debris) were placed in the drop and incubated in a moist-chamber for about 30 min. The material was then rinsed in seawater in Petri dishes, mounted in seawater and observed under an Olympus BH2 RFL epifluorescence microscope, using a blue-light excitation and 515-nm barrier filters.

Results

The antiserum was first tested against six isolates of thraustochytrids, comprising five different species. *Thraustochytrium aggregatum* (NIOCC 89), *Schizochytrium* sp. (NIOCC 90), *Corallochytrium limacisporum* (NIOCC 70) and *T. striatum* (NIOCC 66) did not cross-react with the antiserum. However, two isolates of *T. motivum*, NIOCC 84 and 89, cross-reacted strongly with the antiserum, similar to *Ulkenia visurgensis* NIOCC 83, against which the antiserum had been prepared. Neither *T. motivum* nor *U. visurgensis* revealed any autofluorescence.

A thorough scan of live and dead filaments of *Cladophora* sp. collected from September 1985 to January 1986 did not reveal fluorescing cells, apart from autofluorescing cell walls of the alga and chlorophyll. However, in a sample collected in February 1986, a hydroid polyp that was present in the algal samples revealed the presence of numerous globose, fluorescing cells of *Ulkenia visurgensis* in its body. The hydroid was identified as belonging to the subfamily Obeliinae (P. F. S. Cornelius personal communication). Starting from January 1986, monthly samples of the hydroid were collected and observed till November 1986, except during the monsoon months from June to October. Details of the observations are presented below.

In carefully prepared microscopic mounts of the hydroid, after FA staining, many fluorescing cells of *Ulkenia visurgensis* were observed to be regularly arranged in the coelenteron, often in a row (Fig. 1a), suggesting their presence along the wall of the coelenteron. Mostly, the coelom collapsed under a coverslip and light microscopy then revealed the presence of numerous globose cells (Fig. 1b). Only some of these fluoresced with FA staining, while the others, presumably cells of the hydroid, did not. However, throughout the study it was not possible with light microscopy alone to conclude which one of the globose cells belonged to the thraustochytrid and which to the hydroid (Fig. 1b, d, g, h; Fig. 2b, f). Fig. 1c reveals the hydranth, containing several fluorescing cells. Fig. 1d is a light micro-

graph of a hydranth. The interior of a hydranth containing retracted tentacles revealed fluorescing globose cells (Fig. 1e). Whenever there were numerous cells of the hydroid, as in Fig. 1d and e, strong background fluorescence due to non-specific adsorption of the fluorescent dye made observations difficult. With some practice, however, the intensely fluorescing cells of the thraustochytrid could be clearly distinguished (arrows in Fig. 1c, e). Young, incompletely formed polyps showed very strong fluorescence and numerous, conspicuous cells, frequently present between the perisarc and the coelenteron (arrows in Fig. 1f, g, h).

Whole mounts of the polyps (Fig. 2a) normally did not give any clue regarding the presence of *Ulkenia visurgensis*. By crushing the hydroid under a coverslip, one could recognise numerous globose cells (Fig. 1b). However, in the absence of FA staining, host cells and possible cells of the thraustochytrid could not be distinguished. Often, hydroids did not reveal any fluorescing cells of *U. visurgensis* after FA staining. After staining, if fluorescing cells of *U. visurgensis* were present, these could be squeezed out of the hydranth by pressure on the coverslip (Fig. 2c). An enlarged light micrograph of the fluorescing cell shown in Fig. 2c (arrow) is presented in Fig. 2d. Such cells were globose, without an ectoplasmic net, often, but not always with globules and ranged from 6.0 to 12.0 μm in size. Light micrographs of cells appearing like those of the thraustochytrid inside the hydroid are also shown in Fig. 2f.

Small bits of the hydroid (ca 0.5 mm long), including the mouth parts were removed, washed thoroughly in sterile seawater, observed under a microscope to verify that no thraustochytrid thalli were present on the outside and were transferred to small 5 cm Petri dishes with sterile seawater. The polyps were then crushed using a blunt needle to release the contents. The Petri dish was then baited with sterile pine pollen or larvae of the brine shrimp *Artemia* sp. Thraustochytrids grew on the baits thus provided. Four isolates were thus obtained and identified as *Ulkenia visurgensis*. Fig. 2e shows the cell of *U. visurgensis* growing on brine shrimp larva. The thraustochytrid also grew inside the detached hydranth itself, which was used for the isolation by baiting (Fig. 2g).

Discussion

The observations made in this study suggest that *Ulkenia visurgensis* lives in an intimate association with the hydroid. Cells were detected in the hydroid not only using the FAT, but their presence was also repeatedly verified by re-isolating the same species from the hydroid. Association of thraustochytrids with invertebrate animals has been reported earlier. Wagner-Merner et al. (1980) reported a number of thraustochytrids from the guts of the sea-urchin *Lytechinus variegatus*. Höhnk and Ulken (1979) and Richter (1985) reported them from sponges. However, it is not clear that the occurrence of thraustochytrids in these animals is not passive and that they occur in significant num-

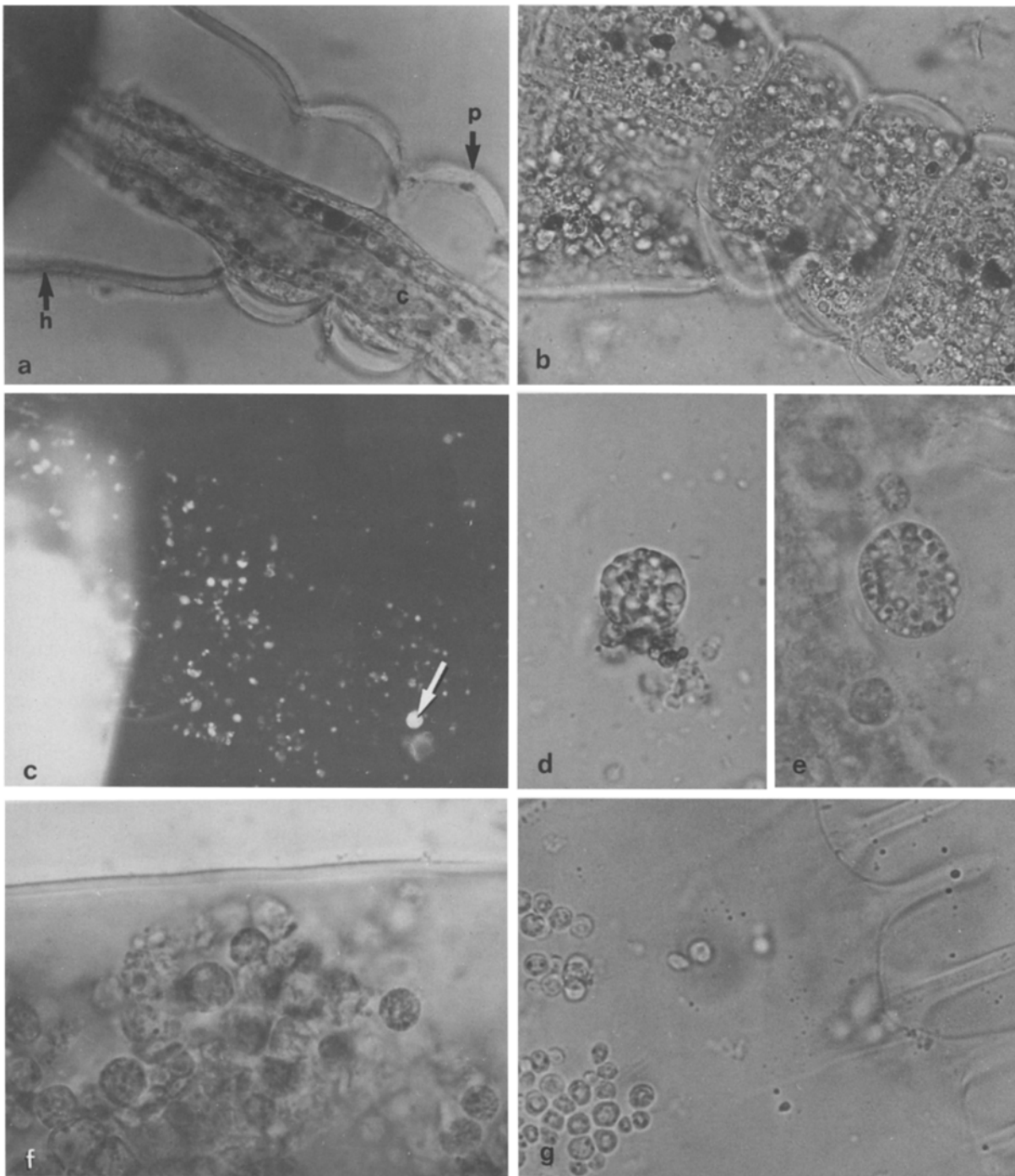


Fig. 2. *Ulkenia visurgensis*. (a) Light micrograph of an intact polyp. $\times 400$; (b) same as 'a', flattened under a coverslip and revealing the presence of cells resembling the thraustochytrid. $\times 400$; (c) fluorescing cells of *Ulkenia visurgensis*, squeezed out of polyp. $\times 250$; (d) enlarged light micrograph of the fluorescing *U. visurgensis* cell shown in 'c' (arrow). $\times 2000$; (e) cell of *U. visurgensis* growing on brine shrimp larva. $\times 1000$; (f) light micrograph of *U. visurgensis*-like cells in the coelenteron of the hydrozoan. $\times 1000$; (g) cells of *U. visurgensis* growing inside a hydrotheca employed to isolate the thraustochytrid. $\times 700$; c: coelenteron; h: hydranth; p: perisarc

bers. On the other hand, the IF technique employed presently demonstrates the abundant presence of a thraustochytrid in the coelenteron and hydranth of the hydroid. The fact that so many cells of *U. visurgensis* were found inside the hydroid, but not in the dead and living algal filaments around it, suggests a specificity regarding its occurrence, at least in the presently investigated site.

Not all the monthly samplings of the hydroid revealed the thraustochytrids. There is probably a seasonality of occurrence, which has to be studied in greater detail. Besides, two other species of hydroids collected from the vicinity did not reveal *Ulkenia visurgensis*. Hence, there is probably also a host specificity. The cells of *U. visurgensis* inside the polyp were different from those in pine pollen/seawater

cultures, in that they lacked ectoplasmic nets. This is not surprising since the formation of ectoplasmic nets is suppressed in cells of thraustochytrids when they are grown in nutrient-rich media. Its habitat inside the polyp apparently does not necessitate the formation of ectoplasmic net elements. The latter have been implicated in nutrient absorption. These observations support our earlier assumptions that the thraustochytrids might occur in substrates hitherto uninvestigated and may look different than in axenic cultures.

There also exists the interesting possibility of thraustochytrids being capable of living in two kinds of habitats, one in which ectoplasmic net elements are not necessary, as in the present case, and the second, in relatively nutrient poor conditions, where the formation of ectoplasmic net elements would be of advantage by increasing the surface area of absorption or to penetrate particulate organic matter.

Background fluorescence due to non-specific adsorption of the fluorescent dye by the animal cells was frequently encountered. This is a common problem in IF studies (Bohlool and Schmidt 1980). However, the thraustochytrid cells could be distinguished by the much more brilliant fluorescence of the entire cells.

The exact nature of the association between *Ulkenia visurgensis* and the hydroid is not certain. The possible existence of cells in the wall of the coelenteron as judged by the regular arrangement of fluorescing cells (Fig. 1a) leads to an interesting speculation. Many hydrozoans are known to harbour symbionts in the endodermis of the cell wall (Hyman 1940). Several groups of algae occur as such symbionts of hydrozoans (Taylor 1974). Particularly, the dinoflagellates (zooxanthellae) in corals have been studied extensively (Loeblich 1984). Further detailed work has to be done to find out if the association between *U. visurgensis* and the hydroid is also symbiotic. The possibility of parasitism of the thraustochytrid on the hydroid cannot be totally ruled out. Polglase (1980) reported the occurrence of thraustochytrids associated with pathological conditions of the lesser octopus. However, in the present case, the polyps containing cells of *U. visurgensis* always appeared healthy and did not seem to be affected by the thraustochytrid. Very often, cells of *U. visurgensis* were found between the coelenteron and the perisarc. It is not clear if the space is also a habitat of the thraustochytrid or if these cells were dislodged from the body wall of the hydroid during microscopic preparation. The observations made in this study might not have been possible without employing the IF technique, particularly since the cells of the thraustochytrid inside its host were indistinguishable. Future studies employing the FAT for other species of thraustochytrids might yield interesting information.

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