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**SESSILE CILIATES WITH BACTERIAL ECTOSYMBIONTS FROM  
TWIN CAYS, BELIZE**

**BY**

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**Figure 1.** SEM photomicrograph of *Zoothamnium niveum* showing microzooids covered by symbiotic bacteria.

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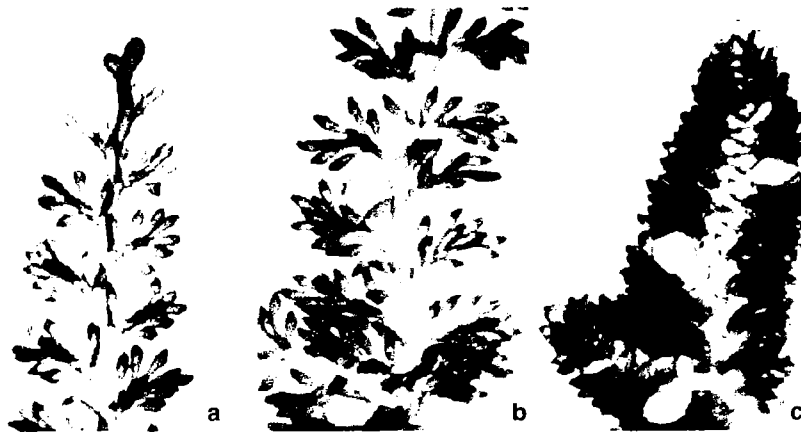
## INTRODUCTION

Two species of sessile peritrich ciliates with bacterial ectosymbionts are common in the mangrove islands of the Belize Barrier Reef. One is the large colonial *Zoothamnium niveum* (Hemprich & Ehrenberg, 1831) Ehrenberg, 1838 (redescribed in Bauer-Nebelsick et al. 1996a). The second species is an as yet undescribed solitary vorticellid, provisionally called *Vorticella* sp. below. Both species occur together on a variety of sulfidic habitats. They are conspicuous because of their white color in incident light which is due to chemoautotrophic sulfur oxidizing bacteria which almost completely cover their surface. The bacteria need both sulfide and oxygen for autotrophic carbon fixation and growth. These compounds, however, coexist in nature only in narrow, changing and often unpredictable micro zones. The association with the protozoans insures a constant supply of these compounds as will be described below.

## SPECIES DESCRIPTION

### *Zoothamnium niveum*

*Zoothamnium niveum* belongs to a large genus of peritrich ciliates which includes marine and freshwater species many of which are epizootic or epiphytic. With the exception of *Z. pelagicum* all species are attached to a substrate. The colonies of *Z. niveum* are feather-shaped, consisting of a central stalk which may be up to 15 mm long from which side branches originate in an alternating arrangement (Fig. 1 and Fig. 2). The branches bear up to 20 microzooids, which are the feeding zooids and are equipped with the complete ciliary apparatus of peritrichs and have a well developed cytopharynx. The tip of the stalk and of those branches which are still growing is occupied by a club-shaped terminal zooid lacking cilia and cytopharynx. At the basis of the branches large globular macrozooids devoid of a cytopharynx are developed, which eventually detach and act as dispersal units (Bauer-Nebelsick et al. 1996a). Older and larger colonies often show one or more secondary feather-shaped fans which apparently originate on the stalk. Such colonies may consist of more than 3000 microzooids and bear up to 80 macrozooids.



**Figure 2.** *Zoothamnium niveum*. A) Tip of colony with dividing terminal zooid B) Middle part of colony showing side branches with microzooids and macrozooids C) Contracted large colony with secondary stalk branching off to the left. Light micrograph.

The stalk is attached to the substrate with a disk and its basal portion is not contractile. The remaining part of the stalk and the branches contain a contractile fiber (spasmoneme) which contracts spontaneously or upon disturbances with extremely high speed ( $0.5 \text{ m}\cdot\text{s}^{-1}$ , Vopel et al. 2002). Except for the non-contractile part of the stalk and the peristomal disc of the microzooids all surfaces are densely covered by a single layer of rod-shaped bacteria  $1.4\mu\text{m} \times 0.4\mu\text{m}$ , which increase in size and change to a more coccoid shape ( $1.9 \times 1\mu\text{m}$ ) towards the distal part of the microzooids (Fig. 1). At the edge of the peristomal disc they appear to form more than one layer and not all bacteria seem to be in contact with the host. The bacteria appear pure white in incident light, which is the reason for the species name of the host, and black in transmitted light (Bauer-Nebelsick et al. 1996b).

According to the 16S rRNA gene the bacteria belong to the  $\gamma$ -proteobacteria (Molnar et al. 2000). Their ultrastructure is similar to free-living thiobacilli, their oxygen uptake decreases upon prolonged exposure to oxygen and is stimulated by incubation in sulfide-containing water. This suggests a sulfur oxidizing chemolithoautotrophic nature of the bacteria, which is further corroborated by the presence of RuBisCO (H. Felbeck, pers. comm.) and the uptake of radiolabelled bicarbonate (Rinke 2002).

*Zoothamnium niveum* occurs regularly on subtidal sulfidic mangrove peat exposed along the walls of tidal channels and on a variety of natural or artificial substrates in the vicinity of sulfide sources (Ott 1996, Ott et al. 2004). It has been observed within dense macroalgal mats (*Avrainvillea* spp, *Halimeda* spp), sea grass leaves and rhizomes on sulfidic mud. Large numbers of colonies have been observed on garbage bags and on rotting vegetables dumped in mangrove ponds (own unpubl. observations).

In the tidal channels groups of up to about 100 colonies are found around disturbed patches on the surface of the peat, where the microbial surface biofilm has been interrupted, e.g. where a rootlet has rotted and fallen out. At such spots, which are commonly just a few mm in diameter, sulfide diffuses into the ambient, oxygen containing water at a higher rate than through the undisturbed peat surface, where a

variety of microbes consume most of it before it enters the boundary layer (Ott et al. 1998). A wave induced mechanism sucks sulfidic water from holes in the peat (Vopel et al., in press), creating a favorable environment for the symbiotic ciliates. The ciliary beat of the microzooids efficiently mixes the ambient oxygen-containing water with the sulfide diffusing from the peat (Vopel et al. 2001). Occasionally the zooids rapidly contract and completely immerse in the sulfidic boundary layer, which is about 3mm thick. During the subsequent slow expansion they drag sulfidic water into the oxic ambient water (Ott et al. 1998, Vopel et al. 2001, 2002).

The life cycle starts with the settlement of a swarmer (macrozooid) which within a few hours grows a 0.5 mm long stalk while transforming into a terminal zooid. This starts to divide, producing 2-3 groups of 1-3 microzooids before the first branch is formed. Growth is rapid and the colonies reach their maximum size within 4 days. From then on the basal parts begin to deteriorate. First the non-contractile part is overgrown by filamentous sulfur bacteria and a variety of other bacteria and diatoms. This epigrowth extends to those parts of stalk and proximal branches that in time lose the microzooids and the original bacterial coat. Mean life span of a colony is 7 days (M. Bright, J. Ott, unpubl. observations).

*Zoothamnium niveum* occupies ephemeral habitats. The disturbed patches on the mangrove peat deliver enough sulfide for approximately 20 days, before they are overgrown by a microbial mat which scavenges the entire sulfide (Ott et al. 1998). Loose debris accumulations are subject to occasional strong wave action, which redistributes the particles and aerates the pore water. The swimmers therefore play a decisive role in finding suitable environmental patches where they can grow into mature colonies, produce swarmers again and thus maintaining the ciliate population in such a changing environment. Swarmers precisely find sulfide sources which may be only a few mm across. Swimming at a speed of about 5 mm.s<sup>-1</sup> which may be maintained for at least 24 hours they may cover a distance of 400 m before settling. The so far measured maximum distance from the nearest source of a swarmer settled on an artificial sulfide producing systems (ASPROS), however, is <5m. During dispersal they seem to stay close to the bottom surface, since ASPROS are readily colonized by *Z. niveum* when exposed flush with the bottom surface, but never when placed at a distance >3cm from it. Macrophyte debris accumulations may act as stepping stones between suitable habitats and even may allow rafting (M. Bright, A. Nussbaumer, J. Ott, unpubl. observations).

*Zoothamnium niveum* was originally described from the Red Sea (Hemprich & Ehrenberg, 1831). In the original description there is no mention of the bacteria, but the white color was noted. The material for the redescription was collected at Twin Cays. Since then it has been found at Blue Ground Range, Tobacco Range and the Pelican Cays. Other reports of this conspicuous species come from the Florida Keys, the Canary Islands and the Eastern (Greece) and Western Mediterranean (Corsica, Elba, Giglio) where it grows on decaying macrophyte debris or hard substrates in close contact with debris accumulations (M. Bright, P. Wirtz, G. Scattolin, J. Pillen, pers. comm. and own unpubl. observations).

*Vorticella* sp.

There is much less data on the second symbiotic peritrich ciliate species and most of the data below are from own unpublished observations. *Vorticella* sp. is solitary like all members of the genus. The zooids have a 350-500 $\mu$ m long stalk which is attached to the substrate with a basal disk. The zooids appear conical when retracted. When fully extended, however, they are disc- or even mushroom-shaped. The aboral side and the fully contractile stalk are densely covered by bacteria which appear similar to those in *Zoothamnium niveum* (Fig. 3). Likewise they appear white in incident light. There are no data as yet on the fine structure, enzyme repertoire and physiology, nor are gene sequences available. The white color which is indicative of sulfur storage and the habitat preference of the symbiosis, however, suggest a chemoautotrophic sulfur-oxidizing nature for the bacteria.

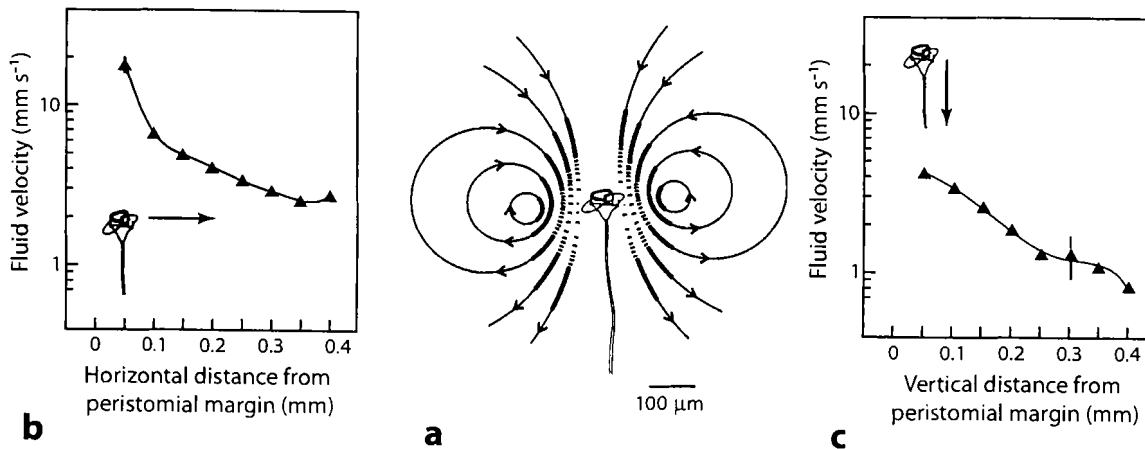


**Figure 3.** *Vorticella* sp. A) Sketch of microbial community with large filamentous sulfur bacteria (*Beggiatoa* sp.) (adapted from Vopel et al. 2001) B) Contracted individual covered by symbiotic bacteria. SEM C) Three individuals seen from the oral side. SEM

*Vorticella* sp. co-occurs regularly with *Zoothamnium niveum* in the vicinity of disturbed peat patches. It seems to be more abundant on older patches where most *Z. niveum* colonies are mature to senescent forming dense lawns of zooids. Due to their small size the zooids are constantly within the sulfidic boundary layer and rely heavily on the current created by beating their cilia (Vopel et al. 2001, 2002) (Fig. 4)

The zooids divide, forming a stalk less dispersal stage which detaches and swims away. It apparently settles not far from the parent zooid, thus rapidly producing dense lawns of individuals. There are no data yet on the life span of the swarmer stages. The dispersal capacity seems to be much less than that of the swarmers of *Z. niveum*, which is indicated by the rather late arrival at sulfide sources. In addition, *Vorticella* sp. was only rarely observed on ASPROS used to attract swarmers both in the laboratory and the field, although once settled on an ASPROS it grows well and reproduces rapidly.

The symbiotic *Vorticella* sp. has so far been observed at all locations of the Belize Barrier Reef system where *Z. niveum* has been found. A similar symbiotic species co-occurs with *Z. niveum* on sea grass debris in Corsica, Western Mediterranean.



**Figure 4.** *Vorticella* sp. a) velocity field around an individual of *Vorticella* sp. b) horizontal velocity profile c) vertical velocity profile (after Vopel et al. 2001, 2002)

### Symbiotic partnership

Benefits of the symbiotic partnership are a constant and rich food supply for the ciliates, which apparently feed largely on the detached symbiotic bacteria and can maintain high growth rates. *Z. niveum* grows several times faster than its non-symbiotic relatives and attains the largest colony size within the genus. The bacteria in turn are supplied with sulfide and oxygen at a predictable and high rate and the motile swarmer stages carry them to new microhabitats when the old ones deteriorate. The bacteria in *Zoothamnium niveum* show a complex cell cycle as adaptation to the symbiotic life (Bright 2002). Both species are extremely patchy distributed wherever they occur. This seems to be correlated with the patchy distribution of sulfide sources. Non-symbiotic *Zoothamnium* and *Vorticella* species occur in low densities in the same habitats but away from the sulfide point sources. The non-symbiotic *Zoothamnium* colonies are most probably *Z. alternans*, which is closely related to *Z. niveum*. Both species have been cultivated in the laboratory for a few generations using ASPROS. Both have, however, resisted long term maintenance and cultivation. Maintaining suitable conditions for the bacteria seems to be critical, since both species first lose the symbionts and are able to survive aposymbiotically for a while. They may even produce dispersal stages and an aposymbiotic generation, which however is incapable of further reproduction. Both ciliates exploit the sharpest sulfide/oxygen gradient of all known sulfide symbioses (Polz et al. 2000). Despite their small size they effectively manipulate the microenvironment within their habitat in order to thrive in an otherwise hostile environment.

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