Project: Ke 287/9 „Untersuchung der Mikrobialithe und Spongien-Assoziation im mit alkalischem Meerwasser gefüllten Kratersee von Satonda (Indonesien) als rezentes Beispiel fossiler Spongiolithfazies", Project Leader: S. Kempe (Darmstadt) & J. Reitner (Göttingen)

New Data on Microbial Communities and Related Sponge Fauna from the Alkaline Satonda Crater Lake (Sumbawa, Indonesia)

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Area of Study: Indonesia, Sunda Islands
Environment: Alkaline crater lake
Stratigraphy: Holocene
Organisms: Microbes and sponges
Depositional Setting: Crater lake
Constructive Processes: Microbial calcification
Destructive Processes: Microbial borings
Preservation: —
Research Topic: Interaction between microbes and metazoans

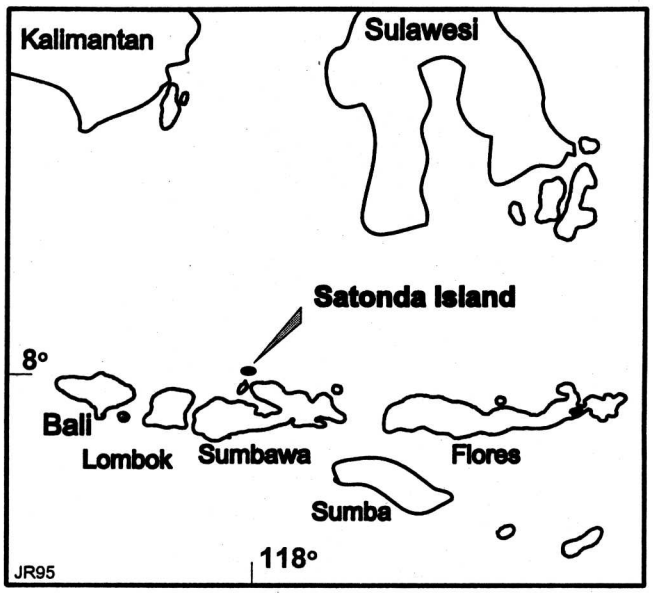

Fig. 1: Location of Satonda Island in the Sunda archipelago, Indonesia.

Abstract

The small crater lake of the Island Satonda is characterized by highly alkaline conditions as a whole probably due to an intense sulfate reduction in the deep anoxic water body. Some portions of the highly alkaline water is penetrating through the uppermost pycnocline and increases the alkalinity in the upper oxic water body (alkalinity pump). The upper water body is beside its slightly increased alkalinity (4-5 meq/l) characterized by a decreased salinity (32 ‰). This special hydrochemical situation let to a very specific and endemic development of the biota. Cyanobacteria and heterotrophic microbes exhibit large diversities in contrast to just one sponge taxon (*Suberites/Polmastia* n. sp.). Common are cyanobacteria of the taxa *Pleurocapsa*, *Phormidium*, *Calothrix*, *Spirulina*, *Microcoleus* and Microcystacea.

1 Introduction

The scientific importance of the Satonda Crater Lake was firstly recognized 1984 by S. Kempe during the Dutch-Indonesian SNELLIUS II expedition. The lake was thoroughly investigated by Kempe and coworkers during the expeditions 1986, 1993 and 1996 (more details in KEMPE et al. 1996). Up to now, no particular work was carried out on the microbes, algae, and metazoan inventory of the lake. The purpose of the recent studies is to close this gap. During the last field trip to Satonda in the end of the wet season G. Arp has cultured cyanobacteria and therefore we are now able to determine in detail various microbial taxa. Not clear at the moment is the calcification potential of the observed biofilms and their role in the construction of the prominent red algae reefs fringing the lake margin.

There is no doubt about the uniqueness of the environment of this lake. It is at the moment the only one known "marine" lake with an increased alkalinity. KEMPE & KAZMIERCZAK (1990, 1993, 1996) have proposed and interpreted that this lake and its inventory of microorganism is maybe an example for late Precambrian oceanic conditions. They have noted a wide spread calcification of pleurocapsan biofilms and the formation of microstromatolites. During the field expeditions in 1993 and 1996 a general calcification of biofilms was not observed. In older parts of the coralline algae reefs well developed microbialites (microstromatolites) were observed which are covering thalli of siphonocladacean green algae. Only few traces of calcifying microbial sheets have been observed in the Recent part of the reefs. However, an early, very rapid silification is common preserving cellular structures including microbes.

SCHUDACK & REITNER (1996) were able to reconstruct the lake history in part by the examination of ostracod assemblages from sediment cores.

REITNER, J., NEUWEILER, F. & GUNKEL, F. (eds., 1996): Global and Regional Controls on Biogenic Sedimentation. I. Reef Evolution. Research Reports. – Göttinger Arb. Geol. Paläont., **Sb2**, 1-7, Göttingen

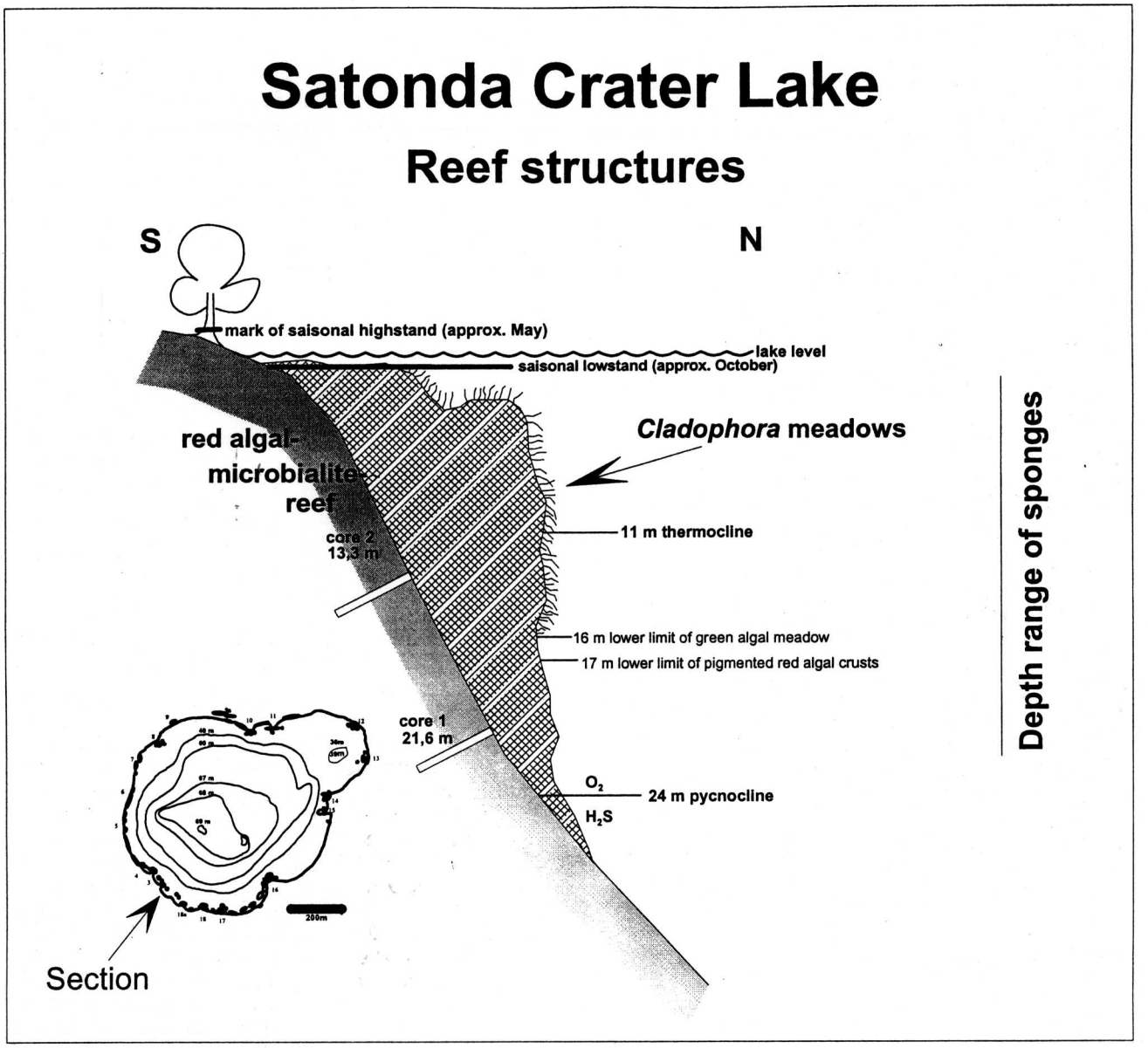

Fig. 2: Sketch of one of the red algal-microbialite-reefs at Satonda Crater Lake, situation in October 1996 (reef station 1). The reef and adjacent soft sediments are drawn in one projection plane. Drawing is not to scale. The inclination of soft sediments does not exceed 30°.

2 Biofilm Composition of Satonda lake Modern Reef Surfaces – Preliminary Report

The surface of red algal-microbialite-reefs of the Satonda Crater Lake and their attached macrophytes are largely covered by complex microbial biofilms and scattered monaxonid demosponges (*Suberites/Polymastia*) (Fig. 2). Biofilms are in average 5-15 µm, occasionally up to 50 µm thick. The contact between encrusting red algae or sponges is always mediated by an intercalated biofilm.

The isolation and identification of present algae, cyanobacteria, and bacteria are at an initial stage. Anaerobic microbes as well as diatoms, dinoflagellates etc. are not treated in this study. Cyanobacterial genera were determined according to morphological and cytological criteria (traditional system), following GEITLER (1932), KOMÁREK & ANAGNOSTIDIS (1986, 1989) and ANAGNOSTIDIS & KOMÁREK (1985, 1988). Presently, enrichment cultures of four genera of cyanobacteria and three genera of microscopic green algae are growing successfully in the laboratory. Beside the conspicuous macroscopic siphonocladacean algae *Cladophoropsis*, *Cladophora* and *Chaetomorpha*, the 2 µm small coccoid chlorophyte *Chlorella* (Pl. 1/1) is obviously an important contributor to the lake's primary production. *Chlorella* has been obtained from the lake surface down to at least 18 m depth from plankton and reef surface samples, although it is not an integral part of the reef biofilms (thin sections).

Cultivable cyanobacteria of reef surfaces comprise the genera *Phormidium*, *Calothrix*, a member of the family Hydrococcaceae (*Pleurocapsa*) and a 1 µm small member of the family Microcystaceae (Pl. 1/2-6).

KEMPE & KAZMIERCZAK (1990, 1993) already stated, that cyanobacteria of the *Pleurocapsa*-group (sensu RIPPKA et al. 1981) cover the reef surfaces, although they did not mention any diagnostic criteria. These authors suggested, that, beside the red algae, growth and calcification of the pleurocapsalean cyanobacteria are responsible for building up essential parts of the "stromatolitic" reefs in Satonda Crater Lake. Our cultivated member of the Hydrococcaceae is growing quite slowly on agar plates, but the formation of nanocytes (endospores) has now been observed unequivo-

cally (Pl. 1/3). The nematoparenchymatous arrangement of cells fits to the genus *Pleurocapsa*, too (Pl. 1/2).

Additionally, three members of the order Chamaesiphonales (*Stichosiphon* among others) and *Heteroleibleinia* have been observed sporadically upon *Cladophora* (fixed material). A *Spirulina*-like species has been detected in few thin sections (Pl. 1/8).

It should be emphasized, that the biofilms constantly exhibit a diverse community of prokaryotes and eukaryotes (Pl. 1/9-11). None of the mentioned genera is dominant at one place. In contrast, various heterotrophic bacteria are omnipresent throughout the mucilaginous biofilms. The physiologic and phylogenetic diversity of these bacterial rods and filaments is not known until now, but their cellulytic and saccharolytic activity is obvious (Pl. 1/11). Fungal hyphae are present in sections (Pl. 1/9) and on agar plates as well. Some observed microborings may result from fungal activity.

Reef growth at Satonda Crater Lake was originally attributed to microbial activity, essentially of pleurocapsalean cyanobacteria, and red algae (KEMPE & KAZMIERCZAK 1990, 1993, KEMPE & KAZMIERCZAK 1996). Our recent observations do not confirm the existence of in situ calcifying cyanobacterial biofilms on reef surfaces, except for few examples of decaying green algal filaments (Pl. 1/12), gastropod shells and faecal pellets (October 1993). New samples taken this year (June 1996), a few weeks after the end of the rainy season, have not been investigated yet. Possibly, they may show calcifying biofilms due to an increased Ca^{2+}-input, but, in any case, their relevance for present reef growth is negligible compared to the carbonate constructions by red algae and sessile foraminifera (*Nubecularia* sp.). This might have been different in the past, when several dm of microbialite (encrusting filamentous green algae) accumulated forming the older reef parts.

3 Sponges

Sponges are represented by different morphotypes of the hadromerid taxon *Suberites* which is characterized by tylostyle megascleres only. The dermal layer of the sponge is constructed of plumose bundles of short tylostyles (150-200 µm), the choanosomal spicules are randomly orientated and much larger than the dermal ones (300-500 µm) (Pl. 2/8-9). Most of the observed sponges exhibit a lateral, encrusting growth habit and therefore show a well developed exhalant canal system (Pl. 2/1+3-4). The exhalant system is differentiated into star-shaped units ("astrorhizae"-pattern). In each unit the main exhalant canals conjugate in one large osculum (Pl. 2/1+3). A second type of this hadromerid sponge type is observed. It exhibits a more or less erect growth habit and does not show any star-shaped outer exhalant system. The sponge is forming tubes with a central osculum also known from the taxon *Polymastia*, which is phylogenetically closely related to *Suberites* (REITNER 1992) (Pl. 2/2) The spicular inventory and spicule arrangements are more or less similar. Whether both morphotypes are separate species or not is still not known because detailed histological work is not yet finished. Both morphotypes exhibit the same basic yellow color, generally more intense in the *Polymastia*-type, probably due to a larger thickness of the soft tissue (Pl. 2/1-2). A lot of color variations are visible from dark green, brown, yellow/brown, and yellow. The different colors are related to microorganisms within the soft tissue. The dark green color is restricted to specimens in extremely shallow water (20-50 cm) and is related to the unicellular green algae *Chlorella* (Pl. 1/1) These algae are part of the plankton and filtered by the sponge. The algae lives within the mesohyle of the sponge. Similar behavior is known from the freshwater sponge *Spongilla* (SALLER 1991). In nutrient poor freshwater *Chlorella* is a symbiont and helps the sponge to survive. The brownish color variation is restricted to few specimens from deeper water (18-20 m) and is related to a higher amount of still unknown mesohyle bacteria (Pl. 2/3). The symbiotic bacteria of the sponge itself are rare and very small (less then 1 µm – nanobacteria) (Pl. 2/5). Size and abundance are comparable to those observed in the marine hadromerid coralline sponge *Spirastrella* (*Acanthochaetetes*) (REITNER & GAUTRET 1996). In many cases the encrusting sponges form very thin films (ca. 50 µm) growing in interspaces of dead red algae heads. The sponges penetrate large spaces of the dead portions of the algae reef surfaces (Pl. 2/4). They prefer light protected areas, except the *Chlorella*-bearing specimens. The steep slopes of the red algae reefs are entirely covered by a dense curtain of *Cladophora* colonies down to 15-16 m (Fig. 2). Sponges are growing underneath this curtain where light amounts range from 200 to 300 lux. The depth limit of the sponges, noticed in 1993, was 20 m short above the pycnocline.

The investigated sponges are particle feeders. Within vacuoles of archaeocytes remains of diatoms were observed. In all observed cases the sponges are growing on active heterotrophic biofilms. There is a close relationship between the biofilms and the sponge, because ostia are very common in the basopinacoderm (Pl. 2/5). We assume that the biofilms release metabolic products consumed by the sponge. This behavior may explain the enormous lateral growth of thin sponge sheets.

Of further significance for the sponge is the ability to build resting bodies (Pl. 2/6-7). In freshwater sponges this ability is commonly realized (gemmulae formation). In marine sponges this character is very rare and restricted to few taxa only. It is known from chaetetid coralline sponges (*Merlia*, *Acanthochaetetes*) and the pharetronid Calcarea taxon *Petrobiona* (REITNER 1992) Resting bodies are also known from haplosclerid demosponges (*Acervochalina loosanoffi*) and the hadromerid *Suberites domuncula* (SIMPSON 1984). The observed resting bodies are located in small protected cryptic niches between coralline algae or small caverns of 200-500 µm. The resting bodies are hemispherical or sack-shaped and filled with archaeocytes/thesocytes. The bodies itself exhibit a strong basic red fuchsin staining behavior. After staining, the fluorochrome calcein is also enriched and indicates an enrichments of Ca-ions in the walls of the resting cysts (Pl. 2/6). In one case a release of the archaeocytes inside the mesohyle was observed. The sponge itself was not reduced or damaged. This indicates that these bodies are nutrient reservoirs.

The sponge fauna is perfectly adapted to this extreme environment. Referring to additional ultrastructural studies we assume that these sponge types are new taxa restricted to this special environment.

Acknowledgements

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Plate 1: Microscopic green algae, cyanobacteria, and heterotrophic bacteria of reef surface biofilms, Satonda Crater Lake, Indonesia.

Fig. 1: The coccoid green alga *Chlorella* is a substantial part of the phytoplankton down to at least 18 m water depth. Each of the 2 µm wide cells contains one bent parietal chloroplast. Enrichment culture from surface water of the lake center. Phase contrast. Sample Sat 96/29. Scale: 10 µm.

Fig. 2: Nematoparenchymatous colony of *Pleurocapsa*. This cyanobacterium is distributed on reef surfaces, but in situ calcification was not observed in natural samples of the Satonda expedition 1993 (October) and 1996 (June). Enrichment culture from reef surface at 60 cm depth. Bright field. Sample Sat 96/23. Scale: 10 µm.

Fig. 3: Two small *Pleurocapsa* colonies. Colony on the right hand side (arrow) is releasing nanocytes (endospores) of 2-3 µm diameter (small arrows). Enrichment culture from reef surface at 60 cm depth. Bright field. Sample Sat 96/23. Scale: 10 µm.

Fig. 4: Solitary filament of the cyanobacterium *Calothrix* (Rivulariaceae), showing thin firm sheath and a basal heterocyst (arrow). Enrichment culture from reef surface at 30 cm depth. Phase contrast. Sample Sat 96/14. Scale: 50 µm.

Fig. 5: Filament tips of a *Phormidium* species with clearly visible sheath (arrow). Enrichment culture from reef surface at 60 cm depth. Phase contrast. Sample Sat 96/23. Scale: 10 µm.

Fig. 6: Ensheathed Pseudoanabaenaceae or Phormidiaceae. The very thin sheath is only visible after trichome breakage (insert). Enrichment culture from reef surface at 30 cm depth. Phase contrast. Sample Sat 96/14. Scale: 10 µm.

Fig. 7: Coccoid cyanobacterium of the Microcystaceae with peripheral arrangement of thylakoids. Ultrathin section of a reef surface biofilm. Note the unidirectional production of the layered sheath. Sample Sat 93/37-2. TEM micrograph #6395. Scale: 500 nm.

Fig. 8: *Spirulina*-like cyanobacteria? occasionally occur within diffuse biofilms upon *Cladophora* and red algal crusts. Sample Sat 93/#JR. TEM micrograph #JR. Scale: 2 µm.

Fig. 9: Complex biofilm upon the green alga *Cladophora*. Beside attached filamentous cyanobacteria (*Phormidium*?) many heterotrophic bacteria thrive within the mucilaginous biofilm. Note fungal hypha penetrating a decaying cyanobacterial filament (arrow). Sample Sat 93/37-2. TEM micrograph #6389. Scale: 1 µm.

Fig. 10: Small colonies of coccoid cyanobacteria (Microcystaceae) (a) and disintegrating filamentous cyanobacteria (b) are distributed between bacterial rods and filaments. No traces of carbonate precipitation were observed within the mucilaginous biofilms upon the siphonocladalean algae *Cladophora*, *Cladophoropsis*, and *Chaetomorpha*. Note fibrillary structure of the cell wall of *Cladophora* (c). Sample 93/37-2. TEM micrograph #6392. Scale: 2 µm.

Fig. 11: Heterotrophic bacteria decomposing the cell wall of a dead *Cladophora*. The fibrillary ultrastructure of cellulose is destroyed (arrows) by extracellular enzymes. Sample 93/37-2. TEM micrograph #6377. Scale: 1 µm.

Fig. 12: races of calcium carbonate precipitates within a thick mucilaginous biofilm surrounding a decayed green algal filament. This is one of the few exceptions of in situ calcification of biofilms at the reef surfaces. Sample 93/#JR. TEM micrograph #JR. Scale: 2 µm.

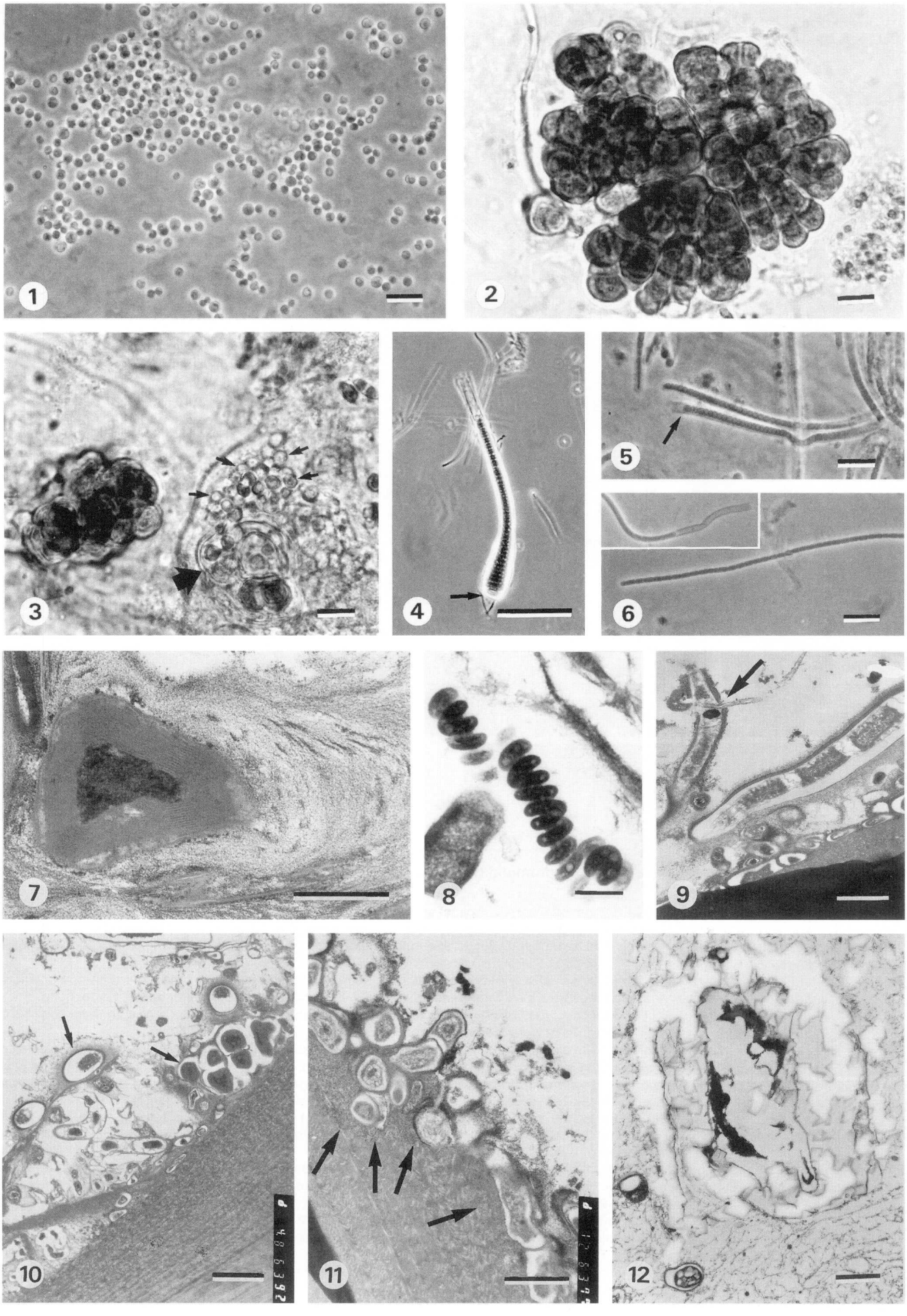
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Plate 2: Porifera, sponge-biofilm-interaction. Hadromerid taxon *Suberites/Polymastia* n. sp.

Fig. 1: Encrusting type of Suberites with a prominent exhalant canal system. The main exhalant canals conjugate in one large opening. The entire system reminds of an astrorhizal system of calcified demosponges (e.g. stromatoporoids). The sponge is bright yellow and grow on top of the living coralline red algae (*Lithoporella*) and between the short thalli of the green alga *Cladophoropsis*. Shallow water 1-3 m water depth. Scale 3 cm.

Fig. 2: Second type of the hadromerid sponge with affinities to *Polymastia*. No astrorhizal-shape exhalant canals are developed but long tubes with a central opening. The sponge grows between *Cladophora* thalli. Shallow water, 1-3 m water depth. Scale 1 cm.

Fig. 3: Deep water representative of the *Suberites* type. This type often exhibits a brownish color. The sponge grows on dead colonies of the red algae *Peyssonelia*. Deep water, 19 m water depth, 2 m above the upper pycnocline. Scale 2.5 cm.

Fig. 4: Deep water *Suberites* main colony (18 m water depth). Thin film of the sponge (less then 50 µm) grows between dead colonies of *Peysonellia*. The slightly red color of the algae are porphyrine remains. Scale 1 cm.

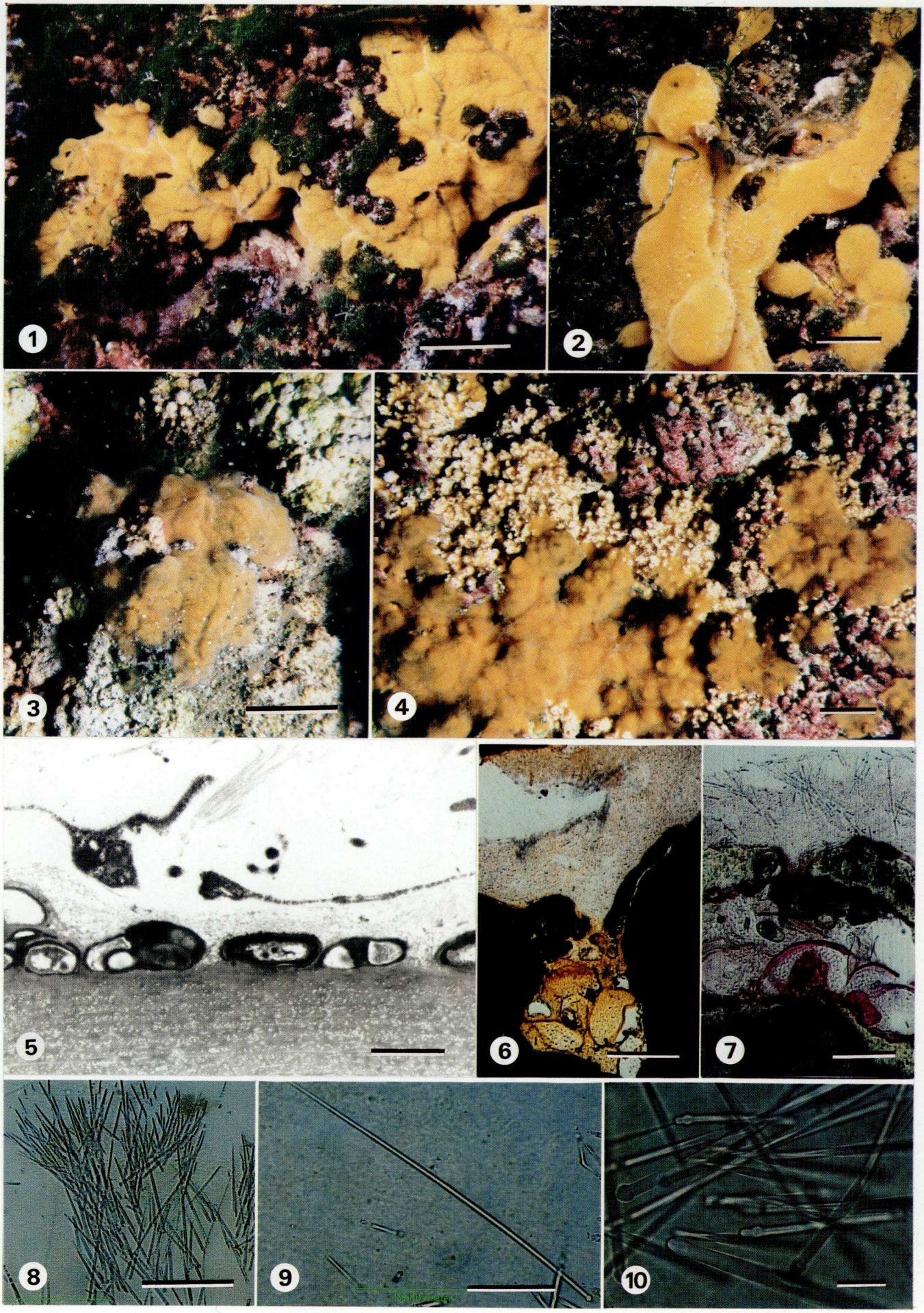
Fig. 5: In all observed cases the sponges are growing on active heterotrophic biofilms. The bacteria are in most cases cellulytic and saccharolytic. In this particular case the biofilm is growing on the dead thallus of a cladophoran green alga. A single layer of bacteria is covered by a thin polysaccharid sheet including EPS. The entire film is overgrown by a sponge. Visible are basopinacocytes at an ostium. The small black dots are sponge related nano-bacteria. The sponge consumes probably metabolic products of the biofilm (farming strategy). Scale 1 µm.

Figs. 6+7: Both types of sponges are forming resting bodies. Resting bodies are very rare in marine sponges and only realized in some coralline sponges (*Merlia*, *Acanthochaetetes*, *Petrobiona*), few haplosclerids and some *Suberites* taxa. The main function of these bodies is to help the sponge to survive during environmental changes. The resting bodies of the Satonda sponges are always located in small cryptic niches. The bodies themselves are hemisphaerical or sack-shaped and filled with archaeocytes (thesocytes). The sheets of the resting bodies exhibit a strong fuchsinophilic staining behavior (Fig. 7) and are enriched in Ca-ions (Fig. 6 calcein stained). Scale: Fig. 6: 150 µm, Fig. 7: 200 µm.

Fig. 8: Plumose arrangement of short tylostyles of the dermal layer. Important character for hadromerids. Scale 200 µm.

Fig. 9: Long tylostyle of the choanosomal part of the sponge. Scale 100 µm.

Fig. 10: Variation of the rounded head of the tylostyles, a typical feature of this sponge type of Satonda. Scale 20 µm.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