Project: Re 665/8 „Steuernde Funktionen und Erhaltungspotentiale moderner und fossiler Porifera“, Project Leader: J. Reitner (Göttingen)

Lake Thetis Domal Microbialites – a Complex Framework of Calcified Biofilms and Organomicrites (Cervantes, Western Australia)

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Area of Study: Western Australia
Environment: Saline lake
Stratigraphy: Holocene
Organisms: Biofilms
Depositional Setting: Microbialites
Constructive Processes: Biofilm calcification, organomineralization
Destructive Processes: Microbial microborings
Preservation: —
Research Topic: Processes of organomineralization

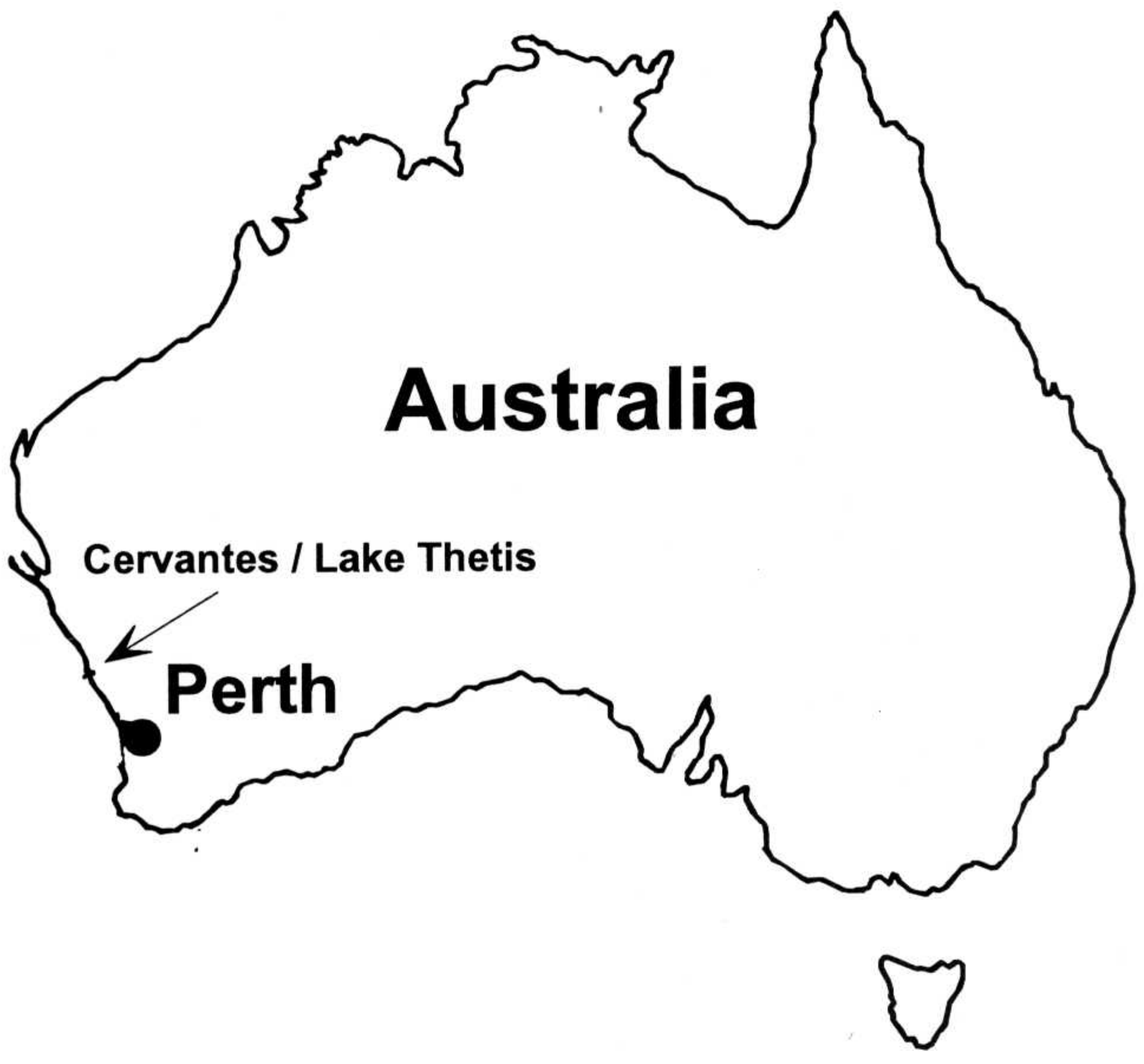

Fig. 1: Locality map.

Abstract

Lake Thetis is a small saline lake with an increased alkalinity and abundant domal microbialites. These microbialites exhibit a rough stromatolitic structure. The recent growth results mainly from calcifying *Entophysalis*-biofilms which are forming a more or less laminated crust. Within the deeper parts of the *Entophysalis*-biofilm the outer basophilic polysaccharide envelopes contain abundant heterotrophic bacteria. Calcification events exactly start at these points. The older, subfossil portions of the microbialites are characterized by plumosely arranged *Scytonema*-filaments which are enclosed by fibrous aragonite. Within small cryptic primary and secondary cavities clearly laminated organomicrites are lining the cavity walls. The formation of this type of "microstromatolites" is related to organic films, which contain no active microbes. These organic films are composed of degraded organic material (polysaccharides, proteins etc.) acting as matrices and templates for nucleation and growth of organomicrites and fibrous aragonite crystals.

1 Introduction

Numerous small saline lakes with varying hydrochemistry are distributed throughout the West Australian coastal plain. Characteristically, all these lakes are substantially fed by groundwater and therefore maintaining permanent water bodies. Supply of divalent cations (e.g. Ca^{2+}, Mg^{2+}) by groundwater influx seems to be a prerequisite for the calcification of their benthic microbial communities, resulting in microbialites (c.f. MOORE et al. 1984). Lake Thetis is situated east of the small town Cervantes, 2 km inland from the Indian ocean (Fig. 1). The lake is 300 m wide and ca. 2 m deep. The lake is situated on a Quaternary limestone pavement. Due to the steep slopes the lake basin is a collapsed doline. The lake basin itself has probably an age of not more than 3,000 yrs BP derived from the bivalve *Katelysia rhytiphora*, which was found in the Holocene basement. This fossil parts of the basin are represented by the Vincent Member of the Herschell Limestone which is 4,800-5,900 yrs BP (PLAYFORD 1988). Lake Thetis biofilms are conspicuous due to their distinct lateral color zonation. Associated domal microbialites have already attracted the attention of few Australian investigators (GREY et al. 1990). This group has done basic work on hydrochemistry and microbiology and has figured out the main belts of benthic microbial communities of the entire lake. No investigations were carried out on the formation processes of the domal microbialites. The purpose of this paper is to demonstrate the different calcification events dependent on various microbial communities and degraded organic matter found in the microbialites.

2 Methods

Samples of stromatolites and biofilms were fixed for 24 h in 4 % glutardialdehyde made up in prefiltered lake water. Cacodylic acid sodium salt was used as buffer. Samples were stained en bloc after fixation and rinsing in filtered lake water. Both fluorescent (chloro-tetracycline, calcein,

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acridine orange) and non-fluorescent dyes (toluidine blue O, basic fuchsine red) were used. After incomplete dehydration in a graded alcohol series, the samples were stored in 70 % ethanol. At home laboratory dehydrated samples were embedded in LR White resin. Thin sections were cut with a LEITZ hardpart microtome. Additionally, some samples were decalcified and LFB-PAS-stained for histological sections. Sections were studied with ZEISS AXIOPLAN and AXIOLAB fluorescence microscopes. For further details of methods see REITNER (1993).

Water samples were filtered and fixed with $HgCl_2$ and suprapure HCl. pH-Measurements were done using a portable pH-meter (HANNA Instruments). Total alkalinity was titrated in field using a HACH-titrator.

3 Biofilm Composition and Growth of Microbialites at Lake Thetis

Lake Thetis stromatolitic bioherms are situated within the littoral zone of the southern to south-western shore, forming some kind of microbial "reef platform". Principally, five types of microbial mats reflect a clear zonation of the lake shore (GREY et al. 1990) (Pl. 1/1). Variations of the lake levels control the seasonal migration of the mats. One of the mat types distinguished by GREY et al. (1990) is considered to form the head-shaped, dm-sized microbialite bioherms, at least their uppermost 5-10 cm. The bioherms represent roughly laminated stromatolites with columnar elements. We investigated thin sections of subfossil portions and fixed, histochemically stained material of biofilm covered surfaces.

Subfossil, more or less columnar parts substantially consist of fibrous aragonite enclosing remains of filamentous cyanobacteria (Pl. 1/2). The aragonite is composed of fan-like to botryoidal aggregates attached perpendicular to the sheath remnants. The tubular sheath remains reach an outer diameter of 20 µm. They are conspicuous due to their brownish color (Pl. 1/3+5). The mode of false branching, characterized by parallel upward grown tips after trichome breakage, closely resembles the genus *Scytonema* (Pl. 1/3).For reason of simplification, we name this stromatolite part "*Scytonema*-dominated", although occasionally coccoid remains and paint-brush-like fabrics (filament traces of *Phormidium*-like tufts) occur, too (Pl. 1/6). Thin filaments of 0.75-1.5 µm diameter, which cross-cut the fibrous aragonite aggregates, clearly resulted from endoliths (fungi? cyanobacteria?). The roughly laminated stromatolitic fabric of the bioherms is the result of the rhythmic alternation of erect and prostrate filament orientation of "*Scytonema*".

The uppermost millimeters of the bioherms display a distinct change in fabric. "*Scytonema*-dominated" stromatolitic

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Plate 1: Microfacies of stromatolitic bioherms and composition of Recent calcifying biofilms, Lake Thetis/Western Australia.

Fig. 1: Zonation of microbial mats on stromatolitic heads at the south-western shore of the slightly alkaline, saline Lake Thetis. Dark-green biofilms fringing the heads at the water line yield colonies of the cyanobacterium *Entophysalis* as characteristic microorganism. Submerged orange-brown mat below contains masses of diatoms. Reddish suspension between the bioherms (and adhering upon bioherm surfaces at the immediate water line) is drifted ashore from the lake bottom due to wind induced current. It consists of diatoms, filamentous cyanobacteria (*Phormidium*, *Spirulina*), and mainly phototrophic bacteria. Circular bioherm in the foreground is approximately 80 cm in diameter.

Fig. 2: Thin section of the uppermost bioherm part. *Scytonema*-dominated, older stromatolitic part (a) is overlain by alternating fibrous and micritic laminae (b) containing subfossil spheres of *Gloeocapsa*/*Entophysalis*. Note fibrous, "sinter-like" crusts which line dissolution voids (arrow). Sample LTHE 95/1. Scale: 1 mm.

Fig. 3: Brownish, tubular sheath remains of cyanobacteria. The mode of false branching is identical to that of *Scytonema*. Sample LTHE 95/1. Scale: 100 µm.

Fig. 4: Organic spheres of calcified *Gloeocapsa*/*Entophysalis* within carbonate crust of a bioherm top. Brownish halos result from organic remains of mucilaginous sheaths. Sample LTHE 95-3/A14c. Scale: 10 µm.

Fig. 5: Transition from older, *Scytonema*-dominated stromatolite (a) to younger crust (b) containing relictic spheres of coccoid cyanobacteria (dark spots). The void in the center is occupied by calcifying *Gloeocapsa*-colonies (stained). Left part of this colony is already heavily calcified (arrow). Sample LTHE 95/6.Acr 1/2, stained with acridine orange. Scale: 100 µm.

Fig. 6: Intercalation of lamina with paintbrush-like fabric in subfossil *Scytonema*-dominated stromatolite. The tufts of filament traces resemble of *Phormidium*-like cyanobacteria. Sample LTHE 95/1. Scale: 100 µm.

Fig. 7: Sinter-like, laminated crust lining a dissolution void of subfossil *Scytonema*-dominated stromatolite. Micritic intercalations of the fibrous crusts show a notable autofluorescence (arrow) caused by relictic organic material. Additionally, some layers still contain large amounts of organic material and therefore are strongly fluorescing due to acridine orange staining. It is suggested that organic templates play a critical factor for carbonate nucleation in these sinter-like crusts. A pure abiotic, physicochemical formation is obviously precluded. Sample LTHE 95/5 Acr, stained with acridine orange. Wide band pass filter green BP 515-565/LP 590, ZEISS 487714; Scale: 100 µm.

Fig. 8: Microbialite surface biofilm showing lateral small-scale variations. Depression on right side is occupied by calcifying *Entophysalis* colonies (a), whereas the left side is strongly bored by the cyanobacterium *Hyella* (b). Note pennate diatoms vertically attached to the biofilm surface (arrow). Sample LTHE 95-3/A14a, stained with calcein. Scale: 100 µm.

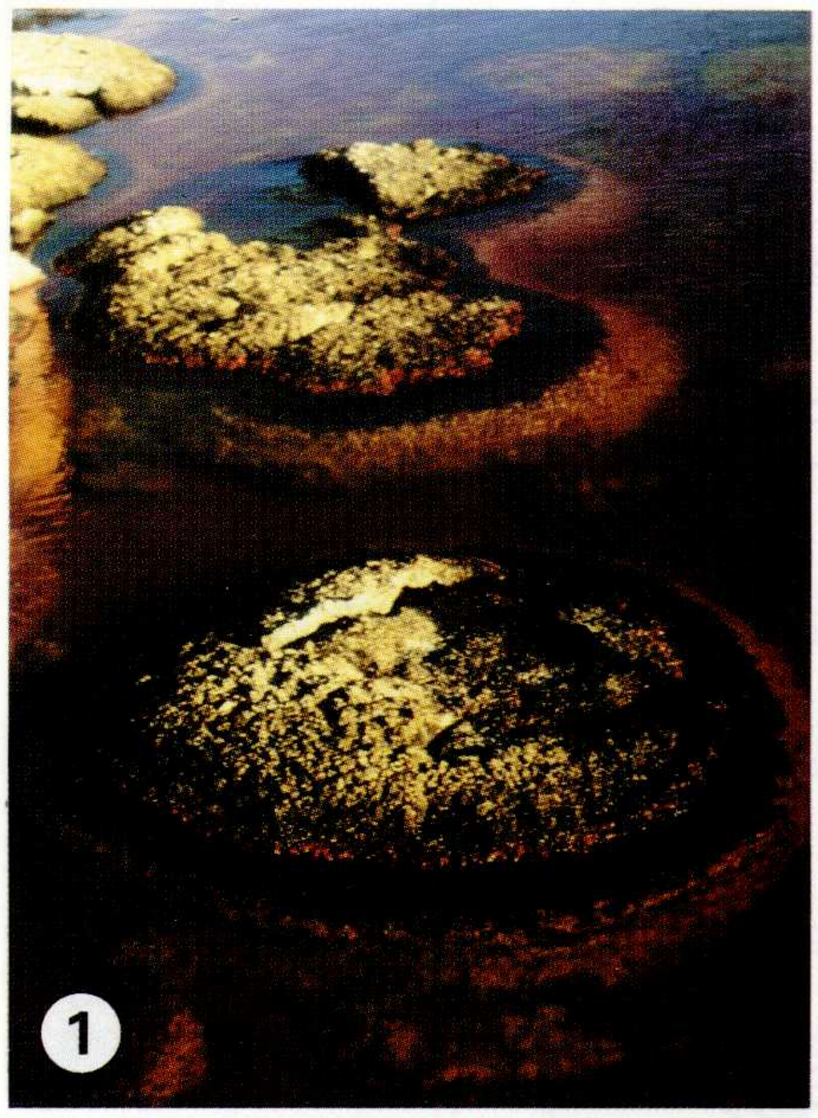
Fig. 9: Thin section of a decalcified microbialite crust demonstrating the large amount of organic material encased in carbonate precipitates of microbial mats. Red stained parts are acidic mucosubstances. Blue colors indicate protoplasm substances. Sample LTHE 95-22, LFB-PAS stained. Scale: 10 µm.

Fig. 10: Colony of *Gloeocapsa* (a) within a small cavity below the bioherm surface. Calcification is proceeding from lower left to the upper right (b). Large parts of the newly precipitated carbonate can be stained with acridine orange due to enclosed organic material. Sample LTHE 95-3/A14c, stained with calcein. Scale: 10 µm.

Fig. 11: Actively calcifying, *Entophysalis*-dominated microbial mat of a bioherm surface ("nodular mat"). Fluorescence of tetracycline-stained mucilaginous substances is clearly increasing downwards, indicating an increase of Ca^{2+}-attracting groups. Below that, carbonate precipitation takes place and encloses deceased microbial colonies. Sample LTHE 95/5, stained with tetracycline. Filter blue-violet, BP 395-440/LP470, ZEISS 487705; Scale: 100 µm.

Fig. 12: Thick *Entophysalis*-dominated microbial mat. *Entophysalis* cell rows and cluster are encapsulated by weakly stained polysaccharide sheaths, which grades into red stained, strongly acidic mucosubstances. Sample LTHE 95-22, LFB-PAS stained. Scale: 50 µm.

Fig. 13: Detail of Fig. 12 showing blue stained bacteria (arrow) in strongly basophilic mucilaginous material between polysaccharide encapsulated *Entophysalis* colonies. Physiologic activity of these bacteria may trigger carbonate precipitation. Bacteria and *Entophysalis* cells are stained blue by hemalaun. Sample LTHE 95-22, LFB-PAS stained. Scale: 10 µm.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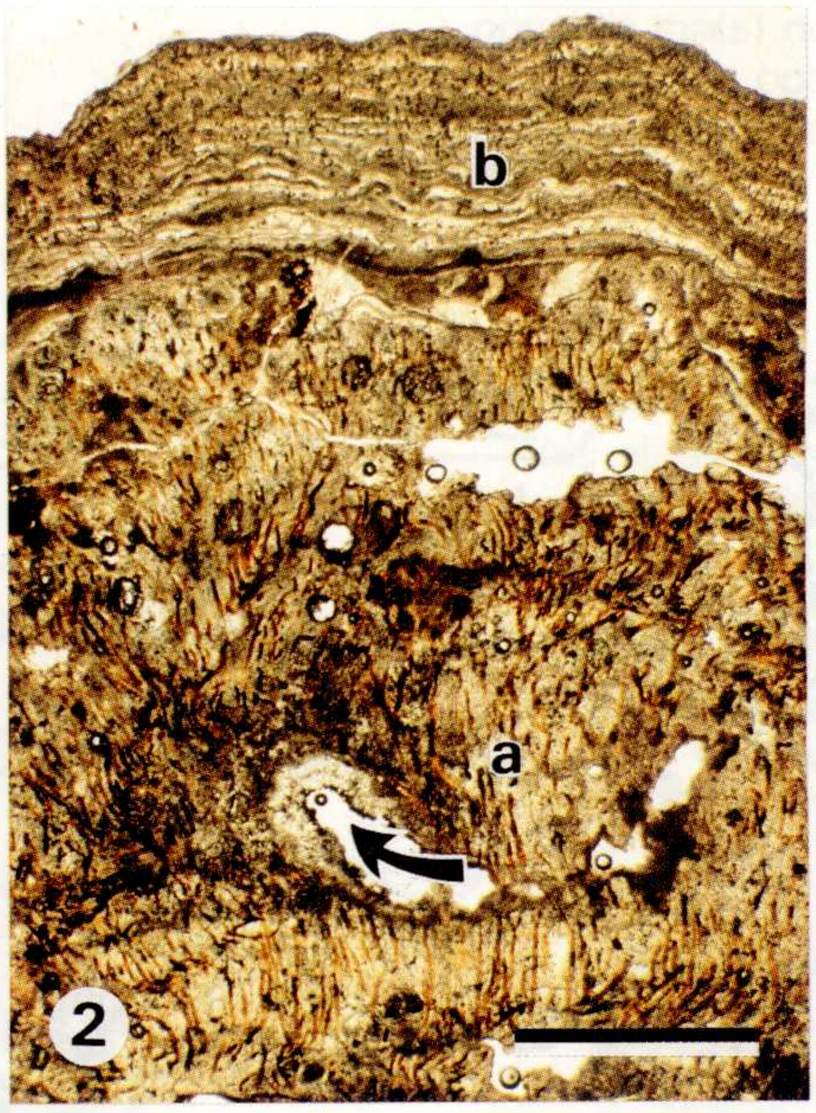

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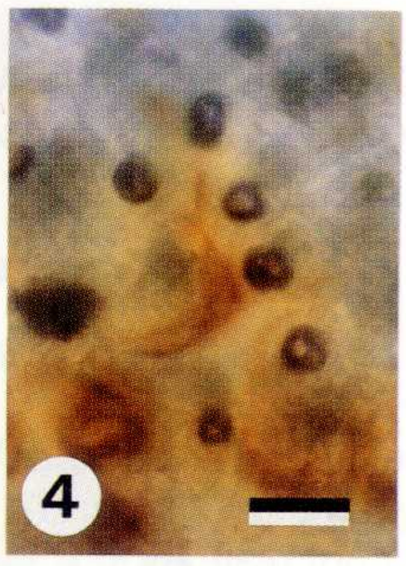
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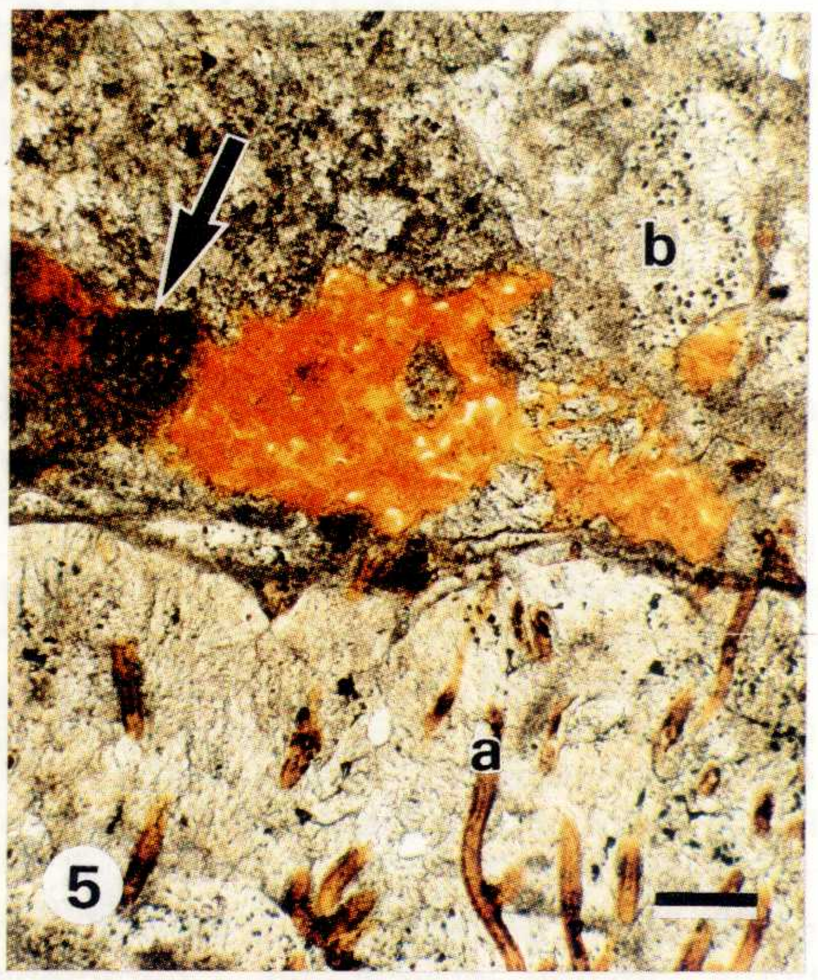

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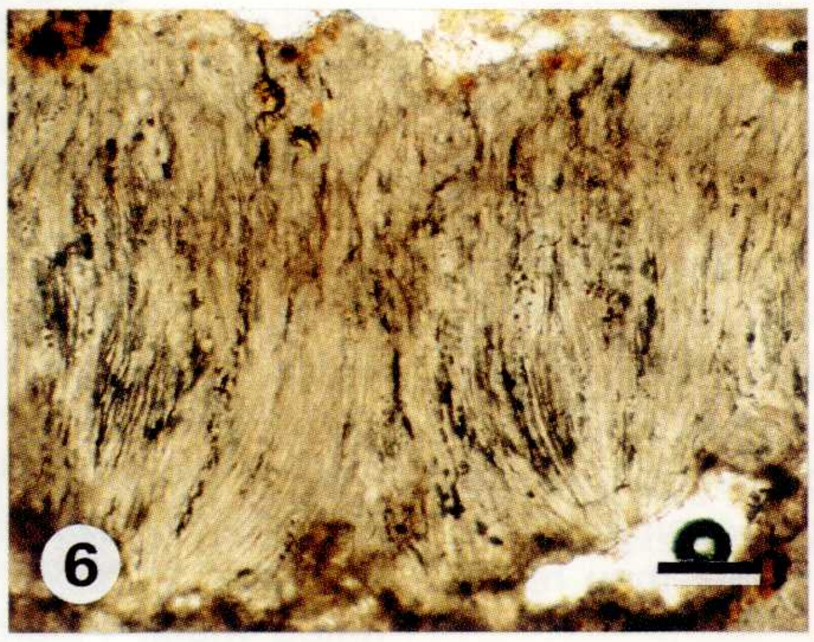
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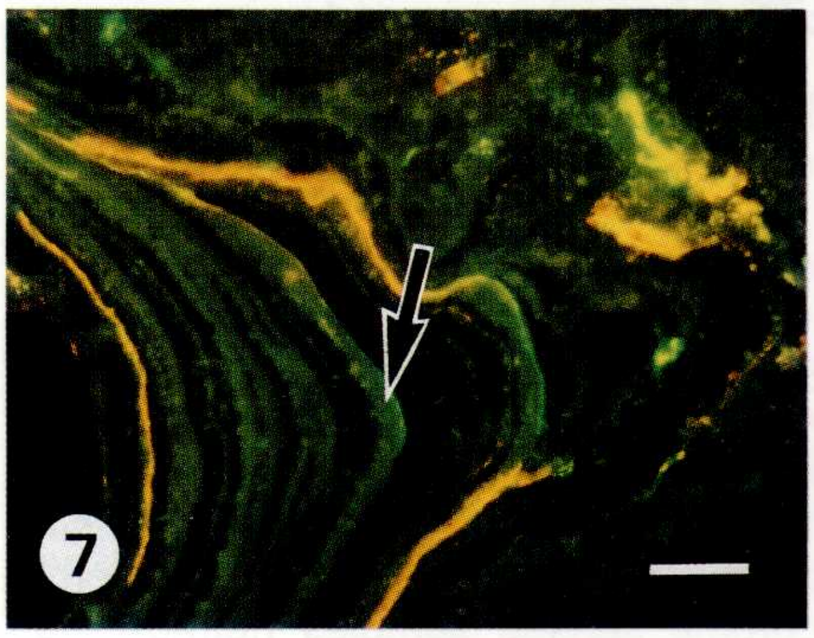
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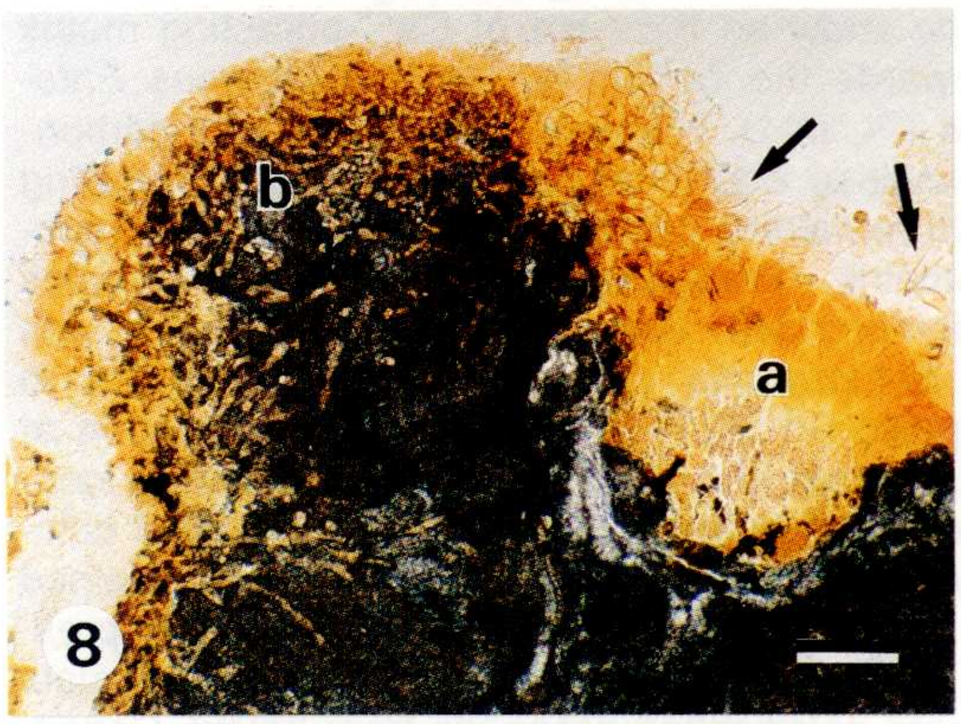

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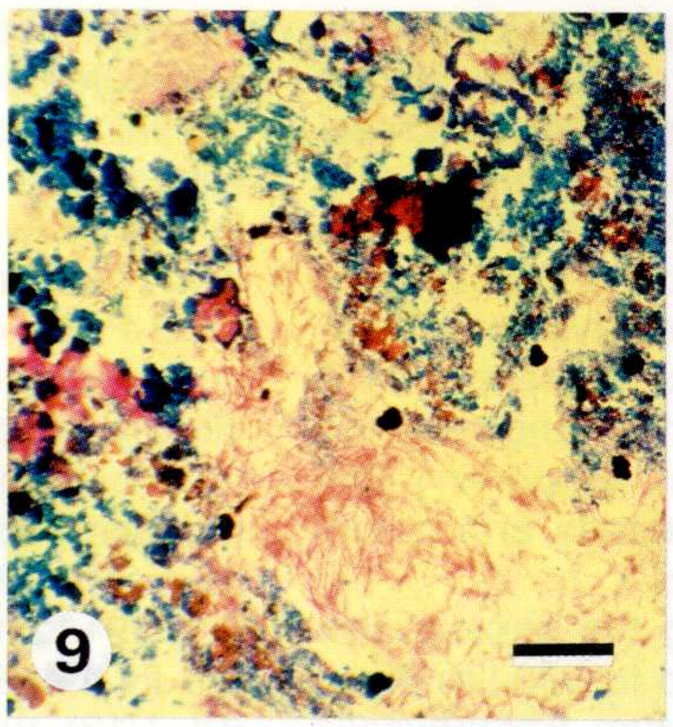
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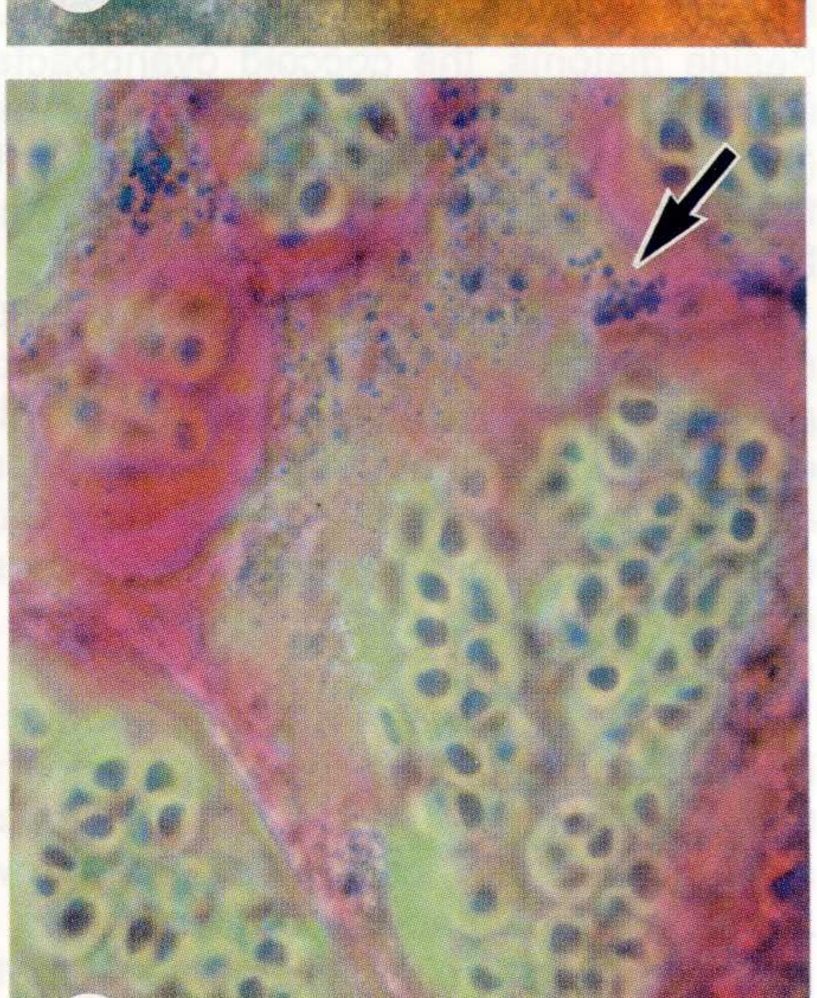

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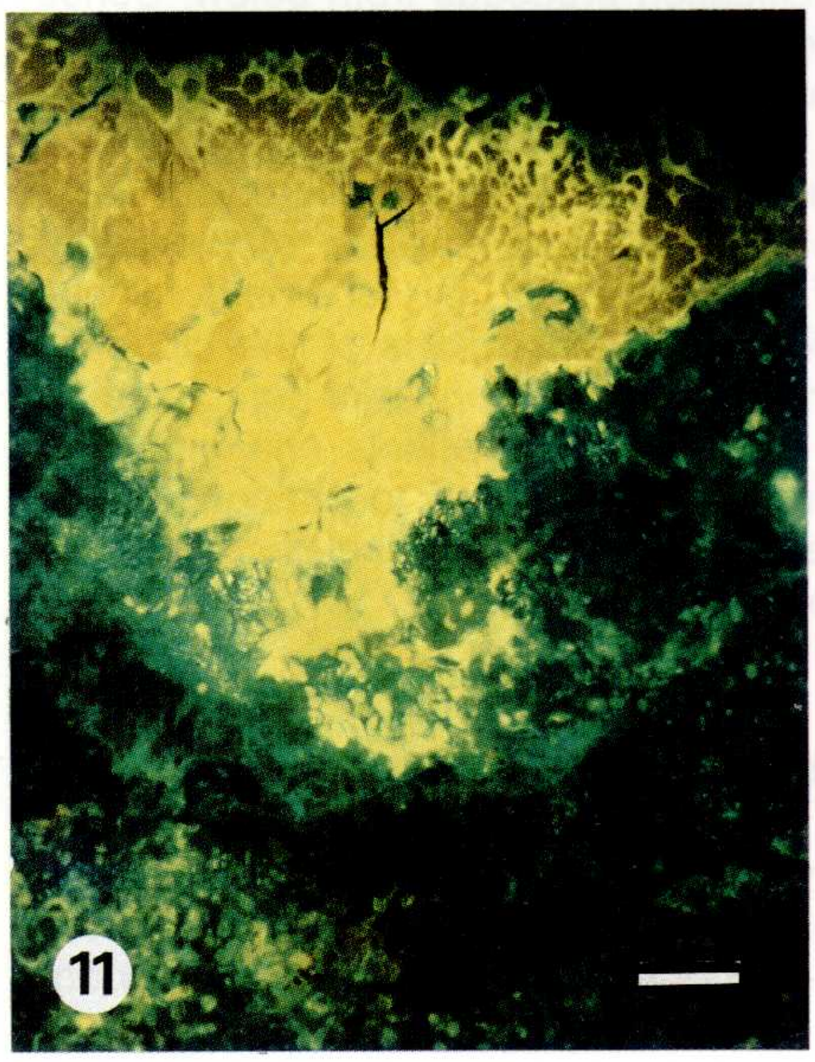
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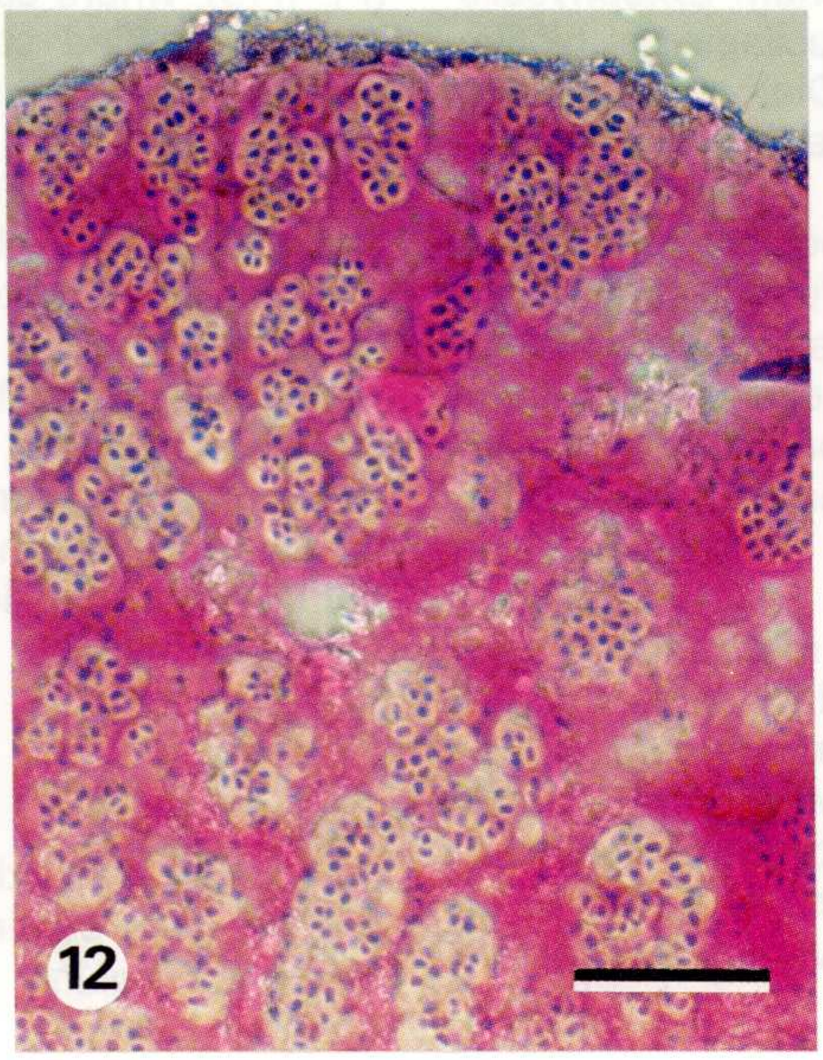
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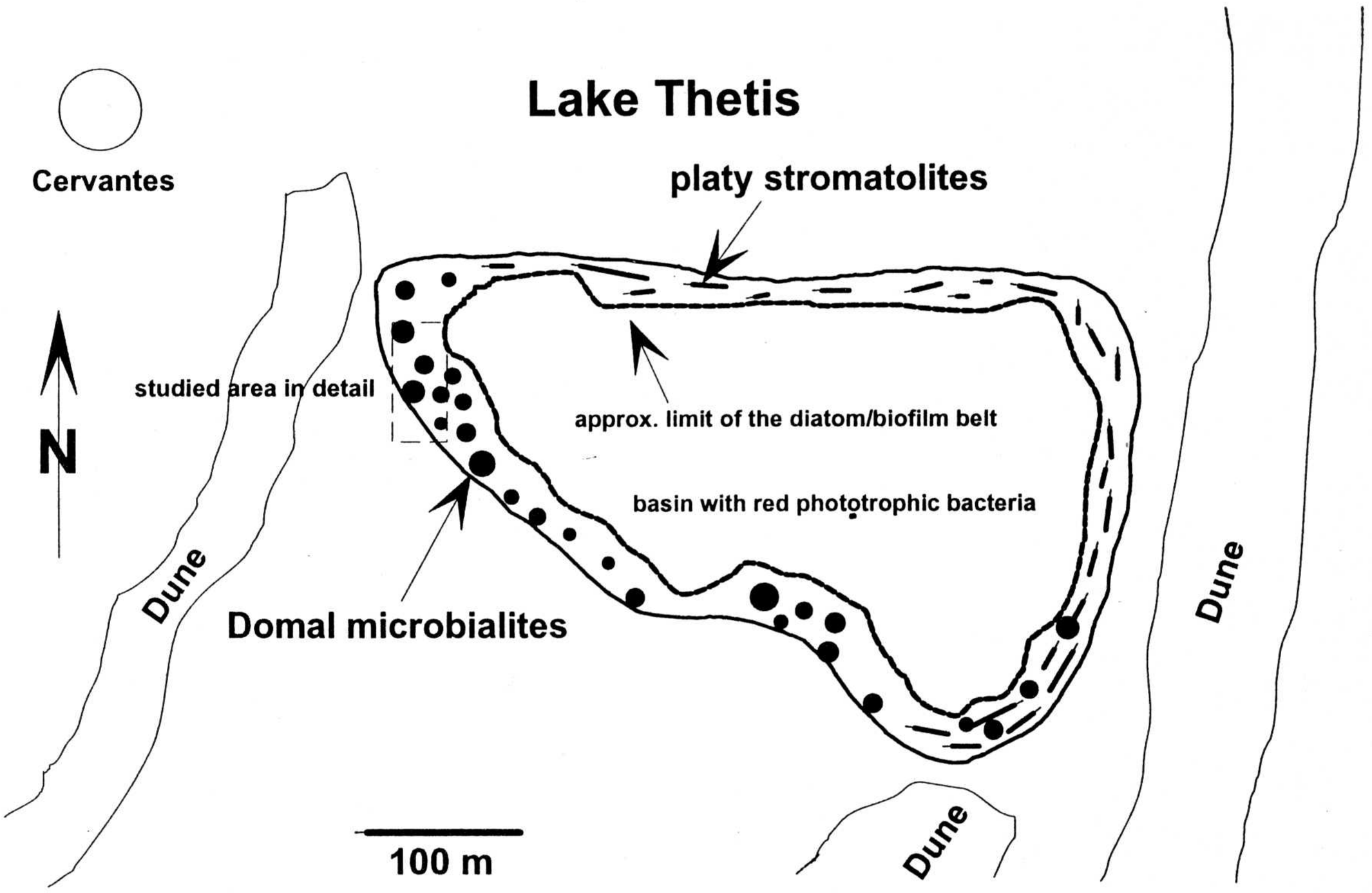

Fig. 2: Shape of Lake Thetis and distribution of microbialites/stromatolites.

carbonate is grading into a laminated micrite with thin fibrous laminae intercalated. Characteristic are numerous coccoid remains, usually dark walled spheres (Pl. 1/4-5). They are considered subfossil cells of the cyanobacteria *Gloeocapsa* and *Entophysalis*. Brownish halos may result from former sheath capsulae.

The high porosity of subfossil bioherm parts results from primary cavities and laminae-parallel to irregular dissolution voids. The cavities are lined by sinter-like crusts ("microstromatolites") composed of alternating fibrous and micritic layers. Some "sinter crusts" of the Miocene Ries crater lake, which are considered lacustrine-vadose precipitates (ARP 1995), are probably fossil equivalents. Fluorescence microscopy reveals that micritic layers exhibit a notable autofluorescence (Pl. 1/7). Acridine-orange-stained samples prove the presence of (at least temporary) thin biofilms on their surface, but only few bacteria and nearly no cyanobacteria or diatoms were detected.

In contrast, dark-green biofilms fringing the stromatolitic heads immediately at the lake level are up to 500 µm thick. Beside diatoms, the coccoid cyanobacterium *Entophysalis* is the dominant microorganism in the mucilaginous biofilm ("nodular mat" sensu GREY et al. 1990). Diatom abundance further increases in submerged areas, grading into orange-brown "diatomaceous mats".

Lateral small-scale variations in composition reflect the nodose, small-scale relief (Pl. 1/8). Protruding stromatolite parts are subject to degradation by radiating microborings of the cyanobacterial genus *Hyella*. Depressions and small cavities are preferentially occupied by *Gloeocapsa* colonies. Carbonate precipitation encloses successively these colonies leading to micrite patches with coccoid remains (Pl. 1/10).

Well developed areas of the "nodular mat" show a coalescence of *Entophysalis* colonies forming up to 500 µm thick biofilms (Pl. 1/12). The vertical arrangement of cell rows occasionally causes digitate growth forms. Locally abundant empty sheaths of filamentous cyanobacteria represent a transition to "filamentous mats" of GREY et al. (1990). On top pennate diatoms are vertically attached within the biofilm. Eukaryotic coccoid algae and protozoa are present, too.

We consider the process of carbonate precipitation within the *Entophysalis* mat, as revealed by histochemical staining methods, to be of general validity for cyanobacteria-dominated mats. Fluorochrome labeled samples (tetracycline, calcein) clearly show an increase of Ca^{2+}-attracting matrix molecules towards the bottom of the mucilaginous *Entophysalis* mats (Pl. 1/11). Nucleation and growth of carbonate crystals enclose and mineralize the colonies, resulting in micrite (with remaining autofluorescence) containing coccoid cell remains. Periodic-acid-Schiff treated samples (LFB-PAS) demonstrate, that the inner sheath envelopes surrounding *Entophysalis* colonies are only neutral to slightly basophilic (Pl. 1/12). This hampers precipitation at this point. In contrast, the basophilic mucosubstances concentrate especially in spaces between cyanobacterial colonies. The PAS positive substances are red-pink stained (Pl. 1/9+12-13). Numerous heterotrophic bacteria are present in these interspaces of the deeper parts of the mats (Pl. 1/13), where calcification proceeds from below. Consequently, lithified stromatolitic crusts of the bioherm tops show a large amount of residual organic material, when decalcified (Pl. 1/9).

Calcification of *Entophysalis*-dominated microbial mats is obviously responsible for the accumulation of the uppermost millimeters of the bioherms. *Scytonema*-dominated carbonates, which form substantial parts of the bioherms, were replaced by the present *Entophysalis*-dominated microbial community. This obviously reflects changing environmental conditions, probably related to changes in lake levels and hydrochemistry. Large parts therefore grew under different conditions than today.

4 Hydrochemistry

To cover the hydrochemical framework, data of alkalinity and pH were obtained from microbial mats and the overlying water column of the open lake, restricted lagoons,

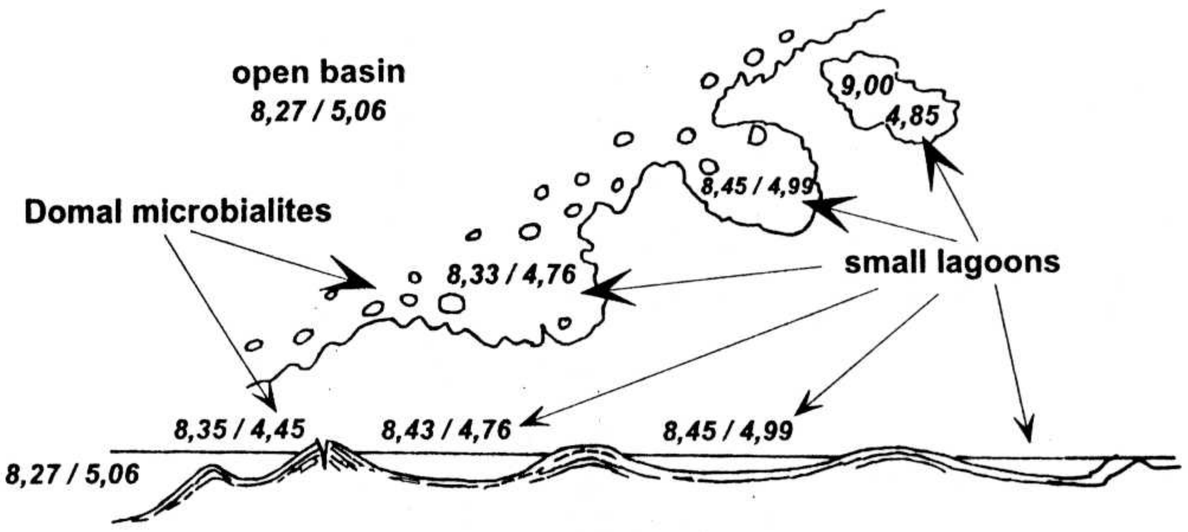

Fig. 3: Measurements of pH and total alkalinity of Lake Thetis waters (March 1995).

closed ponds between, and fractures within the microbialites. During our field trip in March 1995, the alkalinity was generally about two times elevated (4.45-5.06 meq/l), if compared to normal seawater. In contrast, GREY et al. (1990) reported only slightly elevated values. PH-values usually varied between 8.27 and 8.50. Exceptionally low pH (7.79) and high alkalinity (6.30 meq/l) were recorded from soft lake sediment 2 m off the shore. The salinity of the lake varies from 39-53 gTDS/l, inversely corresponding with the lake levels.

A clear tendency from open lake water to evaporating, closed ponds is recognized (Fig. 3). pH rises from 8.27 to 9.00, coupled with a decline in alkalinity from 5.06 to 4.85 meq/l. Our measurements of pH within microbial mats revealed no or only slightly different values compared to the immediately overlying lake water, but microelectrodes probably would give a more differentiated picture. The pH of the water between domal stromatolites (covered by *Entophysalis* mats) near the shore is only slightly raised (8.37) relative to the open lake water (8.27). We expect that small-scale gradients in the *Entophysalis* mats play a significant role in promoting their calcification.

Acknowledgements

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