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European Journal of Protistology 42 (2006) 3–7
European Journal of PROTISTOLOGY
www.elsevier.de/ejop

Cochliopodium gallicum n. sp. (Himatismenida), an amoeba bearing unique scales, from cyanobacterial mats in the Camargue (France)

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Received 30 April 2005; received in revised form 17 August 2005; accepted 22 August 2005

Abstract

Cochliopodium gallicum n. sp., isolated from cyanobacterial mats in the Camargue (France) is the smallest marine species of *Cochliopodium* to date. Its unusual tectum consists of flat plate-shaped scales with honeycomb-like centres, underlain by a layer of filamentous structures connected to each other in the basal and apical parts. The tectum is very fine and can be easily lost under inappropriate EM fixation. In its light-microscopical features, this species resembles *Ovalopodium carrikeri* Sawyer, 1980, a himatismenid that is believed to possess a scaleless, fuzzy or hairy ''glycocalyx''. We suggest that *O. carrikeri* might have been a similar species that lost scales under fixation. Our finding makes desirable a re-investigation of the genus *Ovalopodium*.

Keywords: Amoebae; *Cochliopodium*; *Ovalopodium*; Systematics; Ultrastructure; Scales

Introduction

Amoebae of the genus *Cochliopodium* Hertwig and Lesser, 1874 (Himatismenida) possess a tectum, a single flexible layer of complex carbohydrate scales, which covers the dorsal surface of the adhering cell, while the ventral one remains free. Scales are usually underlain by a thin layer of amorphous material. The general structure of a scale is rather uniform across the genus, with the exception of *C. larifeili* (Kudryavtsev 1999). The genus comprises about 20 valid species, out of which only three, *C. gulosum*, *C. clarum*, and *C. spiniferum*, (Schaeffer 1926; Kudryavtsev 2000, 2004) were so far found exclusively in marine or brackish-water habitats. Two more species, *C. bilimbosum* and *C. minus*, usually inhabit fresh water, but sometimes occur in brackish-water habitats (Garstecki and Arndt 2000). This paper presents a description of a fourth marine *Cochliopodium* which has an unique structure of the tectum, consisting of two distinct layers, one of which is made up by atypical scales and the other one is a thick, well-developed network of filaments.

Material and methods

The strain initially designated as *Cochliopodium* Cam40 was isolated from the cyanobacterial mats in a saline pool with a salinity of 42‰ in the Camargue, France, in August 2000. The clonal culture was initially maintained on 1.5% non-nutrient agar prepared with

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doi:10.1016/j.ejop.2005.08.001

artificial seawater (Wiegandt GmbH, Germany) at a salinity of 40–42‰. To purify it from other eukaryotes, the strain was transferred to the same medium, prepared with 60–62‰ artificial seawater and further maintained at this salinity. The accompanying eukaryotes did not survive the salinity shift, while amoebae were found to be tolerant to high salinity, which influenced neither their size nor morphology. All the light microscopical observations and measurements reported here were made on living amoebae with a 100 × oil-immersion objective, as recommended by Smirnov and Brown (2004). Permanent preparations were stained with Heidenhain's iron haematoxylin (Romeis 1948) and Nuclear Fast Red (Page 1988). For transmission electron microscopy amoebae were fixed either (1) overnight with 1% solution of osmium tetroxide buffered with 62‰ artificial seawater or (2) in a 1% solution of osmium tetroxide in 0.1 M phosphate buffer (pH 7.4) for 15–20 min, dehydrated in an ethanol series and embedded in Epon 812 or Taab 812 resins. The whole mounts of scales were prepared using the modified Sadakane et al. (1996) method. Scales were shadowed with copper at an angle of 15° to the horizontal.

Results and discussion

Diagnosis

Cochliopodium gallicum n. sp. Length in locomotion 10–18 μm (average 14 μm), breadth 10–21 μm (average 16 μm), length:breadth ratio 0.69–1.2 (average 0.9). Locomotive form oval, often drawn out posteriorly into a drop shape, frequently with one or several long trailing filaments. Anterior and lateral margins without subpseudopodia or lobes. Single vesicular nucleus 2–4 μm in diameter (average 3 μm), nucleolus 1–2 μm in diameter (average 1.4 μm). Tectum consists of two distinct layers. The basal layer, ca. 85–120 nm thick, is a palisade of fine filaments, perpendicular to the cell membrane, packed in a hexagonal array and cross-linked proximally and distally. Distal to this is an apical layer made up by scales, 0.7–0.9 μm long, 0.5–0.6 μm broad, in the shape of elongated hexagonal plates curved around their long axes with curled-over margins and honeycomb-like centres.

Type material: The type culture is deposited with the Culture Collection of Algae and Protozoa (Dunstaffnage Marine Laboratory, UK), accession number CCAP 1537/6.

Observed habitat: Cyanobacterial mats in saline pools in the Camargue, France, at a salinity about 40‰.

Differential diagnosis: Differs from all *Cochliopodium* species studied with EM in the structure of the tectum. Locomotive form smaller than that of any other described marine species of *Cochliopodium*.

Description

Light microscopy: Locomotive amoebae were rounded or oval (Figs. 1 and 2). Some cells were irregularly triangular, with the base directed anteriorly. In a plastic culture dish, locomotive amoebae often adopted a drop-shaped form with the length greater than breadth (Fig. 3), but this form was rarely seen on a glass surface. The surface coat of amoebae was never visible with LM. Anterior and lateral margins of the hyaloplasmic veil, surrounding the central granuloplasm, were always smooth. The posterior edge of the cell produced numerous short adhesive filaments (Fig. 1) and a few long filaments (Figs. 2 and 3), sometimes as long as the cell itself. Stationary amoebae, and those involved in non-directed movements, were flattened, rounded or oval in outline. Their hyaloplasmic veil was often very narrow or completely retracted. In culture dishes, amoebae frequently adopted a floating form (Fig. 4). During detachment from the substratum, the cell contracted, the hyaloplasm became folded and produced several narrow hyaline pseudopodia, located compactly in a restricted area of the cell opposite to the spherical granuloplasmic mass.

The single vesicular nucleus was spherical or ovoid with a large central nucleolus. In both haematoxylin-stained preparations and preparations stained with Nuclear Fast Red (Fig. 5), a densely stained ring was seen along the periphery of the nucleus. No structure similar to this ring was observed in TEM sections. No contractile vacuole was ever present. The granuloplasm contained numerous opaque granules less than 1 μm in diameter. The cells from older cultures often contained 1–2 bipyramidal or truncated bipyramidal yellowish crystals about 1–3 μm in length, and several transparent vacuoles of various sizes in the cytoplasm. Cysts were never observed in our cultures. Amoebae fed on bacteria and organic particles of similar size.

Electron microscopy: Of the two fixation protocols applied, protocol (1) gave the more complete picture of the tectum structure, while protocol (2) yielded better preservation of the nucleus and cytoplasm. The dorsal surface of amoebae was covered by a tectum of two distinct layers (Figs. 6–8). The basal layer consisted of fine filamentous structures about 10 nm thick, connected to each other in the basal and apical parts and radiating from the plasma membrane. Tangential sections showed that these filaments were packed at a spacing of about 38–50 nm in a hexagonal array (Figs. 7; reconstruction in Fig. 13), and in vertical sections the filaments appeared to be gathered into blocks up to 0.55 μm wide, each probably corresponding to a scale of the