

***Conferva duplisecta* Pollini: rediscovery in Euganean Thermal Springs (Italy) and new assignment to the *Oscillatoria* genus**

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Abstract — In this paper we report the presence on the thermal muds of the Euganean District of a filamentous cyanobacterium, with morphological features comparable with those of *Conferva duplisecta* Pollini, described in 1817 and never more signalized. Morphological, ultrastructural and molecular analyses led us to define more precisely the taxonomy of this cyanobacterium, ascribing it to the genus *Oscillatoria* and named by us *Oscillatoria duplisecta* (Pollini) Moro et Andreoli.

Key words: 16S rDNA, Cyanobacteria, morphology, *Oscillatoria duplisecta*, thermal muds, ultrastructure.

INTRODUCTION

The thermal muds of the Euganean District are abundantly colonized by cyanobacteria and diatoms which, during the mud maturation process, can be involved in the production of bioactive compounds with therapeutic properties.

A recent survey, for example, evidenced that a cyanobacterium, identified by molecular phylogenetic analyses as *Phormidium* sp. ETS-05 (CESCHI-BERRINI *et al.* 2004), produces glycolipids and sulphoglycolipids with anti-inflammatory action similar or even higher to that of traditional substances like indometacin or betametazone (LALLI *et al.* 2004). Thus, the beneficial effects of the Euganean thermal mud seem to be due not only to the heat and electrolytes present in the thermal water, but also to bioactive compounds produced by the microorganism communities developing during mud maturation.

In this paper we report the morphological, ultrastructural and molecular features of a filamentous cyanobacterium, which we have shown, by molecular analyses, to belong to the genus *Oscillatoria*. This microorganism exhibits morphological features identical to those of a cyanobacterium noticed by POLLINI (1817) in the Euganean Springs and described as *Conferva duplisecta* (= *Oscillaria duplisecta*, *sensu* TREVISAN 1871).

On the basis of the obtained results and of the more recent cyanobacterium taxonomy (GUTHRIE 2006), we propose for this isolate the name *Oscillatoria duplisecta* (Pollini) Moro et Andreoli.

MATERIALS AND METHODS

The cyanobacterium strain utilized in this study was isolated from the thermal mud tanks of the Garden Hotel of Montegrotto Terme (Padua, Italy). The temperature of the water in the tanks was about 40°C and the pH was 6.8. Cultures were set up using BG11 medium (RIPPKA *et al.* 1979) and maintained in a growth chamber at 30 °C, with a light intensity of 35 µmol photons m⁻² s⁻¹ and a 12 h photoperiod. Light microscope observations were made with a Leica Dialux 22 microscope equipped with a Wild MPS51 camera. The electron microscopy (TEM and SEM) and molecular analyses were carried out according to MORO *et al.* (2003) and CESCHI-BERRINI *et al.* (2004) respectively.

RESULTS AND DISCUSSION

The investigated strain shows very long cylindrical straight and unbranched trichomes, with a very thin sheath (Fig. 1). Trichomes are brown-black to greenish and at the light microscope have a right and left-handed rotation. At both the tri-

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chome ends the apical cell, with calyptra, is hemispheric and smaller (28-30 μm wide and 15-16 μm long) than the intercalary cells. These latter are discoidal, wider (40-50 μm) than long (9-10 μm) (Figs 1-2). The trichomes are slightly constricted at the cross-walls, conferring to the single cells a "barrel" aspect (Figs 1-3). Cells divide transversally and grow up to the original size of the mother cell before next division. Reproduction

occurs by the trichome disintegration, throughout necridia (Fig. 3a), into more or less long motile hormogonia, showing, at the ends, equal and hemispheric cells (Fig. 3b). Numerous parallel thylakoids run through the cells, occupying most of the cytoplasm volume (Fig. 4).

Genomic DNA was extracted from axenic cultures of this microorganism, and subsequently the 16S rRNA gene was amplified from DNA extracts

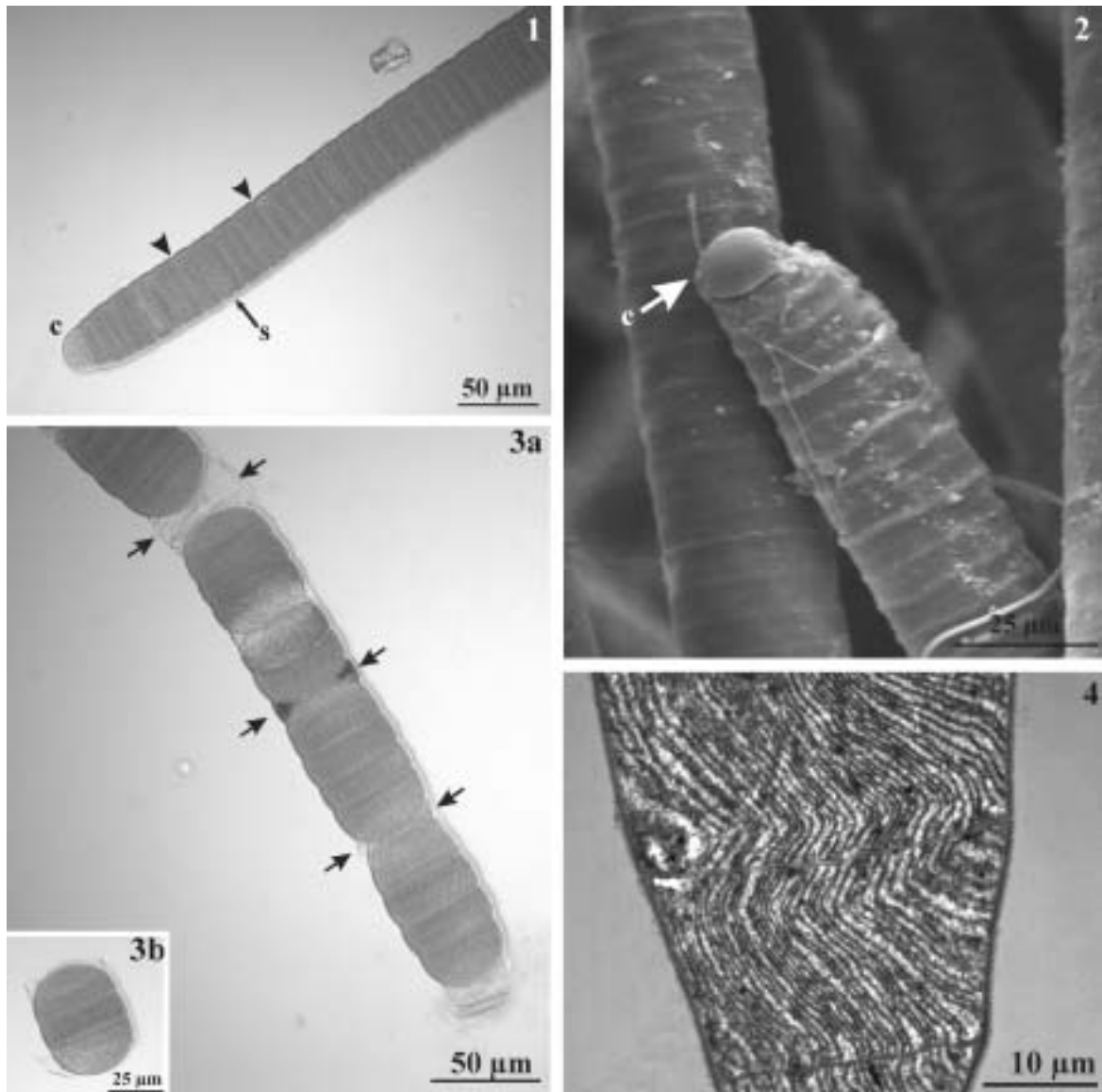


Fig. 1 — Light micrograph of a trichome of *Oscillatoria duplisecta*. Note the thin sheath (s), the calyptra (c) and the constrictions at the cross-walls (arrow heads).

Fig. 2 — Scanning electron micrograph of *Oscillatoria duplisecta* trichomes. An apical cell with calyptra (c) can be seen.

Figs 3a, b — Light micrographs showing the trichome disintegration. Fig. 3a — Necridia (arrows) are present along the trichome. Fig. 3b — A short hormogonium with equal and hemispheric end cells.

Fig. 4 — Transmission electron micrograph of *Oscillatoria duplisecta* showing numerous parallel thylakoids inside the cells.

by PCR. The 16S rDNA sequence of 1371 bp was checked to be sure to have sequenced the exact primary structure of the coding region. The identity of the new sequence was checked using the BLAST program available at the NCBI web server (ALTSCHUL *et al.* 1990). These analyses led us to verify that the 16S rDNA of the investigated strain showed a high identity (98%) with the sequence of *Oscillatoria princeps* Vaucher ex Gomont strain NIVA-CYA 150, isolated from the waters of a river in Thailand (SUDA *et al.* 2002). According to SUDA *et al.* (2002), this strain has to be considered the type one of *O. princeps*, which, in its turn, is the type species of the genus *Oscillatoria* (KOMÁREK and ANAGNOSTIDIS 2005). Therefore our isolate is surely a species belonging to this genus.

The high identity (98%) between the sequence of our isolate and that of the strain NIVA-CYA 150 might suggest that these two strains are morphotypes of the same species. However, in *O. princeps* the trichomes are not constricted at the cross-walls. This feature is an interspecific peculiarity discriminating the species of the genus *Oscillatoria* (KOMÁREK and ANAGNOSTIDIS 2005). Moreover according to KOMÁREK and ANAGNOSTIDIS (2005), *O. princeps* is lacking calyptra and its presence in hot thermal environments is very doubtful.

Our strain is also morphologically like *O. bonnemaisonii* Crouan ex Gomont and *O. margaritifera* Kützing ex Gomont. Both these strains, in fact, have trichomes constricted at the cross-walls. However, differently from the Euganean strain, their common habitats are marine or brackish waters, and their olive-green trichomes are usually thinner than 40 µm (KOMÁREK and ANAGNOSTIDIS 2005). In *O. margaritifera* the apical cells show the calyptra but, unlike our isolate, the trichomes are arcuate towards the ends (KOMÁREK and ANAGNOSTIDIS 2005).

In 1817 POLLINI described in the Euganean Springs some filamentous cyanobacteria, some of them proposed by him as species of the genus *Conferva* (= *Oscillaria*). Among them, *Conferva duplisecta* is the species that, owing to the trichome morphology, is nearest to our isolate. Both the strains, in fact, exhibit similar rotation and large brownish-greenish trichomes, visible to the naked eye, some centimeters long, with identical roundish ends. The cells, wide twice as much as long, have a "barrel" shape.

On the basis of the obtained results and of the more recent cyanobacterium taxonomy (GUIRY 2006), we propose for this species, belonging to

the genus *Oscillatoria*, the name *Oscillatoria duplisecta* (Pollini) Moro et Andreoli. The ultrastructural and molecular data, provided by this study, supplement the previous morphological information by POLLINI (1817).

Interestingly, it has to be pointed out that, after the finding by POLLINI (1817), this cyanobacterium has no more been noticed in the researches carried out on the biogelea of the thermal muds of the Euganean District (BRITO *et al.* 1972; ANDREOLI and RASCIO 1975; TOLOMIO *et al.* 2002 and 2004).

The EMBL/GenBank accession number for the 16S rDNA gene sequence of *Oscillatoria duplisecta* is AM398647. The name of this strain is ETS-06.

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