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Phragmonema sordidum Zopf var. *desanctisianum* Cennamo et De Luca (Phragmonemataceae, Porphyridiales), a new red algal variety from tuff cisterns

P. Cennamo[1], P. De Luca[2]

[1]Facoltà di Lettere dell'Università degli Studi Suor Orsola Benincasa di Napoli, Via S. Caterina da Siena, 80135 Napoli, Italy. [2]Orto Botanico, Università degli Studi di Napoli Federico II, Via Foria 223, 80139 Napoli, Italy
pcennamo@unina.it pdeluca@unina.it

Abstract. *Phragmonema sordidum* Zopf (Phragmonemataceae, Porphyridiales, Rhodophyta), an unicellular terrestrial red alga, is usually reported from very shady and warm caves. We isolated a strain of this alga from tuff cisterns in University of "Suor Orsola Benincasa", Naples (Italy). A comparative examination between this and the reference strain from the ACOI algal collection showed that several differences exist between the newly found strain and the other one in terms of form and size. On this new material, authors established the new variety *Phragmonema sordidum* Zopf var. *desanctisianum*.

Riassunto. *Phragmonema sordidum* Zopf (Phragmonemataceae, Porphyridiales, Rhodophyta), un'alga rossa unicellulare terreste ad habitat generalmente "cavernicolo", è stato identificato ed isolato nelle cisterne della cittadella dell'Università degli Studi Suor Orsola Benincasa di Napoli. Uno studio comparativo tra il ceppo isolato e il ceppo di riferimento della collezione algale ACOI ha mostrato che la nuova alga ha dimensioni e forma differenti rispetto al ceppo di riferimento. Sulla base di queste caratteristiche è stata istituita la nuova varietà *Phragmonema sordidum* Zopf var. *desanctisianum*.

Key words: Algae, Phragmonemataceae, *Phragmonema sordidum*, Rhodophyta, Taxonomy, Tuff

Introduction

The interest in microfloral biodiversity of ancient and archaeological sites, such as historical buildings, monuments and caves, has been increasingly growing in recent years. Among the reasons for this, the most important is that such sites are an invaluable evidence of our historical and cultural past. As it is widely known, microbiological communities develop on the surface of the walls of these sites, giving rise to coloured patinas and incrustations and directly participate in decay processes, causing not only aesthetic damage but also structural deterioration of the surface by production of acids or other metabolites (Dornieden & Gorbushina 2000; Urzì & Krumbein 1994).

There is still little research ongoing on algal flora of cisterns and caves, with the exception of caves with prehistoric paintings, in which Cyanobacteria were reported as abundant (Cifferi 1999).

Cisterns and caves deteriorate as a result of environmental factors. High temperatures, lack of natural light and high relative humidity increase both contamination due to anthropogenic and natural sources, and to the action of micro- and macro- biological communities. The said chemical-physical parameters act as controlling factors on the microbiological communities, regulating both species composition and their distribution. Algae and Cyanobacteria are the most frequent organisms found on walls and are considered primary colonizers. They are abundant as unicellular and colonial forms, and also as filamentous forms, although the latter show less diversity.

A wide research programme on the archaeological area of Naples has evidenced a rich cultural heritage of cisterns and caves. These are largely due to the natural abundance of volcanic tuff rocks in the area, out of which they were built.

In this study, results of investigations aimed to assess the kind and variety of algae affecting

the deterioration in the tuff cisterns in Naples (Italy) are presented.

A cistern in the "Suor Orsola Benincasa" monastic citadel (now, the location of University of Naples "Suor Orsola Benincasa") has demonstrated its usefulness as a model site to investigate the structure and dynamics of algal communities present in this kind of habitats. It is an ideal site as it is easily accessible for sampling and it is also within the university; thus, it is exposed to anthropogenic factors on a daily basis (Fig. 1).

During the investigation of the algal flora in the cisterns, a population of red algae identified as *Phragmonema sordidum* Zopf was found.

Phragmonema sordidum (Phragmonemataceae, Porphyridiales) is the only species of this genus. It is characterised by absence of pyrenoids and presence of endospores. Chromatophore and nucleus are placed parietally, as a consequence of the presence of a large central vacuole. Small grains of starch are dispersed within cells, around the vacuole. This alga presents palmelloidal, pseudo-autosporogenous, pseudo parenchymatous and dendroidal stages.

Genus *Phragmonema* was described by Zopf (1882) from the Berlin botanic garden, where it formed an epiphyte strains of a muddy brown–yellow colour on the leaves of *Ficus barbata*. Geiter (1942) found it in the hothouse of the botanical garden of the University of Vienna, on walls on which *Trentepohlia lagenifera* was growing; he collected it also in the Schönbrunn park in Austria (1942/43). Friedmann (1956) found the alga in two Israel caves: in the prehistorical cave of Guwrin and in the Sanhendriya crypt cave in Jerusalem. Sieminska (1962) collected it in the Sybilla Cave in the archaeological park of Cuma (Naples). Recently, Ricci & Pietrini (2004) found it on the frescoes of the "Colombario degli Scipioni" in Rome.

In this report, the organisms characterized with only unicellular stages and different

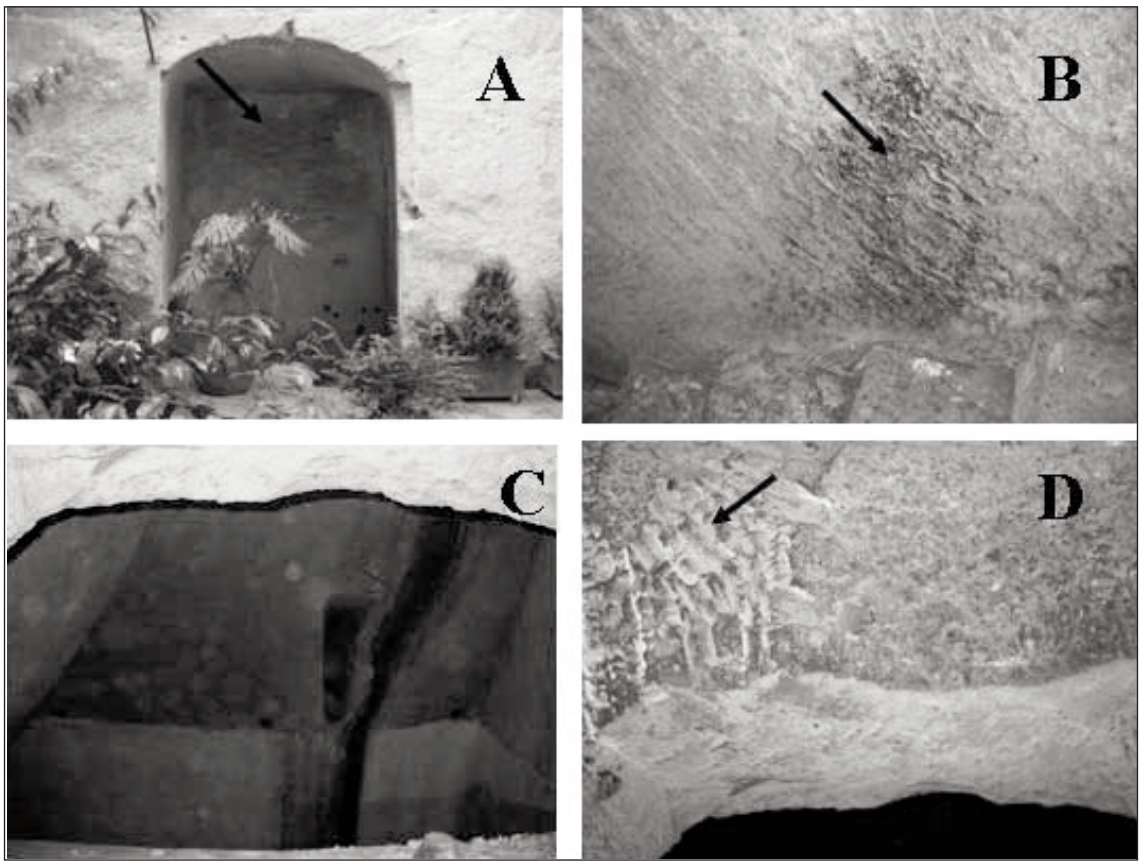

Fig. 1 - Details of the three sampling sites. A: The walls around the entrance of the cistern. B: The rock surrounding the opening of the cistern. C-D: The tuff wall around the mouth of the cistern.

dimensions of the cells are presented and their description as a new variety of species *Phragmonema sordidum* is proposed.

Materials and Methods

Description of the sites

The "Suor Orsola Benincasa" monastic citadel is located in the centre of Naples. Its beautiful cloister and gardens climb the hillside of St. Elmo. The complex was founded by "Suor Orsola" in 1581 and consisted of a monastery and two adjoining churches. The "Suor Orsola" churches were an important point of reference for the local people. Nowadays, the monastic citadel is home to the university of the same name and is used by hundreds of people every day. Two cisterns occur in the site. They had been excavated from local tuff rock (Fig. 1a) and were probably used in the past by the nuns for collecting water. Over the years the cisterns became disused and were finally abandoned.

Three different microenvironments have been sampled: A) the walls around the entrance of the cistern; B) the rock surrounding the opening of the cistern; C) the tuff wall around the mouth of the cistern.

Sampling and analyses

Two different seasons were chosen for collecting: early spring (April 2004), with an atmospheric temperature of 20-24 °C and early autumn (late October 2004) at 15-20 °C. In these periods the photosynthetic communities are usually abundant. The samples were taken from the surface of the cisterns in different sites. The material was collected aseptically in sterile Petri dishes and analysed one day after collection. The algal samples were suspended into T_2-S_2 ACOI medium at 25 °C, under continuous fluorescent light. After four days, cells were separated by serial dilutions and unialgal cultures were obtained within one week. For algal counts, 1 g of fresh sample soil was first suspended and fixed in 20 ml aqueous solution of formaldehyde 3 % and then counted with a blood-counter chamber. Cells were observed every day with a Nikon Eclipse E800 microscope, equipped with Nomarski interference optics. For SEM analysis, mats were collected from each sampling site and fixed in formaldehyde (4%). To preserve the whole structure of cells, they were successively dehydrated in a graded alcohol series and critical point dried. Sub-samples of this material were mounted on aluminium stubs, sputter-coated with gold and examined at an accelerating voltage of 20 kV. Qualitative and quantitative microanalyses were carried out on the other sub samples collected on carbon-coated, graphite stubs. A ZAFPB quantitative microanalysis system was used to detected the main chemical elements.

Results

SEM and ESEM results

Tuff is a volcanic rock made up of rock and mineral fragments in a volcanic ash matrix, formed of pyroclastic material. Tuffs contain little or no quartz but much orthoclase and oligoclase feldspar, often with biotite, augite and hornblende. As a consequence of weathering, they often change to soft red or yellow clay-stones, rich in kaolin with secondary quartz. Tuff is a good substratum for the growth of the microorganisms both for its friable composition and the presence of various nutrient elements. Chemical analysis of the mats collected in the twenty point sampling revealed a high content of Si, S, Al, K and Na. These elements are predominant, probably as a consequence of the presence of opal and alunite in the tuff rock. The sulphate mineral paragenesis includes native sulphur and Al-sulphate hydrate minerals. No significant mineralogical differences have been detected in the different samples.

Biofilm samples

ESEM analysis was carried out on the microbial films sampled in different sites, identified on the basis of the conformation of the cisterns. ESEM profiles around the particles present in the green-brown crust obtained from sampling sites A and B revealed a major

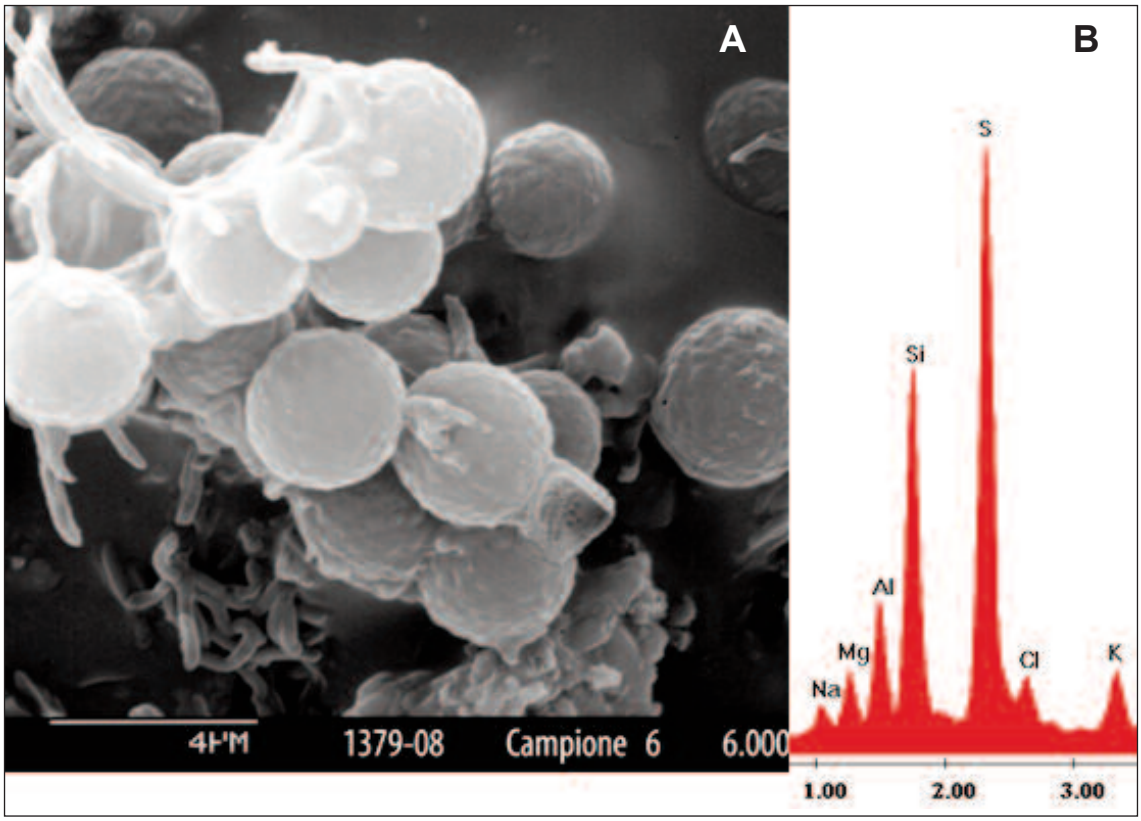

Fig. 2 - A: SEM micrograph of cells from the wall in the cistern (site C). B: ESEM analysis of microelements found in the samples shown in A.

peak for sulphur, potassium and calcium, probably both the latter associated to the components of the rock an to those of the microbial cell walls. Samples from the site C, where the crust is black, showed a higher peak for sulphur, but the other peaks were overlapping (Fig. 2).

Light microscopy

Microorganismal biodiversity basically depends on the temperature and light exposure that gradually decreases with the distance from the entrance of the cisterns. *Phragmonema sordidum* is present in all sites of the cisterns (Fig. 1a,c): near the entrance, where the light intensity is high, and in the deepest part of the site, where the light is dim or, at some hours of the day, actually absent, but where there is more water and therefore relative humidity is higher.

In all samples of cisterns, *P. sordidum* was sufficiently abundant to be found in all microscope observations of the collected mat. We compared this strain to the ACOI 969 culture collection, in the same growth conditions, and we found some differences. In the cistern's samples only coccoid cells were observed, but chloroplast morphology was sufficient to place this alga within Phragmonemataceae. We observed 8–32 autospores arising from a single autosporangium. Cells also appeared to divide by vegetative cell division to produce two to four cells included in compact, thick-walled, mother cells. Zopf (1882), however, in his original description of *P. sordidum*, considered such binary cell division to be equivalent to autospore formation. The cistern's culture cells have different size ranges (15-20 µm) from ACOI 969 (5-10 µm). The cistern's culture has a darker more brownish coloration than the ACOI sample (Fig. 3).

Phragmonema sordidum* Zopf var. *desanctisianum*, var. *nova

Descriptio: Cellulae 15-20 µm, rotundae, inderdum coloniales (2-4 cellulares). Paries

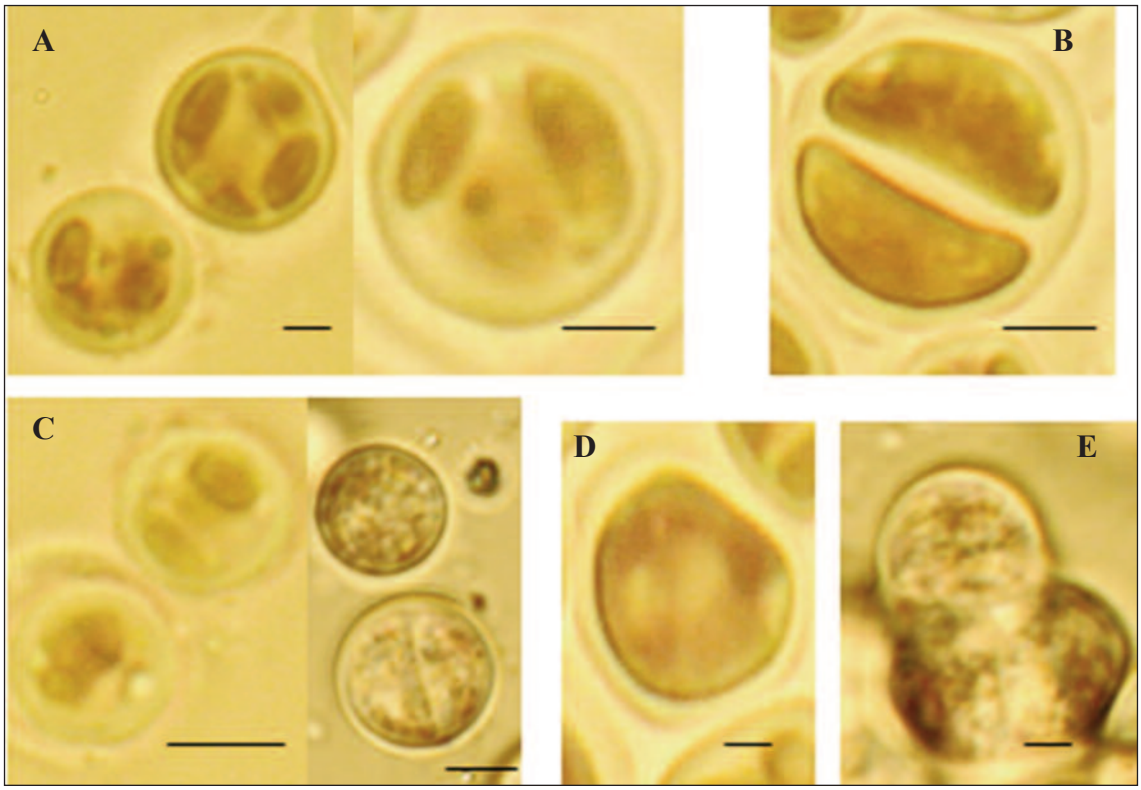

Fig. 3 - A: Optical micrographs of *Phragmonema sordidum* Zopf var. *desanctisianum* strain SOB 101; vegetative cells. Scale bar: 5 µm. B: Two celled sporangium. Scale bar: 5 µm. C-E: Optical micrographs of *Phragmonema sordidum* Zopf var. *sordidum* 969 ACOI. C: Vegetative cells. D: Status palmelloideus. E: Status autosporogenus. Scale bar: 5 µm.

cellularis mucilaginoso strato praedita; chromatophorus parietalis; vacuola singola centralis; nucleus parietalis; a specie typica habitu semper coccoidali, numquam palmellato, dendritico, filamentoso vel pseudoparenchimatoso et dimensionibus cellularum valde maioribus tantum differt. Ceteri characteres eodem modo ac in descriptione speciei a Zopf (1882) composita sunt. Haec varietas excellentissimo viro Francisco De Sanctis, rectori magnifico neapolitanae universitatis Suor Orsola Benincasa, praeclaro professori iuris philosophiae dicata est.

Description: The vegetative cells are spherical of greyish to red colour. The dimensions of cells are 15-20 µm. The chromatophore, in the shape of a plate, and a nucleus placed parietally surround a large central vacuole. Cells divide by vegetative cell division to produce two to four cells within a compact, thick-walled, mother cell.

Habitat: tuff cisterns; found in the cisterns at "Suor Orsola Benincasa" University, in Naples.

This variety is dedicated to Prof. Francesco De Sanctis, rector magnificus of the said university and prominent scholar of law philosophy.

Type culture: PS 101 SOB

Discussion and Conclusions

Cave environments represent interesting sites for studying the taxonomic diversity of photosynthetic microoganisms, especially if we consider that these habitats can be classified as extreme habitats. Combined mineralogical and biological studies constitute a useful tool for detecting possible interactions between the environment and algal communities. In "Suor Orsola Benincasa" cisterns, the occurrence of *Phragmonema* in a surface layer of the tuff rocks is to be related to its ability to live in this extreme habitat at limited light con-

ditions and to colonize tuff rock. According to Sieminska (1962), this alga shall prove to be characteristic for cave habitat. In particular, a strong mineral-microbe relation has been observed, with typical morphologic characters, different size and the exclusive presence of coccoid stage which are, in our opinion, valid characters for creating a new variety of *Phragmonema sordidum*.

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