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Epiphytic Diatom Communities on Sub-Fossil Leaves of *Posidonia oceanica* Delile in the Graeco-Roman Harbor of *Neapolis*: A Tool to Explore the Past

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Abstract

The Graeco-Roman harbor of *Neapolis* (Naples, Italy), chronologically constrained between the late 4th century BC and the 6th century AD, offers a unique sight on relationship between past and present into the history of the marine biodiversity. In fact, the digs expose fossil leaf of *Posidonia oceanica* with epiphytic communities of microorganisms. *Posidonia oceanica* is a seagrass endemic to the Mediterranean that forms large meadows whose remains can persist in the sediment for thousands of years. In this communication, we report results of analyses carried out using both molecular and morphological techniques on sub-fossil leaves of *P. oceanica* and their associated epiphytic communities.

Keywords

***Posidonia oceanica*; Epiphytic Communities; Ancient DNA**

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1. Introduction

Posidonia oceanica (Delile) has been present in the Mediterranean Sea since the Miocene. During the last glaciation, it survived in refugia from which it re-colonized the basin [1] [2]. It presently grows along the coast of the Eastern and Western basins into which the Mediterranean is divided, developing associated communities characterized by conspicuous species diversity [3] Several studies on the distribution and conservation of this plant by employing morphological or molecular data have been carried out, including one regarding ancient *Posidonia* plants presented in the marine sediment in the bay of Naples dated 300 yr ago [4]. The Graeco-Roman harbor of *Neapolis* was discovered in 2004 in the area of Piazza Municipio (Naples, Italy), about 500 m from inland as compared to the modern docks of Naples [5]. The excavations brought to light huge amounts of stratigraphic and archaeological data which allowed the harbor sedimentary record to be chronologically constrained between the late 4th century BC and the 6th century AD [5]. The infilling succession is made up of marine, transitional and continental deposits overlaying a volcanic bedrock consisting of tuffs and pyroclastics of the Neapolitan Yellow Tuff [6] [7]. The most representative sections exposed in the digs were sampled for sedimentological, pa- leoenvironmental and paleobotanical analyses [6]-[8]. In particular, a 7-m thick succession, representing the in- filling of a protected inlet in the ancient harbor, was brought to light and sampled for pollen analysis [17]. This site represents an important archive of marine eukaryotic biodiversity. As such, the Greco-Roman harbor offers a unique opportunity to study sub-fossil samples of *Posidonia oceanica* and to analyze the remains of the epi- phytic communities of microorganisms. No archeobotanical studies are available analyzing aquatic plants re- mains together with their associated epiphytes on both morphological and molecular standpoints. Morphological analysis of botanical remains is comparatively simple since an extensive reference dataset is available to help interpretation, but it also has some limitations, in this case mainly related to plant material degradation. In the present work, we analyze sub-fossil leaves of *P. oceanica* from the Graeco-Roman harbor of *Neapolis* with their associated epiphytic communities, using both molecular and morphological techniques.

2. Material and Methods

Samples collected from the lagoon layers (**Figure 1(a)**) of the Graeco-Roman harbor dated to the 5th century AD, were compared with leaves of *Posidonia oceanica* obtained from herbaria or directly collected on shores and dried (sources are listed in **Table 1**). The samples were dropped in ethanol for 3 - 4 days at room temperature and dehydrated in an ethanol series, critical point dried and sputter-coated with approximately 30 nm of gold. Specimens were examined using a FEI-Quantas 200 ESEM at an accelerating voltage of 20 kV. The DNA region chosen for molecular analyses was the 18S nuclear DNA. Total genomic DNA was isolated from approximately 100 mg of sub-fossil leaves following a modified CTAB procedure [4]. Molecular markers were amplified using primers from literature [9]. Complete sequences of each PCR product were obtained, aligned, and inspected with the AB DNA Sequencing Analysis software ver. 5.2 (Applied Biosystems, Life Technologies), Sequence Navigator ver. 1.0.1 software (ABI Prism, Perkin Elmer) and BioEdit ver. 7.0.9.0 software [10].

Table 1. List of the species *Posidonia oceanica* obtained from herbaria or directly collected.

Taxon	Origin
P. oceanica	P6.1us7282e Naples (Italy) 2012
P. oceanica	Linosa (Italy) 1906
P .oceanica	Ischia (Italy) 2012
P. oceanica	Rodi (Greece) 1925
P. oceanica	Tripoli (Libya) 1919
P. oceanica	Bengasi 1921
P. oceanica	Bengasi (Libya) 1919
P. oceanica	Elba Island (Italy) 1981

3. Results and Discussion

Posidonia oceanica samples have been identified on the grounds of macro- and micro-morphological characters. All the studied leaves (sub-fossil and reference samples) are yellow-brownish, about 0.8 - 1.2 cm wide and show numerous veins. The sub-fossil and herbarium samples are fragments 3 - 4 cm long. At the micro-morphological level, the epidermis is covered by a smooth cuticle, lacks stomata, shows rectangular pavement cells with straight cell walls and perpendicular to oblique transverse cell walls (**Figures 1(b)-(e)**). Fibre strips are visible in samples where the epidermis is damaged (**Figure 1(f)**). Hyphae and epiphytic diatoms are clearly visible on the sub-fossil leaves, whereas members of the bacterial community, usually represented by *Marinomonas posi- donica* [11] and other similar species, are less frequent and more difficult to observe. On the contrary, these bacteria are widely visible on the references samples collected in nature or obtained from herbaria (**Figure 1(e)**).

Diverse epiphytic diatoms are evident on the sub-fossil leaves (**Figures 2 (a)-(f)**). They are all recent benthic species [12]-[15] and namely: the adnate species *Cocconeis stauroneiformis* (Rabenhorst) Okuno (**Figure 2(a)**) and *Amphora* cfr *helenensis* Giffen (**Figure 2(b)**), individuals of *Nitchia* cfr *punctata* Grunow (**Figures 2(c)-(e)**) and a broken frustule of the motile species *Navicula directa* (W. Smith) Ralfs (**Figure 2(f)**). All these species are commonly present on living *P. oceanica* plants which, however, usually show a much complex epiphytic diatom community, often including hundreds of species [16].

The 18 S sequences from the subfossil specimens were 100% identical to those of *P. oceanica* AY491942.

The vertical extent of the *P. oceanica* remains in the lagoon layers (**Figure 1(a)**) is indicative of matte formations that usually occurs when the meadow is buried by an amass of sediments in a relatively short time. This support the hypothesis that the sedimentary infilling and final closure of the bay brought about the decline in

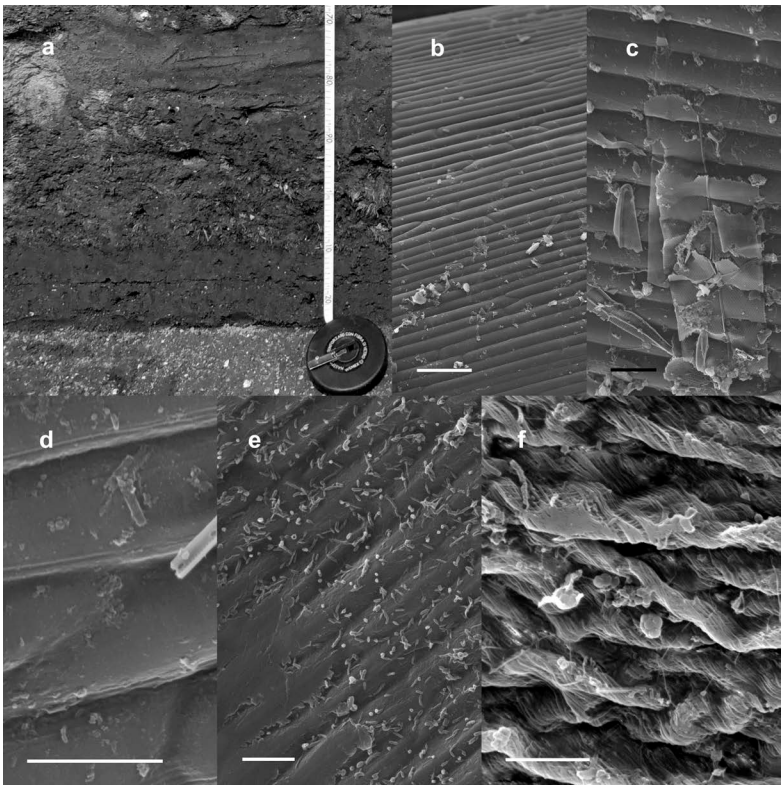

Figure 1. a: Detail of the section exposed in the Piazza Municipio (PM) dig. b: SEM of *Posidonia oceanica* leaf surface (PM). c: SEM detail of *P. oceanica* leaf (PM) surface showing epiphytic hyphae and diatoms. d: SEM detail of *P. oceanica* leaf (PM) surface showing scanty presence of epiphytic bacteria. e: SEM of *P. oceanica* leaf surface (sample collected at Linosa island, Sicily, Italy) showing considerable presence of *Marinomonas posidonica*. f. Fibres on *P. oceanica* (PM) samples. Scale bars b 50 µm, c - f 10 µm.

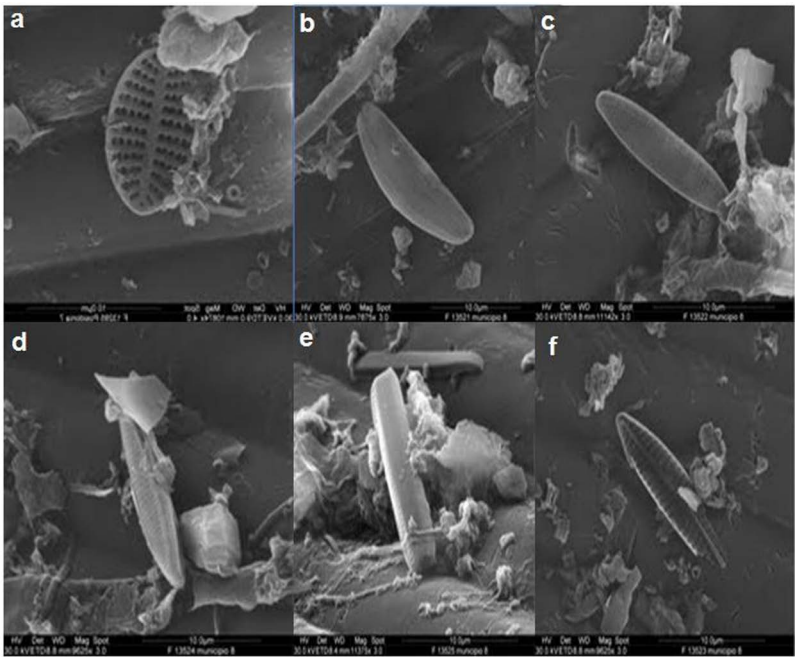

Figure 2. a: *Cocconeis stauroneiformis*. b: *Amphora* cfr *helenensis*. c-e: *Nitchia cfr punctata*. f: broken frustule of motile species *Navicula directa*.

harbor activities until their complete abandonment [17].

4. Conclusion

The Graeco-Roman harbor of *Neapolis* has an archeological area full of material to be investigated. Studies are in progress to extend our knowledge of the algal biodiversity and understand whether the observation of reduced epiphytic communities on the *Posidonia* sub-fossil samples is indeed the record of a locally reduced occurrence of diatom and bacteria or it is related to the sedimentary infilling and burial process of matte.

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