

Cocconeis Ehrenberg (Bacillariophyta), a genus dominating diatom communities associated with *Posidonia oceanica* Delile (monocotyledons) in the Mediterranean Sea

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ABSTRACT

A fine-scale analysis of diatoms associated with *Posidonia oceanica* in selected locations was carried out along a longitudinal gradient in the Mediterranean Sea (00°21'W–27°07'E) to investigate the composition and diversity of epiphytic communities and to determine the distribution and relative importance of their components with special reference to the most common and abundant genus, *Cocconeis*. *Posidonia oceanica* was sampled twice (once per year for two years) at 19 sites. At each site, forty samples of *P. oceanica* shoots separated by several metres were taken. Epiphytic diatom communities were studied with SEM (scanning electron microscopy) and TEM (transmission electron microscopy). Fifty-two diatom genera were identified. In every studied case, adnate *Cocconeis* spp. cells dominated the community. Among 32 *Cocconeis* species and varieties found, recently described *C. neothumensis* var. *marina* and *C. scutellum* var. *posidoniae* were almost always the most numerous. The structure of *Cocconeis* assemblages changed across the various geographic regions both in terms of the species' presence–absence and their relative abundance. In general, the most abundant species appeared to be broadly distributed, while less abundant species had a more distinct biogeographic pattern. In the course of this study, several *Cocconeis* species described as Indo-Pacific were found in the Mediterranean Sea for the first time. Potential relationships between different *Cocconeis* species and genetically distinct *P. oceanica* populations are discussed.

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1. Introduction

Epibiosis is known to be an ubiquitous phenomenon among benthic marine organisms, both uni- and multicellular, from all biological kingdoms (Armstrong, 2001). Several authors reported that complex communities of high degree of species-specificity epiphytized blades of *Posidonia oceanica* (L.) Delile (Van Der Ben, 1971; Scardi et al., 1986; Mazzella, 1999), a marine angiosperm endemic to the Mediterranean Sea (den Hartog, 1972). In the euphotic zone, dense meadows of *P. oceanica* that overgrow the seabed, host copious assemblages of macro- and microalgae (e.g. Mazzella et al., 1989; Buia et al., 1992). In particular, obligate benthic genera of pennate diatoms proliferate extensively on *Posidonia* leaves. Among them, the most commonly represented are *Amphora* Ehr. (Thalassiosiphysales), *Cocconeis* Ehr. (Achnanthales), *Diploneis* Ehr. (Naviculales), *Fragilaria* Lyngbye, *Synedra* Ehr. (Fragilariiales), *Gomphonemopsis* Medlin (Cymbellales), *Mastogloia* Thwaites in W.

Smith (Mastogloiales), and *Navicula sensu lato* (Mazzella, 1983; Mazzella and Spinoccia, 1992; Mazzella et al., 1994).

The diatom–*Posidonia* interaction is thought to be driven and strengthened by biological factors (e.g. life strategies, morpho-functional features, adhesion systems of diatom cells) rather than the actual environmental conditions (Edgar and Pickett-Heaps, 1983, 1984; Hudon and Legendre, 1987). Indeed, the multifaceted structure and dynamics of diatom epiphytic communities owes its coexistence to specimens representing different growth forms, i.e.: adnate diatoms, scarcely motile, adhering strongly to the substrate surface (*Achnantes* Bory, *Cocconeis*); erect diatoms, either single cell or colonial forms, attached to the substrate by means of mucous pillows (*Fragilaria*, *Synedra*, *Tabularia* [Agardh] Snoeijis) or peduncles (*Gomphonemopsis*, *Grammatophora* Ehr., *Licmophora* Agardh); and motile diatoms, biraphid forms living in the community matrix, able to move in a smooth gliding motion (*Navicula* Bory, *Mastogloia*, *Gyrosigma* Hassall, *Pleurosigma* Smith). Under favourable conditions, a certain part of peryphytic community biomass can be also accumulated in overstory typically composed of semi-planktonic diatoms and other microorganisms loosely attached to the substrate (Hudon and Legendre, 1987). The dynamics of development of epiphytic diatom communities on *Posidonia* follow a common

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progression of stages. At the very beginning, coccoid and filamentous bacteria colonize the newly formed leaves. Then, encrusting diatoms (mainly *Cocconeis*), after a short coexistence with bacteria, form pauci- or monospecific assemblages which soon may cover almost the whole leaf surface. Finally, at the climax stage, various diatom forms, including erect and highly motile species, colonize the remnant available microenvironments. It has been documented that in case of *Posidonia*, growth form as well as species diversity of epiphytic diatom community increases in the basal and intermediate parts of adult and senescent leaves, where the community is at a higher developmental stage. However, whereas erect, motile and loosely attached diatoms present a patchy distribution, clumping on certain parts of the host plant, individuals of *Cocconeis* spp. are distributed far more evenly and seem to dominate all other diatom genera in terms of total cell abundance (e.g. Mazzella, 1983; Hudon and Legendre, 1987; Buia et al., 1992; De Stefano et al., 2000).

Numerous studies indicate that in seagrass systems epiphytic microalgae are the most important primary producers, being responsible for up to over 60% of total system production, while seagrass tissues are much less desirable food source for many of the grazers resident in seagrass meadows (Moncreiff and Sullivan, 2001; Borowitzka et al., 2006). In *P. oceanica* meadows, a significant part of microalgal biomass is contributed by *Cocconeis* species due to their high rates of production, opportunistic colonization strategy, and preferences for flat surfaces of seagrass leaves which constitute the bulk of the host plant body (personal observation; Mazzella and Spinoccia, 1992; Gacia et al., 2009 and references therein). The evolutionary and adaptive success of the genus *Cocconeis* is reflected in its great inter- and infraspecific morphological diversity that becomes evident while examining ultrastructural features of exoskeleton (frustule) of single specimen by SEM. Analysis of the available literature suggests that the highest number of marine *Cocconeis* species was recorded from pan-tropical and equatorial regions, though this distribution pattern may be strongly biased by uneven sampling effort across geographical regions and over time. Recent studies indicate that individuals of *Cocconeis* spp. can dominate benthic diatom communities at high latitudes (e.g. Thomas and Jiang, 1986; Wulff et al., 2009; Majewska et al., 2012, Majewska et al., 2013). However, even diatom flora of many far more easily accessible locations of temperate regions remain to be identified. In regards to studies on *Cocconeis* genus, the emerging paradox is that almost 50% of thorough examinations using electron microscopy focus exclusively on three species, *C. scutellum*, *C. placentula*, and *C. pediculus*, and their infraspecific taxa (e.g. Holmes et al., 1982; Poulin et al., 1984; Kobayasi and Nagumo, 1985; Romero, 1996; De Stefano et al., 2000, 2003, 2006, 2008; De Stefano and Marino, 2001, 2003; De Stefano and Romero, 2005). Numerous studies have been carried out in the Mediterranean region and several new *Cocconeis* species have been described in the last few years. Yet, most of them are known from their type localities only and no information on the actual range of the species exist. *Posidonia oceanica* meadows have drawn new attention lately because of the reports of their genetic diversity. Despite theoretical potential of seed dispersal, the gene flow in Mediterranean populations seems to be highly restricted. Based on genetic differences different Mediterranean groups were distinguished (Micheli et al., 2005; Arnaud-Haond et al., 2007; Serra et al., 2010). Factors responsible for reduction of gene flow amongst *Posidonia* populations may also affect associated epiphytic organisms if their dispersal effectiveness is not high enough. No data has yet been reported however, to allow a critical assessment of this hypothesis.

The original aim of the presented study was to describe the epiphytic diatom communities colonizing *Posidonia oceanica* with special attention to genus *Cocconeis* – their most abundant

Table 1
Sampling stations and geographic coordinates.

Station no.	Location	Geographic coordinates
1	Alicante (Spain)	38°18'N 00°21'W
2	Majorca Island (Spain)	39°26'N 02°36'E
3	Medas Islands (Spain)	42°11'N 03°09'E
4	Gulf of Genoa (Spain)	44°26'N 08°27'E
5	Western Sardinia (Italy)	40°41'N 08°00'E
6	Eastern Sardinia (Italy)	40°54'N 09°33'E
7	Ventotene Island (Italy)	40°50'N 13°02'E
8	Ischia Island (Italy)	40°39'N 13°50'E
9	Western Calabria (Italy)	39°41'N 15°32'E
10	Eastern Sicily (Italy)	38°15'N 15°15'E
11	Malta Island (Malta)	35°48'N 14°27'E
12	Istria (Slovenia)	47°32'N 13°27'E
13	Dalmatia (Croatia)	43°36'N 16°24'E
14	Western Salento (Italy)	40°31'N 17°18'E
15	Eastern Salento (Italy)	40°10'N 18°31'E
16	Gulf of Saronico (Greece)	37°55'N 23°37'E
17	Gulf of Edremit (Turkey)	39°31'N 26°37'E
18	Gulf of Kusadasi (Turkey)	37°51'N 27°07'E
19	Patmos Island (Greece)	37°23'N 26°19'E

component. We aimed to compare epiphytic communities found at nineteen sites localized along a longitudinal gradient in the Mediterranean Sea (00°21'W–27°07'E) analyzing the relative contribution of *Cocconeis* genus to the total number of epiphytic diatoms, the specific composition of *Cocconeis* assemblages and the relative abundance of each species within them. Numerical values of ecological indexes (Simpson, Shannon-Weaver, Pielou) representing each sampled locality have been calculated, with the final intention to link structure and diversity of *Cocconeis* assemblages to their geographical distribution in the Mediterranean basin.

2. Materials and methods

2.1. Material collection and preparation

Sampling was carried out once per year for two consecutive years (2000 and 2001) in spring (from end of March to mid-June), when the macrophytic component of the vegetal community associated with *Posidonia* was generally low and concentration of *Posidonia* blades was not at its maximum. This minimized the potential effect of self-shading. Spring was chosen as a period when diatom communities could be regarded as climax communities growing on fully developed but not yet senescing seagrass. Nineteen sampling sites were chosen in order to give a spatial overview and to reflect differences between epiphytic diatom communities in various parts of the Mediterranean Sea (Table 1, Fig. 1). For logistical reasons, African coasts were not sampled. Forty orthotropic shoots (leaves, vertical rhizomes, and roots) were collected from each site at a reciprocal distance of about 5–8 m along the 10 m isobath. In some cases, due to varied bottom morphology sampling was performed down to ca. 20 m depth. Fine-scale floristic analysis of the whole epiphytic diatom community was carried out in six selected localities that represented the main Mediterranean basins under investigation, i.e. Alicante, Gulf of Genoa, Ventotene Island, Malta, Gulf of Kusadasi, and Dalmatia. Samples from other stations were screened for *Cocconeis* species only.

To assess the community development stage fragments of 1–2 cm² were cut from different parts of the *Posidonia* leaf blade (basal, intermediate, apical) and fixed by 1-h immersion in a mixture of 4% formaldehyde and 2.5% glutaraldehyde solutions in filtered seawater. In order to preserve the epiphytic diatom cells intact as well as all the elements of their complex attachment mechanism, samples have been treated following the “sandwich critical

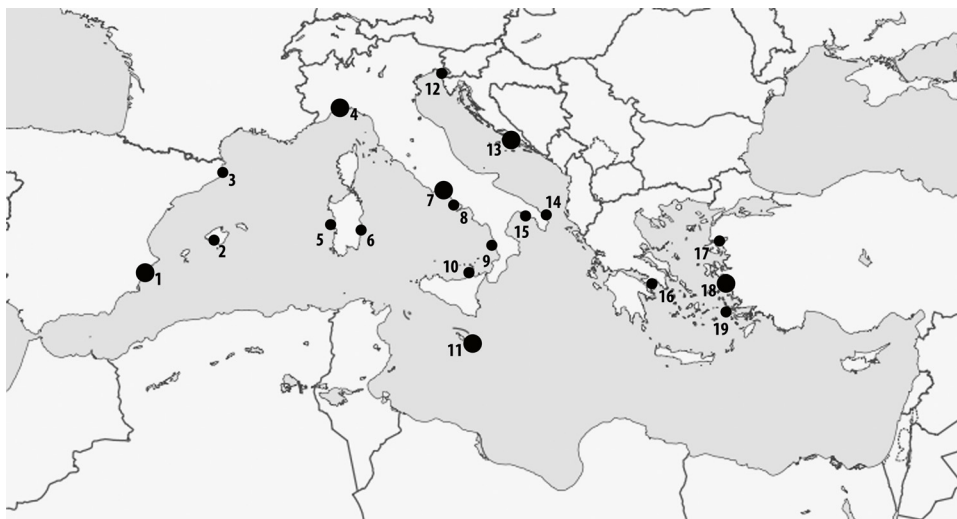


Fig. 1. The Mediterranean Sea map with the sampling locations. Bigger dots (numbers: 1, 4, 7, 11, 13, 18) indicate locations where the fine-scale floristic analysis was carried out for all diatom species found.

point drying method” described by Totti et al. (2003): to remove the excess of fixative each sample was rinsed several times with distillate water and placed on a “Nucleopore” polycarbonate filter (Costar, Corning) in a Swinnex filtration apparatus (Millipore), while a second filter was placed over the first to form a sandwich in which the sample was trapped. Subsequently, each of the prepared seagrass pieces was dehydrated individually by immersion in ethanol–water solution at increasing alcohol concentration (10, 25, 50, 60, 80, 90, 95 and 100%) and treated in a critical point dryer (Polaron CPD7501) using liquid carbon dioxide as infiltrating medium. Finally, dried samples were mounted on aluminium stubs using adhesive carbon tabs, coated with Au–Pd (Polaron SC7640), and examined by scanning electron microscopy (SEM, Jeol 6060LV) operating at 25 kV.

Additionally, in order to facilitate diatom counting and examination of the ultrastructure of the frustules, a second set of samples was prepared. Preliminary examination (carried out with SEM) showed that differences between samples collected at the same sampling site in different years were not statistically significant. Therefore, we decided to focus exclusively on differences created by location. Epiphytic diatoms from all parts, i.e. leaves (inner and outer surfaces) and rhizomes, of all 40 *Posidonia* shoots (20 shoots collected each year of the study) sampled at 19 stations were scraped off with a scalpel and thoroughly mixed to homogenize the samples and to minimize the influence of factors that could possibly affect the diatom composition (e.g. different age of leaves, differences in surface topography between leaves and rhizomes, slightly different depth of sampling) as well as the randomness of the results. The obtained 19 samples were subsequently cleaned following the von Stosch’s method (Hasle and Syvertsen, 1997): nitric acid (64%) and sulphuric acid (97%) were added at a 1:1 and 1:3 ratio to the sample volume respectively, and the mixture was stirred and heated for 3 min until the solution became transparent. After cooling, the precipitate was washed several times with distillate water in a centrifuge and the obtained material was fixed in DDW (double deionised water) with a few drops of acetic acid. Permanent light microscope slides and SEM stubs were prepared. Each sample was analyzed under the SEM as two replicates examined through sequential horizontal transects. A minimum of 3000 *Cocconeis* individuals (at least 1500 in each of the replicates) was counted for each sample. For the samples where the diatom concentration was extremely low, additional replicates (stubs) were prepared and analyzed.

2.2. Microscopical observations and statistical analyses

The identification of *Cocconeis* species was carried out by careful observation of both valves (raphe and rapheless) composing the diatom frustule in outside and inside view in scanning electron microscope (SEM). If necessary, transmission electron microscope (TEM) was used to analyze fine-scale ultrastructural details at high magnification (20,000–50,000×). This thorough study included examination of broken frustules which often offered the possibility of viewing the frustule in various cross sections. In addition, the type material of *Cocconeis* specimens deposited in the Hustedt Diatom Collection (Bremerhaven, Germany; Table 2) was studied and used for suitable comparisons. The investigation was focused especially on subtle morphological differences between the specimens considered as belonging to one species but collected from different regions as well as on species rarely noted from *Posidonia* blades.

Several mathematical indices were used to assess the biodiversity and predominance of *Cocconeis* species in examined samples. The Simpson index (Simpson, 1949) was calculated according to the formula: $\lambda = \sum_i p_i^2$, where i ranges from 1 to the total number of *Cocconeis* species in the community and p_i is the relative abundance of each species. According to Simpson’s parameterization,

Table 2

List of examined samples from the Hustedt Diatom Collection (Bremerhaven, Germany).

Sample code	Locality
Am 37	Bessels Bay, Nares Exp., P.T. Cleve, 12/1881
As 530	Sendai (Japan)
E 5308	Nardsot (Norway)
As 1322	Celebes, Mahalone, Wallacea Exp.
E 74	Lissa, Rovinj (Croatia)–7–1940
Am 792	Beaufort, N.C (U.S.A.)
E 4581	Plon, Madebroken Lake 2–10–1921
E 95	Posillipo, Gulf of Naples (Italy)
E 10772	Gulf of Naples (Italy)
As 37	Java
As 507	Fasana (Italy)
E 69	Neuharlingersiel 19–7–1940
E 3951	Sandwich Islands
At 198	Memmert, 2–9–1938
E 514	Grip (Norway)
E 255	Beaufort, N.C (U.S.A.)
Am 792	Vera Cruz (Mexico) 19–4–1956

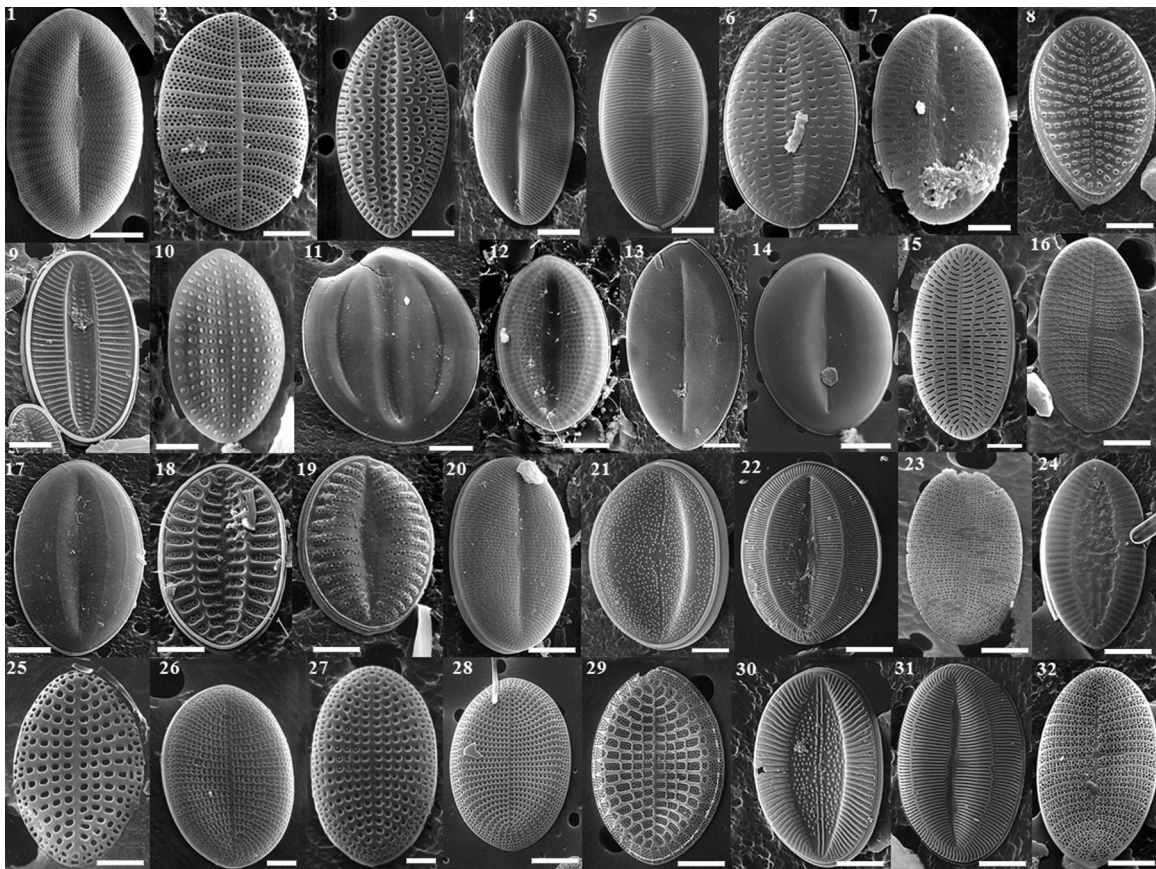


Fig. 2. *Cocconeis* taxa identified in the Mediterranean Sea: (2-1) *C. britannica* Naegeli ex Kützing, (2-2) *C. costata* Gregory, (2-3) *Amphicocconeis debesi* (Hustedt) De Stefano, (2-4) *C. dirupta* var. *flexella* Janisch & Rabenhorst, (2-5) *C. diruptoides* Hustedt, (2-6) *Amphicocconeis disculoides* (Hustedt) De Stefano, (2-7) *C. disculus* Cleve, (2-8) *C. distans* Gregory, (2-9) *C. fluminensis* (Grunow) Per. & Per., (2-10) *C. guttata* Hustedt & Aleem, (2-11) *C. heteroidea* Hantzsch (in Rabenh.), (2-12) *C. maxima* (Grunow) Per. & Per., (2-13) *C. molesta* Kützing, (2-14) *C. multiperforata* De Stefano et al., (2-15) *C. neothumensis* var. *marina* De Stefano et al., (2-16) *C. notata* Petit, (2-17) *C. pellucida* Hantzsch, Grunow in Rabenhorst, (2-18) *C. peltoides* Hustedt, (2-19) *C. pinnata* Gregory ex Greville, (2-20) *C. placentula* var. *placentula* Ehrenberg, (2-21) *C. pseudograta* Hustedt, (2-22) *C. pseudomarginata* Gregory, (2-23) *C. pseudonotata* De Stefano & Marino, (2-24) *C. quarnerensis* Schmidt, (2-25) *C. scutellum* var. *parva* (Grunow) in Van Heurck, (2-26) *C. scutellum* var. *posidoniae* De Stefano et al., (2-27) *C. scutellum* var. *scutellum* Ehrenberg, (2-28) *C. scutellum* var. *baldjikianae* De Stefano et al., (2-29) *C. scutellum* var. *scutellum* f. *decussata* De Stefano et al., (2-30) *C. sovereignii* Hustedt, (2-31) *C. subtilissima* Meister, (2-32) *C. stauroneiformis* (Rabenhorst) Okuno. Scale bars: 2-20, 2-28: 20 μm ; 2-1, 2-3, 2-4, 2-5, 2-6, 2-9, 2-11, 2-13, 2-14, 2-17, 2-20, 2-21, 2-22, 2-24, 2-26, 2-27, 2-29, 2-30, 2-31, 2-32: 10 μm ; 2-2, 2-7, 2-8, 2-10, 2-15, 2-16, 2-18, 2-19, 2-23, 2-25: 5 μm .

the higher the λ value, the greater the dominance of one or few species, and the less diverse the community. The Shannon–Weaver index of diversity (Weaver & Shannon, 1949) was calculated as $H = -\sum_i P_i \ln(P_i)$. Here, $H=0$ when the community is represented by only one species and $H=H_{\max}$ for those communities in which all the species are equally abundant. The Pielou's index of evenness (Pielou, 1969) was calculated as follows: $J = H/H_{\max}$, where H is the observed diversity value and H_{\max} represents the theoretical maximum diversity (H) value possible (i.e. if all species in the sample were equally abundant).

In order to evaluate the degree of similarity between *Cocconeis* assemblages colonizing *Posidonia oceanica* sampled at different sites, the Bray–Curtis coefficient of similarity has been used. Similarities were estimated between every pair of examined communities resulting in a matrix of root-transformed abundance data (Clarke and Warwick, 2001) and visualized by hierarchical agglomerative clustering and non-metric multidimensional scaling (nMDS).

3. Results

3.1. Epiphytic diatom communities associated with *P. oceanica* in the Mediterranean Sea

The generic composition of diatom communities from the six selected Mediterranean localities is shown in Supplementary

material 1. The number of genera ranged from 37 to 44, with the minimal values for Western stations and maximal for the Eastern ones. Most of the genera (about 72%) were found at all sites, however only seven of the noted genera (*Amphora*, *Cocconeis*, *Diploneis*, *Lichmophora*, *Mastogloia*, *Navicula*, *Nitzschia*) were represented by more than 8 species, with others often being significantly less species rich. A “stable” and a “specific” component of the epiphytic diatom communities investigated was identified. The stable component, i.e. diatom genera typically found as epiphytes on seagrasses, was identified based on related literature (e.g. Janisch and Rabenhorst, 1863; Humm, 1964; Harlin, 1975, 1980; Giraud, 1977; Sand-Jensen, 1977; Sullivan, 1977, 1979; Harrison and Chan, 1980; Jacobs and Noten, 1980; Mazzella, 1983, 1999; Hudon and Legendre, 1987; Borowitzka et al., 1990, 2006; Buia et al., 1992). The specific component was composed by genera found in this study but generally rarely present in the communities epiphytic on seagrasses. The stable component comprised mainly the adnate forms of *Amphora*, *Cocconeis*, and *Mastogloia* together with the sessile erect *Gomphonemopsis*, *Grammatophora*, *Lichmophora*, *Synedra* and the epipellic or epipsammic motile genera *Diploneis*, *Navicula*, *Nitzschia*, *Surirella*. Among them, individuals of *Cocconeis* genus dominated in terms of relative abundance, followed by specimens assigned to *Diploneis*, *Mastogloia*, *Lichmophora*, *Grammatophora* and *Gomphonemopsis* genera. The former three genera also dominated in terms of species richness, while

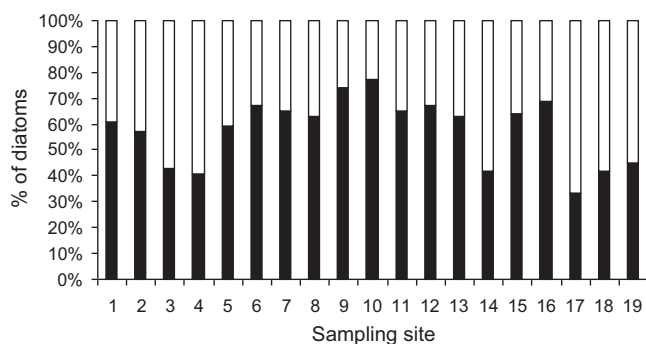


Fig. 3. *Cocconeis* relative abundance in whole diatom community at nineteen sampling sites. Black bars: *Cocconeis* spp.; white bars: other diatom genera.

Grammatophora, *Gomphonemopsis*, and *Synedra* were represented by fewer species (see Supplementary material 1).

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.aquabot.2013.07.008>.

3.2. Diversity of the genus *Cocconeis* in the epiphytic communities

Thirty-two *Cocconeis* taxa were identified within the nineteen Mediterranean sites investigated (Fig. 2). The relative contribution of *Cocconeis* spp. cells to the total diatom abundance in each epiphytic community investigated is reported in Fig. 3: in thirteen out of nineteen localities sampled, the genus *Cocconeis* accounted for more than 50% of the whole diatom community. Among *Cocconeis* taxa, *C. multiperforata*, *C. pseudonotata*, *C. neothomensis* var. *marina*, *C. scutellum* var. *posidoniae*, *C. scutellum* var. *baldjikiana*, *C. scutellum* var. *posidoniae* f. *decussata*, *Amphicocconeis disculoides* and *A. debesi* are the newly described species which have only recently been reported for the Mediterranean region (De Stefano et al., 2000, 2006, 2008; De Stefano and Marino, 2003; De Stefano and Romero, 2005).

3.3. Composition of *Cocconeis* assemblages

Among the thirty-two *Cocconeis* taxa identified, *C. molesta*, *C. neothomensis* var. *marina*, *C. scutellum* var. *posidoniae*, *C. scutellum* var. *scutellum*, *C. stauroneiformis* were ubiquitous, being found in all the locations surveyed (Figs. 2-13, 2-15, 2-26, 2-27, 2-32; Fig. 4), whereas some species were limited to the Western Mediterranean basin (e.g. *C. costata*, *A. disculoides*, *C. disculus*; Figs. 2-2, 2-6, 2-7, respectively; Fig. 4), the Adriatic Sea (e.g. *C. scutellum* var. *scutellum* f. *decussata*; Fig. 2-29), or the Eastern Mediterranean basin (e.g. *C. heteroidea*, *C. notata*, *C. pellucida*, *C. pseudograta*, *C. subtilissima*; Figs. 2-11, 2-16, 2-17, 2-21, 2-31, respectively; Fig. 4). Three species seemed to have a bipolar distribution (*A. debesi*, *C. fluminensis*, *C. quarnerensis*; Figs 2-3, 2-9, 2-24; Fig. 4) rather than being distributed evenly throughout the Mediterranean Sea, while the others showed a more heterogeneous pattern (Fig. 4). Within the five ubiquitous *Cocconeis* taxa, only *C. neothomensis* var. *marina* and *C. scutellum* var. *posidoniae* (Figs. 2-15 and 2-26, respectively; Fig. 4) were clearly dominant in terms of relative abundance, accounting at the various sampling sites for 8–57% and 13–86% of the whole *Cocconeis* community respectively. The other ubiquitous taxa, *C. scutellum* var. *scutellum*, *C. molesta*, and *C. stauroneiformis* (Figs. 2-13, 2-27, and 2-32; Fig. 4), accounted for 1–18%, 0.2–10%, and 0.4–35% of the community. *Cocconeis britannica* (Fig. 2-1; Fig. 4), though absent in the northern localities of the western basin (stations No. 3 and 4, namely Medas Island and Gulf of Genoa, respectively), can be considered well represented in

Table 3

Number of *Cocconeis* species and varieties (s) and values of Simpson (λ), Shannon-Weaver (H), and Pielou (J) indices calculated for each sample.

Sampling station	Location	s	λ	H	J
1	Alicante (Spain)	10	0.32	1.91	0.57
2	Majorca Island (Spain)	17	0.49	1.66	0.41
3	Medas Islands (Spain)	10	0.40	1.93	0.58
4	Gulf of Genoa (Italy)	9	0.51	1.59	0.50
5	Western Sardinia (Italy)	12	0.29	2.08	0.58
6	Eastern Sardinia (Italy)	13	0.54	1.53	0.41
7	Ventotene Island (Italy)	19	0.53	1.67	0.39
8	Ischia Island (Italy)	19	0.50	1.85	0.43
9	Western Calabria (Italy)	8	0.56	1.39	0.46
10	Eastern Sicily (Italy)	9	0.75	0.84	0.27
11	Malta Island (Malta)	9	0.41	1.73	0.55
12	Istria (Slovenia)	16	0.54	1.63	0.41
13	Dalmatia (Croatia)	15	0.27	2.40	0.61
14	Eastern Salento (Italy)	15	0.21	2.71	0.69
15	Western Salento (Italy)	8	0.54	1.44	0.48
16	Gulf of Saronico (Greece)	12	0.30	2.08	0.58
17	Gulf of Edremit (Turkey)	19	0.41	2.10	0.50
18	Gulf of Kusadasi (Turkey)	16	0.30	2.50	0.63
19	Patmos Island (Greece)	16	0.34	2.20	0.55

the other diatom communities investigated with values of relative abundance ranging from 0.7 to 4.4%. All of the remaining twenty-five *Cocconeis* species did not exceed 2% of the *Cocconeis* community (Fig. 4).

3.4. *Cocconeis* distribution pattern

Cocconeis assemblages found in the central Tyrrhenian Sea and Turkish shores displayed the highest specific richness, while the Adriatic stations showed intermediate values (Table 3). Due to the presence of few dominant taxa, the species evenness (Pielou index, J) was relatively low, ranging from 0.4 to 0.6 for the majority of the examined stations. Higher J values, associated with lower Simpson index (λ) values were obtained for the stations of the eastern Mediterranean basin (stations No. 16, 17, 18, 19) and the Adriatic Sea (stations No. 13, 14). The reverse was true for the stations of the western Mediterranean basin, with the only exception being western Sardinia (station No. 5). The comparative analysis of the indices suggested a higher *Cocconeis* taxa diversity in the eastern Mediterranean regions and a lower diversity in the western ones (Table 3).

A dendrogram for hierarchical agglomerative clustering of species abundance data collected at 19 Mediterranean locations was generated. Five clusters were defined based on the various relative contribution of *Cocconeis* taxa to overall similarity within the communities (Fig. 5). The most distinct cluster was composed of communities from the Aegean Sea (stations No. 17, 18, 19 indicated as “AE”), while the closest (the most similar to the latter) group included communities sampled in the Adriatic region (stations No. 12, 13, 14; “A”). Communities from the north-western Mediterranean region (“NW”; stations No. 3 and 4) were quite distinct from those from the central Mediterranean-Tyrrhenian (“CM-T”; stations No. 5, 6, 7, 8) and the southern Tyrrhenian-Ionian sites (“ST-I”; stations No. 9, 10, 11, 15). The latter group was clustered relatively close to the group including communities detected at stations No. 1, 2 and 16, which could not be univocally associated with a given basin. To better illustrate the obtained results, a nMDS analysis was applied to the same initial matrix (Fig. 6). As a 2-dimensional nMDS plot revealed, the station in the Gulf of Saronico (sampling station No. 16) holds an intermediate position between clusters A and AE, while Alicante and Majorca Island stations (sampling stations No. 1 and 2) are located in the neighbourhood of CM-T and ST-I groups, respectively.

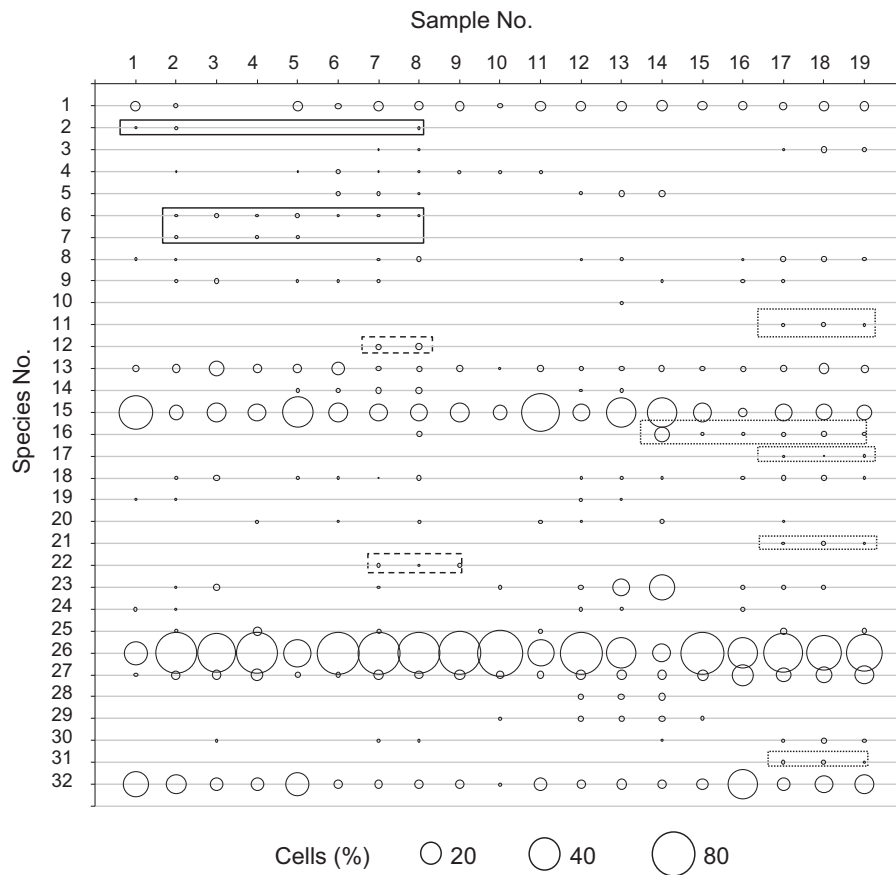


Fig. 4. *Cocconeis* species relative abundance at nineteen sampling sites. Solid-line boxes: species exclusive to the Western Mediterranean (species No. 2, 6, 7); interrupted-line boxes: species exclusive to the Middle Mediterranean (species No. 12, 22); dotted-line boxes: species exclusive to the Eastern Mediterranean (species No. 11, 16, 17, 21, 31).

4. Discussion

Copious and diverse communities of epiphytic diatoms are associated with *Posidonia oceanica* meadows in the Mediterranean Sea. The structure of these communities, when taking into account the sole generic composition, does not vary extremely among the different basins. Our results supplement the description of diatom community structure provided by authors (Mazzella, 1983; Mazzella and Spinoccia, 1992; Mazzella et al., 1994) investigating *Posidonia oceanica* leaves, which shows a pattern of colonization subjected to the motility, life strategies, morpho-functional features and adhesion systems of the epiphytic diatoms (Edgar and Pickett-Heaps, 1983, 1984; Hudon and Legendre, 1987). Eight diatom genera (*Amphora*, *Cocconeis*, *Gomphonemopsis*, *Grammatophora*, *Licmophora*, *Mastogloia*, *Nitzschia*, *Synedra*) dominated the epiphytic communities associated with Mediterranean *P. oceanica*. Among them, individuals of *Cocconeis* genus were still the most numerous, dominating in over 60% of the communities analyzed, with *C. neothumensis* var. *marina* and *C. scutellum* var. *posidoniae* accounting for more than 70% of the whole *Cocconeis* assemblage.

Various studies suggested that the growth form as well as taxonomic structure of epiphytic diatom communities is strongly related to the morphology and micro-topography of the surface of host plant (Majewska et al., 2012, 2013 and references therein). In studied case, smooth and highly homogenous surfaces of *P. oceanica* leaves favoured even development of tightly attached forms such as *Cocconeis*.

4.1. Distribution patterns of Mediterranean *Cocconeis* species

In the course of this study, Indo-Pacific *Cocconeis* species have been found for the first time in the eastern Mediterranean communities, suggesting that allochthonous benthic microalgae could disperse through the nearby Suez Channel to the Mediterranean Sea. The scarcity of available data on the geographic distribution of many species and varieties of *Cocconeis* allows only in a few cases to clearly discriminate autochthonous Mediterranean taxa from those which have perhaps immigrated from other basins. The presence of *C. heteroidea*, *C. pseudograta*, *C. pellucida* and *C. subtilissima*, generally reported in the Indian and Pacific Oceans (Cleve, 1895; Giffen, 1980; Foged, 1987; Suzuki et al., 2000; De Stefano and Romero, 2005), in the eastern Mediterranean region, could be attributed to Lessepsian migration, while the occurrence of the Atlantic species *C. costata* (Romero, 1996; Romero and Rivera, 1996) in the western Mediterranean zone may be due to migrations through the Strait of Gibraltar.

The structure of *Cocconeis* assemblages changed across the various geographic regions both in terms of the species' presence-absence and their relative abundance. The emerging biogeographic pattern inferred from the occurrence of distinct differences between *Cocconeis* communities inhabiting different Mediterranean basins (Eastern Aegean, Adriatic, southern Tyrrhenian-Ionian, central Mediterranean-Tyrrhenian and north-western Mediterranean) is all the more remarkable when considering the continuity between these zones. For instance, *Cocconeis* communities living in the Eastern Aegean and Adriatic

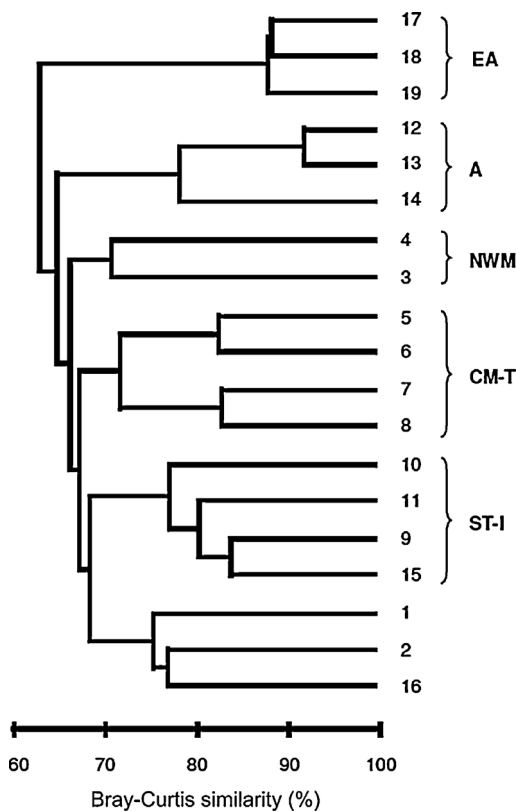


Fig. 5. Similarity dendrogram established for *Cocconeis* spp. communities based on species richness, Simpson, Shannon-Weaver and Pielou indices. Numbers refer to sampled locations (see Fig. 1 and Table 1). Clusters: (EA) Eastern Aegean communities (stations 17, 18, 19 – intra-group similarity = 87.29%); (A) adriatic communities (stations 8, 13, 15 – intra-group similarity = 82.02%); (ST-I) Southern Tyrrhenian-Ionian communities (stations 10, 11, 12, 14 – intra-group similarity = 78.0%); (CM-T) Central Mediterranean-Tyrrhenian communities (stations 4, 6, 7, 9 – intra-group similarity = 74.7%); (NWM) Northern Western Mediterranean communities (stations 3, 5 – intra-group similarity = 70.0%); communities from stations 1, 2, 16 (similarity = 75.2%).

regions showed the highest diversity and were significantly different from those found in other Mediterranean zones.

Marine epibiontic organisms are widely reported as generalists, since they usually cover the surfaces of both living and non-living

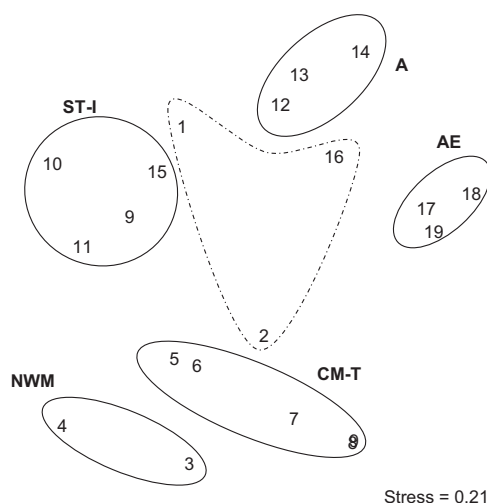


Fig. 6. Non-metric multidimensional scaling plot of *Cocconeis* communities sampled at different sites. EA – Eastern Aegean region; A – Adriatic region; ST-I – Southern Tyrrhenian-Ionian region; CM-T – Central Mediterranean-Tyrrhenian region; NWM – North-Western Mediterranean region.

substrates of various origins present in the same system (Wahl and Mark, 1997). Generalist species with their enhanced colonization and adaptation opportunities are definitely favoured in spreading across the seas and could be ultimately prone to cosmopolitanism. Some of the *Cocconeis* species are most likely cosmopolitan in the Mediterranean Sea (e.g. *C. scutellum* var. *posidoniae*) and there is a reasonable probability they are generalist too. On the other hand, some of the species were found in one basin only (e.g. *C. maxima* in the Southern Tyrrhenian and *C. notata* in the Aegean sea) and, since we have performed a detailed fine-scale analysis, this result should not be considered an artefact of an inappropriate survey method, inadequacy of sample size or species misidentification. Yet, the marine microorganism endemism worldwide is one of the most controversial topics of modern micro-ecology. Since the Mediterranean Sea, in regards to certain parameters, is a relatively homogeneous water body of limited volume, it may be hard to imagine that even the slightest degree of endemism among microalgae may exist there. It has been reported that endemic marine macroalgae represent only a small fraction of the total flora at any site, including vast oceans, inland seas, and diversified coastal areas (Kerswell, 2006). Microbial biodiversity, phylogeny, and evolution are the subjects of ongoing debate (Vincent, 2000). A current general lack of data on rates of dispersal and speciation, as well as constraints on speciation for marine microalgae makes this issue probably unresolvable at this stage of global investigation. New observations and thorough analysis however, may shine some light on the problem. Interestingly, the biogeographic pattern of the investigated *Cocconeis* communities showed an evident east-west cleavage, which overlaps a pattern of the peculiar genetic differentiation of Mediterranean populations of *P. oceanica* described by Arnaud-Haond et al. (2007). This observation may suggest a host-specific interaction between different species of *Cocconeis* and genetically distinct *P. oceanica* populations.

4.2. Is it likely that close association with various *Posidonia oceanica* populations favours local endemism of epiphytic *Cocconeis* forms?

The life history and evolution of *P. oceanica* in the Mediterranean Sea has been recently reconstructed by means of an integrative molecular approach (Arnaud-Haond et al., 2007). The investigation revealed a significant genetic differentiation within an endemic seagrass species and a great divergence between its populations from the eastern and western zones of the Mediterranean Sea. It was stated that *P. oceanica* population dynamics are strongly linked to asexual reproduction (Kubitzki, 1998): the more successful clones undergo massive spreading at a local scale, whereas the intermittent sexual process does not keep pace with population differentiation. Two interplaying factors can potentially modulate the spreading of benthic organisms in the sea: (i) the water circulation and (ii) certain life cycle strategies, which promote dispersal. In this case, potential similar evolutionary patterns and convergent biogeography of endemic *Posidonia* and *Cocconeis* species in the Mediterranean Sea might have been triggered by specific water circulation, which to a great degree prevents western and eastern surface waters from mixing, whereas stable cyclonic eddies (e.g. Lyon and Rhodes gyres) create relatively isolated “islands” (e.g. Aegean Sea, Alboran Sea) by excluding a certain zones from the main circulation (Pinardi and Masetti, 2000). This seems to effectively isolate *Posidonia* populations from the eastern and western Mediterranean basins and fix a probable critical genetic boundary in the Siculo-Tunisian Strait (Arnaud-Haond et al., 2007). Hypothetically, isolation of macroscopic host plants might have effected in isolating associated microscopic epibiontic populations (Caron, 2009). Assuming a similar level of dispersal for organisms of such different dimensions may seem objectionable, yet it must be taken

into account that neither *Posidonia* nor *Cocconeis* has life cycle strategies, which could effectively counteract dispersal barriers and potential genetic isolation in the Mediterranean Sea.

The planktonic sexual stages (fruits and seeds) of *Posidonia* can float up to several days only, and their dispersal range rarely exceeds a few tens of kilometres (Thiel and Gutow, 2005 and references therein). This limits sexual recombination and enhances the genetic separation between clonal meadows. Diatoms, in turn, have a diplontic life cycle with a long-lasting clonal growth and an ephemeral sexual phase occurring once every 2 to ca. 40 years (Mann, 1988; D'Alelio et al., 2011). The infrequency of sexual events seems to positively influence reproductive isolation between epiphytic *Cocconeis* assemblages and, consequently, their speciation (e.g. Amato et al., 2007; D'Alelio et al., 2009). Moreover, benthic diatoms do not have a planktonic stage, spending their entire life cycle associated with solid substrate (Round et al., 1990). Released *Posidonia* fruits and seeds can carry microorganisms, however only for a limited time and over short distances, and their tracks depend substantially on water circulation. Similarly, seagrass leaves falling off the rhizomes at the end of the growth season and floating on the sea surface can carry the whole benthic diatom communities, though their buoyancy and longevity are highly limited and a considerable part of this material is accumulated on the beaches (Thiel and Gutow, 2005 and references therein).

We must recognize that there are many additional aspects that are beyond the present study. It is still unclear whether the genetic differences observed in some *P. oceanica* populations are manifested as phenotypic differences as well. Various biogeochemical factors affect diatom communities in different Mediterranean basins. It is known that the salinity and temperature gradients exist in the Mediterranean Sea. They may be, however, not as important as other factors. The Mediterranean Sea is a latitudinal basin and the range of its mean annual surface temperature is relatively small ($\sim 4^\circ$). Excluding the most western part of Alboran Sea (where we did not sample) the salinity ranges from 37 to 40 PSU (Zavatarelli and Mellor, 1995). Moreover, coastal zones are especially strongly exposed to sudden short-term changes in temperature or salinity and it should not be assumed that found diatom species were particularly sensitive to changes of these two factors. Studies of other authors (especially those of Mazzella or Sullivan) working on *Posidonia* and its epiphytes report that diatom microflora presents well-defined morphological features and its species composition is very similar on all seagrass species, regardless of geographical zone. This may indicate that the substrate is the primary factor in determining the composition of associated diatom flora.

The potential influence of grazers (both grazers of algae growing on blades and grazers of *P. oceanica* itself) on the diatom communities established on *P. oceanica* is another open question. Several publications dealing with *P. oceanica* and its epiphytic flora grazers describe certain elements of herbivorous fauna which feed on *Posidonia* and/or associated microalgae, but in most cases their feeding behaviours and preferences are largely unknown (e.g. Pergent et al., 1994; Alcoverro et al., 1997; Tomas et al., 2005). Some authors indicate the existence of depth-related faunistic zones (Gambi et al., 1992). However, this cannot be an issue in presented studies, as the samples were collected from the very similar depths. It has been proved that some selective diatom grazers feed preferentially on erect and chain-like, colonial forms, which are often easier to grab and detach than e.g. adnate forms (like *Cocconeis*, e.g. Hudon, 1983). Moreover, bigger grazers may prefer bigger diatom cells often belonged to the loosely associated fraction (Peterson, 1987). While this might indeed somewhat influence the ratio between certain diatom growth forms living on the substrate surface, it is highly unlikely (impossible?) that some specific grazer selected actively

some certain *Cocconeis*, influencing the ratio between different *Cocconeis* species found in the samples.

Considering all of the above, it should be assumed that the possibility exists that endemic microorganisms may use specific niches provided by endemic host macroorganisms. A complex study must be carried out, however, to evaluate whether the *Cocconeis* communities have co-evolved along with their hosts.

With this study we wish to contribute to the knowledge on diatom flora in the Mediterranean Sea. The two most dominant *Cocconeis* species observed were recently described by one of the authors. The most abundant species appeared to be broadly distributed, while a small number of less abundant species had a more specific biogeographic pattern. Moreover, the study revealed that the pattern in spatial variance of some *Cocconeis* species is consistent with distribution of genetically distinct *P. oceanica* populations. These findings may be a valuable indication for the future investigations on relations between epiphytes and their host plants. The present survey allowed us to identify rare, potentially endemic *Cocconeis* species. It seems clear, however, that at this moment the literature is not even representative of the most common *Cocconeis* species associated with *Posidonia oceanica* and the floras of species in some communities are hardly well known. Further thorough studies must be therefore undertaken to truly distinguish between *Cocconeis* biogeographies and ecohabitats.

Acknowledgments

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