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## PARTICULAR STRUCTURE OF AN EPIPHYTIC DIATOM ASSEMBLAGE LIVING ON *Ploclamium cartilagineum* (LAMOROUX) DIXON (RHODOPHYCEAE: GIGARTINALES)

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**ABSTRACT**. This is the first record of an epiphytic diatom assemblage of *Ploclamium cartilagineum*. Fifteen years ago observations on a specimen of *P. cartilagineum* revealed that large diatom of certain taxa occurred abundantly on its branches, and suggested also that it may harbor a particular diatom assemblage. Thus, we set out objective into identifying all the diatom taxa in the assemblage represented on the same *P. cartilagineum* specimen, and to determine their proportional abundances. Overall 46 taxa of epiphytic diatoms were identified. The diatom assemblage was characterized by abundant large forms such as *Gephyria media*, and *Hyalodiscus punctatus* a new record for the region, and the small form *Cocconeis californica*. Also include is, *Grammatophora macilenta* a new record for the region. Hitherto rare taxa in the region were very common such as *Rhabdonema adriaticum*, *Campylopyxys garkeana*, and *Melosira polaris/Melosira sol*. Although the estimated species richness and diversity values are high as in other assemblages of benthic diatoms, its basic structure (floristics) in which large forms rival the smaller forms in number, makes this assemblage particular.

Keywords: Large diatoms, rhodophyte, Baja California peninsula, new records.

# Estructura particular de una asociación de diatomeas epifitas sobre *Ploclamium cartilagineum* (Lamoroux) Dixon (Rhodophyceae: Gigartinales)

**RESUMEN**. Se presenta el primer registro de diatomeas epifitas de *Ploclamium cartilagineum*. Hace quince años se observó que sobre las ramas de un espécimen de *P. cartilagineum* habitaban abundantes taxa de diatomeas de gran tamaño, lo que sugirió que hospedaba una asociación particular de diatomeas. Bajo el objetivo de identificar todos los taxa representados de diatomeas en el mismo espécimen y determinar sus abundancias proporcionales en la asociación, se identificaron 46 taxa, de las cuales dos formas grandes resultaron abundancies: *Gephyria media*, y *Hyalodiscus punctatus* un nuevo registro para la región; así como *Cocconeis californica*, una forma pequeña. Asimismo, se agregó un nuevo registro para la región con *Grammatophora macilenta*. Varios taxa considerados raros hasta antes de este estudio fueron muy comunes, como *Rhabdonema adriaticum*, *Campylopyxys garkeana*, y *Melosira polaris/Melosira sol*, entre otros. Aunque la riqueza y diversidad de especies fueron elevadas (típicas) como en otros hábitats bentónicos, su estructura básica (florística) en donde formas grandes son tan abundantes como las pequeñas define una asociación particular.

Palabras clave: Formas grandes, rodofita, península de Baja California, nuevos registros.

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## INTRODUCTION

Because of the abundance and diversity of the microalgae that they harbor macroalgae are considered a favorable substrate for diatoms. This phenomenon has been documented regionally for the southern Baja California peninsula where more than two hundred epiphytic diatom taxa have been recorded for the Gulf of California (Siqueiros Beltrones & Hernández-Almeida, 2006) and likewise for the west (Pacific) coast (Siqueiros Beltrones *et al.*, 2002; Siqueiros Beltrones & López-Fuerte, 2006). These studies include observations on macroalgae hosts of different species.

More recent research revealed that epiphytic diatom assemblages vary significantly in correspondence with the macroalgae genus on which they thrive, although differences at division level (rhodophyte, chlorophyte, ochrophyte) are also evident. And, on the contrary an abundant diatom may account for a significant similarity between hosts of different genera of the same taxonomic division (Hernández-Almeida & Siqueiros Beltrones, 2012). This suggests that epiphytic diatom assemblages may be characteristic of their particular macroalgal host in terms of floristics and structure. For example, on blades of *Macrocystis pyrifera* L. (C. Ag.) the species richness of diatoms surpasses 170 taxa and, besides a high diversity of species, various associations between epiphytic diatoms have been observed to occur (Siqueiros Beltrones *et al.*, 2002).

Incidental observations nearly fifteen years ago on a specimen of *Ploclamium car-tilagineum* (Lamoroux) Dixon revealed that diatoms occurred abundantly on its branches.

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This rhodophyte has a wide distribution and it is considered among the most frequent macroalgae along the west coast of Baja California Sur (BCS) associated to the regional abalone banks (Serviere-Zaragoza *et al.*, 2003). Readily identifiable diatoms included large conspicuous forms such as *Gephyria media* Arnott and *Rhabdonema adriaticum* Kützing, and a centric form tentatively identified as *Porosira* sp. that until then had been recorded rarely within gut contents of abalone (*Haliotis* spp.) and chitons (*Stenoplax* spp.) (Siqueiros Beltrones *et al.*, 2004).

The primary observations showed that *P. catilagineum* was a very favorable substrate for certain diatom taxa, and suggested that these taxa could be sole colonizers and/or the dominant epiphytes on *P. cartilagineum*. Thus, our objective was to make a precise identification of the epiphytic diatom taxa on the collected *P. cartilagineum* specimen and to determine their proportional abundances, providing an approximate view of its structure which can be later compared to other *P. cartilagineum* and different rhodophyte taxa.

#### METHOD

In order to describe the epiphytic diatom assemblage harbored by *P. cartilagineum*, we examined the same specimen (preserved dry) found stranded at Bahía Magdalena beach in 1999, a bushy fertile specimen heavily epiphytized by diatoms. We first made microscope observations of sub-samples of rehydrated branches mounted on semi-permanent slides sealed with nail polish. Likewise, we observed sub-samples of abundant fine (dust) material detached from the dry specimen mounted on semi-permanent slides.

To taxonomically identify the diatoms, six sub-samples were cleaned by oxidation with nitric acid and alcohol (Siqueiros Beltrones, 2002) including three of fine material and three consisting of coarsely diced branches. Then, two double permanent slides of each one were mounted using synthetic resin (Pleurax). The slides were examined under the microscope with phase contrast and planapo-chromatic optics at different magnifications. All identifications were made at 1000× following the regional references by Sigueiros Beltrones (2002), Sigueiros Beltrones et al. (2004), and Sigueiros Beltrones and Argumedo-Hernández (2005), as well as classic literature: Hustedt (1959; 1961-66), Peragallo & Peragallo (1908), Round et al. (1990), Schmidt et al. (1874-1959), and Witkowski et al. (2000). A micro-photographic record of the diatom taxa was made.

At least 500 valves were counted in each of the six slides (N>3000) at 1000×. Based on their relative abundances (RA) taxa were classified as: abundant (>400 valves); very common (100-399); common (20-99); uncommon (4-19); rare (1-3). With the RA, ecological indices were computed to further describe the structure of the diatom assemblage on the basis of species diversity (Shannon's H'), equitability (Pielou's J'), both with log<sub>2</sub>, and dominance (Simpson's  $\lambda$ ) (Brower *et al.*, 1998). All computations were done using program Primer 6 v 6.1.6.

## **RESULTS AND DISCUSSION**

After fifteen year in storage the specimen of P. cartilagineum remained in good condition. It still showed heavy epiphytism in spite of having a great amount of diatoms detached after years of drying. Slides of re-hydrated *P. cartilagineum* branches showed heavy epiphytism by large diatom forms (>100µm), whilst the slides of dehydrated branches permitted the observation of numerous small diatoms that were otherwise invisible in wet preparations (Figs. 1-6). Most were adnate (cocconeiform) forms but also stalked small colonies of naviculoids were common; all formed patches frequently within the angles of the branches. Although all diatoms were identified taxonomically from the permanent slides, unlike with the larger forms whose abundances were evident in the permanent preparations, in these the smaller forms were underestimated with was observed in the fresh mountings. In few occasions, clumps of small mixed taxa remained in the permanent slides, attached to undigested host tissue.

Conspicuous forms. The diatom assemblage was dominated by three abundant taxa. The most numerous, Gephyria media, a large araphid form measuring between 65-250µm long accounted for >26% of the valves. It adheres to the thallus of *P. cartilagineum* by a mucilaginous pad and goes to form chains of around six individuals. Smaller forms of G. media may also be epiphytic on larger ones (Figs. 4, 6, 8). We had first recorded G. media from Isla Magdalena off the coast of Baja California Sur (México) on rocky substrate within an abalone habitat and from Haliotis spp. and Chiton spp. gut contents, both times as rare (Sigueiros Beltrones, 2000; Siqueiros Beltrones, & Valenzuela-Romero. 2004). However, Tiffany (2002) studied a dense population of live G. media growing on a specimen of the red algae Pterosiphonia sp. from California.

Equally conspicuous although less abundant as the former taxon was *Hyalodiscus punctatus* A. Schmidt (18%), also a large spe-



**Figures 1-6**.- Aggregations of epiphytic diatoms on host (fresh mountings). At 1000× unless noted. 1) *Cocconeis californica*; 2) *Halamphora acutiuscula*; 3) Tubular colony of *Navicula rusticensis*; 4) Chain of *Gephyria media*, 400×; 5) *Navicula* cf. *incerta*; 6) Small *G. media* epiphytic on large cell 400×. Bar = 10 μm (figs. 1-3, 5); 25 μm (figs. 4, 6).

cies reaching between 85 - 210 µm in diameter (Figs. 15, 29). This taxon had been misidentified and recorded as Porosira sp., rare from both abalone and chiton gut contents, and as abundant in this same *P. cartilagineum* speci-men (Siqueiros Beltrones *et al.*, 2004). However, our specimens do not show a conspicuous rimoportula (labial process) like Porosira but many scattered small rimoportula throughout the valve and aligned around the valve margin. It does however show the distinctive hyaline central flat area (Figs. 15, 29) characteristic of Hvalodiscus. The frustules of H. punctatus adhere to the host by thick mucilage pads that are apparently secreted through rimoportula accumulated on a specific part of the edge of the valve (Figs. 16, 18), unlike the other Hyalodiscus described by Round et al. (1999) where mucilage secretion is through the central area; also in *H. punctatus* cells do not form chains but are solitary; paired specimens were observed only once (Siqueiros Beltrones et al., 2004).

The third abundant taxon (>12%) was *Cocconeis californica* (Grunow) Cleve, a small form hitherto recorded as rare from sediments in San Francisco, California (Laws, 1988) and as an epiphyte of *Macrocystis pyrifera* C. Agardh (Siqueiros Beltrones & Argumedo-Hernández, 2005). It was observed copiously in fresh preparations distributed extensively on the host and, although in the permanent slides it appeared only as a common taxon when loose, it occurred abundantly still attached to the host undigested tissues (Figs. 1, 58, 62).

Two other small forms, *Navicula rusticensis* Lobban and *Navicula* cf. *incerta* Grunow (Figs. 3, 5, 82, 83) were very common, occurring in multispecies clumps but seldom as loose specimens. *N. rusticensis* seems to form tubular colonies where other diatom taxa dwell, *v. gr. Amphora exilitata* Giffen (Figs. 84, 85); parts of the tubes remain adhered to undigested host tissue after oxidation (Fig. 3). *N. incerta* has proven to be readily cultivated as food-source for rearing abalone young (Correa Reyes *et al.*, 2001).

Another conspicuous taxon is *Rhabdonema adriaticum* (Figs. 9, 11, 12), a large form measuring between 45-145 µm long that has been recorded as rare from gut contents of abalone (Siqueiros Beltrones *et al.*, 2004). It occurred commonly together with *Climacosphenia moniligera* Ehrenberg (Fig. 7), a species that reaches over 800 µm, and *Grammatophora macilenta* W. Smith, also a large form, represents another new record for the region (Figs. 36-40). This taxon was uncommon and only valves were observed. It probably had been misidentified earlier as *Grammatophora oceanica* Ehrenberg (Figs. 41-44). However, our specimens of *G. macilenta* are much larger: 80-140  $\mu$ m long, and its striae are finer: 25-31 striae/10  $\mu$ m; while the latter reaches only 50  $\mu$ m in length, with 20-24 striae/10  $\mu$ m. All three taxa stand out due to their size and, along with the abundant large taxa, contribute to the impressive degree of epiphytism shown by *P. cartilagineum*.

Another taxon recorded earlier as rare or uncommon in the region but that was now common on P. cartilagineum was Campylopyxis garkeana (Grunow) Medlin (Figs. 69-74), a stalked form that was readily visible in the fresh mountings but also found loose in the permanent slides. Likewise, Melosira polaris Grunow occurred commonly. However, the identification of this taxon rises an issue, inasmuch it seems to be confused with Melosira sol (Ehrenberg) Kützing. Although the images and different sizes (46 µm vs. 56 µm, respectively) suggest certain taxonomic difference, these may be explained by normal growth variation of the chain colonies (Figs. 30-35). Thus, it is required to determine if one or two taxa should be reported and whether or not both names are legitimate. Both taxonomic names are here retained until further research is done. Either way, as with the other conspicuous diatoms, these seem to find a favorable substrate on *P. cartilagineum*.

Assemblage structure. Overall 46 taxa of epiphytic diatoms were identified on the examined specimen of *P. cartilagineum*, albeit twelve taxa did not show in the quantitative analysis (Table 1). The epiphytic diatom assemblage on *P. cartilagineum* showed a typical benthic diatom structure with few abundant taxa and many rare and uncommon ones (Siqueiros Beltrones, 2005). This becomes more precise as we add the 12 rare taxa not included in the quantitative analysis.

The observed species richness as well as the other values depicting species diversity showed high values (Table 2). These fall within the normal spectrum for samples of diatom assemblages with high species diversity (Siqueiros Beltrones, 2005), and below the average values of H' estimated for the red algae *Laurencia pacifica* (H'= 4.14) and *Laurencia johnstonii* (H'= 4.58) for seasonal samplings that yielded 123 and 142 epiphytic diatom taxa, respectively (Siqueiros Beltrones & Hernández-Almeida, 2006). A later study showed that such values could vary according to season and locality (Hernández-Almeida & Siqueiros Beltrones, 2008) but in general were higher than in the present study.

In both of the above studies the most numerous species found on red algae hosts were **Table 1**.- Epiphytic diatom taxa observed on a *Ploclamium cartilagineum* specimen from the southern Baja California peninsula (México). R= rare; UC=uncommon; C=common; VC=very common; A=abundant. N=3633 valves. \*Observed but not quantified. N = new record.

Achnanthes groenlandica (Cleve) Grunow (R). Fig. 79	Gomphonemopsis pseudexigua (Simonsen) Medlin. (C). Fig. 81		
Actynoptychus senarius (Ehrenberg) Ehrenberg (R)* Fig. 19	Grammatophora hamulifera Kützing (C). Figs. 45-46, 48		
Actynoptychus minutus Greville (R)* Fig. 17	Grammatophora macilenta W. Smith (R)* N. Figs. 36-40		
Amphicocconeis disculoides (Hustedt) Stefano & Marino (UC)	Grammatophora oceanica Ehrenberg (UC). Figs. 41-44		
Amphora angusta Gregory (R)*	Grammatophora undulata Ehrenberg (C). Fig. 47		
Amphora exilitata Giffen (C). Figs. 84-85	Halamphora acutiuscula (Kützing) Levkov (UC). (R). Fig. 2		
Amphora proteus var. contigua Cleve (UC). Figs. 86-87	Hyalodiscus punctatus A. Schmidt (A) N. Figs. 15, 16, 18, 29		
Aulacodiscus affinis Grunow (UC) *. Figs. 26-28	Hyalodiscus scoticus (Kützing) Grunow (R). Figs. 20-21		
Campylodiscus crebecostatus var. speciosa Eulenstein $(R)^*.$ Fig. 13	Licmophora communis (Heiberg) Grunow in Van Heurck $(R)^\star$		
Campylopyxis garkeana (Grunow) Medlin (C). Figs. 69-74	Licmosoma squamosum Round & Alexander (UC). Fig. 10		
Climacosphenia moniligera Ehrenberg (C). Fig. 7	Melosira polaris Grunow (C). Figs. 30-31, 34-35		
Cocconeis californica (Grunow) Cleve (A). Figs. 1, 59, 63	Melosira sol (Ehrenberg) Kützing (R)*. Figs. 32-33, 34-35		
Cocconeis costata var. hexagona Grunow in Van Heurck (C). Fig. 56	Navicula cf. incerta Grunow (VC). Fig. 83		
Cocconeis costata var. pacifica Grunow (R)*	Navicula directa (W. Smith) Ralfs (UC). Fig. 68		
Cocconeis dirupta W. Gregory (UC). Figs. 49-52, 64	Navicula rusticensis Lobban (VC). Figs. 3, 82		
Cocconeis dirupta var. flexella (Janisch & Rabenhorst) Grunow (C). Figs. 53, 65	Parlibellus hamulifer (Grunow) De Toni (R)		
Cocconeis neodiminuta Kramer. Figs. 58, 60-62	Podosira stelligera (Bailey) Mann (R). Figs. 24-25		
Cocconeis peltoides Hustedt. Fig. 57	Rhabdonema adriaticum Kützing (VC). Figs. 9, 11-12		
Cocconeis scutellum Ehrenberg (VC). Figs. 66-67	Rhoicosphenia genuflexa (Kützing) Medlin (VC). Figs. 75-78		
Cocconeis sp. (R). Figs. 54-55	Surirella fastuosa (Ehrenberg) Kützing (R)*. Fig. 14		
Coscinodiscus sp. (R)*. Figs. 22-23	Tabularia gaillonii (Bory de Saint-Vincent) Bukhtiyarova (R)		
Gephyria media Arnott (A). Figs. 4, 6, 8	Tabularia investiens (W. Smith) Williams & Round (C)		
Gomphoseptatum aestuarii (Cleve) Medlin (VC). Fig. 80	Trachyneis aspera (Ehrenberg) Cleve (R)*		

typical small forms of Amphora, Mastogloia, Nitzschia, Cocconeis, Navicula and Achnanthes. Thus, the basic structure of our P. cartilagineum diatom assemblage differs from most other assemblages hitherto observed in that the larger conspicuous forms are highly numerous, more even (apparently) than the smaller common forms. Although, it has to be considered that the small forms that occurred in clumps (in spite of the alternate techniques followed) could have been underestimated during quantification, thus affecting the estimated values of diversity. Although the estimated species richness and diversity values are high as in other assemblages of benthic diatoms, its basic structure (floristics) in which large forms rival the smaller forms in number, makes this assemblage particular.

Another remark is that the abundant forms and several of the very common ones had been recorded earlier as rare taxa (Table 1) for the region. Although this supports the hypothesis that epiphytic diatom assemblages are characteristic of their particular macroalgal host (Hernández-Almeida & Siqueiros Beltrones, 2012), the extent of this has to be examined by observing other rhodophytes and other macroalgae from the same area preferably in terms of beta diversity, inasmuch macroalgae species composition varies along the BCS coasts (Serviere-Zaragoza *et al.*, 2003).

Several fleshy rhodophyte species including *P. cartilagineum*, have been recorded as part of the diet of green abalone (*Haliotis fulgens* Philippi 1845) during El Niño conditions off the southern coast of the Baja California



**Figures 7-14**.- At 1000× unless noted. GV = girdle view; all others = valve view. 7) *Climacosphenia moniligera*, 400× (GV); 8) *Gephyria media* (GV); 9) *Rhabdonema adriaticum*; 10) *Licmosoma squamosum*; 11, 12) *R. adriaticum*, 400× (GV) and close up; 13) *Campylodiscus crebecostatus* var. *speciosa*; 14) *Surirella fastuosa*. Bar = 25 µm (figs. 7-8, 11); 10 µm (figs. 9-10, 12-14).



**Figures 15-21**.- At 1000× unless noted. 15) *Hyalodiscus punctatus*, 400×; 16) *H. punctatus* showing cumulus of rimoportula at margin; 17) *Actynoptychus minutus*; 18) Fresh mounting of *H. punctatus* attached to host by mucilage pad 400×; 19) *Actynoptychus senarius*; 20, 21) *Hyalodiscus scoticus*. Bar = 25 µm (figs. 15, 18); 25 µm figs. (16-17, 19-21).



**Figures 22-29**.- At 1000× unless noted. 22) *Coscinodiscus* sp. 400×; 23) *Coscinodiscus* sp. center close up; 24, 25) *Podosira stelligera*; 26) *Aulacodiscus affinis*, 400×; 27, 28) *A. affinis* close up of center and margin; 29) Center close up of *Hyalodiscus punctatus*. Bar = 25 μm figs. 22, 26; 10 μm figs. 23-25, 27-29.



**Figures 30-35**.- At 1000× unless noted. 30, 31) *Melosira polaris*; 32, 33) *Melosira sol*; 34, 35) Different size stacks (GV) of either *M. polaris* or *M. sol*. Bar = 10 μm figs. 30-33; 25 μm figs. 34-35.



Figures 36-48.- At 1000×. 36-40) Grammatophora macilenta; 41-44) Grammatophora oceanica; 45, 46, 48) Grammatophora hamulifera; 47) Grammatophora undulata. Bar =  $10 \ \mu m$ .



**Figures 49-67**.- At 1000×. 49-52, 64) Cocconeis dirupta; 53, 65) C. dirupta var. flexella; 54, 55) Cocconeis sp.; 56) Cocconeis costata var. hexagona; 57) C. peltoides; 58, 60, 61, 62) C. neodiminuta; 59, 63) Cocconeis californica; 66, 67) Cocconeis scutellum. Bar = 10 μm



**Figures 68-87.**- At 1000×. 68) Navicula directa; 69-74) Campylopyxis garkeana; 75-78) Rhoicosphenia genuflexa; 79) Achnanthes groenlandica; 80) Gomphoseptatum aestuarii; 81) Gomphonemopsis pseudexigua; 82) Navicula rusticencis; 83) Navicula cf. incerta; 84, 85) Amphora exilitata; 86, 87) Amphora proteus var. contigua. Bar = 10 μm

 Table 2.- Estimated diversity values for the epiphytic diatom assemblage growing on a Ploclamiun cartilagineum specimen collected in BCS.

Sample	Ν	S	J'	H'	λ	1- К
A (dust)	535	21	0.78	3.44	0.134	0.865
B (diced)	526	25	0.66	3.10	0.199	0.800
C (diced)	586	16	0.63	2.53	0.240	0.759
D (dust)	717	25	0.79	3.69	0.109	0.890
E (diced)	523	22	0.68	3.06	0.173	0.826
F (dust)	748	22	0.71	3.16	0.146	0.853
Integrated	3635	34	0.69	3.52	0.134	0.865

peninsula (Mazariegos-Villarreal et al., 2012). Over 300 taxa are known to be a potential part of the diet of *Haliotis* spp. mostly epiphytes (Siqueiros Beltrones, 2004). This is the most conspicuous event of epiphytism by diatoms ever recorded in the region, and goes to show that herbivores such as abalone and chitons may be substantially enriching their diet when consuming these heavily epiphytized macroalgae. However, it is unknown how these epiphytic diatoms are distributed among the many macroalgae taxa that are grazed by abalone and other mollusks. Considering the numerous macrophyte taxa found along the California and Baja California peninsula coasts, it is evident that studies on epiphytic diatoms from the eastern Pacific are still lacking. Thus, a close relation between these abundant and very common taxa and their fleshy red algae hosts is strongly suggested and requires investigation in order to determine how the assemblage structure of epiphytic diatoms varies from host to host and in time.

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