#### HOST MICROBE INTERACTIONS



# Shared Epizoic Taxa and Differences in Diatom Community Structure Between Green Turtles (*Chelonia mydas*) from Distant Habitats

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Abstract The first reports of diatoms growing on marine mammals date back to the early 1900s. However, only recently has direct evidence been provided for similar associations between diatoms and sea turtles. We present a comparison of diatom communities inhabiting carapaces of green turtles *Chelonia mydas* sampled at two remote sites located within the Indian (Iran) and Atlantic (Costa Rica) Ocean basins. Diatom observations and counts were carried out using scanning electron microscopy. Techniques involving critical point drying enabled observations of diatoms and other microepibionts still attached to sea turtle carapace and revealed specific aspects of the epizoic community structure. Species-poor, well-developed diatom communities were found on all examined sea turtles. Significant differences be-

tween the two host sea turtle populations were observed in terms of diatom abundance and their community structure (including growth form structure). A total of 12 and 22 diatom taxa were found from sea turtles in Iran and Costa Rica, respectively, and eight of these species belonging to *Amphora*, *Chelonicola*, *Cocconeis*, *Navicula*, *Nitzschia* and *Poulinea* genera were observed in samples from both locations. Potential mechanisms of diatom dispersal and the influence of the external environment, sea turtle behaviour, its life stage, and foraging and breeding habitats, as well as epibiotic bacterial flora on epizoic communities, are discussed.

**Keywords** Biofilm · Epizoic diatom · Marine epibiosis · Persian Gulf · Sea turtle · Tortuguero

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#### Introduction

Numerous studies have indicated that the development of epibiotic diatom communities is strongly influenced by the morphology, roughness, micro-topography, chemical composition and physiological state of the living substrate [1–12]. Several diatom taxa, including entire genera, such as Epiphalaina R.W. Holmes, Nagasawa et Takano [13]; Tursiocola R.W. Holmes, Nagasawa et Takano [13]; Bennetella R.W. Holmes [14]; and Plumosigma T. Nemoto [15], have only been observed growing on aquatic animals and are thus presumably exclusively epizoic. It has also been suggested that some epiphytic diatoms may exhibit host specificity towards different aquatic plant or seaweed species [7, 16]. These studies highlight the importance of substrate type for diatom community development, suggesting a specific nature of the relationship between the host organism and its epibionts. However, it has also been shown that the epibiotic diatom community may vary greatly depending on geographical location and external environmental conditions [7, 8, 17, 18].

Diatoms growing on marine mammals have long been documented, with the first reports on these associations dating back to the beginning of the twentieth century [19, 20]. Nevertheless, only very recently have several studies provided direct evidence for similar associations between diatoms and reptiles, and specifically sea turtles [21-25]. The sea turtleassociated diatom communities appear to be composed of few species that sometimes reach very high abundances [24]. Many of the newly observed taxa exhibit unique morphology. One further outcome of recent surveys examining sea turtle microflora is descriptions of new diatom species and genera, such as Chelonicola Majewska, De Stefano & Van de Vijver [23]; *Poulinea* Majewska, De Stefano & Van de Vijver [23]; and Medlinella Frankovich, Ashworth & M.J. Sullivan [21]. Although the diversity of marine epizoic diatoms is still largely unknown, ongoing studies indicate that different diatom communities may exist on both different sea turtle species and different body parts of the same individual (carapace vs. skin, R. Majewska unpubl., T.A. Frankovich, personal communication).

As all sea turtles are highly migratory, it is unclear to what extent the habitats of different species or populations overlap. It is known that different sea turtle species share nesting areas during the breeding season [26]. Much less, however, is known about their pelagic life stages and foraging areas. According to Robinson et al. [26], macroepibiont communities on conspecific sea turtles from different nesting sites located within the East Pacific Ocean were more similar than those observed on different host turtle species from the same nesting beach. The authors concluded that the observed diversity of macroepibionts might be related not only to breeding habitats but also to the host turtle foraging

behaviours and diversity of foraging habitats. The same may be true for turtle microepibionts, such as epibiotic diatoms. To date, however, no data exist to test this hypothesis.

The present study explores and compares the diatom communities growing on green turtles (*Chelonia mydas* Linnaeus 1758) from Hengam Island (Persian Gulf, Iran) and Tortuguero (Atlantic coast of Costa Rica) located within two different ocean basins. Our aim was to assess the abundances, species composition and ecology (including the growth form structure) of green turtle epizoic diatoms and to investigate how the diatom communities differ between the conspecific sea turtle populations nesting at two remote sites. This study is the first to describe the green turtle diatom community.

# **Materials and Methods**

#### **Material Collection and Treatment**

Diatom samples used in this study were collected in October 2014 from green turtles in Tortuguero National Park (10° 32′ 33" N, 83° 29′ 59" W) on the Atlantic coast of Costa Rica and in June 2015 in the vicinity of Hengam Island in the Persian Gulf (Iran; 26° 47′ 32″ N, 55° 47′ 51″ E; Fig. 1). In Tortuguero, approximately 5– 15 cm<sup>2</sup> of randomly chosen carapace sections of several green turtle females were scraped off when the animals came ashore to lay eggs. In total, 190 samples from 95 turtles were collected. Samples of epizoic diatoms were taken by scraping individual turtle carapaces with a razor or a scalpel (Fig. 2). This method is not invasive, as it is limited to the most external part of the turtle carapace scutes, and it does not cause the animal suffering. In Iran, the same method was used to sample 30 green turtle specimens that were captured in shallow coastal waters by SCUBA divers. In total, 60 samples (two from each individual) from posterior and lateral parts of the turtle carapaces were collected. Although in most cases the carapace length was not measured, all turtles sampled in the Persian Gulf appeared to be adult or subadult specimens. Sampling procedures took place as approved by the Costa Rican Ministry of Environment and Energy (MINAE) or the Hormozgan Environmental Organization (Iran) under the close supervision of park rangers and experienced research station personnel. All techniques involved respect the ethical standards in the Declaration of Helsinki [27], as well as all applicable national laws.

After collection, wet carapace fragments were immediately transferred into plastic containers with 4% formaldehyde solution in seawater. To observe diatoms in their original position on the substrate surface and to preserve all the



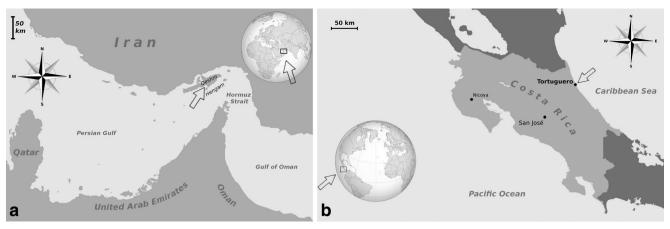


Fig. 1 Location of the sampling areas where green turtles (Chelonia mydas) were found: a Persian Gulf, b Tortuguero

mucilaginous structures produced by the epibiotic community, ca. 1 cm² subsamples were cut from each of the carapace pieces selected for further analyses. Subsequently, the sections were dehydrated through a 25, 50, 60, 70, 80, 90, 95 and 100% alcohol series and treated with a critical point dryer (K850 EMITECH). For taxonomic analyses of the diatom frustules, a set of subsamples was digested with 37% H<sub>2</sub>O<sub>2</sub> heated to ca. 80 °C following van der Werff [28]. After 1 h, the reaction was completed by adding KMnO<sub>4</sub>. Cleaned material was then centrifuged and rinsed at least three times. For scanning electron microscopy (SEM), both the carapace sections and oxidized diatom suspension were placed on aluminium stubs and sputter-coated with platinum using a DESK V HP TSC Cold Sputter Coater.

Samples and SEM stubs are stored at the Unit for Environmental Sciences and Management, North-West University in Potchefstroom (South Africa), housing the South African National Diatom Collection, and in the BR-collection, property of the Belgian federal government and given in permanent loan to the Botanic Garden Meise (Belgium).



Fig. 2 Collection of the sea turtle scutes

# **Microscopic Observations and Diatom Counts**

Using the same approach as Majewska et al. [24], preliminary observations using light microscopy suggested that diatom communities on different turtle specimens from the same location were very similar in terms of both growth form structure and species composition. Therefore, for the purpose of this study, 38 randomly selected sea turtle samples from each locality were used for a further detailed observation and analysis under either a Zeiss Supra 40 (Centro Grandi Apparecchiature, University of Campania "Luigi Vanvitelli", Naples) or a JEOL 6700F (Stazione Zoologica Anton Dohrn, Naples) scanning electron microscope. Diatoms were identified and counted on a surface area of ca. 2 mm<sup>2</sup> for each of the three subsamples derived from the 76 sea turtle samples at magnifications ranging between ×400 and ×60,000. Comparative statistical analyses were performed using Excel (Microsoft) with the Analysis ToolPak add-in and PRIMER Ver. 6 [29] software. Non-metric multidimensional scaling (nMDS) was used to assess and illustrate dissimilarities among all samples. To build a matrix, the Bray-Curtis similarity index, calculated on square root transformed species abundance (number of diatoms on 1 mm<sup>2</sup> of the carapace) data was used. A three-dimensional nMDS plot was generated to visualize the resemblance patterns observed among the examined diatom communities. Analysis of similarities (ANOSIM) was run to test for significant differences between epizoic communities associated with green turtles from different populations. To assess the level of dissimilarity within and between the sample groups and to identify the main diatom taxa responsible for these differences, a similarity percentage analysis (SIMPER) was performed. In addition, Margalef's species richness d ( $d = (S - 1)/(\log_e N)$ , where S = number of species, N = number of individuals) and Shannon-Wiener diversity H' (log<sub>e</sub>) indices were calculated for each of the samples. The F test and t test were used to test whether variances and means of parameters characterizing diatom communities differed between the two turtle populations.



#### Results

#### **Diatom Abundances and Growth Form Structure**

Diatoms were one of the main elements of epizoic microflora found on carapace fragments from sea turtles at both locations (Figs. 3 and 4). However, their overall abundance differed significantly between the sampling sites (t test, p < 0.0001), being higher in Costa Rica  $(782 \pm 250 \text{ cells } 1 \text{ mm}^{-2})$  than in Iran  $(348 \pm 140 \text{ cells})$ 1 mm<sup>-2</sup>). Bare areas with no epibionts were present in samples from both locations, but the epizoic diatom cover was visibly sparser in samples from Iran (Fig. 3a-c). Carapaces of turtles from Tortuguero were locally covered by dense mucilaginous mats and high densities of bacteria (Fig. 3d-f, arrows). The same was not observed in samples from Iran, where stalked diatoms with lightly silicified and fragile frustules were the predominant group of microorganisms found (Figs. 3b and 4i–n). In terms of growth form structure, a clear difference between the diatom communities collected at two different locations was also observed (Fig. 5). While erect diatoms (mainly Chelonicola costaricensis and Poulinea lepidochelicola) clearly dominated all samples from Iran reaching on average  $93.9 \pm 8\%$  of the total diatom number, the growth form structure of diatom communities observed in Costa Rica was more diversified with motile (Haslea amicorum, Labellicula lecohuiana, Navicula spp., Nitzschia spp. and *Proschkinia* sp.) and erect (mainly *Achnanthes* elongata, C. costaricensis and P. lepidochelicola) forms contributing on average  $48.4 \pm 13$  and  $45.8 \pm 15\%$  of the total cell abundance, respectively (Fig. 5). Some planktonic forms (including tychoplankton) were observed in Costa Rica (up to 13.4% of total diatom abundance; 1.4% on average), and tube-

Fig. 3 Biofilms on green turtle carapaces. a and b Sparse biofilm on green turtles from Iran (note very long mucilaginous stalks produced by *Chelonicola costaricensis*, *arrows*). c-f Dense biofilm on green turtles from Costa Rica. d Specimens of *Nitzschia* sp. 1 immersed partly in mucilaginous matrix and bacteria (*arrows*). e and f Examples of bacteria living in the biofilm (e = uncoated sample). *Scale bars* 10 μm (a-c), 2 μm (d), 1 μm (e, f)

dwelling *Parlibellus* sp. (up to 8.9%; 0.9% on average) occurred in eight samples from Iran (Fig. 5). In Costa Rica, *H. amicorum* and *Navicula* spp. were often observed partly immersed in mucilaginous matrix. However, proper tubes were not observed and thus these species are classified here as "motile".

### **Diatom Species Composition**

A total of 26 diatom taxa belonging to 20 genera were observed (Table 1). Among them, 22 species (17 genera) were found in samples from Costa Rica and 12 (nine genera) in those from Iran. Eight diatom species (Amphora sp. 2 sensu Majewska et al. [24], Amphora sp. 3, Chelonicola costaricensis, Cocconeis cf. neothumensis, Navicula sp. 1 sensu Majewska et al. [24], Nitzschia fasciculata, Nitzschia sp. 1 sensu Majewska et al. [24] and Poulinea lepidochelicola) were present on turtles from both locations (Fig. 4), contributing 72 and 96% of the overall (zero abundances included) mean diatom abundance in Costa Rica and Iran, respectively (Table 1). Fourteen taxa were restricted to Costa Rican and four to Iranian turtles. Three species, Amphora sp. 2, C. costaricensis and P. lepidochelicola, contributed >10% of total diatom abundance in at least one of the samples from each location, the latter being the only taxon present in all examined samples (Table 1).

In Costa Rica, 13 species exceeded 3% of total diatom abundance and four, *Labellicula lecohuiana*, *Achnanthes elongata*, *Nitzschia* sp. 1 and *P. lepidochelicola*, contributed >10% of total cell count in at least one of the samples (Table 1). The latter three taxa occurred in all samples. Seven species, *A. elongata*, *Amphora* sp. 2, *C. costaricensis*, *L. lecohuiana*, *Navicula* sp., *Nitzschia* sp. 1 and

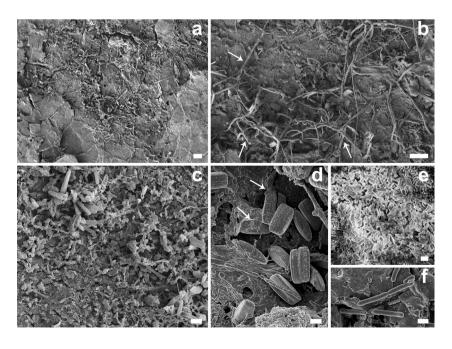
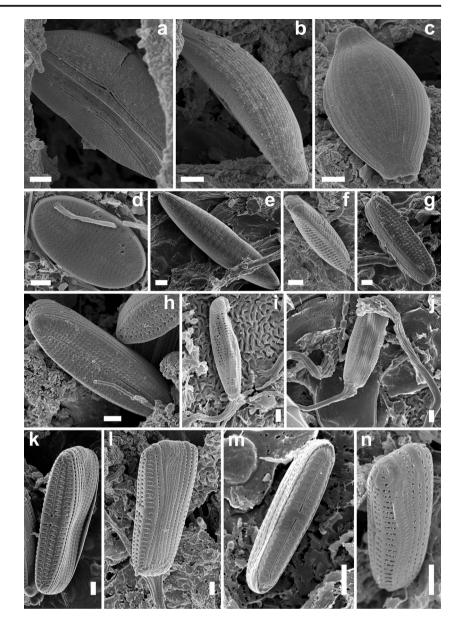




Fig. 4 Sea turtle-associated diatom species found in both sampled locations. a-c Amphora sp. 3. d Cocconeis cf. neothumensis. e Navicula sp. 1 sensu Majewska et al. [24]. f-g Nitzschia sp. 1 sensu Majewska et al. [24]. h-j Specimens of Chelonicola costaricensis from Costa Rica (h) and Iran (i, j). k-n Specimens of Poulinea lepidochelicola from Costa Rica (k, l) and Iran (m, n). Scale bars 1 um



P. lepidochelicola, exceeded 1% of the overall mean relative diatom abundance (Table 1). In turtle material collected from Iran, only four species, Amphora sp. 2, Parlibellus sp., C. costaricensis and P. lepidochelicola, contributed >3% of the total diatom abundance in at least one of the samples, the latter two species exceeding 70% of total diatoms and being present in all 38 samples (Table 1). Only three species, P. lepidochelicola, C. costaricensis and Amphora sp. 2, contributed >1% of the overall mean diatom abundance (Table 1).

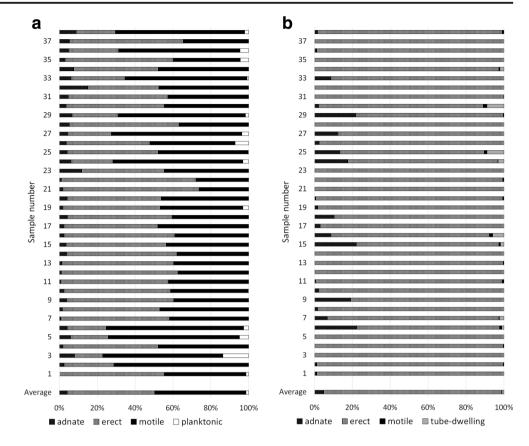
Species number per sample ranged from 5 to 14 (6.9 on average) in Costa Rica and from 2 to 9 (3.7 on average) in Iran (Supplementary Table S1). Similarly, both evenness and diversity indices were higher for Costa Rican samples, ranging from 1 to 3.2 (1.4 on average; evenness) and from 1.4 to 2.4 (1.7 on average; diversity), than those collected in Iran, where

the indices ranged from 0.3 to 2.1 (0.8 on average; evenness) and from 0.7 to 1.7 (1.0 on average; diversity; Supplementary Table S1). All the differences observed were statistically significant (t test, p < 0.0001 for each pair of means).

Although a major proportion of the most important species occurred in samples from both locations, nMDS analysis revealed important differences between the two groups of diatom communities clearly separating them from each other in the three-dimensional plot (Fig. 6). The high level of compositional dissimilarity between the sample sets was further confirmed by ANOSIM (global R = 0.987, p = 0.001). The total average dissimilarity between the diatom communities from the two different green turtle populations was 68.9%, and the four taxa, *Nitzschia* sp. 1, *A. elongata*, *C. costaricensis*, and *Amphora* sp. 1, were indicated as being responsible for >50% of that



Fig. 5 Diatom growth form percent contribution to total diatom abundance. a Costa Rica, b Iran



dissimilarity (SIMPER). Within the groups, the level of similarity among samples was slightly higher for communities collected from Iran (73.9%) than for those from Costa Rica (70.3%; SIMPER).

#### Discussion

Almost all known species of assumed truly epizoic diatoms that have been described so far have been found almost exclusively on marine taxa. The small number of freshwater animal-associated diatoms can be explained by relatively high availability of both hard substrate and nutrient resources found normally in shallow inland freshwater bodies. When neither attachment surface nor nutrients are sufficiently limiting, investment in adaptation to epizoic life and growth on chemically and kinetically uninert substrate may not necessarily be the most efficient way of survival. Thus, the wide range of hard substrates often accessible in lakes and rivers makes exclusive epibiosis and species specificity in surfaceassociated diatoms less advantageous. In the open water marine environment, firm substrate within the photic zone is extremely scarce and therefore constitutes a major limitation to all benthic primary producers. In such habitats, every newly emerging surface, including animal external tissues, is of great importance, and successful adaptation to conditions potentially unfavourable to other species is immediately rewarded.

The present study demonstrated that all green turtle individuals sampled in two remote locations hosted diatom communities, which in each case constituted a major element of the turtle carapace microflora. Significant differences in cell density, growth form structure, and species composition were also observed between the diatom communities of sea turtles from either location. Undoubtedly, the external environment affects epibiotic flora to some degree, and this distinction is not unexpected. Diatom communities associated with turtles from the Persian Gulf were less abundant and less diverse in terms of both species composition and growth form structure than those found in Costa Rica. It is notable that eight species, among 12 and 22 taxa found in Iran and Costa Rica, respectively, occurred in samples from both locations. Further, six of these species, Amphora sp. 2 sensu Majewska et al. [24], Chelonicola costaricensis, Cocconeis cf. neothumensis, Navicula sp. 1 sensu Majewska et al. [24], Nitzschia sp. 1 sensu Majewska et al. [24] and Poulinea lepidochelicola, were observed on the carapace of the olive ridley turtle (Lepidochelys olivacea) from Ostional, on the Pacific coast of Costa Rica [24].

Despite significant interest and a growing number of studies focused on the biology of all seven existing sea turtle species and their protection, many aspects of sea turtle life history, including natal origins, migration routes, and foraging behaviour, remain only poorly documented. Recent genetic



Table 1 List of the sea turtle-associated diatom taxa with the minimal, maximal and mean relative abundances (RA) found and percentages of samples in which the taxa were recorded

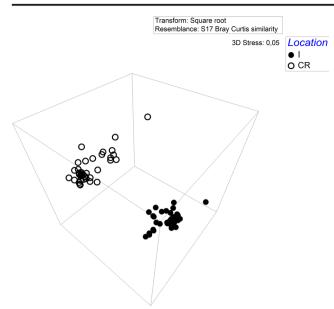
Taxa	Costa Rica				Iran			
	RA when present (%)	Mean RA when present (%)	Frequency (%)	Overall RA (%)	RA when present (%)	Mean RA when present (%)	Frequency (%)	Overall RA (%)
Achnanthes elongata Majewska & Van	3.9–50.3	23.8	100	23.8	_	_	_	_
de Vijver  Amphora ef. bigibba Grunow ex  A.Schmidt	_	-	_	_	0.2-0.9	0.5	15.8	0.1
Amphora sp. 1 sensu Majewska et al. 2015c	4.1–7.4	5.8	5.3	0.3	-	_	-	_
Amphora sp. 2 sensu Majewska et al. 2015c	1.1–21.5	10.3	94.7	9.8	0.8–15	4	42.1	1.7
Amphora sp. 3	1-2.3	1.7	13.2	0.2	0.6-2.1	1.4	7.9	0.1
Azpetia sp.	0.1 - 3.3	0.8	13.2	0.1	_	_	_	_
Chelonicola costaricensis Majewska, De Stefano & Van de Vijver	2.3–12.5	5.5	23.7	1.3	27.2–74.7	46.2	100	46.2
Cocconeis cf. neothumensis De Stefano, Marino & Mazzella	0.2-0.8	0.4	7.9	0.0	0.5–2.5	1	28.9	0.3
Dimeregramma sp.	_	_	_	_	0.2	0.2	2.6	0.0
Fragilaria sp.	0.3 - 3	1.2	13.2	0.2	_	_	_	_
Haslea amicorum Herwig, Tiffany, Hargraves & Sterrenburg	1.7–3.7	2.7	23.7	0.6	-	-	=	_
Labellicula lecohuiana Majewska & Van de Vijver	4.2–32.7	24.2	26.3	6.4	_	_	_	_
<i>Navicula</i> <b>sp. 1</b> sensu Majewska et al. 2015c	0.4–7.7	3	60.5	1.8	0.6–2.2	1.2	21.1	0.3
Navicula ef. rusticensis Lobban	0.9 - 5.6	3.3	10.5	0.3	_	_	_	_
Nitzschia fasciculata Grunow	0.3	0.3	2.6	0.0	0.2	0.2	2.6	0.0
<i>Nitzschia</i> <b>sp. 1</b> sensu Majewska et al. 2015c	20.6–71.2	38.4	100	38.4	0.3–1.2	0.7	23.7	0.2
Nitzschia sp. 2	0.5 - 0.9	0.7	18.4	0.1		_	-	-
Parlibellus sp.	-	_	-	_	1-8.9	4.2	21.1	0.9
Poulinea lepidochelicola Majewska, De Stefano & Van de Vijver	9.1–34	20.5	100	20.5	25.3–70.8	47.6	100	47.6
<i>Proschkinia</i> sp. sensu Majewska et al. 2015c	0.3–3	1.6	31.6	0.5	_	_	-	_
Pteroncola inane (Giffen) Round	_	_	_	_	0.2 - 0.9	0.4	7.9	0.0
Skeletonema sp.	2-3.1	2.3	10.5	0.2	_	_	_	_
Stephanodiscus sp.	0.7	0.7	2.6	0.0	_	_	=	_
Thalassionema nitzschioides (Grunow) Mereschkowsky	0.8–7.9	2.5	34.2	0.9	_	_	_	_
Thalassiosira sp.	0.7	0.7	2.6	0.0		_		-
Thalassiothrix sp.	0.2	0.2	2.6	0.0	_	_	_	_
Shared taxa				72.0				96.3

Species found in both Costa Rica and Iran are given in bold

studies have advanced the existing concepts of sea turtle phylogeography, providing new insights into their breeding strategy and evolutionary success [30–33]. It is assumed that the distribution range of habitats utilized by green turtles is controlled mainly by the habitat preference and thermal regime [30]. As primarily herbivorous, green turtles forage in shallow coastal waters [26, 30]. Although quite robust evidence supporting natal homing [34] in green turtles exists, it

has also been shown that feeding pastures can be shared by several green turtle populations inhabiting the same ocean basin [30–32, 35, 36]. The potential for overlapping of feeding areas in populations breeding within different ocean basins is generally unknown. It seems unlikely that thermophilic marine green turtles pass the natural geographic barriers, such as the Isthmus of Panama and Southern Africa, where warm Indian Ocean waters encounter the cold Benguela





**Fig. 6** Non-metric multidimensional scaling (nMDS) plot based on diatom species abundance data showing the relationship between the sea turtle-associated diatom communities from Iran (*I*) and Costa Rica (*CR*)

Current in the Atlantic [30]. However, studies based on ocean circulation models and genetic analysis suggest that links between the South Atlantic and southwestern Indian Ocean exist [31]. Tagging studies have indicated that only a very small percentage of females ever nest far from their native beaches [30]. Nonetheless, these occasional shifts in nesting areas seem to be crucial for species survival in a changing world, as beaches that were suitable for nesting a hundred, thousand or million years ago may not be suitable ones today. As a consequence of natal homing, nesting green turtle colonies show a strong population structure manifested by maternally inherited markers [30]. Breeding behaviour in males is much less understood. It is assumed that mating may occur outside the breeding areas and males originating from several different populations may be involved [30, 37]. If so, males may constitute an important natural vector in turtle-associated diatom dispersal among green sea turtle populations nesting in different geographical locations. Furthermore, multidirectional hybridization among almost all members of Cheloniidae has recently been documented [30] and it is therefore plausible that mating behaviour facilitates diatom dispersal among different sea turtle species.

Information derived from various genetic analyses indicates that the separation between Indo-Pacific and Atlantic populations of green turtle occurred several million years ago [30, 38]. Although that divergence was not sufficient to induce reproductive isolation, genetic differences between populations inhabiting the two regions are still significant, which suggests that mating events involving Indo-Pacific and Atlantic turtles are rare [30]. As no molecular analyses

of the diatom species could be performed, it is unknown how closely related the morphologically similar/identical species observed on turtles from the two remote regions are. Considering their much shorter life cycle and much higher reproductive rate, epizoic diatoms, unlike their host turtle, have the potential to have undergone an allopatric speciation event in the last 10–15 million years. Unquestionably, however, the newly discovered turtle-associated diatom taxa observed on green turtles from both Indian and Atlantic Oceans, as well as those found living on olive ridley turtles from the eastern Pacific, are closely related taxa and we speculate that their evolution in time and space overlapped with that of sea turtles. Genetic analyses between these different diatom communities could therefore be a very interesting avenue for future research.

According to Robinson et al. [26], the diversity of macroepibionts associated with several sea turtle species was related to the diversity of individual turtle foraging habitats. Thus, turtles with a lower diversity of foraging habitats might be expected to have a lower diversity of epibiotic diatoms. The significantly lower number (12) of diatom taxa found on turtles from Iran, as compared to nesting green turtle females from Tortuguero (22 taxa) or the olive ridleys at Ostional (21), might be related to the particularly challenging environmental conditions occurring in the Persian Gulf. The adverse effects of naturally high salinity, temperature and summer-winter temperature amplitude are further exacerbated by numerous pressures from human activities including transport, residential and commercial uses, industrial development, water desalination, fishing, agriculture and recent Gulf wars [39, 40]. As a result of these, species richness in the Persian Gulf is relatively low and compares poorly with that of other warm seas [40, 41].

A previous study that investigated the diatom flora of nesting olive ridleys from the Pacific coast of Costa Rica found very high numbers, exceeding those from most other substrates, of diatoms living within a thick biofilm encapsulating all examined carapace surfaces [24]. Diatom abundances on olive ridleys exceeded 27,000 cells mm<sup>-2</sup> and were substantially higher than those reported in this study (on average 782 and 348 cells mm<sup>-2</sup>, for green turtles from Costa Rica and Iran, respectively [24]). It is presumed that Pacific populations of olive ridleys utilize both oceanic and coastal foraging habitats including highly productive upwelling areas along the western coast of the South American continent [42, 43]. These nutrient-rich upwelling waters may have a direct effect on turtle-associated diatom communities stimulating strongly their growth. Nonetheless, potential differences in swimming and diving behaviour and/or carapace shape and roughness between the two sea turtle species cannot be excluded as factors stimulating or inhibiting epizoic diatom growth.

One of the interesting differences observed in the course of the present study was the presence of a rich bacterial



community in the Costa Rican samples and their almost complete absence in material collected in Iran. Numerous studies have documented specific interactions between diatoms and bacteria [44-47], and it has been shown that diatoms, especially those producing large amounts of extracellular polymeric substances (EPS), may promote and stimulate development of bacterial flora [44]. It is assumed that marine bacteria use chemotaxis to benefit from the algal excretions concentrated within the so-called phycosphere [48]. Conversely, algal growth may be enhanced by products of bacterial activity (including vitamins, hormones or siderophores), and there is convincing evidence that presence of bacteria is desired to ensure a proper development of "slime"-forming diatom communities [47]. Thus, we assume that the high number of bacteria observed in samples from Tortuguero was a direct effect of the relatively high percentage of diatom taxa belonging to Achnanthes, Amphora, and Navicula genera, which are often found in slime covering various substrates [44, 45]. Biofilm composed of agglomerations of adhered diatoms and bacteria, and the products of their activity, promotes adhesion of other particles including macroepibionts and planktonic organisms [49], which explains why planktonic diatoms were found on Costa Rican sea turtles and not on those from Iran. Diatom communities associated with green turtles from the Persian Gulf were composed mainly of the two recently described diatom species C. costaricensis and P. lepidochelicola. The fact that these two taxa were present on the carapace surface even when bacteria were not may further support the hypothesis that these species, unlike the previously mentioned "slime producers", are particularly closely associated with the host animal.

# **Concluding Remarks**

Marine vertebrates, including sea turtles, appear to be mobile islands of surface-associated diatom life, providing a hard substrate for any organism that can attach and endure the extreme conditions and competition for space and resources on the animals' body surfaces. It has recently been proposed that taxonomic analysis of these hitch-hiking diatom assemblages may reveal important information pertaining to the sea turtle life history and single individual migration routes [24, 25]. In the present study, few diatoms known from other habitats contributed to the total diatom number found on sea turtle carapace, while much of the differences in the diatom community structure between the two sea turtle populations was related to the degree of development of the biofilm, likely affected greatly by external environmental factors. In both sea turtle populations, diatom communities were composed of similarly dominant shared taxa with the addition of other species that occurred when the biofilm became denser. The attached erect diatoms, Chelonicola costaricensis and Poulinea lepidochelicola, exhibited much reduced relative abundances in the denser biofilm on sea turtles from Costa Rica. As the biofilm accumulates, the available, uncolonized substrate surface decreases and relative abundance of forms that may require a direct attachment to the sea turtle carapace declines. This may suggest that at least some of the observed diatoms are truly epizoic. The fact that sea turtle-associated diatom flora seem to be unique and composed mainly of several previously unknown species suggests an important new research direction in studies on both sea turtle and diatom phylogeny, biogeography, and evolution.

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