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RESEARCH ARTICLE

Scanning electron microscopic studies on epiphytic diatoms on pneumatophores of *Avicennia marina* in Puducherry mangroves

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SUMMARY

Present study is processed on the epiphytic diatom growing on the aerial roots of *Avicennia marina* growing in Puducherry young mangroves. Species diversity, index of dominance, species richness have been arrived at. Further, images of epiphytic diatom were taken using Scanning Electron Microspcope for the first time in *Avicennia marina*. The factors influencing such epiphytic distribution have been discussed.

Key Words **:** Epiphytic diatom, Mangroves, Species diversity, SEM images

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Many studies have been made on epiphytic diatom assemblages in coastal waters and wetlands (Sullivan, 1981; Du and Jin 1983; Almeida and Siqueiros-Beltrones, 2008). However, most of these studies were on epiphytic diatoms growing on macroalgae and seagrasses and few from mangrove roots. Mangroves are salt-tolerant trees that grow on sheltered tropical coastlines throughout the world (Ellison, 2008; Bouillon *et al*., 2008). Their strong roots (stilt roots, prop roots, buttress roots, aerial roots / pneumatophores) offer suitable substrates for a variety of epiphytic diatoms (Lai and Wang, 2004; Ellison, 2008; Bouillon *et al.,* 2008; Chen *et al.,* 2010). Almost all studies on epiphytic diatoms in mangrove regions have been confined to large tropical to subtropical lagoons in Bahamas (Sullivan, 1981), Louisiana (Maples, 1983), Florida (Navarro and Torres, 1987), Singapore and southern Malaysia (Wah and Wee, 1988), Japan (Nagumo and Hara, 1990) and in the temperate regions of the southern hemisphere, such as Australia (Foged, 1979). Much of the epiphytic floras growing on marine macrophytes are diatoms (Kita and Harada, 1962; Jacobs and Noten, 1980). Studies show that few species may be occupying the surface of pneumatophores of black mangrove (Maples, 1983, Siqueiros-Beltrones and Lopez Fuerte, 2006) worked on samples of epiphytic diatom assemblages found on prop roots of red mangrove (*Rhizophora mangle*). Benjamin *et al*. (2006) have examined diatom assemblages and dominance in *Rhizophora* species. Chen *et al.* (2010) have studied the geographical and seasonal patterns of epiphytic diatoms on a subtropical mangrove (*Kandelia candel* L.) from four sites in southern China and identified 103 species of epiphytic diatom belonging to 40 genera. Naidoo *et al.* (2008) had studied the nature of the epiphytism of red algae and also the occurrence and possible role of other epiphytic micro-organisms within the superficial tissues of pneumatophores of *Avicennia marina* (Forssk.) Vierh.

To acquire more scientific information presently an attempt has been made to examine the epiphytic diatom assemblage on the aerial roots of *Avicennia marina* growing in the backwater of Pondicherry coast.

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MATERIALS AND METHODS

Study area :

Geographically the study area is lying within the boundaries of latitudes 11°46'03" to 11°53'40" North and longitudes 79°49'45" to 79°48'00" East. It is encircled by three villages *viz.,* Ariankuppam, Murungapakkam, Veerampattinam and two islets namely Thengaithittu and Ashramthittu. The mangrove exists as fringing vegetation distributed along the sides of Ariankuppam estuary / backwaters. Seven true mangrove flora (Class: Dicotyledonae) species belonging to three families were recorded here in the Punducherry mangrove ecosystem. *Avicennia marina*, was first reported by Blasco (1975) is the extensively growing true mangrove distributed throughout the inundated area. It forms very dense stand to the western and northern side of Thengaithittu, near the bridge and also at the small creek as indicated in the Fig. A. Though the water way is a tributary of the river Gingee, for the last many years no freshwater reaches into this mangrove area except the municipal and agricultural discharges. This tide-dominated estuary opens into the Bay of Bengal on the Coromandal coast. The tidal amplitude averages to 20-70cm and differs accordingly to the lunar period, reaching its maximum during the northeast monsoon. The climate is sub-humid ranging with 65-75 per cent relative humidity and with an average temperature of 28.8°C. The annual rainfall reaches to 1200mm with an average of 105mm.

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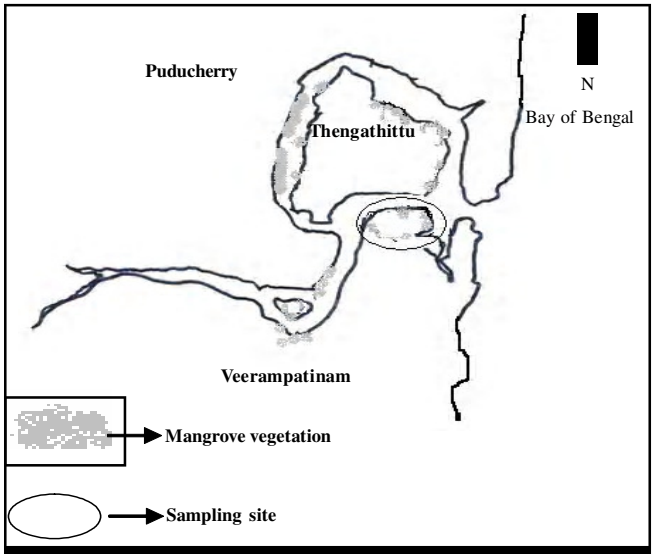

Fig. A : Map showing the study area

Samples of respiratory roots (pneumatophores) were collected from Pondicherry coastal mangroves during January 2011 (sample 1) and February 2011 (sample 2). Samples were collected during low tide. Aerial root segments 30 cm^2 in area (2 by 15 cm) were cut off. Each sample composed of three segments. Totally 8 root samples were collected. All visible epiphytic macroalgae found were removed from each bark strip under a dissecting microscope Methods described by Siqueiros-Beltrones and López Fuerte (2006) with slight modification. Fresh material was observed in fresh mounts to observe the approximate proportion of empty frustules (dead) and live cells at the time of sampling. The root segments were then brushed using a H toothbrush and rinsed with tap water to obtain a concentrate 5of sediments, organic matter, and diatoms. The organic matter inside the concentrate with diatom frustules was oxidized using a 1:3:1 mixture of sample, nitric acid, and commercial ethanol (Siqueiros-Beltrones, 2000) to obtain clean diatom to facilitate identification and enumeration. The floristic analysis was carried out separately from the quantitative analysis to identify as many taxa as possible in the assemblages. Digital photos were taken using the cannon A495 powershoot camera provided in Zessus Primostar microscope available in the department of Ecology and Environmental Sciences.

To examine the ultra structure of the root samples and also to find out the mode of attachment of diatom on aerial roots, SEM study was undertaken. In the SEM studies the root materials were freeze-fractured following immersion in liquid nitrogen. Fixation was done in a mixture of 1 per cent glutaraldehyde and 1 per cent paraformaldehyde, followed by serial dehydration in ethanol/water mixtures up to 100 per cent ethanol. Final dehydration was done in hexamethyldisilazane overnight. Fixed samples were gold-coated for 1 min (sputter-coated with gold prior) to viewing 20kV and a magnification up to X15000 on a Pondicherry University Instruct Name=S-3400 Scanning Electron Microscope available CIF of Pondicherry university, as described by (Ojeda and Chapparro, 2004; Ali Miserez *et al.,* 2009).

Taxonomic determinations were accomplished based on Manual on methodology for biological parameters (Anonymous, 1998).

Species diversity was calculated using the formula given by Margalef (1968) as:

$$\mathbf{H' = -\sum (n_i/N) In(n_i/N)}$$

where H' = Shannon-Weiner index of general diversity; n_i = importance value index of species i; N= importance value index of the community.

The index of the dominance of the community was calculated by Simpson's index (Simpson, 1949) as:

$$\mathbf{C = \sum (n_i/N)^2}$$

where C = index of dominance n_i and N being the same

as in the Shannon index of general diversity.

The index of species richness (d) was calculated following Menhinick (1968):

$$D = s/\sqrt{n}$$

where s = number of species ; n = number of individuals.

The evenness index of the community (e) was calculated following Pielou (1966) as:

$$e = H'/\log S$$

where S = number of species, H'= Shannon index.

RESULTS AND DISCUSSION

A total of 31 species belonging to 18 genera under 7 families were recorded from aerial root samples of *Avicennia marina* collected from Pondicherry Mangrove region. Table 1 shows the percentage distribution of epiphytic diatom isolated from the aerial root surfaces. Among 31 species identified, *Bacteriastrum delicatulum* and *Nitzschia sigma* are the most abundant species. Rhizosolenia is the genus that supported more diatom species (Fig. 1) and Naviculoidae is the most dominant family (Fig. 2) in terms of number of species represented. The data are tested statistically for Shannon diversity index, index of dominance, species evenness and species richness. Index of dominance is 0.0469 (Table 1). Shannon's diversity index was found to be 3.2014 (Table 1). Evenness index and species richness are found to be 2.146 and 1.3365, respectively. The Scanning Electron microscopic images showing the epiphytic diatoms on the surface of the aerial roots are given (Fig. 3).

Much of the epiphytic micro flora growing on marine macrophytes are diatoms (Kita and Harada, 1962; Jacobs and Noten, 1980) and may contribute significantly to the primary production of mangrove systems (Siqueiros-Beltrones *et al*., 2005). Studies pertaining to epiphytic diatom assemblage on prop roots of different mangrove plants have been studied by various investigators (Maples, 1983; Wah and Wee, 1988; Nagumo and Hara, 1990; Siqueiros-Beltrones *et al.,* 2005, Chen *et al.,* 2010). But studies pertaining to epiphytic micro algal assemblage on pneumatophores of *Avicennia marina* are very much limited except for the work of Naidoo *et al.* (2008) who had studied on the epiphytic organisms on the pneumatophores of *Avicennia marina;* however studies on ultra structure of epiphytic diatoms growing on pnematophores using SEM has not been done.

Fig. 2 : Classification based on family order

In the present study, species composition and ultra structure of root surfaces have been attempted. A total of 31 species belonging to 18 genera and 7 families were recorded from 8 aerial root samples of *Avicennia marina* collected from Pondicherry Mangrove region where as Maples (1983) has recorded only 23 species from the pneumatophores of *Avicennia germinans* collected from Louisiana, USA. *Bacteriastrum delicatulum* and *Nitzschia sigma* are the most abundant species recorded among 31 species. *Nitzschia fasciculate* was the most abundant species recorded by

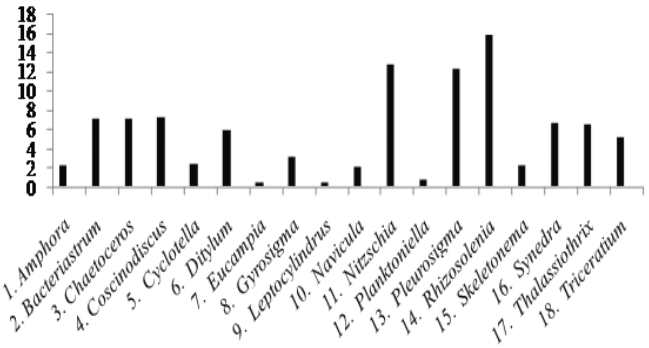

Fig. 1 : Genus- wise diatom recorded

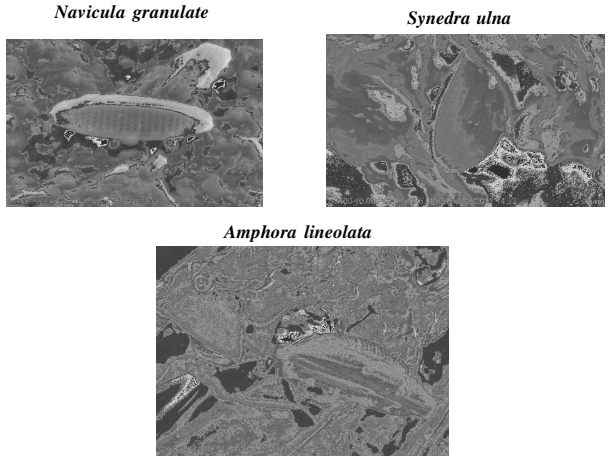

Fig. 3 : SEM images of Diatoms

Table 1: Index of dominance and Shannon's diversity index

Sr. No.	Species name	Number (n)	(n/N)*ln(n/N)
1.	*Amphora leneolata* Ehrenberg	12	-0.08482
2.	*Bacteriastrum delicatulum* Cleve	38	-0.18719
3.	*Chaetoceros affinis* Lauder	7	-0.05649
4.	*Chaetoceros curvisetus* Cleve	31	-0.16444
5.	*Coscinodiscus centralis* Ehrenberg	13	-0.08996
6.	*Coscinodiscus gigas* Ehrenberg	3	-0.02894
7.	*Coscinodiscus jonesianus* (Greville) Ostenfeld	5	-0.04348
8.	*Coscinodiscus oculus* iridis Ehrenberg	18	-0.11367
9.	*Cyclotella striata* (Kuetzing) Grunow	13	-0.08996
10.	*Ditylum brightwellii* (West) Grunow	32	-0.16786
11.	*Eucampia zoodiacus* Ehrenberg	2	-0.0208
12.	*Gyrosigma balticum* (Ehrenberg) Rabenhorst	17	-0.10916
13.	*Leptocylindrus danicus* Cleve	2	-0.0208
14.	*Navicula granulata* Berb. and Her.	11	-0.07953
15.	*Nitzschia acuta* Cleve	3	-0.02894
16.	*Nitzschia closterium* (Ehrenberg) Smith	12	-0.08482
17.	*Nitzschia seriata* Cleve	15	-0.09981
18.	*Nitzschia sigma* (Kuetzing) W. Smith	38	-0.18719
19.	*Planktoniella sol* (Wallich) Schutt	4	-0.03644
20.	*Pleurosigma angulatum* (Kuetz.) W. Smith	30	-0.16097
21.	*Pleurosigma elongatum* W. Smith	36	-0.18096
22.	*Rhizosolenia alata* (Cleve) Grunow	32	-0.16786
23.	*Rhizosolenia cylindrus* Cleve	15	-0.09981
24.	*Rhizosolenia robusta* Norman	12	-0.08482
25.	*Rhizosolenia setigera* Brightwell	11	-0.07953
26.	*Rhizosolenia styliformis* Brightwell	15	-0.09981
27.	*Skeletonema costatum* (Grev.) Cleve	12	-0.08482
28.	*Synedra ulna* (Kuetz) Ehrenberg	36	-0.18096
29.	*Thalassiothrix frauenfeldii* (Grunow)	35	-0.17777
30.	*Triceratium favus* Ehrenberg	13	-0.08996
31.	*Triceratium reticulatum* Ehrenberg	15	-0.09981
		N=538	-3.2014

Index of dominance C=0.0469 Shannon's diversity index = 3.2014

Maples (1983). The genus Rhizosolenia supported more species among the epiphytic forms on the aerial roots of *Avicennia marina* (Fig.1). Naviculoidae is the most dominant family (Fig.2) among the recorded 7 families. Index of dominance is found to be 0.0469 (Table 1). Species diversity (H') index was found to be 3.2014 (Table 1). The species diversity (H') values estimated presently are the highest recorded in similar studies for benthic diatoms in other mangrove systems (Sullivan, 1981; Navarro, 1987; Siqueiros-Beltrones and Sanchez Castrejon, 1999). From Fig. 1, it is evident that *Rhizosolenia* is the most abundant genus. Evenness index of the community is 2.146. Species Richness is found to be 1.3365. Studies elsewhere on mangrove roots by Sullivan (1981), Maples (1983) and Navarro and Torres (1987) recoreded: 83, 23, and 107 diatom taxa, respectively. Navarro (1982), on the other hand, recorded 226 taxa in 234 samples during a year cycle. The present observations appeared significant although as it has posed questions about the possible role of the association between mangroves and epiphytes and these can only be addressed with further investigations. The variations in the number of species, diversity index, species richness and evenness are largely influenced by regional/site specific edaphic and hydrological factors including the status of pollution of the given study area.

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