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Diversity and distribution of ciliated protozoans on the mangrove leaf litters of *Rhizophora apiculata*

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Abstract

The diversity and distribution of ciliated protozoans on the mangrove leaf litters of *Rhizophora apiculata* with physicochemical parameters were studied for one year (June 2014 - May 2015) from ten selected stations of the Ayiramthengu mangrove ecosystem in Kerala, India. Taxonomic studies recorded 115 species of ciliates, belonging to 16 subclasses among them Peritricha represents the subclass having the maximum species number and abundance followed by Suctoria. *Vorticella companula* Ehrenberg, 1831 records the maximum abundance and dominance index followed by *Euplotes minuta*. Multivariate statistical analysis and analysis of variance revealed seasonal difference in the distribution of mangrove ciliates, where the highest number of species were distributed in post-monsoon and minimum in pre-monsoon. Canonical correspondence analysis explains the importance of dissolved oxygen, temperature, conductivity, salinity, sulphate, phosphate and nitrate in the distribution and abundance of ciliates protozoans. The study highlights the seasonal difference in the distribution, diversity and abundance of ciliated protozoans on the mangrove leaf litters of *Rhizophora apiculata* in the Ayiramthengu mangrove ecosystem, Kerala, India.

Keywords: Ciliates, Leaf litter, Peritricha, Spirotrichea

Introduction

Mangroves are a group of salt tolerant flowering trees and shrubs, which are circumglobal in distribution and are specially adapted to grow in the intertidal regions of the tropical and subtropical coastlines (Tomlinson 1986; Duke 1993; Hogarth 1999; Saenger 2002). Five per cent of world's mangrove vegetation is distributed in India, spreading over 4,500 km^2 along the coastal states. Earlier, Kerala was abundant with 700 km^2 of mangrove forest and the deterioration started in the middle of the 20th century. At present, the mangrove vegetation has been reduced to a mere 17 km^2 that comes under the control of state government (Mini *et al.* 2014).

Total of 15 pure and 33 semi mangrove species had been recorded from entire costal area of Kerala (Vidyasagaran *et al.* 2014). Mangrove flora of Ayiramthengu comprise of totally nine species belonging to six families (Myrsinaceae, Avicenniaceae, Rhizophoraceae, Euphorbiacea, Combretaceae and Sonneratiaceae), Avicenniaceae is the largest family in Ayiramthengu region followed by Rhizophoraceae (Vijayan *et al.* 2015). The amount of leaf litter produced by mangrove plants will not be same as in every species, it will vary with leaf size, wide and dense leaves of Rhizophora apiculata produce a large amount of litter compared to species with small and narrow leaves (Efriyeldi *et al.* 2021). Leaf litterers deposited on the mangrove soil mainly consisted of ungrazed material largely processed through a detritus-based food chain and accounts for considerable near-shore secondary productivity (Odum & Heald 1975). Heavy lingo cellulose deposition is an adaptation possessed by the

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leaves of *Rhizophora apiculata* to survive in any harsh environmental fluctuations; these depositions make the leaf hard and slow down the degradation process (Nielsen & Andersen 2003). Degradation of fallen mangrove vegetation starts immediately after its colonization by fungi and bacteria (Alongi *et al.* 1989; Moran & Hodson 1989). Fungal saprophytic enzymes deteriorate the lignocellulose compounds of mangrove leaves (Findlay 1986) and the degraded mangrove vegetative materials support the colonization of heterogeneous microbial communities (Odum & Heald 1975; Bano *et al.* 1997). Microscopic examination of vegetative matter reveals the presence of a complex community composed of fungi, bacteria, protozoa, and microalgae (Odum & Heald 1975) for their growth mangroves absorb the essential nutrients released by these organisms (Alongi *et al.* 2003; Reef *et al.* 2010; Srisunont *et al.* 2017). Therefore, nutrient cycling by litterfall does not only promote nutrient recycling in mangroves but also strengthen the bonds between different trophic levels in the mangrove chain (Srisunont *et al.* 2017).

Ciliates associated with mangrove leaf litters such as Oligotrichia, Hypotrichia and Peritricha were the important detritivores that showed a characteristic role in nutrient cycling (Utz 2008; Li *et al.* 2010). They are the main bacterial consumers who showed some special characteristics such as modified oral cilia, short generation times, and rapid multiplication (Li *et al.* 2010). Ekelund *et al.* (2002) experimentally showed that the organic matter released by plants could stimulate bacterial and ciliate activity in the root zone leading to mineralization of organic soil nitrogen and assimilation by plants. The activity of ciliates may be important in mangrove plant nutrition; on other hand, the growth of plants may significantly affect the ciliate community. Kathiresan & Bingham (2001) have extensively studied the role of benthic communities on the growth of the mangrove ecosystem in the Pichavaram mangrove ecosystem in India.

Major factors influencing ciliate diversity are tidal inundations, local environmental conditions and physicochemical characteristics of water (Dorothy *et al.* 2003). Each of these factors individually contributes to the diversity and abundance of mangrove ciliates (Chithra & Sunil Kumar 2015, 2018, 2019). Ciliates are found attached to different microhabitats of the mangrove ecosystem, but their diversity and abundance depend on the nature of substrate they attach. Other than the nature of substrate, decomposition time of the attached substrate and leaching should play a significant role in determining the initial colonizers and later the protozoan species especially ciliates Dorothy *et al.* (2003). Most of the studies discussing the ciliate diversity were reported from the northern regions of India (Das *et al.*, 1993; Piyali & Das, 1997; Mahajan & Nair 1965; Das 1971; Bindu 2010). There are no previous studies examined the diversity of ciliates on the sedimented leaf litters of the southern coast of India. Thus, the objectives of this study were to investigate the species composition, diversity and density of ciliates on the sedimentary leaf litters with respect to the seasonal hydrological parameters.

Materials and Methods

Study area

The study area was located in Ayiramthengu mangrove situated (lat. 9^0 6′ to 9^0 8′ N long. 76^0 2’ to 76^0 29’ E) in Kollam district of Kerala in the part of the Kayamkulam estuary, which is the narrow stretch of tropical backwater on the southwest coast of India (Figure 1a). The area covers about 20 acres, and most of the inner regions of mangrove forest patches were left untouched by humans. Two streams and a canal enter the lake during the rainy season, which links the rivers Pamba and Achankoil. Ten sampling sites were considered for the study, and each site was fixed at an interval of 50 m.

Hydrology

The water samples were collected in a properly labelled clear airtight container. The temperature was measured on-station using portable mercury thermometer (-20^0-110^0 C). Water samples were transferred to the lab in dark conditions, maintaining at 4^o C for the determination of other physico chemical parameters. pH of the sample was analyzed using a pH meter (MK 6) and a conductivity meter (EQ 660A) was used to analyse the presence of electrolytes in the sample. The Winkler method was used to determine the amount of dissolved oxygen in the sample. Mohr’s method was used to analyze the presence of chloride and the Phenol disulphonic acid method was used to analyze the presence of nitrate concentration in mangrove water. The stannous chloride method was used to analyze the presence of phosphate and turbidimetric method was used to analyze the presence of sulphate concentration in mangrove water. All the above-mentioned analysis was carried out according to the standard method prescribed in American Public Health Association *et al.* (1998).

Collection, identification and enumeration of epibenthic ciliates

The methods described by Maybruck & Rogerson (2004) were adopted for the collection of epibenthic organisms from mangrove leaf litter. Mangrove leaf litters were covered with a heterogeneous mixture of trapped material (Figure 1b). Samples were collected by taking one cm^2 patch of the moist film of trapped

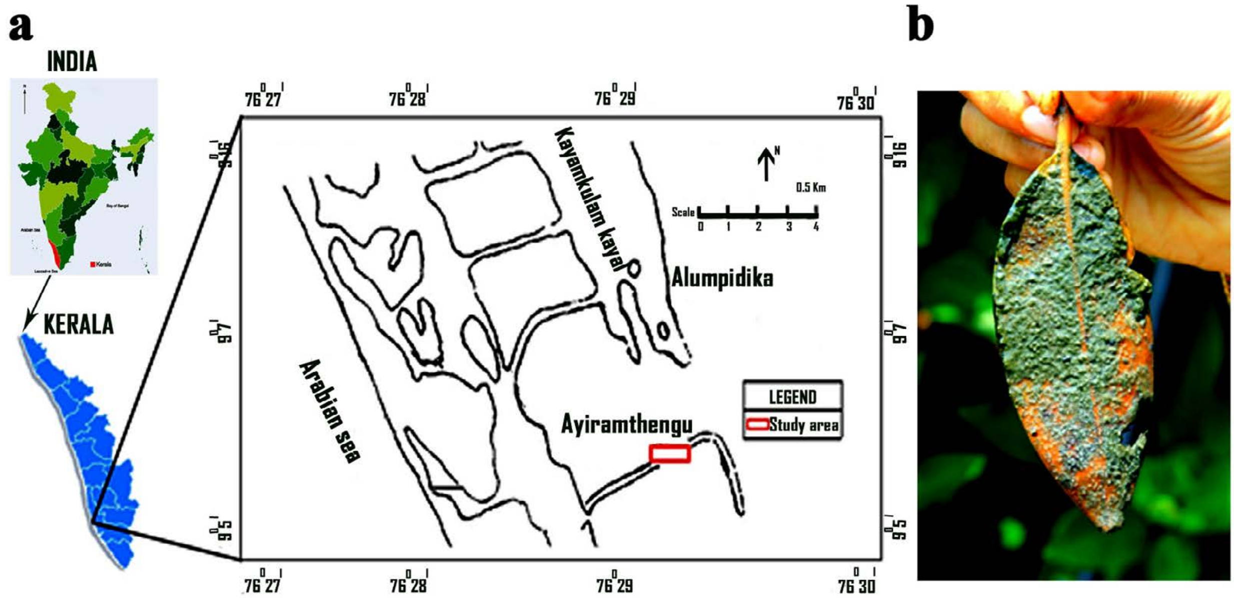

Figure 1. Map of Ayiramthengu mangrove ecosystem (a); Mangrove leaf litter with heterogeneous trapped material (b)

material to a sterile 15 ml sampling bottle. Continuous one-year sampling (June 2014 - May 2015) was carried out to analyze the composition and distribution of epibenthic organisms in mangrove leaf litter. Biometrical characteristics of the epibenthic organisms collected from the mangrove leaf litter were carefully examined under live condition. For detailed analysis, samples were treated with silver carbonate technique proposed by Fernandez-Galiano (1976). The data collected were analyzed using PAST 4.03 (Hammer *et al.* 2001), statistical software packages for Social Sciences (SPSS) software version 16.0 (Handcock *et al.* 2008) and Biodiversity pro (McAleece *et al.* 1997).

Results

Environmental factors

The overview of hydrological parameters analysed during the study period is provided in Tab. 1. Temperature and dissolved oxygen record their minimum values in post-monsoon. Temperature exhibits its maximum values in pre-monsoon, and dissolved oxygen records its maximum values in monsoon. Conductivity, pH and salinity exhibit their minimum values in the monsoon and maximum values in the pre-monsoon. Nitrate, phosphate and sulphate exhibit their minimum values in pre-monsoon and maximum values in monsoon. The average annual precipitations relative to the baseline time periods of June 2014 to May 2015 are presented in Table 1. The data reveals that the maximum average rainfall (374.97 mm) was recorded in the monsoon and the minimum (100.47 mm) recorded post-monsoon.

Species composition, dominance index and density

An aggregate of 115 species spread over 16 subclasses was identified during the one-year study period. Among them, Peritricha represents the subclass having the maximum species number (64) followed by Suctoria (11). Heterotrichea, Hypotrichia, Nassophorea and Stichotrichia are the subclasses having only one species record during the entire study period. *Vorticella companula* Ehrenberg, 1831 records the maximum abundance (1480 no./cm^2) and dominance index (0.0460) followed by *Euplotes minuta* (1349 no./cm^2 & 0.04198). *Epiphyllum soliforme*, *Acanthocystis aculeate*, *Cothurnia recurvate*, *Pyxicola annulata*, *Vorticella campanulate*, *Cohnilembus* sp.1, *Tintinnopsis sacculus*, *Acineta calkinsi* and *Ephelota plana* are with the rarest distributional records from Ayiramthengu mangroves. *Cothurnia* represents the genus with a maximum number of species (18) followed by *Zoothamnium* (11) (Table 2).

Out of 32,132 individuals counted during the one year survey, maximum numbers of ciliates were recorded during post-monsoon (14431) trailed by monsoon (10403) and pre-monsoon (7298). Peritricha represents the subclass having the maximum number of individuals (19778) followed by Suctoria (4026) and Spirotrichea (3143) (Table 3). Percentage distribution pattern also highlights that 62% of the total ciliate composition in the Ayiramthengu mangrove ecosystem is occupied with Peritricha followed by Suctoria (13%) and Spirotrichea (10%) (Figure 2). Among the recorded peritrichs, 45 % of them are distributed in post-monsoon, 32% in monsoon and 23% in pre-monsoon (Figure 2a). Investigating the species dispersion pattern, 93 species

Table 1. Overview of hydrological parameters

	Monsoon (M)	Post- monsoon (P.M)	Pre-monsoon (Pr. M)	Minimum	Maximum
Temperature	28.99 ± 0.32	27.85 ± 0.92	29.05 ± 1.36	26.5^0 C (P.M)	32.7^0 C (Pr. M)
pH	6.85 ± 0.43	7.14 ± 0.09	7.53 ± 0.09	6.2 (M)	8.2 (Pr. M)
Conductivity	12.94 ± 8.62	26.64 ± 10.37	27.45 ± 11.91	0.2 s/m (M)	37.4 s/m (Pr. M)
D.O	4.62 ± 0.39	3.80 ± 0.85	4.59 ± 1.03	0.8 mg/l (P.M)	7.6 mg/l (M)
Salinity	12.15 ± 1.28	14.00 ± 1.04	17.56 ± 2.86	10 ppm (M)	25.2 ppm (Pr. M)
Nitrate	2.47 ± 0.65	2.32 ± 0.59	1.64 ± 0.98	0.09 mg/l (Pr. M)	7.74 mg/l (M)
Phosphate	2.55 ± 0.05	2.49 ± 0.06	2.33 ± 0.02	2.26 mg/l (Pr. M)	2.87 mg/l (M)
Sulphate	267.36 ± 41.02	126.41 ± 59.17	104.10 ± 77.20	7.94 mg/l (Pr. M)	295.18 mg/l (M)
Average Rainy Days	17	7	6		
Average Rain Fall (mm)	374.97	100.47	183.4		

were recorded during the monsoon, 111 during the post-monsoon and 105 during the pre-monsoon.

Diversity indices

The maximum Shannon and Simpson diversity was observed in the monsoon (0.637, 0.398) and the minimum recorded during the pre-monsoon (0.588, 0.432). Analyzes of species richness by Margaleff index, showed maximum species richness in pre-monsoon (4.142) and the lowest recorded in post-monsoon (3.847). Maximum value of Shannon Evenness index was observed in monsoon (0.529) and minimum was recorded during pre-monsoon (0.488) (Table 4).

Analyzing the subclass wise diversity index, Suctoria (1.017), Stichotrichia (1.016) and Peritricha (1.015) were with the maximum Shannon diversity and Heterotrichea (0.843) and Cyrtophoria (0.853) with the minimum diversity. Maximum Simpson diversity was observed in the case of Stichotrichia (0.091) and the minimum diversity was observed in the case of Cyrtophoria (0.176). Similar to the Shannon diversity index, Shannon evenness also shows a similar trend,

Table 2. Abundance and dominance index of ciliates counted during the study period

SI. No.	CILIATES	Abundance	Dominance index
	Cyrtophoria (3)		
1	*Chilodonella cucullulus*	59	0.001836176
2	*Chlamydodon sp1*	106	0.003298892
3	*Dysteria proraefrons*	196	0.006099838
	Haptoria (5)		
4	*Acineria incurvata*	80	0.00248973
5	*Epiphyllum shenzhenense*	127	0.003952446
6	*Epiphyllum soliforme*	49	0.00152496
7	*Litonotus paracygnus*	88	0.002738703
8	*Litonotus petzi*	50	0.001556081
	Heliozoa (3)		
9	*Acanthocystis aculeata*	3	9.33649E-05
10	*Actinophrys eichhorni*	97	0.003018797
11	*Actinophrys sol*	108	0.003361135
	Heterotrichea (1)		
12	*Blepharisma lateritium*	73	0.002271879
	Holotrichia (3)		
13	*Coleps hirtus*	689	0.021442798

SI. No.	CILIATES	Abundance	Dominance index
14	*Holophyra oblonga*	92	0.002863189
15	*Prorodon Sp1*	246	0.007655919
	Hymenostomatia (2)		
16	*Uronea acutum*	247	0.007687041
17	*Uronea caudata*	160	0.00497946
	Hypotrichia (1)		
18	*Diophrys hystrix*	141	0.004388149
	Nassophorea (1)		
19	*Nassula Ornata*	347	0.010799203
	Oligotrichia (2)		
20	*Strobidinopsis elongata*	76	0.002365243
21	*Strobidinopsis paracalkinsi*	341	0.010612474
	Peniculia (2)		
22	*Frontonia subtropica*	354	0.011017055
23	*Frontonia tchibisovae*	149	0.004637122
24	*Paramecium brusaria*	489	0.015218474
25	*Paramecium polycaryum*	80	0.00248973
	Peritricha (64)		
26	*Cothurnia acuta*	79	0.002458608
27	*Cothurnia angusta*	141	0.004388149
28	*Cothurnia annulata*	266	0.008278352
29	*Cothurnia anomala*	212	0.006597784
30	*Cothurnia asymmetrica*	582	0.018112785
31	*Cothurnia carinogammari*	412	0.012822109
32	*Cothurnia coarctata*	354	0.011017055
33	*Cothurnia elegans*	168	0.005228433
34	*Cothurnia kahli*	261	0.008122744
35	*Cothurnia kiwi*	267	0.008309473
36	*Cothurnia limnoriae*	183	0.005695257
37	*Cothurnia recurva*	355	0.011048176
38	*Cothurnia recurvata*	31	0.00096477
39	*Cothurnia Sp 1*	121	0.003765716
40	*Cothurnia Sp 2*	87	0.002707581
41	*Cothurnia trophonicola*	171	0.005321798
42	*Cothurnia vaga*	350	0.010892568
43	*Cothurnia variabilis*	182	0.005664135
44	*Epistylis coronata*	329	0.010239014
45	*Epistylis hentscheli*	95	0.002956554
46	*Epistylis nayagarae*	365	0.011359393
47	*Epistylis plicatilis*	267	0.008309473
48	*Epistylis procumbens*	245	0.007624798
49	*Paravorticella sp*	457	0.014222582
50	*Platycola mollis*	104	0.003236649

Sl. No.	**CILIATES**	Abundance	Dominance index
51	*Platycola truncata*	91	0.002832068
52	*Pseudovorticella banatica*	364	0.011328271
53	*Pseudovorticella clampi*	444	0.013818001
54	*Pseudovorticella stilleri*	553	0.017210258
55	*Pyxicola annulata*	44	0.001369351
56	*Pyxicola constricta*	139	0.004325906
57	*Pyxicola eforiana*	86	0.00267646
58	*Pyxicola ligae*	52	0.001618324
59	*Pyxicola limbata*	115	0.003578987
60	*Thuricola folliculata*	107	0.003330014
61	*Thuricola gracilis*	193	0.006006473
62	*Thuricola incisa*	83	0.002583095
63	*Thuricola valvata*	84	0.002614216
64	*Vaginicola ceratophylli*	63	0.001960662
65	*Vaginicola compressa*	84	0.002614216
66	*Vaginicola elongata*	112	0.003485622
67	*Vaginicola gigantea*	76	0.002365243
68	*Vaginicola inclinata*	51	0.001587203
69	*Vaginicola ingentia*	64	0.001991784
70	*Vaginicola vestita*	120	0.003734595
71	*Vorticella alba*	1181	0.036754637
72	*Vorticella annulata*	564	0.017552596
73	*Vorticella bidulphae*	733	0.02281215
74	*Vorticella campanulate*	48	0.001493838
75	*Vorticella companula*	1480	0.046060002
76	*Vorticella longitricha*	525	0.016338852
77	*Zoothamnium duplicatum*	867	0.026982447
78	*Zoothamnium foissneri*	429	0.013351176
79	*Zoothamnium hentscheli*	436	0.013569028
80	*Zoothamnium kentii*	788	0.024523839
81	*Zoothamnium mengi*	418	0.013008839
82	*Zoothamnium mucedo*	242	0.007531433
83	*Zoothamnium parahentscheli*	450	0.01400473
84	*Zoothamnium pararbuscula*	730	0.022718785
85	*Zoothamnium plumula*	699	0.021754015
86	*Zoothamnium procerius*	188	0.005850865
87	*Zoothamnium ramosissimum*	161	0.005010581
88	*Zoothamnium sinense*	658	0.020478028
89	*Zoothamnopsis liui*	172	0.005352919
	Phyllopharyngia (3)		
90	*Aegyriana oliva*	152	0.004730487
91	*Chlamydodon mnemosine*	167	0.005197311
92	*Chlamydodon pedarius*	56	0.001742811

SI. No.	CILIATES	Abundance	Dominance index
	Scuticociliatia (3)		
93	*Cohnilembus verminus*	158	0.004917216
94	*Cohnilembus sp.1*	44	0.001369351
95	*Pleuronema wilberti*	55	0.001711689
	Spirotrichea (8)		
96	*Euplotes antarcticus*	328	0.010207892
97	*Euplotes eurystomus*	107	0.003330014
98	*Euplotes minuta*	1349	0.04198307
99	*Euplotes muscicola*	111	0.0034545
100	*Stentor roeseli*	314	0.00977219
101	*Tintinnopsis rotundata*	647	0.02013569
102	*Tintinnopsis sacculus*	43	0.00133823
103	*Tintinnopsis schotti*	244	0.007593676
	Stichotrichia (1)		
104	*Stichotricha secunda*	106	0.003298892
	Suctoria (11)		
105	*Acineta biloba*	726	0.022594299
106	*Acineta calkinsi*	32	0.000995892
107	*Acineta compressa*	212	0.006597784
108	*Acineta papillifera*	96	0.002987676
109	*Crossacineta annulata*	216	0.006722271
110	*Ephelota gemmipara*	724	0.022532055
111	*Ephelota mammillata*	708	0.022034109
112	*Ephelota plana*	29	0.000902527
113	*Ephelota turncata*	438	0.013631271
114	*Metacineta mystacina*	71	0.002209635
115	*Podophrya macrostyla*	774	0.024088136
			1

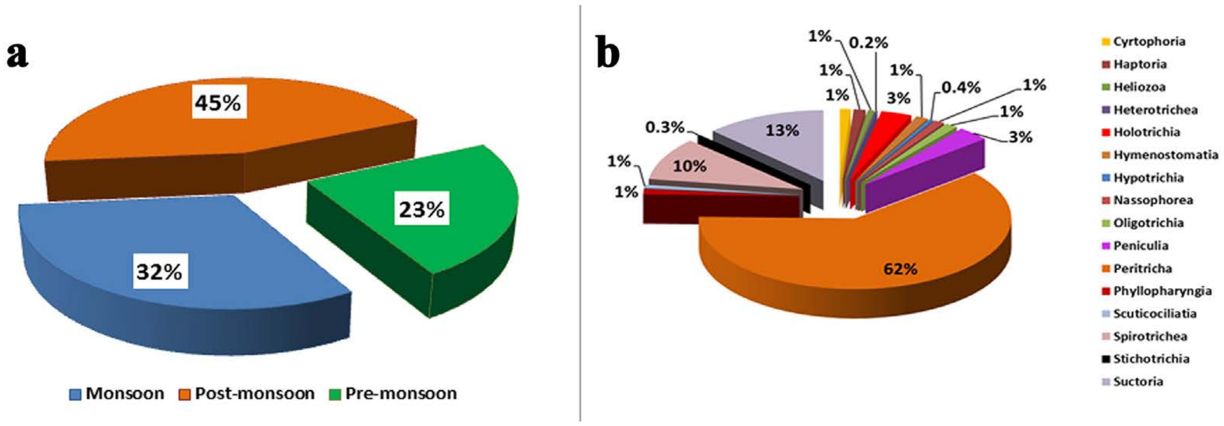

Figure 2. Percentage composition of the epibenthic ciliates on the study area (a); Percentage composition of the peritrich ciliates on the study area (b)

Table 3. Monthly distribution of different sub classes of ciliates during the study period

Sub class	Monsoon	Post-monsoon	Premonsoon	Total
Cyrtophoria	91	246	24	361
Haptoria	115	196	83	394
Heliozoa	44	123	41	208
Heterotrichea	38	16	19	73
Holotrichia	568	230	229	1027
Hymenostomatia	182	204	21	407
Hypotrichia	66	57	18	141
Nassophorea	122	184	41	347
Oligotrichia	89	283	45	417
Peniculia	261	557	254	1072
Peritricha	6293	8847	4638	19778
Phyllopharyngia	79	126	170	375
Scuticociliatia	93	100	64	257
Spirotrichea	807	1633	703	3143
Stichotrichia	45	37	24	106
Suctoria	1510	1592	924	4026
	10403	14431	7298	32132

where also the maximum evenness was observed in the case of Suctoria (0.8446) followed by Stichotrichia (0.8438) and Peritricha (0.8429). Minimum evenness was observed in the case of Heterotrichea (0.7001). The subclass Heterotrichea showed the maximum species richness value (5.537) and the minimum observed in the case of Peritricha (2.344) (Table 4).

Relationship between ciliates and environmental factors

Analysis of variance (ANOVA) showed the relationship between different subclasses of ciliates with environmental factors. The monthly variances in the distribution of ciliates were analysed by considering $p < 0.05$ as significant. The observed p-value (2.7E-106) is lesser than the significance level 0.05, and showed that most of the months have different means (Table 5).

Multivariate statistical analysis

Cluster analyzes clearly showed two general clusters: monsoon and post-monsoon period as one cluster, and the other cluster refers to the community structure in other months of the year (Figure 3). Detailed analysis revealed five distinct groupings, which appeared to reflect differences in the monthly distribution of ciliates in the Ayiramthengu mangroves ecosystem (Figure 3). Cluster 1 consisted of the month of June, July and August, which is the monsoon season, with high diversity and evenness of organisms. Cluster 2 consisted of the months of September and October, represented by the late monsoon period and early post-monsoon with unique climatic and physicochemical characteristics, with a similar population density. Cluster 3 consisted of the months of November, December and January with maximum population density and species number, Cluster 4 consisted of the months of February and March, with similar population density values as in Cluster 3. Cluster 5 consisted of the months of April and May with minimum population density, maximum richness and lowest diversity.

Canonical correspondence analysis (CCA)

CCA revealed sixteen distinct ciliate subclass groupings, which appeared to reflect differences in environmental characters within the Ayiramthengu mangroves (Figure 4). Eigen value of axis 1 and axis 2 itself explains 70.28 % of the relationship between environmental variables and ciliate communities. This result shows the close association between environmental variables and ciliate community.

In the tripod of CCA, vectors dissolved oxygen, temperature and sulphate have maximum length, and strongly influences the ciliate diversity. The ordination diagram of CCA revealed a strong negative loading of axis 1 with conductivity and salinity. Oligotrichia and Peniculia showed a negative association with axis 2 and illustrate the importance of conductivity and salinity

Table 4. Sub class wise diversity indices recorded during the study period

Index	Monsoon	Post-Monsoon	Pre-monsoon
Shannon H' Log Base 10.	0.637	0.635	0.588
Simpsons Diversity (D)	0.398	0.404	0.432
Margaleff M Base 10.	3.983	3.847	4.142
Shannon Evenness	0.529	0.527	0.488

Sub class wise diversity indices recorded during the study period

	Cyrtophoria	Haptoria	Heliozoa	Heterotrichea	Holotrichia	Hymenostomatia	Hypotrichia	Nassophorea	Oligotrichia	Peniculia	Peritricha	Phyllopharyngia	Scuticociliatia	Spirotrichea	Stichotrichia	Suctoria
Shannon H' Log Base 10.	0.85	1.00	0.95	0.84	0.98	0.87	0.89	0.94	0.86	0.96	1.01	1.01	0.97	1.00	1.01	1.01
Shannon Evenness	0.70	0.83	0.79	0.70	0.81	0.72	0.74	0.78	0.71	0.80	0.84	0.83	0.81	0.83	0.84	0.84
Simpsons Diversity (D)	0.17	0.10	0.12	0.14	0.11	0.15	0.13	0.13	0.17	0.12	0.10	0.10	0.11	0.10	0.09	0.10
Margaleff M Base 10.	3.94	3.89	4.31	5.53	3.38	3.89	4.70	4.00	3.83	3.35	2.34	3.91	4.23	2.87	5.10	2.80

Table 5. ANOVA table showing the monthly distributional variations of epibenthic ciliates in relation to hydrological parameters

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	31112248	23	1352706	82.20345	2.7E-106	1.570294
Within Groups	4344276	264	16455.59			
Total	35456524	287				

in their abundance and distribution. The lowest level of phosphate, nitrate, salinity and conductivity favors the influx of Spirotrichea, Nassophorea, Haptoria and Peritricha in mangrove leaf litter. Holotrichia reaches its maximum abundance in higher sulphate concentration and Stichotrichia reaches its maximum number in a moderate temperature range. Similarly, Phyllopharyngea reaches its maximum abundance in mid-low pH and Peniculia reaches its maximum number in moderate salinity condition. Oligotrichia reaches its maximum number in mid-low conductivity.

k-dominance

A k-dominance plot curve was drawn based on the community structure of ciliates in the Ayiramthengu mangrove ecosystem. In the present research, the data collected during the one-year survey was fed into the dominance plot (Figure 5). The highest dominance was seen in the month of May, where the ciliate assemblage had the highest diversity.

Discussion

The present investigation describes species composition, distribution, diversity and abundance of epibenthic ciliates from the sedimentary leaf litters of Ayiramthengu mangroves in relation to the abiotic factor prevailing in the ecosystem. Several studies have been conducted on the physicochemical analysis of mangrove water from various parts of India, and only a few studies have been reported on the taxonomy and community structure of ciliates from Indian mangroves (Dorothy *et al.* 2003; Nandi *et al.* 1993; Sarkar 2014; Chithra & Sunil Kumar 2015, 2018, 2019a, 2019b; Chithra *et al.* 2018). Monthly variations in the distribution of epibenthic ciliates in mangrove leaf litters were well documented by analyzing the level of significance through one-way ANOVA and CCA. The analysis of the data revealed that the epibenthic ciliates attach to the mangrove soil exhibit monthly variations according to the changes in hydrological conditions. Chen *et al.* (2009) had made a similar study in the tropical mangrove ecosystem and recorded that the changes in hydrological parameters determine the distribution of mangrove species in each habitat.

Mangrove soils are typically saline, anoxic, acidic, and frequently waterlogged. These soil properties directly affect the distribution of mangrove ciliates (Li *et al.* 2010). In the present study, conductivity and dissolved oxygen exhibited high negative correlations with ciliates. The conductivity and the dissolved oxygen frequently affect ciliates distribution (Ekelund & Ronn 1994; Opravilová & Hájek 2006; Ehrmann *et al.* 2012) and it affects ciliate species composition, as well as ciliate species diversity and density in the soil (Lara *et al.* 2016). Very few studies have been reported to investigate the ciliate community structure and physicochemical properties (Chao *et al.* 2006; Aguilera *et al.* 2006; Li *et al.* 2010; Ting *et al.* 2012; Fokam *et al.* 2015; Debastiani *et al.* 2016) and the information is still lacking from India.

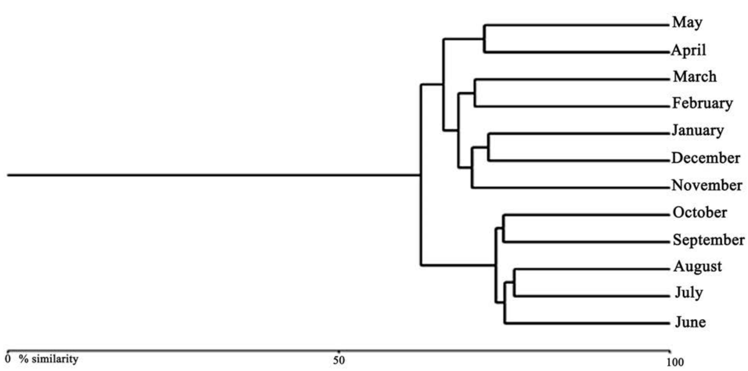

Figure 3. Cluster analysis dendrogram showing grouping of different months sampled during the study period

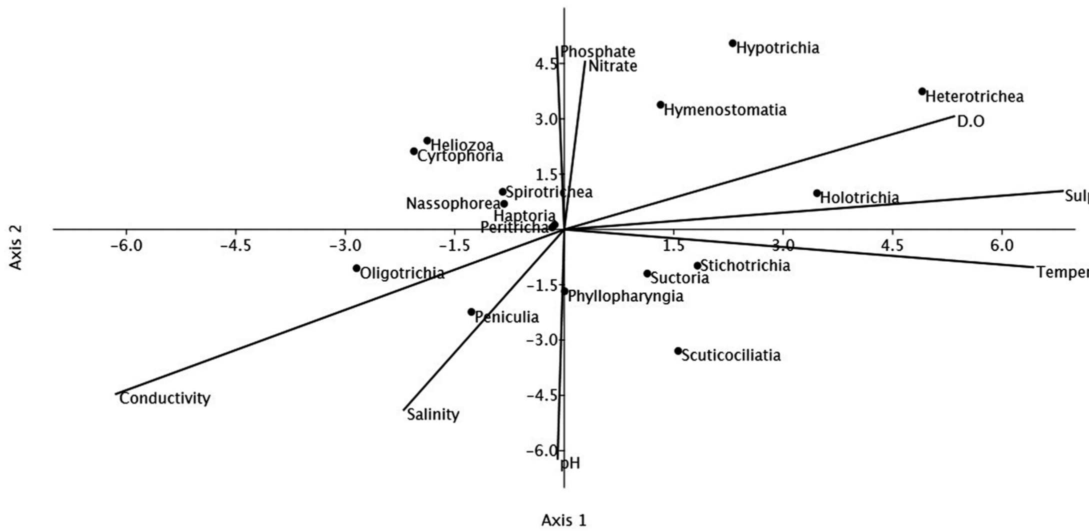

Figure 4. CCA plot similarity showing grouping of sub classes sampled during different seasons along with environmental factors

Peritrichs are the dominant ciliate subclass recorded from the study area. They are adapted to a wide range of habitats. Sedentary forms are abundant in marine, brackish and freshwater habitats attaching to a wide variety of substrates, plant, animals and inanimate (Warren 1983). Most of the peritrichs recorded from the study are attached to algal communities, mostly on Spirogyra. Interspecific factors such as growth pattern and cyst formation may also be highly significant in the case of peritrichs. Pseudo colony formation and solitary life in peritrichs may provide survival value by enhancing feeding efficiency, surface dominance, conjugation and predator avoidance (Spoon 1974).

Suctorians are the second dominant epibionts observed from the study habitat, similar to peritrichs, most of the suctorians are attached to a living or nonliving substratum. Suctorians are very common both in marine and freshwater environments (Foissner *et al.* 2007). They attach to their hosts directly or by using stalks, and they feed mainly on other ciliates (Verni & Guatieri 1997). While analyzing the species-wise abundance, *Vorticella companula* Ehrenberg, 1831 shows the maximum abundance and dominance index. During unfavourable conditions, they develop a posterior ring of cilia to become a telotroch. It separates away from the stalk and swims away to some favourable place where it develops a stalk and starts a normal life. The telotroch helps in its dispersal. *Vorticella companula* Ehrenberg, 1831 is a solitary species and typically form pseudocolonies because of telotrochs settling on the same substrate and very close to the mature organisms (Paranjape 1987). *Euplotes minuta* is the second most dominant species found to be dominant in the Ayiramthengu mangrove ecosystem. It is a highly diversified and cosmopolitan genus, with a large number of species that have been observed and investigated in all kinds of biotopes (Chen *et al.* 2013). *Zoothamnium* can be found in freshwater, brackish and marine waters between 0 – 8 meters deep and typically form a symbiotic relationship with a wide variety of animals, although some may be free-living and attaching to aquatic plants and inanimate substrates (Clamp *et al.* 2006).

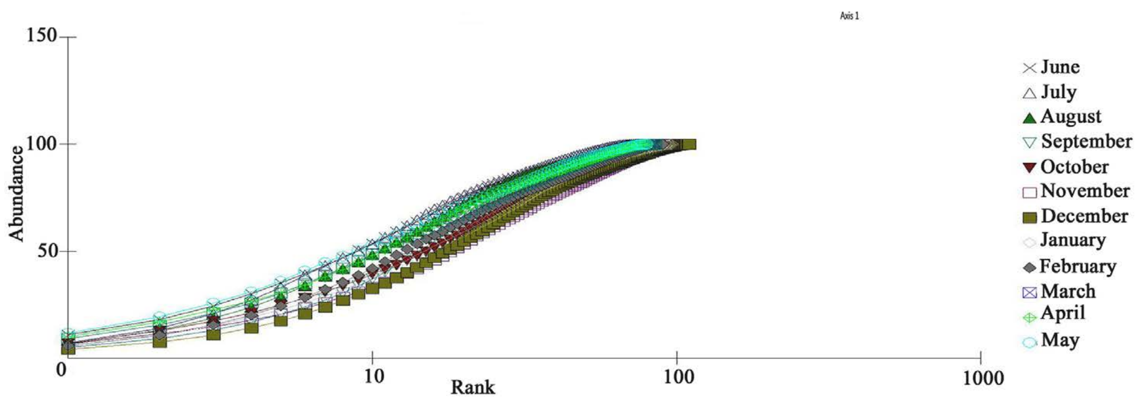

Figure5. K dominance plot showing the abundance of ciliates in study year

Analyzing the species composition of ciliates with respect to three seasons, post-monsoon represents the season with maximum species number followed by pre-monsoon. Thus, the dominant occurrence of the above-mentioned ciliates in each season may be closely associated with the species-specific environmental conditions that are required for encystment or excystment (Godhantaraman 2002). Especially in biological and other characteristic features of these ciliates support their dominance in the dynamic mangrove habitat. The present study reports the occurrence of 31 species of ciliates which were evenly distributed throughout the study period. It is because of their ability to switch to a different diet throughout the three seasons (Pierce & Turner 1992). During the study period, maximum Evenness, Shannon and Simpson diversity values were observed in monsoon and minimum recorded in pre-monsoon, due to the impact of the highest species richness in pre-monsoon. Similar studies were reported from the Vellar mangrove waters, where the total species richness of ciliates was highest in pre-monsoon and lowest during monsoon, as commonly observed in many marine coastal and estuarine waters (Capriulo & Carpenter 1983; Verity & Lagdon 1984; Verity 1987; Paranjape 1987; Burkill *et al.* 1995; Kamiyama & Tsujino 1996). Similar seasonal variations have also been reported in the Parangipettai coastal waters (Krishnamurthy & Santhanam 1975; Naidu *et al.* 1977; Mahajan & Nair 1965). Cluster analysis and k-dominance index also revealed the similar diversity, abundance and distribution pattern of ciliates during one-year study period. Studies on ANOVA records a station-wise heterogeneity during the one-year study period. Analysing the species diversity and evenness, Suctoria, Stichotrichia and Peritricha showed maximum diversity values, while Heterotrichea and Cyrtophoria the minimum diversity. Suctorians can be found in all types of water bodies on a wide diversity of hosts and substrates. Larger ciliate subclasses such as Heterotrichea and Cyrtophoria were found occasionally in the sampling area because the species of this subclass are more adapted to freshwater habitat (Mahajan & Nair 1965). The majority of these ciliates are commensals of various water invertebrates or vertebrates (Dovgal 2002). Taxonomic studies highlighting the distributional records of the above-mentioned groups were reported earlier from the study habitat (Chithra & Sunil Kumar 2015, 2018, 2019a, 2019b; Chithra *et al.* 2018). When analyzing the CCA, vectors for strong positively and negatively associated variable showed an acute angle and were close to each other. Values of positive and negative loading axis 1 and 2 of CCA helps to understand the effect of environmental variables to the distribution of mangrove ciliates. Analysing the ordination plot for CCA, vectors such as conductivity, temperature, sulphate and dissolved oxygen showed maximum length that, strongly influence ciliate composition and abundance. Habitat preference of ciliate was according to the changes in suspended materials and the changes favors the occurrence of bacterivores, algivorous and omnivorous ciliates in the study habitat (Foissner & Berger 1996).

Conclusion

The present study highlighted the role of physicochemical parameters in structuring the ciliate community in the Ayiramthengu mangrove ecosystem. Ciliates, generally considered among the most evolved and complex protozoa, which can perceive and react to a wide variety of environmental stimuli. The study also revealed that the ecological variations recorded from the mangrove habitat were very significant to make a change in the ciliate distribution. Meanwhile, in an aspect of ciliate as ecological indicators, evenly distributed community structure reflects the existence of a healthy food web in the present ecosystem. The literature survey revealed the fact that little work has been done on the diversity and distribution of ciliates on mangrove leaf litters of *Rhizophora apiculata*, and our current finding may provide some information for researchers in the field of mangrove ciliates.

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