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Community structure and diversity of epibenthic soil ciliates in a mangrove ecosystem on the southern coast of India

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Original Article

Abstract

The present study evaluated the diversity of epibenthic ciliates in the topsoil habitat of the Ayiramthengu mangrove ecosystem on the southwest coast of India. The study was carried out from June 2014 to May 2015. Ecological parameters like water temperature, pH, dissolved oxygen, conductivity, salinity, nitrate, phosphate and sulphate were also analyzed. The mangrove soil consisted of 84 species of ciliates belonging to 13 subclasses, namely Bicosidae, Cyrtophoria, Haptoria, Heterotrichea, Holotrichia, Nassophorea, Peniculia, Peritricha, Phyllopharyngea, Protocruziidia, Scuticociliatia, Spirotrichea and Suctoria. Among them, Spirotrichea occupied the dominant portion (43%) followed by Phyllopharyngea (24%) and Peniculia (9%). *Chlamydodon mnemosyne* was the most dominant ciliate (1989 no./cm^2) followed by *Paramecium brusaria* (814 no./cm^2). The effects of environmental parameters on the community structure of ciliates were evaluated. The maximum number of ciliates (2787 no./cm^2) and maximum Shannon diversity index (1.692) were recorded in January. The minimum number of ciliates (1803 no./cm^2) and minimum Shannon diversity were in May (1.477). Similarly, maximum species richness was recorded in May (25.79) and minimum in January (24.38). Canonical correspondence analysis explained the importance of dissolved oxygen, temperature, conductivity, salinity, sulphate, phosphate and nitrate in the distribution and abundance of ciliates. Availability of food, rate of precipitation and the variations in physicochemical characteristics of the mangrove were the factors that determined the distribution of epibenthic ciliates.

Keywords: *Ciliates, epibenthic organisms, Ayiramthengu, spirotrichea*

Introduction

Epibenthic communities are the most important biotic factor that influences mangrove growth (Ellison *et al.,* 1996). Mangrove soil is a combination of silt and sand that supports a diverse faunal and floral life. Meanwhile, the epibenthic communities inhabiting the soil surface directly influence the mangrove growth. Higher species richness and substantial biomass of epibenthic protozoans are critical for maintaining the structure and functioning of mangrove ecosystems (Cannicci *et al.,* 2008; Rogerson and Gwaltney, 2000; Godhantaraman, 2002). Increasing microbial activity in mangrove wetland (Bonkowski, 2004) or by changing the composition of microbial community structure (Clarholm, 1981; Vickerman, 1992; Liao *et al*., 2009), benthic protozoa play a significant role in the decomposition (Dorothy *et al*., 2003). Meanwhile, benthic protozoans occupy an essential position in the mangrove benthic food web and are usually consumed by meiofauna or macrobenthic fauna (Alongi, 1988; Biswas *et al*., 2013). The diverse assemblage of protozoans was recorded in mangrove surface soil and which plays a significant role in mineralization, regulating algal and bacterial populations and controlling diseases by feeding on pathogens (Hoorman, 2011).

Soil ciliates are one of the important epibenthic communities that showed a characteristic role in nutrient recycling (Christoffersen and González, 2003; Janssen and Heijmans, 1998). It also accelerates the mineralization processes of soil nutrients (Ekelund and Ronn, 1994). The prominent effect brought by soil ciliates may be important in mangrove plant nutrition; on another hand, the growth of plants may also significantly affect the soil quality and ciliate community (Li *et al.,* 2010). Moreover, they are good bioindicators of soil environments (Foissner, 1987, 1997). Components of the mangrove benthos have been extensively studied by many authors (Kathiresan and Bingham, 2001; Lovelock, 2008; Tatoi *et al.,* 2013; Kardol *et al.,* 2006; Frouz *et al.,* 2008; Dickie *et al.,* 2011). The present study aims to investigate the species composition, diversity and density of soil ciliates concerning the seasonal hydrological parameters.

Material and methods

Study area

The study area was located in Ayiramthengu mangrove situated (lat. 9^{o} $6^{'}$ to 9^{o} $8^{'}$ N long.76^{o} $28^{'}$ to 76^{o} $29^{'}$ E) in Kollam District of Kerala, a part of Kayamkulam Estuary, which is the narrow stretch of tropical backwater on the southwest coast of India (Fig. 1). The area covers about 20 acres, and most of the inner regions of mangrove forest patches were left untouched by humans. The mangrove forest shares boundaries with three panchayats in Karunagapally Taluk of Kollam District. They are Clappana, Alappad, and Devikulangara. Seventy per cent of the mangrove forest areas are within the Clappana and Alappad Panchayats, while the rest are in with Devikulangara Panchayat. Two streams and a canal enter the lake during the rainy season, which links the rivers Pamba and Achankoil. The estuary opens into the Arabian Sea at Valiazheekal. 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 and clear airtight container. The temperature of the water was studied at the site itself. Dissolved oxygen is fixed *in situ*, samples for the analysis of other parameters such as pH, conductivity, salinity, nitrate, phosphate, and sulphate were carried to the laboratory, and the experiments were done on the day itself. The temperature was measured using a portable mercury thermometer (-20^{o} -110^{o} C). The pH of the sample was analyzed using a pH meter model (MK 6). A conductivity meter (Model EQ 660A) was used to analyse the presence of electrolytes in the sample. Dissolved oxygen was determined by the Winkler method, Chloride by Mohr's method, Nitrate by phenol di-sulphonic acid method, phosphate by stannous chloride method and sulphate by the Turbidimetric method. All the above-mentioned analysis was carried out according to the standard method prescribed in APHA (1998).

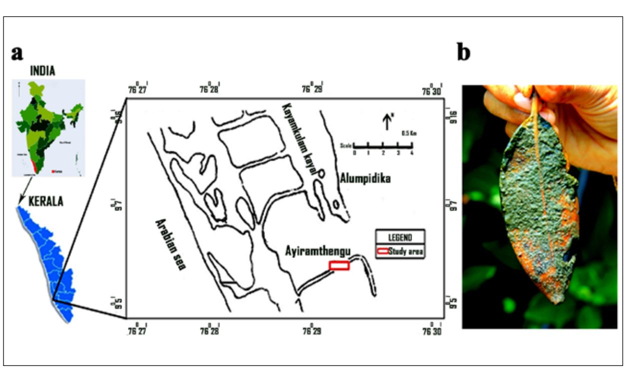

Fig. 1. Map of Ayiramthengu mangrove ecosystem

Collection, identification and enumeration of ciliates

The fresh surface soil layer was collected for identification and enumeration of epibenthic ciliates. Quantitative analysis of soil epibenthic organisms was done using the "non- flooded petri dish method" described by Foissner (1987). 100 g of fresh soil was transferred to a petri dish and diluted with 20 ml of mangrove water but not flooded. After settling the sample, added 1 ml supernatant solution into the Sedgwick rafter-counting chamber for enumeration. During counting, it is also convenient to make a preliminary species list, which may help to select the counting strategy. If the distribution is visually uneven, the sample should be discarded. For uniform colony count, the number of cells in the colony was multiplied with factor 4. For non-uniform colonies cells that cover an area are multiplied by the total grids occupied by that colony. All other non-colonial unicellular species were counted in each chamber. (Moncheva *et al.*, 2010). Biometrical characteristics of the epibenthic organisms collected from the mangrove soil were carefully examined under live conditions. Live cell observations were done by the method described by Foissner (2014). The preferable method is thus to select and pick cells using an appropriate micropipette and place them on a slide in a very small drop of fluid. Excess water is removed and the slide is covered with a coverslip after applying petroleum jelly at the corners of the coverslip. The petroleum jelly corners are pressed with a mounted needle until the ciliates become slightly squeezed between the slide and the coverslip. For detailed analysis, preserve it in a 3% formalin solution. For further details, samples were subject to the silver carbonate technique proposed by Fernandez-Galiano (1976). For the identification and classification of phylum Ciliophora, three main classification systems are used. Corliss (1979), which is based on morphological characters and the other by Lynn (2008), which is inferred mainly from ultra-structural characters, and the species identified were cross-referred with the website for World Registrar of Marine Species (WoRMS). Sampling was done in alternate months in a year (June 2014 to May 2015) to analyze the composition and distribution of epibenthic organisms. Diversity (Shannon and Simpson), Richness (Margaleff) and Evenness (Shannon) were used to analyse the diversity and distribution pattern of ciliates in each season. The relationship between environmental parameters with ciliate subclasses was analysed by performing a one-way ANOVA. The seasonal distribution of epibenthic ciliates was well documented by using correlation analysis CCA and cluster analysis. The data collected were analyzed using statistical software packages for Social Sciences (SPSS) software version 16.0 and Biodiversity pro (Mc Aleece *et al.*, 1997).

Results

Environmental factors

The overview of hydrological parameters analysed during the study period is presented in Table 1. Temperature and dissolved oxygen recorded their minimum values in post-monsoon, temperature exhibited its maximum values in pre-monsoon, and dissolved oxygen its maximum values in monsoon. pH, conductivity and salinity recorded their minimum values in pre-monsoon and maximum values in pre-monsoon. Minimum values for nitrate, phosphate, and sulphate were in pre-monsoon and maximum values in monsoon. The average annual precipitations relative to the baseline periods of 2014 June–2015 May are presented in Table 2. The data reveals that the maximum average rainfall (374.97 mm) was recorded during the monsoon and the minimum (100.47 mm) was recorded post-monsoon.

Table1. Overview of hydrological parameters

No.	Hydrological parameter	Minimum	Maximum
1	Temperature	26.5° C	32.7° C
2	pH	6.2	8.2
3	Conductivity	0.2 s/m	37.4 s/m
4	Dissolved oxygen	0.8 mg/l	7.6 mg/l
5	Salinity	10 ppm	25.2 ppm
6	Nitrate	0.09 mg/l	7.74 mg/l
7	Phosphate	2.26 mg/l	2.87 mg/l
8	Sulphate	7.94 mg/l	295.18 mg/l

Table 2. Average monthly rainfall (mm) during the study period

Year	Month	Rainfall	No. of rainy days	Season	Average rainy days	Average rainfall (mm)
	June	346.3	19			
	July	488.6	24	Monsoon	17.25	374.97
	August	507.1	14			
2014	September	157.9	12			
	October	337.4	17			
	November	42.7	8	Post monsoon	7	100.47
	December	20.0	3			
	January	1.8	Nil			
	February	3.7	Nil			
2015	March	136.6	4	Pre Monsoon	5.5	183.4
	April	321.8	9			
	May	271.5	11			

Species composition, dominance index and density

The mangrove soil consisted of 84 species of epibenthic ciliates (Table 3) belonging to 13 subclasses namely Bicosidae, Cyrtophoria, Haptoria, Heterotrichea, Holotrichia, Nassophorea, Peniculia, Peritricha, Phyllopharyngea, Protocruziidia, Scuticociliatia, Spirotrichea and Suctoria. Among them, Spirotrichea dominated (43%) followed by Phyllopharyngea (24%) and Peniculia (9%) (Fig. 2). The free-living ciliate *Chlamydodon mnemosyne* was the most dominant ciliate (1989 no./cm^2) showed the maximum dominance index value of 8.14 followed by *Paramecium brusaria* (814 no./cm^2) 6.04, *Euplotes minuta* (744 no./cm^2) 5.52, *Chlamydodon obliquus* (571 no./cm^2) 4.24, *Acineta tuberosa* (470 no./cm^2) 3.49, *Euplotes antarcticus* (397 no./cm^2) 2.95, *Stentor roeseli* (394 no./cm^2) 2.92, *Aegyria olive* (378 no./cm^2) 2.80, *Ephelota truncata* (343 no./cm^2) 2.54, *Tintinnopsis cylindrica* (300 no./cm^2) 2.22 *Tintinnopsis rotundata* (255 no./cm^2) 1.89 and *Codonellopsis schabi* (251 no./cm^2) 1.86 (Table 3, Fig. 3). Maximum ciliate density was observed in post-monsoon (37%) and minimum observed in pre-monsoon (29%) (Fig. 4). The seasonal distribution of dominant ciliates is presented in Fig. 5. Distribution pattern is different in each species, *Chlamydodon mnemosyne* is the dominant species that show maximum abundance in premonsoon, a similar pattern of distribution was shown by *Aegyria olive* and *Ephelota truncata. Paramecium brusaria, Euplotes minuta, Chlamydodon obliquus, Stentor roeseli* and *Codonellopsis schabi* was maximum in monsoon. *Acineta tuberosa, Euplotes antarcticus, Tintinnopsis cylindrica* and *Tintinnopsis rotundata* showed their maximum abundance in post-monsoon. Among the 12 dominant species, seven of them included the sub-class spirotrichea, one belonging to heterotrichea and two of them included the subclass suctoria. A total of 13457 no. /cm^2 ciliates were counted during the study period and the maximum number of ciliates

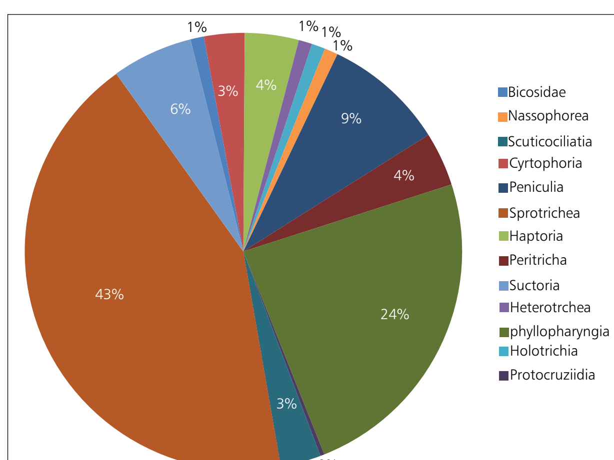

Fig. 2. Percentage composition of the epibenthic ciliates in the study area

Table 3. Species composition, bimonthly population density (no./cm²) and dominance index of epibenthic ciliates.

Species	July	Sep	Nov	Jan	Mar	May	Total	Dominance Index
Ciliates								
Acineria incurvata	38	20	21	14	9	-	102	0.75796983
Acineta tuberosa	45	95	103	74	80	73	470	3.492606079
Aegyria oliva	70	34	62	50	85	77	378	2.808947016
Amphileptus eigneri	-	-	6	19	-	-	25	0.185776919
Amphileptus houi	17	7	6	21	21	7	79	0.587055064
Amphisiella milnei	-	17	22	127	30	5	201	1.493646429
Blepharisma sinuosum	25	33	14	14	14	16	116	0.862004905
Chaenea teres	-	6	11	17	13	6	53	0.393847068
Chlamydodon mnemosyne	204	267	405	327	402	384	1989	14.78041168
Chlamydodon obliquus	113	129	135	92	35	67	571	4.243144832
Chlamydodon triquetrus	-	-	37	21	42	7	107	0.795125214
Chlamydonella pseudochilodon	-	6	16	10	16	19	67	0.497882143
Chlamydonellopsis calkinsi	-	-	9	12	24	6	51	0.378984915
Cinetochilum ovale	25	18	30	26	46	67	212	1.575388274
Codonellopsis schabi	102	72	-	66	11	-	251	1.865200268
Condylostoma curva	24	39	-	-	-	-	63	0.468157836
Condylostoma granulosum	-	-	2	12	15	7	36	0.267518763
Cothurnia kiwi	28	24	23	24	32	35	166	1.233558743
Didinum gargantua	5	10	6	13	7	9	50	0.371553838
Dysteria brasiliensis	33	20	39	37	8	3	140	1.040350747
Dysteria derouxi	-	6	14	18	2	2	42	0.312105224
Dysteria procera	34	21	31	4	1	-	91	0.676227985
Dysteria proraefrons	46	6	-	-	-	-	52	0.386415992
Ephelota truncata	47	72	46	54	36	88	343	2.54885933
Epistylis coronate	-	-	8	21	6	-	35	0.260087687
Euplotes antarcticus	53	84	90	70	38	62	397	2.950137475
Euplotes encystylus	45	16	14	29	25	29	158	1.174110129
Euplotes eurystomus	-	-	-	30	69	8	107	0.795125214
Euplotes minuta	175	102	60	214	105	88	744	5.528721112
Euplotes parabalteatus	78	46	12	12	-	-	148	1.099799361
Euplotes parawoodruffi	25	10	-	-	-	-	35	0.260087687
Euplotes raikovi	20	15	20	53	28	12	148	1.099799361
Euplotes rariseta	-	-	3	12	1	-	16	0.118897228
Euplotes sinicus	19	34	81	50	4	9	197	1.463922122
Euplotes vannus	28	41	32	62	37	34	234	1.738871963
Eutintinnus apertus	7	5	-	-	-	-	12	0.089172921
Favella ehrenbergi	-	-	37	80	-	-	117	0.869435981
Frontonia canadensis	22	39	37	21	18	24	161	1.196403359
Frontonia didieri	-	-	15	12	11	9	47	0.349260608
Frontonia multinucleata	-	-	-	9	6	-	15	0.111466151
Frontonia sinica	18	22	28	23	25	29	145	1.077506131
Frontonia subtropica	-	-	8	37	19	-	64	0.475588913
Hartmannula derouxi	12	11	35	7	-	-	65	0.48301999

Species	July	Sep	Nov	Jan	Mar	May	Total	Dominance Index
Kentrophyllum verrucosum	10	4	6	4	6	8	38	0.282380917
lacrymaria marina	6	6	7	9	18	5	51	0.378984915
Loxophyllum helus	-	-	12	6	-	-	18	0.133759382
Loxophyllum jinni	21	3	6	11	7	11	59	0.438433529
Loxophyllum rostratum	6	6	-	-	-	3	15	0.111466151
Loxophyllum shini	-	-	10	10	12	3	35	0.260087687
Metopus sp.	21	34	44	15	19	10	143	1.062643977
Nassula ornata	29	25	27	18	28	31	158	1.174110129
Paramecium brusaria	157	199	99	76	118	165	814	6.048896485
Placidae sp.	16	14	11	9	8	10	68	0.50531322
Placus salinus	31	31	25	36	21	34	178	1.322731664
Pleuronema crassum	18	8	8	-	-	-	34	0.25265661
Pleuronema grolierei	46	9	22	23	-	-	100	0.743107676
Protocruzia contrax	17	-	-	15	8	12	52	0.386415992
Saprodinium dentatum	4	10	13	15	11	17	70	0.520175373
Spirostomum teres	8	15	13	10	11	13	70	0.520175373
Spirostomum minus	21	3	15	11	8	4	62	0.460726759
Stentor coeruleus	15	34	31	60	35	7	182	1.352455971
Stentor roeseli	85	77	48	63	62	59	394	2.927844245
Stentor sp.	-	-	-	24	-	-	24	0.178345842
Thuricola innixa	-	-	-	15	10	-	25	0.185776919
Thuricola kellicottiana	5	-	-	6	-	-	11	0.081741844
Tintinnopsis cylindrica	55	37	30	81	59	38	300	2.229323029
Tintinnopsis failakkiaensis	-	-	-	62	15	5	82	0.609348295
Tintinnopsis fimbriata	-	20	34	50	54	67	225	1.671992272
Tintinnopsis gracilis	68	88	14	8	27	28	233	1.731440886
Tintinnopsis nucula	-	-	-	17	22	20	59	0.438433529
Tintinnopsis parvula	52	5	-	-	-	-	57	0.423571375
Tintinnopsis radix	36	40	40	36	-	-	152	1.129523668
Tintinnopsis rotundata	43	23	17	92	75	5	255	1.894924575
Tintinnopsis sacculus	53	28	3	1	-	-	85	0.631641525
Tintinnopsis schotti	-	13	75	56	-	-	144	1.070075054
Tintinnopsis tentaculata	13	26	-	-	-	2	41	0.304674147
Tintinnopsis tocantinensis	20	25	33	52	12	3	145	1.077506131
Tintinnopsis undella	22	15	16	37	15	10	115	0.854573828
Tintinnopsis acuminate	18	23	-	-	-	-	41	0.304674147
Tintinnopsis ampla	32	28	24	32	19	27	162	1.203834436
Trochilia petrani	11	-	-	3	-	2	16	0.118897228
Vorticella companula	32	27	10	9	10	13	101	0.750538753
Vorticella kenti	13	11	14	11	13	9	71	0.52760645
Zoothamnium commune	21	8	15	10	18	-	72	0.535037527
	Monsoon		Post monsoon		Pre-monsoon			
	4585		5027		3845		13457	100
Number of species	67		76		66			

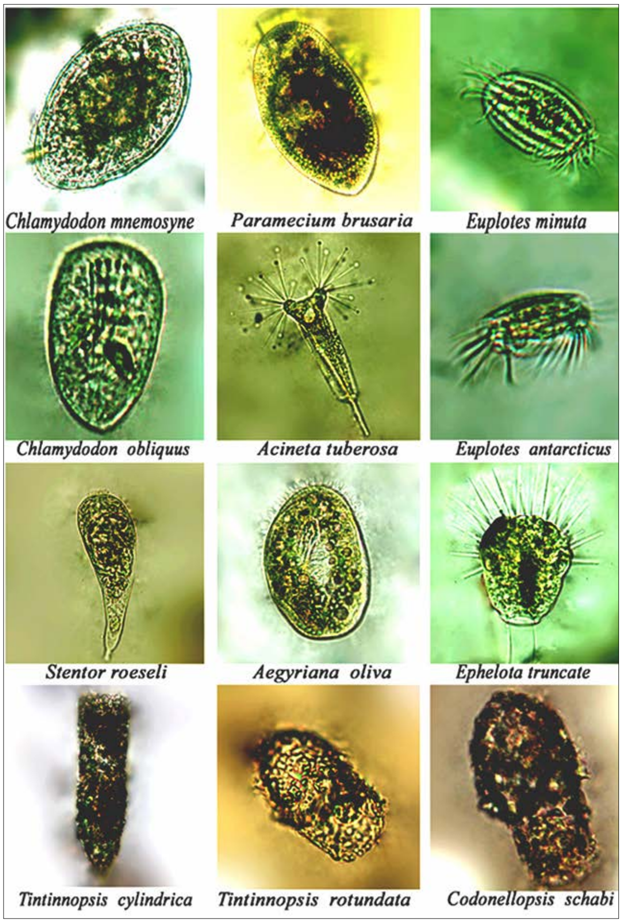

Fig. 3. Dominant ciliates recorded during the study period

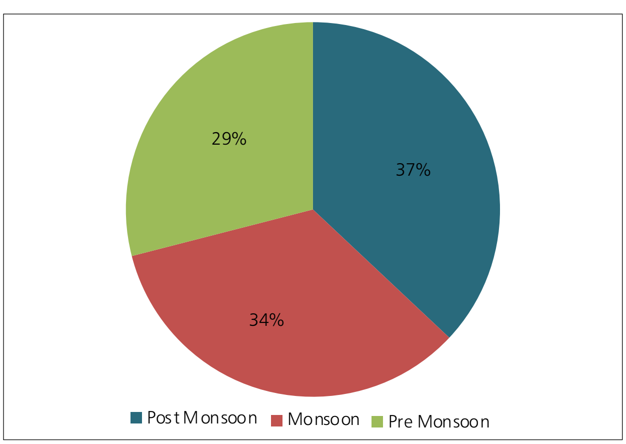

Fig. 4. Density-wise distribution of epibenthic ciliates in the study area

was recorded during post-monsoon (5027 no/cm^2) and the minimum recorded during pre-monsoon (3845 no./cm^2). Analyzing the species distribution pattern 68 species were recorded during monsoon, 78 during post-monsoon and 67 during pre-monsoon. Forty-one species out of a total of 85 were distributed in all the months surveyed.

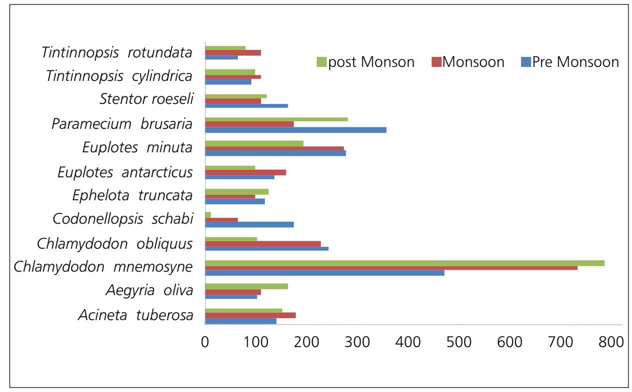

Fig.5. Seasonal distribution of dominant ciliates

Diversity indices

The maximum number of ciliates (2,787 no. /cm^2) and maximum Shannon diversity index (1.692) were recorded in January and the minimum number of ciliates (1,803 no. / cm^2) and minimum Shannon diversity was observed in May (1.477) (Table 4). Margaleff richness index analyzes the variations in species richness and the values are normally higher in an area having lower dominance. The maximum value was recorded in May (25.79) and the minimum was recorded in January (24.38) (Table 4). Shannon evenness is a commonly used diversity tool, which analyses how numerically equal, is the community. The maximum evenness (0.854) was observed in January and the minimum was recorded in May (0.745) (Table 4).

Table 4. Diversity, richness and evenness of epibenthic ciliates on mangrove soil

Index	July	September	November	January	March	May
Shannon H' Log Base 10.	1.647	1.612	1.609	1.692	1.587	1.477
Margaleff M Base 10.	24.9	25.099	25.127	24.382	25.377	25.799
Shannon Evenness	0.831	0.813	0.812	0.854	0.801	0.745

Relationship between ciliates and environmental factors

Analysis of variance (ANOVA) showed the relationship between different subclasses of ciliates with environmental factors. The distribution variance of ciliates in different months was performed considering $p < 0.05$ as significant. Analyzing the monthly data of soil ciliates by one-way ANOVA, the p-value is 3.32E-15, lesser than the significance level of 0.05, and most of the months had different means (Table 5).

Correlations revealed the importance of hydrological parameters on the distribution of epibenthic ciliates on mangrove soil (Tables 6, 7 and 8). During monsoon, hydrological parameters did not affect significantly the distribution of ciliates in mangrove

Table 5. ANOVA table showing the seasonal distributional variations of epibenthic ciliates concerning hydrological parameters

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	52983121	8	6622890	163.4866	3.32E-15	2.510158
Within Groups	729185.2	18	40510.29			
Total	53712306	26				

Table 6. Correlation matrix between epibenthic ciliates of mangrove soil and environmental parameters in monsoon

Parameters	Temperature	pH	Conductivity	D.O	Salinity	Nitrate	Phosphate	Sulphate	Ciliates
Temperature	1								
pH	-0.059								
Conductivity	0.518	0.229							
D.O	0.289	0.535	.678*						
Salinity	0.23	0.265	0.571	0.617					
Nitrate	-0.239	0.284	0.276	0.241	.724*				
Phosphate	0.592	-0.264	-0.247	-0.41	-0.502	-.633*			
Sulphate	0.56	-0.205	-0.293	-0.392	-0.519	-.650*	.987**		
Ciliates	-0.189	-0.283	-0.437	-0.408	-0.471	-0.339	0.312	0.265	1

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

Table 7. Correlation matrix between epibenthic ciliates of mangrove soil and environmental parameters in post-monsoon.

Parameters	Temperature	pH	Conductivity	D.O	Salinity	Nitrate	Phosphate	Sulphate	Ciliates
Temperature	1								
pH	.923**	1							
Conductivity	.798**	.751*	1						
D.O	.658*	.638*	.730*	1					
Salinity	.650*	.774**	.731*	.674*	1				
Nitrate	-.645*	-0.527	-0.486	-0.224	-0.354	1			
Phosphate	-0.469	-0.591	-0.4	-.800**	-0.629	-0.134	1		
Sulphate	.912**	.821**	.637*	0.6	0.463	-.769**	-0.355	1	
Ciliates	-0.488	-0.521	-.868**	-.780**	-.658*	0.078	0.549	-0.295	1

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

Table 8. Correlation matrix between epibenthic ciliates of mangrove soil and environmental parameters in pre-monsoon.

Parameters	Temperature	pH	Conductivity	D.O	Salinity	Nitrate	Phosphate	Sulphate	Ciliates
Temperature	1								
pH	.743*	1							
Conductivity	.727*	0.612	1						
D.O	.761*	.664*	0.579	1					
Salinity	-0.331	-0.376	0	-0.38	1				
Nitrate	-.744*	-0.631	-.689*	-.680*	-0.031	1			
Phosphate	-.753*	-0.466	-.767**	-.642*	0.342	0.493	1		
Sulphate	.929**	.643*	.889**	.766**	-0.261	-.760*	-.851**	1	
Ciliates	-.743*	-0.532	-0.306	-.740*	0.452	0.369	.700*	-0.6	1

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

soil (Table 6). During post-monsoon conductivity (-0.868) and dissolved oxygen (-0.78) exhibited a high negative correlation with ciliates and a low negative correlation with salinity (-.658) (Table 7). During premonsoon temperature (-0.743) dissolved oxygen (-0.74) and phosphate (-.7) exhibit a low negative correlation with ciliates (Table 8).

The CCA revealed twelve distinct ciliate species, which appeared to reflect differences in environmental characteristics within the Ayiramthengu mangroves (Fig. 6). Eigenvalue of axis 1 and axis 2 itself explains 76.91% of the relationship between environmental variables and ciliate communities. This result showed the close association between environmental variables and the ciliate community. In the triplot of CCA, vectors temperature, sulphate, dissolved oxygen and pH had the maximum length and strongly influence the ciliate diversity. The ordination diagram of CCA revealed a strong negative loading axis of 1 with nitrate (r= -0.8556), phosphate (r= -0.8869) and sulphate (r= -0.9782). *Codonellopsis schabi, Chlamydodon obliquus, Euplotes minuta* and *Euplotes antarcticus.* showed a negative association with axis 1 and illustrate the importance of nitrate, phosphate and sulphate on their abundance and distribution. pH (r=0.9616, conductivity (r=0.9595) and salinity (0.9331) have positive loading with axis 1. *Chlamydodon mnemosyne, Acineta tuberosa, Tintinnopsis rotundata, Ephelota truncata* and *Aegyria olive* showed a positive association with axis 1 and illustrate the significance of pH, conductivity and salinity in the distribution and abundance of ciliates. CCA triplot on axis 2 revealed a strong positive correlation with temperature (r= 0.9866) and dissolved oxygen (r= 0.971). *Paramecium brusaria* and *Stentor roeseli* are positively correlated with axis 2 and which clearly defines the role of nitrate and temperature in their abundance distribution.

Multivariate analysis

Cluster analyses clearly showed three general clusters: monsoon and early post-monsoon period as one cluster, and the other

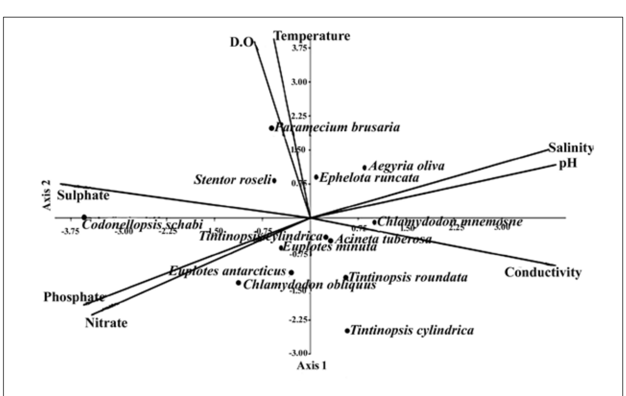

Fig. 6. CCA plot similarity showing the grouping of dominant ciliates sampled during different seasons along with environmental factors.

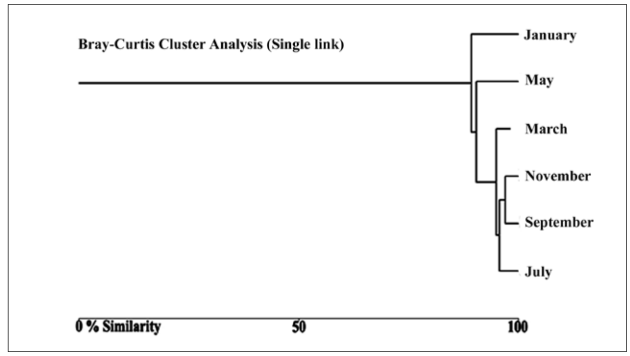

Fig. 7. Cluster analysis dendrogram showing the grouping of different months sampled during the study period

cluster refers to the community structure in other months of the year (Fig. 7). Detailed analysis revealed four distinct groupings, which appeared to reflect differences in the monthly distribution of ciliates in the Ayiramthengu mangroves ecosystem. Cluster 1 consisted of July, September and November, which is the monsoon and early post-monsoon season, with high diversity and evenness of organisms. Cluster 2 consisted of March, represented by the early pre-monsoon period with unique climatic and physicochemical characteristics, with a similar population density. Cluster 3 consisted of May with minimum population density and species number, Cluster 4 consisted of January with maximum population diversity.

Discussion

Several studies have been conducted on physical and chemical analysis of mangrove water from various parts of India (Vijaya Kumar and Kumara, 2014; Ayyadurai *et al.*, 2017; Dattatreya *et al.*, 2018), and only a few studies have reported the taxonomy and community structure of ciliates from Indian mangroves (Nandi, 1993; Dorothy *et al.*, 2003; Sarkar, 2014; Chithra and Sunil Kumar, 2015, 2018, 2019a, 2019b; Chithra *et al.*, 2018). Maximum temperature (37.2 °C) was observed during pre-monsoon and the minimum (26.5 °C) during post-monsoon. During pre-monsoon temperature showed an impact on the distribution of the ciliate community. Surface temperature in mangrove water is influenced by many ambient conditions. These elements include the intensity of solar radiation, rate of evaporation and freshwater influx (Sarvanakumar *et al.*, 2008; Prabu *et al.*, 2008). Most of the months surveyed maintain a slightly alkaline pH. Previous observation from mangroves supports the above findings (Praseetha and Rajani, 2015; Satheeshkumar *et al.*, 2011). Ayiramthengu ecosystem supports a huge diversity of molluscan and arthropod populations (Amina *et al.*, 2013). Dissolved calcium shells of these organisms make the water alkaline. Conductivity showed an impact on the distribution of ciliates in the Ayiramthengu mangrove ecosystem. Maximum conductivity (37.4 s/m) was recorded during pre-monsoon and minimum values in monsoon. During monsoon, Ayiramthengu experienced heavy rainfall and surrounding low-lying areas were flooded. Due to the heavy rainfall and minimum dissolved solids, the lowest conductivity values were recorded during monsoon and the maximum recorded during pre-monsoon due to heavy evaporation. Rita and Ramanathan (2008) made a similar finding in Bhitarkanika mangroves, Orissa. Corresponding to conductivity, changes in rain patterns also affect the dissolved oxygen content in mangrove water and which in turn affects the distribution of mangrove ciliates. Minimum values of dissolved oxygen were observed in post-monsoon (0.8 mg/l) and maximum observed in monsoon (7.6 mg/ ml). Saravanakumar *et al.* (2008) made a parallel finding that the changes in dissolved oxygen in mangrove water are due to the combined effect of heavy rain and wind. Salinity also showed an impact on the distribution of ciliates on mangrove soil. Maximum salinity (25.2 ppt) was observed during pre-monsoon and the minimum (10.0 ppt) was recorded during monsoon. According to Aksornkoae (1993) increased evaporation rate, seasonal differences in rainfall and lean summer flows are the factors that increase the mangrove salinity.

During the monsoon, phosphate, nitrate, and sulphate level in the water increase considerably due to the entry of seawater and land drainage. Manju *et al.* (2012) reported a related finding in the mangrove ecosystems along the northern coasts of Kerala. Salt-water intrusion with limited nitrate concentration, high rate of planktonic photosynthetic activities, decreased runoff, adsorption to sediments, and utilization by phytoplankton may be the reason for the low value of nitrate (Ramakrishnan *et al.*, 1999). Agricultural runoff from the river, sewage, increased utilization of cement for construction purposes, and aquaculture activities were the factors responsible for the increasing concentration of sulphate in mangrove water (Rahman *et al.*, 2013). Rainfall is the most important cyclic phenomenon, which brings important changes in the hydrological characteristics of a tropical mangrove ecosystem (Dattatreya *et al.,* 2018). During the study period, the maximum number of species and density recorded during post-monsoon (Table 3) experienced a minimum percentage of rainfall (Table 2). So, the maximum ciliate diversity was observed during post-monsoon and the minimum during pre-monsoon. Temperature fluctuations and concentrations of available nutrients are the factors that determine the distribution of ciliates in an ecosystem (Gligora *et al.*, 2007). Availability of food, rate of precipitation, and variations in physical and chemical characteristics of mangrove water were the factors that determine the distribution of epibenthic ciliates. Ciliates depend on bacteria as their feed. Dorothy (2003) stated that the abundance of ciliate in a mangrove ecosystem was due to the influence of the bacterial community as a feed for the dependent community. Later, Satheesh Kumar *et al.* (2011) also reported that post-monsoon represents the season, having the highest diversity, and monsoon shows the lowest. Seasonal variations in the distribution of epibenthic ciliates in mangrove soil were well documented by analyzing the level of significance through one-way ANOVA. The analysis of the data revealed that the epibenthic ciliates attached to the mangrove soil exhibit seasonal variations according to the changes in hydrological conditions. Cluster analysis and k-dominance index also revealed similar diversity, abundance and distribution patterns of ciliates during the study period. Chen *et al.* (2009) made a similar study on 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, the conductivity and dissolved oxygen exhibited high negative correlations with ciliates. The conductivity and the dissolved oxygen frequently affect ciliate distribution (Ekelund and Ronn, 1994; Opravilová and 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 correlation studies have been reported to investigate the ciliate community structure and physicochemical properties (Chao *et al.*, 2006; Aguilera *et al.*, 2006; Li *et al.*, 2010a; Li *et al.*, 2010b; Ting *et al.*, 2012; Fokam *et al.*, 2015; Debastiani *et al.*, 2016) and the information is still lacking from India. Monthly variations in the distribution of epibenthic ciliates in mangrove soil 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) made a similar study on the tropical mangrove ecosystem and recorded that the changes in hydrological parameters determine the distribution of mangrove species in each habitat. These soil properties directly affect the distribution of mangrove ciliates (Li *et al.*, 2010). In the present study, vectors temperature, sulphate, dissolved oxygen and pH have the maximum length and strongly influence the ciliate diversity. These factors frequently affect ciliate distribution (Li *et al.*, 2010; Ekelund and Ronn, 1994; Opravilova and Hajek, 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.

Among the ciliates, Spirotrichea occupied the dominant portion followed by Phyllopharyngea. Spirotrichea can encyst when the soil moisture decreases and excyst promptly to recover their normal morphological characteristics when the soil moisture increases. Besides, with their flattened bodies, the Spirotrichea ciliates can creep into adjacent soil granules or litter and can escape harsh conditions (Rosati *et al.*, 2008). In the present study, free-living *Chlamydodon mnemosyne* was the most dominant ciliates that showed the maximum dominance index value. *Paramecium brusaria* is another dominant ciliate found in Ayiramthengu mangrove and it is an exceptional ciliate with a mutualistic endosymbiotic relationship with green algae called Zoochlorella. *Euplotes minuta* and *Euplotes antarcticus* are the ciliates belonging to the sub-class spirotrichea that were 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). Adaptations to cold temperatures (La Terza *et al.,* 2001), salinity fluctuations (Syberg-Olsen *et al.*, 2016) and intracellular bacterial symbioses (Vannini *et al.*, 2012) are the features that make them dominant over mangrove habitats. Suctorian ciliates are very common both in marine and freshwater environments, frequently as epibionts on a variety of animals (Foissner *et al.*, 1999). *Acineta tuberosa* and *Ephelota truncata* are the representative suctorian ciliates found to be dominant in the study habitat. Most of the suctorians are sedentary in their habitat and lost their cilia in the trophic stage. Instead, they are not free-swimming; possess some special adaptation to survive in harsh environmental conditions. Among the observed Heterotrichea ciliates*, Stentor roeseli* is the dominant one reported from the habitat studied. *Aegyria olive* is the dominant ciliate belonging to the subclass Spirotrichea. Compared with other ciliates their morphological characters were exclusively adapted to live in marine and brackish environments (Song and Wilbert, 2002). *Tintinnopsis cylindrica* and *T. rotundata* are the most successful planktonic ciliate found to be dominant in the habitat studied. They are well adapted to living in open water and responding quickly to changes in environmental conditions (Laybourn, 1992). Seasonal analysis of the species composition of ciliates revealed post-monsoon to have maximum diversity. The diversity of the above-mentioned ciliates may be closely associated with suitable environmental conditions that are required for the excystement of ciliate spores (Godhantaraman, 2002). Moreover, the speciality in biological and other characteristic features of these ciliates supports their dominance in the dynamic mangrove habitat.

Conclusion

The present study highlighted the role of physicochemical parameters in structuring the ciliate community in the Ayiramthengu mangrove ecosystem and the differences in species diversity is due to differences in habitat heterogeneity, specifically, the habitats that provided more habitat heterogeneity and refuge often promoted the structural and functional diversity of the benthic community. The study also revealed that the ecological variations recorded from the mangrove habitat were much significant to make a change in the ciliate distribution. Though the ciliate species act as ecological indicators, an evenly distributed community structure reflects the existence of a healthy food web in the described ecosystem.

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