



Insights into protist diversity and biogeography in intertidal sediments sampled across a range of spatial scales

Yongbo Pan,¹ Jun Yang^{1b},² George B McManus^{1b,3}, Senjie Lin^{1b,3}, Wenjing Zhang^{1b,1*}

¹State Key Laboratory of Marine Environmental Science, Marine Biodiversity and Global Change Research Center, Xiamen University, Xiamen, China

²Aquatic EcoHealth Group, Fujian Key Laboratory of Watershed Ecology, Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen, China

³Department of Marine Sciences, University of Connecticut, Groton, Connecticut

Abstract

The relative importance of different ecological processes that shape community structure is a central but poorly understood topic in protist ecology. This study used an 18S rRNA gene sequencing approach to examine the relative contributions of environmental selective (environmental filtering) and neutral processes (dispersal and ecological drift) in the community assembly of three diverse protist groups (Bacillariophyta, Cercozoa, and Ciliophora) from intertidal sediment samples spanning a geographical distance up to 12,000 km. All three protist communities exhibited similar and distinct biogeographical patterns, and followed strong distance–decay relationships at continental scale (ca. 12,000 km), regional scale (ca. 1500 km), and local scale (ca. 50 km). Network analysis showed that temperature, salinity, nitrite and nitrate nitrogen, total nitrogen, and 0.1–0.25 mm grain size together associated with 60.8%, 55.5%, and 50.0% of the OTUs, which represented 68.1%, 58.5%, and 59.2% of sequence abundances for Bacillariophyta, Cercozoa, and Ciliophora co-occurrence networks, respectively, indicating that these environmental variables played the central roles in influencing community composition. On the other hand, a neutral community model explained 73.6%, 64.2%, and 70.2% of community variation for Bacillariophyta, Cercozoa, and Ciliophora, respectively. More importantly, variation partitioning and partial Mantel tests showed that environmental selection exhibited a slightly greater influence on Ciliophora compared to spatial factors, but both components were roughly equivalent in Bacillariophyta and Cercozoa communities. Taken together, these results demonstrate that both environmental selection and neutral processes play important roles in creating the biogeographical patterns of protist communities in intertidal sandy beach ecosystems.

It is well known that protists exhibit very high biodiversity (Locey and Lennon 2016), but how such high diversity and biogeographic patterns are generated and maintained are long-standing puzzles to microbiologists. Traditionally, the identification of protists is based on morphological features, which can lead to taxonomic misclassification because of insufficient resolution (Gentekaki and Lynn 2010; Katz et al. 2011). The presence in protists of both polymorphism (a single species with multiple morphotypes) and crypticity (multiple species with

indistinguishable morphology) can exacerbate this problem and cause under- or overestimates of diversity (Dolan 2016). However, in the past decade, protist biogeography research has made great progress, as high-throughput sequencing (HTS) combined with metabarcoding can provide extensive distributional data, including even the rare members of a community. Several studies have described geographical patterns in 18S rRNA gene sequences from diverse ecosystems, including shallow freshwater systems (Simon et al. 2015), the deep ocean (Pernice et al. 2015), coastal waters and sediments (Massana et al. 2015) as well as soils (Fiore-Donno et al. 2018). These studies provide evidence that protists are not spatially homogeneous, but display significant structure just like many plant and animal communities do.

In addition to species composition and geographical distribution, ecologists have recently begun investigating the mechanisms that control community diversity and biogeography. These are core, but sometime controversial, topics in ecology. On the one hand, niche-based or selective processes involve

*Correspondence: zhangwenjing@xmu.edu.cn

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deterministic factors (e.g., environmental conditions, species traits, and interactions of species) that shape community composition (Fargione et al. 2003). On the other hand, neutral processes which can be related to random fluctuations in species abundances (ecological drift) and limited dispersal are independent of species traits that shape the communities (Chave 2004). It is now widely accepted that both selective and neutral processes are involved in the assembly of microbial communities, but their relative importance remains intensely debated (Zhou et al. 2014; Stegen et al. 2015; Mo et al. 2018). For example, Zhou et al. (2018) found that microeukaryote communities were shaped by environmental selection and biological interactions, which are the main deterministic driving factors governing the distribution patterns of marine plankton at global scales. In contrast, Bahram et al. (2016) provide empirical evidence that neutral processes play the dominant role in the distribution of soil microeukaryotes at local levels. Additionally, Berdjeb et al. (2018) argued that environmental factors provide limited predictability for marine protist community variability, while interspecific relationships and stochastic processes are relevant in explaining changes in the composition of protist communities at short temporal scales. However, Xue et al. (2018) demonstrated that both deterministic and stochastic processes play important roles in the assembly of eukaryotic plankton communities. This debate continues because the data available to disentangle these two drivers of microbial community composition are limited and often conflicting. Therefore, analyzing these two processes in different taxa and different geographical scales but in the same sampling sites will enhance comparability, which is needed for better understanding the relative contribution of environmental selective and neutral processes. Indeed, simultaneous sampling is crucial for comparative research, because the relative strength of dispersal limitation and species sorting varies across environmental conditions (Yeh et al. 2015).

Sandy beaches are coastal ecosystems that are tightly linked to human impacts, with growing relevance in the face of environmental pollution and global change (Nel et al. 2014). Despite the well-recognized ecological importance of sandy beaches, only a few studies have been conducted so far on their microbial biogeography (Massana et al. 2015), and the relative importance of environmental selective and neutral processes in the assembly of protist communities has not been reported for continental scales in this ecosystem. The present study provides comprehensive insight into distribution patterns of three important protist taxa (i.e., Bacillariophyta, Cercozoa, and Ciliophora) in intertidal sands at a continental scale (North America and Asia, along a latitudinal gradient ranging from 24°N to 42°N). These three unicellular protist groups were selected because (1) they are very diverse in intertidal sediments, and (2) their biogeographical patterns and the mechanisms that underlie and control their diversity in intertidal sediments are still unclear. By using the HTS method, bioinformatic tools and multivariate statistical analyses, this study aims

to answer two questions: (1) How do the alpha and beta diversities of Bacillariophyta, Cercozoa, and Ciliophora vary in sandy beaches across 35 intertidal samples? (2) What are the relative roles of environmental selective and neutral processes in determining protist community composition and biogeography in these ecosystems?

Materials and methods

Study region and data set

The genomic and environmental data used in this study were collected in early September 2014. Thirty-six benthic samples were collected from 12 intertidal stations located in four regions (Connecticut [CT]; and Qingdao [QD], Zhoushan [ZS] and Xiamen [XM] in China). Detailed sampling strategy and methodology have been described in a previous study (Zhang et al. 2018a). In short, samples of intertidal sands were transported to the laboratory and immersed in 5 L of filtered, sterile seawater and shaken to extract the microbes. Subsequently, the sand was separated with a 200 μm pore-size sieve. The microbes were collected by filtering the seawater onto a 0.22 μm pore size polycarbonate membrane (47-mm-diameter, Millipore). Genomic DNA was extracted from the membranes using the FastDNA SPIN Kit (MP Biomedicals) following the manufacturer's instructions. Libraries of the V9 region of the 18S ribosomal DNA were made using the primers 1380F and 1510R (Amaral-Zettler et al. 2009), and sequenced on an Illumina MiSeq (Illumina) following a paired-end approach (Caporaso et al. 2012).

Bioinformatics

Paired-end sequence reads were processed with FLASH and contig reads between 135 and 152 bp were preserved (Magoč and Salzberg 2011). Primers and low-quality reads were removed using QIIME (Caporaso et al. 2010). Reads with an average Phred score < 25 were discarded, as were reads with any consecutive runs of low-quality bases > 3. The minimum of continuous high-quality bases was 75% of total read length, the maximum number of ambiguous bases was 0, and the lowest quality score allowed was 3 (Xue et al. 2018). Chimeras were identified and removed using UCHIME (Edgar et al. 2011). The remaining high-quality sequences were taxonomically assigned using the PR2 4.10 database (Guillou et al. 2013). Finally, sequences were clustered into OTUs with a 97% sequence similarity cutoff. Singleton OTUs (OTUs with only one read in the combined data set) were discarded prior to further analyses.

The Bacillariophyta, Cercozoa, and Ciliophora sequences were collected from each sample for subsequent analysis. One sample with fewer than 500 sequences was discarded, therefore leaving 35 samples in this study. So that all samples would contain the same number of sequences for subsequent analysis, randomly sampled subsets were collected for each of the three protist groups. These subsets were sampled at the minimum number of sequences observed for that group among all samples. Thus, the final data set for Bacillariophyta, Cercozoa, and

Ciliophora retained 33,005, 19,705, and 20,160 high-quality sequences, respectively. The V9 sequence data used in this study are available from the NCBI Sequence Read Archive (SRA) database under the accession number SRP099521 and BioProject number PRJNA362750.

Physicochemical analyses

In total, nine pore-water physicochemical properties and two sedimentary properties were measured at each sampling site. These variables included water temperature, salinity, pH, total carbon (TC), total organic carbon (TOC), total nitrogen (TN), nitrite and nitrate nitrogen ($\text{NO}_x\text{-N}$), total phosphorus (TP), phosphate phosphorus ($\text{PO}_4\text{-P}$), sedimentary organic matter content (ORG), and grain size. Detailed methods have been described in a previous study (Zhang et al. 2018a).

Statistical analyses

Alpha diversity indices and rarefaction curves were computed for each sample and the entire set using the *diversity* function in the “vegan” package of R software (R Core Team 2015). Good’s coverage was calculated in MOTHUR v.1.33.3 (Schloss et al. 2009). Effect of environmental factors on alpha-diversity was analyzed by one-way ANOVA using SPSS v.22.0 (IBM Corp.). Non-metric multidimensional scaling ordination was used to visualize community composition based on the Bray–Curtis dissimilarity matrix. To evaluate differences in community composition among the four sampling regions, an analysis of similarity (ANOSIM) was performed. These analyses were carried out in PRIMER 7.0 (Clarke and Gorley 2015). The links between the community Bray–Curtis dissimilarity and the geographical distances among sampling sites were determined using Spearman’s rank correlations.

In order to assess the relationships between OTUs and environmental factors, integrated networks were constructed for each protist group. OTUs present in $> 1/3$ of the total samples were used for this analysis (Liu et al. 2019), and the “psych” R package was used to calculate the pairwise Spearman’s rank correlations (r) between OTUs and environmental factors. Only p -values < 0.01 and $|r| > 0.4$ correlations were included in the network analyses (Liu et al. 2019). Network visualizations were made with Gephi version 0.9.1.

A redundancy analysis (RDA) was carried out to evaluate the relationships between protist communities and environmental and spatial variables; prior to the RDA, detrended correspondence analysis was performed to calculate the gradient lengths. Spatial variables were defined as the eigenvectors with associated positive eigenvalues extracted based on the longitude and latitude of sampling sites using principal coordinates of neighbor matrices (PCNM) analysis (Borcard and Legendre 2002). All continuous environmental variables were $\log(x + 1)$ transformed (with the exception of pH), while the proportions of different sediment grain sizes (a categorical environment variable) were Arcsin transformed to improve homoscedasticity and normality. Forward selection was conducted to select significant variables

using the “ordiR2step” function from the “vegan” package (Blanchet et al. 2008). All significant variables were included in RDA and further analyses. To test the contribution of spatial factors (neutral processes) and environmental selection in assembling communities, a variation partitioning analysis (VPA) with an adjusted R^2 coefficient was performed (Borcard et al. 1992; Wang et al. 2015). The relative contributions of both components were assessed for spatial variables (S), environmental variables (E), pure spatial variables (S|E), pure environmental variables (E|S), and the combined effects of both spatial and environmental variables (S∩E). In this analysis, the residual proportion represents the unexplained variance. To compare the VPA results, both Mantel and partial Mantel tests for relationships between community dissimilarity and environmental/spatial variables were also conducted (Legendre and Legendre 2012).

Furthermore, Sloan’s neutral community model (NCM) was also constructed to evaluate the contribution of neutral processes in community structure (Sloan et al. 2006). The model used here is an adaptation of the neutral theory (Hubbell 2001) adjusted to fit large microbial populations, and the analysis was performed in R software. In this analysis, Nm is an estimate of dispersal between communities while the R^2 determines the overall fit to the neutral community model (Chen et al. 2017).

Results

Species richness and alpha diversity

Among the 35 samples, a total of 439, 661, and 732 OTUs were obtained from Bacillariophyta, Cercozoa, and Ciliophora, respectively (Tables S1–S3). The estimated species accumulation curves based on the pooled data (35 samples) were nearly saturated for all three taxa (Fig. 1), although individual samples did not show full saturation (Fig. S1), and their Good’s coverages ranged from 99.30% to 99.76% (Tables S1–S3). These results indicated that the majority of the OTUs of the three protist taxa had been recovered from the studied metacommunity at global level.

Among sites, the species richness (OTU number) varied from 81 to 143 for Bacillariophyta, from 75 to 162 for Cercozoa, and from 91 to 172 for Ciliophora, with an average of 113, 120, and 127 OTUs, respectively (Tables S1–S3). One-way ANOVA indicated that a variety of environmental variables significantly influenced taxa richness (Table S4). Specifically, one environmental factor (i.e., temperature) was significantly associated with Bacillariophyta richness, whereas four environmental factors (temperature, Salinity, $\text{NO}_x\text{-N}$, and $\text{PO}_4\text{-P}$) and five environmental factors (temperature, pH, TOC, $\text{NO}_x\text{-N}$, and TP) were significantly correlated with Cercozoa and Ciliophora richness, respectively. Additionally, other alpha-diversity indices (Chao 1, ACE, Shannon-Wiener index, Simpson index, and Pielou’s evenness) were also significantly affected by environmental variables (Table S4), suggesting that environment selection may be a key determinant of α -diversity in the study areas.

The Bacillariophyta OTUs were grouped into four principal groups (Fig. S2a), with the raphid-pennate diatoms showing the

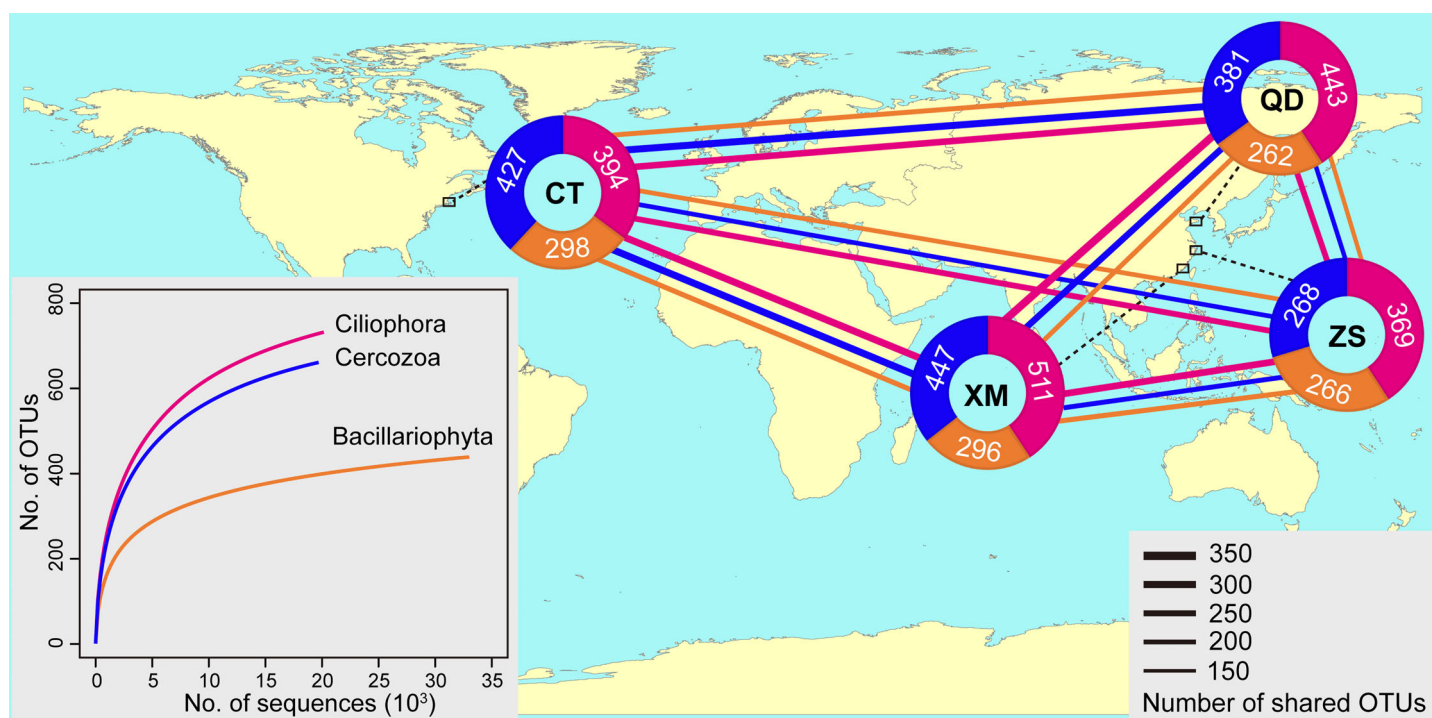


Fig. 1. Map of OTU richness (operational taxonomic units, clustered at 97% sequence identity) for three unicellular protist groups in four study regions, from a total of 35 intertidal sediment samples (CT: 11 samples; QD: 6 samples; ZS: 6 samples; XM: 12 samples). Line widths indicate the number of shared OTUs between pairs of regions. Numbers indicate the number of OTUs found in each region. Inset: Rarefaction curves for the whole OTU collection for each group. CT, Connecticut in U.S.A.; QD, Qingdao in China; ZS, Zhoushan in China; XM, Xiamen in China.

highest diversity (171 OTUs) and the polar-centric diatoms being the most abundant (50.8% of total sequences). The Cercozoa were grouped at the class level (Fig. S2b), in which Thecofilosea was the most diverse (218 OTUs) and abundant (53.8% of sequences). For the Ciliophora, 10 of the 12 currently recognized classes were detected (Fig. S2c), with Spirotrichea being the most diverse (298 OTUs) and abundant (68.1% of total sequences). Of the three protist groups, the Ciliophora had the smallest percentage of unclassifiable sequences.

Geographical patterns

At the OTU level, the three protist communities showed distinct and similar biogeographical distribution patterns, clustering into four groups based on sampling regions (CT, QD, ZS, and XM, respectively) (Fig. 2a). This was confirmed by ANOSIM analysis, which showed that the four sampling regions were significantly separated at $p = 0.001$ level (Table 1), indicating that community composition differed among the four study regions. However, in pairwise comparisons, a large number of Bacillariophyta, Cercozoa, and Ciliophora OTUs were shared between sampling regions (Fig. 1). Even among all four sampling regions, the shared number reached 148 (33.7%), 150 (22.7%), and 170 (23.2%) OTUs of Bacillariophyta, Cercozoa, and Ciliophora, respectively, while only 127 (28.9%), 222 (33.6%), and 245 (33.5%) OTUs were unique to one region, respectively (Fig. 2b).

Spearman correlograms comparing community Bray–Curtis dissimilarity and geographical distances among samples indicated significant and positive correlations for all three communities at the continental scale (all samples), regional scale (within China), and even at local scale (within each of the four sampling regions) (Fig. 2c). These significant distance–decay relationships are evidence of biogeographical patterns in these protist taxa. Interestingly, the relative abundance of an OTU was significantly correlated with the number of sites at which it was found ($p < 0.001$, Fig. S3) for all of the three taxa, suggesting that the more abundant species had higher dispersal than the rare ones.

Relationships between environmental variables and protist communities

The networks constructed from the Spearman correlations consisted of 1535, 1107, and 1100 edges connecting 130, 119, and 128 nodes (OTUs and environmental variables) for Bacillariophyta, Cercozoa, and Ciliophora, respectively (Fig. 3). These show the significant and strong pairwise correlations among OTUs and between OTUs and environmental variables (Spearman's $|r| > 0.4$, $p < 0.01$). Interestingly, the percentage of positive edges was generally higher than the negative ones in the OTU–OTU relationships (Table 2), especially in the Cercozoa network, which had 80% positive edges and only 20% negative edges. These results suggest that facilitation rather than competition was more common within these protist

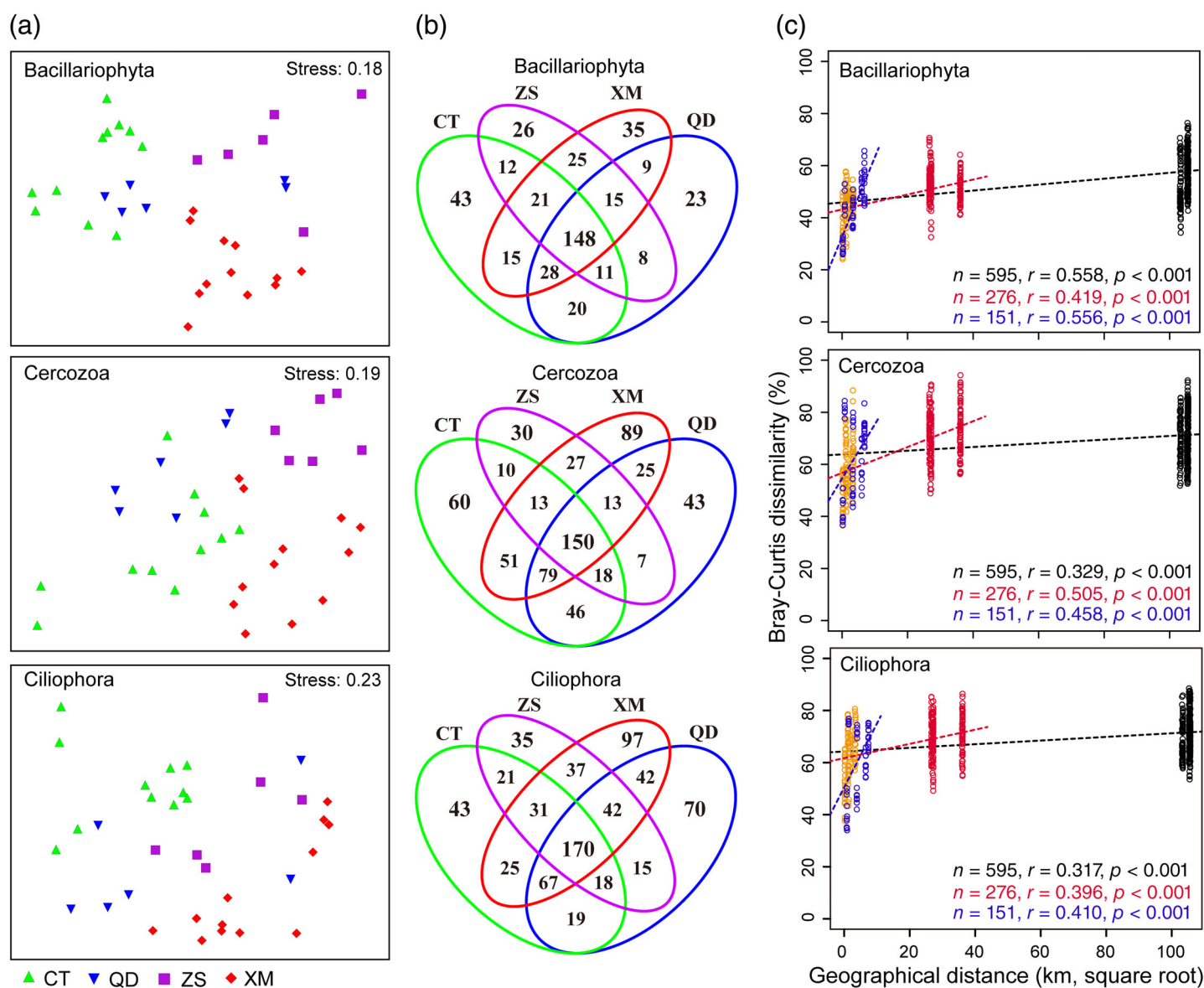


Fig. 2. Community distribution of Bacillariophyta, Cercozoa, and Ciliophora. **(a)** Non-metric multidimensional scaling ordinations (NMDS) of the three protist groups based on Bray–Curtis dissimilarity. **(b)** Venn diagrams showing the numbers of unique and shared OTUs among the four sampling regions. **(c)** Spearman's rank correlations between the pairwise Bray–Curtis dissimilarity between samples and the geographical distance (square root transformed) between sampling sites. The n is the number of comparisons for all samples (black text; all symbols), just Chinese samples (red text; red + yellow symbols) and within each of the four sampling regions (blue text; blue + yellow symbols).

communities. However, a different scenario occurred between environmental variable–OTU relationships, which had more negative edges than positive edges (Table 2). This suggests that environmental filters play an important role in community assembly. Five variables (temperature, salinity, TN, $\text{NO}_x\text{-N}$, and the 0.1–0.25 mm grain size fraction) all showed a high degree of connectivity in all three protist networks (Fig. 3). Statistical analysis confirmed that these variables associated with 60.8%, 55.5%, and 50.0% of the nodes (OTUs), which occupied 68.1%, 58.5%, and 59.2% of sequence abundances for the

Bacillariophyta, Cercozoa, and Ciliophora networks, respectively (Table S5), indicating that these five variables played central roles in connecting the protist OTUs in these co-occurrence networks.

Environmental selective and neutral processes in community variation

The RDA showed that Bacillariophyta, Cercozoa, and Ciliophora communities were significantly correlated with temperature, salinity and $\text{NO}_x\text{-N}$ ($p < 0.05$; Fig. 4a; total nitrogen was closely correlated with $\text{NO}_x\text{-N}$ and was therefore removed

Table 1. Analysis of similarities (ANOSIM) of intertidal communities for Bacillariophyta, Cercozoa, and Ciliophora among four different regions.

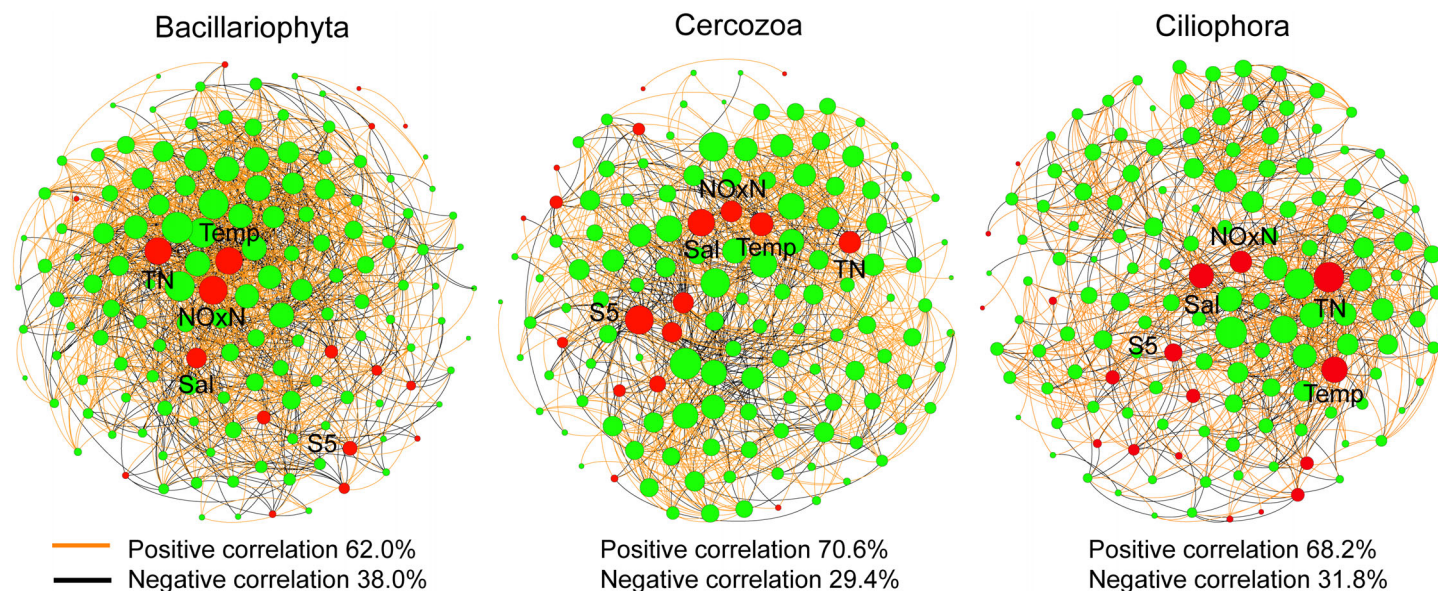
	Bacillariophyta	Cercozoa	Ciliophora
CT vs. QD	0.342*	0.297*	0.303*
CT vs. ZS	0.794**	0.549**	0.482**
CT vs. XM	0.840**	0.518**	0.581**
QD vs. ZS	0.581**	0.896**	0.502**
QD vs. XM	0.617**	0.706**	0.417**
ZS vs. XM	0.668**	0.679**	0.471**
Global test	0.671**	0.582**	0.471**

CT, Connecticut in U.S.A.; QD, Qingdao in China; ZS, Zhoushan in China; XM, Xiamen in China.

* $p < 0.05$; ** $p < 0.01$.

based on the forward selection model). These communities were also correlated with different sediment grain sizes and spatial variables. For example, three grain size variables (S1: 2–5 mm,

S2: 1–2 mm, and S5: 0.1–0.25 mm) and five spatial variables (PCNM Nos. 1–2, PCNM Nos. 4–5, and PCNM No. 19) were significantly correlated with the Bacillariophyta; three grain size variables (S3: 0.5–1 mm, S4: 0.25–0.5 mm, and S5) and four spatial variables (PCNM Nos. 1–3 and PCNM No. 5) were significantly correlated with the Cercozoa, and three grain size variables (S2, S4, and S5) and four spatial variables (PCNM Nos. 1–3 and PCNM No. 5) were significant predictors for the Ciliophora ($p < 0.05$; Fig. 4a). VPA was performed to quantify the impact of environmental and spatial factors on community variation. Spatial, environmental, and grain size variables together explained 65%, 55%, and 46% of the Bacillariophyta, Cercozoa, and Ciliophora communities, respectively (Fig. 4b). Among these, environmental factors and grain size independently explained 25%, 25%, and 24% for the three protist communities, respectively, suggesting the important role of environmental selection in community assembly. On the other hand, since spatial factors are related to dispersal, the strong interaction between spatial and environmental factors (12%, 7%, and 6%) and relatively large independent contribution of

**Fig. 3.** Network analysis illustrating the relationships among abundant protist OTUs (green nodes) and environmental factors (red nodes). The size of each OTU or environmental factor is proportional to its number of connections (its degree of centrality). The five environmental variables with the most connections are labeled: Temp, temperature; Sal, salinity; TN, total nitrogen; NO_x-N, nitrite and nitrate nitrogen; S5, 0.1–0.25 mm grain size. The percentages of positive and negative correlations (edges) are for the whole data sets of each group (including OTU–OTU connections, OTU–environmental factor connections, and environmental–environmental connections).**Table 2.** Number of edges between environmental variables and OTUs of the integrated networks in protists from intertidal sediment.

	Total edges		OTU–OTU		Variable–OTU		Variable–variable	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Bacillariophyta	951(62%)	584(38%)	814(66%)	419(34%)	122(44%)	155(56%)	15(60%)	10(40%)
Cercozoa	782(71%)	325(29%)	685(80%)	175(20%)	82(37%)	140(63%)	15(60%)	10(40%)
Ciliophora	750(68%)	350(32%)	649(74%)	230(26%)	86(44%)	110(56%)	15(60%)	10(40%)

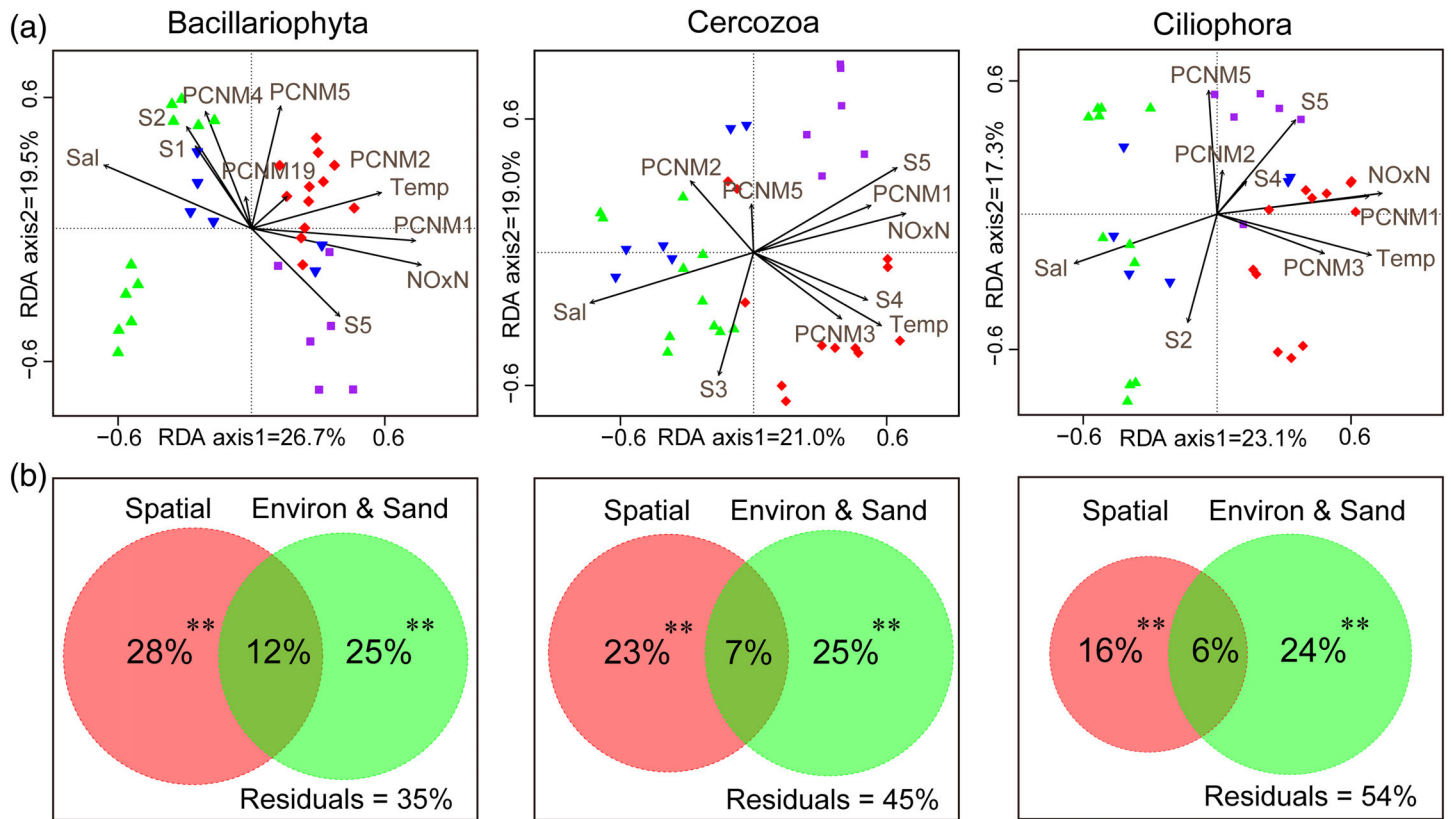


Fig. 4. Effects of environmental and spatial factors on protist community composition. **(a)** RDA ordinations showing community composition in relation to significant spatial, environmental, and sand grain size variables ($p < 0.05$). S1–S5 represent different sand grain sizes (S1: 2–5 mm; S2: 1–2 mm; S3: 0.5–1 mm; S4: 0.25–0.5 mm; S5: 0.1–0.25 mm). Temp, temperature; Sal, salinity; NO_xN, nitrite and nitrate nitrogen. PCNM: Spatial variables (see text for definitions). **(b)** Venn diagram of variation partitioning, showing the effects of spatial, environmental, and sand grain size variables on the community composition. Values indicate the percentage of community variation explained by each fraction, including pure, shared explained, and unexplained variability. Forward selection procedures were used to select the best subset of spatial, environmental and sand grain size variables explaining community variation, respectively. ****** $p < 0.01$.

spatial factors (28%, 23%, and 16%) on community composition indicates the significance of neutral processes in community assembly. The near equivalence of environmental selection

and neutral processes in explanation of community variation was further confirmed by Mantel and partial Mantel tests (Table S6).

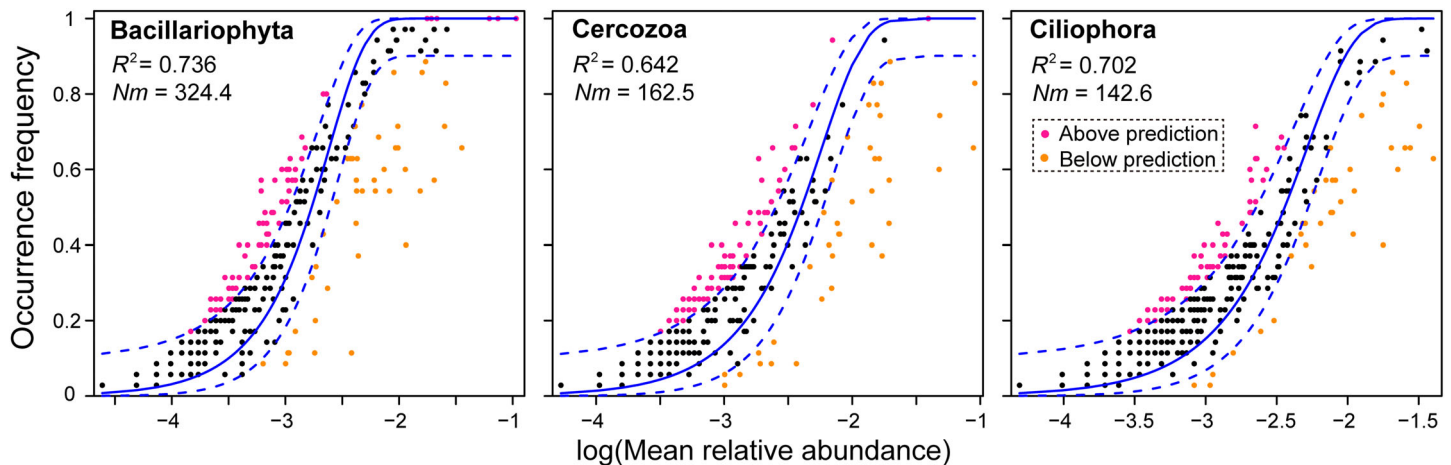


Fig. 5. Fit of the neutral community model (NCM) of three protist taxa. The solid blue lines indicate the best fit to the NCM as in Sloan et al. (2006) and dashed blue lines represent 95% confidence intervals around the model prediction. The OTUs that occur more or less frequently than predicted by the NCM are shown in pink or yellow colors, respectively. R^2 indicates the fit to the NCM. Nm indicates metacommunity size times immigration.

Fit to the NCM

The NCM successfully described the frequency distributions of OTUs for the Bacillariophyta ($R^2 = 0.736$), Cercozoa ($R^2 = 0.642$), and Ciliophora communities ($R^2 = 0.702$, Fig. 5), confirming that the processes of passive dispersal and ecological drift have an important impact on the distribution of all three protist groups.

Discussion

Diversity and biogeographical patterns

This study evaluated species diversity and biogeographical patterns of intertidal Bacillariophyta, Cercozoa, and Ciliophora in samples from marine sandy sediments. In the plankton, these protist taxa have been considered cosmopolitan in the ocean, with global distributions driven by ocean currents and circulation (Weisse 2008). However, in the present study, a different pattern was observed in marine sandy sediments, which exhibited similar and distinct biogeographical patterns for all three taxa (Fig. 2 and Table 1). This suggests the presence of distinct regional- or continental-scale species pools that are subject to different dispersal processes than are plankton assemblages.

Bacillariophyta (Diatoms) are a lineage of the Stramenopiles that are highly diverse in marine waters and sediments (Tirichine et al. 2017). Pelagic and benthic diatoms together represent one of the ecologically most important microalgal groups in the oceans (Karsten et al. 2012). They are estimated to contribute about 40% of primary production in the ocean (Field et al. 1998). They are an important part of marine food webs, supplying organic carbon to higher trophic levels in both plankton and benthos, and are an important component of the vertical flux of organic carbon in the sea (Tirichine et al. 2017). A previous study demonstrated that diatoms are the dominating group (by sequence number) among the 43 deep-branching lineages of microeukaryotes (Zhang et al. 2018a), indicating their important role in ecosystems. In total, 439 Bacillariophyta OTUs were detected in this study which was higher than previous work focusing on diatoms in intertidal sediments that was based on microscopy and/or PCR-DGGE methods (Sahan et al. 2007; Ribeiro et al. 2013; Siqueiros-Beltrones et al. 2017). For example, Sahan et al. (2007) found 158 diatom taxa in the Westerschelde estuary located in the Netherlands; Ribeiro et al. (2013) found 183 diatom taxa in the Tagus estuary; and Siqueiros-Beltrones et al. (2017) recovered 232 diatom taxa in the Guerrero Negro Lagoon located on the Baja California peninsula. However, the Tara Oceans expedition has estimated the diversity of marine planktonic diatoms at 4748 OTUs in sunlit ocean waters (Malviya et al. 2016), which is about 10-fold greater than the current study (439 OTUs). The rarefaction curves based on the pooled data set were near saturation in this study (Fig. 1), thus the great difference in OTU numbers between sunlit ocean waters and intertidal sediment may indicate

fundamental differences in the ways planktonic and benthic diatom assemblages are structured.

Cercozoa is a large group of protists, encompassing high morphological, functional, and ecological diversity. It is a well-studied lineage adapted to terrestrial, marine benthic, and anaerobic habitats (Bass et al. 2009; Ploch et al. 2016), but its environmental diversity remains underexplored (Fiore-Donno et al. 2018), especially in the intertidal zone. This study provides the first detailed insights into the diversity and biogeography of Cercozoa in intertidal sandy ecosystems. In total, 661 Cercozoa OTUs were recovered, comprising 5.1% of the total microeukaryote OTUs (Zhang et al. 2018a). A previous study found that Cercozoa comprised between 9% and 24% of all assigned eukaryotic OTUs on the ocean floor of the Arctic and Southern Oceans (Pawlowski et al. 2011). Even greater diversity was found in soil; for example, Harder et al. (2016) recovered 1585 OTUs of Cercozoa from a heathland in Denmark. Furthermore, more new species of this large group will likely be found now that new barcoding primers for efficient recovered of cercozoan sequences are available (Fiore-Donno et al. 2018).

For Ciliophora, 10 class-level lineages were found in this study (Fig. S2a). Of the 12 described ciliate classes, only Armophorea and Cariacotrichea were not found. Both Armophorea and Cariacotrichea are well known for living in anoxic environments (Lynn 2008; Orsi et al. 2012), therefore, it is not surprising that both clades were not found in marine sandy sediments. Altogether, 732 Ciliophora OTUs were recovered in this study, which was higher than findings focused on offshore shallow waters (313 OTUs, Grattepanche et al. 2016), and deep-sea hydrothermal vents (353 OTUs, Zhao and Xu 2016), but lower than a larger sample size study of surface ocean waters (1274 OTUs, Gimmler et al. 2016). Several studies have demonstrated that marine Ciliophora dominate benthic microeukaryote communities, including in European coastal sediments (Massana et al. 2015), Yellow Sea surficial sediments (Gong et al. 2015), and a deep-sea methane seep ecosystem (Pasulka et al. 2016). A recent study recovered 483 ciliate OTUs from 11 intertidal sediment sites in the Yellow Sea, China (Zhao and Xu 2017), roughly two thirds of what the present study found. The difference may be due to the fact that this study included more samples across a larger spatial scale. Spirotrichea comprises one of the most morphologically diverse classes in the Ciliophora phylum. Spirotrichs play key ecological roles as nutrient recyclers and trophic links to mesozooplankton and fish (Calbet and Saiz 2005; Dolan et al. 2012). This taxon was the most diverse and abundant group of ciliates, accounting for 40.5% of the observed ciliate richness and 67.9% of sequences in this study, similar to what was observed in sunlit ocean waters sampled by the Tara Oceans expedition (Gimmler et al. 2016) and in intertidal sediments of the Yellow Sea (Zhao and Xu 2017). The present study confirms that this taxon also has an important function in sandy sediment ecosystems as well.

Controlling factors for geographical distributions

Quantifying the relative contributions of environmental selection and neutral processes in the assembly of microbial communities is a core challenge in ecology. This study provides evidence that both processes are important in structuring all three protist groups in sandy beach sediments.

Environmental conditions are important selective factors that varied among the sampling sites. Temperature, salinity, and nitrite and nitrate nitrogen ($\text{NO}_x\text{-N}$) were the three most important environmental factors that were significantly related to the community composition of all three taxa (Fig. 4a). Previous studies have found that temperature influences protist growth, survival, and feeding, and thus could control their densities and community composition (Weisse et al. 2002; Barboza and Defeo 2015; Tveit et al. 2015). Salinity is a major environmental factor across many ecosystems at local-scale (Yu et al. 2015; Zhang et al. 2017, 2018b) as well as continental-scale analyses (Zhang et al. 2018a). For instance, the changes in planktonic ciliate diversity from shallow to deep waters and from inshore to offshore on the continental shelf are significantly associated with increasing salinity (Grattepanche et al. 2016). Nitrite and nitrate nitrogen ($\text{NO}_x\text{-N}$), an important nutrient for autotrophs such as diatoms, has been associated with the temporal variability of eukaryotic community composition (Wang et al. 2013, 2014; Xue et al. 2018). In the present study, $\text{NO}_x\text{-N}$ was associated with variations in community composition for all three protist groups, even the primarily heterotrophic ciliates. Possibly, nitrite and nitrate nitrogen affects heterotrophic communities via changes in their autotrophic prey. Grain size also had significant effects on the variation of community composition in this study (Fig. 4a). In intertidal zones of sandy beaches, species richness is often negatively related to sediment grain size (Barboza and Defeo 2015; Lallias et al. 2015). One possible reason is that the biological communities and the physicochemical properties of benthic systems can be influenced by grain size in direct or indirect ways, via changes in oxygen, organic matter, tortuosity, pore size, and presence of deposit-feeding macrofauna. Similar to these findings, the current study found that temperature and other variables were significantly related to the OTU richness of the three protist groups (Table S4), suggesting that some of taxa had high temperature preferences. Additionally, network analysis suggested that temperature, salinity, TN, $\text{NO}_x\text{-N}$, and S5 (0.1–0.25 mm) associated with more than 50.0% of the OTUs, and that these OTUs dominated the sequence abundances for all three of the protist networks (Table S5), indicating that these environmental variables maintained community structure via being directly connected to most of the protist OTUs.

Normally, neutral processes can also shape biological communities. Neutral community models have been well explored in bacterial community structure, such as in wastewater treatment plants (Ofiteru et al. 2010), bioreactors (Zhou et al. 2013), lakes (Roguet et al. 2015), soils (Powell et al. 2015), and

subtropical bays (Mo et al. 2018). Recently, it was found that this model is also helpful in explaining microeukaryote community structure (Dolan et al. 2007; Chen et al. 2017). Indeed, the current study found that all three protist communities were largely explained by neutral processes (Fig. 5).

The fact that the spatial variables significantly affected the structure of the three communities is evidence of dispersal limitation at continental (ca. 12,000 km), regional (ca. 1500 km), and local scales (ca. 50 km, Fig. 2c). There is still considerable debate over whether microorganisms are dispersal limited, and divergent results have been reported, depending on the sampling scale (Bates et al. 2013; Gimmler et al. 2016). Dispersal cannot be explicitly considered as a deterministic or a neutral process (Lowe and McPeck 2014; Vellend et al. 2014). However, dispersal can be treated as stochastic when dispersal rates are dependent on population size (Zhou and Ning 2017). In this study, it was found that the abundant species had a greater probability of dispersal than rare ones (relative abundance is correlated with frequency of occurrence; Fig. S3), indicating that dispersal rates indeed depend on population size, and supporting the idea that dispersal is stochastic. Therefore, neutral random processes are important in shaping community assembly in sandy beach ecosystems for the three large taxa quantified here.

A principal goal of this study was to quantify the relative importance of environmental selective and neutral processes in defining community composition. Results showed that purely spatial variables (16%) were slightly less influential contributors than the environmental plus grain-size components (24%) in regulating the assembly of Ciliophora, but both components were roughly equivalent in Bacillariophyta (28% vs. 25%) and Cercozoa (23% vs. 25%) communities (Fig. 4b). Partial Mantel tests also confirmed that spatial factors alone were roughly equivalent to environmental variables for explaining community assembly for all three groups (Table S6). These results were similar to those of several previous studies that focused on different species and ecosystems, such as bacterial communities of plateau lakes (Liao et al. 2016), subtropical bays (Mo et al. 2018), or subsurface regions (Stegen et al. 2012), soil fungi communities (Glassman et al. 2017), reservoir eukaryotic plankton communities (Xue et al. 2018), and protist communities from surface sediments in the South China Sea (Wu and Wang 2019). However, a great fraction of the community variation in our VPA was unexplained (Fig. 4b). The unexplained variation may come from other ecological factors and unmeasured environmental variables, or interactions between different taxa, such as competition and predation. Additionally, stochastic processes of growth, death, colonization, extinction, and genetic diversification could also contribute to the unexplained variation (Hanson et al. 2012); these were not quantified in this study. Thus, factors not considered in this study may also be important and inclusion of them may change conclusions about the relative importance of environmental selective and neutral processes in

community assembly, so more study of these processes in natural protist communities is needed.

Conclusion

This study provides insights about three important and diverse protist groups (Bacillariophyta, Cercozoa, and Ciliophora) and their geographical patterns of community composition in marine sandy beach ecosystems. Similar and distinct biogeographical patterns were discovered within the three groups based on intertidal sediment samples separated in space by up to 12,000 km. Both selective (deterministic) and neutral (stochastic) processes shaped community assembly, and both components were roughly equivalent in their impacts on three protist communities. Specifically, temperature, salinity, nitrite and nitrate nitrogen, total nitrogen, and the 0.1–0.25 mm grain size fraction were the five most important environmental variables that structured community composition. All three taxa gave a good fit to a neutral community model of community assembly, indicating that stochastic processes are also important. Altogether, our study highlights the need to consider both environmental selection and neutral processes to understand protist community assembly and biogeography.

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Conflict of Interest

The authors declare no competing commercial interests in relation to the submitted work.

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