



Water mass–driven spatial effects and environmental heterogeneity shape microeukaryote biogeography in a subtropical, hydrographically complex ocean system - A case study of ciliates

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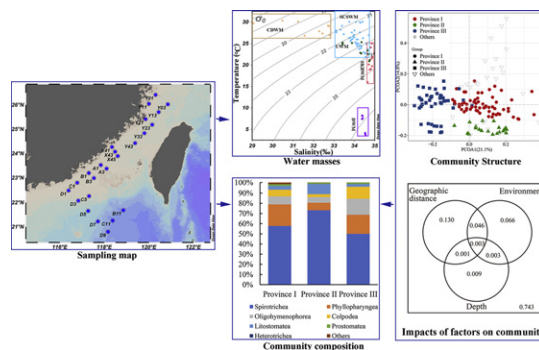
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HIGHLIGHTS

- Determination of water masses occurring in Taiwan Strait
- The ciliates were dominantly comprised of Spirotrichea and Phyllopharyngea.
- Ciliate alpha diversity was associated with water chemistry and food abundance.
- Three biogeographic provinces of ciliates were found on a regional scale.
- Water mass–driven spatial effects and environmental heterogeneity largely shape ciliate biogeography.

GRAPHICAL ABSTRACT



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ABSTRACT

The relative importance of geographic distance and depth in shaping microeukaryote community composition on a regional scale remains unclear, especially how that composition is related to the movement of water masses. Here, we collected 156 water samples across the Taiwan Strait, which is characterized by complex topography and dynamic circulation, to investigate the composition of the ciliate community with high-throughput sequencing of the 18S rRNA gene transcript. Ciliate alpha diversity exhibited strong correlations with water chemistry, food abundance, and geographic distance; approximately 50% of the variance of the diversity could be explained by dissolved oxygen concentrations, chlorophyll *a* concentrations, bacterial abundance, and latitude. The sampling sites could be divided into three provinces based on the compositions of the ciliate communities, which exhibited a distinctly nonuniform spatial distribution pattern on a regional scale (587 km). Geographic distance, environmental conditions, and depth were identified as principal determinants of the ciliate community within the Strait. Geographic distance was the most influential factor. The effect of geographic distance seems to mainly reflect the movement of water masses that strongly constrain dispersal and contribute to environmental heterogeneity that accounts for 86.0% and 5.5%, respectively, of community variance across the Strait. Overall, this study revealed that ciliate biogeography as a function of depth and environmental gradients is linked on a regional scale

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to the water masses that the ciliates inhabit. This result expands our knowledge of the drivers of microeukaryote community composition across regions within which there are water mass movements and strong spatial and environmental gradients.

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1. Introduction

Ciliates (Protist: Alveolata: Ciliophora) are a large group of microbial eukaryotes that inhabit a wide range of aquatic and terrestrial environments (Foissner, 2008). They are morphologically highly diverse and may reach abundances of tens of thousands of cells per liter of water (Lynn, 2008). In marine planktonic ecosystems, they usually dominate the microzooplankton in a variety of environments (Pierce and Turner, 1992), and they form an essential trophic link that channels carbon flow from the microbial loop to the classic food web (Azam et al., 1983; Sherr and Sherr, 1987).

Studies have demonstrated that biological–physical interactions significantly influence the distribution of marine organisms. At regional scales (>100 km), physical processes are particularly powerful and therefore have a strong influence on microbial variability (Djurhuus et al., 2017; Mann and Lazier, 2013). On a regional scale, dispersal is more subject to the influence of physical factors (e.g., geographic distance, depth of the water column, and ocean currents) for microzooplankton than for zooplankton because of the limited mobility of the former (Reid and Stewart, 1989). The variety of physical oceanographic conditions on a regional scale also creates a wide range of environmental gradients that exerts differential selection on the organisms in the region. Studies have indicated that spatial and environmental variability, as well as the movement of water masses, significantly influence the diversity and distribution of microzooplankton (Bachy et al., 2014; Gimmler et al., 2016; Santoferrara et al., 2016). For example, geographic distance plays a crucial role in structuring the communities of oligotrich and choreotrich ciliates; a distinct assemblage is restricted to offshore waters along a 163-km transect off the coast of New England, USA (Grattepanche et al., 2015). Depth shapes ciliate community composition from the surface to abyssal depths over a distance of 1300 km in the western Pacific Ocean (Zhao et al., 2017). Environmental factors, including temperature and the concentration of dissolved oxygen, are essential factors that structure the composition of nanociliate communities at the basin scale in the Bohai and Yellow Seas (Dong et al., 2014). Species of tintinnid ciliates endemic to specific water masses are found in the open waters near Prydz Bay, East Antarctic (Liang et al., 2018), in the western Pacific Polar front (Kato and Taniguchi, 1993), and in the northern East China Sea (Kim et al., 2012). However, few studies have tried to differentiate between the effects of geographic distance and depth on microzooplankton on a regional scale, especially when these effects are confounded by the presence of strong environmental gradients and movement of water masses. Studies of microbial communities across depth strata on a regional scale is important because that is the scale at which different water masses create contrasting physical and chemical conditions that lead to different microbial communities (Bardgett et al., 2008). However, such studies have been rare (Hamilton et al., 2008; Bauer et al., 2018; Christian et al., 2013), especially for microzooplankton. As a result, there are significant knowledge gaps about how geographic distance, depth, environmental conditions, and movement of water masses collectively affect microzooplankton communities on a regional scale.

The Taiwan Strait is located between Taiwan Island and southeast Mainland China. It connects to the continental shelf of the East China Sea in the north and the deep ocean basin of the South China Sea in the south. It is therefore an essential conduit of water and chemical elements between the East China Sea and the South China Sea (Hong et al., 2011). The Taiwan Strait is characterized by complex bottom topography primarily on the continental shelf, where the mean depth is about

60 m. There is an abrupt change of depth between the Taiwan Strait and the South China Sea deep basin, where the greatest water depth exceeds 1000 m (Hu et al., 2010). The Taiwan Strait is impacted by subtropical monsoon winds. Wind-driven currents are mostly southwestward in winter but northeastward in summer. Hence there are strong seasonal variations in the circulation in the strait because of the changing wind field (Hu et al., 2010). Circulation is complex in the Taiwan Strait; the major currents are the China Coastal Current, the extension of the South China Sea Warm Current, and the intrusion from the Kuroshio (Guan and Fang, 2006). Besides, the prevailing southwest monsoon in summer facilitates the development of upwelling along the west coast of the strait, which runs parallel to the coast due to Ekman transport (Hu et al., 2003). The complex physical and chemical conditions thus make the Taiwan Strait an ideal system to investigate microzooplankton distributions on a regional scale and elucidate the influence of geographic distance, depth, water masses, and environmental conditions on the communities.

In this study, we used 18S rRNA gene transcript-based high-throughput sequencing to investigate the diversity and distribution of ciliate communities based on samples collected from coastal to oceanic regions and from the surface down to mesopelagic depths. Our goal was to delineate the horizontal and vertical distribution of ciliate communities across the Taiwan Strait and to relate their distributions to the water masses and environmental conditions in which they live. We hypothesized that there was a distinct pattern of the spatial distribution of both the ciliate alpha and beta diversities and that on a regional scale those diversities were co-structured by spatial and environmental factors as well as by currents. Acceptance of this hypothesis led us to assess the relative importance of geographic distance, depth, water masses, and environmental conditions in maintaining ciliate communities on a regional scale.

2. Materials and methods

2.1. Sampling and environmental factors analyses

We collected samples from 25 sites that ranged from 20.5°N to 27.05°N and from 115.47°E to 122°E; the sites encompassed almost the whole region of the Taiwan Strait (Fig. 1A). The sampling transects were from a routine-monitoring project of the Taiwan Strait funded by the Natural Science Foundation. This project conducts cruises each year, and the investigated transects, which are also the ones we studied in the present study, cover the full region of the Taiwan Strait. Due to the cost and limited human resources, water column samples were collected at every other sampling station, which (except for Y33, Y34, B11, and C11) were regularly distributed along the transects we investigated. The names of the sampling sites used in the routine monitoring work were retained to facilitate comparison of the data in future studies. Altogether, 156 samples (74 for nano- and 82 for micro-ciliates, respectively) were collected from the R/V Yanping II at discrete water depths ranging from the surface (3 m) down to the mesopelagic zone (1000 m) during summer (13–24 July) of the year 2016. Five liters of seawater were pre-filtered with 200- μ m nylon mesh (Sefar Nitex) to remove mesozooplankton. The water was then sequentially filtered through 20- μ m nylon mesh (Millipore, USA) and a 3- μ m polycarbonate filter (Millipore, USA). All filters were preserved at -80°C until extraction. Depth, temperature, salinity, and dissolved oxygen (DO) concentrations were measured with a SeaBird conductivity-temperature-depth (CTD) profiler (SeaBird 917, USA). A sample of 300–1000 ml of

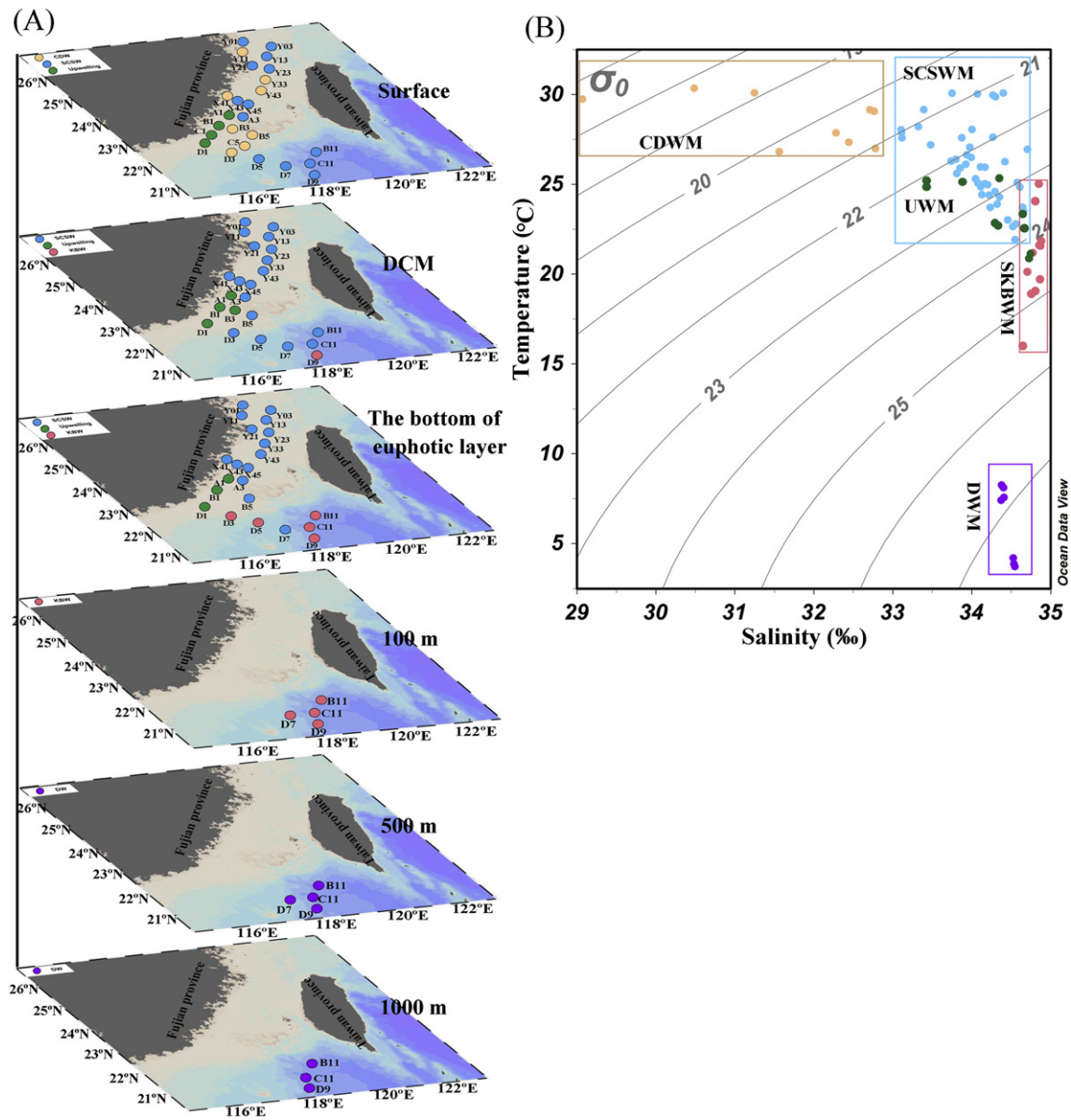


Fig. 1. (A) Map of Taiwan Strait highlighting the sampling sites and the distribution of water masses across depth strata in the present study; (B) five water masses identified based on properties of temperature and salinity. The lines across the panel B represent isopycnal. Orange dots represent Coastal Diluted Water Mass (CDWM); blue dots represent South China Sea Water Mass (SCSWM); red dots represent Subsurface Kuroshio Branch Water Mass (SKBWM); green dots represent Upwelling Water Mass (UWM); purple ones represent Deep Water Mass (DWM).

water was treated according to Huang et al. (2010), and the chlorophyll *a* (Chl *a*) concentration in the water was then determined with a Trilogy fluorometer (Turner Designs, USA) at a wavelength of 436 nm (Parsons, 1984). Concentrations of dissolved nutrients, including dissolved inorganic nitrogen (DIN), soluble reactive phosphorus (SRP), and dissolved silicate (DSi), were measured with a nutrient analyzer (QUATTRO, Seal, Germany) according to Du et al. (2013). A 1.8-ml seawater sample was pre-filtered through 20- μ m mesh and then was fixed with ice-cold glutaraldehyde at a final concentration of 1%. Samples were stored at -80°C and were later assayed via flow cytometry (Beckman Coulter, Epics Altra II) to determine bacterial abundance (Jiao et al., 2014). For nanoflagellate enumeration, 50 ml of seawater was filtered through 20- μ m nylon mesh to exclude larger plankton, fixed with a 1% final concentration of ice-cold glutaraldehyde, stained with DAPI (4',6-diamidino-2-phenylindole; Sigma, USA) for 10 min, and counted via fluorescence microscopy (Nikon ECLIPSE 90i, Nikon, Japan) (Sherr et al., 1993).

2.2. Nucleic acid extraction, sequencing, and processing of sequences

RNA was extracted using the RNeasy Mini Kit (Qiagen, USA) according to the manufacturer's instructions and was then reverse transcribed with the QuantiTect Reverse Transcription Kit (Qiagen, USA). The hyper-variable V4 region of the ciliate 18S rRNA gene transcript was amplified with a nested polymerase chain reaction (PCR) approach. First, PCR amplification was performed with ciliate-specific primers (Dopheide et al., 2008). Subsequently, a second PCR was performed with eukaryote-specific primers that covered the hypervariable V4 region of the 18S rRNA gene transcript (Stoeck et al., 2010). All PCR products were pooled and then were purified using the Wizard® SV Gel and PCR Clean-Up System kit (Promega, USA). All purified PCR products were sent to Novogene Bioinformatics Technology Co. Ltd. (Beijing, China) for sequencing using an Illumina MiSeq platform.

Briefly, paired-end sequences were merged using FLASH (Fast Length Adjustment of Short reads) software with a minimum of 10

base pair (bp) overlap and a maximum mismatch ratio of 0.1 (Magoč and Salzberg, 2011). QIIME (Quantitative Insights Into Microbial Ecology) v.1.8.0. software (Caporaso et al., 2010) was then employed to filter low-quality sequences according to the following criteria: maximum number of consecutive low-quality bases = 3; minimum of continuous high-quality bases = 75% of total read length. After removing low-quality sequences, the USEARCH v10 (Edgar, 2013) sequence analysis tool was used to discard chimera and singletons and to delineate Operational Taxonomic Units (OTUs) with a sequence similarity of 97% following Massana et al. (2015). Taxonomic assignments were performed with the UBLAST search tool against the Protist Ribosomal Reference database v4.7.1 (PR2) (Guillou et al., 2012). Finally, ciliate-only OTUs were retained, and all samples were normalized by randomly resampling to 19,165 reads per sample for further analyses.

2.3. Statistical analyses

Alpha diversity indices were calculated for each sample using QIIME v.1.8.0. (Caporaso et al., 2010). A comparison of alpha diversity indices was performed with one-way ANOVA and Student's *t*-test. Association between environmental factors and alpha diversity indices was calculated with Spearman's rank correlation using the 'corr. test' function of the "psych" package in R (Revelle, 2019). The maps of the sampling stations were created with the free software program Ocean Data View (ODV) version 4.4.2 (Schlitzer, 2013). The geographic distance used in this study was the distance between sampling sites. Pairwise geographic distances between sampling sites were computed by QIIME using the script distance_matrix_from_mapping.py with the data of Longitude and Latitude. Environmental factors were z-score transformed, and sequence data were $\text{Log}(x + 1)$ transformed before downstream analyses. Differences in environmental parameters between samples were analyzed by calculating the Euclidean distances between the samples. Permutational multivariate analysis of variance (Adonis) in the vegan package was performed to test differences on environmental samples between groups, that are water masses, depth strata, and latitudes (Chapman and Underwood, 1999). Partitioning of beta diversity was investigated by an unweighted pair-group method with arithmetic means (UPGMA) cluster analysis based on the Bray-Curtis dissimilarity matrix, which was computed by PRIMER 6 (Clarke and Gorley, 2009). Based on the Bray-Curtis dissimilarity matrix, the ordination of the communities from the 156 samples was visualized with a principal coordinate analysis (PCoA) plot using the "vegan" package in R (Oksanen et al., 2010). Phylogenetic dissimilarities (unweighted UniFrac distance) of ciliate communities were also computed using the "GUniFrac" package in R (Chen, 2012) to verify the community structure revealed by Bray-Curtis similarity-based PCoA. Analysis of similarity (ANOSIM) in the "vegan" package was performed to test differences on ciliate community structures between groups (Chapman and Underwood, 1999). Simple and partial Mantel tests (9999 permutations) were used to explore the effects of geographic distance, depth, and environmental factors on the total beta diversity (Legendre and Legendre, 1998). The significantly discriminant taxa in each ciliate province were determined using the linear discriminant analysis (LDA) effect size (Segata et al., 2011), which employs the factorial Kruskal-Wallis sum-rank test ($\alpha = 0.01$) to identify taxa with significantly different relative abundances between groups (using one-against-all comparisons). Significant taxa were used to plot taxonomic cladograms illustrating differences between three ciliate provinces. To identify the specific OTUs that characterized each of the ciliate provinces, we used the Indicator Species Analysis run in R using the package "Indicspecies" (Dufrene and Legendre, 1997). Only OTUs with indicator values (IV) > 0.3 and $p < 0.05$ were considered good indicators. The contributions of geographic distance, depth, and environment to the variances of ciliate communities were assessed with variance partitioning analysis (VPA) based on canonical correspondence analysis (CCA). VPA was performed using the "vegan" package in R (Oksanen et al., 2010).

Quantification of ecological processes (i.e., selection, dispersal, and drift) was made in accord with Stegen et al. (2013). We first used the phylogenetic turnover between communities to determine the influence of selection; then, we used OTU turnover to determine the influences of dispersal and drift.

3. Results

3.1. Determination of water masses

Four clusters were identified based on the temperature and salinity datasets collected from the water column of twenty-five sampling sites across the Taiwan Strait (Fig. 1A). Cluster 1 was identified as a Coastal Diluted Water Mass (CDWM) based on its hydrographic properties of relatively high temperature (26.83–30.63 °C) and low salinity (29.07–32.44‰) (Fig. 1B). In summer, the CDWM mixes with freshwater from the Pearl, Jiulong, and Min rivers as it flows northwards and therefore has relatively low salinity. Cluster 2 was identified as a South China Sea Water Mass (SCSWM); its temperature (21.93–30.09 °C) and salinity (32.71–34.70‰) were relatively high (Fig. 1B). In cluster 2, an Upwelling Water Mass (UWM) could be further separated from the SCSWM based on its distinctly lower temperatures (20.89–27.01 °C) and higher salinities (32.78–34.74‰) than those of otherwise similar sites at the same water depth when profiles of temperature and salinity on each transect were examined (Fig. S1). Cluster 3 corresponded to sampling sites at water depths of 50–200 m in the southeastern strait and was characterized by lower temperatures (16.04–25.04 °C) and the highest salinities (34.64–34.88‰) (Fig. 1B). It was therefore identified as a Subsurface Kuroshio Branch Water Mass (SKBWM). Cluster 4 had the lowest temperatures (3.79–8.31 °C) and high salinities (34.37–34.54‰) (Fig. 1B). These characteristics corresponded to water at depths of 500–1000 m in the southeastern strait, and Cluster 4 was therefore identified as a Deep Water Mass (DWM).

3.2. The environment of the sampling region

There were gradients of environmental characteristics of water collected across the Taiwan strait in several dimensions: horizontally from north to south and from coastal to oceanic waters, and vertically from the surface to great depths. Across distances of about 587 km in any horizontal direction and about 1000 m in the vertical direction, water temperature ranged from 3.79 to 30.36 °C, salinity from 29.07 to 34.88‰, the concentration of dissolved oxygen (DO) from 1.15 to 8.53 mg/L, the concentration of Chl *a* from 0 to 9.96 µg/L, abundances of bacteria from 69.6 to 2890 × 10³ cells/ml, abundances of heterotrophic nanoflagellates (HNF) and phototrophic nanoflagellates (PNF) from 203 to 3833 cells/L and from 0 to 7165 cells/L, respectively, the concentration of dissolved inorganic nitrogen (DIN) from 0.143 to 27 µmol/L, the concentration of soluble reactive phosphorus (SRP) from 0 to 2.35 µmol/L, and the concentration of dissolved silicate (DSi) from 0.25 to 95.96 mg/L (Table 1). Changes of environmental characteristics between water masses were examined with principal component analysis (PCA) (Fig. S2). Terms used to describe spatial variations of environmental conditions are defined here to facilitate discussion. Sampling sites were divided into northern and southern regions at the 24° N parallel (Dai et al., 2014). Water at a depth of 3 m was defined as surface water; the upper 100 m of the water column was defined as the euphotic zone; and the water at depths of 200–1000 m was defined as the mesopelagic zone. Overall, the greatest heterogeneity of environmental factors was between water masses (Adonis, $R^2 = 0.533$, $p = 0.001$), followed by different depth strata (Adonis, $R^2 = 0.278$, $p = 0.001$). The smallest heterogeneity was associated with latitudinal comparisons (Adonis, $R^2 = 0.022$, $p = 0.041$). Hence there were multiple environmental gradients across the strait.

Table 1
Environmental parameters of sampling sites throughout study (mean \pm SD).

	Depth			Water mass					Province		
	Surface	Euphotic	Mesopelagic	CDWM	SKBWM	SCSWM	UWM	DWM	Province I	Province II	Province III
DO (mg/L)	3.90 \pm 1.68	3.24 \pm 1.18	2.07 \pm 1.01	4.16 \pm 1.54	3.02 \pm 0.96	3.37 \pm 1.48	3.78 \pm 0.92	1.75 \pm 0.58	3.40 \pm 1.83	4.12 \pm 0.85	2.85 \pm 1.08
DIN (μ mol/L)	2.92 \pm 2.67	3.56 \pm 1.99	18.84 \pm 5.3	4.24 \pm 3.50	4.61 \pm 2.72	2.79 \pm 1.85	4.22 \pm 1.45	20.21 \pm 4.14	3.94 \pm 2.82	2.98 \pm 1.46	6.42 \pm 7.88
SRP (μ mol/L)	0.10 \pm 0.09	0.20 \pm 0.15	1.76 \pm 0.49	0.08 \pm 0.05	0.36 \pm 0.22	0.15 \pm 0.11	0.24 \pm 0.15	1.89 \pm 0.38	0.19 \pm 0.12	0.12 \pm 0.09	0.56 \pm 0.76
DSi (μ mol/L)	5.12 \pm 4.05	5.20 \pm 3.43	44.49 \pm 34.61	4.48 \pm 3.05	4.52 \pm 2.44	4.65 \pm 3.26	8.86 \pm 4.5	50.17 \pm 33.33	5.85 \pm 3.74	6.15 \pm 4.64	14.19 \pm 25.76
Temperature ($^{\circ}$ C)	28.02 \pm 1.77	24.02 \pm 2.32	7.45 \pm 3.72	28.61 \pm 1.28	20.85 \pm 2.37	26.12 \pm 2.12	23.15 \pm 1.48	6.22 \pm 1.94	25.51 \pm 2.39	25.71 \pm 2.57	21.10 \pm 8.75
Salinity (‰)	33.04 \pm 1.27	34.36 \pm 0.4	34.47 \pm 0.09	31.7 \pm 1.19	34.79 \pm 0.07	34.07 \pm 0.41	34.32 \pm 0.47	34.45 \pm 0.07	33.88 \pm 0.65	33.65 \pm 1.09	34.17 \pm 1.20
Chl <i>a</i> (μ g/L)	1.03 \pm 2.17	0.56 \pm 0.52	0.00 \pm 0.00	1.54 \pm 3.00	0.22 \pm 0.16	0.48 \pm 0.4	1.52 \pm 1.71	0.00 \pm 0.00	0.80 \pm 1.83	1.22 \pm 1.48	0.25 \pm 0.30
Bacteria ($\times 10^5$ cells/mL)	16.19 \pm 6.49	10.17 \pm 4.44	1.29 \pm 0.35	19.14 \pm 6.39	4.42 \pm 2.23	11.93 \pm 3.86	14.72 \pm 6.87	1.23 \pm 0.32	12.53 \pm 3.89	16.41 \pm 7.53	6.92 \pm 5.46
HNF ($\times 10^3$ cells/L)	1.53 \pm 0.75	1.15 \pm 0.51	0.53 \pm 0.25	2.04 \pm 0.93	0.86 \pm 0.43	1.21 \pm 0.48	1.25 \pm 0.52	0.57 \pm 0.26	1.23 \pm 0.57	1.37 \pm 0.79	1.09 \pm 0.58
PNF ($\times 10^3$ cells/L)	2.98 \pm 1.78	1.81 \pm 1.08	0.07 \pm 0.05	4.07 \pm 1.45	0.82 \pm 0.40	1.76 \pm 0.79	3.86 \pm 1.59	0.06 \pm 0.04	2.00 \pm 1.08	3.51 \pm 1.99	1.21 \pm 1.13
NF ($\times 10^3$ cells/L)	4.52 \pm 2.18	2.96 \pm 1.24	0.61 \pm 0.27	6.12 \pm 2.07	1.68 \pm 0.64	2.97 \pm 0.97	5.12 \pm 1.44	0.62 \pm 0.29	3.24 \pm 1.39	4.89 \pm 2.40	2.30 \pm 1.60

3.3. Distribution of alpha diversity

Alpha diversity showed a distinct horizontal distribution pattern. The southern region possessed higher alpha diversity than the northern region (Fig. 2A, D). Alpha diversity did not vary significantly between depth strata (Fig. 2B, E) and between water masses (Fig. 2C, F). Correlation analysis showed that alpha diversity was a function of several environmental factors (Table S1). In general, ciliate OTU richness correlated significantly with water chemistry (i.e., dissolved oxygen concentration, salinity, temperature, SRP concentrations, and SRP:DSi ratios) and food sources (i.e., Chl *a* concentrations and abundances of bacteria). Besides, spatial factors (i.e., latitude, longitude, and depth) were important factors correlated with alpha diversity (Table S1). Multivariate regression tree (MRT) analysis was used to visualize relationships between alpha diversity and environmental factors in a tree with one split based on DO concentration, Chl *a* concentration, bacterial abundance, latitude, and SRP:DSi ratios (Fig. S3). The primary contributor to alpha diversity variation was DO (contribution of 17.2%), followed by Chl *a* (11.3%), bacteria (10.9%), latitude (9.8%), and SRP:DSi (7.9%). Diversity variation was more strongly correlated with environmental variations than variations of depth, salinity, and temperature between water masses (Table S2). Environmental variations still had a significant effect on alpha diversity, irrespective of depth and water masses, whereas the effects of depth and water masses on alpha diversity were minor/insignificant when environmental characteristics were controlled for (Table S2).

3.4. Distribution of ciliate communities

Altogether, 2,989,740 sequences belonging to 1065 OTUs were observed for the final dataset. These OTUs were assigned to seven classes (Fig. 3A) that originated from 73 families and 119 genera. Spirotrichea was the most dominant class; it accounted for more than half of both OTU (51.2%) and sequence (57.6%) abundances, followed by Phyllopharyngea, which contributed 17.1% and 18.5% of all OTUs and sequences, respectively. The third most abundant subclass was Oligohymenophorea (12.9% of OTUs and 9.9% of sequences), followed by Colpodea (9.7% of OTUs and 7.9% of sequences), Litostomatea (5.8% of OTUs and 3.9% of sequences) and Prostomatea (1.9% of OTUs and 1.4% of sequences), each of which contributed 1–13% of diversity and

sequence abundance (Fig. 3A). The subclass Heterotrichea accounted for <1% of the total OTUs and sequences (Fig. 3A). The significant contributors at subclass levels to each dominant class (i.e., Spirotrichea, Phyllopharyngea, and Oligohymenophorea) are shown in Fig. S4. Within the Spirotrichea, Choreotrichia, which accounted for 63.2% of OTUs and 62.0% of sequences, and Oligotrichia, which accounted for 32.1% of OTUs and 37.7% of sequences, were the most diverse and abundant subclasses (Fig. S4A). Within the Phyllopharyngea, the subclass Cyrtophoria accounted for most of the observed diversity and sequence abundance (78.30% of OTUs and 93.27% of sequences) (Fig. S4B). Within the class Oligohymenophorea, the subclass Scuticociliatia was the most diverse and abundant (72.90% of OTUs and 60.60% of sequences), followed by Peritrichia (6.45% of OTUs and 37.87% of sequences) (Fig. S4C).

Clustering analysis (Fig. 4), PCoA (Fig. 3B–G), and ANOSIM (Table 2) were used to identify the ciliate community structure. Partitioning of beta diversity revealed that ciliate communities generally fell into three regional clusters based on their composition, though some samples were dispersed over the clustering tree/PCoA plots. They were cluster I (corresponding to the samples in the northern region, i.e., transects from Y to X), cluster II (corresponding to the samples in nearshore of southern region, i.e., transects from A to C), and cluster III (corresponding to the samples in the southernmost region, i.e. transect D and offshore site of B11) (Fig. 4A, B). This division suggests that the sampling sites across the Taiwan Strait can be classified into three major ciliate provinces (Figs. 3B–G, 4; Table 2), with the eigenvalues among the three provinces being significantly different in axes PCoA1 and PCoA2 (Fig. 3C, D, F, G). The differences in community composition among depth strata and water masses were weak (Table S3); therefore, these differences were not apparent in the PCoA plots (Fig. S5). Four classes—Spirotrichea, Phyllopharyngea, Oligohymenophorea, and Colpodea—generally dominated in each ciliate province, and their combined contribution ranged from 88.98% to 96.35% in each dataset (Fig. 3A; Table S4). Indicator analysis revealed high variability in the number of strict indicator OTUs across the three ciliate provinces (ranging from 62 in cluster I, 93 in cluster II, and 148 in cluster III) (Table S5). The taxonomic compositions of the strict indicators differed significantly among the three ciliate provinces (Table S5). The LDA effect size analysis revealed similar results (Fig. 5). The Prostomatea, Chlamydomontidae, Dictyocystidae, Favella, Apokeronopsis, and Tintinnopsis dadayi were more abundant in cluster I; Choreotrichia and Plagiocampa sp. were more abundant in cluster II; and Colpodea,

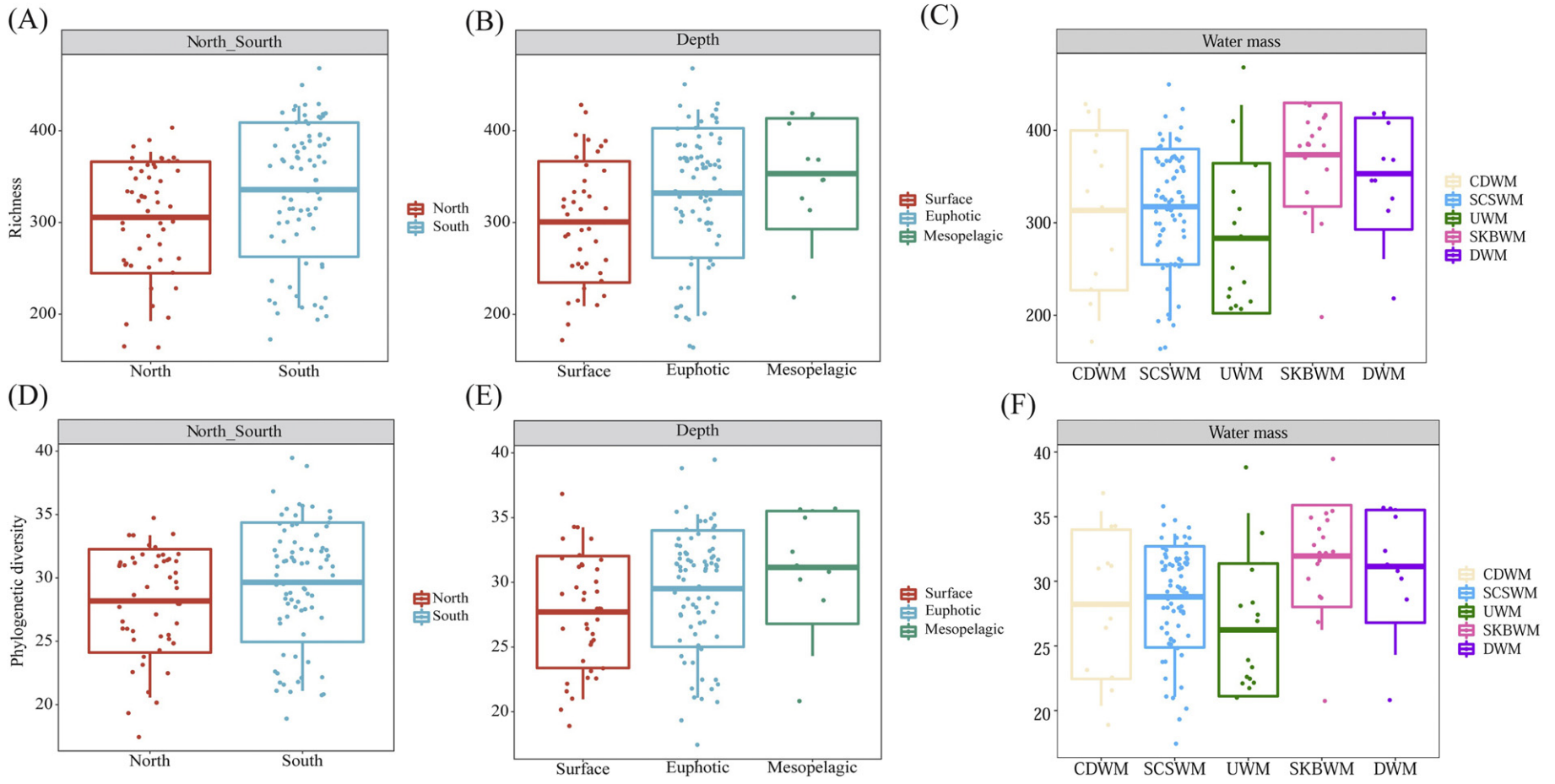


Fig. 2. Comparison of alpha diversity (OTU richness, A–C; phylogenetic diversity, D–F) in horizontal (A, D), vertical (B, E) dimensions, and among water masses (C, F) as demonstrated by boxplots. The error bars indicate SD, and bars without labels indicate significant differences at the level of $p > 0.05$.

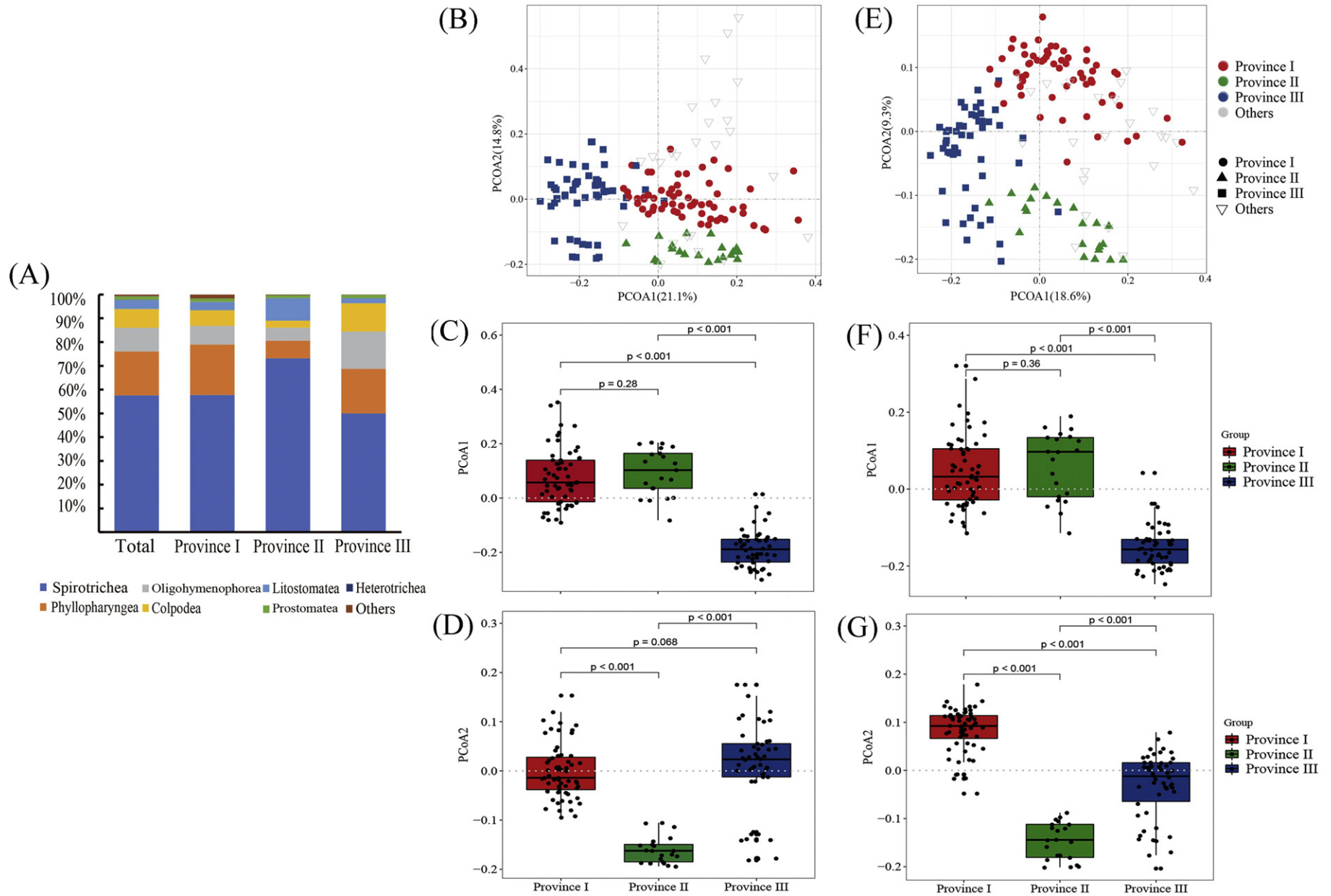


Fig. 3. (A) Bar chart shows community composition at the class level of the total and the three provinces; (B, E) principal coordinate analysis (PCoA) plots based on Bray-Curtis dissimilarity matrix (B), and phylogenetic distance (unweighted Unifrac distance, E) show the community structure of ciliates across Taiwan strait. Three provinces represent: province I (corresponding to the samples in northern region, transects from Y to X), province II (corresponding to the samples in nearshore of southern region, transects from A to C), and province III (corresponding to the samples in southernmost region, transect D and offshore site of B11); (C, D, F, G) Boxplot demonstrated comparisons of the eigenvalues among the three provinces in axes PCoA1 and PCoA2. Pairwise comparisons are tested using one-way ANOVA.

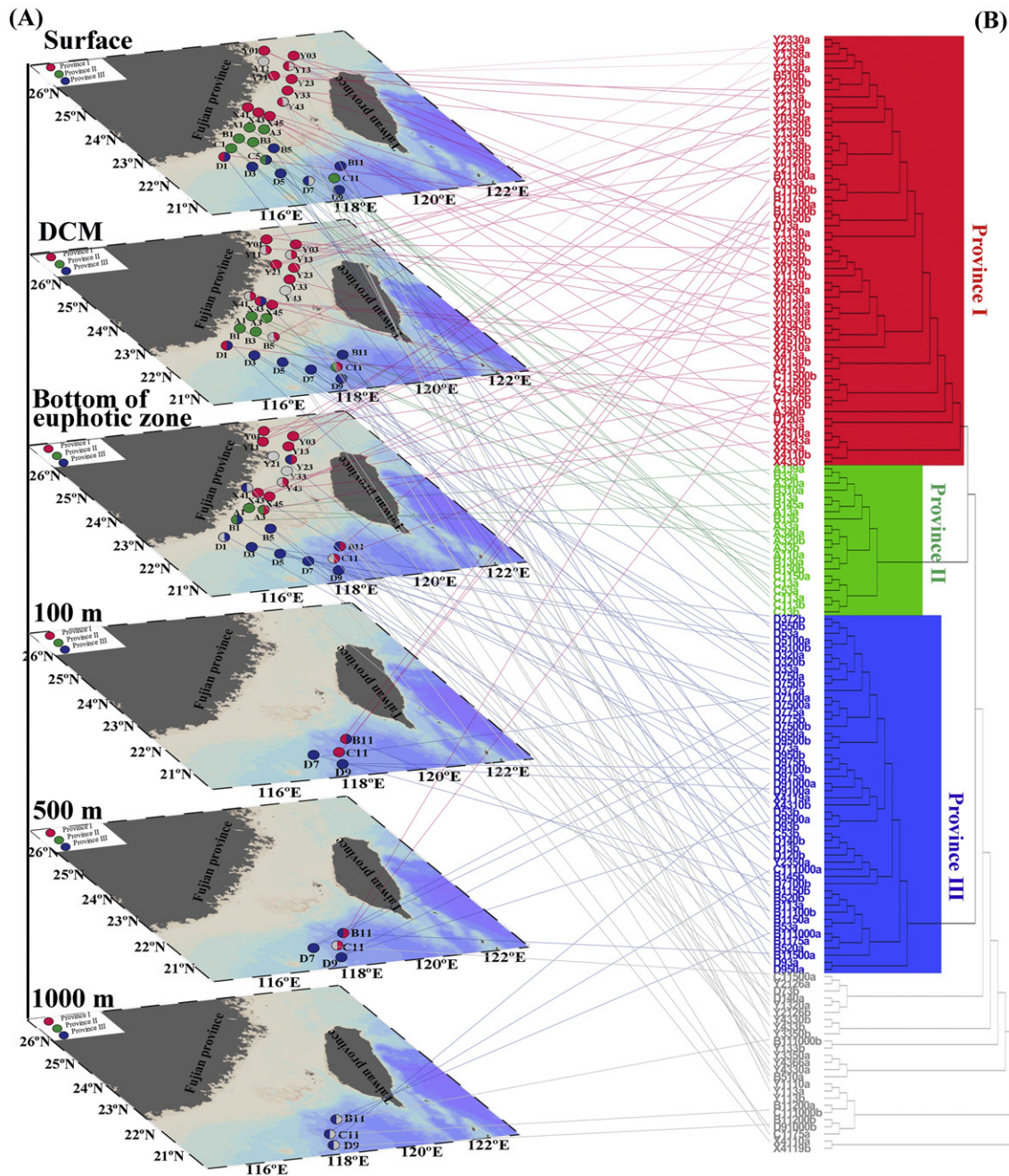


Fig. 4. (A) Map of Taiwan Strait highlighting the sampling sites and the distribution of ciliate provinces across depth strata in the present study; (B) Three ciliate provinces were identified based on clustering analysis. Red branches represent Ciliate Province I; green branches represent Ciliate Province II; blue branches represent Ciliate Province III. Dots on panel A were colored by the ciliate provinces they represent. Dots were divided into two halves representing nano- and micro-sized ciliate samples, respectively.

Oligohymenophorea, Oligotrichia, Hartmannulidae, Lynnellidae, *Amphorides*, *Eutintinnus*, *Salpingella*, and *Parastrombidinopsis minima* were more abundant in cluster III (Fig. 5). At the class level, five discriminant taxa among the three provinces were revealed by linear discriminant analysis effect size (LEfSe) analysis, i.e., Prostomatea for Province I, Choreotrichia for Province II, and Colpodea, Oligohymenophorea, and Oligotrichia for Province III (Fig. 5).

3.5. Partition of geographic distance, depth, water masses, and environments based on ciliate community structure

The demonstration by Mantel and Partial Mantel tests that community composition covaried significantly with geographic distance,

depth, water mass (represented by temperature and salinity) and environment (represented by all biotic and abiotic factors except temperature and salinity) suggested that spatial factors (i.e. geographic distance and depth), water mass, and environment co-shaped ciliate community structure across the Taiwan Strait (Table 3). Among these factors, geographic distance was the most important (Table 3). This result is mirrored by the Variance Partitioning Analysis (VPA), which revealed that geographic distance alone explained 13.6% of community variance, environment explained 6.8%, and depth explained only 1.0% (Fig. 6A). Multivariate regression tree (MRT) analysis was used to visualize relationships between community structure and environmental factors (Fig. S6). It revealed that latitude and DO together explained 22.5% of the

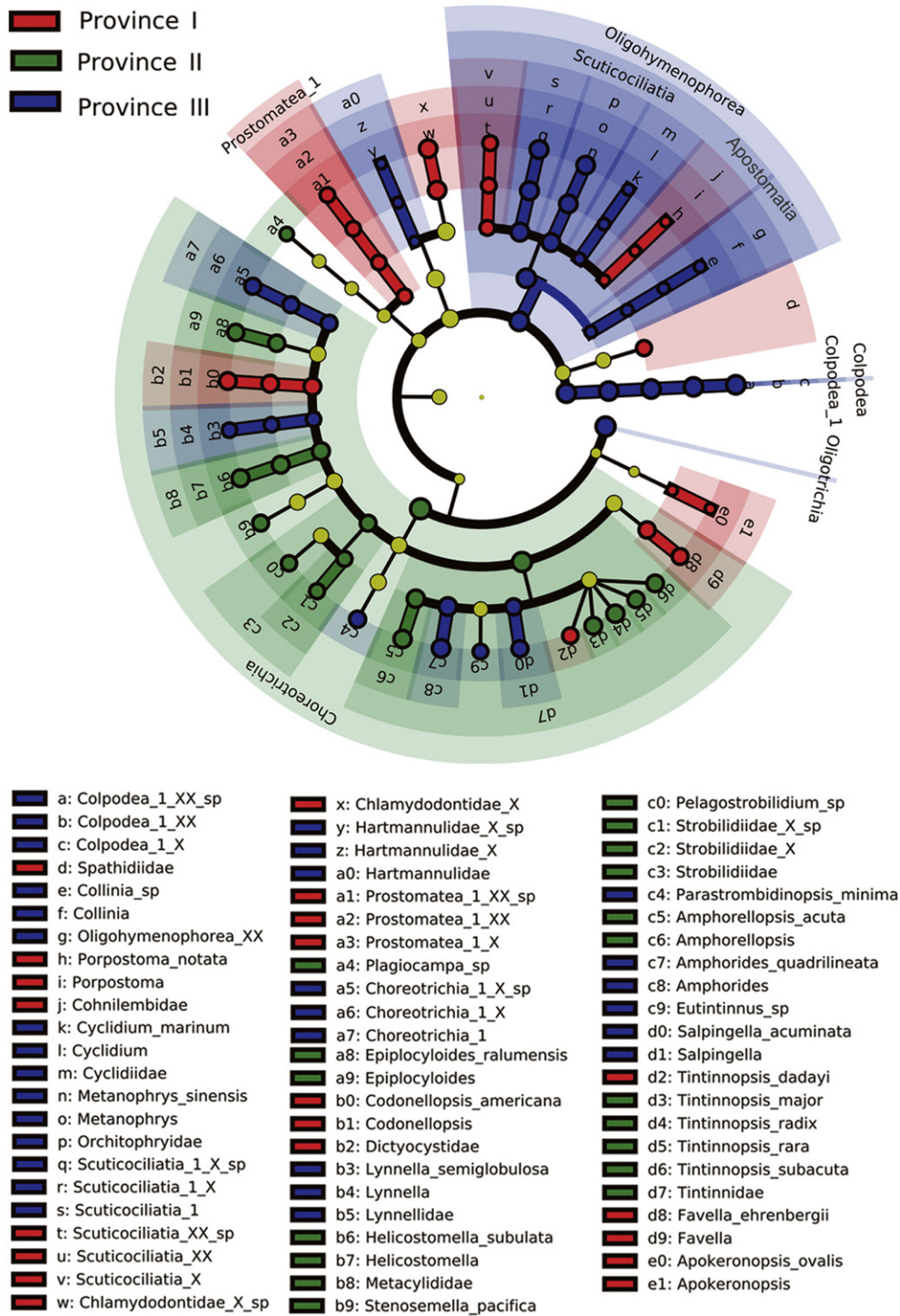


Fig. 5. Least Discriminant Analysis (LDA) derived taxonomic cladogram compares all samples categorized by three ciliate provinces. Significantly discriminant taxon nodes are colored, and branch areas are shaded according to the highest-ranked group for that taxon. If the taxon is not significantly differentially represented among sample groups, the corresponding node is colored in yellow.

variation in community composition (Fig. S6). As indicated by the hierarchical order of splits, latitude was the major contributor to community variation; its contribution was 16.1% (Fig. S6). Thus, the strong association between geographic distance and community composition confirmed the pattern of the three ciliate provinces in the Taiwan Strait (Figs. 3B–G, 4; Table 3). To further clarify which ecological processes structured ciliate distributions, we applied a phylogenetic null model. The null model indicated that dispersal limitation accounted for most (86.0%) of the variance of community

composition across the Taiwan Strait. The rest was contributed by ecological drift (8.3%), heterogeneous selection (5.5%), and homogeneous selection (0.1%) (Fig. 6B). Dispersal limitation accounted for most (48.8% to 95.3%) of the variance within each ciliate province; the highest percentage was associated with cluster III (Fig. 6B). Ecological drift (1.6% to 48.8%), heterogeneous selection (1.9% to 3.8%), and homogeneous selection (0 to 0.1%) accounted for relatively small percentages of the variance of community composition in each ciliate province (Fig. 6B).

Table 2

ANOSIM tests of the groupings of ciliate communities by the three provinces. Community turnover is based on the Bray-Curtis distance.

Pairs	ANOSIM	
	R	p
Total	0.693	0.001
Province I vs. Province II	0.713	0.001
Province I vs. Province III	0.707	0.001
Province II vs. Province III	0.798	0.001

4. Discussion

4.1. Regional alpha diversity

The present study revealed an association of alpha diversity with water chemistry, food abundance, and geographic distance (Fig. S3; Table S1). Ciliates are commonly assumed to be obligate aerobes (Finlay, 1981), though some members have been reported to be anaerobes, obligate anaerobes, or microaerophiles (Finlay and Fenchel, 1989; Fenchel and Finlay, 1991). Their diversity and community structure are sensitive to oxygen levels (Xu et al., 2014; Medina et al., 2016; Fenchel, 2012; Triadó-Margarit and Casamayor, 2015). Chl *a* concentrations and bacterial abundance could be regarded as proxies for ciliate foods (Ota and Taniguchi, 2003; Chiang et al., 2003). The higher concentrations of DO and Chl *a* and higher abundance of bacteria in the northern than southern region may facilitate the rapid growth of certain species that become abundant under suitable conditions and suppress the growth of other species in the communities of two regions. The possible relationship between these environmental conditions and diversity across the Taiwan Strait could explain the strong coupling of ciliate diversity and the concentrations of DO, Chl *a*, and bacteria in the study. Also, there are only two water masses in the northern region, the SCSWM and CDWM, whereas there are five water masses in the southern region, the CDWM, SCSWM, UWM, SKBWM, and UWM. These multiple water masses likely bring numerous species of ciliates into the region and may therefore contribute to the distribution of diversity. The positive correlation between alpha diversity and SRP:DSi ratios in the Taiwan Strait contrasts with the results of a previous study in the Bohai Sea and the Yellow Sea, which showed that the diversity of nanociliates was associated with SRP:DSi ratios, but the correlation was negative rather than positive (Dong et al., 2014). The association between ciliate diversity and SRP:DSi ratios may be the result of a cascade effect. The SRP:DSi ratio may be an indicator of nutrient limitation, which would impact the phytoplankton community and in turn, ciliate diversity and

Table 3

Simple and partial Mantel tests were performed to show the effects of geographic distance/depth/environment/water mass on the beta diversity of ciliate communities.

	r	p
Geographic distance	0.189	0.001
Environment	0.185	0.001
Water mass	0.189	0.001
Depth	0.169	0.002
Geographic distance-environment & water mass & depth ^a	0.172	0.001
Environment-water mass & depth ^b	0.086	0.025
Water mass-environment & depth ^c	0.094	0.008
Depth-environment & water mass ^d	0.089	0.025

Water mass was represented by temperature and salinity; environment was represented by all biotic and abiotic factors except temperature and salinity.

^a Correlation between ciliate beta diversity and geographic distance controlled by environment, depth and water mass derived from partial-Mantel test.

^b Correlation between ciliate beta diversity and environmental variability controlled by depth and water mass derived from partial-Mantel test.

^c Correlation between ciliate beta diversity and water mass controlled by depth and environment derived from partial-Mantel test.

^d Correlation between ciliate beta diversity and depth controlled by environment and water mass derived from partial-Mantel test.

assemblages through microbial trophic links. Despite the fact that depth and water mass were controlled in the statistical analysis, the variation of ciliate diversity across the region was significantly associated with the differences of environmental parameters between samples. In contrast, the correlations between alpha diversity and depth, geographic distance, and water mass were either low or insignificant without the inclusion of environmental variations (Table S2). Further analysis indicated that environmental factors were most strongly correlated with water mass ($r = 0.557, p = 0.001$), followed by depth ($r = 0.340, p = 0.001$) and location ($r = 0.091, p = 0.002$). Overall, these results suggest that environmental conditions structured by water mass, depth, and geographic distance likely defined ciliate diversity across the Strait. The results, therefore, revealed the intricate mechanisms that shape the distribution of ciliate diversity in the Taiwan Strait.

4.2. Regional biogeography

Previous studies of the distribution of ciliate communities have been restricted mainly to either small (0–1 km) (Doherty et al., 2010; Sun et al., 2017, 2019) or very large (>5000 km) spatial scales (Gimmler et al., 2016; Dolan et al., 2007). However, the pattern of the distribution of ciliates across substantial depth and environmental gradients on a regional scale (>100 km) has rarely been explored. Different water masses could create contrasting physical conditions and microbial communities on a regional scale and could therefore confound elucidation of distribution patterns. In this study, we studied ciliate communities across the Taiwan Strait, which is characterized by sharp depth and environmental gradients and complex movements of water masses. Our discovery of three ciliate provinces across the Taiwan Strait (Figs. 3B–G, 4; Table 2) revealed strong biogeographic provincialism in this marine ecosystem. The most abundant discriminant class in province I was Prostomatea (0.26% in province I, Fig. 5). This class of ciliates exhibit distinctive morphologies, and their very different ecological roles differentiate them from other ciliates (Lynn, 2008). Prostomes are encounter feeders or raptors, and they have been reported to be a significant consumer of algae and flagellates. Some members of the Prostomatea have been implicated in controlling the growth of some red-tide algae (Jeong et al., 2002; Weisse and Montagnes, 1998; Klaveness, 1984). This role is consistent with the correlation between the concentration of chl *a* ($r = -0.255, p = 0.003$), abundance of 2–5- μm PNF ($r = -0.254, p = 0.003$) and the discriminant class Prostomatea in province I (Fig. 5). Altogether, five OTUs of Prostomatea were found in province I, i.e., OTU97, OTU141, OTU2023, OTU600, and OTU2342, among which the former two dominated (with a contribution of 99.14%) the class Prostomatea, and the rest were minor contributors. When blasting with sequences deposited at the NCBI, OTU97 showed a high similarity to an environmental ciliate sequence (KJ763140, coverage 100%, similarity 100%) that was collected from a depth of 5 m at the San Pedro Ocean Time-series station in the eastern North Pacific, and OTU141 was close to an environmental ciliate sequence (AY665049, coverage 100%, similarity 100%) that was collected from the deep chlorophyll maximum in a Sargasso Sea mesoscale eddy. The two environmental sequences that OTU97 and OTU141 corresponded with were therefore all from typical marine environments. Indeed, an examination of the distribution of prostome OTUs between water masses in province I revealed that they made a minor contribution to prostomes in the CDWM (4.9% only) but were massively present in SCSWM. The fact that CDWM occurred in the nearshore sampling sites of province I and that the concentration of DO generally decreased with longitude ($r = -0.614, p < 0.001$) could thus explain the strong correlation between longitude ($r = 0.441, p < 0.001$), the concentration of DO ($r = -0.444, p < 0.001$) and the discriminant class Prostomatea in province I.

Choreotrichia is a major assemblage of planktonic ciliates in the ocean worldwide (Lynn, 2008) and was the most abundant discriminant class in ciliate province II (57.4%; Fig. 5). Extensive studies have demonstrated that choreotrichs are dominant grazers in microbial

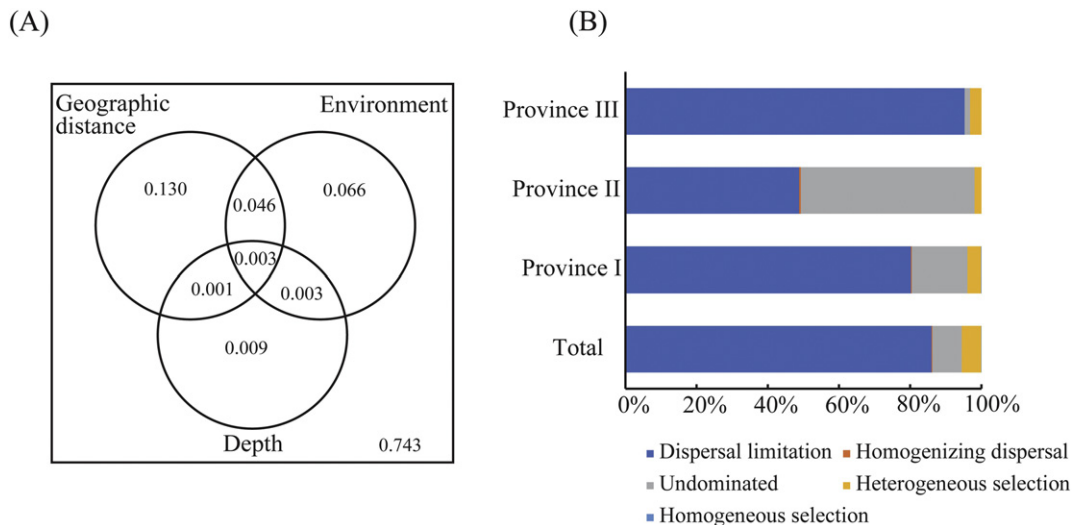


Fig. 6. (A) Variation partitioning analysis shows the effects of geographic distance, depth, and environment on the community structure of ciliates; (B) summary of the relative contributions of the ecological processes which determine community assembly in the total community and each ciliate province.

food webs and can consume up to 27% of annual primary production in coastal waters (Capriulo and Carpenter, 1983). The average concentration of chl *a* and average abundance of bacteria were higher in ciliate province II (1.22 $\mu\text{g/L}$ and 16.41×10^5 cells/mL, respectively) than in ciliate provinces I (0.80 $\mu\text{g/L}$ and 12.53×10^5 cells/mL, respectively) and III (0.25 $\mu\text{g/L}$ and 6.92×10^5 cells/mL, respectively). Therefore, the high chl *a* concentrations and high abundance of bacteria in province II could favor the growth of choreotrich ciliates. This line of reasoning could explain the strong association of Choreotrichia with concentrations of chl *a* ($r = 0.456$, $p < 0.001$) and abundance of bacteria ($r = 0.387$, $p < 0.001$) in ciliate province II.

The discriminant taxa in ciliate province III were dominated by the classes Oligotrichia (22.4%), Oligohymenophorea (15.7%), and Colpodea (11.9%). Oligotrichs have been extensively studied in a variety of marine environments, including both neritic and eupelagic regions (Lynn and Montagnes, 1991; Pierce and Turner, 1992). They contribute to a complex microbial food web by feeding on bacteria and autotrophic and heterotrophic nanoplankton. (Agatha, 2011). Oligotrichs generally dominate planktonic ciliate communities; they are approximately four times as abundant as loricated choreotrichs (Abboud-Abi Saab, 1989). The relative abundance of oligotrichs to a ciliate community tends to increase from coastal to oceanic waters. The fact that there was a lower proportion of coastal versus oceanic sampling sites in province III than the other two provinces could thus explain the dominance of oligotrichs in the province III. Among the class Oligohymenophorea, the subclasses Scuticiliatia and Apostomatia were the two major contributors to the variations of the oligohymenophoran community among the three provinces (Fig. 5). Scuticociliates have a high tolerance to a wide range of salinities and are particularly abundant in habitats with high levels of organic matter, nutrients, and bacteria (Urrutxurtu et al., 2003). The higher abundance of scuticociliates in province III might be related to the more significant depth gradient in the region versus the other two provinces. In province III, several samples were collected at water depths of 200–1000 m (i.e., mesopelagic zone), where particles that sink from the euphotic zone and dissolved organic matter that is advected downward are extensively reprocessed (Robinson et al., 2010). The fact that scuticociliates can actively feed on particles and bacteria in the mesopelagic zone therefore explains the strong association between depth ($r = 0.407$, $p < 0.001$), bacteria ($r = 0.484$, $p < 0.001$) and Oligohymenophorea in this ciliate province (Fig. 5). Apostomatia have been reported to be parasitic symbionts, primarily with crustaceans, but they can cause severe mortalities of their hosts (Bradbury, 1996; Gómez-Gutiérrez et al., 2003). It has previously been reported

that the diversity and abundance of parasitic ciliates are higher in the mesopelagic zone than in overlying water in the western Pacific Ocean (Weisse, 2017; Zhao et al., 2017) and northern South China Sea (Sun et al., 2019). Together with the results of this study, these findings indicate that parasitic oligohymenophoreans are an essential component of mesopelagic food chains. Because Colpodea generally inhabit soil and freshwater environments, their dominance in this province was surprising. This province encompasses multiple water masses, including the CDWM, UWM, SCSWM, SKBWM, and DWM, whereas Province II and I contain fewer water masses—CDWM, UWM, and SCSWM in Province II and CDWM and SCSWM in Province I. Because of the vast freshwater input of the Pearl River, which flows into the southern Taiwan Strait (Bai et al., 2015), large numbers of soil/freshwater colpodean ciliates are expected to dominate the local ciliate communities in the Taiwan Strait. However, only two of 128 colpodean OTUs could be associated with a particular species, namely *Colpoda inflata* and *C. steinii*, both of which are well known terrestrial species (Foissner, 1993). These two species accounted for only 0.27% and 0.006%, respectively, of the total colpodean community. The remainder of the 126 colpodean OTUs could be assigned to only a taxonomic class and were very similar to uncultured eukaryote sequences in GenBank found with the basic local alignment search tool. The implication is that unknown and highly diverse colpodean species dominate the colpodean community in the Taiwan Strait. Recent studies based on next-generation sequencing have changed our view of colpodeans as purely terrestrial and freshwater organisms (Dunthorn et al., 2014; Gimmler et al., 2016; Zhao et al., 2017). Horizontally, colpodean OTUs are found distributed worldwide in the euphotic zone of marine waters, even in polar regions (Gimmler et al., 2016). Vertically, Colpodea ciliates have been reported to be abundant at depths of 2000 m in the western Pacific Ocean based on both rDNA and rRNA sequencing (Zhao et al., 2017). The implication is that the diversity and range of the distribution of colpodean ciliates may have been greatly underestimated in previous investigations. Future studies employing light microscopy, scanning electron microscopy, and sequencing should be performed to explore the taxonomy and phylogeny of these unknown colpodean ciliates in marine ecosystems.

These results indicate that there is a nonrandom provincial distribution of ciliates in the water column across the Taiwan Strait. The statistical association of the discriminant taxa of each province with spatial factors and specific environmental characteristics suggests that local biotic and abiotic conditions likely shape the distribution of the ciliate provinces. However, there is significant covariance between environmental factors (Table S6). This covariance confounded the identification

of the underlying factors responsible for the nonrandom distribution of ciliate taxa. The implication is that multiple interactive factors underlie the complex processes by which conditions in different regions are determined.

4.3. Mechanisms shaping ciliate biogeographic pattern

Mantel and Partial Mantel tests (Table 3), and VPA (Fig. 6A) have shown that geographic distance, depth, and environment characteristics are essential factors responsible for the variation of ciliate communities across the Taiwan Strait. Among these factors, geographic distance is the most important (Table 3; Fig. 6A). This conclusion is consistent with the fact that the distribution pattern of the ciliate community is nonrandom on a regional scale. In other words, the three ciliate provinces reflected the fact that the dynamics of ciliates across the Taiwan Strait were governed primarily by spatial variability (Figs. 6A, S6). A true spatial effect could be related to historical events, such as environmental conditions in the past, the legacies of which have been maintained due to dispersal limitation (Ge et al., 2008). Spatial effects could also be related to the fact that in a highly connected ecosystem, poor competitors can escape from local competitive exclusion by emigration (Van der Gucht et al., 2007) because of their high dispersal rate. The effects of this dispersal cannot be separated out by variance partitioning analysis (Lindström and Langenheder, 2012). Multiple water masses exist in the Taiwan Strait (Fig. 1B), and their movement may facilitate high rates of dispersal of microorganisms that inhabit them but strongly limit dispersal among water masses across the Strait. This hypothesis is consistent with the results of the phylogenetic null model analysis, which revealed that dispersal limitation, which causes the community composition to be dissimilar when there are few exchanges between communities, contributed 86.0% of the community variance across the Strait (Fig. 6B). The percentage of the variance explained by geographic distance could also be related to unmeasured biotic and abiotic factors, such as the abundance of mesozooplankton, which is an important factor that shapes ciliate communities via top-down control in many parts of the ocean (Johansson et al., 2004; Nielsen and Kiørboe, 1994). The implication is that the importance of geographic distance per se may have been overestimated to some extent by the fact that not all environmental parameters related to ciliate communities were measured. Use of the phylogenetic null model showed that environmental selection played a minor role in community variations in the Strait (Fig. 6B). Water masses differ from each other in terms of their physicochemical and biotic characteristics (Hu et al., 2010). Local habitat conditions could select for specific taxonomic groups and could therefore be the primary source of a heterogeneous selection of ciliate assemblages across the Taiwan Strait. Overall, dispersal limitation overrode species sorting in determining the composition of the ciliate community across the Taiwan Strait. This conclusion is consistent with the results of previous studies of ciliate distributions in some other environments. For example, it has been found that two classes of ciliates, Oligotrichia and Choreotrichia, follow a log-series distribution pattern along an estuary in Long Island Sound, despite strong environmental gradients from the estuary to the ocean (Doherty et al., 2010). In the southeastern tropical Pacific Ocean, the observed distribution of tintinnid ciliates at most stations most closely matched a log-series distribution, consistent with the neutral theory of random colonization from a large species pool (Dolan et al., 2007). In the northern South China Sea, dispersal limitation was found to be the essential ecological process shaping the ciliate community during an observation period of two years (Sun et al., 2019). We infer that the dominance of stochastic processes in the ciliate community in the present study was due mainly to dispersal limitation, which could be mainly the result of the movement of water masses.

5. Conclusions

Microbial eukaryotes play a vital role in marine food chains and global nutrient cycling. However, few studies have tried to tease apart the effects of geographic distance and depth on the microeukaryote community on a regional scale, especially when those effects are confounded by the presence of strong environmental gradients and movement of water masses. The underlying mechanisms that account for the assembly of microeukaryote communities in dynamic habitats with different water masses on a regional scale therefore remain unclear. The Taiwan Strait is a complex marine ecosystem with a complex topography and dynamic circulation, where the distribution and determinants of ciliate communities remain unclear. This study revealed that ciliate communities show a provincial distribution across the Strait. The biogeographic patterns of the ciliate community are very much a function of water mass-driven spatial effects and environmental heterogeneity. The results offer new insights into the spatial distributions of ciliate communities and their shaping factors in the Taiwan Strait, and they expand knowledge of the relative impacts of deterministic and stochastic processes on community assemblages of microeukaryotes across a region within which there are water mass movements and strong spatial and environmental gradients.

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Data accessibility

NCBI Sequence Read Archive, accession code PRJNA560553.

Author contributions

PS designed the experiments. YW performed the experiments. PS and YW analyzed the data. All authors wrote the paper.

Declaration of competing interest

We declare that the submitted work was conducted in the absence of any commercial or financial relationships that could potentially be construed as a conflict of interest.

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