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Diversity and distribution of small-sized planktonic ciliate communities in the East China Sea

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Ciliates are an important ecological component in the microbial food web, but few studies have been conducted on the spatial distribution of small-sized planktonic ciliate communities in the East China Sea (ECS). Investigating ciliate communities using conventional morphological approaches is particularly difficult for the small, fragile, and naked species. Therefore, we applied DNA metabarcoding analysis to explore the spatial pattern of small-sized planktonic ciliate community structure within the surface, deep chlorophyll maximum (DCM), and bottom layers. Results showed the cosmopolitan species, *Leegaardiella* sp., was dominant and widespread in the ECS. The relative abundance of the mixotrophic family Tontonnidae decreased in the deeper layer. We characterized water masses of the ECS using environmental variables. In nano-sized ciliate communities, non-metric multidimensional scaling (NMDS) plots revealed a correlation with temperature, salinity, density, and depth. The circulation patterns were similar to cluster analysis results, suggesting that hydrographic conditions shaped small-sized ciliate community composition.

KEYWORDS

planktonic ciliate, metabarcoding analysis, SSU rDNA, spatial distribution, East China Sea (ECS)

Introduction

Ciliates are highly diverse unicellular eukaryotes found in various aquatic environments. Planktonic ciliates, dominated by oligotrich, choreotrich, and tintinnid ciliates (Alveolata, Ciliophora, Spirotrichea), vary in size from tens to hundreds of micrometers (Lynn, 2008). In pelagic ecosystems, they are crucial components in

microbial food webs, transferring energy to higher trophic levels while grazing on smaller plankton, such as pico- and nanoplankton (Azam et al., 1983; Pierce and Turner, 1992).

The East China Sea (ECS) is located in the Northwest Pacific Ocean, one of the world's largest and most productive marginal seas. It is a system with complex hydrological dynamics resulting from the interaction of various water masses (Ichikawa and Beardsley, 2002; Lee and Chao, 2003; Yang et al., 2011; Zhu et al., 2022). In short, Yellow Sea Current flows from the north to the south, Changjiang diluted water (CDW) is along the coastal area, Kuroshio intrusion passes through the east of Taiwan from the south to the north, and Taiwan Current Warm Water (TCWW) flows from the south to the north (Isobe, 2008; Chen et al., 2009; Liu et al., 2021a, b; Kang and Na, 2022).

The Changjiang River generally discharges the highest in July or August, carrying large amounts of terrestrial nutrient inputs into the aquatic environments (Wu et al., 2019). A positive relationship between ciliate density and increased bacteria availability in the plume area has been reported (Chiang et al., 2003). Ciliate communities also transfer organic carbon to higher trophic levels by consuming phytoplankton and smaller plankton in the ECS (Suzuki and Miyabe, 2007; Choi et al., 2012).

Until now, more detailed surveys of tintinnid communities were studied than aloricate, oligotrich, and choreotrich ciliate communities, as their loricae were more accessible to be identified and stored by traditional taxonomic methods (Bachy et al., 2014; Dolan et al., 2009; Santoferrara and Alder, 2009; Dolan and Pierce, 2013; Dolan et al., 2013, 2016; Li et al., 2018). Moreover, aloricate ciliates are more abundant, especially in small sizes in the ECS, which increases the difficulties in traditional research under the microscope (Pitta and Giannakourou, 2000; Zheng et al., 2015; Yang et al., 2020). These small-sized ciliates represent an important grazer for picoplankton (Kim et al., 2021; Romano et al., 2021). An alternative way to survey the biodiversity and spatial distribution of ciliate communities is the metabarcoding approach, which can reveal more taxa than microscopic observations, particularly in small, fragile, and aloricate species (Bachy et al., 2013; Santoferrara et al., 2013; Gimmler et al., 2016; Santoferrara et al., 2018; Ganser et al., 2021). In this study, we characterized the species composition and distribution of small-sized planktonic ciliate communities using metabarcoding on vertical and horizontal scales. We also revealed the relationships between planktonic ciliates and environmental factors in this complex hydrological environment.

Materials and methods

Sample collection

Seawater samples were collected using Go-Flo bottles mounted on a conductivity, temperature, and depth (CTD) rosette (Sea-Bird 91 Electronics, Bellevue, WA, USA). A total of 30 stations with different depths were sampled in the ECS during a summer cruise in July 2019 (Figure 1). Hydrographic data were measured using a CTD profiler. The nutrient variables, including dissolved inorganic nitrate, phosphate, silicate, and chlorophyll *a* (Chl *a*) concentration,

were measured according to the standard methods developed by Gong et al. (2003). The precision for the determination of nitrate, phosphate, silicate, and Chl *a* were $\pm 0.3 \mu\text{M}$, $\pm 0.5 \mu\text{M}$, $\pm 0.01 \mu\text{M}$, and $\pm 0.02 \text{ mg/m}^3$, respectively.

For DNA samples, 10 L of three-layered seawater (surface, deep chlorophyll maximum (DCM), and bottom) were pre-filtered through a 200- μm nylon mesh, followed by filtering through a 20- μm nylon filter (90 mm diameter, 100 Millipore, Billerica, MA, USA), and then subsequently filtered through 3- μm and 0.2- μm pore size polycarbonate membranes (142 mm diameter, Millipore, USA) using a peristaltic pump. The filters were immediately preserved in liquid nitrogen and stored at -80°C until DNA extraction.

DNA extraction, amplification, and sequencing

DNA was extracted using the DNeasy PowerWater Kit (Qiagen, Valencia, CA, USA). The V4 region of rDNA was amplified using TAREuk454FWD1 (5'-CCA GCA SCY GCG GTA ATT CC-3') as the forward primer and the modified TAREukREV3 (5'-ACT TTC GTT CTT GAT YRA-3') as the reverse primer (Stoeck et al., 2010). PCR conditions were as described in Lin et al. (2022). Amplicons were sequenced on an Illumina MiSeq platform, generating 300-bp paired-end reads. Data have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database under the project number PRJNA738614.

Sequence processing

The sequence primers were removed using cutadapt (Martin, 2011) and then underwent quality filtering and denoising using the standard pipeline of the DADA2 package (Callahan et al., 2016) under R software. Reads were filtered with the following parameters: truncLen and minLen = c (240, 180), truncQ = 2, and maxEE = c (2, 2). Taxonomic assignments of amplicon sequence variants (ASVs) were conducted using the Protist Ribosomal Reference Database (PR2) version 4.12.0 (Guillou et al., 2013). Planktonic ciliate amplicons were selected corresponding to the orders Tintinnida, Choreotrichida, and Strombidiida, among the division Ciliophora in PR2. Ciliate communities were subsampled with 250 reads for 100 times.

Data analysis and visualization

All statistical analyses were conducted using software R (Team, 2013). Alpha diversity indices (Shannon index H' and richness) and the Bray-Curtis dissimilarities were calculated using the Vegan package (Oksanen et al., 2019). The non-metric multidimensional scaling (NMDS) plot was performed to visualize the patterns and identify potential environmental drivers for the ciliate community composition based on the log-transformed ASV abundances and scaled environmental variables. Venn diagram was made using the ggVennDiagram package (Gao et al., 2021). Ciliate communities

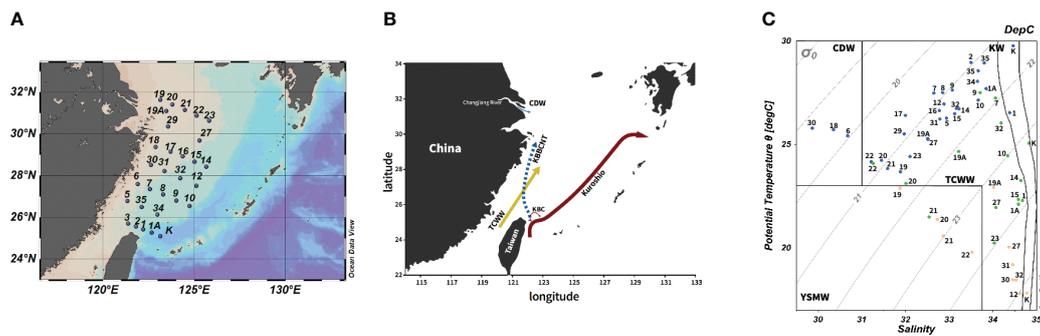


FIGURE 1

(A) Sampling sites from this cruise. (B) The schematic map of circulation in the ECS adapted from Yang et al. (2011). (C) The T-S diagram of ciliate samples from this cruise and water masses were defined by Gong et al. (1996). The blue, green, and orange circles represent water samples at the surface, DCM, and bottom layer, respectively. CDW, Changjiang diluted water; TCWW, Taiwan Current Warm Water; YSMW, Yellow Sea Mixed Water; KW, Kuroshio water. The schematic map shows the shelf current system of the ECS in summer. KBBCNT, Kuroshio Bottom Branch Current to the northeast of Taiwan; KBC, Kuroshio Branch Current; CDW, Changjiang diluted water; TCWW, Taiwan Current Warm Water; ECS, East China Sea; T-S, temperature–salinity; DCM, deep chlorophyll maximum.

were grouped by hierarchical cluster analysis using Ward's minimum variance method and square-rooting Bray–Curtis distances with cluster package (Maechler et al., 2022). To understand the relationship between the environment and each cluster, differences in environmental variables among clusters were examined using Tukey's honestly significant difference (HSD) *post-hoc* test, followed by an analysis of variance (ANOVA). Graphics were created using the ggplot2 package (Wickham, 2016). The plots of sampling stations, temperature–salinity (T-S) diagram, and vertical profiles of environment variables were generated in the Ocean Data View (Schlitzer, 2022). Small-sized planktonic ciliates (<20 μm) indicated sequences obtained from the nano-sized fraction. The pico-sized reads were counted for the presence of ASVs to reveal the distribution.

Results

Hydrographic conditions

During this cruise, seawater temperature and salinity ranged from 17.8°C to 29.7°C and 29.8 to 34.8, respectively. Four water masses observed in the study area followed the definition by Gong et al. (1996): TCWW, Yellow Sea Mixed Water (YSMW), CDW, and Kuroshio water (KW) (Figure 1). The YSMW influenced the bottom of the northern transect in the ECS, while the coastal surface area was influenced by the low-salinity CDW ($S < 31$; Supplementary Figure S1B). The KW intruded in northeastern Taiwan, involving a topographic upwelling at St. 1 (Supplementary Figure S1). The TCWW dominated the shelf area of most of the sampling sites (Figure 1C).

Overall diversity across three size fractions

We obtained a total of 15,980 ASVs represented by ~11,465,721 DNA reads. Alveolata contributed largely to total reads, particularly

in nano-sized fraction (73.9%, data not shown). A total of 314 ASVs (21,336 reads) remained as planktonic ciliates across three size fractions after subsampling (19, 36, and 29 samples from micro-sized, nano-sized, and pico-sized fractions, respectively). A complete list of ciliate taxa from this dataset is reported in Supplementary Table 1. Overall, nano- and pico-sized fractions share more similarities than micro-sized where the Bray–Curtis distances were closer in the NMDS plot (Supplementary Figure S2D). The alpha diversity and species richness were lower in the micro-sized fraction when compared to both the nano- and pico-sized fractions (Supplementary Figure S2A). Tintinnida accounted for more than 50% of the ciliate relative abundance in the micro-sized fraction, in which *Salpingella* sp., *Amphorellopsis acuta*, and *Stenosemella pacifica* were found to be the major contributors (Supplementary Figure S3). Strombidiida dominated in two small-sized fractions, which were *Strombidiida_G_XX_sp.*, *Strombidiidae_H_X_sp.*, and *Strombidiidae_G_X_sp.* in the nano-sized fraction, and *Strombidiida_G_XX_sp.*, *Spirotontonia_sp.*, and *Tontonnidae_B_X_sp.* in pico-sized fractions (43.97% and 52.93%, respectively; Supplementary Figures S2B, S3). *Leegaardiella* sp. was the most abundant taxon across three size fractions in the ECS.

Nano-sized ciliate communities and relationship with environmental variables

Hierarchical cluster analysis based on the Bray–Curtis dissimilarities was performed on nano-sized ciliate communities. These clusters were subsequently organized into two groups, defined by a distance threshold of 1.7, resulting in one group containing three clusters and another with two clusters (Figure 2). In nano-sized samples, both cluster analysis and NMDS revealed a strong correlation with depth (Figures 2, 3). The first group, which included Clusters I, II, and IV, was influenced by the deeper water column. Cluster IV, which comprised St. 20 and St. 21 across all depths in the water mass of TCWW and YSMW, was dominated by Choreotrichida. The taxonomic compositions in Cluster I were

mostly from deeper water samples in TCWW, with only one exception in YSMW. Although Clusters II and IV shared similar taxonomic compositions according to the analysis of Bray–Curtis dissimilarity, the former was mainly influenced by TCWW and KW (Figure 2), whereas the latter was mainly influenced by YSMW. The second group containing Clusters III and V was primarily composed of samples collected from shallow depths, particularly the surface layer. These shallow water samples were mostly from TCWW and positively correlated to temperature but significantly different in salinity (Supplementary Figure S4; Table 1). Clusters III and V, both from shallow water, were higher in temperature and oxygen concentration. The biological parameters indicated the potential prey (*Synechococcus*, photosynthetic picoeukaryotes, and bacteria), and only *Synechococcus* was higher in Cluster III (Supplementary Figure S4).

Distribution of small-sized ciliates

The top four taxa in nano-sized fraction, *Leegaardiella* sp., *Strombidiida_G_XX_sp.*, *Strombidiidae_G_X_sp.*, and *Strombidiidae_H_X_sp.*, were widespread and across all three layers (Figures 4, 5). *Lynnella semiglobulosa*, *Pelagostrobilidium minutum*, *Rimostrobilidium venilia*, *Strombidium capitatum*, *Strombidium*

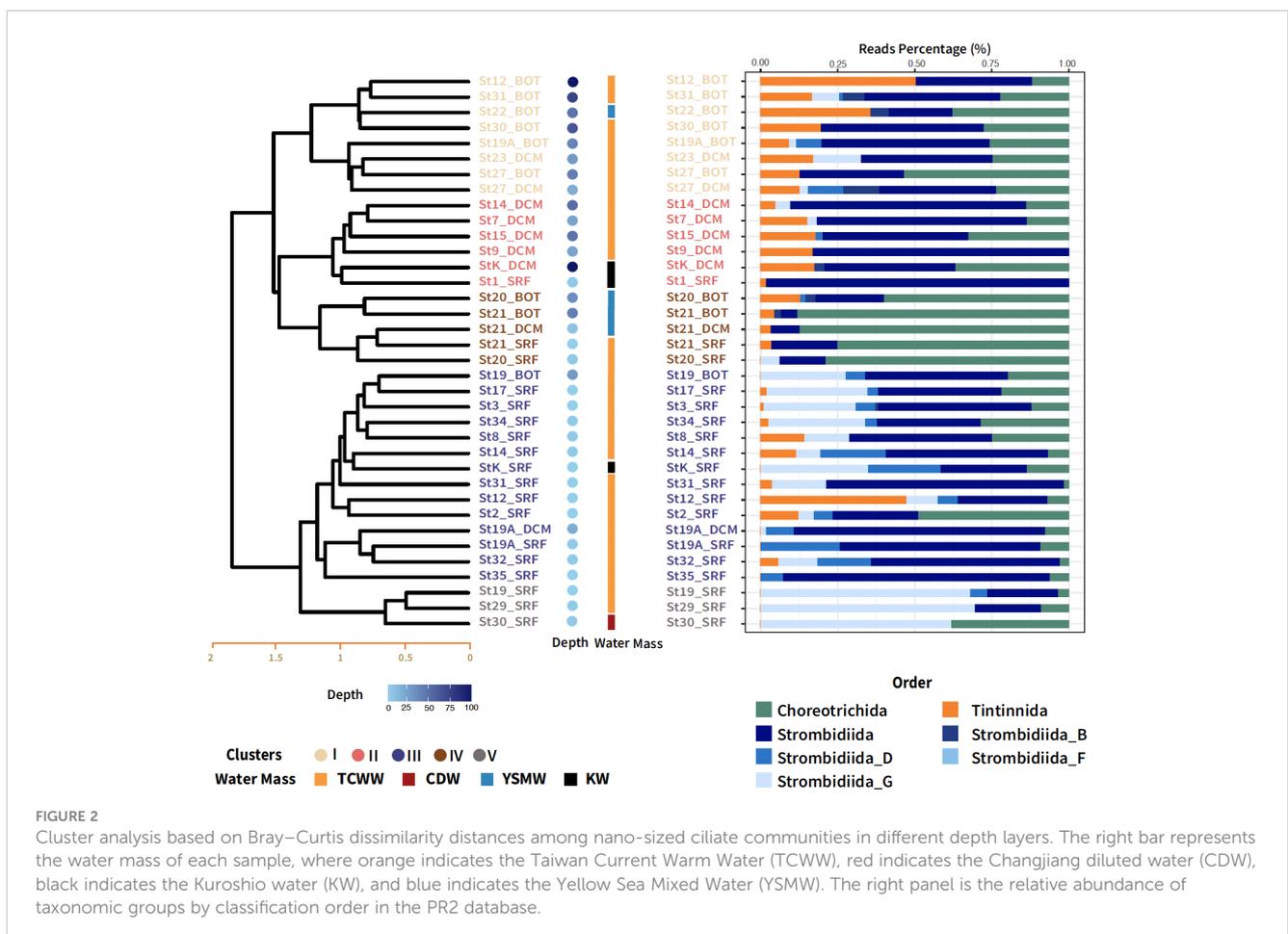
caudispina, and *Strombidium triquetrum* were only found in the surface layer (data not shown).

Multiple vertical distribution patterns were observed based on different annotated species. The presence of the mixotrophic family Tontonnidae, *Pseudotontonia simplicidens*, *Pseudotontonia_sp.*, *Spirotontonia grandis*, *Spirotontonia_sp.*, *Spirotontonia turbinata*, *Tontonnidae_A_X_sp.*, and *Tontonnidae_B_X_sp.*, decreased in the deeper layer. There was an opposite pattern with the Tontonnidae, with *Tintinnidae_X_sp.* and *Parastrombidinopsis shimi* in low abundance/absent in the surface layer (Figure 5). *Strobilidium caudatum* and *Strombidium_M_sp.* were found across three layers but on the north transect (Figure 4).

Discussion

Metabarcoding

Research on microbial community composition and diversity using metabarcoding has been more prevalent globally but relatively scarce in the ECS (Bik et al., 2012; De Vargas et al., 2015; Gimmler et al., 2016; Malviya et al., 2016; Noan et al., 2016; Cordier et al., 2022). Metabarcoding has the potential to reveal large-scale patterns in plankton diversity and community structure that were largely



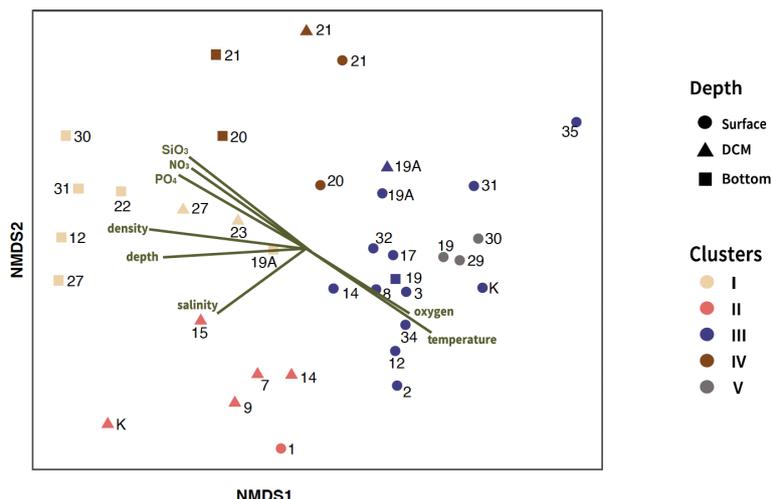


FIGURE 3 Non-metric multidimensional scaling (NMDS) plot using Bray–Curtis dissimilarity distances of nano-sized ciliate communities, overlaid with environmental vector fitting ($p < 0.05$). The plot indicates five different clusters of ciliate communities, each represented by a different color. The stress value for the plot was 0.17.

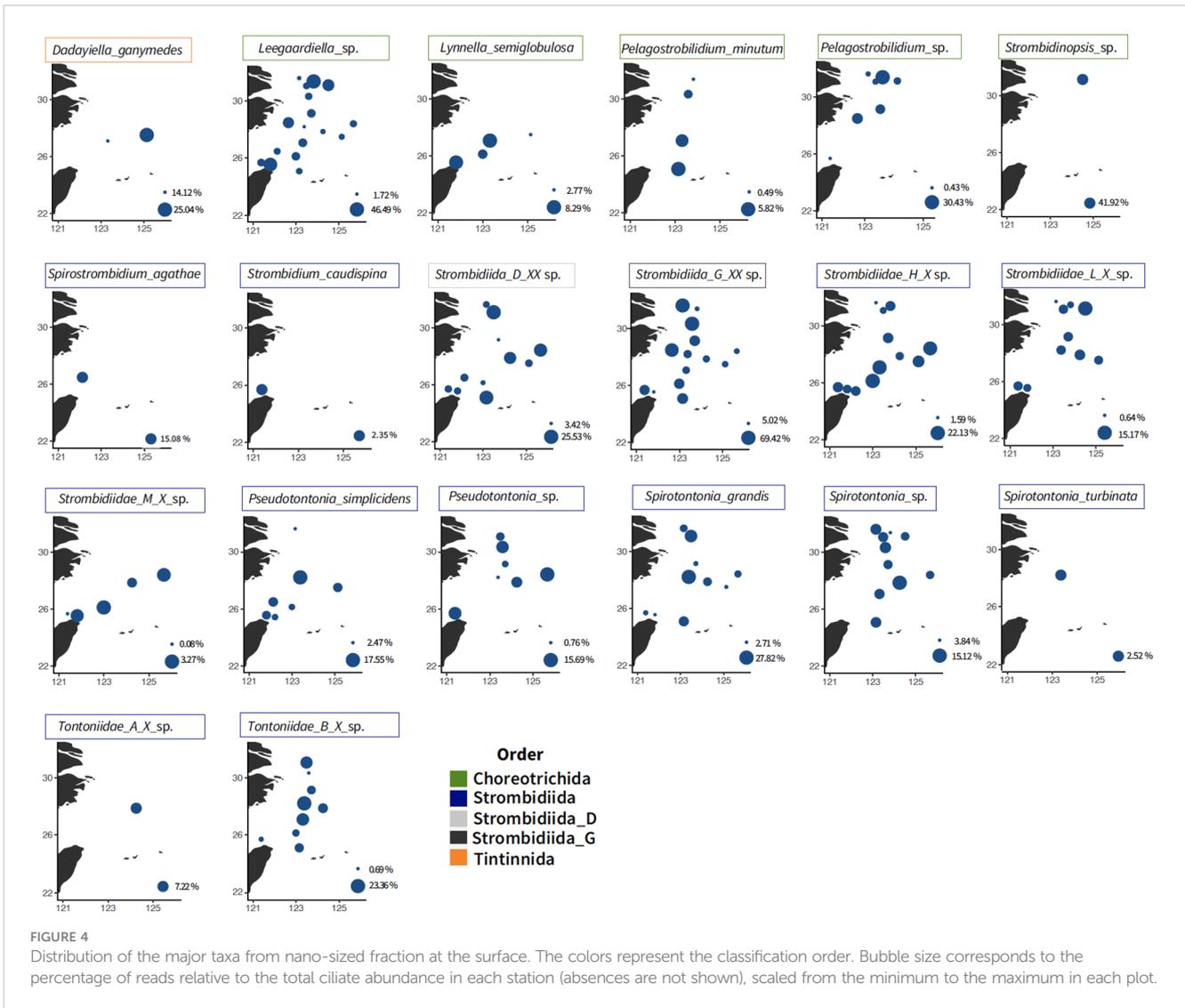
invisible in morphological analyses due to large numbers of undescribed species and the difficulty in characterizing the species present in the plankton community (Massana et al., 2006; Ichinomiya et al., 2016; Morard et al., 2018; Tragin and Vaultot, 2019). Studies focusing on aloricate ciliate communities often face challenges in the resolution of morphological characteristics due to differences in fixation methodologies, which results in fewer studies based on morphology, as staining requires specific expertise (Wang et al., 2013; Sun et al., 2019; Huang et al., 2021; Kim et al., 2021). Identification of tintinnids could be easier and more complete for tintinnid communities by their external loricae compared to aloricate

ones (Dolan et al., 2012; Dolan and Pierce, 2013; Zhang et al., 2017; Romano et al., 2021; Wang et al., 2021b; Li et al., 2023; Mieczan et al., 2023). However, ciliate community investigations can be more accessible without elaborate staining procedures and subjective observation under microscopy once the standardized pipeline of molecular sequencing and data filtering was followed (Bachy et al., 2013; Santoferrara et al., 2020; Burki et al., 2021). Metabarcoding provides rigor standards comparing dissimilarities among plenty of communities. Small-sized aloricate ciliates (<20 μm) were up to 50% in planktonic ciliate communities in many regions, including the ECS by morphological data (Yang et al., 2020; Wang et al., 2023a, b). Thus, this

TABLE 1 Environmental variables fit on NMDS plot using permutation tests.

	Nanoplankton				
	NMDS1	NMDS2	r ²	Pr(>r)	
Temperature	0.80863	-0.58832	0.7906	0.001	***
Salinity	-0.78347	-0.62143	0.4236	0.001	***
Density	-0.991	0.13389	0.7593	0.001	***
Fluorescence	0.89859	0.4388	0.1473	0.077	
Oxygen	0.80498	-0.5933	0.6614	0.001	***
Depth	-0.99782	-0.06604	0.676	0.001	***
NH ₄	-0.99792	0.06443	0.0663	0.344	
NO ₃	-0.79614	0.60511	0.6829	0.001	***
NO ₂	-0.08581	0.99631	0.0981	0.174	
PO ₄	-0.84712	0.5314	0.7451	0.001	***
SiO ₃	-0.76535	0.64362	0.7714	0.001	***
Chl a	0.99923	-0.03923	0.1174	0.136	

NMDS, non-metric multidimensional scaling; Chl a, chlorophyll a. Significance: 0 '***', Permutation: 999.



study can improve the understanding of planktonic ciliate community structure, particularly for small and naked species.

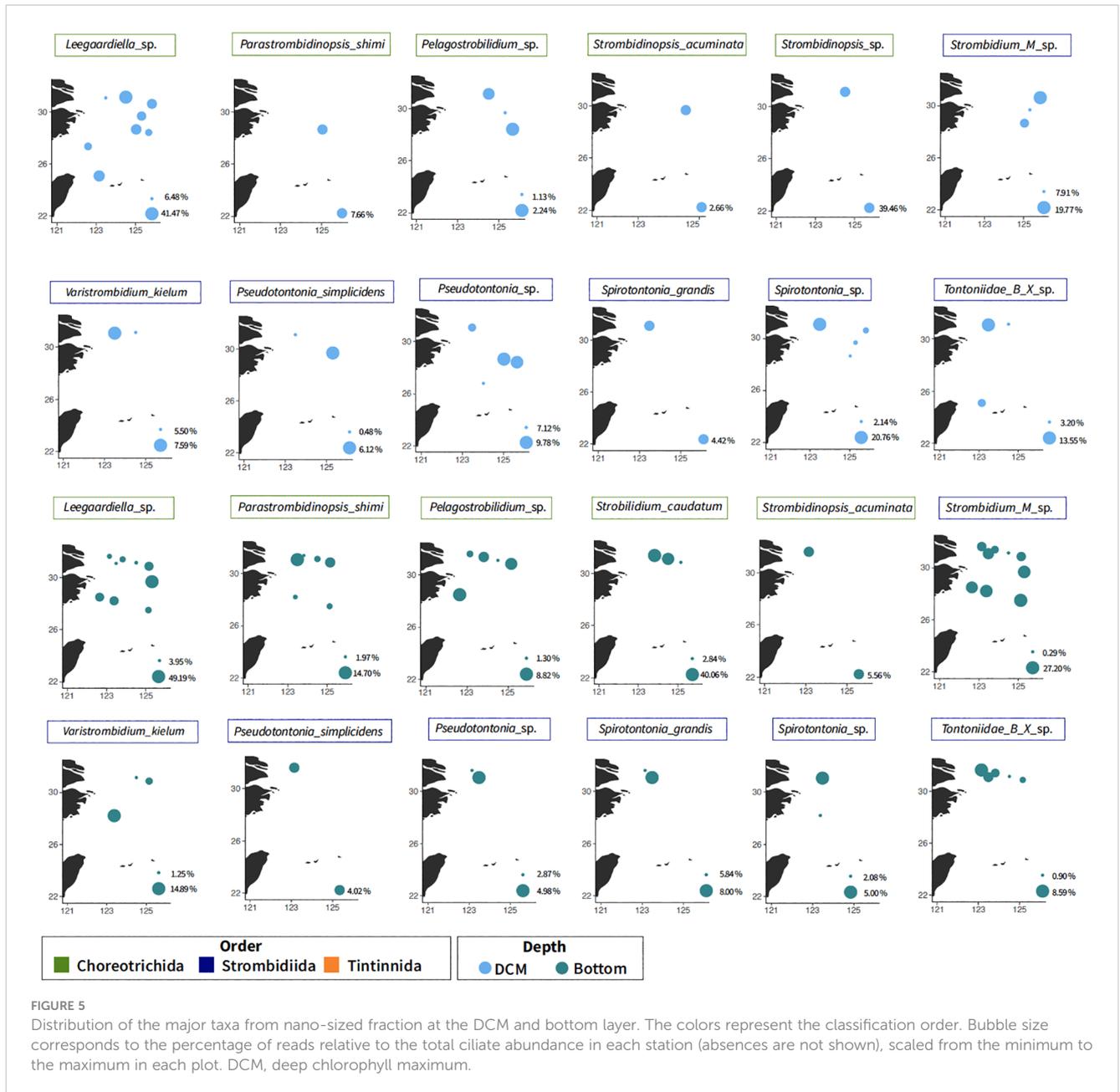
Ciliate sequences in pico-sized fraction

To date, many surveys by metabarcoding have shown ciliate sequences in the pico-sized fraction (Romari and Vaulot, 2004; Wu et al., 2017; Xu et al., 2017; Grattepanche et al., 2022; Flegontova et al., 2023). Canals et al. (2020) indicated that these sequences provided useful information about ciliate communities where over 94% of Ciliophora abundance from the Tara Ocean expedition in each size fraction corresponded to operational taxonomic units (OTUs) detected in the three fractions. There are hypotheses to explain these sequences in the pico-sized fraction: a) cell breakage during sequential filtration or b) membrane flexibility of ciliates to pass the small pores on filters (Cheung et al., 2008). It is possible for ciliates with a minute oral diameter (i.e., 2–5 μm) to pass through 3

μm , but molecular data are lacking (Yang et al., 2020). Comparing reads in nano- and pico-sized fraction from the same sampling sites, only *Strombidiida_G_XX_sp.* differed significantly in read abundance (Supplementary Figure S5, $p < 0.05$). Our results supported ciliate sequences in the pico-sized fraction from the cell breakages during filtration.

Overall diversity across three size fractions

Leegaardiella sp., a cosmopolitan species reported by Agatha (2011), was dominant across three size fractions in this cruise (Supplementary Figure S4). Additionally, with morphological data, *Leegaardiella ovalis* was identified in the same region (Yang et al., 2020). However, more sequences from the database are needed to establish a connection between the two species. In the micro-sized fraction, tintinnid contributed to half of the relative abundance, which decreased as size decreased, which was similar to



previous studies in both coastal and pelagic samples (Supplementary Figure S2B; Grattepanche et al., 2016b; Canals et al., 2020). Tintinnid loricae are larger (50–400 μm in length) than aloricate oligotrich and choreotrich ciliates, facilitating the retention of cells on 20- μm filters. Most tintinnid ciliates were reported with morphological records except *S. pacifica*, *Tintinnopsis platensis*, and *Ascampbelliella acuta* (Xu et al., 2013; Li et al., 2016; Feng et al., 2018; Li et al., 2018; Sun et al., 2019; Yang et al., 2021; Wang et al., 2021b; Yu et al., 2022; Li et al., 2023). In both nano-sized and pico-sized fractions, dominant taxa were similar but different in relative abundance (Supplementary Figure S3). *P. minutum* was absent in the micro-sized fraction, which corresponded to its morphological characteristics (Chen et al., 2017).

Horizontal and vertical distribution of small-sized ciliates

We focused more on ASVs, which were annotated with aloricate morphospecies (Figure 3). *Dadayiella ganymedes* was found in offshore stations in the ECS and recorded on a large scale in the western Pacific region (Li et al., 2021; Feng et al., 2022; Li et al., 2023). *Strombidiopsis acuminata*, *S. caudatum*, *P. shimi*, *Strombidium_M_sp.*, and *Varistrombidium kielum* were found only on the north transect across different layers, which might be related to the Changjiang River discharge-induced aloricate ciliate abundance in the summer (Chiang et al., 2003; Tsai et al., 2011; Yu et al., 2016). The mixotrophic family Tontonnidae was found

vertically and horizontally in the ECS, whereas several species were frequently recorded in adjacent areas (Yang et al., 2020, 2021). However, a common species, *Loboea strobila*, was not detected during this cruise (Sun et al., 2019; Huang et al., 2021).

Hydrographic conditions shape small-sized ciliate community composition

The hydrology of the ECS was intricate and influenced by factors such as the Changjiang River outflow, Taiwan Strait, and Kuroshio. In general, the clustering pattern in nano-sized communities was similar to the current circulation patterns in summer (Figure 1B; Yuan et al., 2008). Kuroshio Bottom Branch Current to the northeast of Taiwan (KBBCNT), which is bifurcated from Kuroshio subsurface water (KSSW), formed year-round upwelling off northeast Taiwan with its collision on the shelf (Liu et al., 1992; Wu et al., 2008; Kao et al., 2023). We observed upwelling in St. 1 and St. 1A by decreasing temperature (Supplementary Figure S1A; Chen et al., 2015). Then, KBBCNT flowed northwestward along the 60-m isobath, carrying high-salinity and nutrient water and mixing with TCWW, toward the Changjiang River mouth (Yang et al., 2011). This circulation influenced Clusters I and II, in which Tintinnida and Strombidiida were dominant in both clusters (Figure 2). In addition, the TCWW flowed northward in the upper layer mixed with Kuroshio surface water (KSW) and CDW (Ichikawa and Beardsley, 2002). It might reflect the distribution as Cluster III.

The Changjiang River discharge carried a large amount of terrestrial nutrients in summer, which moved northeastward, subsequently mixing with saline ambient waters (Chen et al., 2009). However, only St. 30 in Cluster V was classified under CDW (Figure 1C). Considering the location of St. 19 and St. 29 and the highest Chl *a* concentration measured at the surface in St. 19 (3.39 mg/m³), which were possibly influenced by CDW and TCWW, resulting in the lowest salinity among all clusters (Supplementary Figure S4). The highest average Chl *a* value observed in Cluster V. *Strombidiida_G_XX_sp.* was dominant in Cluster V, indicating potential predation on phytoplankton (66%, Supplementary Table S3). Moreover, the two mixotrophic genera, *Spirotonionia* and *Pseudotonia*, up to 12% in this cluster suggested the bottom-up environment. Cluster IV, which was from St. 20 and St. 21, was influenced by YSMW. *Strombidinopsis sp.* and *S. caudatum* were distinct and abundant species that implied different niches of YSMW, as in lower temperature and salinity (25% and 14%, Supplementary Table S3). However, different ASVs contributed to different clusters, which indicated the possibility of cryptic species of different niches (data not shown). The strong correlation between the ordination and depth ($R^2 = 0.68$) and particularly density ($R^2 = 0.76$), coupled with significant differences in temperature and salinity between clusters, implied that cluster dissimilarity was influenced by the currents (Table 1).

The definitions of the water masses are not consistent in the ECS (Gong et al., 2000; Ichikawa and Beardsley, 2002; Yang et al., 2011; Quan et al., 2013; Zhou et al., 2018). Surface water in the ECS is mainly mixed by the CDW, KSW, and TCWW, while the deeper layer is

influenced by KSSW, Shelf Mixing Water (SMW), and TCWW by the Optimum Multiparameter analysis (Zhou et al., 2018). Ciliate community composition and diversity reported differ markedly with depth (Countway et al., 2010; Grattepanche et al., 2016a, b; Santoferrara et al., 2023; Tucker et al., 2017; Zhao et al., 2017; Wang et al., 2021a), water masses (Sun et al., 2020; Yang et al., 2020; Gu et al., 2021), and geographical position (inshore/offshore) (Grattepanche et al., 2015; Santoferrara et al., 2016; Grattepanche et al., 2016b). While Yang et al. (2020) indicated that planktonic ciliates were potentially reliable indicators of water masses in the ECS, this was not universally consistent, possibly due to methodological differences in definitions of water masses or ciliate analysis approaches (Zhang et al., 2015). The NMDS plot revealed a correlation with multiple environmental variables, particularly density (Figure 3, Table 1). Additionally, the distribution of clusters was similar to current circulation patterns, suggesting that currents influence community composition. Here, we presented evidence that the composition of nano-sized ciliate communities varied with the circulation of currents.

In summary, detailed planktonic ciliate community composition was reported by metabarcoding in the ECS. The specified vertical and horizontal distributions of major small-sized taxa were first revealed. We supported hydrographic conditions shaping community structure by the similarities of cluster distribution. Building a more comprehensive database of reference sequences in future studies can provide more information for ciliate communities.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

W-TC: Writing – original draft, Writing – review & editing. Y-CL: Data curation, Formal analysis, Writing – review & editing. S-FT: Writing – review & editing. K-PC: Funding acquisition, Project administration, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2024.1349707/full#supplementary-material>

SUPPLEMENTARY TABLE 1

List of the number of annotated ciliate species, the total number of reads, and the presence (+) or absence (-) across three size fractions.

SUPPLEMENTARY TABLE 2

Nano-sized planktonic ciliate ASV table in each sample.

SUPPLEMENTARY TABLE 3

Percentage of top ten nano-sized ciliate taxa in each cluster.

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