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RESEARCH PAPER

Spatial distribution of planktonic ciliates in the western Pacific Ocean: along the transect from Shenzhen (China) to Pohnpei (Micronesia)

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Abstract

Planktonic ciliates have been recognized as major consumers of nano- and picoplankton in pelagic ecosystems, playing pivotal roles in the transfer of matter and energy in the microbial loop. However, due to the difficulties in identification, the species composition of ciliate assemblages, especially for the small, fragile, and naked species that usually dominate the ciliate communities in the oceanic waters, remains largely unknown. In the present study, 22 stations along the transect from Shenzhen (China) to Pohnpei (Micronesia) were sampled for the enumeration of picoplankton and nanoflagellates. In addition, pigment analysis of major phytoplankton groups along with the measurements of environmental variables including temperature, salinity, and nutrients were also carried out. Ciliates were identified at species level using quantitative protargol stain to reveal the species composition and their distribution patterns from off-shore to open ocean. Ciliate abundance was positively correlated with phosphate, silicate, and pico-sized pigmented eukaryotes (PPEs), whereas the biomass was closely related with PPEs, heterotrophic nanoflagellates, and chlorophytes. The combination of silicate and pigmented nanoflagellates was identified as the major factor driving the ciliate community composition. The close relationship between silicate and ciliate abundance and community structure needs further validation based on more data collected from oceanic waters. Our study showed the necessity of using techniques that can reveal the community composition at higher taxonomic resolutions in future studies on ciliates.

Keywords Community structure · Distribution pattern · Diversity · Nanoflagellates · Quantitative protargol stain

Introduction

Microbial eukaryotes play a variety of crucial roles in marine ecosystems as primary producers, predators, decomposers, and parasites (Caron et al. 2017; Fenchel 2008). The geographic distribution and diversity of microbial eukaryotes have long been a topic of research interest in the scientific community (Finlay 2002; Foissner 2008). Compared to larger and more motile zooplanktonic organisms, the spatial distributions of micro-organisms are more strongly influenced by physical factors due to their comparatively limited motility (Reid and Stewart 1989). The distribution patterns based on community structure have been found to be pivotal in understanding the mechanisms that drive community diversity, functional succession, and biogeography in microbial ecology (Jaillard et al. 2014; Zhou and Ning 2017).

Marine planktonic ciliates (Alveolata, Ciliophora) are a morphologically diverse group, and are ubiquitous in the oceans (Lynn 2008). As one of the most important

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components in the microbial food web, planktonic ciliates have been recognized as major consumers of nano- and picoplankton (Burkill et al. 1993; Sherr and Sherr 1987), which are the dominant size fractions in terms of biomass and primary productivity in pelagic ecosystems (Azam et al. 1983; Stoecker et al. 1994). Consequently, ciliates play an important role as mediators in the transfer of matter and energy from the primary production to higher trophic levels (Calbet and Saiz 2005; Landry and Calbet 2004; Stoecker and McDowell-Cappuzzo 1990), and therefore, in the functioning of marine food webs (Gifford 1991). To date, studies on the spatial distributions of planktonic ciliates have focused mainly on tintinnids because of their robust and relatively easily identifiable lorica (Modigh et al. 2003; Pierce and Turner 1993; Zhang et al. 2015) despite the fact that lorica plasticity exists in some species (Bachy et al. 2012; Kim et al. 2010; Xu et al. 2012, 2013). In contrast, aloricate oligotrichs, which usually dominate planktonic ciliate communities both in coastal waters and open oceans (Sun et al. 2017, 2020; Yang et al. 2015, 2020; Yu et al. 2015), are less known as they are typically small, fragile, and difficult to identify without infraciliature. As a result, studies on ciliate community based on cell abundance and biomass data with high taxonomic resolution are rare. This is especially true for the oligotrophic open oceans, where the role of microbial components may be more important (Suzuki et al. 1998). In recent years, sequencing techniques, including high throughput sequencing (HTS) of SSU rRNA genes, have increasingly been applied to assess the biodiversity and distribution patterns of ciliates (Grattepanche et al. 2014; Li et al. 2019; Santoferrara et al. 2016; Sun et al. 2017, 2019, 2020; Zhao et al. 2017). However, due to the large variations of rRNA gene copy number among microbial eukaryotes including ciliates (Gong et al. 2013), such approaches do not provide direct information on cell abundance and biomass. Thus, the conventional methods, e.g., microscopical identification and enumeration, still play key roles in the ecological studies on ciliates.

The tropical/subtropical western Pacific Ocean is considered as oligotrophic and unproductive compared with the eastern Pacific and other oceans (Olson 2001). Several studies have been carried out on the spatial distributions of pico- and nanoplankton communities in this region (e.g., Liang et al. 2016; Tsai et al. 2005; Xu et al. 2018). However, studies on the spatial distributions and diversity of planktonic ciliates have been scarce, and little is known about the factors that determine their distribution (Gómez 2007; Wang et al. 2019; Zhao et al. 2017). To improve our knowledge in this context, investigations on planktonic ciliates were conducted during a cruise in summer 2016, along the transect from Shenzhen (China) to Pohnpei (Micronesia). This is approximately 4993 km across, as a linkage between the South China Sea and the western Pacific Ocean. The quantitative protargol staining (QPS) method was used as it can reveal the identity of ciliates mostly at species level (Montagnes and Lynn 1987; Skibbe 1994).

The main objectives of this study were to: (1) characterize the diversity, community structure, and spatial distribution patterns of planktonic ciliates; and (2) reveal their potential relationship with environmental variables to identify the main physicochemical and biological factors that significantly influence the spatial distribution and diversity of planktonic ciliates in the western Pacific Ocean.

Results

Hydrographical conditions

The ranges of physicochemical parameters at the 22 sites (Fig. 1) are summarized in Supplementary Table S1. The surface seawater temperature ranged from 27.5 °C (DY-8 and DY-9) to 29.7 °C (DY-16 and DY-23) with an average of 28.6 °C. The salinity remained quite consistent at ca. 35.3 except that at station DY-1, which was slightly lower than those of other stations. The inorganic nutrients exhibited similar horizontal distribution patterns to that of salinity, with the maximum concentrations all occurring at DY-1 (0.755, 0.159, and 1.821 μmol/L for NO_2+NO_3, PO_4, and Si, respectively) and decreasing from off-shore to open ocean. The average concentrations of NO_2+NO_3, PO_4, and Si were 0.125 ± 0.206, 0.017 ± 0.038, and 0.758 ± 0.307 μmol/L, respectively.

The highest surface Chl *a* concentrations occurred at DY-4 (0.204 μg/L) and DY-18 (0.199 μg/L), whereas those of other sites ranged from 0.009 to 0.057 μg/L. The relative contributions of the eight major phytoplankton groups (diatoms, dinoflagellates, haptophytes, chlorophytes, cryptophytes, prasinophytes, *Synechococcus*, and *Prochlorococcus*) to the total Chl *a* are shown in Supplementary Fig. S1 (see Supplementary Table S2 for concentrations of pigments). At most stations, haptophytes and cyanobacteria including *Synechococcus* and *Prochlorococcus* were clearly the dominant groups, whereas at DY-18, cryptophytes contributed the highest to the phytoplankton pigment and at DY-11, followed by dinoflagellates and other groups.

Spatial distribution of picoplankton and nanoflagellates

The horizontal abundance distributions of picoplankton groups showed a distinct trend decreasing from off-shore to open ocean areas. The highest abundance of heterotrophic bacteria, *Prochlorococcus* and pico-sized pigmented eukaryotes (PPEs) all occurred at DY-1 (Supplementary Fig. S2a, c, d), whereas the maximum abundance

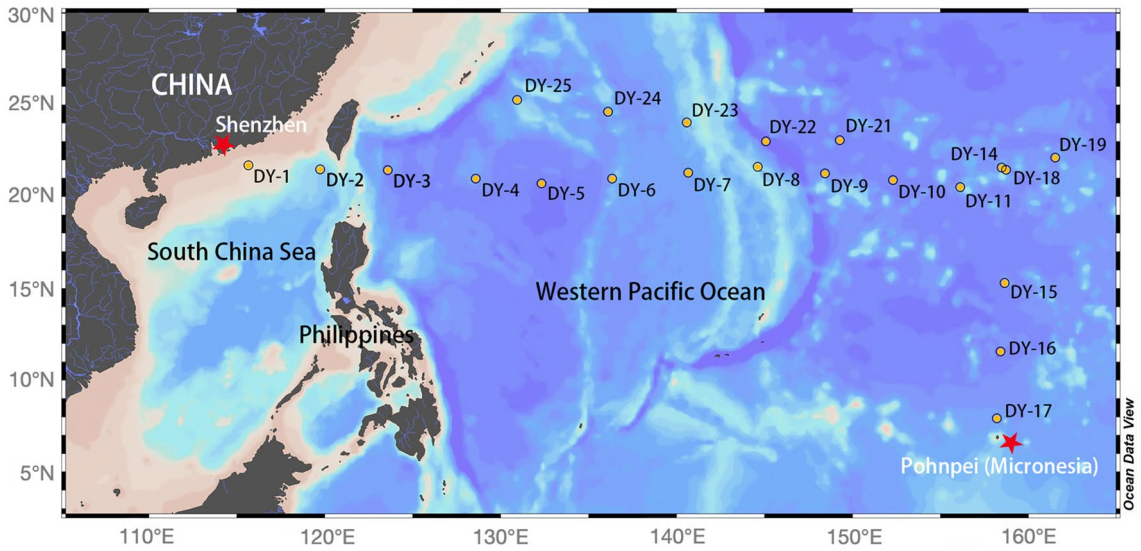

Fig. 1 Sampling stations along the transect from Shenzhen (China) to Pohnpei (Micronesia) in the western Pacific Ocean

of *Synechococcus* occurred at DY-3 where it was slightly higher than that of DY-1 (Supplementary Fig. S2b). High abundances of *Prochlorococcus* were observed also at DY-3 and DY-7 (Supplementary Fig. S2c), and the distribution of PPEs exhibited relatively higher variability compared with other picoplankton groups (Supplementary Fig. S2d). The average abundances of heterotrophic bacteria, *Synechococcus*, *Prochlorococcus*, and PPEs were $5.56 \pm 1.09 \times 10^5$, $2.35 \pm 4.75 \times 10^2$, $5.18 \pm 6.53 \times 10^3$, and $0.73 \pm 0.36 \times 10^2$ cells/ml, respectively.

The abundance of heterotrophic nanoflatellates (HNFs) ranged from 9.36 to 74.86 cells/ml, averaging 36.16 ± 18.10 cells/ml. The 2–5 μm size fraction dominated HNF abundance at all sites except at DY-1, and showed much stronger fluctuation compared with the 5–10 μm size fraction (Fig. 2a). In contrast, the biomass of HNFs ranged from 0.29 to 2.27 μg C/L, and the 2–5 μm size fraction was much less variable (Fig. 2a). Furthermore, the biomass of 2–5 μm sized HNFs were dominant at most of the sites; the exceptions being DY-1, DY-14, DY-15, DY-16, and DY-21, where the biomass of 5–10 μm size fraction surpassed that of the 2–5 μm (Fig. 2b). The abundances of pigmented nanoflatellates (PNFs) were higher than those of HNFs at all sites, ranging from 93.58 to 252.66 cells/ml with an average of 186.73 ± 66.70 cells/ml. The 2–5 μm size fraction dominated PNF abundance at all stations, and showed an increasing trend from west to east (Fig. 2c). The biomass of PNFs ranged from 0.01 to 2.29 μg C/L, and exhibited higher variability compared with that of HNFs, and the 2–5 μm size fraction dominated the PNF biomass at all sites except DY-1 and DY-17 (Fig. 2d).

Spatial distribution of planktonic ciliates

A total of 41 ciliate species were recorded, including 32 species of aloricate oligotrichs and choreotrichs, two species of tintinnids, and seven species of other groups including *Pseudokeronopsis* sp., *Urotricha cyrtonucleata*, *Tiarina fusus*, *Tiarina poopsia*, *Balanion comatum*, *Didinium gargantua*, and *Myrionecta rubra* (see Fig. 3c for representative species after staining). The highest species number was observed at DY-1 (32) followed by DY-2 (31), and the lowest (5) was found at DY-11 and DY-19 (Supplementary Table S3). The average Shannon–wiener index (H') and Simpson's diversity index (D) were 2.172 and 0.843, respectively, and the lowest were found at DY-16 (Supplementary Table S2). The abundance of ciliates varied between 20 and 720 cells/L, averaging 164 cells/L, with the highest found at DY-1 and the lowest at DY-19 (Fig. 3a). The biomass of ciliates ranged between 0.009 and 0.878 μg C/L (on average 0.192 μg C/L), with the highest and the lowest found at DY-1 and DY-19, respectively (Fig. 3b). The sizes of ciliates showed high inter- and intraspecific variations and most cells were within 20–40 μm size fraction (Fig. 4). The species with the largest cell size was *Laboea strobila*, ranging from 43.3 to 123.6 μm (on average 76 μm). *Strombidium pollostomum* and some cells of *Leegaardiella ovalis*, *Lohamanniella oviformis*, *Strombidium dalum*, and *Strombidium epidemum* were found to be smaller than 20 μm (Fig. 4).

Based on the dominance index (DI), the seven most dominant species were *Strombidium emergens* (DI = 0.204), *Tontonia gracillima* (DI = 0.093), *S. epidemum* (DI = 0.092), *Strombidium wulffi* (DI = 0.076), *L. ovalis* (DI = 0.059),

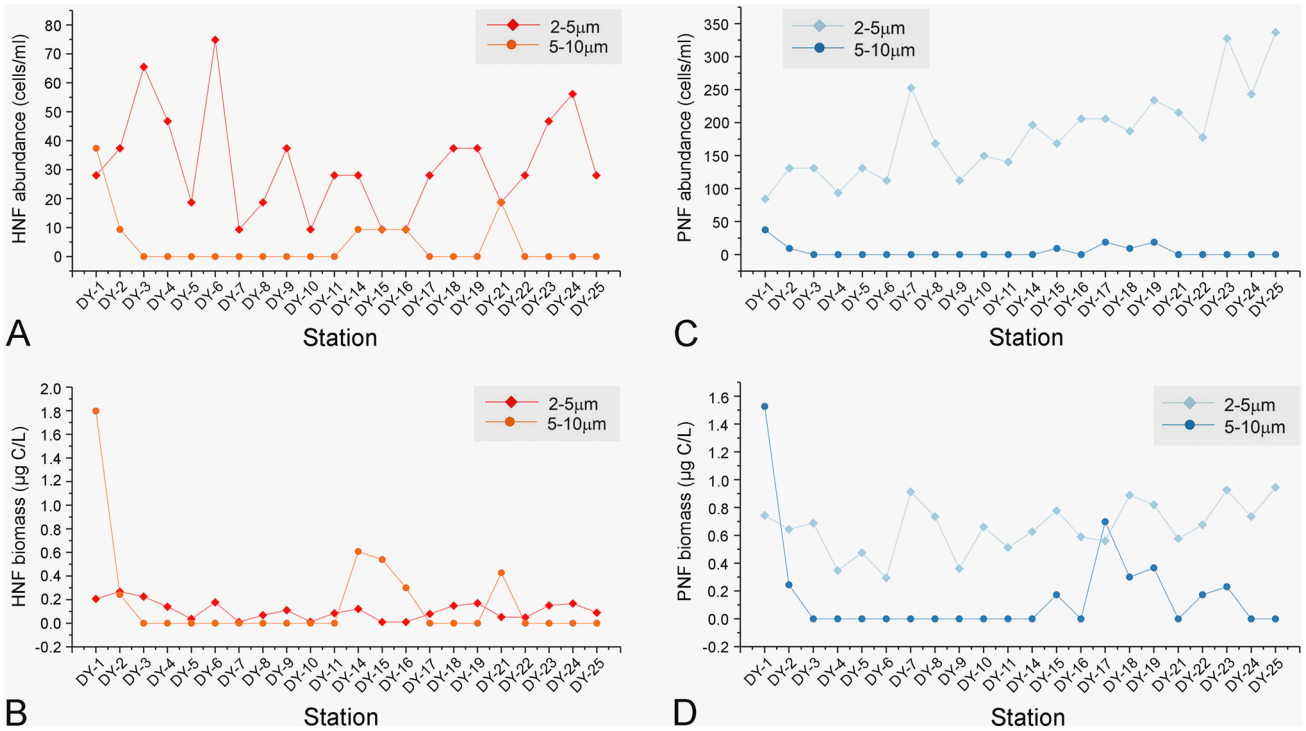

Fig. 2 Distribution of heterotrophic nanoflagellates (HNFs) and pigmented nanoflagellates (PNFs) abundances (**a**, **c**, respectively) and biomasses (**b**, **d**, respectively) along the transect from Shenzhen (China) to Pohnpei (Micronesia) in the western Pacific Ocean

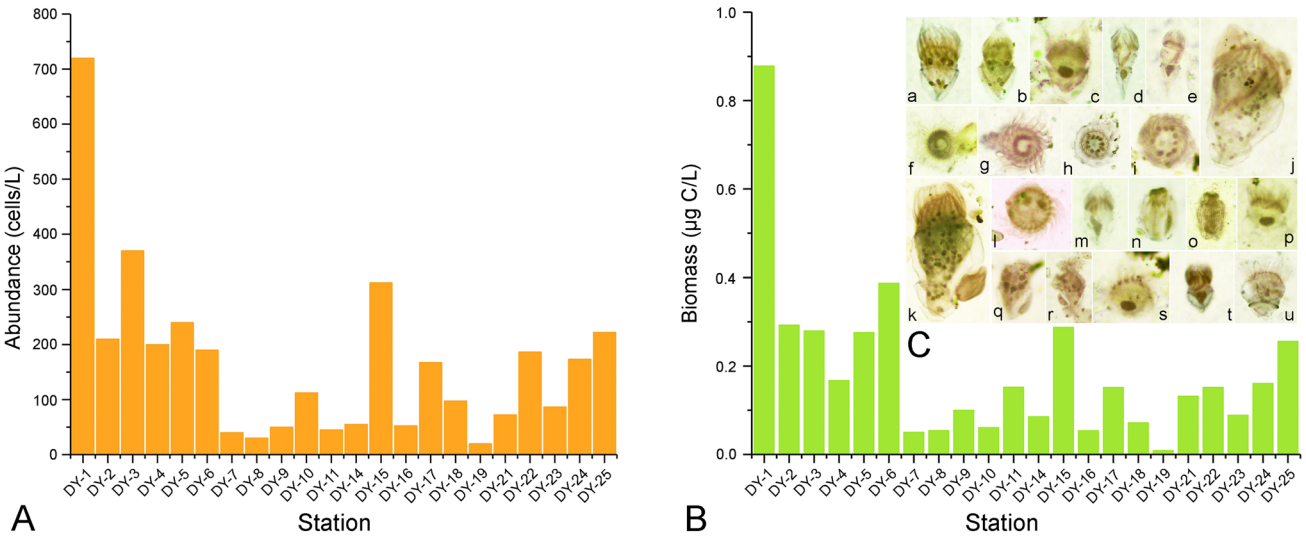

Fig. 3 Abundance (**a**), biomass (**b**) of planktonic ciliates and representative species (**c**) after quantitative protargol staining along the transect from Shenzhen (China) to Pohnpei (Micronesia) in the western Pacific Ocean. (a, b: *Strombidium wulffi*; c: *Strombidium epidemum*; d, e: *Strombidium emergens*; f, g: *Pelagostrobilidium neptunii*; h, i: *Rimostrombidium multinucleatum*; j: *Laboea strobila*; k: *Spirotontonia turbinata*; l: *Leegaardiella sol*; m, t: *Strombidium dalum*; n, o: *Urotricha cyrtonucleata*; p: *Lohamanniella oviformis*; q, r: *Tontonia gracillima*; s: *Leegaardiella ovalis*; u: *Strombidium capitatum*)

Strombidium capitatum (DI = 0.051), and *L. oviformis* (DI = 0.034), all of which were found at more than 50% of the sites (Fig. 5). The horizontal distribution of the most dominant species, *S. emergens,* which was also the only species that occurred at all stations (Fig. 5), exhibited much higher variability compared with other species (Fig. 6).

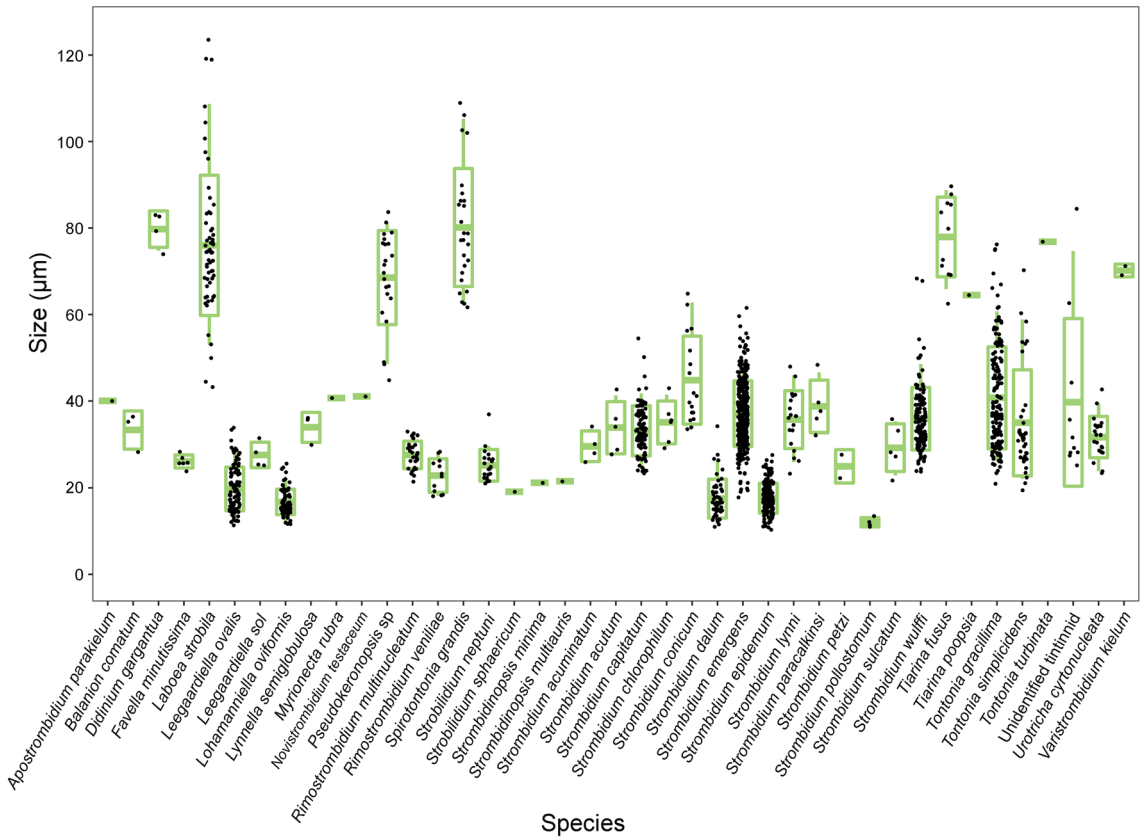

Fig. 4 Box plots showing size distribution of each recovered ciliate species. The line in each box plot indicates the median, the box delimits the 25th and 75th percentile

The ciliate communities were separated into four groups by both clustering dendrogram and principle coordination analysis (PCoA) based on the Bray Curtis distance (Fig. 7). Group A included DY-1 and DY-3 which were dominated by *S. wulffi*, *S. emergens*, and *T. gracillima* (Fig. 8a). Group B included DY-2, DY-4, DY-5, DY-6, DY-22, DY-23, DY-24, and DY-25, which were dominated by *S. emergens* and *S. epidemum* (Fig. 8b). Group C included DY-7, DY-8, and DY-9, which were dominated by *S. emergens* and *T. gracillima* (Fig. 8c). Group D included the rest of sites, in which *S. emergens* contributed a much higher proportion compared with other species (Fig. 8d).

Relationships between ciliate communities and environmental variables

Ciliate abundance exhibited positive correlations with phosphate, silicate, and the abundance of PPEs and negatively correlated with the 2–5 μm sized PNFs (Table 1). Ciliate biomass was positively correlated with the abundances of PPEs, HNFs, and chlorophytes, and negatively correlated with the abundances of PNFs and total NFs. With regard to the abundance of the dominant species, significant positive correlations were found between *S. emergens* and temperature; *S. epidemum* and Chl *a*, PPEs and HNF abundances, diatoms, chlorophytes and cryptophytes; *T. gracillima* and phosphate, silicate, and NF biomass; *S. wulffi* and temperature, phosphate, and PNF biomass; *L. ovalis* and silicate, heterotrophic bacteria and PPE abundances. The abundance of *L. oviformis* was positively correlated with PPE abundance, and negatively correlated with NF abundance. No significant correlation was found between the abundance of *S. capitatum* and any environmental variable (Supplementary Table S4).

Correlations between ciliate community composition and environmental variables were revealed by the multivariate biota-environmental (BIOENV) analysis (Table 2). The best matches of the combinations of environmental variables with the variations in ciliate communities involved the combinations of silicate, PNFs, haptophytes, $NO_2 + NO_3$, heterotrophic bacteria, *Prochlorococcus*, temperature, and PPEs, especially the combination of silicate and PNFs.

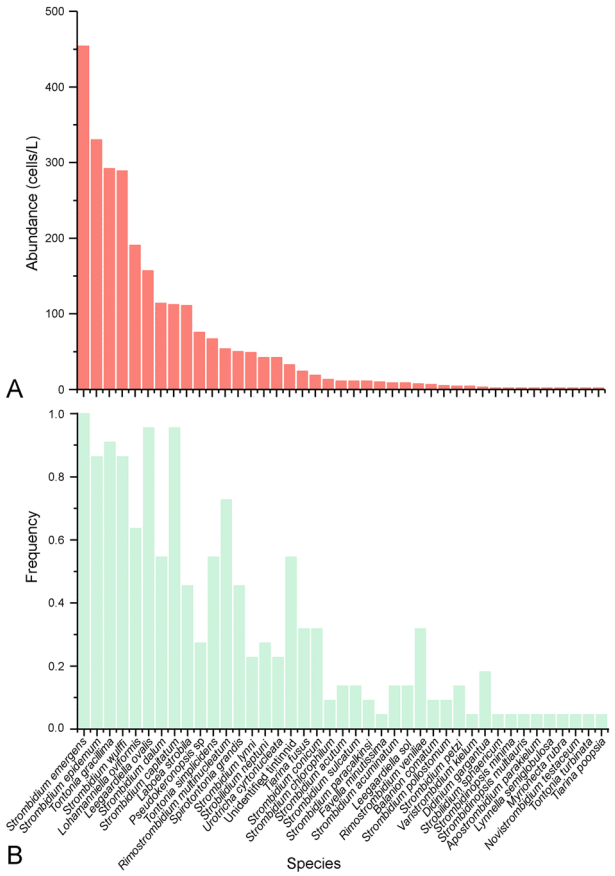

Fig. 5 Average abundance and frequency of each identified ciliate species at each sampling site

Discussion

Hitherto, most studies on the diversity and spatial distribution of planktonic ciliates have focused on coastal regions and/or continental shelves (e.g., Sun et al. 2017; Yang et al. 2020; Zhang et al. 2015). In contrast, there have been few such studies in open oceans in general, and tropical and subtropical open oceans in particular, and most of which focused mostly on loricate tintinnids (Modigh et al. 2003; Wang et al. 2019). Consequently, knowledge of community compositions of aloricate oligotrichs and choreotrichs, which are the dominant group of pelagic ciliates, is scant. This strongly restricts our understanding of the global diversity and geographic distribution of planktonic ciliates. It has been proposed that changes in the taxonomic composition of ciliate communities can have a profound impact on their ecological function (Levinsen and Nielsen 2002). Therefore, investigating the community composition and spatial distribution patterns of ciliate communities based on high taxonomic resolution is of considerable importance for appreciating their role in the functioning of open ocean ecosystems.

Environmental characteristics of the sampling sites

The subtropical/tropical western Pacific Ocean is characterized by the relatively stable environmental conditions. Our measurements on surface water temperature, salinity, and inorganic nutrients were consistent with the ranges reported in previous studies (e.g., Gómez 2007; Wang et al. 2019; Xu et al. 2018; Zhao et al. 2017), though with slight differences, which may be mainly due to the variations in sampling period. Nevertheless, the surface Chl *a* concentrations in the present study showed a wider range with the maxima higher than those reported in the previous studies in the same region, e.g., Zhao et al. (2017), who recorded a range of 0.052–0.133 μg/L in the surface layer (vs. 0.009–0.204 μg/L in the present study). These differences may result from a broader sampling area of the present study. In terms of pigment composition, the dominance of haptophyte- and cyanobacteria-affiliated pigments in the other oceanic waters has also been reported (Liu et al. 2009; Not et al. 2008; Xu et al. 2018).

In the epipelagic zones of the open oceans, which are characterized by low concentrations of inorganic nutrients, heterotrophic bacteria and *Prochlorococcus* often numerically dominate the picoplankton communities (Li et al. 2014; Liang et al. 2016), which is consistent with the present study. Nanoflagellates (NFs) are proposed to be the main consumers of picoplankton and potential prey for planktonic ciliates (Sanders et al. 2000). Our results showed that PNFs dominate the NF community in terms of both abundance and biomass, and 2–5 μm size fraction surpassed the 5–10 μm size group at all stations in terms of abundance and biomass at most stations. Similar results have also been reported in the continental shelf waters of the East China Sea (Tsai et al. 2016; Yang et al. 2020), northern South China Sea (Zhang et al. 2017), and the western Pacific Ocean (Xu et al. 2018).

Spatial distribution of planktonic ciliate communities

Ciliate abundances and biomass recorded in this study were mostly at the same levels commonly reported in previous studies of open waters of the Pacific (Gómez 2007; Suzuki et al. 1998; Wang et al. 2019, 2020), Atlantic (Rychert et al. 2014), and Indian (Leakey et al. 1996) Oceans, although much higher values were occasionally reported in some studies (Chavez et al. 1996; Vørs et al. 1995).

Our results showed that aloricate oligotrichs and choreotrichs were the most dominant groups within the ciliate communities in terms of species richness, abundance, and biomass, with the top seven dominant species all belonging to either of these two groups (Fig. 6). Our study is consistent with previous reports showing that aloricate oligotrichs and choreotrichs are major components of ciliate

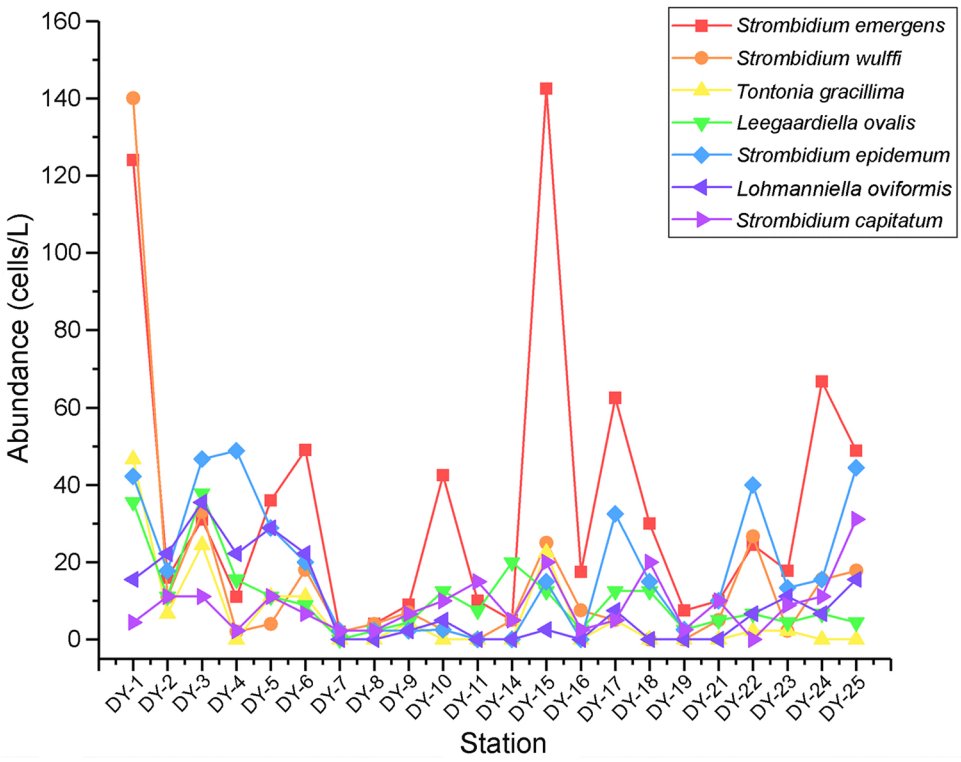

Fig. 6 Abundances of the seven dominant ciliate species at each sampling station

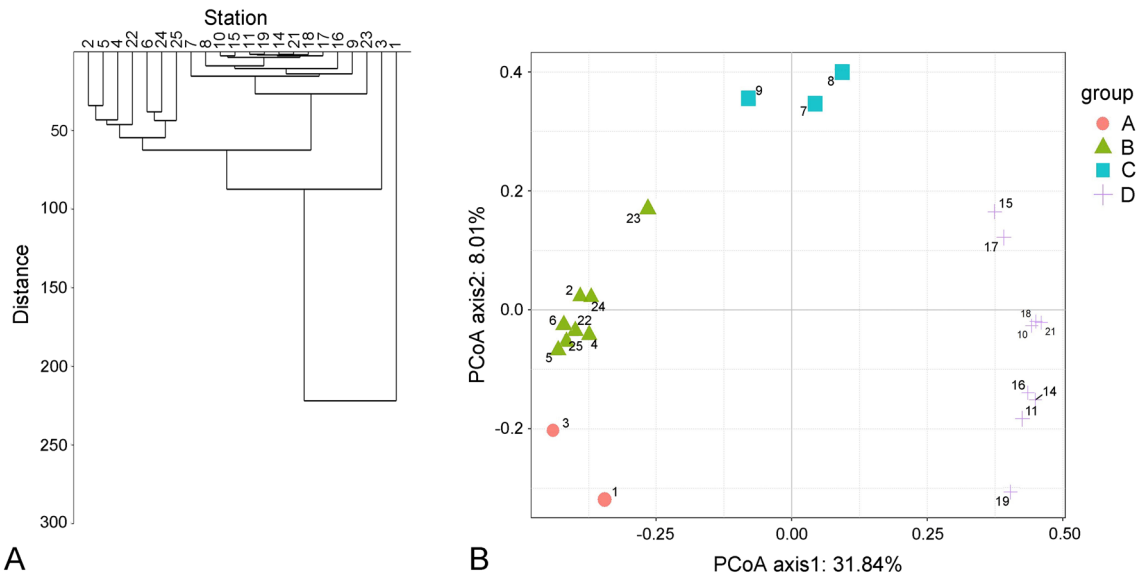

Fig. 7 The clustering dendrogram (**a**) and principle coordination analysis (PCoA) plot (**b**) of the ciliate communities based on Bray–Curtis distance matrices from log-transformed ciliate abundance data

communities in coastal and shelf waters (Chiang et al. 2003; Sun et al. 2019, 2020; Yang et al. 2020) and open oceans (Gómez 2007; Jiang et al. 2015; Leakey et al. 1996; Rychert et al. 2014). It is well known that many species within these two groups are mixotrophic (or potentially mixotrophic) as they can retain the chloroplasts of their prey which can remain functional for several days (Johnson 2011). It may be the flexible nutrition mode of these mixotrophic ciliates that allows them to prevail in dynamic coastal waters or oligotrophic open ocean waters. Further studies are necessary to accurately quantify the contributions of pelagic ciliates to primary production and the

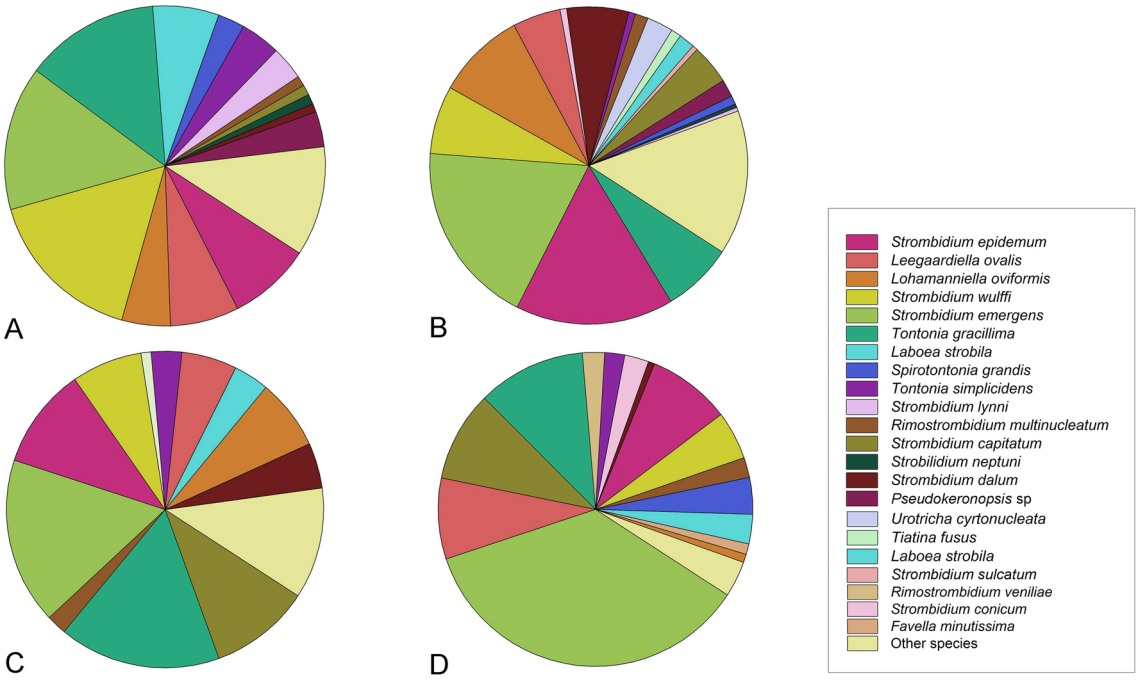

Fig. 8 Community composition of ciliate groups **a**–**d** as clustered in Fig. 7

transfer of energy and materials to higher trophic levels in open ocean ecosystems.

It is not feasible to compare the community composition of ciliates among studies as the species composition of aloricate ciliates is poorly known in open ocean waters. The reason was because identification to the species level was typically impossible due to the limitation of fixation and sophisticated staining procedures required for revealing the infraciliature. Rychert et al. (2014) investigated the composition of ciliate communities in the surface waters of the Atlantic Ocean with ciliates only identified to the genus level. Wang et al. (2020) reported the spatial distribution of aloricate ciliates under different size fractions in the tropical western Pacific without community composition information. To date, there has been no report on the species composition of aloricate ciliates based on high taxonomic resolution (e.g., employing the quantitative protargol stain method) in the subtropical and tropical waters of the western Pacific Ocean. In contrast, more information on the diversity and distribution of loricate tintinnids is available. For example, Gómez (2007) identified a total of 43 tintinnids, representing 10–20% of the aloricate ciliate abundance in the western and central equatorial Pacific Ocean. Wang et al. (2019) recorded a total of 56 tintinnid species in the western Pacific Ocean. Nevertheless, the species number of tintinnids and their proportion in the ciliate communities were much higher than in the present study. Different methodologies of sampling may be one of the reasons for such differences. In our study, 500 ml water samples were fixed at each site, and the limitation of our sampling effort (11 L seawater in total) may underestimate the diversity of tintinnids with low abundances. In addition, tintinnids tend to be restricted to the deep chlorophyll maximum layer, whereas the aloricate ciliates were more widely distributed in the upper pelagic zones (Gómez 2007). Despite these facts, a variation in the community structure of tintinnids may occur in this region considering that Wang et al. (2016) reported a total of 40 tintinnid species in the surface waters of the tropical western Pacific based on 25 L samples compared to only two tintinnids based on 11 L samples in our study.

With regard to the dominant species in this study, four belonged to *Strombidium*, i.e., *S. emergens*, *S. epidemum*, *S. wulffi*, and *S. capitatum*, contributing on average 30% of the ciliate abundance. Similarly, Sun et al. (2019) found that *Strombidium* was the most abundant genus among the 29 genera recovered, contributing more than 30% of the total abundance in mesopelagic waters of the northern South China Sea. Some *Strombidium* species have often been reported to constitute a substantial part of the ciliate communities in a wide range of marine waters worldwide, such as in the Amundsen Sea (Jiang et al. 2014), the southern North Sea (Yang et al. 2015), the northern South China Sea (Sun et al. 2019), and the western Arctic Ocean (Jiang et al. 2015). Yang et al. (2020) reported that *S. emergens* was one of five aloricate species that dominated the coastal and shelf waters of the East China Sea. In our study, *S. emergens* occurred at all stations, with the highest total abundance. Our findings suggest that these common and

Table 1 Spearman correlation between ciliate abundance, ciliate biomass, and biotic, abiotic factors

	Ciliate abundance	Ciliate biomass
Salinity	− 0.361	− 0.387
T	0.313	0.264
NO_2+NO_3	− 0.095	0.067
PO_4	0.445*	0.432
Si	0.623**	0.376
Chl *a*	0.288	0.205
Prochlorococcus abundance	− 0.187	− 0.074
Synechococcus abundance	− 0.038	0.025
Abundance of PPEs	0.540**	0.655**
Abundance of heterotrophic bacteria	0.351	0.221
Total abundance of PNFs	− 0.382	− 0.675**
Abundance of PNFs (2–5 μm)	− 0.600**	− 0.736**
Abundance of PNFs (5–10 μm)	0.239	0.182
Total biomass of PNFs	0.351	0.409
Biomass of PNFs (2–5 μm)	− 0.119	− 0.340
Biomass of PNFs (5–10 μm)	0.255	0.179
Total abundance of HNFs	0.354	0.562*
Abundance of HNFs (2–5 μm)	0.215	0.383
Abundance of HNFs (5–10 μm)	0.275	0.262
Total biomass of HNFs	0.074	− 0.116
Biomass of HNFs (2–5 μm)	0.276	0.434
Biomass of HNFs (5–10 μm)	0.276	0.253
Abundance of NFs	− 0.347	− 0.473*
Biomass of NFs	0.195	0.063
Pigments		
Dinoflagellates	0.086	− 0.063
Diatoms	0.186	0.030
Haptophytes (T8)	0.252	0.323
Haptophytes (T6)	0.104	− 0.075
Chlorophytes	0.406	0.508*
Cryptophytes	0.376	0.344
Prasinophytes	0.172	0.129
Prochlorococcus	− 0.005	− 0.160
Synechococcus	0.078	0.268

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

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

abundant planktonic ciliates may have a wide ecological niche, leading to a cosmopolitan distribution in the world oceans. However, it does not imply that all pelagic ciliates tend to have such a distribution as some species, especially the rare species, have been recorded only in some regions. Further efforts are needed to reveal the species composition of planktonic ciliate communities in diverse marine environments, especially the vast open oceans.

Factors driving the spatial distribution of planktonic ciliates in the western Pacific Ocean

The results of the correlation analysis in this study showed significant positive correlations between silicate and ciliate abundance and community structure (Tables 1, 2). It is commonly accepted that planktonic ciliates do not utilize silicate, and they are not primary grazers on diatoms. Also, no significant correlations between diatom affiliated pigments and ciliate abundance and biomass were found (Table 1). Therefore, we believe that the positive correlation between silicate and ciliate abundance may be just side effect without ecological significance. However, due to the fact that not many studies focusing on ciliates and their environmental driving parameters have been carried out especially in open oceanic waters, we are open to the possibility that there may be currently unknown coupling relationship between ciliate and silicate, which needs further validation in future studies. Also, the present study disclosed closely positive correlations of planktonic ciliates with PPEs and HNFs, indicating that: (1) PPEs and HNFs may be important food sources of ciliates; and (2) the "bottom-up" control effect could be a key factor in affecting the spatial distribution of planktonic ciliates in the subtropical and tropical waters of the western Pacific Ocean. Negative correlations between ciliates and PNFs suggest that they may compete for inorganic nutrients and/or food sources as mixotrophy is quite common in aloricate ciliates (Johnson 2011). Positive correlations between phosphate and the dominant species *S. emergens*, *S. wulffi*, and *T. gracillima* further imply that these species are potentially mixotrophs, and may rely more on phototrophy than heterotrophy in oligotrophic open oceans. In addition, positive correlations between PPEs and the dominant species *S. epidemum*, *L. oviformis*, and *L. ovalis* indicate close trophic coupling between these groups. A previous study showed that *Strombidium sulcatum* could feed effectively on prey items ranging in size from 0.6 to 6.6 μm, and most efficiently on prey 2.5 μm in size, which is ~ 10% of their body size (Bernard and Rassoulzadegan 1990). The sizes of PPEs fall just within the optimal food size range of these dominant ciliate species with equivalent spherical diameters of about 20 μm, which is approximately the size of *S. sulcatum*.

Environmental heterogeneity and geographical distance have been considered as the main driving forces in shaping microeukaryote biogeography and diversity. Zhao et al. (2017) reported that geographic distance had little to no effect on the dispersal of planktonic ciliates over a spatial distance of 1300 km in the western Pacific Ocean, and indicated that environmental factors are a stronger force in shaping marine planktonic ciliate communities than the geographic distance. However, Sun et al. (2020) identified geographic distance, environmental conditions, and depth as principal determinants of ciliate communities in the Taiwan

Table 2 Results of the multivariate biota-environment (BIOENV) analysis between the ciliate community composition and environmental variables, showing the best matches of the combinations of environmental variables with variations in ciliate communities

Rank	Best combinations of environmental variables	Correlation	*p*
1	Si, PNF	0.194	< 0.01
2	PNF, Hapt (T8), Hapt (T6)	0.194	< 0.01
3	NO_2+NO_3, Heterotrophic bacteria, PNF, Hapt (T8), Hapt (T6)	0.192	< 0.01
4	NO_2+NO_3, *Procholorococcus*, PNF, Hapt (T8), Hapt (T6)	0.190	< 0.01
5	Temperature, NO_2+NO_3, PNF, Hapt (T8), Hapt (T6)	0.187	< 0.05
6	Heterotrophic bacteria, PNF, Hapt (T8), Hapt (T6)	0.184	< 0.05
7	NO_2+NO_3, Heterotrophic bacteria, PNF, Hapt (T8)	0.184	< 0.05
8	NO_2+NO_3, PPEs, PNF, Hapt (T8), Hapt (T6)	0.182	< 0.05
9	*Procholorococcus*, PNF, Hapt (T8), Hapt (T6)	0.182	< 0.05
10	PNF	0.180	< 0.05

Strait, among which geographic distance was the most influential factor. The results of the present study showed that the spatial distribution of planktonic ciliate communities was strongly related to PPEs, NFs, and nutrients, suggesting that environmental conditions, especially those linked to the "bottom-up" control effect, could be an important factor shaping the ciliate communities in the open oceans. Furthermore, our study based on samples across nearly 5000 km showed that ciliate community similarity negatively correlated with geographic distance ($r = -0.286$, $p = 0.003$), indicating that geographic distance might be responsible for the observed partitioning of ciliate communities (Supplementary Fig. S3). Zhao et al. (2017) proposed that a high proportion of common ciliate species in the surface water layer might mask the effect of geographic distance on the dispersal of planktonic ciliates. In our study, only five ciliate species were found at more than 80% of the stations, whereas 22 species occurred at less than 20% of the stations (Fig. 5). Therefore, it seems that the sampling area was sufficiently large to reveal the effect of geographic distance on the ciliate communities in open oceans. Further studies based on broader survey areas and larger sample collections are needed to confirm the findings of the present study.

Conclusion

Based on the quantitative protargol stain method, the spatial distribution of planktonic ciliate communities in the western Pacific Ocean was investigated. In total, 41 ciliate species were recorded, 32 of which were affiliated with aloricate oligotrichs and choreotrichs. Species numbers at individual sampling stations ranged from 5 to 32. The abundance of ciliates varied between 20 and 720 cells/L averaging 164 cells/L, and the biomass ranged from 0.009 to 0.878 µg C/L averaging 0.192 µg C/L. The composition of ciliate communities showed an off-shore to open ocean distribution pattern with *S. emergens* being the most dominant species, occurring at all stations. Ciliate abundances were positively correlated with phosphate, silicate and pico-sized pigmented eukaryotes (PPEs), whereas the biomass was closely correlated with PPEs, heterotrophic nanoflagellates (HNFs) and chlorophytes. The combination of silicate and pigmented nanoflagellates (PNFs) was identified as the major factor driving the ciliate community composition. The close relationship between siliate and ciliates need further validation based on more data collected from open ocean waters.

Materials and methods

Sample collection

Sampling was conducted on board R/V Xiang Yang Hong (leg DY40B) from 5 August to 16 October in 2016, in the subtropical/tropical western Pacific Ocean along a transect from Shenzhen (China) to Pohnpei (Micronesia) (Fig. 1). A total of 22 stations were sampled. At each station, surface water samples were collected using a plastic barrel tied onto a long rope to determine ciliate, nanoflagellate, and picoplankton abundances and for the measurements of physicochemical variables. Water temperature and salinity were measured using a water quality analyzer (YSI Pro2030, YSI Life Sciences, OH, USA). Water samples (500 ml) for measuring nutrients including nitrite + nitrate (NO_2+NO_3), phosphate (PO_4), and silicate (Si) were collected and frozen at – 20 °C onboard and analyzed in the laboratory using a Technicon AA3 Auto-Analyzer (Bran-Lube, Germany). Water samples (5 L) for phytoplankton pigment analysis were filtered through a ca. 0.7 µm pore size, 47 mm GF/F (Gleman) glass fibre filter without pre-filtration and immediately frozen in liquid nitrogen prior to analysis in the laboratory.

Phytoplankton pigment composition

Pigment analysis followed the protocol described previously (Huang et al. 2010). Briefly, phytoplankton pigments were extracted in *N*,*N*-dimethylformamide and analyzed using a

High Performance Liquid Chromatography (Agilent Series 1100, Agilent Technologies) system fitted with a 3.5-mm Eclipse XDB C8 column (100 × 4.6 mm; Agilent Technologies) following a modification of the method of Mantoura and Llewellyn (1983) and Van Heukelem and Thomas (2001). Chl *a* was obtained as the sum of monovinyl and divinyl Chl *a*. Based on pigment data, the phytoplankton community was calculated using CHEMTAX program (Mackey et al. 1996).

Enumeration of picoplankton and nanoflagellates

For flow cytometry analysis of picoplankton (heterotrophic bacteria, *Synechococcus*, *Procholorococcus*, and pigmented pico-sized eukaryotes, PPEs), five replicate 1.8 ml subsamples prefiltered through 20 μm meshes were fixed onboard with ice-cold glutaraldehyde (final concentration 0.1%), flash frozen in liquid nitrogen and then stored at – 80 °C until analyzed in the laboratory. Methods used for flow cytometry (Epics Altra II, Beckman Coulter) analysis followed procedures described previously (Marie et al. 1999).

For nanoflagellate abundance determination, 50 ml seawater were preserved in glutaraldehyde (final concentration 1%), fixed at 4 °C in the dark for 2–4 h, filtered onto 0.8 μm pore-size black polycarbonate membrane filters and stained with DAPI (final concentration 10 μg/ml) for 10 min and kept at − 20 °C until analysis. The membrane filters were then examined in the laboratory under an epifluorescence microscope (Olympus BX51). Heterotrophic nanoflagellates (HNFs) and pigmented nanoflagellates (PNFs) were identified separately according to Caron et al. (2017). To further determine the size structure of NFs, NFs were divided into three size groups (2–5, 5–10, and 10–20 μm). No NFs within 10–20 μm size fraction were observed in the present study, thus only 2–5 and 5–10 μm size fractions were included. The nanoflagellate biovolume was estimated using the same method as that for ciliates (see below). A conversion factor of 0.22 pg C/μm^3 was used to estimate the carbon biomass of nanoflagellates (Borsheim and Bratbak 1987).

Identification and enumeration of ciliates

Seawater samples (500 ml) were fixed with Bouin's solution (10% final concentration) with addition of ice-cold acetic acid (1% final concentration) prior to fixation, and then stored in the cold (4 °C) and dark. Samples were processed within three months after the cruise. Ciliate species composition and abundances were determined using the quantitative protargol staining (QPS) method (Skibbe 1994), with cells counted and identified under a light microscope (Olympus BX51) at magnifications of 400×. Aloricate ciliates were identified mainly based on Montagnes and Lynn (1991) and Song et al. (2009). Tintinnids were identified based on lorica features according to Kofoid and Campbell (1929). The biovolume of each taxon was determined by measuring cell dimensions assuming appropriate geometric shapes according to Hillebrand et al. (1999). A conversion factor of 0.19 pg C/μm^3 was used to estimate the carbon biomass of ciliates (Putt and Stoecker 1989).

Data processing

Multivariate analysis was performed using the PRIMER v 6.1 package (Clarke and Gorley 2006). Principle coordination analysis (PCoA) was conducted based on Bray–Curtis distance computed on log-transformed ciliate abundance data to achieve the clustering of ciliate communities. The submodule Biota-Environment (BIOENV) within the PRIMER program was used to explore the potential relationships between ciliate communities and environmental variables, and ten best matches of environmental variables were identified. Spearman's correlation analysis between ciliate abundances and environmental variables was conducted using the statistical software SPSS v19.0 with a significance level of 5%. The dominance index was calculated for each ciliate species according to the following equation: $Y=(n_i/N_0)\times f_i$, where Y is the dominance index, n_i is the number of individuals of species i at all stations, N_0 is the total number of individuals at all stations, and f_i is the frequency of species i at all stations. The species with a dominance index more than 0.02 was considered as the dominant species of the community. The alpha diversity indices including Shannon–wiener index (H') and Simpson's diversity index (D) were computed using PSAT software (Hammer et al. 2001). The geographical maps were created using ODV software (Schlitzer 2013).

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Author contributions DX conceived and designed the study. SH conducted sampling. SH, HH, BG and LW performed laboratory work. HH, JY, and YW analyzed the data and drafted the manuscript. All authors made further revisions and approved the final version of manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interests.

Animal and human rights statement No animal and human rights are involved in this article.

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