Boreal Tintinnid Assemblage in the Northwest Pacific and Its Connection with the Japan Sea in Summer 2014

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Abstract

Tintinnids are planktonic ciliates that play an important role in marine ecosystem. According to their distribution in the world oceans, tintinnid genera were divided into several biogeographical types such as boreal, warm water, austral and neritic. Therefore, the oceanic tintinnid assemblage could be correspondingly divided into boreal assemblage, warm water assemblage and austral assemblage. The purpose of this study was to investigate the characteristics of boreal tintinnid assemblage in the Northwest Pacific and the Arctic, and to identify the connection between boreal tintinnid assemblage and neighboring assemblages. Surface water samples were collected along a transect from the East China Sea to the Chukchi Sea in summer 2014. According to the presence of boreal genera and warm water genera, three tintinnid assemblages (the East China Sea neritic assemblage, the Japan Sea warm water assemblage, and the boreal assemblage) were identified along the transect. The boreal assemblage extended from the Chukchi Sea to the waters north of the Sōya Strait. Densities peaks occurred at stations in the two branches of the Alaska Current and decreased both northward and southward. The densities were <10 ind./dm³ at most stations in Arctic region. The dominant genera (Acanthostomella, Codonellopsis, Parafavella, and Ptychocylis) accounted for 79.07±29.67% (n = 49) of the abundance in the boreal assemblage. The densities of the dominant genera covaried with strongly significant positive correlations. Tintinnids with lorica oral diameter of 22–26 μm and 38–42 μm were dominant and contributed 67.35% and 15.13%, respectively, to the total abundance in the boreal assemblage. The distribution and densities of tintinnids in the study area suggest that the S ya Strait might be a geographical barrier for tintinnids expansion.
Introduction

Tintinnids are planktonic ciliates with shells (lorica). Taxonomically, tintinnids belong to the subclass Choreotrichia, class Spirotrichea [1]. As planktonic ciliates, tintinnids play an important role in the transfer of matter and energy between the microbial food web and the classic food chain in the marine planktonic ecosystem [2]. The morphology and size of the lorica have conventionally been used as taxonomic criteria despite plasticity of the lorica in some genera [3]. The lorica oral diameter (LOD; diameter of the mouth end of the lorica) is related to the size of food items: the size of the largest prey is about 45% of the LOD and the size of the preferred prey (removed at maximum rates) is about 25% of the LOD [4].

Tintinnid genera have been classified as cosmopolitan, neritic, warm water, boreal, and austral biogeographical types based on their distribution in the global ocean [5,6]. Correspondingly, according to the presence of each biogeographical genera type, there should be boreal, warm water and aurast tintinnid assemblages in the oceanic water from Arctic to Antarctic. In the neritic waters are the neritic assemblages. There are also mixed assemblage at the edges of neighboring assemblages. Since there are only three oceanic assemblages extending from the Arctic to the Antarctic, each of the assemblage extends on a scale ≥3000 km (mega-scale) [7]. There are some studies about the aurast assemblage and its mixing with warm water assemblage in the southwestern Atlantic Ocean [8–11], the warm water assemblage in Indian Ocean [12] and southeastern Pacific Ocean [13]. There are also some studies on tintinnid diversity at such large geographical scales in Mediterranean Sea [14–16]. In the Mediterranean Sea, both tintinnid species richness (10–25) and community averages of LOD (28–38 μm) increased from west to east. The dominant species were different in different areas, too [16]. Most tintinnid species showed a clear regional distribution and a well-defined tintinnid assemblage characterized each oceanic province along a transect from 42°N to 43°S across the Mediterranean Sea, the Red Sea, the Arabian Sea, the Indian Ocean and the Tasman Sea. For example, Xystonella and Xystonellopsis were considered as typical for the Mediterranean Sea. Epiplocyloides reticulate was considered as typical for the Red Sea [12].

Data on boreal tintinnid assemblages are scarce. There are few studies in the boreal assemblage from the northern Pacific [17] to Arctic [17,18]. Dolan et al. [18] found that Ptychocylis urnula or Salpingella faurei was the most abundant species in Arctic region. Acanthostomella norvegica, Codonellopsis frigida, C. contracta, Parafavella subrotundata, P. ventricosa, and P. obtusa were important components of tintinnid abundance in the oceanic waters, while Ptycho- cylis spp. and Tintinnopsis spp. were important in the shelf waters in Arctic and subarctic Pacific oceans [17]. These studies are limited in space coverage and low resolution in station numbers and distances between stations. In this paper, we studied tintinnid assemblages along a transect across the whole boreal assemblage from the Arctic to the eastern end of the Oyashio Current and south to the Japan Sea. The high resolution of station arrangement revealed the spatial variation of the assemblage. The aims of this study were (1) to describe the characteristics of the boreal tintinnid assemblage from 77.13°N in the Chukchi Sea to the northwest subarctic gyre in the Pacific, and (2) to investigate the connection between boreal tintinnid assemblage and that in the Japan Sea.

Materials and Methods

Tintinnids in surface waters were sampled during “The 6th Chinese National Arctic Research Expedition” (11 July–31 August, 2014) on board R/V XUELONG along a transect (10290 km long) that started in the East China Sea, passed through the Japan Sea, Okhotsk Sea, and Bering Sea, and finished in the Chukchi Sea. There were 68 stations approximately evenly distributed
along the transect, the distances between the adjoining stations were in the range of 60–480 km (on average 180±90 km, n = 67, Fig 1). No specific permissions were required to collect water samples because the stations were located in international waters. The field studies did not involve or impact on any endangered or protected species. The stations in the Japan Sea were in the path of the Tsushima Current, a branch of the Kuroshio Current, which influences the East China Sea and the Japan Sea, while the stations north of the Sōya Strait were influenced by two major North Pacific currents (the Oyashio Current and the Alaska Current) and currents flowing from the Bering Sea to the Chukchi Sea [19–22].

At each station, 80 dm³ of surface (5 m depth) seawater was collected using an underway sampling system. The water was then gently filtered through a small net (mesh pore size 10 μm). The samples in the cod end of the net (about 150 cm³) were transferred into sample bottles and immediately fixed with Lugol’s solution (1% final concentration). The samples were placed at cool, dark environment for preservation. At the same time, the temperature and salinity of the surface seawater and chlorophyll a (Chl a) concentration were measured using a CTD sampling system (Seabird 21).

In the laboratory, one subsample (25 cm³ or a larger volume if tintinnids were scarce) from each concentrated sample was settled in an Utermöhl counting chamber for at least 24 h and examined using an Olympus IX 71 inverted microscope (100× or 400×). At least 20 individuals (if possible) of each species were photographed and measured. Tintinnid species were identified based on lorica morphology and size according to the literatures [23–25]. SPSS version 16 statistical software was used to perform a correlation analysis.
Results

Hydrography

The surface water temperature ranged from –0.68°C (St. 55) to 24.25°C (St. 8). There were two sharp drops in temperature along the transect. The temperature increased slightly from St. 1 to St. 8, and then dropped rapidly from St. 9 (23.66°C) to St. 22 (10.49°C). It remained stable in the North Pacific and Bering Sea, then dropped rapidly from St. 47 (9.36°C) and remained below 0°C after St. 55 (Fig 2). The surface water salinity increased gradually from the East China Sea to the Japan Sea, and then remained at a high level (30.88–34.66) between the Japan...
Sea and the Bering Strait. The salinity dropped at St. 53 and remained at a low level (<27.38) in the Chukchi Sea (Fig 2). According to the T–S diagram, the water mass along the transect could be divided into East China Sea Water, Japan Sea Water, Subarctic Pacific Water and Coastal Arctic Water. St. 1–St. 3 located in East China Sea Water with high temperature but the salinity was relatively low. The stations between St. 4 and St. 15 located in the Japan Water with high temperature and high salinity. St. 53–St. 68 located in the Coastal Arctic Water with low temperature and low salinity (Fig 3).

The surface Chl a concentration ranged from 0.06 μg/dm³ (St. 65) to 8.32 μg/dm³ (St. 22). It increased gradually from the East China Sea to the northeastern edge of the Okhotsk Sea and reached a maximum at St. 22 before quickly falling to a relatively low level (<1.44 μg/dm³). Chl a concentration were high (3.17–5.80 μg/dm³) in the south part of the Bering Sea. Chl a concentration in the Chukchi Sea was very low (<0.2 μg/dm³; Fig 2).

Species richness, densities of tintinnids and their relationship with environmental factors

In total, 54 tintinnid species from 21 genera were identified (Figs 4 and 5; Table 1). There was an undetermined species (Fig 5) with hyaline, cylindro-conical in shape, and open at both ends. The loria length was about 44.88±3.97 μm, the LOD was 10.70±1.19 μm, and the aboral diameter was 3.62±0.77 μm (n = 20). We discussed it as a separate undetermined species and genus in this study. Thirty three species occurred at less than five stations and most (22) of them had low densities (<1 ind./dm³; Table 1).

Tintinnid densities ranged between 0 and 849.21 ind./dm³ (Fig 2). There were generally high densities between the Okhotsk Sea and southwestern Bering Sea, especially at stations St. 24–St. 27 and St. 35–St. 41. No tintinnid was found at St. 3, St. 56, St. 58, St. 59, and St. 61. Tintinnid densities were extremely low (<13 ind./dm³) in the Chukchi Sea (Fig 2).

The tintinnid species richness at individual stations ranged from 0 to 12, and was relatively high (3–12 species per station) in the northeastern East China Sea, the southwestern Japan Sea, and the northern Bering Sea. Tintinnid species richness declined after St. 55 (<4 species per station; Fig 2).
Correlation analysis identified significant positive correlations between tintinnid species richness and densities and temperature, salinity, and Chl $a$ concentration (all $P<0.01$; Table 2). Tintinnid species richness and densities were very low when the temperature was $<5^\circ$C, but were high when the temperature was between $8^\circ$C and $14^\circ$C. Tintinnid species richness increased as salinity increased. Tintinnid species richness initially increased with an increase in Chl $a$ concentration and then stabilized, while densities increased steadily as Chl $a$ concentration increased (Fig 6).

Distribution patterns of tintinnid genera

Among the 21 tintinnid genera, there were 2 boreal genera, 8 cosmopolitan genera, 4 neritic, and 3 warm water genera. Two genera and the undetermined species were not grouped into any biogeographical type (Table 1). The occurrence of each tintinnid genus by station was shown in Fig 7. Five genera (Ascampbelliella, Climacocylis, Coxliella, Protorhabdonella, and Ste nosemella) were found at only one or two stations and their densities were $<1$ ind./dm$^3$ (Table 1). Density distribution of other genera were shown in Fig 8.

Prolectella and Rhabdonella were warm water genera, Prolectella contained one species (Prolectella expolita) and Rhabdonella contained two species (Rhabdonella cornucopia and Rhabdonella sp.). They occurred at stations in the Japan Sea (Fig 8).
Table 1. List of tintinnid species, the station number of occurrence (n) and maximum density (Max, ind./dm³).

| Species | n   | Max  | Species | n   | Max  | Species | n   | Max  |
|---------|-----|------|---------|-----|------|---------|-----|------|
| **Boreal genera** |     |      | **Warm water genera** |     |      |
| Eutintinnu sapertus | 2 | 2.05 | T. baltica | 4 | 0.93 |
| Parafavella denticulata | 9 | 32.08 | E. fraknoii | 3 | 1.46 | T. beroidea | 6 | 16.27 |
| P. elegans | 4 | 0.66 | E. lusus-undae | 8 | 3.08 | T. japonica | 2 | 0.53 |
| P. faceta | 26 | 53.47 | E. mirabilis | 2 | 0.27 | T. kofoidi | 1 | 0.13 |
| P. gigantea | 4 | 1.95 | E. pacificus | 6 | 2.83 | T. mayeri | 4 | 0.53 |
| P. hadai | 5 | 19.96 | E. rectus | 1 | 1.29 | T. nana | 1 | 15.80 |
| P. jorgensenii | 37 | 198.08 | E. stramentus | 7 | 27.94 | T. radix | 2 | 4.11 |
| P. pacifica | 5 | 79.26 | Protorhabdonella striatura | 1 | 0.26 | T. rapa | 3 | 1.68 |
| P. promissa | 4 | 0.66 | Salpingella curta | 1 | 1.30 | T. tubulosoides | 1 | 0.30 |
| P. ventricosa | 9 | 32.08 | S. faurei | 16 | 8.37 | T. urnula | 1 | 0.13 |
| Psychoclyis obtusa | 42 | 59.88 | Steenstrupiella gracilis | 1 | 0.13 | Ascampbelliella retusa | 1 | 0.25 |
| **Cosmopolitan genera** |     |      | **Untergruppen** |     |      |
| S. robusta | 1 | 0.08 | S. steenstrupii | 3 | 0.66 | Proplectella expolita | 6 | 6.44 |
| A. minutissima | 2 | 1.03 | Neritic genera |     |      |
| A. norvegica | 44 | 478.09 | Leprotintinnus pellucidus | 14 | 63.38 | Rhabdonella cornucopia | 3 | 0.40 |
| Amphorides brandti | 1 | 1.11 | Favella azorica | 3 | 199.18 | Rhabdonella sp. | 1 | 0.12 |
| A. minor | 1 | 0.25 | F. panamensis | 2 | 0.77 | Climacosyclis scalaroides | 1 | 0.08 |
| A. quadrilineata | 13 | 55.95 | Helicostomella subulata | 7 | 51.29 | Coxiella cymaticoides | 1 | 0.98 |
| Codonellopsis frigida | 33 | 45.16 | Stenosemella nivalis | 2 | 0.26 | Undetermined | 20 | 34.48 |
| Dadayiella ganymedes | 9 | 3.47 | Tintinnopsis acuminata | 11 | 15.80 |     |     |     |

doi:10.1371/journal.pone.0153379.g005
Favela, Leprotintinnus, Helicostomella and Tintinnopsis were neritic genera. Favela occurred at 4 stations in the Japan Sea and the East China Sea. Leprotintinnus and Helicostomella occurred at coastal stations east of the Kamchatka Peninsula and the Chukchi Sea. Tintinnopsis was found at coastal stations along the whole transect (Fig 8).

Parafavella and Ptychocylis belonged to boreal genera. Ptychocylis obtusa was the only species in genus Ptychocylis. It occurred north of the Sōya Strait (St. 16 – St. 66). Nine tintinnid species of the genus Parafavella were identified. The distribution of Parafavella faceta and P. jorgenseni was similar. Both species were first detected at St. 17. Their densities initially increased along the transect, then decreased in the Arctic. P. elegans occurred in the Bering Strait with low densities of <0.66 ind./dm³ (Table 1). P. denticulata occurred between St. 28 and St. 40 where most tintinnid species of the genus Parafavella were highly abundant. Most species in Parafavella only occurred at stations north of the Sōya Strait with the exception that P. pacifica was only found in the northern Japan Sea (Fig 9).

The cosmopolitan genera Acanthostomella, Amphorides, Codonellopsis, Dadayiella, Eutintinnus, Salpingella, and Steenstrupiella contained 3, 3, 1, 1, 7, 2, and 3 species, respectively. Dadayiella ganymedes and Eutintinnus stramentus were identified in the Japan Sea but were not found north of the Sōya Strait. Codonellopsis frigida distributed at stations between St. 20 and St. 63. Acanthostomella norvegica mainly distributed north of the Sōya Strait. It also occurred at St. 15 in the Japan Sea with extremely low density (0.26 ind./dm³). The genus Salpingella distributed in the southern (St. 4–St. 9) and middle (St. 23–St. 37) sections of the transect (Fig 8).

**Tintinnid assemblages division**

We used the occurrence of boreal genera and warm water genera as objective criteria of tintinnid assemblage division. The 5 stations with zero density (St. 3, St. 56, St. 58, St. 59, and St. 61) were excluded of assemblage division. The stations with both boreal and warm water genera were set according to T–S water mass division.

**Table 2.** Spearman’s rank correlation coefficient between environmental and biological variables.

|               | T   | S   | Chl a |
|---------------|-----|-----|-------|
| Species richness | 0.362** | 0.471** | 0.373** |
| Densities      | 0.384** | 0.534** | 0.784** |

T: temperature, S: salinity, Chl a: chlorophyll a concentration.

**: Correlation is significant at the 0.01 level (2–tailed).

doi:10.1371/journal.pone.0153379.t002

**Fig 6.** Responses of tintinnid species richness and density with the variation of temperature (T), salinity (S) and chlorophyll a (Chl a) concentration.

doi:10.1371/journal.pone.0153379.g006
Neither warm water genera nor boreal genera tintinnid was identified at the two stations (St. 1 and St. 2) in the East China Sea. We considered the tintinnid assemblage at these two stations as East China Sea neritic assemblage. Warm water genera occurred in the Japan Sea (St. 4 to St. 15). At the stations between St. 11 and St. 15, warm water genera and \textit{P. pacifica} belonging to boreal genera mixed. In the T–S diagram, these stations belong to the Japan Sea Water with high temperature (Fig 3). Therefore the tintinnid assemblage at St. 4 to St. 15 was

![Fig 7. Occurrence of tintinnid genera at each station.](image)

Note that the undetermined species was listed as a genus. Blue filled circles: boreal genera; pink filled circles: cosmopolitan genera; green filled circles: neritic genera, red filled circles: warm water genera, black filled circles: ungrouped genera. (Serial number of tintinnid genera. 1: \textit{Tintinnopsis}; 2: \textit{Parafavella}; 3: \textit{Ptychocylis}; 4: \textit{Acanthostomella}; 5: \textit{Codonellopsis}; 6: \textit{Leprotintinnus}; 7: \textit{Helicostomella}; 8: \textit{Stenosemella}; 9: \textit{Coxliella}; 10: undetermined; 11: \textit{Salpingella}; 12: \textit{Eutintinnus}; 13: \textit{Amphorides}; 14: \textit{Favella}; 15: \textit{Dadayiella}; 16: \textit{Propilectella}; 17: \textit{Protorhabdonella}; 18: \textit{Steenstrupiella}; 19: \textit{Rhabdonella}; 20: \textit{Climacocylis}; 21: \textit{Ascampbelliella}).

doi:10.1371/journal.pone.0153379.g007

![Fig 8. Density distribution of tintinnid genera which occurred at more than 2 stations.](image)

Blue filled circles: boreal genera; pink filled circles: cosmopolitan genera; green filled circles: neritic genera, red filled circles: warm water genera, black filled circles: ungrouped genera.

doi:10.1371/journal.pone.0153379.g008
considered as Japan Sea warm water assemblage. Tintinnid assemblage at stations north of St. 16 was considered as boreal assemblage with boreal genera occurred.

**Characteristics of boreal assemblage**

The boreal assemblage covered the Subarctic Pacific Water and Coastal Arctic Water and has a difference in densities in the two waters. Total densities decreased sharply at stations in the Coastal Arctic Water and was <10 ind./dm³ at most stations north of the St. 49 (Figs 2 and 10).

**Fig 9. Density distribution of tintinnid species in genus *Parafavella*.**

doi:10.1371/journal.pone.0153379.g009

**Fig 10. Species richness and density at stations in boreal assemblage.**

doi:10.1371/journal.pone.0153379.g010
There were 30 species with 14 LOD size–classes in the boreal assemblage. Species in LOD of 22–26 μm (A. norvegica, C. frigida, Stenosemella nivalis, Tintinnopsis beroidea, and T. rapa) accounted for 67.35% of the total abundance. The second LOD size-class with high contribution to abundance (15.13%) was 38–42 μm (Amphorides quadrilineata and P. jorgenseni). The other 23 species in LOD size–classes 10–14 μm, 18–22 μm, 26–30 μm, 30–34 μm, 34–38 μm, 42–46 μm, 46–50 μm, 50–54 μm, 54–58 μm, 58–62 μm, 66–70 μm, and >70 μm accounted for 17.52% of boreal assemblage abundance (Fig 11).

With maximum densities of 478.09, 445.16, 229.87, and 59.88 ind./dm³, respectively, the genera Acanthostomella, Codonellopsis, Parafavella, Ptychocylis, had higher maximum densities than other genera. On average, they contributed 79.07±29.67% (n = 49) to the abundance in the boreal assemblage (Fig 12). They also had consecutive distributions. The distribution patterns of Acanthostomella, Codonellopsis, Parafavella, Ptychocylis, and the undetermined species were similar. They centered in the Bering Sea with extensions both southward and northward. Their high densities centered in the Bering Sea and north of the Okhotsk Sea (St. 18–St. 41) and decreased both southward and northward. The undetermined species occurred between St. 16 and St. 47 with densities of 0.27–23.43 ind./dm³ (Fig 8). The correlations among densities of them were strongly significant positive (all P<0.01; Table 3; Fig 13).

According to whether neritic genera tintinnid occurred, the boreal assemblage could be divided into two parts: boreal assemblage without neritic genera (B1 assemblage) and boreal assemblage with neritic genera (B2 assemblage). There were 12 species with 7 LOD size–classes in B1 assemblage, and these species were considered as core boreal species. In the B1 assemblage, two species (A. norvegica and C. frigida) with a LOD of 22–26 μm accounted for 69.65% (49.16% and 20.49%, respectively) of the total abundance. The second highest contributor to abundance (13.16%) was P. jorgenseni with a LOD of 38–42 μm (Fig 11). Core boreal species had high species richness at stations between St. 29 and St. 42, and decreased northward and southward, and extremely low in Arctic region (Fig 10).

Twenty nine species with 14 LOD size–classes were found in B2 assemblage. Among them, 11 species were core boreal species. Eighteen other species (added species) occurred in B2 assemblage, including 12, 4, and 1 species belong to neritic, cosmopolitan, and boreal genera, respectively.
Though the number of species and LOD size–classes increased in the B2 assemblage, the most abundant species in B2 assemblage were similar with those in B1 assemblage: species in LOD of 22–26 μm and 38–42 μm contributed to 63.74% and 18.22% to total abundance, respectively (Fig 11). The added species increased species richness in B2 assemblage, especially at stations in the Coastal Arctic Water. However, compared with core boreal species, densities of the added species were low and made up a small portion of total abundance at most stations in B2 assemblage. High portion mainly occurred at stations in the Arctic water where the total densities were low (Fig 10). If the core species were excluded, the added species with LOD size–classes of 34–38 μm, 18–22 μm, and 30–34 μm were dominant and accounted for 43.88%, 25.51%, and 16.51% of abundance in B2 assemblage, respectively (Fig 11).

Table 3. Spearman’s rank correlation coefficient among density distribution of genera Parafavella, Acanthostomella, Codonelopsis, Ptychocylis and the undetermined species.

|                | Acanthostomella | Codonelopsis | Parafavella | Ptychocylis | Undetermined species |
|----------------|-----------------|--------------|-------------|-------------|---------------------|
| Acanthostomella| 1               |              |             |             |                     |
| Codonelopsis   | 0.786 **        | 1            |             |             |                     |
| Parafavella    | 0.799 **        | 0.798 **     | 1           |             |                     |
| Ptychocylis    | 0.720 **        | 0.677 **     | 0.689 **    | 1           |                     |
| Undetermined species | 0.698 **      | 0.573 **     | 0.641 **    | 0.651 **    | 1                   |

**: Correlation is significant at the 0.01 level (2–tailed).
Discussion

Hydrography and assemblages division

The division of tintinnid assemblage corresponded well with water mass division by T–S diagram. The East China Sea neritic assemblage and the Japan Sea warm water assemblage located in the East China Sea and the Japan Sea, respectively. The boreal assemblage was in the waters north of the Sōya Strait where the T–S diagram identified two water masses (Subarctic Pacific Water and Coastal Arctic Water).

The transect in this study passed through the following biological provinces: the North Pacific Epicontinental Sea Province (the Bering Sea and the Okhotsk Sea), the Pacific Subarctic Gyre Province, the Kuroshio Current Province (the Japan Sea), and the China Sea Coastal Province [20,26]. Our division of the East China Sea neritic assemblage and the Japan Sea warm water assemblage corresponded with the China Sea Coastal Province and the Kuroshio Current Province, respectively. The boreal assemblage in this research covered the North Pacific Epicontinental Sea Province and the Pacific Subarctic Gyre Province [20,26]. Our result was in consistent with Priede [26] who described the region north of the westerly current in the Northern Hemisphere as the polar (or boreal) biome. In the present study we used the term boreal to describe the assemblages in this biome.

The Japan Sea warm water assemblage was at the brim of the warm water assemblage. The East China Sea neritic assemblage only had 2 stations. Therefore these two assemblages in our study were not typical. The transect covered the boreal assemblage from its north end to south end. Our research is the first study of the boreal assemblage in a transect across the assemblage.

Characteristics of the boreal assemblage

There were very few previous studies of tintinnids in the Arctic [17,18] and subarctic Pacific [17] regions. The study in subarctic Pacific [17] region only gave total densities and species names of dominant species in 15 stations.

The densities of tintinnids in the boreal assemblage had a sharp decrease from the Subarctic Pacific Water to Coastal Arctic Water (Figs 2 and 10). This decrease in densities were consistent with findings of previous studies [17,18]. Meanwhile, all tintinnid species occurred in the Chukchi Sea had been reported at other areas other than Arctic [17,18]. Therefore tintinnids might intrude into the Chukchi Sea through the Bering Strait by the help of current from the Bering Sea [19]. But most tintinnids cannot adapt to the extreme environment in the Chukchi Sea, this caused the low densities and tintinnid species richness in the Arctic region [17]. This also proved that there was no indigenous tintinnid species in the Arctic Ocean [17]. This is different from the case in Antarctic. Some species in genera Cymatocylis, laackmanniella, and...
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*codonellopsis* only distributed in Antarctic region south of 58°S, and were considered as Antarctic indigenous tintinnids [10].

The positions of the high densities (St. 24–St.27, St. 35–St. 41) were corresponding with the two branches of the Alaska Current located in the Bering Sea. But we did not have current data, we were not sure whether the high densities were related with the current branches at the time of sampling. Previous study showed that tintinnid destiny was high in the area where water masses mixed [12]. Therefore we thought the high densities were caused by the mixing of branches of the Alaska Current and the Oyashio Current.

It was reported that *Ptychocylis urnula* or *Salpingella faurei* was the most abundant species in Arctic region [18], while the species in genera *Acanthostomella, Codonellopsis, Ptychocylis, Parafavella*, and *Tintinnopsis* were the most abundant in subarctic Pacific [17]. The similar abundant genera were found in our study with *Acanthostomella, Codonellopsis, Ptychocylis*, and *Parafavella* accounted for 79.07±29.67% (n = 49) of abundance at stations in the boreal assemblage (Fig 12).

One undetermined species that has not been reported in previous study was found in this study. According to its lorica characteristics, it may belong to the genus *Salpingella*. But it was different with *S. acuminata* or *S. faurei* that had been reported in the Arctic [18], and subarctic regions [17].

The distribution patterns of *Acanthostomella, Codonellopsis, Parafavella, Ptychocylis*, and the undetermined species were similar (Fig 13). Those genera was similar to “species group” proposed by Fager and Mcgowan [27]. Their positive correlation might be because that they had similar reactions to properties of the environment.

LOD is a valuable characteristic of tintinnid feeding activity. The maximal prey size ingested is about 45% of the LOD, while the preferred prey size is about 25% of the LOD [4]. Species in two LOD size–classes (22–26 and 38–42 μm) contributed 82.81% to the total abundance in B1 assemblage (Fig 11). This means that the prey size was limited to a small number of size–classes. Species in those two LOD size–classes were still the main contributors to total abundance in B2 assemblage, but the number of LOD size–classes increased. This indicated that the added species in B2 assemblage had different prey size–classes with the core species in boreal assemblage.

Boreal assemblage in our study was at the downstream of the Alaska Current, the characteristics of the assemblage in this transect should also apply to the area in the east part of the Alaska Current and to the entire Bering Sea.

### Obstruction of the Sōya Strait to tintinnid expansion

The Sōya Strait is about 40 km long and 20–40 m deep. The boreal assemblage and the Japan Sea warm water assemblage were separated by the Sōya Strait, which might be a natural geographic barrier for tintinnid species. Species including *Dadayiella ganymedes, Eutintinnus stramentus*, and *Proplectella expolita* were identified in the Japan Sea but were not found north of the Sōya Strait. *Acanthostomella norvegica* and *Codonellopsis frigida* mainly distributed north of the Sōya Strait. *S. faurei* was reported to be the most abundant species in the Arctic region [18]. In this study, it was identified in areas south of the Japan Sea and in the Northwest Pacific, but were not found in the northern Japan Sea or the Okhotsk Sea. We did not know the connection of the two distribution areas.

Lorica morphology of some tintinnid species might vary following the environmental change, especially species in genus *Parafavella* [28]. The variation of tintinnid lorica morphology made species classification inaccurate just based on lorica morphology. In our study, *Parafavella pacifica* was the only species in genus *Parafavella* occurred in the Japan Sea warm water assemblage. It may be an intraspecific variation of the species north of the Sōya Strait. The lorica shape changed to adapt to the Japan Sea environment.

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*PLOS ONE | DOI:10.1371/journal.pone.0153379 April 7, 2016*
As the Sōya Strait is a natural geographic barrier, it was not possible to observe mixing between warm water and boreal tintinnid assemblages where the Oyashio and Kuroshio Currents meet. There are limited biological data available for this transitional zone created by the mixing of the Oyashio and Kuroshio Currents [29]. *D. ganymedes* was distributed the farthest north from the East China Sea into the Japan Sea, whereas *Steenstrupiella* spp. were confined to the south. This pattern was consistent with the intrusion of the Kuroshio Current into the East China Sea [30]. Therefore, we suggest that *D. ganymedes* is the most likely species to exist in the region east of Japan where the Kuroshio and Oyashio Currents meet, whereas *Steenstrupiella* spp. are likely to be the first to disappear.

Conclusions

With large space coverage and high resolution in station distribution, we identified three tintinnid assemblages (East China Sea neritic, Japan Sea warm water, and boreal assemblages) along the study transect. The tintinnid assemblage north of the Sōya Strait was boreal assemblage. There were peaks in densities at stations approximately between the two branches of the Alaska Current. The densities decreased both northward and southward. In Arctic region, the densities were extremely low (<10 ind./dm³ at most stations). *Acanthostomella, Codonellopsis, Parafavella*, and *Ptychocylis* were the dominant genera in this boreal assemblage. There were strong positive correlations between the densities of these four genera. In coastal waters, more species occurred and made the boreal assemblage much more complex both in tintinnid composition and LOD size–classes. The Sōya Strait forms a physical barrier between the boreal assemblage and the Japan Sea warm water assemblage.

Supporting Information

S1 File. All original data. (XLS)

Author Contributions

Conceived and designed the experiments: WCZ TX. Performed the experiments: ZQX SQW GTZ. Analyzed the data: HBL WCZ TX. Contributed reagents/materials/analysis tools: HBL WCZ. Wrote the paper: HBL WCZ.

References

1. Lynn DH. The ciliated protozoa: characterization, classification, and guide to the literature. Springer Science & Business Media; 2008.
2. Pierce RW, Turner JT. Ecology of planktonic ciliates in marine food webs. Reviews in Aquatic Sciences. 1992; 6(2): 139–181.
3. Williams R, McCall H, Pierce RW, Turner JT. Speciation of the tintinnid genus Cymatocylis by morphometric analysis of the loricae. Marine Ecology Progress Series. 1994; 107(3): 263–272.
4. Dolan JR. Morphology and ecology in tintinnid ciliates of the marine plankton: correlates of lorica dimensions. Acta Protozoologica. 2010; 49(3):235–244.
5. Pierce RW, Turner JT. Global biogeography of marine tintinnids. Marine Ecology Progress Series. 1993; 94: 11–11.
6. Dolan JR, Montagnes DJ, Agatha S, Coats DW, Stoecker DK. The Biology and Ecology of Tintinnid Ciliates: Models for Marine Plankton. Wiley–Blackwell, Oxford; 2013.
7. Haury LR, McGowan JA, Wiebe PH. Patterns and Processes in the Time–Space Scales of Plankton Distributions. In: Steele JH, editor. Spatial Pattern in Plankton Communities. Springer US, 1978. pp: 277–327.
8. Thompson GA, Alder VA, Boltovskoy D, Brandini F. Abundance and biogeography of tintinnids (ciliophora) and associated microzooplankton in the Southwestern Atlantic Ocean. Journal of Plankton Research. 1999; 21(7):1265–1298.

9. Thompson GA. Tintinnid diversity trends in the southwestern Atlantic Ocean (29 to 60°S). Aquatic Microbial Ecology. 2004; 35(1): 93–103.

10. Thompson GA, Alder VA. Patterns in tintinnid species composition and abundance in relation to hydrological conditions of the southwestern Atlantic during austral spring. Aquatic Microbial Ecology. 2005; 40(1):85–101.

11. Santoferrara L, Alder V. Abundance trends and ecology of planktonic ciliates of the south-western Atlantic (35–63°S): A comparison between neritic and oceanic environments. Journal of Plankton Research. 2009; 31(8):837–851.

12. Modigh M, Castaldo S, Saggiorno M. Distribution of tintinnid species from 42°N to 43°S through the Indian Ocean. In: Jones MB, Ingólfsson, Ólafsson AE, Helgason GV, Gunnarsson K, Svaravsson J, editors. Migrations and Dispersal of Marine Organisms. Springer Netherlands; 2003. pp. 251–262.

13. Dolan J R, Ritchie M E, Ras J. The “neutral” community structure of planktonic herbivores, tintinnid ciliates of the microzooplankton, across the SE Tropical Pacific Ocean. Biogeosciences Discussions. 2007; 4(3):297–310.

14. Dolan JR, Claustre H, Carlotti F, Plounevez S, Moutin T. Microzooplankton diversity: relationships of tintinnid ciliates with resources, competitors and predators from the Atlantic Coast of Morocco to the Eastern Mediterranean. Deep–Sea Research Part I. 2002; 49: 1217–1232.

15. Dolan JR, Claustre H, Vidussi F. Planktonic ciliates in the Mediterranean Sea: longitudinal trends. Deep–Sea Research Part I. 1999; 46: 2025–2039.

16. Dolan JR. Tintinnid ciliate diversity in the Mediterranean Sea: longitudinal patterns related to water column structure in late spring–early summer. Aquatic Microbial Ecology. 2000; 22: 69–78.

17. Taniguchi A. Microzooplankton biomass in the Arctic and Subarctic Pacific Ocean in summer. National Institute of Polar Research. 1984; 32: 63–76.

18. Dolan JR, Yang EJ, Kim TW, Kang SH. Microzooplankton in a warming Arctic: A comparison of tintinnids and radiolarians from summer 2011 and 2012 in the Chukchi Sea. ActaProtozoologica. 2014; 53: 101–113.

19. Springer AM, McRoy CP, Flint MV. The Bering Sea Green Belt: shelf–edge processes and ecosystem production. Fisheries Oceanography. 1996; 5(3–4): 205–223.

20. Longhurst A. Ecological geography of the sea. San Diego, CA, USA: Academic Press, 1998.

21. Senjyu T. The Japan Sea intermediate water; its characteristics and circulation. Journal of Oceanography. 1999; 55(2): 111–122.

22. Steele M, Morison J, Ernold W, Rigor I, Ortmeyer M, Shimada K. Circulation of summer Pacific halocline water in the Arctic Ocean. Journal of Geophysical Research: Oceans (1978–2012). 2004; 109 (C2).

23. Kofoid CA, Campbell AS. A conspectus of the marine and fresh–water Ciliata belonging to the suborder Tintinnioinea: with descriptions of new species principally from the Agassiz expedition to the eastern tropical Pacific 1904–1905. University of California press, California, 1929.

24. Kofoid CA, Campbell AS. Reports on the scientific results of the expedition to the eastern tropical Pacific, in charge to Alexander Agassiz, by US Fish commission steamer “Albatross”, from October 1904 to March 1905, Lieut.–Commander LM Garrett, USN commanding: XXXVII. The Ciliata: The Tintinnioinea. Bulletin of the Museum of Comparative Zoology of Harvard College, Harvard, 1939.

25. Zhang WC, Feng MP, Yu Y, Zhang CX, Xiao T. An illustrated guide to contemporary tintinnids in the world. Science Press, Beijing, 2012.

26. Priede IG. Biogeography of the oceans: a review of development of knowledge of currents, fronts and regional boundaries from sailing ships in the sixteenth century to satellite remote sensing. Pure and Applied Geophysics. 2014; 171(6): 1013–1027.

27. Fager EW, Mcgowan JA. Zooplankton Species Groups in the North Pacific. Science. 1963; 140 (3566):453–460. PMID: 17829536

28. Davis CC. Variations of the lorica in the genus Parafavella (Protozoa: Tintinnida) in northern Norway waters. Canadian Journal of Zoology. 1978; 56(8):1822–1827.

29. PICES. Marine Ecosystems of the North Pacific. PICES Special Publication 1, 2004.

30. Li HB, Zhao Y, Chen X, Zhang WC, Xu JH, Li J, et al. Interaction between neritic and warm water tintinnids in surface waters of East China Sea. Deep Sea Research Part II. 2016; 124: 84–92.