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BIODIVERSITY OF Tintinnids (Tintinnida) IN KHANH HOA - BINH THUAN WATERS

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ABSTRACT: Tintinnids are an important protozoan group in the aquatic food web and had been widely studied in various waters. There are about 1000 known species in the world. However, there have been very few taxonomic studies in Viet Nam and therefore the number of Tintinnid taxa and their distribution are poorly known. The present study documents 65 tintinnids species belonging to 30 genera and 13 families in samples collected from Khanh Hoa - Binh Thuan waters in 2016 and 2017. There were 17 new taxa records for Viet Nam protozoan fauna, raising the number of tintinnids recorded in Viet Nam to 125 taxa. Tintinnid assemblages in Khanh Hoa-Binh Thuan waters shared about 17 species with Ha Long Bay, 32 species with Con Co island and 26 species with coastal waters of South Viet Nam. Analysis of species diversity shows that the Shannon diversity index H' varied from 1.5 to 2.6. Distribution of species numbers and diversity in the Khanh Hoa - Binh Thuan waters revealed possible combined effects of hydrographical activities (e.g. upwelling), Mekong river influent (e.g. salinity), and food available on tintinnid communities.

Keywords: Loricate ciliate, Tintinnid, South Central, Viet Nam.

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INTRODUCTION

Tintinnid ciliates are a group of microzooplankton that range from 20 to 200 µm in size and are found living mostly in surface waters of global marine systems (Dolan, 2013). This group plays an important role in the aquatic food web by feeding on various nanosize organisms, such as diatoms and dinoflagellates (Montagnes, 2013) and in turn being consumed by larger zooplankton such as copepods (Stoecker, 2013). The hardened, vase-like shell of tintinnids is a unique taxonomic characteristic varying widely among different species and genera. This group is more species than other ciliates and is fairly well known taxonomically. For example, the early monographs of Kofoid & Campbell (1929, 1939) listed over 734 tintinnid species. This list increased to 750 species by the late 1940s and there are now about 1000 species described (Agatha & Strüder-Kypke, 2013).

Tintinnid taxonomy and classification have been studied extensively since the late nineteenth and early twentieth centuries. Early works included those of Fol (1883), Kofoid & Campbell (1929, 1939), and Hada (1932, 1938). This group of ciliates was classified and remained in a single order, Tintinnida, since 1929 (Kofoid & Campbell, 1929). However, recent phylogenetic analysis revealed some conflicts with the proposal of Kofoid & Campbell (1939) about the evolution of this group (Agatha & Strüder-Kypke, 2013) as well as helping to identify relationships among the species within the genera and families in this order (e.g. Strüder-Kypke & Lynn, 2008). This group includes taxa that are similar

morphologically at the species level while still showing appreciable variation of lorica within a single species, providing an ideal system for addressing fundamental questions of biological variability in time, space and species composition (Dolan, 2013). Recent reports on the diversity, biogeography and composition of tintinnids assemblages in different waters, e.g. Chihara & Murano (1997), Al-Yamani et al. (2011), Zaid & Hellal (2012), Wang et al. (2013), Durmuş & Balkis (2014), Yu et al. (2015) are advancing our knowledge of tintinnids while addressing questions of broad biological interest.

In Viet Nam, studies on tintinnids have to date been limited to a few publications and species lists (e.g. Rose, 1926; Dawydoff, 1936; Shirota, 1966). A few recent papers have reported tintinnid species occurrence and distribution in Ha Long Bay (Dinh Van Nhan et al., 2014) and Con Co Island (Dinh Van Nhan et al., 2016) in North Viet Nam, but for South Viet Nam, only a species list by Shirota (1966) for coastal waters has been published.

In South Central Viet Nam, coastal upwelling is present during the southwest monsoon period when the Mekong river also has an impact on coastal and offshore waters. During this time, different water masses are present (Dippner & Loick-Wilde, 2011) and play an important role in structuring the biological communities of Vietnamese waters (Loick-Wilde et al., 2017). In the present study, we explored the interaction between different water types and tintinnid diversity and composition in Khanh Hoa - Binh Thuan waters during the southwest monsoon period. This study represents the first detailed account of the distribution and diversity of tintinnids from this area, especially in offshore waters and thus addresses the gap in knowledge of tintinnid taxonomy and ecology in this waters.

MATERIAL AND METHODS

Study area

The sampling was carried out in June 2016 at 14 stations in Khanh Hoa-Binh Thuan waters, during cruise FK160603 of the R/V *Falkor*. Location of the sampling sites is shown in figure 1. During September 2016 to October 2017, an additional sampling was monthly carried out at station NT20 (fig. 1).Water column parameters such as temperature, salinity, dissolved oxygen saturation, pH and chlorophyll-*a* were measured with a CTD-rosette system (SBE 9+, Sea-Bird Electronics Inc., USA).

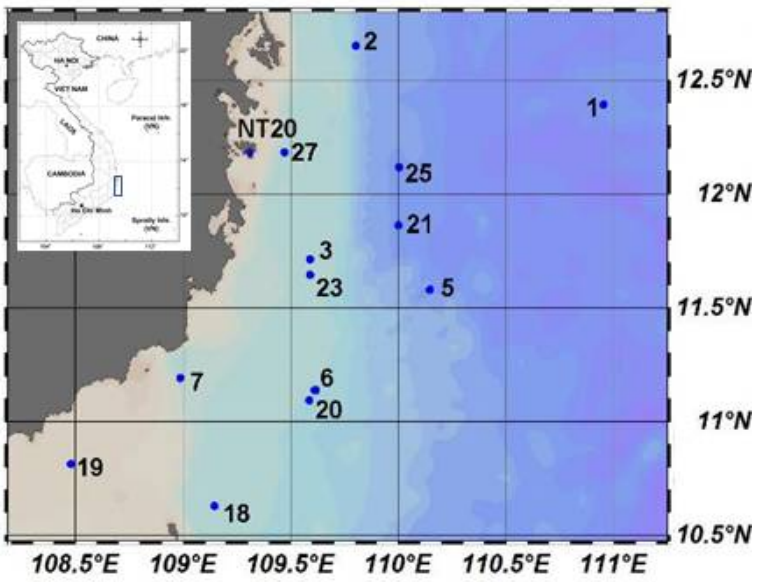

Figure 1. Map of study areas in the Khanh Hoa-Binh Thuan waters shows sampling stations (solid circles).

Qualitative and quantitative tintinnid analysis

Qualitative plankton samples were collected in vertical tows (100 m to the surface) of a Juday net (45µm mesh). The samples were fixed with Lugol's solution (5% final concentration) and stored in dark bottles (500ml) at room temperature. Samples were concentrated by settling in the laboratory in 500 ml cylinders and transferred to 30ml bottles. After species composition analysis, formaldehyde solution was added to the samples (4% to final concentration) for long-term storage.

Quantitative samples were collected at different depths at each station using the CTD-rosette system. Typically, 4 depths were sampled at each station (e.g., 1, 10, 30, 50, and 100 m depth). A volume of 5 liters of water was collected from the rosette, then gently passed through a 20 µm sieve. The concentrated sample on the sieve was transferred to a 15 ml vial, then fixed with Lugol's solution (5% final concentration) and stored under dark/cool conditions. In the laboratory, the samples were allowed to settle for>48 hours in graded centrifuged tubes and concentrated to 5-10 ml by siphoning out the supernatant.

Tintinnids were enumerated at the species level using a Sedgwick-Rafter counting chamber and a Olympus CX41 microscope (100x magnification). At least 300 individuals were counted for the least 11.5% of counting errors.

Standard references were used for identifying species, including the works by Kofoid and Campbell (1929, 1939), Shirota (1966), Chihara & Murano (1997), and Marshall (1934, 1969). The samples were examined using a Leica LDMB microscope with phase contrast and differential interference contrast optics. A digital camera, Olympus DP71, was used for microphotography.

Data analyses were performed using the PRIMER v6 software package, and MS Excel 2010. Counting data were used for diversity analysis and log-transformed for cluster analysis.

RESULTS AND DISCUSSION

General features of temperature and salinity in the survey area

Stations in our study area can be divided into three categories based on the distribution of salinity and temperature, upwelling waters (UpW), offshore waters (OSW) and onshore waters (OnSW).

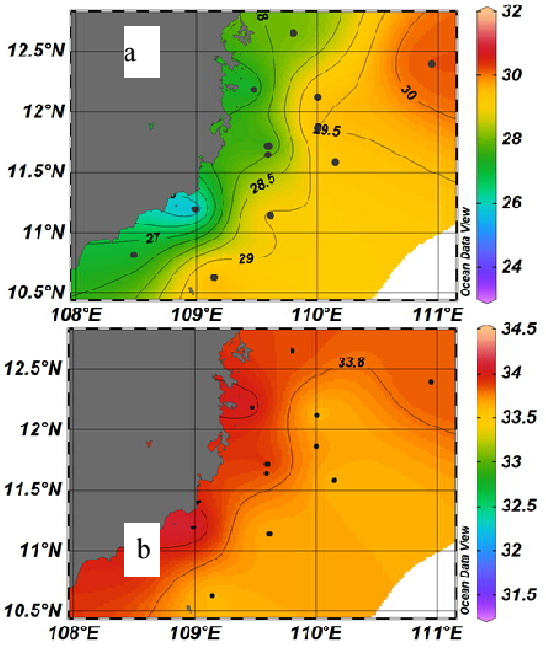

Figure 2. a. Distribution of temperature (°C); b. salinity (psu) at the surface in 6/2016.

Figure 2 shows the area with low temperature and high salinity, reflecting up welling, near the coast from Khanh Hoa to Binh Thuan. This water type, UpW, characterized stations FK002, FK003, FK007, FK019, FK023, and FK027. The second water type, OnSW, included stations with temperature 28.5-30°C and with salinity < 33.8 psu (FK005, FK006, FK018, FK020, FK021, FK025). This water type is a mixture of near-shore waters and offshore waters influenced by the Mekong outflow (Dippner & Loick-Wilde, 2011). The third water type, OSW (station FK001) has high temperature and salinity. The survey was conducted in June 2016 during the Southeast Monsoon and during a post El Niño year when

the SW monsoon and upwelling activity are both expected to be weaker than average (Dippner et al., 2008).

Species composition of Tintinnids

We identified 65 species of tintinnids belonging to 30 genera and 13 families. Of these, 4 tintinnid genera (*Acanthostomella, Brandtiella, Parundella* and *Xystonellopsis*) and 17 tintinnid species were new records for Viet Nam (table 2, figs. 3-5).

Table 2. List of species recorded in Khanh Hoa - Binh Thuan waters, including occurrence in North Viet Nam (Ha Long Bay and Con Co island) and in coastal waters of South Viet Nam. [I] - Shirota (1966); [II]&[III] - Dinh Van Nhan et al. (2014, 2016).

Ord.	Taxa (this study)	Coastal waters of South Viet Nam [I]	Ha Long Bay [II]	Con Co island [III]
	Family Ascampbelliellidae Corliss, 1960			
1	*Acanthostomella conicoides* Kofoid & Campbell, 1929*			
2	*A. norvegica* Daday, 1887*			
3	*Ascampbelliella retusa* Hada, 1935			+
	Family Codonellidae Kent, 1881			
4	*Codonella amphorella* Biedermann, 1893	+		
5	*C. galea* Haeckel, 1873 *			
6	*Poroecus annulatus* Kofoid & Campbell, 1929 *			
7	*P. apicatus* Kofoid & Campbell, 1929	+		
8	*Tintinnopsis beroidea* Stein, 1867	+	+	+
9	*T. cylindrica* Daday, 1887		+	
10	*T. dadayi* Kofoid, 1905		+	
11	*T. karajacensis* Brandt, 1896			+
12	*T. nucula* Fol, 1884	+	+	+
13	*T. parvula* Jörgensen, 1912		+	
14	*T. radix* Imhof, 1886	+	+	+
15	*T. tocantinensis* Kofoid & Campbell, 1929		+	+
16	*T. urnula* Meunier, 1910		+	
	Family Codonellopsidae Kofoid & Campbell, 1929			
17	*Codonellopsis morchella* (Cleve) Jörgensen, 1924	+	+	+
18	*C. orthoceras* (Haeckel, 1873) Jörgensen, 1924			+
19	*C. ostenfeldi* (Schmidt, 1902) Kofoid & Campbell, 1929	+	+	+
20	*C. schabi*(Brandt, 1906) Kofoid & Campbell, 1929			+
21	*Codonellopsis* sp.			
	Family Cyttarocylididae Kofoid & Campbell, 1939			
22	*Cyttarocylis ampulla* Bachy et al. 2012	+		
	Family Dictyocystidae Haeckel, 1873			
23	*Dictyocysta lepida* Ehrenberg, 1854	+		
24	*Wangiella dicollaria* Nie, 1934		+	+
	Family Epiplocylididae Kofoid & Campbell, 1939			

25	*Epiplocylis undella* (Ostenfeld & Schmidt) Jörgensen, 1927	+		+
26	*Epiplocyloides ralumensis* Brandt, 1906*			
27	*E. reticulata* Ostenfeld & Schmidt, 1901			+
	Family Metacylididae Kofoid & Campbell, 1929			
28	*Climacocylis scalaria* Brandt, 1906*			
29	*C. scalaroides Kofoid & Campbell, 1929*		+	+
30	*Metacylis jorgenseni* (Cleve) Kofoid and Campbell, 1929*			
	Family Ptychocylididae			
31	*Favella azorica* (Cleve, 1900) Jörgensen, 1924	+	+	
	Family Rhabdonellidae Kofoid & Campbell, 1929			
32	*Protorhabdonella curta* Cleve, 1900*			
33	*P. simplex* (Cleve) Jörgensen, 1924	+		+
34	*Rhabdonella amor* (Cleve, 1900) Brandt, 1907	+		
35	*R. cornucopia* Kofoid & Campbell, 1929*			
36	*R. elegans* Jörgensen, 1924			+
37	*R. poculum* Ostenfeld & Schmidt, 1901	+		+
38	*R. spiralis* Fol, 1881			+
39	*Rhabdonellopsis apophysata* (Cleve, 1900) Kofoid & Campbell, 1929	+		+
	Family Tintinnidae Kofoid & Campbell, 1929			
40	*Amphorellopsis acuta* Kofoid & Campbell, 1929		+	+
41	*A. tetragone* (Jörgensen, 1924) Kofoid & Campbell, 1929	+		
42	*Amphorides quadrilineata* Claparède & Lachmann, 1858		+	+
43	*Brandtiella palliata* (Brandt, 1906) Kofoid & Campbell, 1929*			
44	*Dadayiella ganymedes* (Entz, 1884) Kofoid & Campbell, 1929	+		+
45	*D. pachytoecus* Dendy, 1924	+		
46	*Eutintinnus elegans* (Jörgensen) Kofoid & Campbell, 1939	+		
47	*E. fraknoii* Daday, 1887			+
48	*E. lusus-undae* Entz, 1885	+		+
49	*E. pacificus* (Kofoid & Campbell, 1929) Kofoid & Campbell, 1939*			
50	*E. stramentus* Kofoid & Campbell, 1929	+	+	+
51	*Ormosella trachelium* (Jörgensen) Kofoid &Campbell, 1929	+		
52	*Salpingella acuminata* (Claparède & Lachmann, 1858) Jörgensen, 1924	+		+
53	*S. rotundata* Kofoid & Campbell, 1929			+
54	*Steenstrupiella intumescens* (Jörgensen, 1924) Kofoid & Campbell, 1929			+
55	*S. steenstrupii* (Claparède & Lachmann, 1858) Kofoid & Campbell, 1929	+		+
	Family Tintinnidiidae Kofoid & Campbell, 1929			

56	*Leprotintinnus nordqvisti* (Brandt, 1906) Kofoid & Campbell, 1929	+	+	+
	Family Undellidae Kofoid & Campbell, 1929			
57	*Undella claparedei* (Entz) Daday, 1887			+
58	*U. clevei* Jörgensen, 1924			+
	Family Xystonellidae Kofoid & Campbell, 1929			
59	*Parundella aculeata* Ostenfeld, 1899*			
60	*Xystonella treforti* Daday, 1887	+		
61	*Xystonellopsis cymatica* (Brandt, 1906) Jörgensen, 1924*			
62	*X. dicymatica* (Brandt, 1906) Kofoid & Campbell, 1929*			
63	*X. krämeri* (Brandt) Kofoid & Campbell, 1929*			
64	*X. paradoxa* (Cleve, 1900) Jörgensen, 1924*			
65	*X. tenuirostris* Brandt, 1906*			
Total	65	26	17	32

(+): shared species; (*) new record for Viet Nam protozoan fauna.

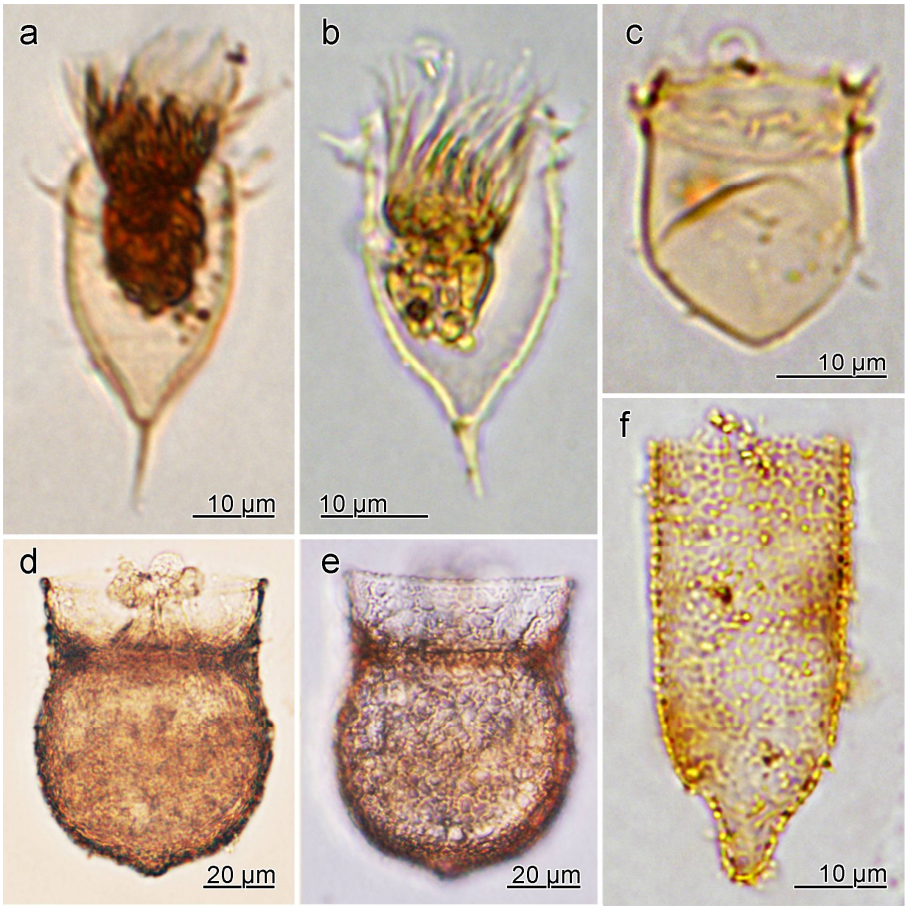

Figure 3a-f. Light microphotographs of newly recorded tintinnid species; a-b. *Acanthostomella conicoides*; c. *A. norvegica*; d-e. *Codonella galea*; f. *Poroecus annulatus.*

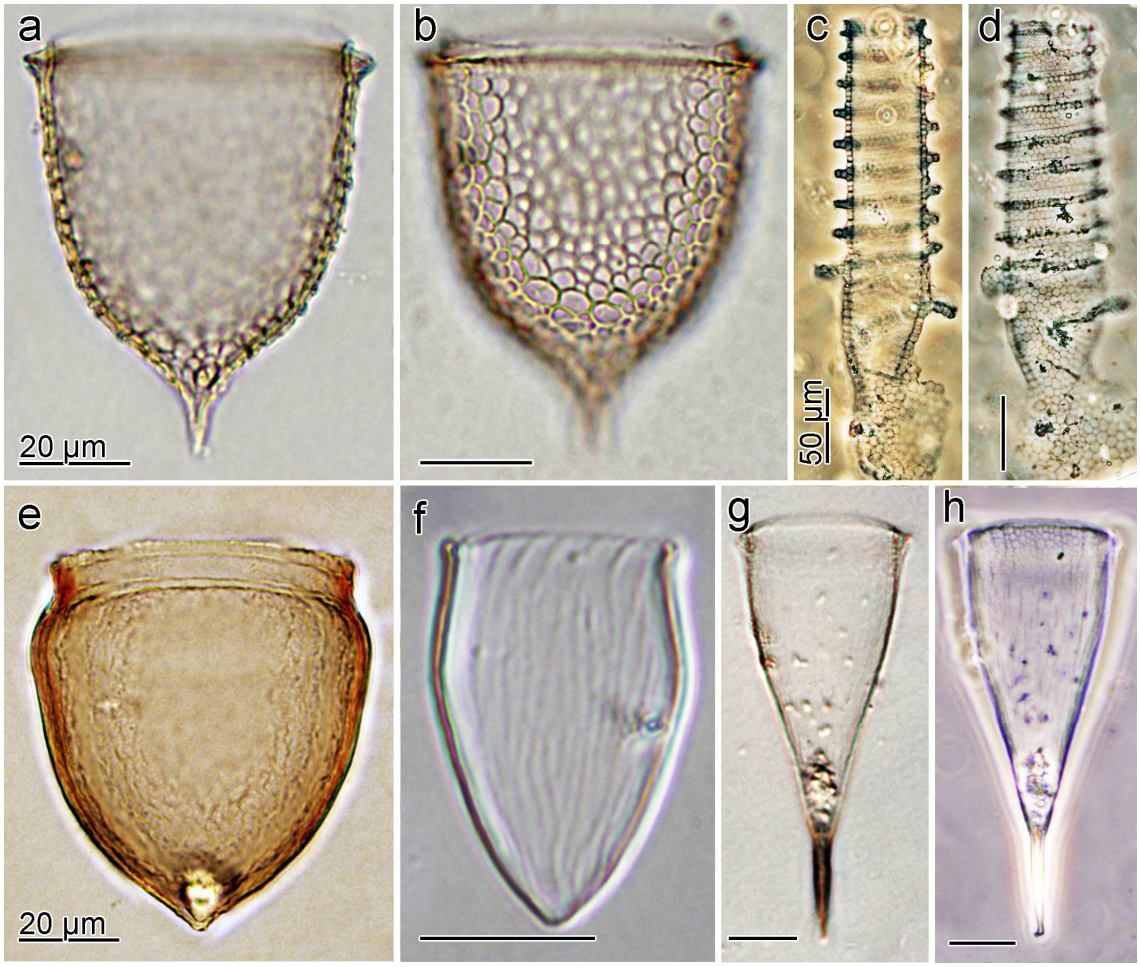

Figure 4a-h. Light microphotographs of newly recorded tintinnid species. - a-b. *Epiplocyloides ralumensis*; - c-d. *Climacocylis scalaria*; - e. *Metacylis jorgenseni*; - f. *Protorhabdonella curta*; - g-h. *Rhabdonella cornucopia.* Scalebars in figs. 4b, f, g, and h are 20 µm; and in Fig. 4d is 50 µm.

With 65 species recorded (table 2), Khanh Hoa-Binh Thuan waters contain the most diverse community of tintinnids yet characterized in Viet Nam. These waters host 13 of 15 families and 30 of 75 genera of tintinnids worldwide. Compared with previous studies, our study region shared some 26 species with coastal areas in the south of Viet Nam (Shirota, 1966), 17 species with Ha Long Bay (Dinh Van Nhan et al., 2014), and 32 species with Con Co island (Dinh Van Nhan et al., 2016).

In the Bien Dong, lower species number was found in different locations. There were 39 species found in Manila Bay including much earlier work in 1941 (Santiago et al., 2017). In East Asian Waters, 20 tintinnid species were recorded during investigation in 1998 (Lee & Kim, 2010). Comparing with the updated species list in the Manila Bay (Santiago et al., 2017) and pervious works in Viet Nam (Shirota, 1966; Dinh Van Nhan et al., 2014, 2016), this present study reports 16 species as new records for the Bien Dong protozoan fauna.

Among the 30 genera found in the study waters, the genus *Tintinnopsis* has the highest diversity, with 9 species accounting for 13.8% of the total species count. Four genera (*Xystonellopsis*, *Codonellopsis*, *Eutintinnus* and *Rhabdonella*) contained five species each,

accounting for 7.7% of the species present. The genera *Acanthostomella*, *Codonella, Poroecus, Epiplocyloides*, *Climacocylis, Dadayiella*, *Protorhabdonella, Ormosella, Salpingella, Steenstrupiella, Amphorellopsis* and *Undella* each has two species present and accounted for 3.1% of the species richness. The rest of the genera found in these waters (*Ascampbelliella*, *Cyttarocylis*, *Dictyocysta*, *Epiplocylis*, *Favella*, *Metacylis*, *Wangiella*, *Rhabdonellopsis*, *Amphorides*, *Brandtiella*, *Leprotintinnus*, *Parundella*, *Ormosella*, and *Xystonella*) were each represented by a single species accounting for 1.5% of the overall species count (fig.6).

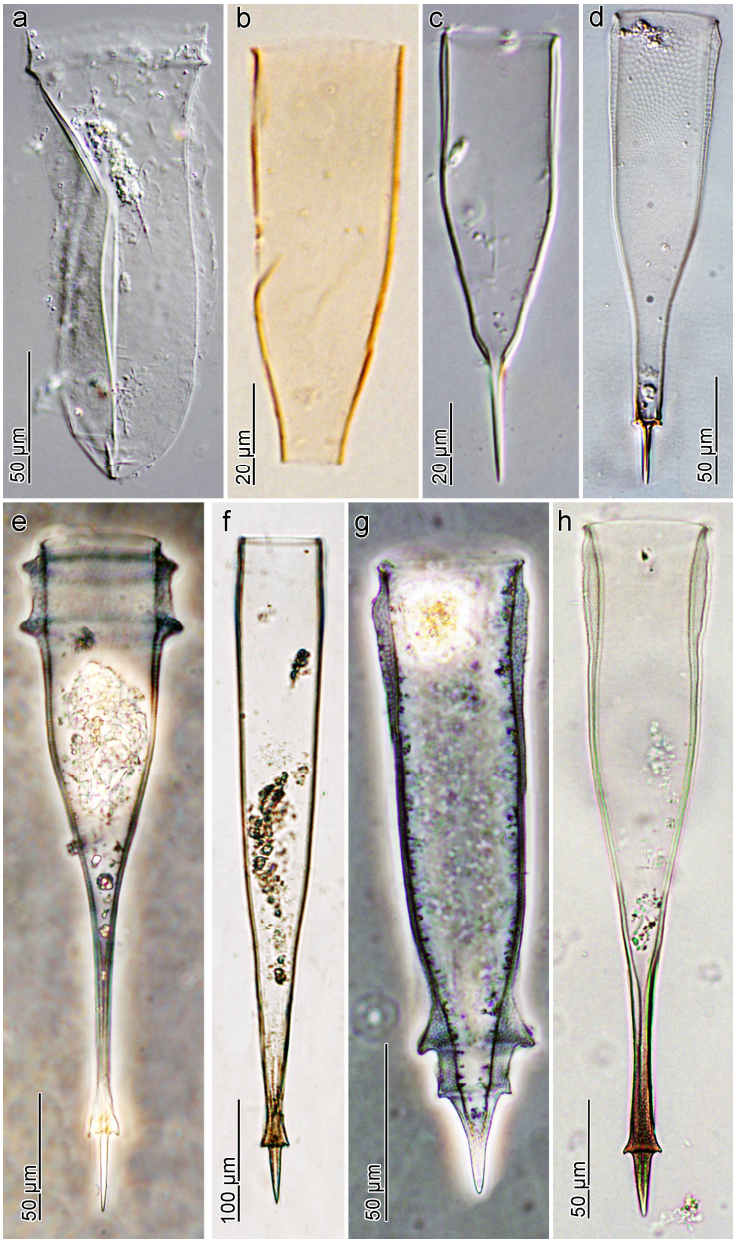

Figure 5a-h. Light microphotographs of newly recorded tintinnid species. a. *Brandtiella palliata*; b. *Eutintinnus pacificus*; c. *Parundella aculeata*; d. *Xystonellopsis cymatica*; e. *X. dicymatica*; f. *X. krämeri*; g. *X. paradoxa*; h. *X. tenuirostris.*

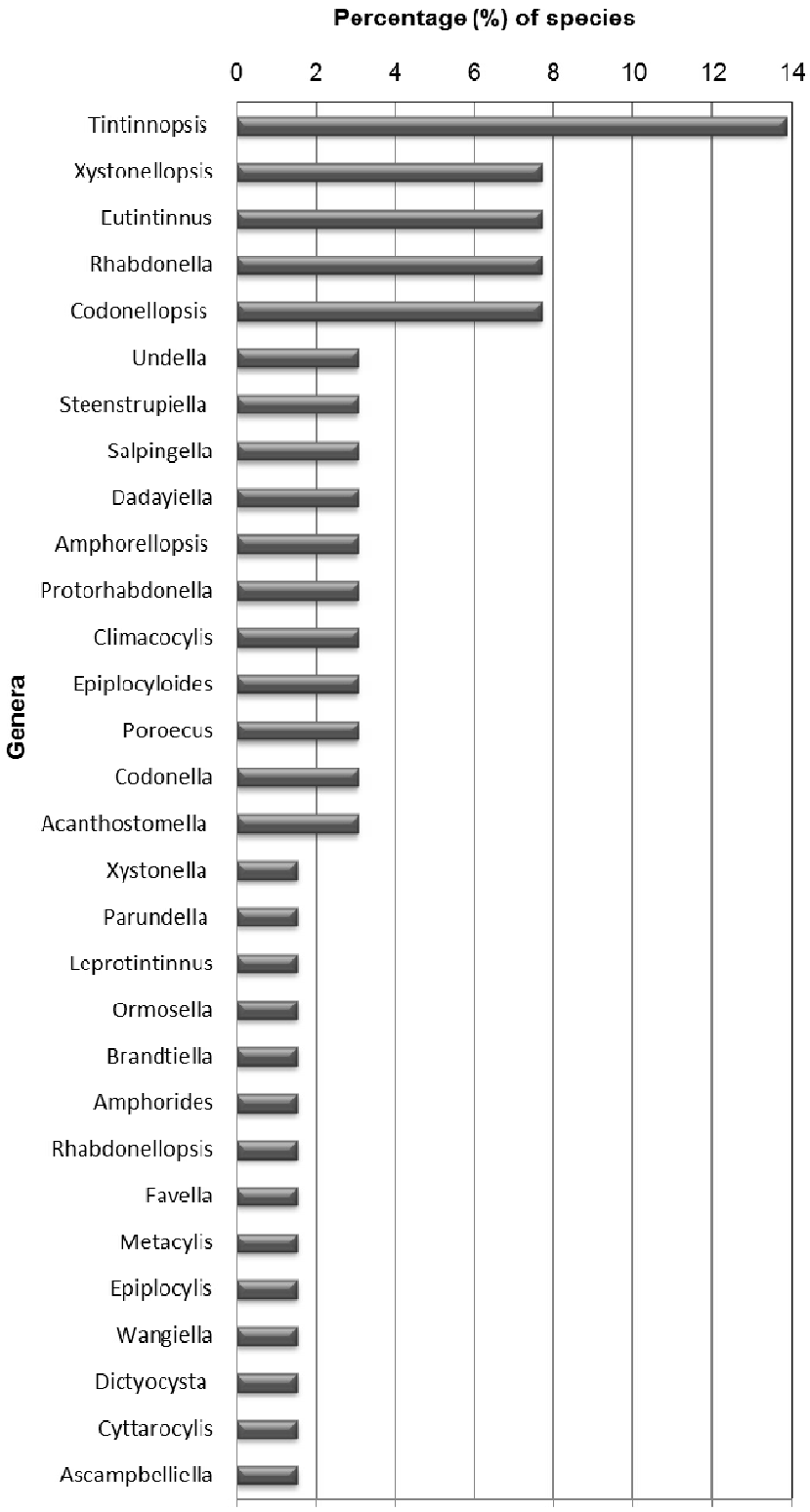

Figure 6. Percentage of species richnessin each genus

Species diversity and variation among water types

The number of tintinnid species varies among the water types. The highest species number was in the OnSW (e.g. FK005, FK006), with 32-37 species present at each station. In the up-welling waters (e.g. FK019, FK007, FK027), species number ranged from 24 to 34 species. Offshore waters have the lowest species number (28 species) (fig.7a).

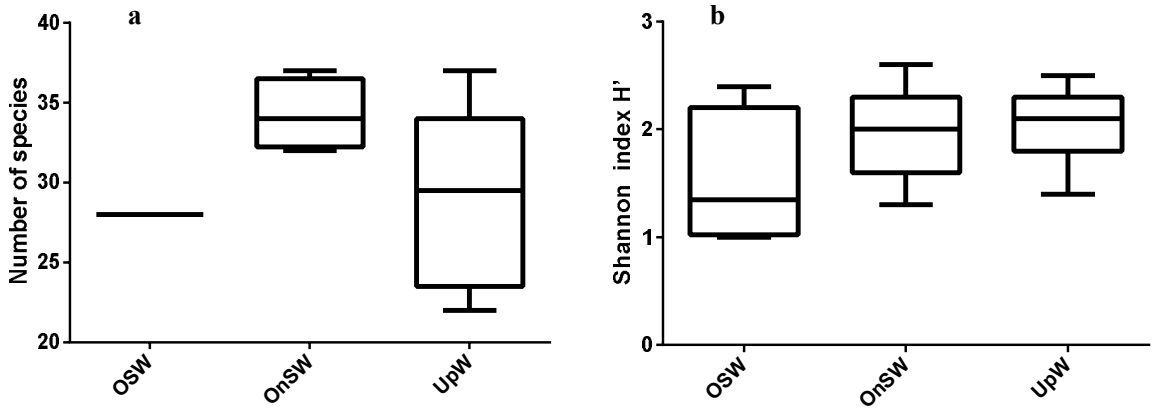

Figure 7a-b. Number of tintinnid species (a), and Shannon diversity index H' (b) among the water types.

The Shannon diversity index (H') of tintinnids varied from 1.5 to 2.6 across our study region. The OnSW water type and upwelling water were least variable in terms of diversity while the offshore waters showed much higher variation in the diversity index (fig. 7b).

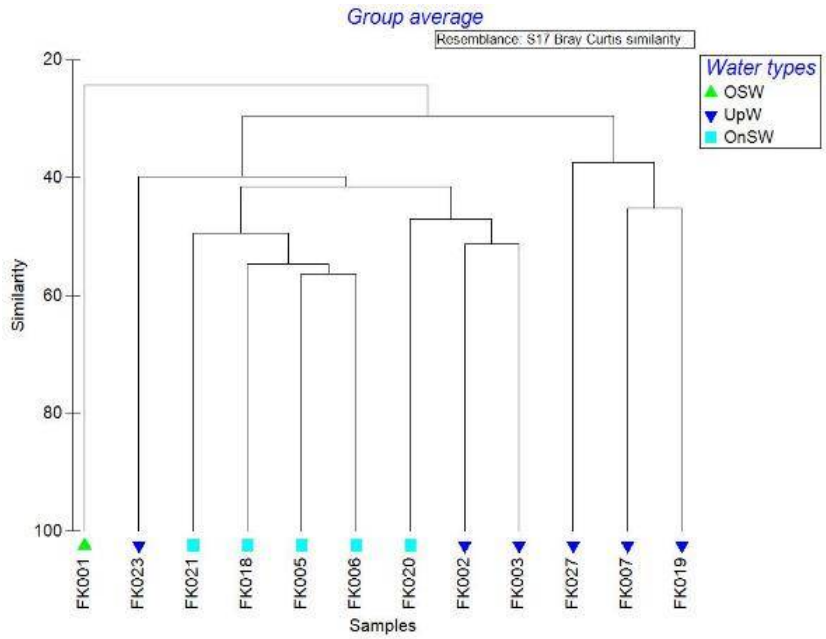

Figure 8. Clustering dendrogram of different tintinnid assemblages using the Bray-Curtis similarity index

An analysis of the species composition and abundance using the Bray-Curtis similarity index showed rather distinct assemblages reflecting water types (fig. 8). The OSW was quite distinct, sharing only some 25% similarity with other two water types. This particular station (FK001) had only 7.5% similarity with an upwelling station (FK019). Within the UpW region, three stations formed a coherent group (FK007, FK019, and FK027) while a fourth was more similar to onshore waters (FK023). This coherent group has the most difference (88.2%) with OSW tintinnid assemblages, and is formed by stations near center of upwelling area. In OnSW, stations FK005 and FK006 have the highest similarity index of 56.6% and this group shared 53,5% similarity with station FK018.

A SIMPER analysis showed a difference between the OSW and UpW tintinnid assemblages of about 78.1% and a smaller difference of 72.9% between the OSW and OnSW communities. At our station offshore FK001 (OSW), which was warm and oceanic, as much as 50% of the species were indicators for warm Kuroshio waters (Kim et al., 2012), including the two most abundant species, *Salpingella acuminata* and *Xystonella treforti*. In our samples, one species characteristic of cold oceanic water, *Acanthostomella norvegica*, (Pierce & Turner, 1993), was found primarily at OnSW stations (e.g. FK005 and FK006). This species was typically found at temperatures of 5-10^{o}C with salinities of 30-35 psu (OBIS, 2017). One of the East Sea indicator species (Kim et al., 2012), *Epiplocyloides reticulata*, was commonly found at OnSW and UpW stations but not at OSW station. The two water types UpW and OnSW shared about 36.0% similarity in their tintinnid assemblages but the dominant species of these two water types are different. Specifically, *Dadayiella ganymedes* and *Protorhabdonella simplex* contributed as much as 20% of tintinnid abundance in UpW waters while *Protorhabdonella curta* and *Acanthostomella conicoides* were the dominant species in OnSW waters.

In this present study, tintinnid taxonomy and distribution on offshore waters were presented for the first time in Viet Nam as well as in the Bien Dong. There were 16 taxa newly recorded for Bien Dong's protozoan fauna. A larger number of genera (30) and families (13) reported in the study waters indicating this is a species rich area for tintinnid ciliates. This present study is providing, however, preliminary knowledge on how different water types would defining different tintinnid assemblages. The results from this work revealed the need of regional research on tintinnid communities in several aspects including taxonomy and genetic, quantitative analysis on impacts of different oceanography/biological processes as well as environmental factors on tintinnid community structure, variable in loricate sizes and shapes, and biological role of this particulate groups in the microbial food web.

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