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RESEARCH ARTICLE

First record of four tintinnid species in the Lebanese marine waters of the eastern Mediterranean Sea

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Abstract

The present paper reports the first record of four tintinnid species from the Lebanese marine waters, Eastern Mediterranean: *Bursaopsis bursa* (Cleve 1900) Kofoid and Campbell 1929, *Eutintinnus haslae* Taniguchi and Hada, 1981, *Favella aciculifera* Jörgensen, 1924 and *Xystonellopsis scyphium* Jörgensen, 1924, which contribute to the regional checklist of ciliates species. These species were collected at an offshore station in the depths between 150 and 600 m on October 11, 2018. Considering the studies in different regions, the newly reported species belong to mesopelagic waters, suggesting that the biogeographical distribution of tintinnids is homogenous in deep waters.

Keywords: Eastern Mediterranean, Lebanese marine waters, tintinnids, microzooplankton, *Bursaopsis bursa*, *Eutintinnus haslae*, *Favellla aciculifera*, *Xystonellopsis scyphium*

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Introduction

Tintinnids are planktonic ciliates and members of the suborder Tintinnina. These organisms are found largely in the surface layer of the sea, where they feed on algae ranging in size from 2 to 20 µm (Dolan *et al.* 2006). They are a vital link in marine planktonic food webs (Alder 1999; Dolan *et al.* 2002; Bojanic *et al.* 2006). Therefore, they play important trophic roles and changes in distribution and phenology of tintinnid taxa could cause mismatches between successive trophic levels (Hinder *et al.* 2012). Short generation times, high abundances, and fast reproduction rates, coupled with a high grazing impact, enhance the importance of tintinnids as a key trophic link between the microbial and the metazoan compartments (Laval-Peuto and Brownlee 1986). Tintinnids are the dominant ciliates and comprise over 90% of the microzooplankton in the upper water column in open waters (Rassoulzadegan 1977). In the Mediterranean Sea, tintinnids are the most investigated component of microzooplankton communities. These organisms were significantly abundant in the upper layers (Sitran *et al.* 2009). Similarly, the surface population presented much higher variability compared to the deep-water population and concentrations tended to increase significantly in the whole column during the mixing period (Dolan *et al.* 2019). In fact, knowledge on the planktonic component is still far more limited in deep-sea habitats and domains than the pelagic counterpart.

Previous studies about tintinnids in the Lebanese marine waters were mostly qualitative and quantitative (Abboud-Abi Saab 1989; 2002; Abboud-Abi Saab *et al.* 2012 and references therein). All identified species and their ecology were listed in the recapitulated monograph (Abboud-Abi Saab 2008) that deals with the taxonomic survey on tintinnids (Order Oligotrichida, Suborder Tintinnina) collected from 1979 to 2006 to cover different studies in neritic and oceanic waters (net tows and water samples). Moreover, these studies have only investigated ciliate tintinnids between the surface and 60 m depth without considering deep water masses. So far, a total of 150 taxa (117 species, 2 varieties and 31 unidentified species) have been reported (Abboud-Abi Saab 2008).

This papers reports for the first time a record of tintinnid species *Bursaopsis bursa* (Cleve 1900) Kofoid and Campbell, 1929, *Eutintinnus haslae* Taniguchi and Hada, 1981, *Favellla aciculifera* Jörgensen, 1924 and *Xystonellopsis scyphium* Jörgensen, 1924 in the Lebanese deep waters, which will contribute to the regional checklist.

Materials and Methods

This study was carried out at a marine station A3, almost 10.5 km off the coast (33º 59'17.83" N, 35º 25'58.64" E) over a depth of ~1250 m using the Lebanese R/V "CANA-CNRS" on October 11, 2018 (Figure 1). Water column profile of temperature and salinity were obtained using a CTD (Idronaut, type Ocean seven 316 plus).

Samples were collected using a Niskin bottle at different depths (0, 80, 150, 250, 400 and 600 m). About seven liters of water were screened through a 20 µm collector and preserved with 4% borate buffered formaldehyde. In the laboratory, samples were gravimetrically settled to aliquots of 100 ml in a combined plate chamber. For tintinnid enumeration of each sample, the entire bottom chamber was examined using a phase contrast inverted microscope Wild M40 following the Utermöhl's method (1958) at 100 X magnifications for most specimens. All identifications were made on the basis of lorica morphology. Identification and counting were completed to the species level, using standard taxonomic references (Jörgensen 1924; Kofoid and Campbell 1929; 1939; Wuchang *et al.*

2012; Dolan *et al.* 2019). Total length (TL), maximum diameter (MD) and oral diameter (OD) were measured.

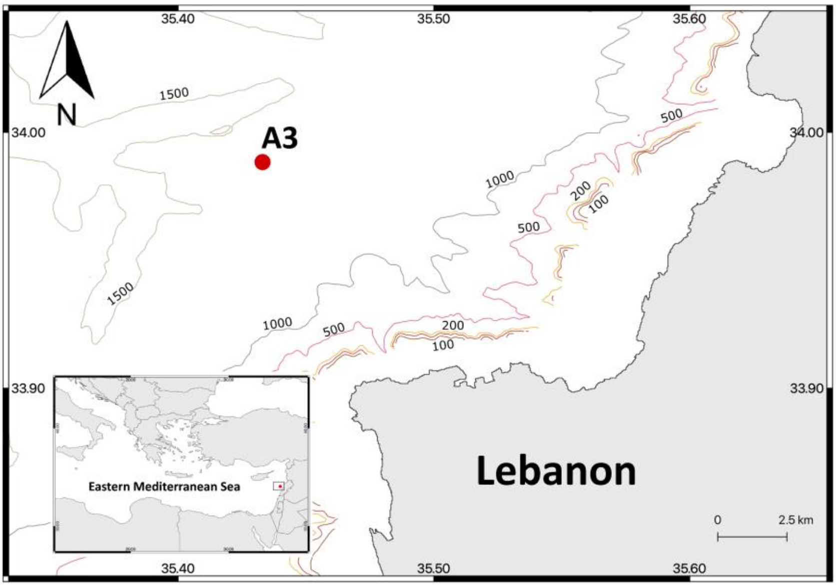

Figure 1. Study site, Station A3, off the Lebanese coast in the eastern Mediterranean Sea

Results and Discussion

The hydrographical variables varied from the surface to 600 m depth at the sampling site. Temperature varied between 27.7 ºC and 13.9 ºC, while salinity ranged between 39.3 ‰ and 38.6 ‰. Large seasonal changes exist in the eastern Mediterranean Sea, especially during summer and in a lesser degree during autumn (period of study) when stratification starts to decrease, enhancing vertical water mixing (Houpert *et al.* 2015).

Four tintinnid species were recorded for the first time in the Lebanese waters during this study. The list and biogeographical distribution of these species are presented below, noting that all species belong to the same suborder (Tintinnina).

1. Family: Tintinnidae Claparède and Lachmann, 1858
Subfamily: Tintinninae Kofoid and Campbell, 1929
Genus: *Bursaopsis* Kofoid and Campbell 1929
Species: *Bursaopsis bursa* (Cleve, 1900) Kofoid and Campbell 1929

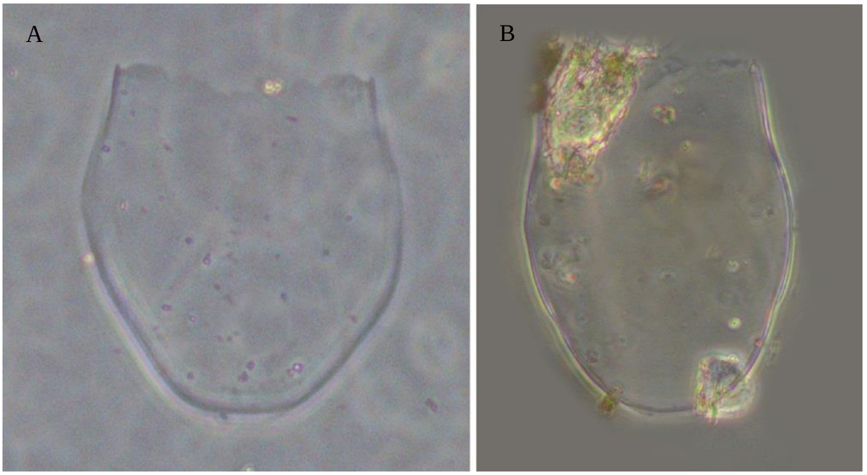

Figure 2: *Bursaopsis bursa* (Cleve, 1900) Kofoid and Campbell 1929 collected at:
A) 80 m depth (TL= 105 µm, MD =102 µm, OD= 81 µm) and
B) 600 m depth (TL= 158 µm, MD= 118 µm, OD= 81 µm).

Two specimens were observed in this study (Figure 2). *Bursaopsis bursa* was detected at 80 m depth (T= 20.1 ºC, salinity= 39.1 ‰) and 600 m depth (T= 13.9 ºC, salinity= 38.6‰) at the study site. This species was previously known as *Tintinnus bursa* and was first described by Cleve (1900). The loricate is ovoid urn-shaped and the oral aperture is more or less small, the wall is thin and transparent. The first specimen had a TL= 105 µm, MD =102 µm and OD= 81 µm at 80 m depth and the second had a TL= 158 µm, MD= 118 µm and OD= 81 µm at 600 m depth. This species is known as stenothermal, cold habitat and stenohaline, inhabiting euhaline waters (Gavrilova and Dovgal 2018). It was detected off the Azores (Kofoid and Campbell 1929) and North Atlantic (Gaarder 1946; Marshall 1969). Marshall (1969) detected the same species with a TL= 70-145 µm and OD= 67-80 µm in the Atlantic waters.

2. Family: Tintinnidae Claparède and Lachmann, 1858
Subfamily: Salpingellinae Kofoid and Campbell, 1939
Genus: *Eutintinnus* Kofoid and Campbell, 1939
Species: Eutintinnus haslae Taniguchi and Hada, 1981

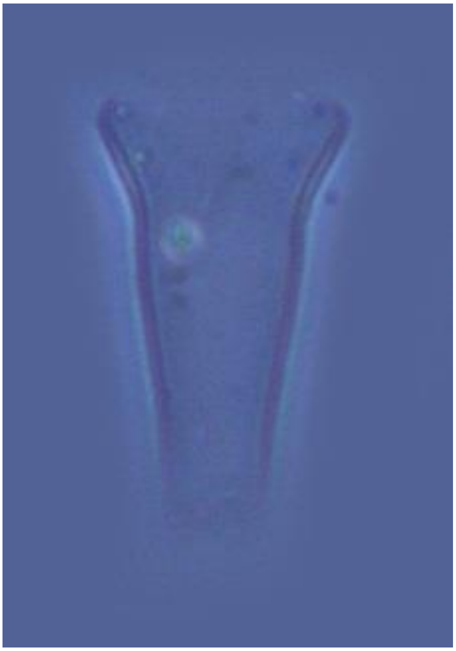

Figure 3. *Eutintinnus haslae* Taniguchi and Hada, 1981 collected at 150 m depth (TL= 55 µm, OD= 31 µm, aboral diameter= 15 µm)

Only one specimen of *Eutintinnus haslae* was detected at 150 m depth (T = 17.4 ºC, salinity = 39.1‰) in the study site (Figure 3). This species has a cylindro-conical shape with a flaring mouth. It is one of the smallest and short tapering truncated cones with a low but broad median bulge. Its oral region develops into a wide funnel-like suboral flaring. Below the bulge, the slope of the contour is gradually decreasing and the posterior end often terminates into a narrow aboral cylinder; the cylinder varies in length. Its wall is hyaline, uniform in thickness or sometimes very slightly thicker at throat (Taniguchi and Hada 1981). This specimen had a TL = 55 µm, OD = 31 µm and aboral diameter = 15 µm. *Eutintinnus haslae* is mainly a tropical species and prefers shade conditions. The first record of this species, named as *Eutintinnus* sp., was originally reported from the equatorial area of the eastern Pacific Ocean by Hasle (1960). Later, it was described as a new species *E. hasleae* by Taniguchi and Hada (1981), Modigh *et al*. (2003) and Gomez (2007) and found in the upper 300 m layer of the Philippines Sea and the Pacific and Indian Oceans. It was also reported from the Ukrainian part of the Black Sea (Gavrilova 2005). Furthermore, Dolan *et al.* (2019) noted this species at 250 m depth in the Ligure-provinçal Basin. Our specimen greatly resembled the form found in the Indian Ocean with slight differences in morphological measurements with TL = 46 µm; OD = 35 µm and aboral diameter = 6 µm (Gomez 2007).

3. Family: Ptychocylididae Kofoid and Campbell, 1929
Genus: *Favella* Jörgensen, 1924
Species: *Favella aciculifera* Jörgensen, 1924

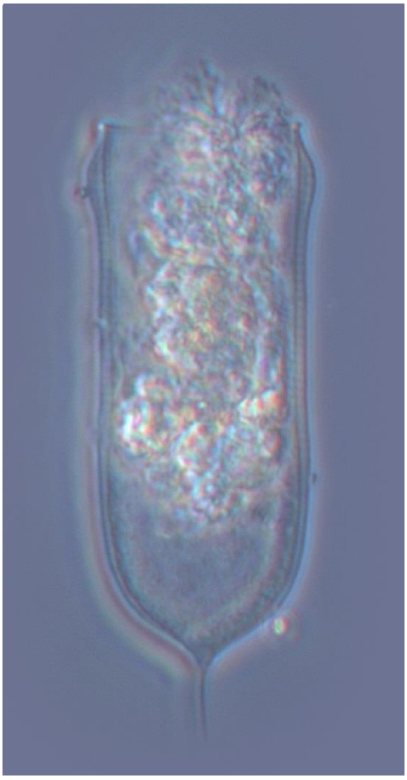

Figure 4. *Favella aciculifera* Jörgensen, 1924 collected at 250 m depth (TL = 121 µm, L without spine = 101 µm, OD = 39 µm)

Only one specimen was observed (Figure 4). *Favella aciculifera* was detected at 250 m depth (T = 15.6 ºC, salinity = 38.9 ‰) at the study site. It was first described by Jörgensen (1924). Later, it was synonymized as *Parundella aciculifera* by Kofoid and Campbell (1929), *Parundella spinosa* by Kofoid and Campbell (1929; 1939), and *Xystonellopsis aciculifera* by Balech (1968). Lorica is swollen below the mouth and it is rapidly rounded to a short protracted point carrying a fine needle or bristle. The two lamellae of the wall are well-developed and separated down to near the protracted point where they merge into a single thin wall. At the mouth, they are closer together and more distant at the swollen part. The wall shows in the middle a secondary maximum of thickness. Meshes are almost regularly hexagonal, larger and more distinctly visible on the swollen portion, smaller towards the mouth and in the lower ⅔ (Jörgensen 1924). This specimen had TL = 121 µm, L without spine = 101 µm and OD = 39 µm. It was found at 250 m depth in the Liguria-provinçal Basin by Dolan *et al.* (2019) with an abundance not exceeding 0.1 cells. L^{-1} all year around and a total absence during the periods of water column mixing. It was abundant between 400 and 600 m depth in the open waters of the South Adriatic Sea (Krsinic and Grbec 2006) and from deep waters of the Gulf of Mexico (Balech 1968). The morphological measurements of our specimen were in the range of values of the same species found in Gulf of Mexico, Caribbean Sea (TL = 104.5-127.5 µm) by Balech (1968) and in Tropical Pacific (TL = 101-132 µm with larger OD = 33-36 µm) known as *Parundella aciculifera* by Kofoid and Campbell (1939).

4. Family: Xystonellidae Kofoid and Campbell 1929
Genus: *Xystonellopsis* Jörgensen 1924
Species: *Xystonellopsis scyphium* Jörgensen, 1924

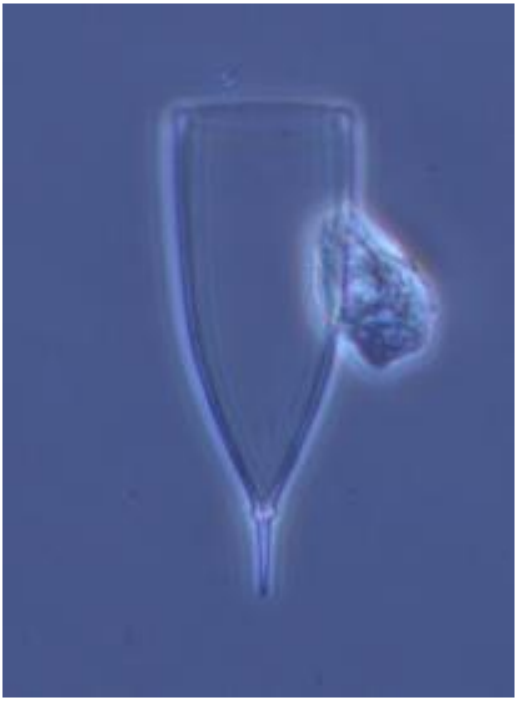

Figure 5. *Xystonellopsis scyphium* Jörgensen, 1924 collected at 150 m depth (TL = 135 µm, L without spine = 111 µm, OD = 46 µm)

One specimen of *Xystonellopsis scyphium* was found at 150 m depth (T = 17.4 ºC, salinity = 39.1 ‰) at the study site (Figure 5). It was originally described by Jörgensen (1924). The lorica has a slight dilated mouth and gradually tends to have converging lateral outlines poculiform. It has a short and broad upper part of caudal prolongation, ending as usual cross-cut, but continues through a strong, single-walled, subula. The wall above the subulate ends double, with - at any rate in the upper half – well separated outer and inner lamellae. At the swollen portion above, it is nearly twice as thick as in the middle and generally less thick below. But, it is slightly thicker just before the upper end of subula (Jörgensen 1924). This specimen had TL = 135 µm, L without spine = 111 µm and OD = 46 µm. This species was found to be in negative correlation with temperature (Njire *et al. 2019*). It was noted in the Gulf of Corinth (Kofoid and Campbell 1929) and Gulf of Mexico in the Caribbean Sea (Balech 1968). It has also been recorded in the deep sea of South Adriatic Sea (between 400 and 1200 m depth) (Krsinic and Grbec 2006) and mesopelagic depth of the Ligure-provinçal Basin (250 m depth) (Dolan *et al. 2019*). Our specimen had approximately the same measurements as the specimen (TL = 133 µm; OD = 46 µm) found in the Gulf of Corinth (Kofoid and Campbell 1929).

Previous studies on the water layer between the surface and 60 m depth showed that the Lebanese coastal waters are rich in terms of tintinnid species, a particularity of tropical and intertropical waters. The presence of few psychrophilic species along with the thermophilic affinity of most species reflected the temperate-warm characteristics of local waters (Abboud-Abi Saab 2008). In terms of the biogeographic distribution, the identified species are neritic, cosmopolitan and oceanic (Dolan and Pierce 2013). Furthermore, Polat *et al.* (2019) found that the diversity of tintinnids was high in the Cilicia Basin and some species can display high abundance. Our results were in agreement with the findings of Dolan *et al.* (2019) who detected a deep-water community of tintinnid ciliates either in the surface layer or restricted and endemic to deep waters. Three out of four records here were also noted in the northwestern Mediterranean (Krsinic and Grbec 2006; Dolan *et al.* 2019). The latter authors found that species from the mesopelagic Mediterranean Sea also exist in the North Atlantic Ocean.

It was suggested that the biogeographical distribution of tintinnids is more homogenous in deep waters, supporting the dynamicity of the species with distinct ecologies and the wide distribution of populations in the deep oceans. This still needs verification in sampling during different seasons. The exploration of deeper water layers allowed to find new records. Nonetheless, the present paper reports these species for the first time in the Lebanese waters. The rare occurrence of these species may be related to the lack of studies due to logistic resources reaching greater depths in the region. As a result, *Bursaopsis bursa*, *Eutintinnus haslae*, *Favellla aciculifera* and *Xystonellopsis scyphium* were added to the regional plankton check-list of the Lebanese waters. More samples of deep waters will provide a better understanding of the state of the deep-sea species.

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