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Invasive alien species of tintinnid ciliates from the northeastern Black Sea, Russian and Abkhazian coast

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Summary

The taxonomic composition and dynamics of numerical density of alien tintinnid species in the bays and harbours of the northeastern Black Sea were studied. The highest number of non-indigenous tintinnid species (9 species) were found in the Novorossiysk Harbour, the lowest number of species (4 species) in the Gelendzhik Bay, and two species – in the harbours of Sochi and Sukhum. In the Novorossiysk Harbour, *Amphorellopsis acuta* Schmidt, 1902 (average density 2.1 ± 1×10^6 cells/m^3), *Dartintinnus alderae* Smith, Song, Gavrilova, Kurilov, Liu, McManus and Santoferrara, 2018 (average density 1.34 ± 0.6×10^6 cells/m^3), and *Tintinnopsis tocantinensis* Kofoid and Campbell, 1929 (average density 0.73 ± 0.31×10^6 cells/m^3) were dominant during the late summer-autumn season of 2012–2017. The summarized abundance of tintinnid ciliates was almost six to eight times higher than in the late 1990s. This study report *Rhizodomus tagatzi* Strelkov and Wirketis, 1950 and *Tintinnopsis mortensenii* Schmidt, 1902 for the first time from the northeastern Black Sea. The origin of alien species populations in the northeastern Black Sea have been linked probably to their introduction by shipping.

Key words: invasive species, tintinnid ciliates, harbours, northeastern Black Sea, Russia, Abkhazia

Introduction

One of the main routes of introduction of alien species is their transport by vessels, especially in ballast waters (Carlton, 1985). Ballast water of commercial ships has long been known to be a vector for the introduction of alien species of tintinnid ciliates (Pierce et al., 1997; Selifonova, 2012, 2018). The tintinnids are neritic organisms and this restricts their distribution for long distance in natural pathways. Ten non-indigenous tintinnid species were registered in the Sevastopol Bay and the adjacent coastal regions of the Crimea during 1997–2014 (Gavrilova and Dolan, 2007; Gavrilova and Dovgal, 2016; Gavrilova, 2017). Some of them were recorded from the northwestern coast of the Black

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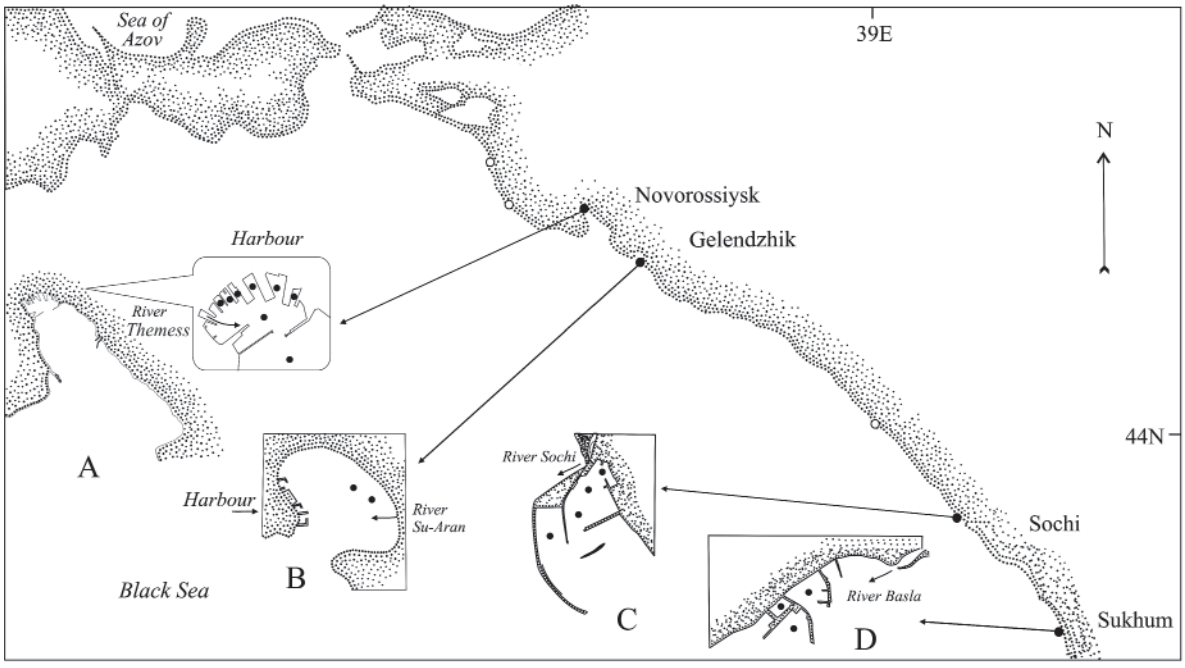

Fig. 1. Location of the stations in bays and harbours of the northeastern Black Sea where invasive alien species of tintinnid ciliates were found: A – Novorossiysk, B – Gelendzhik, C – Sochi, D – Sukhum.

Sea (Kurilov, 2005). The invasive alien species of tintinnid ciliates living along the northeastern coast of the Black Sea – the harbours of Novorossiysk and Tuapse, and the Liman "Zmeinoe ozero" (Bolshoi Utrish) located near the oil terminal – were reported in a few papers (Gavrilova, 2005; Selifonova, 2011, 2012a, 2012b, 2015, 2018). Nowadays, the contribution of alien species to the overall list of the Black Sea tintinnids is considerable and accounts for ca. 33% (Gavrilova and Dovgal, 2016).

The target of this work was to study taxonomic composition and dynamics of numerical density of alien tintinnid species in bays and harbours of the northeastern shelf of the Black Sea, including the Abkhazian coast.

Material and methods

Sampling was conducted in the bays and harbours of the northeastern Black Sea, viz., Novorossiysk (August-November 2012–2017), Sochi, Sukhum and Gelendzhik (August–September 2016–2017). The samples collected at the sampling stations (Fig. 1) during late summer - autumn have been analyzed within the period of mass occurrence of the invasive species in the plankton. Temperature and salinity of seawater (argentometric method) were determined by PhD V.K. Chasovnikov (Chemistry Laboratory of the Southern Branch of Shirshov Institute of Oceanology, Russian Academy of Sciences).

Plankton samples for the qualitative analysis were collected by towing a 40-μm mesh plankton net within the top half-meter of water. The tintinnid ciliates for the quantitative analysis were collected from the surface by a plastic bucket. The 1500 ml samples were preserved with glutaraldehyde solution (2% final concentration) and concentrated using sedimentation to 10–20 ml subsamples. Alien tintinnid species we observed with an inverted microscope in settling chambers. For determination of abundance, the aloricated ciliates were studied alive with stereomicroscope before preservation using Sorokin's counting chamber (Sorokin, 1999).

Results

In the Novorossiysk Harbour, 9 alien species of tintinnid ciliates belonging to 6 genera: *Tintinnopsis mortensenii* Schmidt, 1902, *T. tocantinensis* Kofoid and Campbell, 1929, *Rhizodomus tagatzi* Strelkov and Wirketis, 1950, *Amphorellopsis acuta* Schmidt, 1902, *Eutintinnus lususundae* Entz., 1885, *E. tubulosus* Ostenfeld, 1899, *E. apertus* Kofoid and

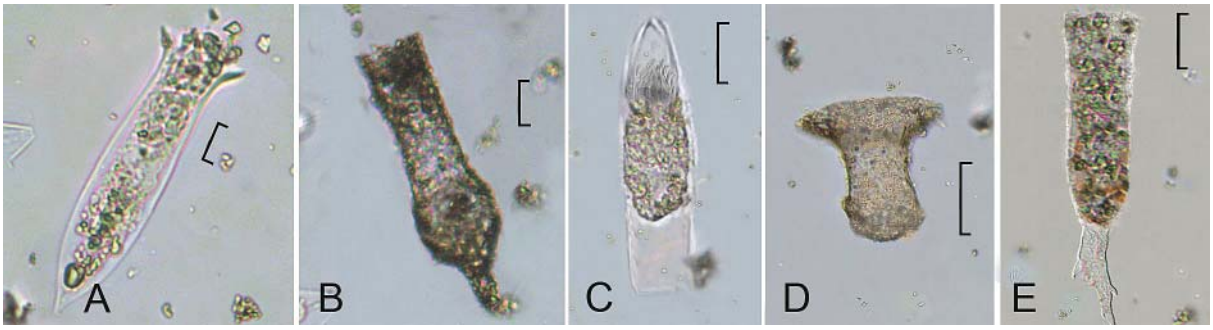

Fig. 2. Dominant invasive tintinnid species from the Novorossiysk Harbour (late summer-autumn): A – *Amphorellopsis acuta*, B – *Tintinnopsis tocantinensis*, C – *Dartintinnus alderae*, D – *Tintinnopsis mortensenii*, E – *Rhizodomus tagatzi*, first record from the northeastern Black Sea. Scale bars: A, B – 20 μm, D, E – 30 μm, C – 10 μm.

Campbell, 1929, *Dartintinnus alderae* Smith, Song, Gavrilova, Kurilov, Liu, McManus and Santoferrara, 2018, *Salpingella decurlata* Jörgensen, 1924, were identified during the period of time from 2012 to 2017 (Table 1).

One of those, *R. tagatzi*, was recorded in the Novorossiysk Harbour for the first time during late summer-autumn 2015 (Fig. 2). *R. tagatzi* reached the maximal abundance of 0.01×10^6 cells/m^3 in October 2015 and 0.05×10^6 cells/m^3 – in August-September 2016.

T. mortenseni were found for the first time at station (44°43.55′N, 37°47.1′E; salinity 16.1‰) located near the estuary of the River Tsemess in August 2017. Non-indigenous tintinnids distribution was restricted to the sites of ballast water dum-ping (port area), confirming the allochtonous origin of the species. The most abundant populations throughout the whole sampling period in the Novorossiysk Harbour were registered for the species *A. acuta* (average density $2.1 \pm 1\times10^6$ cells/m^3), *D. alderae* (average density $1.34 \pm 0.6\times10^6$ cells/m^3), *T. tocantinensis* (average density $0.73 \pm 0.31 \times10^6$ cells/m^3). The maximal density of *A. acuta* ($9.3 – 13.5\times10^6$ cells/m^3) was recorded in September 2015–2016, *D. alderae* ($4.7–7.9\times10^6$ cells/m^3) – in August 2016–2017, *T. tocantinensis* (4.4×10^6 cells/m^3) – in October 2012 (Fig. 3). Other tintinnid species were less abundant. For example, *E. tubulosus* reached maximal density of $0.09 – 0.2\times10^6$ cells/m^3 in August-September 2012–2014; *E. apertus* – 0.005×10^6 cells/m^3 in August 2014; *Salpingella decurlata* – 0.02×10^6 cells/m^3 in October-November 2015–2016. Tintinnids *E. lusundae* and *T. mortensenii* were rare species; only a few specimens of these species were noted. A total number of the invasive tintinnids varied from 0.4 to 14.4×10^6 cells/m^3, averaged $5.4 \pm 4.4\times10^6$ cells/m^3. In the Novorossiysk Harbour, during late summer – autumn the summarized abundance of alien tintinnid ciliates constituted up to 30–40% of the total ciliates abundance, including the aloricated forms (Fig. 3).

Table 1. List of invasive alien species of tintinnid ciliates identified in the northeastern Black Sea.

Species	N	G	So	Su
Tintinnopsis mortensenii Schmidt, 1902	+			
Tintinnopsis tocantinensis Kofoid and Campbell, 1929	+			
Rhizodomus tagatzi Strelkov and Wirketis, 1950	+	+		
Amphorellopsis acuta Schmidt, 1902	+	+		
Eutintinnus lusundae Entz., 1885	+			
E. tubulosus Ostenfeld, 189	+	+	+	+
E. apertus Kofoid and Campbell, 1929	+	+	+	+
Dartintinnus alderae Smith et al., 2018	+			
Salpingella decurlata Jörgensen, 1924	+			

Notes. Novorossiysk Harbour, G – Gelendzhik Bay, So – Sochi Harbour, Sukhum Harbour.

An examination of plankton samples collected from the inshore waters in the other regions of the northeastern Black Sea revealed the presence of a less diverse community of non-indigenous tintinnids. For the first time two alien species of tintinnids belonging to 1 genera (*E. tubulosus* and *E. apertus*) were observed from the harbours of Sochi and Sukhum; 4 alien species belonging to 3 genera (*E. tubulosus, E. apertus, R. tagatzi, A. acuta*) – from the Gelendzhik Bay. Generally, numerical density of each species in August–September 2016–2017 (temperature of water 26–28 °C) did not exceed

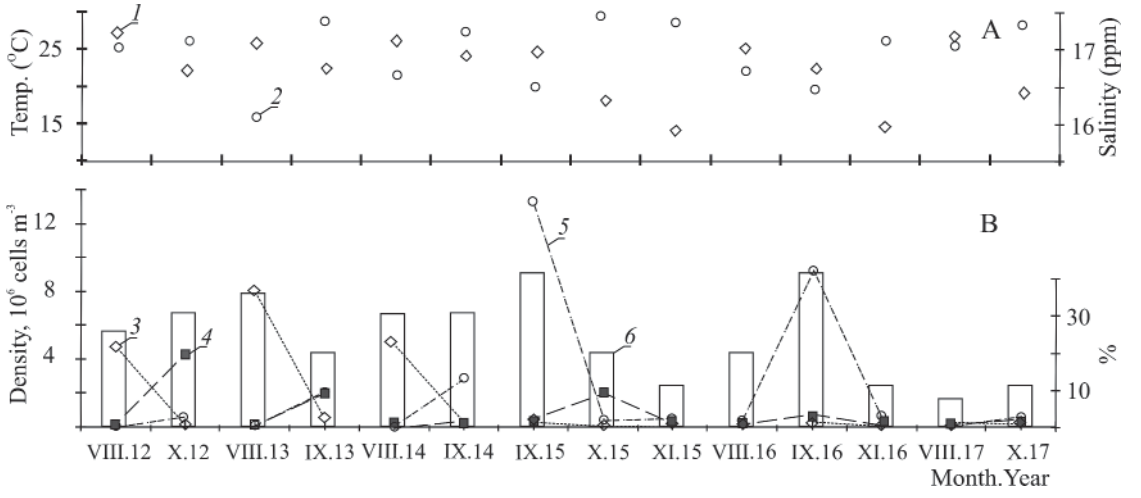

Fig. 3. Dynamics of temperature fluctuations and salinity (A); density of dominant invasive species of tintinnids ($\times 10^6$ cells/m^3) and relative content (%) of invasive species of tintinnids and total ciliates number (B), from the surface water layer of the Novorossiysk Harbour: 1 – temperature, 2 – salinity, 3 – *Dartintinnus alderae*, 4 – *Tintinnopsis tocantinensis*, 5 – *Amphorellopsis acuta*, 6 – percentage of the total density of ciliates.

0.001–0.05×10^6 cells/m^3. They accounted for ca. 15% of total ciliates abundance in the bays and harbours of Gelendzhik and Sochi and 5% – in the Suhkum Bay.

Among the alien tintinnid species of the north-eastern Black Sea, there were recorded the established species – *A. acuta, T. tocantinensis, D. alderae, E. tubulosus, E. apertus, S. decurlata*; cryptogenic species – *R. tagatzi*; and casual species – *E. lusu-sundae* and T. *mortensenii*. The discovery of *E. lususundae* and *T. mortensenii* in the Novorossiysk Harbour could be considered as an accidental finding since only a few specimens were encountered in the areas the of ships' ballast water discharge. Status of non-native species is defined according to Zenetos et al. (2005).

Discussion

Among 20 species of native tintinnid ciliates known for the Black Sea, 18 non-indigenous species belonging to 13 genera: *Codonella aspera* Kofoid and Campbell, 1929, *Tintinnopsis mortensenii* Schmidt, 1902, *Tintinnosis nucula* (Fol., 1884), *Tintinnopsis radix* (Imhof, 1886), T*intinnopsis tocantinensis* Kofoid and Campbell, 1929, *Codenellopsis morchella* (Cleve) Jörgensen, 1924, *Cyttarocylis ampula* Bachy et al., 2012, *Dictyocysta mitra* Haeckel, 1873, *Favella azorica* (Cleve, 1900) Jörgensen, 1924, *Schmidingerella serrata* (Möbius, 1887) Agatha and Strüder-Kypke, 2012, *Rhizodomus tagatzi* Strelkov and Wirketis, 1950, *Amphorellopsis acuta* Schmidt, 1902, *Eutintinnus lusundae* Entz., 1885, *E. tubulosus* Ostenfeld, 1899, *E. apertus* Kofoid and Campbell, 1929, *Dartintinnus alderae* Smith et al., 2018, *Salpingella decurlata* Jörgensen, 1924 and *Xystohella lohmanni* (Brandt, 1906) Brandt, 1906 (Table 2) were registered in the Black Sea over the past few years (Kurilov, 2005, 2007, 2011; Balkis, Koray, 2014; Gavrilova, Dolan, 2007; Gavrilova, Dovgal, 2017; Selifonova, 2011, 2012a, and others). Among those, the species which was identified as *Nolaclusilis* sp. in the Black Sea (Gavrilova, Dolan, 2007; Gavrilova, Dovgal, 2016) is a new species *D. alderae* with dual-ended lorica collapsibility (Smith et al., 2018).

In the areas of intensive navigation of the north-eastern shelf of the Black Sea, Russian and Abkhazian coast, 9 alien species of tintinnid ciliates were established during the period of our investigations (2012–2017). The coastal waters of the Novorossiysk Harbour host very reach fauna of alien tintinnid ciliates since maximal number of those species were found in this ecosystem during a period of the recent 10–15 years. The Novorossiysk Harbour is the largest transport junction in the south of Russia. Its annual capacity exceeds 5 thousand vessels and 100 million tons of cargo (80% – transportation of oil and oil products). During the handling operations, more than 60 million tons of ballast water is discharged into the Novorossyisk Bay. Live individuals of *E. lusundae, Eutintinnus* spp.

Table 2. Invasive species of marine tintinnid ciliates reported from the Black Sea.

Genus	Species	Region
Codonella	*Codonella aspera* Kofoid & Campbell, 1929	SW, Turkish
Tintinnopsis	*Tintinnopsis mortensenii* Schmidt, 1902	Crimean, NE
	Tintinnosis nucula (Fol., 1884)	Turkish
	Tintinnopsis radix (Imhof, 1886)	Turkish
	Tintinnopsis tocantinensis Kofoid & Campbell, 1929	Crimean, NE
Codonellopsis	*Codenellopsis morchella* (Cleve) Jörgensen, 1924	SW, Turkish
Cyttarocylis	*Cyttarocylis ampula* Bachy et al., 2012 *(=Petalotricha ampulla* (Fol, 1881) Kent, 1882)	SE, Turkish
Dictyocysta	*Dictyocysta mitra* Haeckel, 1873	SW, Turkish
Favella	*Favella azorica* (Cleve, 1900) Jörgensen, 1924	SW, Turkish
Schmidingerella	*Schmidingerella serrata* (Möbius, 1887) Agatha & Strüder-Kypke, 2012	SE, Turkish
Rhizodomus	*Rhizodomus tagatzi* Strelkov & Wirketis, 1950 (=*Tintinnopsis corniger* Hada, 1964*)*	Crimean, NE
Amphorellopsis	*Amphorellopsis acuta* Schmidt, 1902	Crimean, NE
Eutintinnus	*Eutintinnus lususundae Entz.,* 1885	SW, NW, NE, Crimean
	E. tubulosus Ostenfeld, 1899	NW, NE, Crimean
	E. apertus Kofoid & Campbell, 1929	NW, NE, Crimean
Dartintinnus	*Dartintinnus alderae* Smith et al., 2018	NW, NE, Crimean
Salpingella	*Salpingella decurlata* Jörgensen, 1924	Crimean, NE
Xystohella	*Xystohella lohmanni* (Brandt, 1906) Brandt, 1906	Turkish

Notes. NW – north-west shelf of the Black Sea; NE – north-east, SW – south-west; SE – south-east.

were found in 2006–2009 in ballast waters of the tankers, which arrived in Novorossiysk to take cargo of petroleum products (Selifonova, 2009; 2018). Their abundance reached 0.05–0.7×10^6 cells/m^3. All organisms had good integrity, most likely being preserved alive. In "live" samples of ballast waters, high number of planktonic aloricate infusorians belonging to the Strombidiidae and Mesodiniidae family were recorded – 17.6–116×10^6 cells/m^3. The first abundance outbreak of alien tintinnid species was registered in the Novorossiysk Harbour in August and September of 2001 (Gavrilova, 2005); those were *E. lususundae, E. tubulosus, E. apertus* and *Eutintinnus* sp. (Gavrilova, 2005). The finding of *S. decurtata* in this region dated back to 2002, *A. acuta* and *T. tocantinensis* – 2010 (Selifonova, 2011, 2012a), *D. alderae* – 2012, *R. tagatzi* – 2015, *T. mortensenii* – 2017. Since then, those species have been regularly observed in the samples during late summer and autumn: some of them were represented by single specimens; others have reached high population density. Non-indigenous tintinnid species, among them *A. acuta, D. alderae, T. tocantinensis*, were the key components among tintinnids. Native tintinnid ciliates *Tintinnopsis tubulosa* Levander, 1900, *T.minuta* Wailes, 1925, *T. campanula* Ehrenberg, 1840 and *Favella ehrenbergii* (Claparede and Lachmann, 1858) Jorgensen, 1924 were found in the offshore regions of the Black Sea (Crimea and Caucasus) in low number (Gavrilova, Dovgal, 2016, Selifonova, 2018). In the Novorossiysk Harbour, the summarized abundance of alien tintinnid ciliates was almost six to eight times higher than in the late 1990s (Selifonova, 2018).

Comparison of the tintinnid fauna in the Novorossiysk Harbour with that of the adjacent areas indicates that the waters of small bays and harbours are poorer and less diverse. The fewest number of species was found in Tuapse – *E. tubulosus* and *T. tocantinensis* (Selifonova, 2012), the Liman «Zmeinoe Ozero» (Bolshoi Utrish) – *T. tocantinensis, E. tubulosus, A. acuta, S. decurlata* (Selifonova, 2015), Sochi and Sukhum – *E. tubulosus* and *E. apertus*, Gelendzhik – *E. tubulosus, E. apertus, R. tagatzi, A. acuta*. However, the abundance of some alien species of tintinnids in the Liman "Zmeinoe Ozero" can be compared to that in the Novorossiysk Harbour. Here, maximal density of *T. tocantinensis* reached 2.1 million cells/m^3, *A. acuta* – 0.9×10^6 cells/m^3, *E. tubulosus* – 0.4×10^6 cells/m^3, *S. decurlata* – 0.4×10^6 cells/m^3. The Liman "Zmeinoe Ozero" is located near the oil terminal "KTK", where plenty of isolated ballast waters are dumped.

To conclude, this work summarizes our current knowledge about the diversity of the invasive tintinnid ciliate species in the northeastern Black

Sea (Russian and Abkhazian coast) and provides a reference database for the future studies. If we consider that the nine non-indigenous species reported in the studied harbours and bays of the northeastern Black Sea were carried to these regions by theballast water tanks of vessels, then even more alien tintinnids could be expected there in the forthcoming years. This finding points to the need for the further comprehensive studies to be conducted in the seaports and bays since they act as acclimatization areas for the ciliate and other invasive species.

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