Taxonomic composition and distribution of soft-walled monothalamid foraminifera in the area of Zernov's Phyllophora Field (NW Black Sea)

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Running head: Black Sea monothalamids

Abstract

We studied assemblages of soft-walled, single-chambered foraminifera (monothalamids) at eight stations in the area of Zernov's Phyllophora Field **(ZPF)** on the NW continental shelf of the Black Sea. This work is based on samples collected during Cruise 70 of the RV *Prof. Vodyanitsky* and provides the first analysis of the taxonomic composition of monothalamids from this region. These delicate, poorly-known foraminifera were found at all stations in the studied area. They were represented by 9 forms that are identified to species or genus level as well as 14 undescribed species, 9 of which are found in the Black Sea for the first time. Four species, *Psammophaga* sp., *Vellaria pellucidus*, *Goodayia rostellatum* and *Krymia fusiformis*, were most abundant, with *Psammophaga* sp. being the dominant species overall. Previous studies have suggested a link between members of this genus and eutrophication. There are a number of taxonomic parallels between these ZPF assemblages and those from the Adriatic Sea, e.g. the occurrence of the genera *Goodayia, Psammophaga* and *Vellaria,* although it is possible that some other taxa are endemic to the Black Sea.

Keywords: Benthic foraminifera, allogromiids, diversity, *Psammophaga, Phyllophora*

Introduction

Single-chambered foraminifera (monothalamids), many of them with delicate, soft-walled tests, are an important, diverse, but often overlooked component of the meiofauna in marine settings ranging from coastal waters to extreme oceans depths (e.g., Gooday 2002; Pawlowski et al. 2002; Todo et al. 2005; Majewski et al., 2007; Habura et al., 2008; Sabbatini et al., 2013). They represent a diverse, heterogeneous and paraphyletic grouping of forms for which there is currently no satisfactory classification system (Pawlowski et al. 2013). In particular, it is clear from molecular data that the two existing orders, the Allogromiida (for organic-walled species) and the Astrorhiziida (for agglutinated species), are artificial taxa (Pawlowski et al. 2003, 2013).

The study of these protists in the Black Sea was initiated during the last century (Sergeeva & Kolesnikova 1996; Golemansky 1999; see Temelkov et al., 2006 for brief review of earlier records from the Bulgarian Black Sea coast). The first publications were devoted to the distribution of monothalamids in the fully oxic coastal waters of the Crimean Peninsula (Anikeeva & Sergeeva 2001; Anikeeva 2003). Later, Sergeeva & Anikeeva (2006) Sergeeva et al. (2010), Sergeeva et al. (2011) investigated the taxonomic composition and distribution of monothalamids in deeper areas of the Black Sea, including those subject to hypoxic conditions, while Sergeeva & Anikeeva (2014) studied monothalamids from normoxic and hypoxic settings in shallow water. Sergeeva & Anikeeva (2004) and Anikeeva (2005) provided the first records of some taxa for this basin and species that were new to science were described by Gooday et al. (2006), Sergeeva & Anikeeva (2008), Gooday et al. (2011) and Anikeeva et al. (2013). A separate paper (Sergeeva & Mazlumyan 2006) dealt with the metazoan meiofauna from the ZPF.

Zernov's Phyllophora Field (ZPF) is an area of the NW Black Sea shelf (20-60 m water depth) that is characterized by high densities of rhodophyte algae. Since the 1960s the ZPF has shrunk dramatically in extent (Zaitsev & Mamaev 1997), leading to its designation as a Marine Protected Area in 2008 (Kostylev et al. 2010) The abundance and distribution of benthic foraminifera, including soft-shelled monothalamids, within the ZPF was analysed by Sergeeva et al. (reviewed in Sergeeva et al., 2015), based on a series of cruises of the RV '*Professor Vodyanitsky*'. These authors concluded that monothalamids were substantially more abundant than hard-shelled multichambered species. However, abundances were depressed in the ZPF compared with those in the eastern part of Karkinitsky Gulf (north-western Crimean Peninsula), where a second smaller and shallower (8-10 m water depth) *Phyllophora* field is situated (Zaitzev & Mamaev 1997). The main objective of the present contribution is to present the first account of the taxonomic composition of soft-shelled monothalamids in this important part of the Black Sea shelf.

Material & Methods

SAMPLE COLLECTION AND PROCESSING

Samples of bottom sediments were collected using the bottom grab "Okean-25" during the 70th cruise of the RV *Professor Vodyanitsky* (August 2011) at 8 stations (Fig. 1, Table 1) in the area of Zernov Phyllophora field. Subsamples for the study of meiobenthos were taken with a perspex tube (cross-sectional area 18.1 cm^2) and the entire upper 5 cm of sediment preserved in 75% alcohol. This preservative is not ideal for soft-shelled foraminifera, but was required for studies of other meiofaunal taxa (nematodes and harpacticoid copepods) that were carried out on the same samples (Revkova in prep.)

In the laboratory each sediment subsample was washed through an upper sieve with a mesh size of 1 mm and a lower sieve with a mesh size of 64 µm, close to the 63-µm lower size limit commonly used in studies of small foraminifera (Phipps et al., 2012), including soft-shelled monothalamids (Gooday 1986). The residues were stained on the sieve using Rose Bengal and analyzed under a binocular microscope in a Bogorov chamber. This device, designed for analysing zooplankton, improves the accuracy with which meiobenthic samples can be sorted. The foraminifera were picked out from the residues using a glass pipette and placed in cavity slides with a mixture of glycerol (50 %) and water (50 %). Specimens were examined in detail using a Mikmed-6 compound microscope and photographs taken with a Canon A620 digital camera.

STUDY AREA

The Zernov Phyllophora Field (ZPF) is situated on the continental shelf in the north-western part of the Black Sea. It is characterised by a unique assemblage of unattached algae dominated by members of the keystone genus *Phyllophora* (Kalugina-Gutnik 1975; Morozova-Vodyanitskaya 1948; Shchapova 1953). The region was named in honor of the famous Russian scientist S. A. Zernov, who discovered this remarkable ecosystem covering almost half of the area to the west of the Crimean Peninsula in 1908 (Zernov 1909). The algal field hosts a rich assemblage of associated marine organisms, including about 47 species of fish and 118 species of invertebrates (Zaitzev & Mamaev 1997). These populations extend out from the ZPF and supplement assemblages in other areas of the Black Sea, while photosynthesis by the phytoplankton and phytobenthos of the ZPF generates huge quantities of oxygen that enrich the sea water and atmosphere (Zaitzev & Mamaev 1997; Kostylev et al. 2010).

More than a century ago, the area of the ZPF reached 11,000 km^2 at depths between 20 and 60 m (Kalugina-Gutnik, Lachko 1966, 1968; Kaminer 1981; Zaitzev & Mamaev 1997). However, during the latter part of the 20th Century, particularly during the period from 1970 to

1980, the ZPF suffered considerable degradation as a result of anthropogenic eutrophication and associated oxygen depletion, leading to a dramatic decline in stocks of *Phyllophora* off the coast of Ukraine and other Black Sea regions (Zaitsev & Mamaev 1997). By the early 1990s, the area had shrunk to 500 km^2 and the biomass had declined from an estimated 7-10 million tons in the 1950s to 300,000 to 500,00 tons (Zaitsev 1992), although the ecosystem has subsequently recovered somewhat (Zaika et al. 2004; Kostylev et al. 2010; Sergeeva et al. 2015). As a response to the catastrophic reduction of *Phyllophora* resources and the degradation of macrophytobenthos in this part of the Black Sea, the "Zernov Phyllophora Field" Marine Protected Area (botanical reserve) was created in 2008 (Kostylev et al. 2010). Regular monitoring of the state of the ZPF in order to promote the restoration of its biodiversity is one goal of the establishment of this marine reserve. The study of deep-water habitats in the north-western Black Sea, including the ZPF, by scientists at the Institute of Marine Biological Research (former Institute of the Biology of the Southern Seas) in Sevastopol was suspended in 1991 due to the cessation of marine research expeditions. The return of the RV *Professor Vodyanitsky* to marine science in 2010 was connected with this monitoring effort.

The habitat (sediment) characteristics at the stations sampled during Cruise 70 of the R/V *Professor Vodyanitsky* are noted in Table 1 and available information on the physical and chemical characteristics of the bottom water summarized in Table 2; unfortunately, data are not available for all the sampling stations.

Results

Physical and chemical data are available for 5 of the 8 sites sampled (Table 2). Salinity (16.57 - 17.96) and pH (8.05 - 8.24) were relatively stable. Both bottom temperatures and dissolved oxygen, on the other hand, varied considerably, from 8°C (Stn 18) to 20°C (Stn 20) and 3.66 ml/L (Stn 20) to 6.14 ml/L (Stn 18), respectively.

Soft-walled monothalamous foraminifera occured at all the stations in the study area. They were represented by a total of 23 morphospecies, 8 of which were identified to species and one to genus level (Table 3). The other 14 species (9 'allogromiids' with organic-walled tests and 5 'saccamminids' with agglutinated tests) are undescribed. Nine of these undescribed morphospecies (Fig. 2) were previously unknown and are recorded here for the first time from the Black Sea. The species richness of monothalamids was maximal at Stn 20, where 15 species were recorded, followed by Stn 19 with 11 species (Table 3). The other 6 stations yielded between 2 and 5 species. Multichambered calcareous and agglutinated species were represented by occasional specimens (e.g. *Ammonia* and *Eggerella* spp.), but not included in the analyses.

Overall, the most abundant species in the area of the ZPF were *Psammophaga* sp., *Vellaria pellucidus*, *Goodayia rostellatum* and *Krymia fusiformis* (Fig. 3). The highest densities (>27,000 ind./m^2 at Stn 26) were observed in the case of *Psammophaga* sp, although the abundance of this species varied considerably between stations, with an average value of ~10,000 ind./m^2. Maximum densities were 13,000 ind./m^2 at Stn 19 (average 4,500 ind./m^2) for *G. rostellatum* and 11,500 ind./m^2 at Stn 19 (average 4,500 ind./m^2) for *V. pellucidus.* These three species occured at 6 or 7 of the 8 sampled stations. *Krymia fusiformis* was less abundant, occuring at densities of <3,000 ind./m^2 at three stations. The remaining species were found at only 1-2 stations (Table 3). In most cases they were represented by <1,900 ind./m^2. However, at Stn 19, four species (Allogromiid spp. E, G, 30, 33) were reached densities between 7180 and 9940 ind./m^2 (Table 4).

Discussion

Our new observations add to existing evidence (reviewed by Sergeeva et al. 2015) that numerous species of soft-walled monothalamid foraminifera live in the shallower waters of the Black Sea. Monothalamids appear to be as abundant and diverse in this relatively isolated basin as they are in other coastal and shelf settings (e.g. Habura et al. 2008). Indeed, at the ZPF sites that we studied, these delicate single-chambered forms were considerably more abundant than the more familiar hard-shelled multichambered taxa, which were represented only by occasional specimens. Similarly, Sergeeva and colleagues reported that hard-shelled multichambered foraminiferal species were almost 12 times more abundant than monothalamous species in samples collected from the *Phyllophora* field during Cruise 64 of the RV *'Professor Vodyanitsky'* (July 2010) and 'encountered only once at several stations' in the ZPF during Cruise 68 (November 2010) (Sergeeva et al. 2015). The abundance of monothalamids, and particularly that of multichambered foraminifera, is higher in the small *Phyllophora* field (Sergeeva et al. 2015), located in the eastern part of Karkinitsky Gulf (Zaika & Mamaev 1997), The limited available environmental data shed little light on the factors controlling the distribution and diversity of monothalamids within the ZPF. It is notable, however, that the most diverse site (Stn 20), which hosts 15 species, is also characterized by a higher temperature and a lower dissolved oxygen concentration than other sites for which environmental data are available (Table 2). In other parts of the Black Sea, monothalamids appear to be more tolerant of hypoxia than calcareous taxa. In particular, they exhibit a peak in abundance in samples taken close to the upper limit of sulphidic waters, where they outnumber the calcareous foraminifera (Sergeeva et al. 2010, 2015).

The 'saccamminid' *Psammophaga* sp. is the dominant species in our ZPF material (Fig. 3). *Psammophaga* is one of the most extensively studied of the monothalamid genera and is reported from localities around the world (e.g. Pawlowski & Majewski 2011; Altin-Ballero et al. 2013). There is evidence for a link between members of this genus and eutrophic conditions. Sabbatini et al. (2012) observed large populations of their *Psammophaga* sp. 1 associated with high-quality organic matter derived from a phytoplankton bloom in the central Adriatic. They suggest that *Psammophaga* species could be used as indicators of eutrophication in coastal and shelf settings. This genus is also very common in sediments impacted by organic matter derived from fish farms (Pawlowski et al. 2014). It is possible, therefore, that the abundance of *Psammophaga* sp. in the area of the ZPF is linked to eutrophication. However, this species is notably absent from Stn 20 (Table 3), which has relatively low dissolved oxygen values and therefore is likely to be the most organically enriched site (Table 2). More environmental data from the ZPF, including over seasonal cycles, would help to clarify the ecology of this important monothalamid.

The wider distributions of species reported here from the Black Sea are unclear. Probably the most relevant comparison is with monothalamid species occurring at similar water depths (<50 m) in the northern part of the Adriatic Sea (Sabbatini et al. 2010, 2013). Species of *Psammophaga* are common in both areas. Molecular data indicate that *Psammophaga* from Kazach'ya Bay (Crimea) is closely related to a single *Psammophaga* sequence from near Southampton (UK) (Gooday et al. 2011) and branches as a sister group to a species from Sapelo Island, Georgia (USA), subsequently described as *P. sapela* Altin-Ballero, **Habura**, Goldstein 2013. Whether the ZPF form represents the same phylotype as the Kazach'ya Bay *Psammophaga*, and it's relationship to the Adriatic species of this genus, must await future molecular analysis.

A particularly interesting parallel between monothalamids from the Black Sea (including the ZPF) and Adriatic monothalamids is the occurrence in both areas of the recently described genus *Goodayia*. The Adriatic species illustrated by Sabbatini et al. (2010, Pl.1, fig.3) and Sabbatini et al. (201**3**, Pl.3, figs 8-10) is similar morphologically to *G. rostellatum*, as illustrated by Sergeeva & Anikeeva (2008, Fig.1) and Sergeeva et al. (2015, Fig.2). Species of *Vellaria* also occur in both basins and *Bathyallogromia* is a widely distributed genus that was first described from the Weddell Sea (Gooday et al. 2004; Cornelius & Gooday 2004) and is reported from various parts of the Black Sea (Sergeeva et al. 2015). Allogromia sp. 32 (Fig. 4C) resembles *Bowseria* sp. of Sabbatini et al. (201**3**, Pl.3, fig.1) and possibly represents the same species, although this cannot be decided based on photographic evidence. On the other hand, some species could be endemic to the Black Sea. These include *Bellarium rotundus*, *Krymia* *fusiformis*, and *Tinogullmia lukyanovae*, all of which were described from this basin (Gooday et al. 2006; Anikeeva et al. 2013). They occur fairly widely in the Black Sea but are currently unknown elsewhere. Conversely, the well-known monothalamid genus *Micrometula*, which is widely distributed in many parts of the world (Pawlowski & Holzmann 2008) including in the Adriatic (Sabbatini et al. 2013), has yet to be reported from the Black Sea.

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Table 1. Locations and sediment characteristics of sites sampled during the 70th cruise of R/V "Professor Vodyanitsky".

Station	Depth (m)	Coordinates		Sediment
		°N	°E	
18	47	45.506	31.401	Dense silt with many mollusc shells
19	38	45.507	30.716	Silt with dead mollusc shells
20	24	45.621	30.629	Silty sand with mollusc shells
21	34	45.624	30.837	Silty, mollusc shells
23	37	45.619	31.055	Large mollusc shells
24	33	46.052	31.222	Silty, dead mollusc shells
26	20	46.058	31.538	Silty, mollusc shells
27	44	45.619	31.636	Silt with dead mollusc shells; H_2S smell

Table 2. Some physical and chemical attributes of the bottom water at the sampling stations (ND = no data)

Station	T (°C)	S ‰	O_2 (ml/L)	pH
18	8	17.92	6.14	8.18
19	ND	ND	ND	ND
20	20	16.57	3.66	8.24
21	ND	ND	ND	ND
23	ND	ND	ND	ND
24	8.5	17.88	5.69	8.13
26	12	17.53	4.48	8.05
27	8.7	17.96	5.92	8.20

Table 3. The distribution of soft-walled foraminiferal morphospecies at the sampling stations. The names 'Allogromiid' and 'Saccamminid' are used in an informal sense.

Species	**Station**							
	18	**19**	**20**	**21**	**23**	**24**	**26**	**27**
Bellarium rotundus			x					
Ovammina opaca			x				x	
Tinogullmia lukyanovae			x			x		
**Psammophaga* sp.	x	x		x	x	x	x	x
Krymia fusiformis		x	x	x				
Goodayia rostellatum	x	x	x	x	x	x		x
Vellaria pellucidus		x	x	x	x	x	x	
Vellaria sacculus			x				x	
Bathyallogromia sp. 2		x					x	
Allogromiid sp. 1						x		
Allogromiid sp. 5			x					
**Allogromiid sp. E		x						
***Allogromiid sp. G		x						
Allogromiid sp. S		x						
Allogromiid sp. 30		x	x					
Allogromiid sp. 31			x					
Allogromiid sp. 32			x					
Allogromiid sp. 33		x	x					
Saccamminid sp. 5			x					
Saccamminid sp. 20			x					
Saccamminid sp. 21			x					
Saccamminid sp. 22								x
Saccamminid sp. 27		x						
Total number of species	**2**	**11**	**15**	**4**	**3**	**5**	**5**	**3**

* Formerly known as *Psammophaga simplora* in Black Sea literature
**Formerly known as Allogromiidae gen. sp. E
***Formerly known as Allogromiidae gen. sp. G

Table 4. Abundance of undescribed monothalamid species the sampling stations. The names 'Allogromiid' and 'Saccamminid' are used in an informal sense.

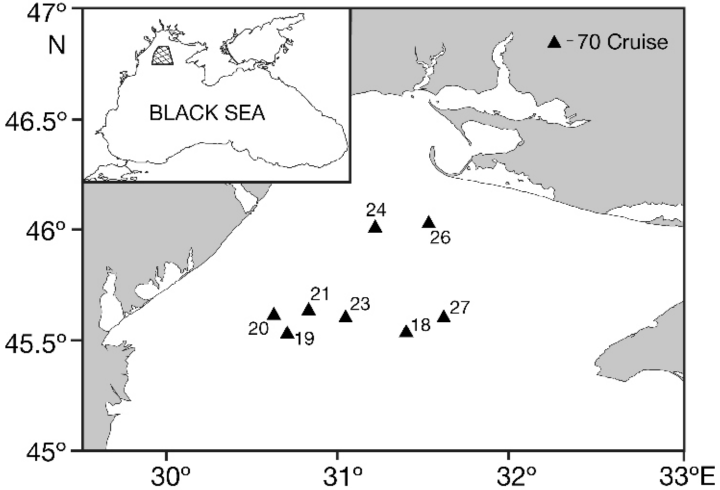
Morphotypes	st. 19	st. 20	st. 24	st. 27
Allogromiid sp. 1	0	0	550	0
Allogromiid sp. 5	0	1100	0	0
Allogromiid sp. E	9940	0	0	0
Allogromiid sp. G	7180	0	0	0
Allogromiid sp. S	550	0	0	0
Allogromiid sp. 30	9940	1100	0	0
Allogromiid sp. 31	0	1300	0	0
Allogromiid sp. 32	0	1650	0	0
Allogromiid sp. 33	7730	1650	0	0
Saccamminid sp.5	0	1100	0	0
Saccamminid sp.20	0	1840	0	0
Saccamminid sp.21	0	1650	0	0
Saccamminid sp. 22	0	0	0	550
Saccamminid sp.27	1100	0	0	0

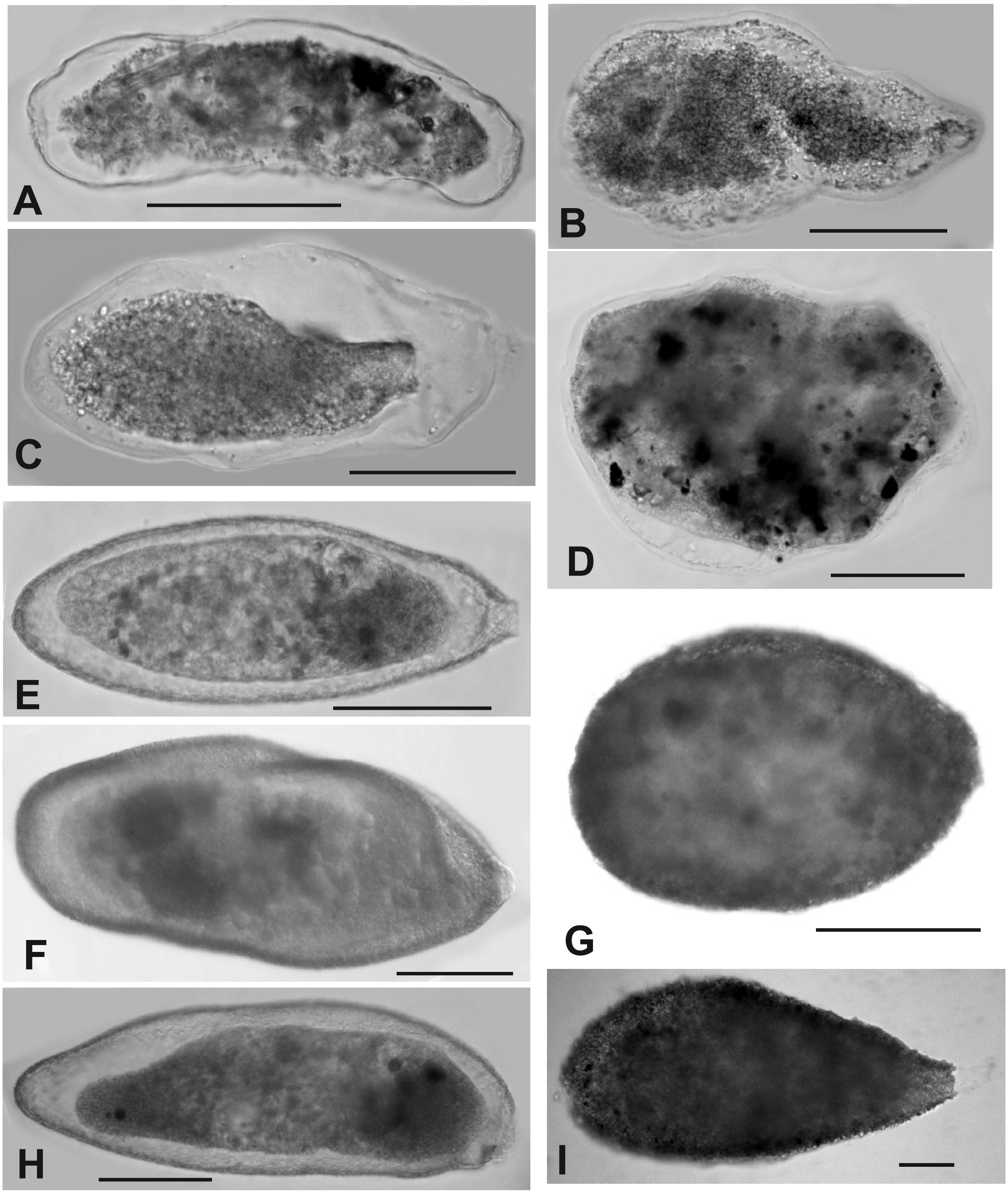
Figure captions

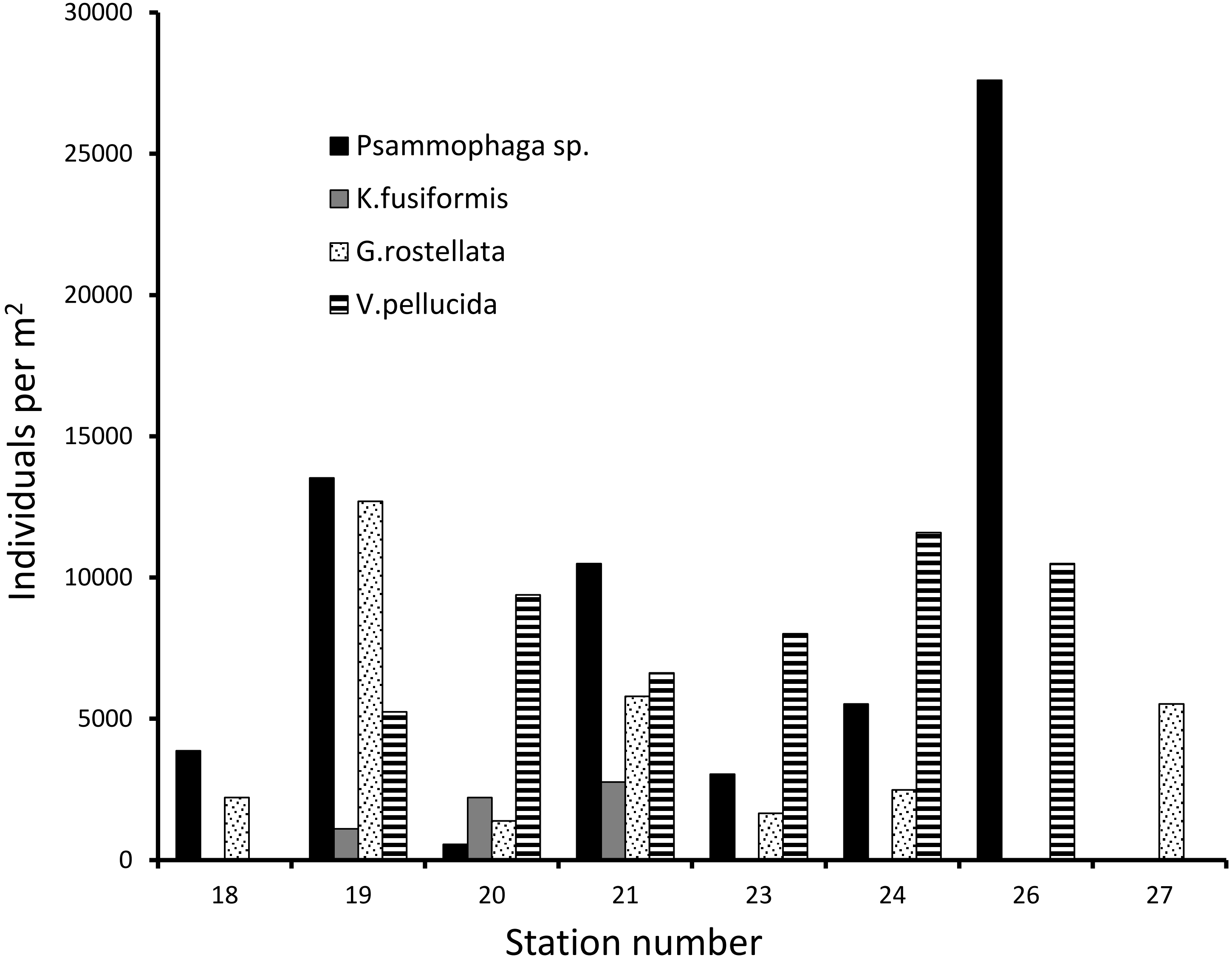
Fig. 1. Stations sampled in the Zernov's Phyllophora Field during Cruise 70 of the RV *Prof. Vodyanitsky*.

Fig. 2. Previously unknown and undescribed species that have been recognised for the first time in Zernov's Phyllophora Field. A) Allogromiid sp. 30. B) Allogromiid sp. 31. C) Allogromiid sp. 32. D) Allogromiid sp. 33. E) Saccamminid sp. 22. F) Saccamminid sp. 20. G) Saccamminid sp. 27. H) Saccamminid sp. 5. I) Saccamminid sp. 21. Scale bars = 100 µm.

Fig. 3. Density of the most abundant monothalamid species collected at different stations in Zernov's Phyllophora Field during Cruise 70 of the RV “Prof. Vodyanitsky”


47°
N
46.5°
46°
45.5°
45°
30°
31°
32°
33°E
▲ - 70 Cruise
BLACK SEA
24
26
21
23
27
20
19
18


A
B
C
D
E
F
G
H
I



Psammophaga sp.
K.fusiformis
G.rostellata
V.pellucida
Individuals per m2
30000
25000
20000
15000
10000
5000
0
18
19
20
21
23
24
26
27
Station number