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Three new species of *Gromia* (Protista, Rhizaria) identified from the Romanian Black Sea shelf

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Abstract

The protist genus *Gromia* was first described in 1835 by Dujardin and while gromiids are prominent in the marine environment, *Gromia oviformis* was, for a long time, the only valid species regularly recorded. To date, 16 species that are morphologically and/or genetically distinct have been described. While recent studies are documenting their diversity and their ecological importance, *G. oviformis* has been the sole gromiid species identified in the Black Sea, although unnamed *Gromia* species have also been recorded. We collected sediment samples from the Romanian continental shelf at varying depths (48 – 58 m) to study the morphological and genetic diversity of gromiids in this part of the Black Sea. Three new species, *Gromia bugnae* sp. nov., *Gromia dianae* sp. nov. and *Gromia fabi* sp. nov., were identified based on an integrative taxonomic approach, thus bringing the total described gromiid species to 19. Analysis of partial SSU rRNA gene sequences confirms that these are distinct species. Additionally, an undescribed species is represented by a sequence from the northern part of the Black Sea (Sevastopol, Kazachya Bay). The study provides further evidence of the diversity of gromiids in the Black Sea and underlines the importance of this little-known group in marginal seas.

Keywords: Gromia; New species; Black Sea; SSU rRNA; Protist; Diversity

Introduction

Gromia is a cosmopolitan genus consisting of macroscopic monothalamous (single-chambered) protists that are characterized by a proteinaceous test with “honeycomb membranes" in the inner layer of the test wall and by the filose pseudopodia that extends from an aperture surrounded by an oral capsule (Hedley, 1960; Hedley and Wakefield, 1969; Ogden and Hedley, 1980). The proteinaceous test encapsulates cytoplasm containing stercomata, waste pellets that comprise fine sediment particles, detritus and other undigested material held together by glycosaminoglycans (Hedley, 1962; Hedley and Bertaud, 1962; Tendal, 1979). Gromiids are benthic marine organisms and are believed to play a key role in benthic food webs by feeding on detritus and recycling nitrogen and carbon (Aranda da Silva, 2005; Gooday et al., 2000; Piña-Ochoa et al., 2010).

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Until the late 1990′s, only one species of the genus *Gromia* was described: *Gromia oviformis* (Dujardin, 1835). *Gromia oviformis* has been recorded in different habitats across a wide latitudinal range from polar to tropical environments (Arnold, 1972, 1951; Bowser et al., 1996; Gooday et al., 1996; Gooday and Bowser, 2005; Hedley, 1962). Gromiids inhabit littoral and sublittoral habitats (Arnold, 1972, 1951; Bowser et al., 1996; Gooday et al., 1996; Hedley and Bertaud, 1962) living on seaweed holdfasts (Arnold, 1972), rocks (Hedley and Bertaud, 1962), and sediment surface (Bowser et al., 1996; Hedley and Bertaud, 1962; Jepps, 1926), in association with tunicates (Lwoff, 1925) or with sponges (Arnold, 1951). They have also been documented in the deep sea (Aranda da Silva et al., 2006; Gooday et al., 2000; Gooday and Bowser, 2005; Matz et al., 2008; Rothe et al., 2009). Despite the characteristics of the genus and the diverse range of habitats, the lack of distinctive morphological features has resulted in similar morphotypes being grouped as a single species (Burki et al., 2002) or, in some cases, the misidentification of gromiid species as allogromiids (Foraminifera) (Hedley, 1958) or as fecal pellets (Nyholm and Gertz, 1973).

The taxonomic position of *Gromia* has undergone various transformations (Rothe et al., 2009). Based on morphological characteristics, they were first grouped with Filosea (Bovee, 1985; Hedley, 1958; Ogden and Hedley, 1980; Rhumbler, 1904) before being assigned to “Amoebae of uncertain affinities” (Patterson et al., 2000). The first molecular genetic studies to include *Gromia* assigned this genus to Cercozoa based on Small Subunit ribosomal RNA (SSU rRNA) sequencing data (Burki et al., 2002). This is a phylum based on molecular data (Bhattacharya et al., 1995; Cavalier-Smith and Chao, 2003) that is sister to Foraminifera and Haplosporidia within the supergroup Rhizaria (Nikolaev et al., 2004). *Gromia* is currently assigned to Endomyxa which is either placed as a sister group or encompasses Retaria, a group including Foraminifera, Acantharea and Polycystinea (Adl et al., 2019).

The growing interest in this often overlooked genus has led to the description of 16 new species over the last two decades based on molecular and morphological characteristics (e.g. Aranda da Silva and Gooday, 2009; Gooday et al., 2022, 2021, 2000; Rothe et al., 2009). The type species *G. oviformis* is a species complex that includes several distinct species from the Mediterranean Sea, the tropical Pacific (Guam) and subtropical Atlantic (Madeira) (Burki et al., 2002). *Gromia oviformis* and a number of distinct unnamed morphotypes have also been identified in the Black Sea in previous studies (Pavel et al., 2021; Revkov et al., 2018, Sergeeva and Mazlumian, 2015, Sergeeva et al., 2013, 2017) with a vertical distribution from 10 m to 300 m corresponding to coastal areas down to the deep-water zone that is marked by permanent hypoxic and anoxic conditions (Sergeeva et al., 2017). Seven undescribed morphotypes as well as *G.* cf. *oviformis* were reported by Sergeeva et al. (2017) from the Black Sea. Morphotypes were distinguished by their spherical, elongate or ellipsoidal shape and ranging in size from 230 µm to more than 2 mm. Sergeeva and Mazlumian (2015) noted that gromiids sampled from the Istanbul Strait (Bosporus) had spherical or ellipsoidal shape ranging in size from a few micrometers to more than 1.5 mm with an abundance maximum at 75 m depth. Pavel et al. (2021) sampled gromiids from 27 stations along the Romanian coast of the Black Sea. They distinguished spherical oval and elongate morphotypes ranging in length between 1 mm and 2 mm and highest density of individuals recorded at 53 m depth.

The present study investigates the diversity of *Gromia* from the Romanian continental shelf of the Black Sea using a combination of molecular and morphological methods. The three new species presented in this paper are the first ones described from a marginal sea and reveal the potential of adaptation in gromiids to live in brackish waters as well as in fully marine areas.

Material and methods

The Black Sea

The Black Sea is less saline (13 – 23 PSU) than the neighboring Mediterranean Sea (38.5 PSU) and the oxygenated surface layer (up to 100 m deep) overlies a deep permanent anoxic layer that extends down to the sea floor (Stewart et al., 2007; Zaitsev, 2008). The Romanian continental shelf is also characterized by large inputs of freshwater from the Danube river and its three main delta arms.

Sampling locations

Samples were collected at three stations (100C, MA11 and SU04) on the Romanian continental shelf of the Black Sea (Fig. 1) between June and August 2021 during the Mare Nigrum expeditions 219 (MN219) and 222 (MN222). Station 100C was sampled on the 14th of June 2021 (MN219) while stations MA11 and SU04 were sampled on the 14th of August 2021 and the 17th of August 2021 respectively (MN222). Stations SU04 and 100C were located on the east of the Sulina Danube river mouth at depths of 53 m and 48 m respectively and MA11 was located to the east of Mangalia at a depth of 58 m.

Bottom-water temperatures recorded across the three stations ranged between 7.47 and 8.38 °C, with cooler temperatures noted further away from the continental shelf. The salinity at the three stations ranged between 18.59 and 18.71 PSU. Sediments collected at station MA11 are of littoral origin and correspond to the *Terebellides stroemii* Sars, 1835 habitat. Sediments at station SU04 were sublittoral and correspond to the *Modiolula phaseolina*

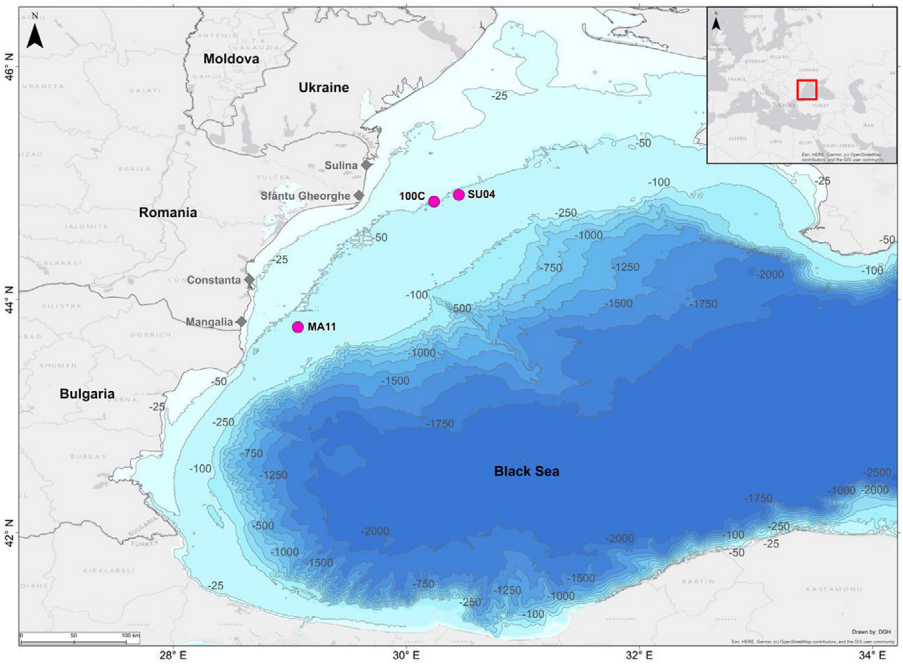

Fig. 1. Site location. *Map of sampling locations on the Romanian continental shelf of the Black Sea.*

R. A. Philippi, 1844 habitat. The substrate found at station 100C comprises muds and mixed sediments, which are part of the habitat of *Modiolula phaseolina* in the offshore circalittoral marine environment (Table 1).

Sample collection and sample processing

The samples were retrieved from the superficial sediment layer (5 cm) taken from one of four tubes of a Multicorer Mark-400 with an opening of 78.5 cm^2 (Ø 10 cm), deployed from the *Mare Nigrum* research vessel. Samples were washed on board through a sieve of 500 µm mesh size on top of a sieve of 125 µm mesh size. Large debris were discarded and residues were transferred into the 125 µm sieve. The greater than 125 µm fractions were stored in laboratory jars with seawater and placed in a fridge at 4 ℃ for a maximum of two days, with the sea water being changed daily. Specimens were sorted on board in Petri dishes filled with sea water that were constantly kept chilled by placing them on freeze packs and counted using an Olympus SZ61 stereomicroscope mounted with an IDS uEye camera and photographed using the uEye Cockpit v4.20 program. Specimens for genetic analyses were transferred individually to Eppendorf tubes containing 200 µl of RNAlater and stored at −20 ℃. Other specimens for were stored in 4% formaldehyde solution (4% formalin) at room temperature. Some of the specimens were then used for morphological analyses and subsequently stored in 95% ethanol. The collected samples resulted in 26 gromiids specimens from station 100C (17 stored in RNAlater and 9 in formalin),

Table 1. Sampling stations: Station parameters and *Gromia* species retrieved from three sampling stations.

Station	Date [mm/yy]	Longitude °E	Latitude °N	Depth [m]	Species
100C	06/21	31°14′15″	44°40′30″	48	*Gromia bugnae*
MA11	08/21	29°04′10″	43°45′50″	58	*Gromia bugnae, Gromia fabi*
SU04	08/21	30°27′00″	44°54′00″	53	*Gromia bugnae, Gromia dianae*
Station	pH	Salinity [PSU]	Conductivity [mS m^{-1}]	Dissolved Oxygen [mg l^{-1}]	Sediment type
100C	8.5	18.69	20.21	8.55	Muds and mixed sediments in the *M. phaseolina* habitat
MA11	8.5	18.59	20.6	7.73	Littoral mixed sediments within the *Terebellides stroemii* habitat
SU04	8.66	18.71	20.62	7.77	Sublittoral mixed sediments within the *M. phaseolina* habitat

24 specimens (14 stored in RNAlater and 10 in formalin) from SU04 and 55 specimens (29 stored in RNAlater and 26 in formalin) from MA11. These specimens were not separated into species at this stage. Within this collection, 25 were selected for the molecular analysis. Water parameters (depth, salinity, temperature, pH, dissolved oxygen and conductivity) were measured using a CTD Rosette (CTD SBE 25 and a rosette model SBE 32 equipped with 12 five liters Niskin bottles) launched prior to the Multicorer to avoid turbulences from lifting sediments.

Morphological description

In the laboratory, gromiids for morphological analysis were sorted, identified and described using a Carl Zeiss Stemi 508 Stereomicroscope with Axiocam 208 color and a Carl Zeiss PrimoStar microscope. Figured individuals were measured using the ImageJ2 Fiji v 2.3.0 program (Schindelin et al., 2012) against a defined scale. Holotypes and paratypes were photographed using scanning electron microscopy at the Grigore Antipa National Museum of Natural History (Bucharest, Romania). Selected specimens stored in 95% ethanol were rehydrated, fixed with 3% glutaraldehyde in sodium cacodylate buffer (0.1 M, pH 7.4). The samples were dehydrated in an ascending alcohol series: 30%, 50%, 70%, 80%, 90% and 100% ethanol (30 min for each change). The gromiids were transferred to a mixed solution of ethanol and hexamethyldisilazane (HMDS) (3:1, 1:1, and 1:3), and finally in 100% HMDS. The samples were left overnight for chemical evaporation and complete drying of the specimens. The specimens were mounted on aluminum stubs covered with conductive double-sided adhesive carbon tabs and then sputter-coated (SEM Coating Unit E5100) with gold for 60 s. The gromiids were analyzed and photographed using a Phenom Pro scanning electron microscope (Phenom-World, Thermo Fisher Scientific, The Netherlands), at 10 kV acceleration voltage. Type specimens, stored in 2 ml glass tubes in ethyl alcohol, were deposited in the Rhizaria Group collection at the National Institute of Marine Geology and Geo-Ecology (Bucharest, Romania).

DNA extraction, PCR amplification and sequencing

Twenty-five specimens of *Gromia* were extracted individually using DNeasy Plant Mini Kit (Qiagen). Each DNA extraction is identified by a unique isolate number (Table 2). Semi-nested PCR amplification was carried out for the 3′ end fragment of the SSU rRNA using eukaryotic primer SSU forward primer s12.2 (GATYAGATACCGTCG) at the first amplification step and the gromiid-specific SSU forward primer s13.3 (CGTTGGATAGGACTC) for the reamplification. The eukaryotic SSU reverse primer s20r (5′GACGGGCGGTGTGTACAA) was used for both amplification steps. Thirty-five and 25 cycles were performed for the first and the second PCR, with annealing temperatures of 50 °C and 52 °C, respectively. Eleven isolates yielded positive amplification results; their numbers are given in Table 2. The amplified PCR products were purified using the High Pure PCR Cleanup Micro Kit (Roche Diagnostics). Sequencing reactions were performed using the BigDye terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The resulting sequences were deposited in the NCBI/GenBank database. Isolate and accession numbers are specified in Table 2.

Phylogenetic analysis

The obtained sequences were added to 61 gromiid sequences (Table 2) that are part of the publicly available 18S database of *Gromia* (NCBI/Nucleotide; https://www.ncbi.nlm.nih.gov/nucleotide/). All sequences were aligned using the default parameters of the Muscle automatic alignment option as implemented in SeaView vs. 4.3.3 (Gouy et al., 2010). The alignment contains 72 sequences with 816 sites used for analysis. The phylogenetic tree was constructed using maximum likelihood phylogeny (PhyML 3.0) as implemented in ATGC: PhyML (Guindon et al., 2010). An automatic model selection by SMS (Lefort et al., 2017) based on Akaike Information Criterion (AIC) was used, resulting in a HKY85 + R substitution model being selected for the analysis. The initial tree is based on BioNJ. Bootstrap values (BV) are based on 100 replicates. The obtained tree is unrooted and represents the branching order.

Results

Systematic descriptions
Kingdom Chromista
Subkingdom Harosa, Cavalier-Smith, 2010
Infrakingdom Rhizaria, Cavalier-Smith, 2002
Phylum Cercozoa, Cavalier-Smith, 1998
Subphylum Endomyxa, Cavalier-Smith, 2002
Class Gromiidea, Cavalier-Smith & Chao, 2003.
Order Gromiida, Claparède and Lachmann, 1856
Family Gromiidae, Reuss, 1862
Genus *Gromia*, Dujardin, 1835.

Gromia bugnae Kreuter, Holzmann & Pavel sp. nov. (Fig. 2 A – D, Fig. 3 A – F)

Zoobank Registration

urn:lsid:zoobank.org:act:482694A7-12B2-4305-9FB1-1C1FD6799B99

Diagnosis

Test almost spherical to ovoid, transparent and smooth, relatively large, 420–1320 μm in length and 328 to

Table 2. *Gromia* DNA isolate and accession numbers: Localities, isolate and accession number, and species names of investigated *Gromia* species.

Isolate	Species	Accession number	Sampling locality	Isolate	Species	Accession number	Sampling locality
21,140	*Gromia amygdaliformis*	MZ468172	South Georgia	5894	*Gromia psammophila*	MT906548	Chile, Rio Amarillo
21,165	*Gromia amygdaliformis*	MZ468173	South Georgia	21,175	*Gromia psammophila*	MZ468160	Falkland Islands
21,167	*Gromia amygdaliformis*	MZ468175	South Georgia	21,176	*Gromia psammophila*	MZ468161	Falkland Islands
20,001	*Gromia botelliformis*	MT906560	Greenland, Nuuk fjords	21,144	*Gromia saoirsei*	MZ468167	South Georgia
20,002	*Gromia botelliformis*	MT906561	Greenland, Nuuk fjords	21,145	*Gromia saoirsei*	MZ468168	South Georgia
20,008	*Gromia botelliformis*	MT906562	Greenland, Nuuk fjords	21,146	*Gromia saoirsei*	MZ468169	South Georgia
20,006	*Gromia brevis*	MT906529	Greenland, Nuuk fjords	9918	*Gromia* sp.	MT906518	Svalbard
20,005	*Gromia brevis*	MT906531	Greenland, Nuuk fjords	9932	*Gromia* sp.	MT906519	Svalbard
20,004	*Gromia brevis*	MT906530	Greenland, Nuuk fjords	9933	*Gromia* sp.	MT906520	Svalbard
21,186	*Gromia cedhageni*	MZ468194	Falkland Islands	20,007	*Gromia* sp.	MT906521	Greenland, Nuuk fjords
21,187	*Gromia cedhageni*	MZ468195	Falkland Islands	10,122	***Gromia* sp.**	MT906578	Sevastopol, Kazachya Bay, 5 – 10 *m*
21,188	*Gromia cedhageni*	MZ468196	Falkland Islands	4385	*Gromia* sp.1	MG519733	Oman margin
19,998	*Gromia cucumiformis*[a]	MT906599, MT906602	Greenland, Nuuk fjords	4411	*Gromia* sp.1	MG519731	Oman margin
20,000	*Gromia cucumiformis*	MT906605	Greenland, Nuuk fjords	4396	*Gromia* sp.1	MG519734	Oman margin
21,506	***Gromia dianae***	OP934039	Romania	4399	*Gromia* sp.2	MG519737	Oman margin
21,510	***Gromia dianae***	OP934040	Romania	4331	*Gromia* sp.3	MG519740	Oman margin
21,500	***Gromia fabi***	OP934036	Romania	4400	*Gromia* sp.3	MG519739	Oman margin
21,503	***Gromia fabi***	OP934037	Romania	4421	*Gromia* sp.4	MG519744	Oman margin
21,507	***Gromia fabi***	OP934038	Romania	4338	*Gromia* sp.5	MG519775	Pakistan margin
21,130	*Gromia landrethi*	MZ468176	South Georgia	4366	*Gromia* sp.5	MG519777	Pakistan margin
21,141	*Gromia landrethi*	MZ468177	South Georgia	4319	*Gromia* sp.6	MG519761	Pakistan margin
21,150	*Gromia landrethi*	MZ468180	South Georgia	4390	*Gromia* sp.6	MG519762	Pakistan margin
9644	*Gromia marmorea*	MG519736	Weddell Sea	4320	*Gromia* sp.6	MG519765	Pakistan margin
9625	*Gromia melinus*	MG519742	Weddell Sea	4260	*Gromia* sp.7[a]	MG519746, MG519747	Pakistan margin
6679	*Gromia oviformis*	MT906522	France, Marseille	4259	*Gromia* sp.7	MG519745	Pakistan margin
6680	*Gromia oviformis*	MT906523	France, Marseille	4353	*Gromia sphaerica*	MG519758	Oman margin
6682	*Gromia oviformis*	MT906522	France, Marseille	4375	*Gromia sphaerica*	MG519757	Oman margin
12,976	*Gromia oviformis*	MG519738	Italy, Naples	4466	*Gromia sphaerica*	MG519756	Oman margin
n.a.	*Gromia oviformis*	AJ457815	Tunisia, Hammamet	21,493	***Gromia bugnae***	OP934041	Romania
n.a.	*Gromia oviformis*	AJ457811	Madeira	21,494	***Gromia bugnae***	OP934042	Romania
n.a.	*Gromia oviformis*	AJ457814	Guam	21,499	***Gromia bugnae***	OP934043	Romania
21,129	*Gromia pashukae*	MZ468198	South Georgia	21,501	***Gromia bugnae***	OP934044	Romania
21,201	*Gromia pashukae*	MZ701628	South Georgia	21,508	***Gromia bugnae***	OP934045	Romania
21,202	*Gromia pashukae*	MZ701629	South Georgia	21,509	***Gromia bugnae***	OP934046	Romania
5794	*Gromia psammophila*	MZ468158	Chile, Rio Amarillo	13,840	*Gromia winnetoui*	MG519735	Weddell Sea

Species printed in bold were investigated for the current study.

[a] PCR products have been cloned prior to sequencing.

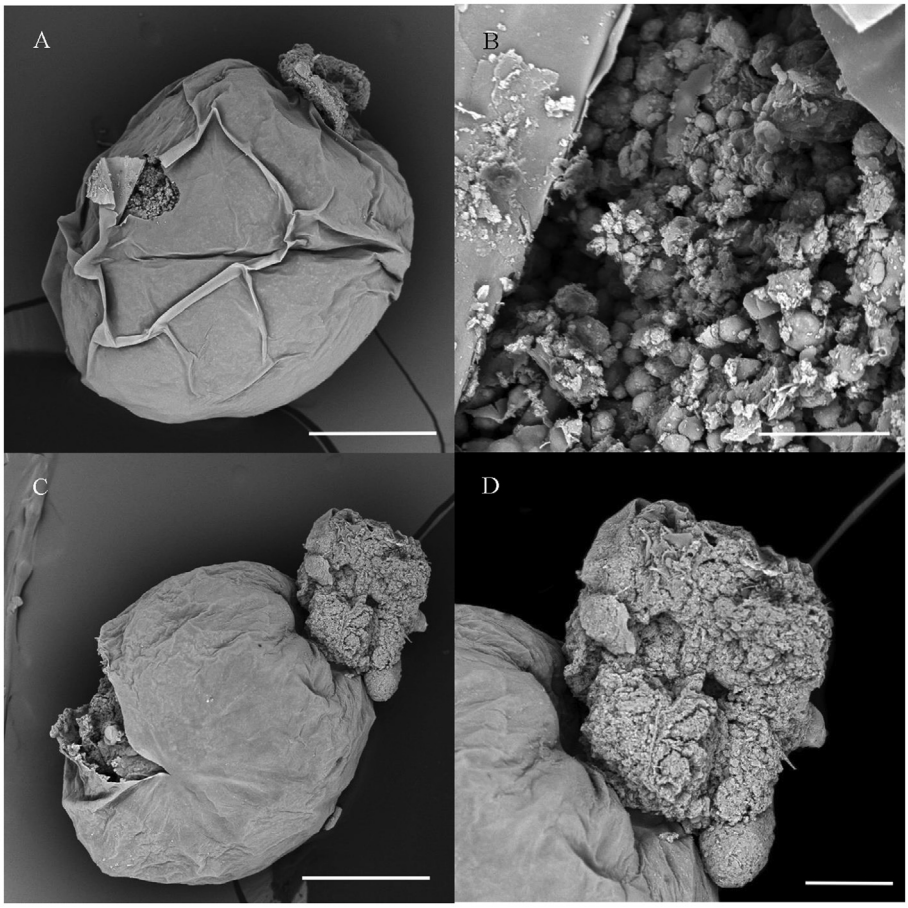

Fig. 2. *Gromia bugnae*, scanning electron microscope images of type specimens. *A) Holotype from station MA11, registration number GROM01. B) Detail of holotype showing test. C) Paratype from station SU04, registration number GROM01_01. D) Detail of apertural end of paratype with accumulation of organic material. Scales* 300 μm *(A,C);* 30 μm *(B);* 100 μm *(D).*

1156 μm in width, with a length/width ratio between 1.1 and 1.4. Apertural end generally flattened with a protruding circular oral capsule, often surrounded by accumulated detritus.

Type Material

Holotype (accession number GROM01) from station MA11, longitude 29°04′10″, latitude 43°45′50″, 58 m; two paratypes (accession numbers GROM01_1 and GROM01_2) from station SU04, longitude 30°27′00″, latitude 44°54′00′, 53 m and three paratypes GROM01_3, GROM01_4 and GROM01_5 from station 100C, longitude 31°14′15″, latitude 44°40′30″, 48 m, stored in 95% ethanol and deposited at the National Institute of Marine Geology and Geo-Ecology, Romania.

Other material

Six specimens for molecular analysis: from station SU04 (DNA isolate 21501), from station 100C (DNA isolates 21,493 and 21494) and from station MA11(DNA isolates 21508, 21499, 21509).

Etymology

Named in the memory of Jean-Pierre Bugnon, grandfather of the first author, for his knowledge and passion about the natural world and its biodiversity passed on to his grandson whom he fully supported in choosing a career path as a biologist.

Description

The collected specimens have a spherical to ovoid test, 420 to 1320 μm in length (mean 861 ± 328 μm) and 328 to 1156 μm in width (mean 730 ± 288 μm), with a length/width ratio between 1.07 and 1.36 (mean 1.19 ± 0.10) (n = 8 in all cases). The wall is thin, delicate and transparent with a reflective, slightly iridescent surface. The test content comprises mainly small round stercomata, with a variable proportion of small, darker, usually brownish particles scattered among them. Clusters of larger

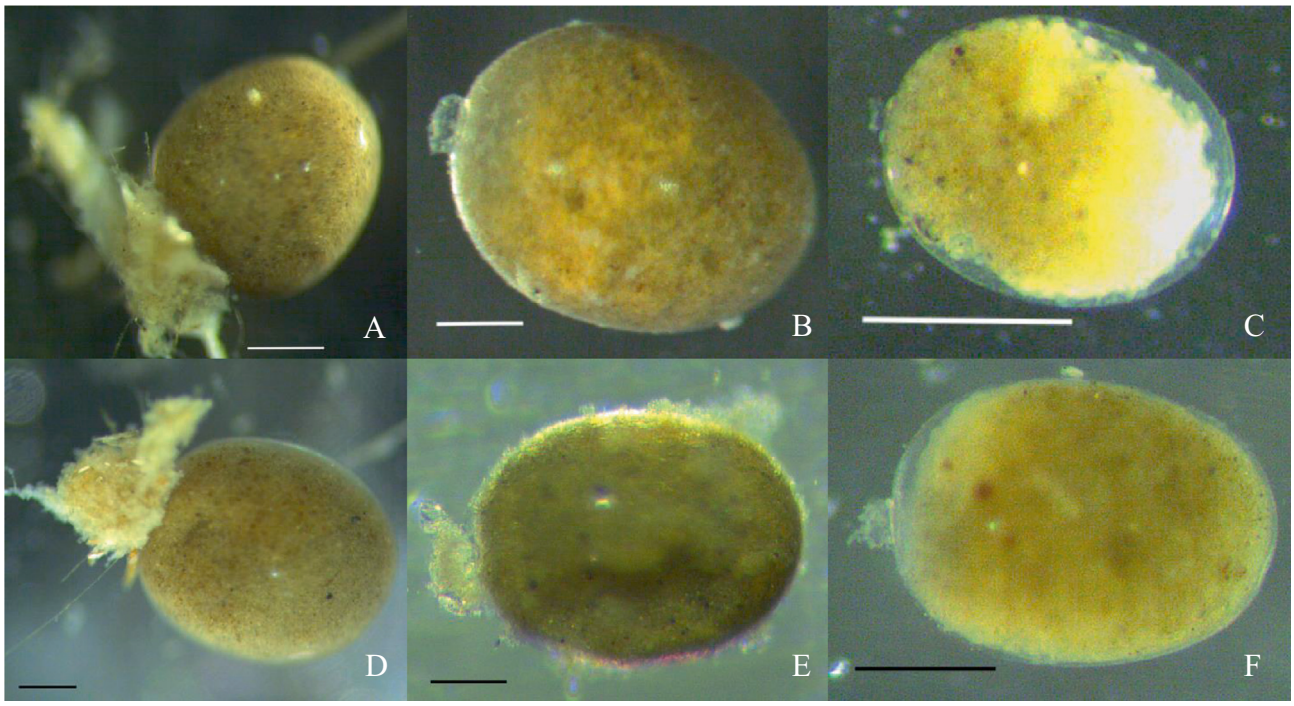

Fig. 3. *Gromia bugnae*, optical images of specimens whose DNA was extracted and subsequently sequenced. *Specimens are referred to by their DNA isolate numbers. A) Isolate 21,501 from station SU04. B) Isolate 21,493 from station 100C. C) Isolate 21,508 from station MA11. D) Isolate 21,499 from station MA11. E) Isolate 21,494 from station 100C. F) Isolate 21,509 from station MA11. Scales* 300 μm *(A–D;F)* 100 μm *(E).*

mineral particles, including quartz, mica and a green mineral, possibly hornblende, are sometimes visible (Fig. 2 B). In specimens corresponding to DNA isolates 21,508 (Fig. 3 C) and 21,509 (Fig. 3 F), the interior part of the test is not completely filled with sediment. The test is generally smooth with no ornamentation (Fig. 2 A, C), except in the specimen corresponding to DNA isolate 21,494 (Fig. 3 E) which has an accumulation of detritus on the surface. The oral capsule typically forms a dome-like structure that is circular in planar view and has a distinct rim. It is relatively large with a diameter of 44 to 209 μm (mean 117 ± 84 μm) and projects 19 to 122 μm (mean 67 ± 0.0 5 μm) beyond the general outline of the test (n = 3 in all cases). Filamentous pseudopodia projecting from the apertural end are visible in some specimens (Fig. 3 A, C) and detritus is sometimes accumulated around the oral capsule (Fig. 2 C, D; Fig. 3 A, D, E).

Molecular characteristics

Gromia bugnae (91% BV) branches as sister to the paraphyletic clade of *G. oviformis*, but the branching is not supported (Fig. 8). The partial SSU rRNA sequences contain 653 (DNA isolate 21509) and 654 (DNA isolates 21493, 21494, 21499, 21501, 21508) nucleotides. The GC content ranges from 46 to 47%.

Distribution

Gromia bugnae is the most abundant gromiid species in our samples with the widest range, being present in each of the three sampled stations across the Romanian Black Sea continental shelf between Mangalia and Sulina.

Habitat

Specimens were collected with debris consisting of empty *Modiolula* shells mixed with mud or sand within the *M. phaseolina* habitat or between the *T. stroemii* habitat and *M. phaseolina* habitat within a bathymetric range of 48 m and 58 m.

Remarks

Specimens of *G. bugnae* have various overall test shapes but are generally more spherical than *G. dianae* or *G. fabi* with a larger overall size. *Gromia bugnae* resembles other spherical to ovoid gromiids collected across the world. In particular, *G. bugnae* has similarities with species including *G. landrethi* (Gooday et al., 2022) and *G. oviformis* (Jepps, 1926; Burki et al., 2002) as well as undescribed species such as *Gromia* sp. 5 in Aranda da Silva et al. (2006) and *Gromia* sp. 5A in Rothe et al. (20211). This species has

resemblances with some specimens of *Gromia* sp. (illustrated in Fig. 4 b, c in Pavel et al. 2021) which were recorded in the same area. However, no genetic data is available to support assigning the specimens to *G. bugnae*.

Gromia dianae Kreuter, Holzmann & Pavel sp. nov. (Fig. 4 A – B, Fig. 5 A – B)

Zoobank registration

urn:lsid:zoobank.org:act:37D6B54F-F980-431E-88B0-294EBF5165DC

Diagnosis

Test slightly elongated, transparent and smooth, containing granular content, 613——1102 µm in length and 501 to 777 µm in width, with a length/width ratio between 1.39 and 1.44. Apertural and adapertural parts evenly rounded with a slightly protruding circular oral capsule.

Type material

Holotype (accession number GROM02) and five paratypes (accession numbers GROM02_1, GROM02_2, GROM02_3, GROM02_4 and GROM02_5) from station SU04, longitude 30°27′00″, latitude 44°54′00″, 53 m stored in 95% ethanol and deposited at the National Institute of Marine Geology and Geo-Ecology, Romania.

Other material

Two specimens from station SU04 for molecular analysis: DNA isolates 21,506 and 21510.

Etymology

Named in honor of Diana Grace Holdsworth for providing continuous advice and support throughout this study.

Description

The specimens have slightly elongate, ovoid tests with evenly rounded adapertural and apertural ends. The test length varies between 613 and 1102 µm (mean 812 ± 257 µm), test width between 501 and 777 µm (mean 573 ± 179 µm) and a length/width ratio between 1.39 and 1.44 (mean 1.4 ± 0.03) (n = 3 in all cases). The wall is thin and transparent with a reflective, usually smooth surface and no ornamentation. The test contents are granular, light brownish in overall color and comprise small round stercomata with a variable proportion of scattered brownish particles. In the specimen corresponding to DNA isolate 21,510 (Fig. 5 B), the adapertural end is not completely filled with test content. The oral capsule does not project from the apertural end (Fig. 4 B). One specimen (Fig. 5 A) was collected with a cluster of detrital material attached to the oral capsule and obscuring it.

Molecular characteristics

Gromia dianae (92% BV) branches as sister to an undescribed gromiid (DNA isolate 10122) from Kazachya Bay (Sevastopol, Black Sea) (Fig. 8). The branching is moderately supported (72% BV). The partial SSU rRNA sequences contain 737 nucleotides. The GC content is 46%.

Distribution

The species was recorded exclusively from the station SU04, offshore of the Danube river mouth of the Sulina arm on the Romanian continental shelf of the Black Sea.

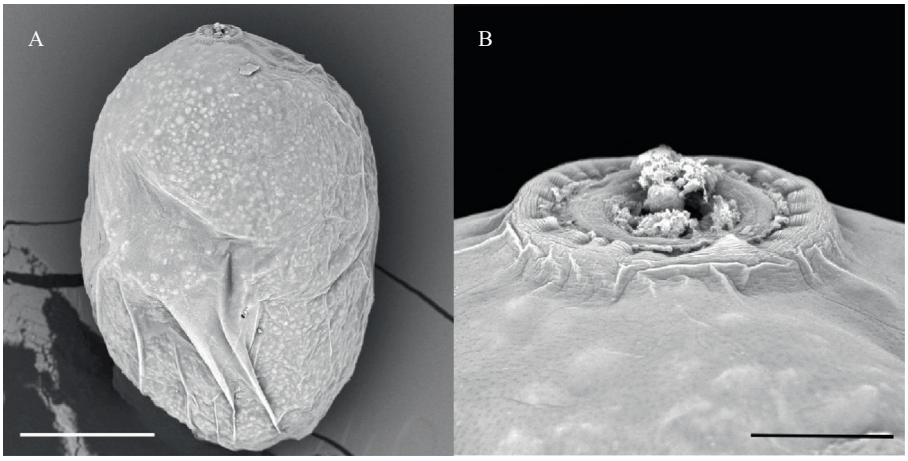

Fig. 4. *Gromia dianae*, scanning electron microscope images of holotype from station SU04, registration number GROM02. *A) Complete specimen. B) Detail showing oral capsule. Scales* 200 µm *(A);* 30 µm *(B).*

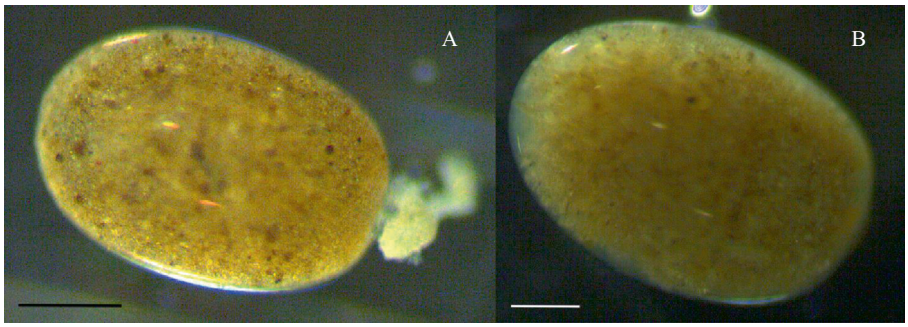

Fig. 5. ***Gromia dianae,*** **optical images of specimens whose DNA was extracted and subsequently sequenced.** *Specimens are referred to by their DNA isolate numbers. A) Isolate 21506. B) Isolate 21510. Scales* 200 μm. *Specimens were sampled from station SU04.*

Habitat

The specimens were collected on debris and sand within the *M. phaseolina* habitat at a depth of 53 m.

Remarks

Gromia dianae has a similar shape as undescribed morphotypes of *Gromia* sp. found in various locations such as in the Black Sea (Fig. 4 a in Pavel et al., 2021; Romania), White Sea (illustrated in Fig. 13d in Gooday et al., 2021) and Weddell Sea (species 5A in Fig. 4 c, e in Rothe et al., 2011) and also resembles *Allogromiidae* gen. sp. A (Fig. 4 in Sergeeva and Anikeeva, 2006; Sevastopol) and undescribed soft-shelled foraminifera sp. A (Fig. 2 in Anikeeva, 2006; Sevastopol) from the Black Sea. *Gromia bugnae*, *G. fabi* and *G. oviformis* are morphologically similar to *G. dianae*; however they generally have a more ovoid shape compared to the more elongated *G. dianae*. The relationship of *G. dianae* with *G. fabi* has also been determined with genetic data that grouped them in one clade with an undescribed gromiid (DNA isolate 10122).

Gromia fabi Kreuter, Holzmann & Pavel sp. nov. (Fig. 6 A – B, Fig. 7 A – C)

Zoobank registration

urn:lsid:zoobank.org:act:2D31C6C3-93C7-4 EB0-83F1-BCAC64922A0D

Diagnosis

Test ovoid, transparent and smooth, relatively small, 400 – 600 μm in length, 300 – 450 μm in width, with a length/width ratio between 1.10 and 1.39. Adapertural end sometimes slightly pointed with a somewhat depressed circular oral capsule.

Type material

Holotype (accession number GROM03) and five paratypes (accession numbers GROM03_1, GROM03_2 and GROM03_3, GROM03_4 and GROM03_5) from station MA11, longitude 29°04′10″, latitude 43°45′50″, 58 m stored in 95% ethanol and deposited at the National Institute of Marine Geology and Geo-Ecology, Romania.

Other material

Three specimens from station MA11 for molecular analysis: DNA isolates 21500, 21503, 21507.

Etymology

Named in honor of Fabio Carbone, godfather and mentor of the first author for the continuous support offered throughout his career including this study.

Description

The collected specimens have an ovoid test that is relatively small, ranging in length from 401 to 537 μm (mean 460 ± 61 μm), width from 344 to 434 μm (mean 373 ± 41 μm) and a length/width ratio between 1.10 and 1.39 (mean 1.23 ± 0.12) (n = 4 in all cases). The apertural end of some specimens (Fig. 6 A; Fig. 7 A, B) is almost flat while the adapertural end is slightly flattened. The specimen corresponding to DNA isolate 21,507 has a slightly more elongated test shape (Fig. 7 C). The overall color is yellowish and the test contents have a granular appearance. The test wall is thin, transparent and smooth. The oral capsule, when not hidden by agglomerated detritus, projects slightly from the apertural end of the test with a height of 30 to 40 μm and a diameter of 69 to 72 μm. The oral capsule is circular, slightly tilted and often obscured by detrital material.

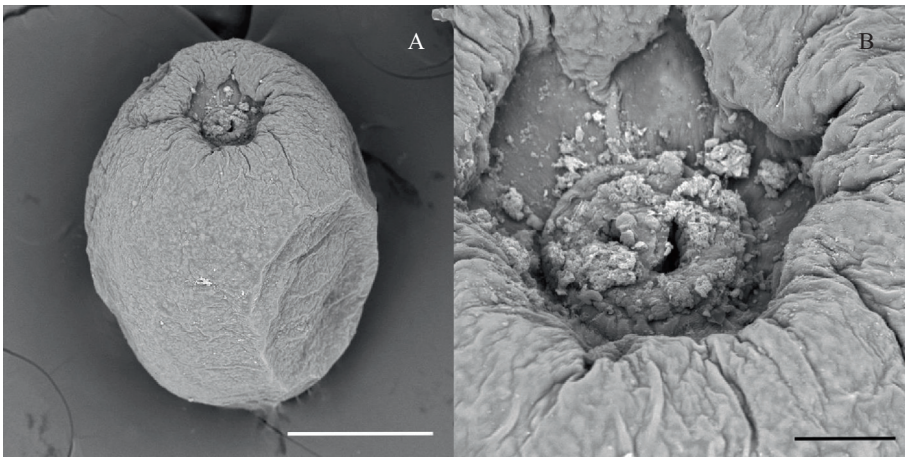

Fig. 6. *Gromia fabi*, scanning electron microscope images of holotype from station MA11, registration number GROM03. *A) Complete specimen. B) Detail showing oral capsule with organic detritus. Scales* 200 μm *(A,C);* 30 μm *(B).*

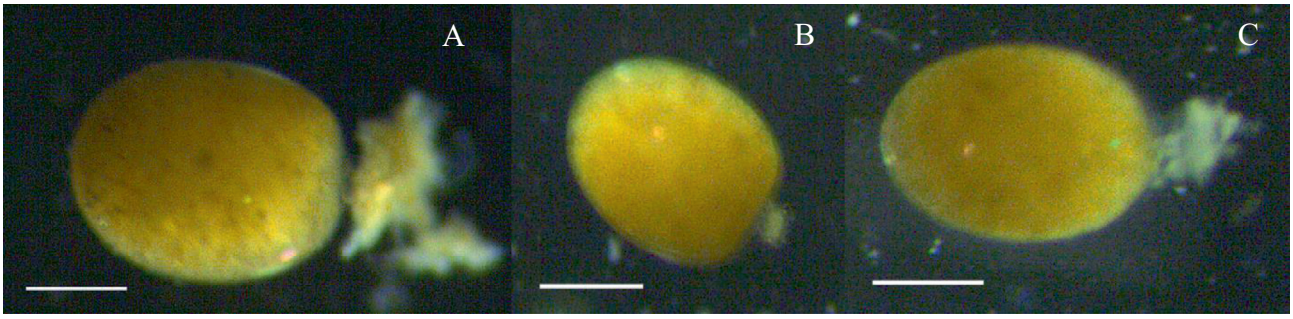

Fig. 7. *Gromia fabi*, optical images of specimens whose DNA was extracted and subsequently sequenced. *Specimens are referred to by their DNA isolate numbers. A) Isolate 21503. B) Isolate 21500. C) Isolate 21507. Scales* 200 μm. *Specimens were sampled from station MA11.*

Molecular characteristics

Gromia fabi (100% BV) branches as sister to *G. dianae* and *Gromia* sp. (DNA isolate 10122) (Fig. 8). The branching is well supported by 81% BV. The partial SSU rRNA sequences contain 696 nucleotides. The GC content is 46%.

Habitat

The species was recorded exclusively from station MA11, off the coast of Mangalia on the continental shelf of the Black Sea.

Ecology

The specimens were collected on a mix of debris composed of empty *Modiolula* shells and mud in between *T. stroemii* and *M. phaseolina* habitats at a depth of 58 m.

Remarks

The ovoid test shape of *G. fabi* looks similar to *G. bugnae*, although the test is smaller (respectively L: 401 – 537 μm/W: 344 – 434 μm compared to L: 420 – 1320 μm/W:328 – 1156 μm). In addition, *G. fabi* shows similarities with *G. oviformis* (illustrated in Fig. 1 in Burki et al., 2002), *G. pashukae* (Gooday et al., 2022), *Gromia* sp. (Goldstein et al., 2011), *Gromia* sp. DNA isolate 20,015 (Fig. 13c in Gooday et al., 2021), *Gromia* sp. (Fig. 20 a in Sergeeva et al., 2017) and *Gromia* sp. (Fig. 4 a in Rothe et al., 2011). There is no genetic evidence of a relationship between *G. fabi* and these species, however *G. fabi* and *G. dianae* are grouped in the same clade.

Molecular characterization

The newly described species are sustained by strong bootstrap values (91, 92 and 100%) (Fig. 8). Two species (*G. dianae, G. fabi*) build a well-supported sister clade (81% BV) that also contains one sequence of *Gromia* sp. (DNA isolate 10122) from Sevastopol. The clade branches as sister to an undescribed *Gromia* from Arctic regions (Svalbard fjords, Nuuk fjords in Greenland) with weak support (71% BV). *Gromia* species from the Falklands (*G. psammophila*) and the deep sea (*G. winnetoui, Gromia* sp. 1) branch at the base of the former clades.

Gromia bugnae (91% BV) branches close to *G. oviformis* from the Mediterranean Sea and Guam, but the

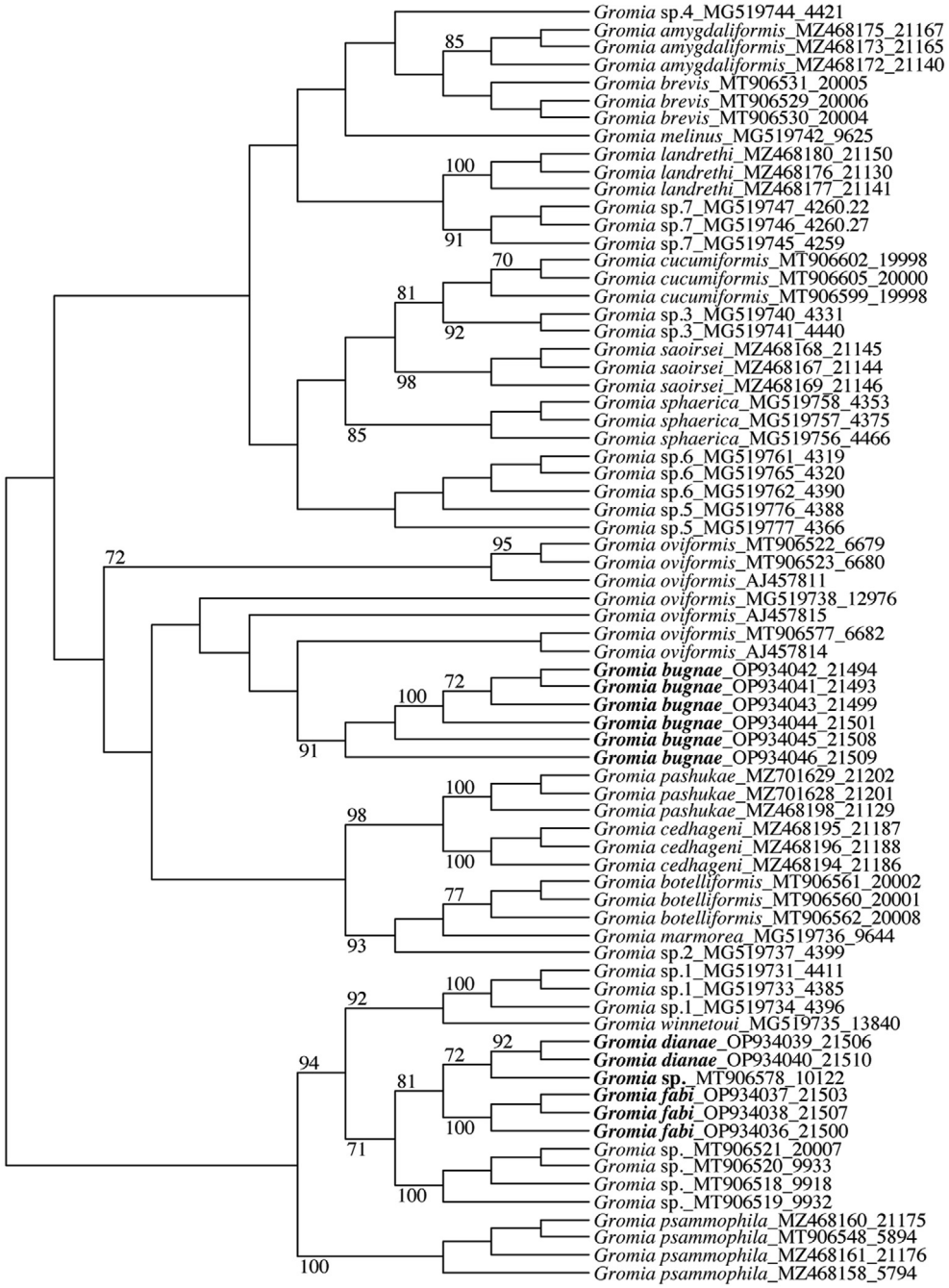

Fig. 8. *Gromia* phylogenetic tree: *PhyMl phylogenetic tree based on the 3'end fragment of the SSU rRNA gene, showing evolutionary relationships of 72 gromiid specimens. Sequences obtained for this study are marked in bold. Specimens are identified by their accession (1st) and isolate numbers (2nd). The tree is unrooted and represents the branching order but does not show ancestry relationships. Numbers at nodes indicate bootstrap values (BV's) greater than 70%.*

branching is not supported. *Gromia* species from South Georgia, the Falklands, the Arctic (Nuuk fjords) and the deep sea (*G. pashukae*, *G. cedhageni*, *G. botelliformis*, *G. marmorea* and *Gromia* sp. 2) branch at their base together with another clade of *G. oviformis* from Madeira and the Mediterranean Sea.

A third group consists of deep-sea species (*G. melinus, G. sphaerica*, *Gromia* sp. 3, *Gromia* sp.4, *Gromia* sp.5, *Gromia* sp.6, *Gromia* sp.7,) and *Gromia* from the Arctic (Nuuk fjords) and South Georgia (*G. amygdaliformis, G. brevis, G. landrethi, G. cucumiformis, G. saoirsei*).

Relationships between these groups are not sustained except for four clades. One of these clades consists of *G. cucumiformis* and *Gromia* sp. 3 (81% BV), another includes *G. pashukae* and *G. cedhageni* (98% BV). The third one comprises *G. botelliformis*, *G. marmorea* and *Gromia* sp.

2 (93% BV) and the fourth one consists of *Gromia* sp.1, *G. winnetoui*, *G. dianae*, *Gromia* sp. (Sevastopol), *G. fabi* and *Gromia* sp. from Svalbard and Nuuk fjords (9% BV).

Discussion

Morphology

While gromiids described from other restricted geographic areas can be distinguished by their test shapes (Gooday et al., 2022, 2021), the three new gromiid species can hardly be differentiated morphologically despite differences in the overall shape of the test with *G. bugnae* being almost spherical, *G. dianae* being slightly elongated and *G. fabi* being ovoid. In general, the morphological characteristics used for identification such as overall shape, size, thickness and ornamentation of the test, and size and shape of the aperture are limited (Gooday et al., 2022). When gromiids are compared across a wider geographical area, very similar morphotypes are represented in different localities and molecular data are needed to clearly distinguish species.

Habitat and diversity

Our integrative taxonomic approach has identified three new species from a limited area along the Romanian continental shelf of the Black Sea. Furthermore, an undescribed species for which one sequence is available was collected earlier from the Crimean area (Sevastopol, Kazachya Bay). We can therefore expect that gromiid diversity will increase with further studies undertaken along Black Sea coasts. Gromiids occur in a wide range of shallow-water marine habitats and have also been reported from deep-sea settings (Gooday et al., 2022, 2021; Gooday and Bowser, 2005; Rothe et al., 2009). A previous study (Pavel et al., 2021) revealed high numbers of gromiids from the oxic zones of the Romanian Black Sea shelf with densities reaching from 133 to 16,758 individuals per square meter. Gromiids are therefore not only well adapted to brackish water conditions but may also play an important role in these local food webs. Other studies in the Black Sea have recorded high abundances of gromiids usually within the top layer of sediments and notably in the oxic/anoxic transition zone where oxygen is depleted (Sergeeva et al., 2013; Sergeeva and Mazlumyan, 2015). In addition, Sergeeva (2000) recorded a considerable number of meiobenthic species along with Foraminifera species up to 2000 m deep. Gromiids have been observed to inhabit anoxic environments when dwelling in deeper sediments (e.g. Gooday et al., 2013) or due to their own respiration activity (Høgslund et al., 2017). The presence of gromiids dwelling around this layer in conditions of low oxygen concentrations aligns with observations from the Pakistan margin of the Arabian Sea where specimens live in low oxygenated waters in the oxygen minimum zone (Gooday and Bowser, 2005) and with the presence of numerous protist species in the suboxic layer of the Black Sea (Wylezich and Jürgens, 2011). One mechanism for gromiids to survive hypoxic and anoxic conditions is suggested to be the accumulation and potentially the use of nitrate for their respiration (e.g. Piña-Ochoa et al., 2010). High abundance of gromiids was also reported from the Weddell Sea (Rothe et al., 2011) where approximately 700 specimens were sampled from a bathyal eutrophic area, suggesting that deep-sea gromiids might play an important role in carbon cycling through ingestion and degradation of fresh organic matter.

Phylogeny

Gromia dianae and *Gromia fabi* build a well-supported sister group that also includes one sequence of *Gromia* sp. from Kazachya Bay (Sevastopol). The species branch next to some undescribed gromiids from shallow water Arctic regions, but the branching is only weakly supported (71% BV). *Gromia bugnae* is not closely related to the former species and branches next to the paraphyletic clade of *G. oviformis.* The type species *G. oviformis* was described by Dujardin (1835) based on specimens collected from the Mediterranean Sea near Marseille, but none of Dujardin's original specimens have survived. Sequenced specimens identified as *G. oviformis* have been sampled from different places in the Mediterranean Sea (Tunisia, Naples, Marseille) as well as from Guam and Madeira. The sequences branch independently and *G. oviformis* therefore clearly represents a species complex (Burki et al., 2002). Our tree contains three *G. oviformis* sequences from Marseille (DNA isolates 6679, 6680, 6682) that branch in different groups with DNA isolate 6682 and *G.oviformis* from Guam branching at the base of *G. bugnae* and DNA isolates 6679 and 6680 and *G. oviformis* from Madeira branching in a separate clade (Fig. 8, Table 2). We do not have morphological data for the sequenced specimens from Marseille and it is not possible to decide whether the original *G. oviformis* is represented by our sequences. In order to solve this problem, the type species of *Gromia* has to be redescribed based on samples from the type area by combining morphological and molecular data (see Table 3).

The three new species could possibly constitute an endemic group as they are currently only known from the Black Sea. However, as knowledge of gromiid biogeography is limited, the restricted distribution of the newly described species could also be an effect of sampling bias. Gathering more knowledge about the diversity and distribution of this wide-spread protist group is important and will further help to understand the function and the services they provide to marine benthic ecosystems.

Table 3. Measurements of *Gromia* specimens: Length, width and ratio of *Gromia* individuals.

Species	ID	Length [mm]	Width [mm]	Length/Width ratio
Gromia bugnae	Holotype GROM01	0.856	0.753	1.1
	Paratype GROM01_1	0.741	0.693	1.1
	Isolate 21493	1.298	1.0345	1.3
	Isolate 21494	0.415	0.328	1.3
	Isolate 21499	1.32	1.156	1.1
	Isolate 21501	0.948	0.876	1.1
	Isolate 21508	0.501	0.406	1.2
	Isolate 21509	0.81	0.597	1.4
Gromia dianae	Holotype GROM02	0.613	0.442	1.4
	Isolate 21506	0.72	0.501	1.4
	Isolate 21510	1.102	0.777	1.4
Gromia fabi	Holotype GROM03	0.401	0.364	1.1
	Isolate 21500	0.423	0.351	1.2
	Isolate 21503	0.537	0.434	1.2
	Isolate 21507	0.478	0.344	1.4

CRediT authorship contribution statement

Sylvain Kreuter: Conceptualization, Methodology, Investigation, Visualization, Writing – original draft, Writing – review & editing, Project administration. **Maria Holzmann:** Conceptualization, Methodology, Investigation, Resources, Data curation, Writing – review & editing, Funding acquisition, Formal analysis. **Diana Grace Holdsworth:** Visualization, Writing – original draft, Writing – review & editing. **Rozalia Motoc:** Visualization, Resources. **Ana Bianca Pavel:** Methodology, Resources, Data curation, Writing – review & editing, Funding acquisition.

Data availability

The data are available on NCBI website and type specimens are available at the National Institute of Marine Geology and Geo-Ecology (Bucharest, Romania).

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References

Adl, S.M., Bass, D., Lane, C.E., Lukeš, J., Schoch, C.L., Smirnov, A., Agatha, S., Berney, C., Brown, M.W., Burki, F., Cárdenas, P., Čepička, I., Chistyakova, L., del Campo, J., Dunthorn, M., Edvardsen, B., Eglit, Y., Guillou, L., Hampl, V., Heiss, A.A., Hoppenrath, M., James, T.Y., Karnkowska, A., Karpov, S., Kim, E., Kolisko, M., Kudryavtsev, A., Lahr, D.J.G., Lara, E., Le Gall, L., Lynn, D.H., Mann, D.G., Massana, R., Mitchell, E.A.D., Morrow, C., Park, J.S., Pawlowski, J.W., Powell, M.J., Richter, D.J., Rueckert, S., Shadwick, L., Shimano, S., Spiegel, F.W., Torruella, G., Youssef, N., Zlatogursky, V., Zhang, Q., 2019. Revisions to the Classification, Nomenclature, and Diversity of Eukaryotes. J. Eukaryot. Microbiol. 66, 4–119. https://doi.org/10.1111/jeu.12691.

Anikeeva, O.V., 2006. Modern state of the soft-shelled foraminiferal fauna in the Sevastopol area (the Black Sea). IGCP 521 Second Plenary Meeting and Field Trip, Odessa, Ukraine, August 20-28, 2006. Odessa, Astroprint. Pp. 15-16.

Aranda da Silva, A., 2005. Benthic protozoan community attributes in relation to environmental gradients in the Arabian Sea (phd). University of Southampton.

Aranda da Silva, A., Gooday, A.J., 2009. Large organic-walled Protista (*Gromia*) in the Arabian Sea: Density, diversity, distribution and ecology. Deep Sea Research Part II: Topical Studies in Oceanography. Benthic Biological and Biogeochemical Patterns and Processes Across an Oxygen Minimum Zone (Pakistan Margin, NE Arabian Sea) 56, 422–433. https://doi.org/10.1016/j.dsr2.2008.12.027.

Aranda da Silva, A., Pawlowski, J., Gooday, A.J., 2006. High diversity of deep-sea *Gromia*from the Arabian Sea revealed by small subunit rDNA sequence analysis. Mar. Biol. 148, 769–777. https://doi.org/10.1007/s00227-005-0071-9.

Arnold, Z.M., 1951. Occurence of *Gromia oviformis* Dujardin in California. J Biol 9, 351–353.

Arnold, Z.M., 1972. Observations on the biology of the Protozoan *Gromia oviformis* Dujardin, University of California publications in zoology, v. 100. University of California Press, Berkeley.

Bhattacharya, D., Helmchen, T., Melkonian, M., 1995. Molecular Evolutionary Analyses of Nuclear-Encoded Small Subunit Ribosomal RNA Identify an Independent Rhizopod Lineage Containing the Euglyphina and the Chlorarachniophyta. J. Eukaryot. Microbiol. 42, 65–69. https://doi.org/10.1111/j.1550-7408.1995.tb01541.x.

Bovee, E.C., 1985. Class Filosea Leidy, 1879. In: An Illustrated Guide to the Protozoa. Society of Protozoologists, Lawrence, pp. 228–245.

Bowser, S.S., Marko, M., Bernhard, J.M., 1996. Occurence of *Gromia oviformis* in McMurdo Sound. Antartic Journal of the United States 31, 122–124.

Burki, F., Berney, C., Pawlowski, J., 2002. Phylogenetic Position of *Gromia oviformis* Dujardin inferred from Nuclear-Encoded Small Subunit Ribosomal DNA. Protist 153, 251–260. https://doi.org/10.1078/1434-4610-00102.

Cavalier-Smith, T., Chao, E.E.Y., 2003. Phylogeny and classification of phylum Cercozoa (Protozoa). Protist 154, 341–358. https://doi.org/10.1078/143446103322454112.

Dujardin, F., 1835. Recherches sur les organismes inférieurs. Ann. Sci. Nat. 2, 345–351.

Goldstein, S.T., Bowser, S.S., Richardson, E.A., 2011. Towards understanding the biodiversity of gromiids: the fine structure of *Gromia* sp. from Zane Grey Creek, Florida Keys. Microsc Microanal 17 (Suppl. 2), 348–349. https://doi.org/10.1017/S1431927611002613.

Gooday, A.J., Bowser, S.S., 2005. The Second Species of Gromia (Protista) from the Deep Sea: its Natural History and Association with the Pakistan Margin Oxygen Minimum Zone. Protist 156, 113–126. https://doi.org/10.1016/j.protis.2004.11.002.

Gooday, A.J., Bowser, S.S., Bernhard, J.M., 1996. Benthic foraminiferal assemblages in Explorers Cove, Antarctica: A shallow-water site with deep-sea characteristics. Prog. Oceanogr. 37, 117–166. https://doi.org/10.1016/S0079-6611(96)00007-9.

Gooday, A.J., Bowser, S.S., Bett, B.J., Smith, C.R., 2000. A large testate protist, *Gromia sphaerica* sp. nov. (Order Filosea), from the bathyal Arabian Sea. Deep Sea Res. Part II 47, 55–73. https://doi.org/10.1016/S0967-0645(99)00100-9.

Gooday, A.J., Kamenskaya, O.E., Soltwedel, T., 2013. Basal foraminifera and gromiids (Protista) at the Håkon-Mosby Mud Volcano (Barents Sea slope). Mar Biodiv 43, 205–225. https://doi.org/10.1007/s12526-013-0148-5.

Gooday, A.J., Holzmann, M., Goetz, E., Cedhagen, T., Korsun, S., Pawlowski, J., 2021. Three new species of *Gromia* (Protista, Rhizaria) from western Greenland fjords. Polar Biol 44, 1037–1053. https://doi.org/10.1007/s00300-021-02858-9.

Gooday, A.J., Holzmann, M., Majewski, W., Pawlowski, J., 2022. New species of *Gromia*(Protista, Rhizaria) from South Georgia and the Falkland Islands. Polar Biol 45, 647–666. https://doi.org/10.1007/s00300-022-03017-4.

Gouy, M., Guindon, S., Gascuel, O., 2010. SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Mol Biol Evol 27, 221–224. https://doi.org/10.1093/molbev/msp259.

Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol 59, 307–321. https://doi.org/10.1093/sysbio/syq010.

Hedley, R.H., 1958. Confusion between *Gromia oviformis* and *Allogromia ovoidea*. Nature 182, 1391–1392. https://doi.org/10.1038/1821391a0.

Hedley, R.H., 1960. The Iron-Containing Shell of *Gromia oviformis* (Rhizopoda). J. Cell Sci. s3–101, 279–293. https://doi.org/10.1242/jcs.s3-101.55.279.

Hedley, R.H., 1962. *Gromia oviformis* (Rhizopodea) from New Zealand with comments on the fossil Chitinozoa. NZJ Sci 5, 121–136.

Hedley, R.H., Bertaud, W.S., 1962. Electron-Microscopic Observations of *Gromia oviformis* (Sarcodina). J. Protozool. 9, 79–87. https://doi.org/10.1111/j.1550-7408.1962.tb02585.x.

Hedley, R.H., Wakefield, J.S.J., 1969. Fine structure of *Gromia oviformis* (Rhizopodea : Protozoa). Bulletin of the British Museum (Natural History). Zoology 18, 67–90. https://doi.org/10.5962/p.314181.

Høgslund, S., Cedhagen, T., Bowser, S.S., Risgaard-Petersen, N., 2017. Sinks and Sources of Intracellular Nitrate in Gromiids. Front. Microbiol. 8, 617. https://doi.org/10.3389/fmicb.2017.00617.

Jepps, M.W., 1926. Memoirs: Contribution to the Study of *Gromia oviformis*Dujardin. J. Cell Sci. s2–70, 701–719. https://doi.org/10.1242/jcs.s2-70.280.701.

Lefort, V., Longueville, J.-E., Gascuel, O., 2017. SMS: Smart Model Selection in PhyML. Mol Biol Evol 34, 2422–2424. https://doi.org/10.1093/molbev/msx149.

Lwoff, A., 1925. La sporogenèse chez une Gromie marine: *Gromia dujardini*. Trav Stn Zool Wimereux 9, 140–145.

Matz, M.V., Frank, T.M., Marshall, N.J., Widder, E.A., Johnsen, S., 2008. Giant Deep-Sea Protist Produces Bilaterian-like Traces. Curr. Biol. 18, 1849–1854. https://doi.org/10.1016/j.cub.2008.10.028.

Nikolaev, S.I., Berney, C., Fahrni, J.F., Bolivar, I., Polet, S., Mylnikov, A.P., Aleshin, V.V., Petrov, N.B., Pawlowski, J., 2004. The twilight of Heliozoa and rise of Rhizaria, an emerging supergroup of amoeboid eukaryotes. Proc. Natl. Acad. Sci. 101, 8066–8071. https://doi.org/10.1073/pnas.0308602101.

Nyholm, K.-G., Gertz, I.N.E.Z., 1973. To the biology of the monothalamous foraminifer*Allogromia marina* n. sp. Zoon 1, 89–93.

Ogden, C.G., Hedley, R.H., 1980. An atlas of freshwater testate amoebae. Oxford University Press [for the] British Museum (Natural History), Oxford.

Patterson, D.J., Simpson, A.G.B., Rogerson, A., 2000. Amoebae of uncertain affinities. In: An Illustrated Guide to the Protozoa. Society of Protozoologists, Lawrence, pp. 804–827.

Pavel, A.B., Menabit, S., Pop, I.C., 2021. New records of soft-shelled monothalamous Foraminifera and gromiids on the Romanian Black Sea shelf. Biologia 76, 2241–2251. https://doi.org/10.1007/s11756-021-00708-x.

Piña-Ochoa, E., Høgslund, S., Geslin, E., Cedhagen, T., Revsbech, N.P., Nielsen, L.P., Schweizer, M., Jorissen, F., Rysgaard, S., Risgaard-Petersen, N., 2010. Widespread occurrence of nitrate storage and denitrification among Foraminifera and Gromiida. Proc. Natl. Acad. Sci. 107, 1148–1153. https://doi.org/10.1073/pnas.0908440107.

Revkov, K.E., Boltacheva, A.N., Timofev, A.V., Bondarev, P.I., Bondarenko, V.L., 2018. Macrozoobenthos of the Zernov's Phyllophora field, northwestern Black Sea : Species richness, quantitative representation and long-term variations. Nature Conservation Research 3 (4), 32–43 https://doi.org/10.24189/ncr.2018.045.

Rhumbler, L., 1904. Systematische Zusammenstellung der recenten Reticulosa. Arch Protistenk 3, 181–294.

Rothe, N., Gooday, A.J., Cedhagen, T., Fahrni, J., Hughes, J.A., Page, A., Pearce, R.B., Pawlowski, J., 2009. Three new species of deep-sea *Gromia*(Protista, Rhizaria) from the bathyal and abyssal Weddell Sea, Antarctica. Zool. J. Linn. Soc. 157, 451–469. https://doi.org/10.1111/j.1096-3642.2009.00540.x.

Rothe, N., Gooday, A.J., Cedhagen, T., Hughes, J.A., 2011. Biodiversity and distribution of the genus *Gromia*(Protista, Rhizaria) in the deep Weddell Sea (Southern Ocean). Polar Biol 34, 69–81. https://doi.org/10.1007/s00300-010-0859-z.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A., 2012. Fiji: an open-source platform for biological-image analysis. Nat Methods 9, 676–682. https://doi.org/10.1038/nmeth.2019.

Sergeeva, N.G., 2000. On biological diversity of hydrogen sulfide zona in the Black Sea. Ecologie Maree 50.

Sergeeva, N.G., Anikeeva, O.V., 2006. Myagkorakovinnye foraminifery (Protozoa, Rhizopoda, Allogromiinae) Chernogo morya: vidovoj sostav I raspredelenie. Ekologya morya. 72, 47–56.

Sergeeva, N.G., Mazlumyan, S.A., 2015. Deep-water hypoxic meiobenthic Protozoa and Metazoa taxa of the Istanbul Strait's (Bosporus) outlet area of the Black Sea. Ecologica Montenegrina 2, 255–270.

Sergeeva, N.G., Mazlumyan, S.A., Çagatay, N., Lichtschlag, A., 2013. Hypoxic meiobenthic communities of the Istanbul Strait's (Bosporus) outlet area of the Black Sea. Turk. J. Fish. Aquat. Sci. 13, 33–41. https://doi.org/10.4194/1303-2712-v13_1_05.

Sergeeva, N.G., Ürkmez, D., Dovgal, I.V., Sezgin, M., 2017. Protists (Ciliophora, Gromiida, Foraminifera) in the Black Sea meiobenthic communities. J. Black Sea/Mediterranean Environment 23, 121–155.

Stewart, K., Kassakian, S., Krynytzky, M., DiJulio, D., Murray, J.W., 2007. Oxic, suboxic, and anoxic conditions in the Black Sea. In: Yanko-Hombach, V., Gilbert, A.S., Panin, N., Dolukhanov, P.M. (Eds.), The Black Sea Flood Question: Changes in Coastline, Climate, and Human Settlement. Springer, Netherlands, pp. 1–21. https://doi.org/10.1007/978-1-4020-5302-3_1.

Tendal, O.S., 1979. Aspects of the biology of komokiagea and xenophyophoria. Sarsia 64, 13–17. https://doi.org/10.1080/00364827.1979.10411356.

Wylezich, C., Jürgens, K., 2011. Protist diversity in suboxic and sulfidic waters of the Black Sea. Environ. Microbiol. 13, 2939–2956. https://doi.org/10.1111/j.1462-2920.2011.02569.x.

Zaitsev, Y., 2008. Introduction to the Black Sea Ecology. Smil Edition and Publishing Agency ltd..