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Psammophaga secriensia sp. nov., a New Monothalamid Foraminifera (Protista, Rhizaria) from the Romanian Black Sea Shelf †

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Abstract: Based on molecular and morphological characters, we describe a new species of monothalamous foraminifera, *Psammophaga secriensia* sp. nov., that was sampled from two coastal locations (48 m and 53 m depth) on the Romanian Black Sea continental shelf. Molecular data further confirm its presence in the northeastern part of the Black Sea (Balaklava Bay, 5–10 m depth). Specimens of *Psammophaga secriensia* sp. nov. are characterized by an elongate to broadly pyriform test and a simple rounded aperture. The wall is translucent and the cytoplasm contains mineral grains of different sizes. The genus *Psammophaga*, including *Psammophaga simplora* and several undetermined morphotypes, has been reported from different areas of the Black Sea. Previous research using an integrative taxonomic approach has identified two additional species (*Psammophaga zirconia*; *Psammophaga* sp., Gooday et al., 2011) occurring in the Black Sea. Monothalamids are an important part of the meiobenthos in the Black Sea and our results increase the knowledge of foraminiferal diversity in this marginal sea.

Keywords: *Psammophaga*; new species; Black Sea; SSU rRNA; protist; diversity

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1. Introduction

Benthic foraminifera are a crucial part of marine ecosystems, inhabiting diverse habitats from intertidal areas to the deepest ocean trenches, from brackish to hyper-saline waters and from the tropics to the poles. Due to their unicellular organization and short life cycles, they have a quick response to environmental changes, making them effective environmental indicators [1].

Monothalamid foraminifera are a prominent and diverse component of the benthic fauna in both the coastal and deep regions of the Black Sea. The focus on benthic soft-walled foraminifera is evident in various studies conducted by Golemansky [2,3], Anikeeva, and Sergeeva [4], Anikeeva [5,6], Sergeeva [7], Revkov and Sergeeva [8], Sergeeva and Anikeeva [9], Sergeeva et al. [10,11], and Gooday et al. [12]. Initially, research focused on examining the distribution of monothalamids in coastal waters of the Crimean Peninsula, as highlighted in the publications by Anikeeva and Sergeeva [4], and Anikeeva [5]. Subsequently, Sergeeva and Anikeeva [9], and Sergeeva et al. [11,13] extended monothalamid investigations to explore their taxonomic composition and distribution in deeper regions of the Black Sea, including areas characterized by hypoxic conditions. In a related study, Sergeeva and Anikeeva [14] examined monothalamid foraminifera in shallow water environments affected by hypoxia.

Sergeeva and Anikeeva [9] and Anikeeva [15] were the first to publish a comprehensive list of monothalamid foraminifera found in the Black Sea that contains 40 taxa. Subsequent research by Gooday et al. [12,16], Sergeeva, and Anikeeva [17], Sergeeva and Anikeeva [18], and Anikeeva et al. [19] led to the description of new species within this group.

Psammophaga is an abundant component of meiofaunal communities in certain areas of the Black Sea. *Psammophaga simplora*, Arnold, 1982 has been documented as one of the dominant species in coastal bays in the southwestern part of the Crimea Peninsula. It has been observed in locations such as Omega Bay, Sevastopol Bay, and Sevastopol outer harbor. The species has also been found in other regions of the Black Sea, including Bulgaria, the Caucasus, Turkey, and various southern and southwestern regions of Crimea [14]. An agglutinated undescribed *Psammophaga* was reported to be abundant in Zernov's Phyllophora Field [11]. *Psammophaga zirconia*, Sabbatini, Bartolini, and Morigi, was described from the central Adriatic Sea and is also present in the Crimean area in Kazachya Bay, near Sevastopol [20]. An integrative taxonomic study combining molecular and morphological data revealed an additional, yet undescribed *Psammophaga* species present in Kazachya Bay [16]. Furthermore, new representatives of two genera, *Vellaria* and *Nellya* that are closely related to *Psammophaga*, were also reported in the same study from Balaklava Bay, and a new species of *Vellaria* has recently been described from Sivash Bay in the Sea of Azov [18].

Pavel et al. [21] documented the presence of *Psammophaga* sp. from 29 sampling stations along the Romanian coast, ranging in depth from 20 to 79 m. The morphology of these specimens matches the characteristics of *Psammophaga secriensia* sp. nov., but most specimens of Pavel et al. [21] are considerably larger, and sequencing results are needed for their identification. *Psammophaga secriensia* sp. nov. described herein confirms the high diversity of the genus and reveals the adaptative potential of these monothalamids that thrive in brackish as well as fully marine areas.

2. Materials and Methods

2.1. The Black Sea

The Black Sea is a roughly elliptical basin connected to the Mediterranean Sea, situated between 27°27′ and 41°42′ east longitude and 40°55.5′ and 46°32.5′ north latitude. The entire basin covers 461,000 km^2 including the Sea of Azov, which is linked to the Black Sea by the narrow Kerch Strait. It contains about 547,000 km^3 of water with around 90% of it being anaerobic. The Black Sea has a coastline stretching approximately 4000 km. Its maximum length is 1050 km and its maximum width, near Odessa Bay, reaches 530 km, while the minimum width, near Crimea, is 263 km. The average depth of the basin is 1300 m, with the deepest point reaching 2212 m [22].

The Black Sea, characterized by its almost-complete isolation from other oceans and featuring a deep bathyal basin reaching a maximum depth of 2212 m alongside a vast continental shelf, particularly in the northwestern region, stands out as one of the most remarkable regional seas [23]. The Black Sea's unique water composition is shaped by the influx of freshwater from major rivers, with the Danube exerting the greatest influence, and the inflow of Mediterranean water through the Bosphorus and Dardanelles straits. As a result, the waters of the Black Sea exhibit a permanent stratification [24,25]. Within this ecosystem, life thrives in the upper 100 m of the water column, which has lower salinity and reduced density. A distinct pycnocline separates this upper layer from the deeper, denser, anoxic, and sulfidic layers that occupy depths below 100–200 m. [26].

2.2. Site Locations

Samples were collected at two stations (100C and SU04) located on the Romanian continental shelf of the Black Sea, east of the Sulina estuary (Figure 1), during two RV Mare

Nigrum expeditions. Site 100C was sampled at 48 m depth on the 14 of June 2021 during expedition MN219, while site SU04 was sampled at 53 m depth on the 17 of August 2021 during expedition MN222. The recorded temperatures of the bottom water at the two sites were 7.47 °C and 8.19 °C, while the salinity was 18.69 and 18.71 PSU, respectively. Both sampling stations, SU04 and 100C, have substrates composed of mixed sediments including mud, sand, and shells, in the offshore circalittoral marine habitat of *Modiolula phaseolina* (Philippi, 1844), classified according to the Commission Decision (EU) 2017/848, using the classification system of the European nature information system (EUNIS). One specimen of the newly described species was collected in 2006 as part of another study in the shallow northern part of the Balaklava Bay, Ukraine [16], at a depth of 5–10 m. In Table 1, environmental parameters are given for the bay as a whole, rather than for a particular station. The bay is characterized by a substrate consisting of sand and silt. For additional environmental parameters, see Table 1.

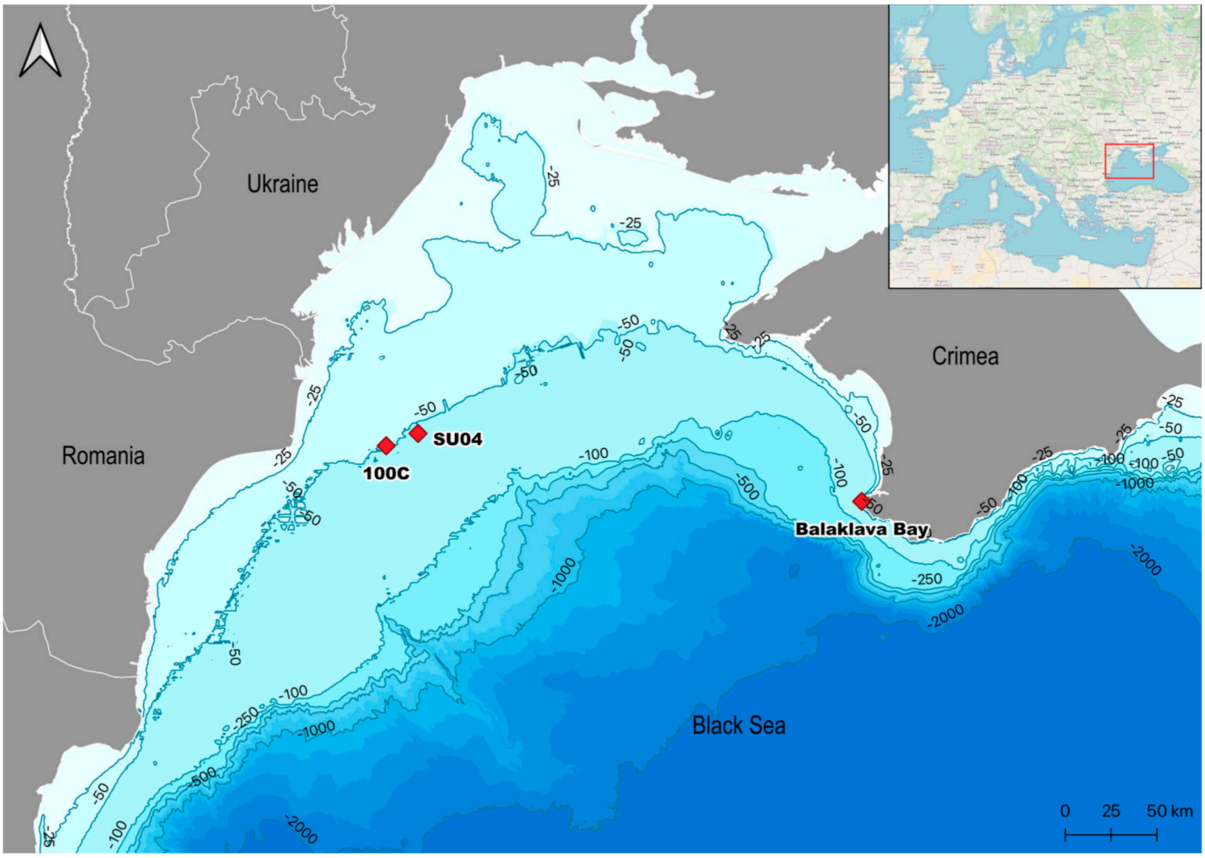

Figure 1. Map of sampling locations on the Romanian continental shelf of the Black Sea.

Table 1. Summary of sampling parameters for stations where *Psammophaga secriensia* sp. nov. occurred. * Parameters for Balaklava Bay are from Gooday et al. [16] and given as ranges.

Station	Date	Lat. °N	Long. °E	Depth [m]	pH	Salinity [PSU]	Conductivity [mS m^{-1}]	Temperature °C
100C	06/21	44°40′30″	31°14′15″	48	8.5	18.69	20.21	7.47
SU04	08/21	44°54′00″	30°27′00″	53	8.66	18.71	20.62	8.19
Balaklava Bay *	06/09	44°29′42″	33°35′39″	4−34	7.43−8.8	14.4−18.3	N/A	6.8−26.8

2.3. Sample Collection and Sample Processing

Meiobenthic samples were collected from the top 5 cm layer of sediment in one of the four tubes of a Multicorer Mark-400 with a cross-sectional area of 78.5 cm^2 (Ø 10 cm), deployed from the Mare Nigrum research vessel. Samples were washed on the ship through a 90 µm mesh sieve. The supernatant was subsequently stored in laboratory jars with seawater and placed in a refrigerator at 4 °C for up to two days, with daily changes of seawater. Specimens were sorted on board, counted using an Olympus SZ61 Stereomicroscope mounted with an IDS uEye camera, and photographed with the uEye Cockpit v4.20 program. Specimens intended for genetic analyses were individually transferred to Eppendorf tubes containing 200 µL of RNAlater solution and stored at −20 °C. Specimens designated for morphological analyses were kept in a 4% formaldehyde solution at room temperature and subsequently stored in 95% ethanol. Water column parameters including depth, salinity, temperature, pH, dissolved oxygen, and conductivity in the water column were measured using a CTD Rosette (CTD SBE 25 and rosette model SBE 32 equipped with twelve 5 L Niskin bottles) launched prior to the Multicorer in order to avoid any resuspension of the sediment.

In the laboratory, foraminifera were sorted under a Carl Zeiss Stemi 508 stereomicroscope equipped with an Axiocam 208 color with Zeiss Image software (ZEN 3.2—blue edition) and a Carl Zeiss PrimoStar microscope and identified based on the classification of Loeblich and Tappan [27]. Individual foraminifera were measured from pictures using the ImageJ v2.3.0 [28] program against a known scale.

Samples from the Balaklava Bay followed a similar methodology [16]. A scuba diver collected the surface sediment layer and the samples were sieved on 63 µm mesh screens. The collected specimen was stored in guanidine lysis buffer for molecular analysis.

2.4. Scanning Electron Microscopy Procedure

For scanning electron microscopy (SEM), selected specimens stored in ethanol were rehydrated and fixed with 3% glutaraldehyde in sodium cacodylate buffer (0.1 M, pH 7.4). The specimens were then dehydrated using an ascending alcohol series (30%, 50%, 70%, 80%, 90%, and 100% ethanol) for 30 min each time. Foraminifera were afterwards transferred to a mixed solution of ethanol and hexamethyldisilazane (HMDS) in various ratios (3:1, 1:1, and 1:3), and finally into 100% HMDS, where they were left overnight to allow for chemical evaporation and complete drying. The dried specimens were mounted on aluminum stubs covered with conductive double-sided adhesive carbon tabs, and then sputter-coated with gold for 60 s using an SEM Coating Unit E5100. The *Psammophaga* individuals were then analyzed and photographed using a Phenom Pro scanning electron microscope (Phenom-World, Thermo Fisher Scientific, The Netherlands) at a 10 kV acceleration voltage [29].

2.5. DNA Extraction, Amplification, and Sequencing

Six *Psammophaga* specimens (Accession number: OQ845893, OQ845894, OQ845895, OQ845896, OQ845897, OQ845898) were extracted individually using guanidine lysis buffer [30]. Semi-nested PCR amplification was carried out for the 18S barcoding fragment of foraminifera [31] using primers s14F3 (ACGCAMGTGTGAAACTTG)-sB (TGATCCTTCTGCAGGTTCACCTAC) for the first and primers 14F1 (AAGGGCACCACAAGAACGC)-sB for the second amplification. Thirty-five and 25 cycles were performed for the first and the second PCR, with an annealing temperature of 50 °C and 52 °C, respectively. 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) and analyzed on a 3130XL Genetic Analyzer (Applied Biosystems). The resulting sequences were deposited in the NCBI/GenBank database. Isolate and accession numbers are specified in Table 2.

Table 2. Information on isolate and accession numbers and sampling locations of sequenced specimens**.** *Psammophaga secriensia* sp. nov. specimens (marked in bold) were investigated for the current study.

Species	Isolate Number	Accession Number	Sampling Location
***Psammophaga secriensia* sp. nov.**	9486	OQ845891, OQ845892	Ukraine, Black Sea, Balaklava Bay
***Psammophaga secriensia* sp. nov.**	21476	OQ845893	Romania, Black Sea, MN219_100C
***Psammophaga secriensia* sp. nov.**	21480	OQ845894	Romania, Black Sea, MN219_100C
***Psammophaga secriensia* sp. nov.**	21482	OQ845895	Romania, Black Sea, MN222_SU04
***Psammophaga secriensia* sp. nov.**	21483	OQ845896	Romania, Black Sea, MN222_SU04
***Psammophaga secriensia* sp. nov.**	21484	OQ845897	Romania, Black Sea, MN222_SU04
***Psammophaga secriensia* sp. nov.**	21485	OQ845898	Romania, Black Sea, MN222_SU04
Psammophaga magnetica, Pawlowski and Majewski, 2011	2976	FN995274	Antarctica, Admiralty Bay
Psammophaga magnetica, Pawlowski and Majewski, 2011	3184	FN995272	Antarctica, Admiralty Bay
Psammophaga fuegia, Gschwend, Majda, Majewski, and Pawlowski, 2016	17392	KU313692	Chile, Patagonia
Psammophaga fuegia, Gschwend, Majda, Majewski, and Pawlowski, 2016	17510	KU313694	Chile, Patagonia
Psammophaga sp., Gooday et al., 2011	10102	FN995301	Ukraine, Black Sea, Kazachya Bay
Psammophaga sp., Gooday et al., 2011	10112	FN995303	Ukraine, Black Sea, Kazachya Bay
Psammophaga sapela, Altin-Ballero, Habura, and Goldstein, 2013	231	AJ317985	USA, Sapelo Island
Psammophaga sapela, Altin-Ballero, Habura, and Goldstein, 2013	not applicable	HM584698	USA, Sapelo Island
Psammophaga crystallifera, Dahlgren, 1962	2361	FN995293	Sweden, Tjaerno
Psammophaga crystallifera, Dahlgren, 1962	8145	FN995300	Antarctic Peninsula, King George Island
Niveus flexilis, Altin, Habura, and Goldstein, 2009	RnGwhiteallo	EU213257	USA, coastal Georgia
Niveus flexilis, Altin, Habura, and Goldstein, 2009	6R	KF841626	USA, coastal Georgia
Psammophaga zirconia, Sabbatini, Bartolini, and Morigi, 2016	18412	LN886768, LN886769	Italy, Adriatic Sea
Vellaria pellucida, Gooday and Fernando, 1992	10165	FN995322	Ukraine, Black Sea, Balaklava Bay
Vellaria pellucida, Gooday and Fernando, 1992	10168	FN995327	Ukraine, Black Sea, Balaklava Bay
Nellya rugosa, Gooday, Anikeeva, and Pawlowski, 2010	10153	FN995337	Ukraine, Black Sea, Balaklava Bay
Nellya rugosa, Gooday, Anikeeva, and Pawlowski, 2010	10154	FN995331	Ukraine, Black Sea, Balaklava Bay

2.6. Phylogenetic Analysis

The obtained sequences were added to 20 sequences belonging to four different genera (*Nellya*, *Niveus*, *Psammophaga*, *Vellaria*,) that are part of the publicly available 18S database of monothalamid foraminifera (NCBI/Nucleotide; https://www.ncbi.nlm.nih.gov/nucleotide/ (accessed on 19 April 2023)). All sequences were aligned using the default parameters of the MUSCLE automatic alignment option, as implemented in SeaView vs. 4.3.3. [32]. The alignment contains 26 sequences with 1201 sites used for analysis.

The phylogenetic tree was constructed using maximum likelihood phylogeny (PhyML 3.0) as implemented in ATGC: PhyML [33,34]. An automatic model selection by SMS [35] based on Akaike Information Criterion (AIC) was used, resulting in a HKY85 + G substitution model being selected for the analysis. The initial tree is based on BioNJ. Bootstrap values (BV) are based on 100 replicates.

3. Results

Systematic description

Due to the ambiguous classification of the monothalamids, we opt to use the informal name Monothalamids Pawlowski et al. [36] instead of the formal term "Monothalamea" for this paraphyletic group, as suggested by Pawlowski et al. [36].

RHIZARIA Cavalier-Smith, 2002

RETARIA Cavalier-Smith, 1999

FORAMINIFERA d'Orbigny, 1826

Psammophaga, Arnold, 1982

Psammophaga secriensia Pavel, Kreuter and Holzmann sp. nov.

Zoobank Registration

urn: lsid: zoobank.org: act:4B4D99D8-E66B-43B8-96CA-5ADB44386627

Diagnosis:

The test is free and monothalamous, with an elongate to broadly pyriform shape, ranging from 569 µm to 750 µm in length, from 250 µm to 371 µm in width, and with a length/width ratio between 1.8 and 2.9. The aperture is simple and rounded, situated at the terminal end of a short neck that is directed distally. The organic test wall is transparent and delicate, with a smooth and shiny surface. In most specimens, few dispersed sediment particles adhere to it. No peduncle has been observed.

Type Material:

Holotype (accession number FORAM01) and paratype (accession number FORAM01_1) from station 100C, longitude 31°14′15″ E, latitude 44°40′30″ N, 48 m depth and paratypes (accession numbers FORAM01_2; FORAM01_3; FORAM01_4; and FORAM01_5) from station SU04, longitude 30°27′00″, latitude 44° 54′00″, 53 m depth are stored in 95% ethanol and deposited at the National Institute of Marine Geology and GeoEcology, Romania.

Other Material: Seven sequenced specimens (Accession numbers—OQ845891, OQ845892, from Balaklava Bay, OQ845893, OQ845894 from station 100C, OQ845895-OQ845898) from station SU04.

Etymology:

Named after the former director from NIRD GeoEcoMar Constanta Branch—Dan Secrieru, who supports genetic studies of foraminifera in the Black Sea.

Description:

The holotype FORAM01 has an elongate test measuring 582 µm in length and 250 µm in width (Figures 2 and 3, Table 3) with a simple rounded terminal aperture located on a short neck that is directed distally. The aperture is flexible enough to allow the passage of sediment grains. The ingested mineral particles are distributed throughout the test interior. Inclusions consist of dark and light transparent particles identified as mica and quartz, respectively. The cell body does not fill entirely the test lumen. The organic test wall is thin and transparent, with a shiny surface, and sparsely dispersed sediment particles covering it. A peduncle could not be observed. The paratype FORAM01_1, which was also obtained from station 100C, measures 750 µm in length and 259 µm in width (Figure 4A, Table 3).

Paratypes FORAM01_2, FORAM01_3, FORAM01_4, and FORAM01_5 were collected at station SU04 (Figure 4B,C, Table 3). They range from 569 µm to 637 µm in length and from 230 µm to 345 µm in width. All paratypes display a terminal rounded aperture placed on a short neck. The ingested mineral particles in the paratypes are concentrated at the adapertural region rather than being distributed throughout the entire test. The paratypes lack sediment particles adhering on their test wall. Sequenced specimens (Accession numbers OQ845893–OQ845898) range from 569 µm to 740 µm in length and 230 µm to 371 µm in width (Figure 5). Overall, specimens have a length between 569 µm and 750 µm (mean = 625 ± 67 µm, n = 12), a width between 250 µm and 371 µm (mean = 286 ± 47 µm, n = 12), and a length/width ratio between 1.8 and 2.9 (mean = 2.2 ± 0.5, n = 12) (Table 3).

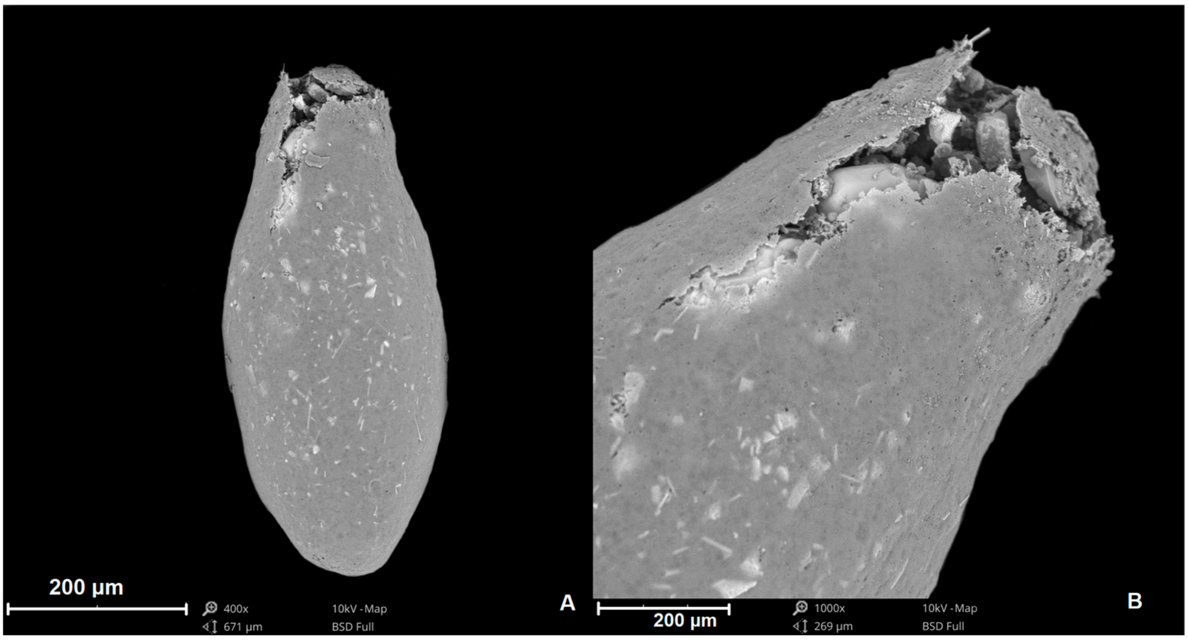

Figure 2. *Psammophaga secriensia* sp. nov. SEM images: (**A**) Holotype FORAM01, view of the whole specimen with sparse mineral grains adhering to the test surface. (**B**) Detail of aperture and test wall partly ripped open; the test is filled with organic matter and mineral grains, the latter whitish to greyish colored. Scale bars 200 µm.

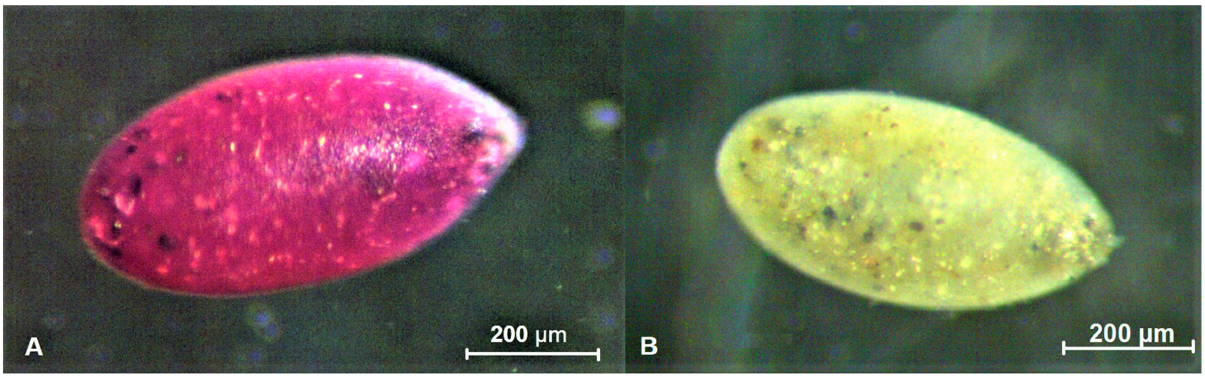

Figure 3. *Psammophaga secriensia* sp. nov.: light microscopic images: (**A**) Holotype FORAM01 colored with Rose Bengal. (**B**) Specimen OQ845897. Ingested mineral grains are distributed throughout the test. Scale bars 200 µm.

Table 3. Morphological measurements for *Psammophaga secriensia* sp. nov.

Species	Accession Number	Length (μm)	Width (μm)	Sampling Station	Depth (m)
Psammophaga secriensia sp. nov.	Holotype FORAM01	582	250	100C	48
	Paratype FORAM 01_1	750	259	100C	48
	Paratype FORAM 01_2	637	345	SUO4	53
	Paratype FORAM 01_3	587	320	SUO4	53
	Paratype FORAM 01_4	577	275	SUO4	53
	Paratype FORAM 01_5	569	230	SUO4	53
	OQ845893	666	315	100C	48
	OQ845894	740	259	100C	48
	OQ845895	573	230	SUO4	53
	OQ845896	569	371	SUO4	53
	OQ845897	637	318	SUO4	53
	OQ845898	597	256	SUO4	53

Figure 4. *Psammophaga secriensia* sp. nov. Light microscopic images: (**A**) Paratype specimen FORAM01_1. (**B**) Paratype specimens FORAM 01_2 and FORAM01_3. (**C**) Paratype specimen FORAM01_4 and FORAM01_5. Ingested mineral grains are accumulated more at the adapertural region and the end of the test. Scale bars 200 μm.

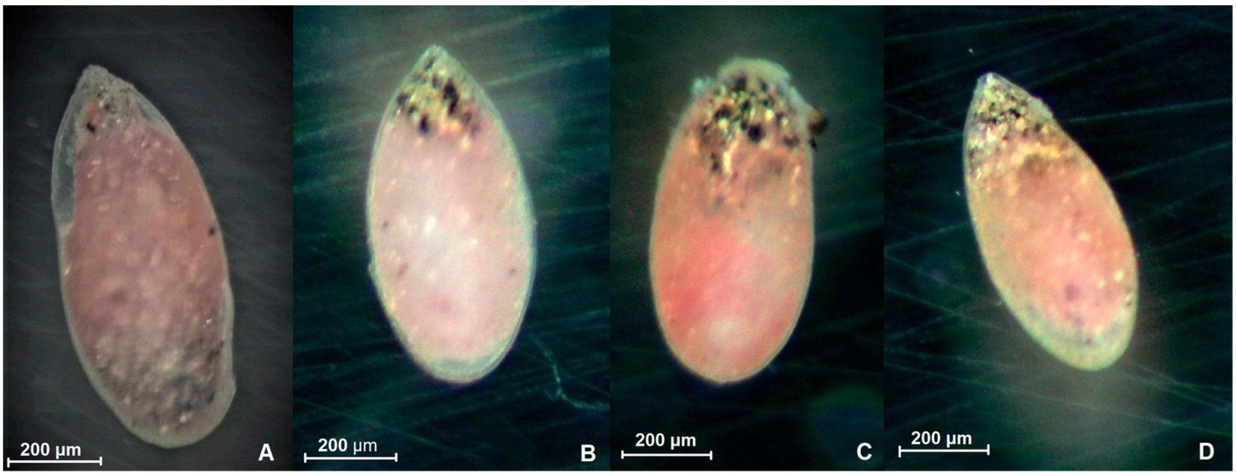

Figure 5. *Psammophaga secriensia* sp. nov. Light microscopic images of sequenced specimens: (**A**) Specimen OQ845894. (**B**) Specimen OQ845896. (**C**) Specimen OQ845898. (**D**) Specimen OQ845895. Scale bars 200 μm.

Molecular characteristics

Psammophaga secriensia sp. nov. (91% BV) branches as sister to *Psammophaga magnetica* with moderate support (78% BV) (Figure 5). The partial SSU rRNA sequences contain 956–962 nucleotides, the GC content ranges from 45 to 46%.

Distribution:

Specimens were present in two sampling stations across the Romanian Black Sea continental shelf between the towns of Mangalia and Sulina and in the Balaklava Bay, Ukraine.

Habitat:

Specimens were sampled from the *Modilula phaseolina* habitat and in mixed sediments including mud, sand, and shells, in the offshore circalittoral marine environment at 48 m and 53 m depth. One specimen (Accession numbers—OQ845891, OQ845892) was collected from Balaklava Bay between 5 and 10 m depth in a previous sampling campaign (Gooday et al. [16], Figure 1). Bottom sediments in the Bay consist of sand, silt, pebbles and shell gravel [16].

Remarks:

Psammophaga secriensia sp. nov. differs from the type species, *Psammophaga simplora* in the general shape, which is elongate to broadly pyriform in the new species and pyriform to ovoidal in *Psammophaga simplora*. The latter species is also smaller, measuring less than 500 µm in length while *Psammophaga secriensia* sp. nov. measures from 569 µm to 750 µm in length. In *Psammophaga simplora*, mineral particles are concentrated around the apertural region while in *Psammophaga secriensia* sp. nov. inclusions are either distributed throughout the test interior or assembled around the adapertural part. *Psammophaga simplora* is finely agglutinated while *Psammophaga secriensia* sp. nov. has only a sparsely agglutinated test wall. A peduncle is absent in both species.

The new species can be distinguished morphologically from *Psammophaga zirconia* by the absence of a peduncle. *Psammophaga zirconia* has a pyriform, elongate or spherical shape and typically ranges in length from 200 µm to 700 µm [20], while *Psammophaga secriensia* sp. nov. is slightly larger, ranging in length from 569 µm to 750 µm.

Sergeeva et al. [13] identified *Psammophaga* sp. specimens from the northwestern Black Sea with a thicker agglutinated test and a cap-like form of the aperture, which are considerably smaller than *Psammophaga secriensia* sp. nov., ranging in length from 300 µm to 330 µm and in width from 115 µm to 145 µm.

Gooday et al. [16] described *Psammophaga* sp. from Kazachya Bay, which has a droplet-like shape compared to the elongate-pyriform shape of *Psammophaga secriensia* sp. nov. *Psammophaga* sp. is also smaller, measuring 270 µm to 470 µm in length, and sequenced specimens (FN995301, FN995303) show that it is a distinct species that does not branch with *Psammophaga secriensia* sp. nov. (Figure 6).

The morphology of *Psammophaga* sp. described by Pavel et al. [21] from the Romanian Continental Shelf closely resembles *Psammophaga secriensia* sp. nov. However, *Psammophaga* sp. of Pavel et al. [21] exhibits different dimensions in terms of length (719–1440 µm; mean = 894 $\pm$ 199 µm, n = 23), and width (462 µm to 765 µm; mean = 532 $\pm$ 93 µm, n = 23) compared to *Psammophaga secriensia* sp. nov. (length: 569 µm to 750 µm; width: 230–371 µm) (Table 3). A molecular analysis of *Psammophaga* sp. sensu Pavel et al. [21] will be needed to clarify whether this is a different species.

Phylogeny

The phylogenetic tree (Figure 6) contains 26 sequences of monothalamid foraminifera belonging to clade E as specified by Pawlowski et al. [36]. The new species also contains two sequences obtained from a specimen (Accession number OQ845891, OQ845892) sampled from Balaklava Bay on the Ukrainian side of the Black Sea. *Psammophaga secriensia* sp. nov. is well supported (86% BV) and branches as sister to *Psammophaga magnetica* with moderate support (78% BV). Four other *Psammophaga* species and *Niveus flexilis* branch at the base of the sister clade. Each species is strongly supported (100% BV), but relationships between these different taxa are not backed up by bootstrap values. *Psammophaga zirconia* (99% BV), *Vellaria pellucida* (70% BV) and *Nellya rugosa* (100% BV) branch at the base.

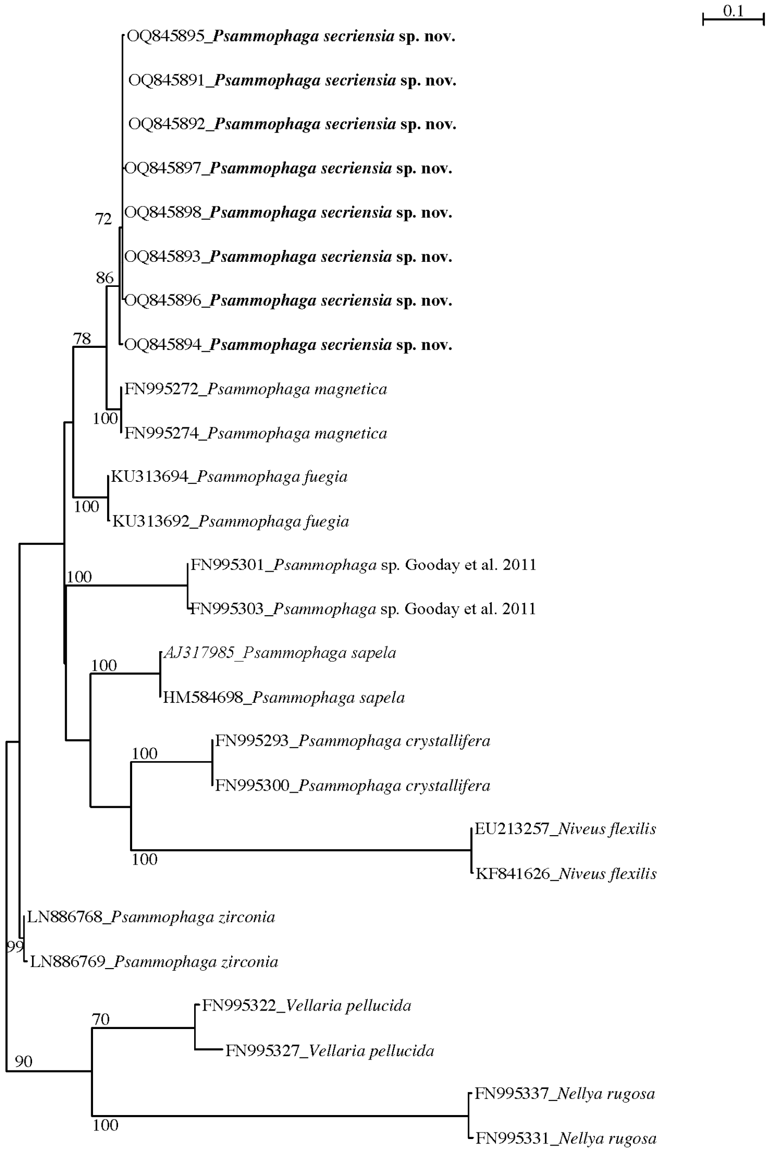

Figure 6. PhyML phylogenetic tree based on the 3′end fragment of the SSU rRNA gene, showing the evolutionary relationships of 26 foraminiferal sequences belonging to Clade E [16,20,37–39]. Specimens marked in bold indicate those for which sequences were acquired for the present study. The tree is unrooted. Specimens are identified by their accession numbers. Numbers at nodes indicate bootstrap values (BV). Only BV > 70% are shown.

4. Discussion

In the Black Sea, monothalamous foraminifera thrive in coastal habitats, forming abundant populations with densities reaching hundreds of individuals per 0.001 m^2 [9]. A recent study has shown that monothalamids in the Black Sea display population densities reaching up to 12.103 individuals/m^2 at depths ranging from 41 to 60 m [21]. On the Crimean Peninsula, *Psammophaga* exhibits higher densities, with an average of 86,000 to 116,000 individuals/m^2 [9,40]. Furthermore, the Crimean Peninsula has a particularly high diversity of monothalamid foraminifera, with over 20 species identified [8]. *Psammophaga* especially inhabits meiobenthic habitats in the southwestern region of Crimea, at a depth range of 142 to 260 m [15]. Additionally, specimens of this genus have been found in areas with methane seeps, at depths ranging from 77 to 172 m [40]. According to Sergeeva and Mazlumyan [40], it indicates that certain organisms such as gromiids, allogromiids, hydrozoa, nematodes, and polychaetes successfully adapted to survive in hypoxic/anoxic and sulfidic conditions within the Black Sea. This fauna is native to the region and not transported from nearby oxygenated areas.

The abundance of monothalamous foraminifera correlates with the availability of organic matter on the seafloor [15]. These foraminifera display opportunistic behavior, responding to pulses of high-quality organic carbon. This suggests a potential role for monothalamids as indicators of shallow-water benthic eutrophication [15].

The three *Psammophaga* species from the Black Sea for which sequences are available, *Psammophaga secriensia* sp. nov., *Psammophaga zirconia*, *Psammophaga* sp. of Gooday et al. [16], are not closely related (Figure 6). *Psammophaga secriensia* sp. nov. branches with *Psammophaga magnetica* [37] that was described from Admiralty Bay in West Antarctica and *Psammophaga fuegia* sampled from the Beagle Channel in South Chile [38,39], but the branching is not supported by bootstrap values. *Psammophaga* sp. [16] from Kazachya Bay and *Psammophaga zirconia* branch separately (Figure 6).

Psammophaga zirconia also occurs in the Mediterranean Sea and sequence data for *Psammophaga* sp., Sabbatini, Bartolini, and Morigi, 2016, show that this type is present in Southampton [16,20]. *Psammophaga secriensia* sp. nov. has so far only been sampled from the Black Sea continental shelf but our knowledge about its biogeographic distribution is restricted and additional investigations will be necessary to determine whether the new species is endemic.

To conclude, our results confirm the high diversity of *Psammophaga* and show that the Black Sea is a promising area for further research on monothalamid foraminifera.

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