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Psammophaga fuegia sp. nov., a New Monothalamid Foraminifera from the Beagle Channel, South America

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Abstract. *Psammophaga fuegia* is a new monothalamid foraminifera discovered in surface sediment samples in the Beagle Channel, South America. The species is a member of the important, globally distributed genus *Psammophaga*, which has the ability to ingest and store mineral particles inside the cytoplasm. Its shape is ovoid to pyriform, the size varies from 250 to 600 µm in length and from 200 to 400 µm in width. Like other *Psammophaga* species *P. fuegia* has a single aperture. It was found in multiple samples across the Beagle Channel area at water depths of 4 to 220 meters and in environments as variable as fjords, the main channel, and the harbour of Puerto Williams (Chile). The occurrences of the new species in environmental DNA and RNA samples correspond well to its distribution inferred from the microscopic study.

Key words: Foraminifera, Monothalamea, taxonomy, molecular phylogeny, eDNA

INTRODUCTION

Monothalamid foraminifera are a paraphyletic group of single-chambered organic-walled or agglutinated species, comprising the former, morphologically characterized orders Allogromiida and Astrorhizida. They are widespread and diverse, probably outnumbering other better-known calcareous foraminifera in their genetic diversity (Pawlowski *et al*. 2013). Research on the diversity and the phylogeny of this group has increased during the last decades (e.g. Gooday 2002; Pawlowski *et al*. 2002; Majewski *et al*. 2005, 2007; Sabbatini *et al*. 2013). Monothalamids are especially abundant and diverse in polar and deep-sea settings; nevertheless they are also common in temperate coastal regions (Habura *et al*. 2008).

Psammophaga are cosmopolitan monothalamous foraminifera that occur in shallow marine habitats. The genus has been reported from Monterey Bay (Arnold 1982), Antarctica (Gooday *et al*. 1996; Majewski *et al*. 2007, 2015; Pawlowski *et al*. 2008; Pawlowski and Majewski 2011), Svalbard (Gooday *et al*. 2005, Majewski *et al*. 2005, Sabbatini *et al*. 2007), the northern Black Sea (Anikeeva 2005), the west Atlantic (Habura *et al*. 2008, Altin-Ballero *et al*. 2013), and from several locations in Europe such as Gullmar Fjord (Dahlgren 1962), the south coast of England (Larkin and Gooday 2004),

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and the Adriatic Sea (Sabbatini *et al*. 2013). Additionally, specimens have been collected at different localities for DNA studies, which include coastal regions of Scotland, Wales, Denmark, New Zealand, and Panama. Despite these abundant records and the fact that *Psammophaga* species are often dominant parts of the monothalamid communities, there are only four species that have been formally described: *Psammophaga simplora* from Monterey Bay (Arnold 1982), *Psammophaga magnetica* from Antarctica (Pawlowski and Majewski 2011), *Psammophaga crystallifera* from Sweden (described as *Allogromia crystallifera* by Dahlgren 1962 and transferred to *Psammophaga* by Pawlowski and Majewski 2011), and *Psammophaga sapela* from Georgia (Altin-Ballero *et al*. 2013). In molecular phylogenetic studies, all *Psammophaga* sequences cluster together in Clade E and form the sister group to *Vellaria* (Pawlowski *et al*. 2002, 2011).

All *Psammophaga* species show similar morphologies, the shape varies from spherical to droplet-shaped and elongate. They have transparent cell walls which are either finely agglutinated or organic. A single aperture symmetrically placed and of varying form opens to the exterior; only rarely individuals with two apertures at opposite ends of the cell have been found (Figure 2I in Pawlowski and Majewski 2011). The eponymous character of the genus and its most visible feature is the presence of numerous mineral particles inside the cytoplasm. Arnold (1982, p.76) has described this nicely in the original genus description: 'Though foraminifera are notorious gourmands, few ingest mineral matter with this one's avidity'.

Here, we describe a new *Psammophaga* species, which was found in the southern limits of South America. We characterize it morphologically and genetically and we analyse its distribution using environmental DNA and RNA data obtained with a high-throughput sequencing approach. Finally, we discuss the global diversity of *Psammophaga*.

METHODS

Sampling

Samples were collected in February 2013 at multiple locations in the Beagle Channel area (Fig. 1). Our target was to sample the most diversity possible and we did not aim to get quantitative data on the organisms' frequencies. The sediment was collected using a Van Veen grab which was operated from the 'Northanger' sailing boat (http://www.northanger.org). This yielded usually undisturbed surface sediments of which we took the upper 2 cm. About 2 g of sediment were immediately put into Life Guard solution for environmental DNA and RNA study. The rest was gently strained with sea water using different mesh-sized sieves (1 mm, 500 µm, 125 µm, 63 µm). The sieved samples were stored separately at cold temperatures (< 5°C). *Psammophaga* specimens were then picked from the 500–125 µm fraction under a stereomicroscope. The living cells were put into guanidine buffer or RNAlater for DNA extraction or fixed in formalin for further morphological study.

Microscopy

Isolated living specimens were photographed with a Leica stereomicroscope before putting them into DNA extraction buffer or formalin. Fixed specimens were photographed by bright field light microscopy in the laboratory in Geneva (Nikon Eclipse Ti, Nikon Instruments). Some specimens were flattened by putting a cover slip directly on the specimens for light microscopic pictures. This step

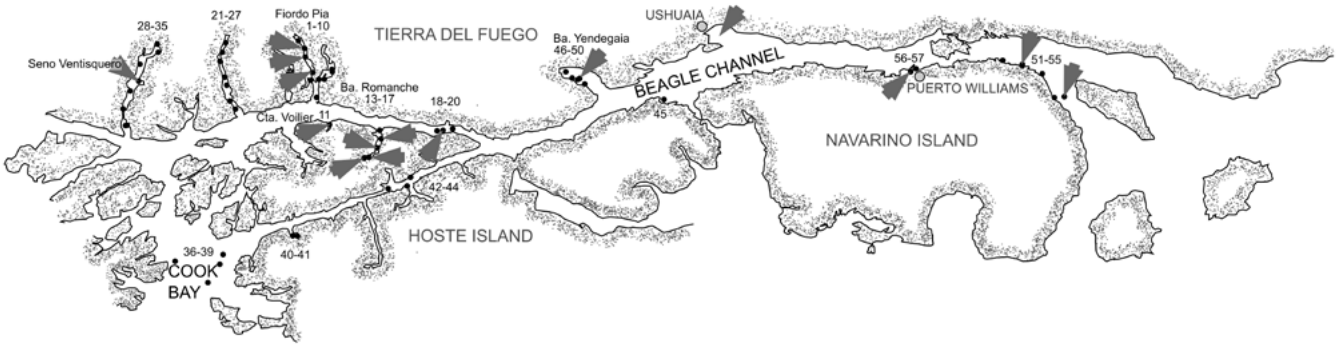

Fig. 1. Map of the Beagle Channel area. The sampling sites are indicated by black dots and their correspondent numbers are shown in groups. *Psammophaga fuegia* was recovered by microscopy and/or environmental sequencing at fifteen sites that are highlighted by grey arrows. Specimens found in Ushuaia were sampled during a previous expedition.

allowed to take better pictures, but slightly distorted the cells, and the aperture of some specimens was damaged.

Mineral particles

For analysing mineral particles a Phillips XL20 scanning electron microscope (SEM) was used in SE and backscattered (BSE) modes, the latter for emphasizing differences in specific gravity. The chemical composition of selected mineral grains was analysed using an energy dispersive X-ray spectrometer (EDS) detector. These results were the basis for mineral analyses using Mineral Element Composition Search at Mineralogy Dataset website (www.webmineral.com/help/Composition.shtml).

Molecular Analyses

DNA from single cells was extracted following the protocol established by Pawlowski (2000). A nested PCR enabled the amplification of part of the SSU rDNA sequence. The primers used were s14F3-newB for the first amplification and s14F1-newB for the nested reamplification. In some cases, we used the 20R primer instead of newB. The amplification with s14F1-20R yields a shorter fragment. Primer sequences are as following: s14F1 (5'-AAG GGC ACC ACA AGA ACG C-3'), s14F3 (5'-ACG CAM GTG TGA AAC TTG-3'), newB (5'-TGC CTT GTT CGA CTT CTC-3'), 20R (5'-GAC GGG CGG TGT GTA CAA-3'). The PCR products were purified with the High Pure PCR Purification Kit (Roche Diagnostics) and cloned using Topo Cloning vectors (Invitrogen) and ultracompetent cells XL2-Blue MRF (Stratagene). For the sequencing reaction we used the BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems) and to sequence a 3130XL Genetic Analyser (Applied Biosystems). We deposited the sequences in GenBank under the accession numbers KU313686–KU313698.

The sequences were manually aligned to 64 other *Psammophaga* sequences and 3 sequences of *Vellaria zucchellii* were added as outgroup. The software SeaView v.4.5.2 (Gouy *et al*. 2010) was used both for the alignment and the construction of the phylogenetic tree; for the latter we chose the implemented PhyML tool with 100 repetitions to calculate the bootstrap values. The best fitting evolutionary model, GTR+G, and the gamma value were evaluated with MEGA software version 6 (Tamura *et al*. 2013).

The extraction of environmental RNA and DNA was done using the PowerSoil Total RNA Isolation Kit and its accessory kit for DNA elution (DNA Elution Accessory Kit, both from MoBio). The kits were used following the manufacturer's instructions. eRNA was cleaned from possible DNA carry-over by a double DNAse treatment. Superscript III RT Polymerase was used for the retrotranscription of eRNA. Each extract of a sample was double tagged by PCR with primers s14F1 and s15 (5'-CCACCTATCACAYAATCATG-3'). The primers were tagged by a five nucleotide-long sequence at their 5' end. The tagged PCR products were pooled equimolarily and sent to Fasteris SA (Plan-les-Ouates, Switzerland) for library preparation and sequencing with MiSeq (Illumina). Computational analysis was done using a pipeline developed in our laboratory. This pipeline as well as further details on the eDNA analysis are explained in Pawlowski *et al*. (2014).

To minimize the effect of possible contaminations and technical errors we thoroughly filtered the reads. Three filtering steps were done: i) an abundance filter (> 10 reads), ii) taking only into account reads of samples where we found them in both the DNA and RNA data, and iii) a mistag filter as developed by Esling *et al*. (2015).

RESULTS

Systematics

Following the newest classification of protists (Adl *et al*. 2012), Foraminifera are classified in the Rhizaria supergroup. Monothalamous foraminifera constitute a highly diverse paraphyletic assemblage of basal lineages (Pawlowski *et al*. 2013) that has not been classified yet on family and order levels.

Supergroup Rhizaria Cavalier-Smith, 2002
Phylum Foraminifera d'Orbigny, 1826
'Monothalamea' (Pawlowski *et al*. 2013)
Clade E (Pawlowski *et al*. 2002)
Genus *Psammophaga* Arnold, 1982
Psammophaga fuegia sp. nov.

Type material: The holotype (Fig. 2; MHNG INVE 92483) and 19 paratypes are fixed in formalin and deposited in the Museum of Natural History of Geneva (MHNG). Eight paratypes from Bahia Romanche (Fig. 3–8, 9, 11, 12, 13; MHNG INVE 92484) and one paratype from Caleta Voilier (Fig. 3–10; MHNG INVE 92485) have been photographed. 10 additional paratypes (MHNG INVE 92486) from Bahia Romanche have been deposited.

Diagnosis: Test free, single-chambered, 0.25–0.6 mm in length, < 0.2–0.4 mm in width, ovoid to pyriform, pointed ends; single aperture, absent or small neck; wall transparent, organic or agglutinated; cytoplasm granular, white to grey, single nucleus visible in some specimens; numerous mineral inclusions, in gen-

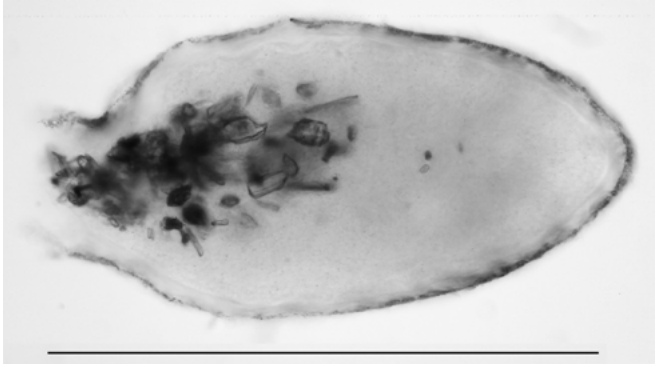

Fig. 2. Holotype of *Psammophaga fuegia* from Bahia Romanche. Scale bar: 500 µm.

eral concentrated at the apertural end; partial 18S gene sequence distinct from other *Psammophaga* species; found in the Beagle Channel area.

Etymology: The species is named after Fuegia Basket, an indigenous woman from the Beagle Channel area, who had been kidnapped and named by Captain Fitz Roy during his first expedition to the area. She was later returned to this place – during the Beagle's most famous expedition with Charles Darwin on board (http://www.gutenberg.org/ebooks/3704).

Description: *Psammophaga fuegia* is an ovoid to pyriform monothalamid with a single aperture at one of the two pointed ends (Fig. 3). The aperture is variable in size and is easily deformed or damaged during manipulations. Sometimes a short neck is present. No internal structures in relation to the aperture are observed. The test varies from 0.25 mm to 0.6 mm in length and from < 0.2 mm to 0.4 mm in width; while the length/width ratio varies from 1.25 to 2. The width measurements have to be considered carefully. Since some specimens were flattened under a coverslip prior to analysis, the original width was somewhat smaller. The organic or agglutinated test wall is flexible. As in other *Psammophaga* species, the organism ingests mineral grains, which are usually accumulated towards the aperture. The morphology of *P. fuegia*, as in other monothalamids, shows high plasticity. Both, the overall shape and the aperture are variable. The overall shape of *P. fuegia* is generally less elongated than *P. magnetica*, yet less rounded than *P. crystallifera*, *P. sapela*, or *P. simplora*. The aperture of *P. fuegia* is often small, with a neck shorter than in *P. magnetica*, and is therefore similar to the aperture of *P. simplora* or *P. crystallifera*. However, we also observe wide apertures without neck in both *P. fuegia* (Fig. 3 10b) and *P. magnetica* (Fig. 2G in Pawlowski and Majewski 2011). Additionally to this considerable morphological plasticity, *Psammophaga* species have a simple morphology and lack characteristic features. Consequently, it is difficult to determine these species based exclusively on their morphology. Genetic or biogeographic data are needed for their identification.

Molecular characterization: 13 partial SSU rDNA sequences of 5 specimens were obtained. The sequences are between 981 and 987 nt long when primer newB was used, and 867 nt long when primer 20R was used. The differences between the sequences, which are smaller than 2%, are mainly due to single substitutions or single indels.

The phylogenetic position of *Psammophaga fuegia* among other *Psammophaga* is uncertain (Fig. 4). All *P. fuegia* sequences group well together, but the basal bootstrap values are too low to discern the true relationships among the described and undescribed species of the genus.

Mineral particles: The mineral composition of grains extracted from a few specimens from sites 16, 17 and 56 show a high diversity. The grains show chemical compositions suggestive of as different minerals as amphibole, cordierite, hematite, ilmenite, ferrigehlenite, pyroxene, titanite, titanoferous magnetite, zircon, and quartz (Fig. 5). A visual inspection of washed sediment residues from the locations of sites 16, 17, and 56 showed abundant quartz grains that were clearly underrepresented within the analysed specimens. Specimens are not magnetic.

Distribution: Specimens were isolated at 10 locations throughout the Beagle Channel area (Fig. 1). They were particularly abundant at 2 stations in Bahia Romanche area and in the harbour of Puerto Williams. High-throughput sequencing of environmental RNA and DNA supports the wide distribution of the species in the area, indicating its high abundance in the same stations as the microscopic studies.

Ecological aspects: The sites at which *P. fuegia* was observed range from 4 to 220 meters in depth and include different habitats. Specimens were found in fjords branching off the main channel as well as in the channel itself. They were also present in an anoxic bay (Caleta Voilier) and in the shallow and apparently human-impacted harbour of Puerto Williams. Unfortunately, no detailed environmental data are available to characterize the species' ecological preferences. Water masses in the area range between Estuarine Water,

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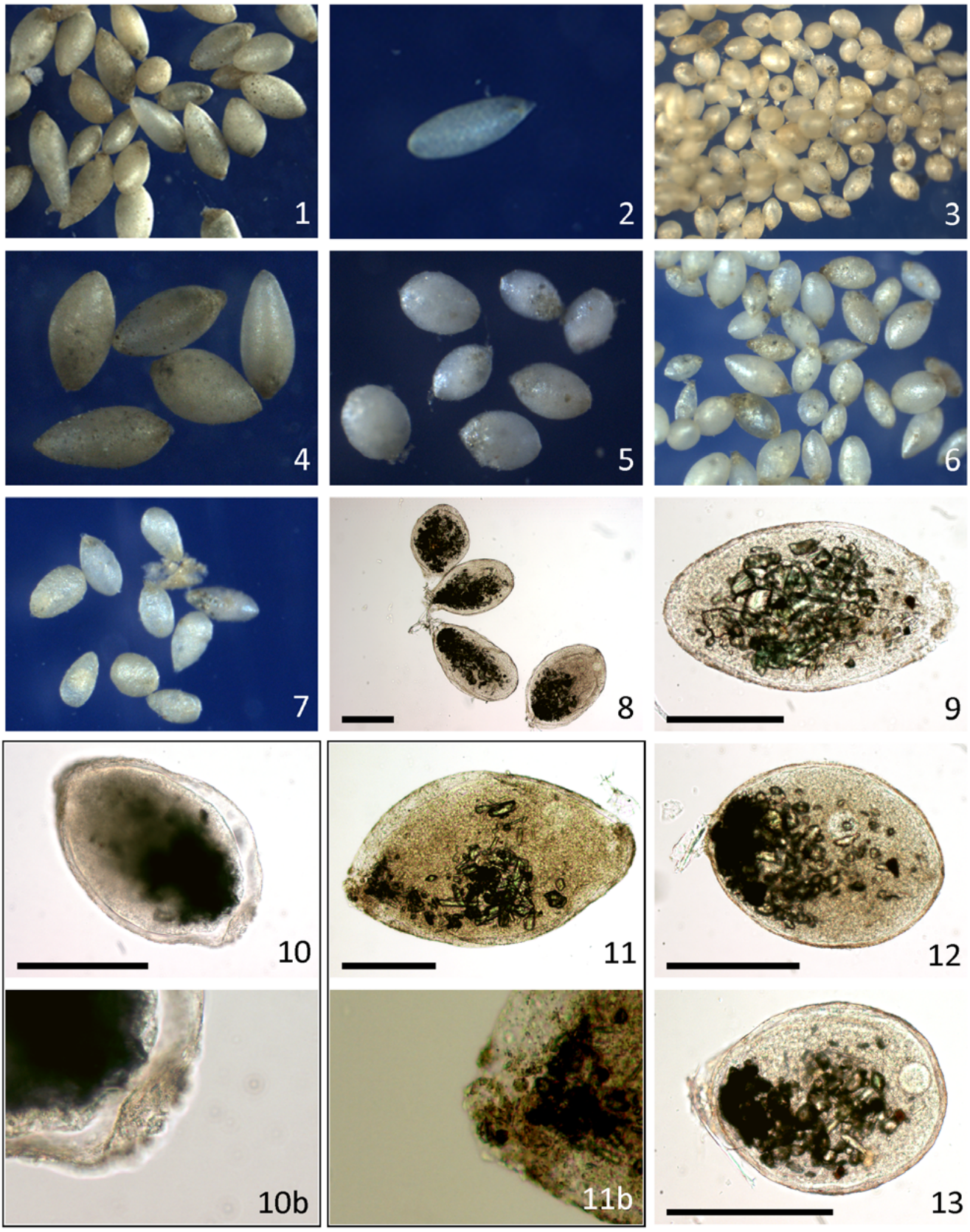
Fig. 3. 1–7 – living *Psammophaga fuegia*; specimens from station 56 (Puerto Williams) are finely agglutinated; **1** and **4** are from station 56 (Puerto Williams); **2** from station 4 (Fiordo Pia); **3** from station 16 (Bahia Romanche); **5** from station 11; **6** from station 17 (Bahia Romanche); **7** from station 9 (Fiordo Pia). **8–13** – fixed specimens of *P. fuegia*, **8**, **9**, **11**, **12** and **13** are flattened, thus they appear more rounded than they naturally, are apertures of **8** and **9** are damaged; **8**, **9**, **11**, **12** and **13** are from station 16 and 17; **10** from station 11; **10b** shows a zoom on the aperture of the specimen shown in **10**; **11b** shows a zoom on the aperture of the specimen shown in **11**. Scale bar: 200 µm.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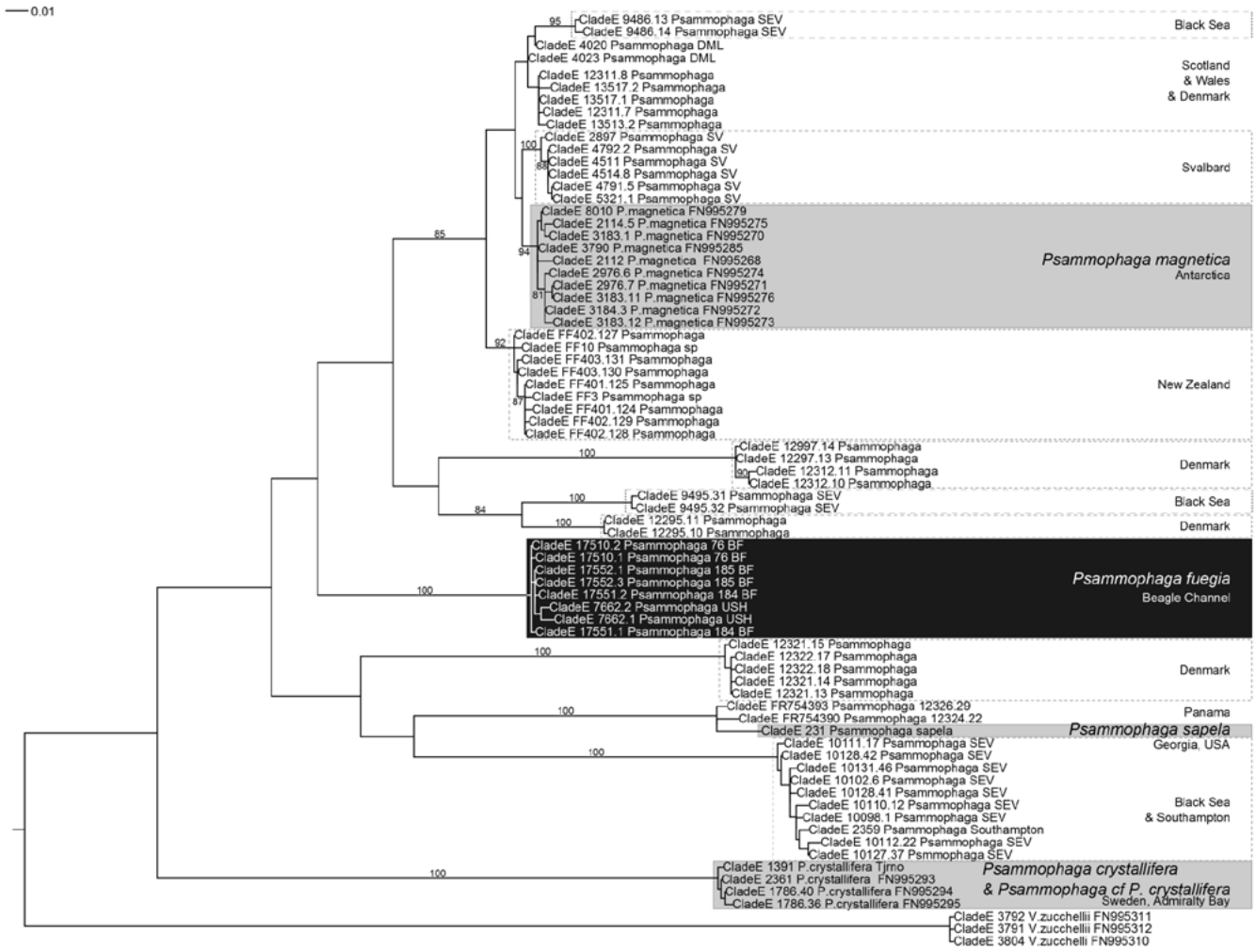

Fig. 4. ML-tree of the genus *Psammophaga*, with *Vellaria zucchellii* as outgroup. Bootstrap values bigger than 80% are shown. Described species are highlighted in grey.

which is a mix of sea and fresh water, and Subantarctic Water (Sievers and Silva 2008), providing a wide range of water temperatures and salinities. It seems that the environmental parameters differ especially between the very shallow harbour of Puerto Williams with only 4 meters of water depth and a significant anthropogenic impact and other of much deeper bathymetry and much less sheltered sites. Specimens found in the harbour of Puerto Williams differ slightly from those recovered at other sites (see Fig. 3). The former have a finely agglutinated cell wall whereas the latter show a transparent organic cell wall. However, as DNA sequences are identical between these two types, they can be regarded as different ecotypes of the same species.

DISCUSSION

The description presented here adds new information to the Molecular Database of Foraminifera (http://forambarcoding.unige.ch/; Pawlowski and Holzmann 2014) and is part of a recently increased, though still limited, interest in monothalamids and their diversity. The integrative taxonomic approach, combining morphological and molecular data is important, as it enables the application of high-throughput sequencing (HTS) techniques to study foraminifera communities or to develop new tools for biomonitoring (Pawlowski *et al*. 2014). Without well-established and comprehensive databases the results of HTS-based studies are dif-

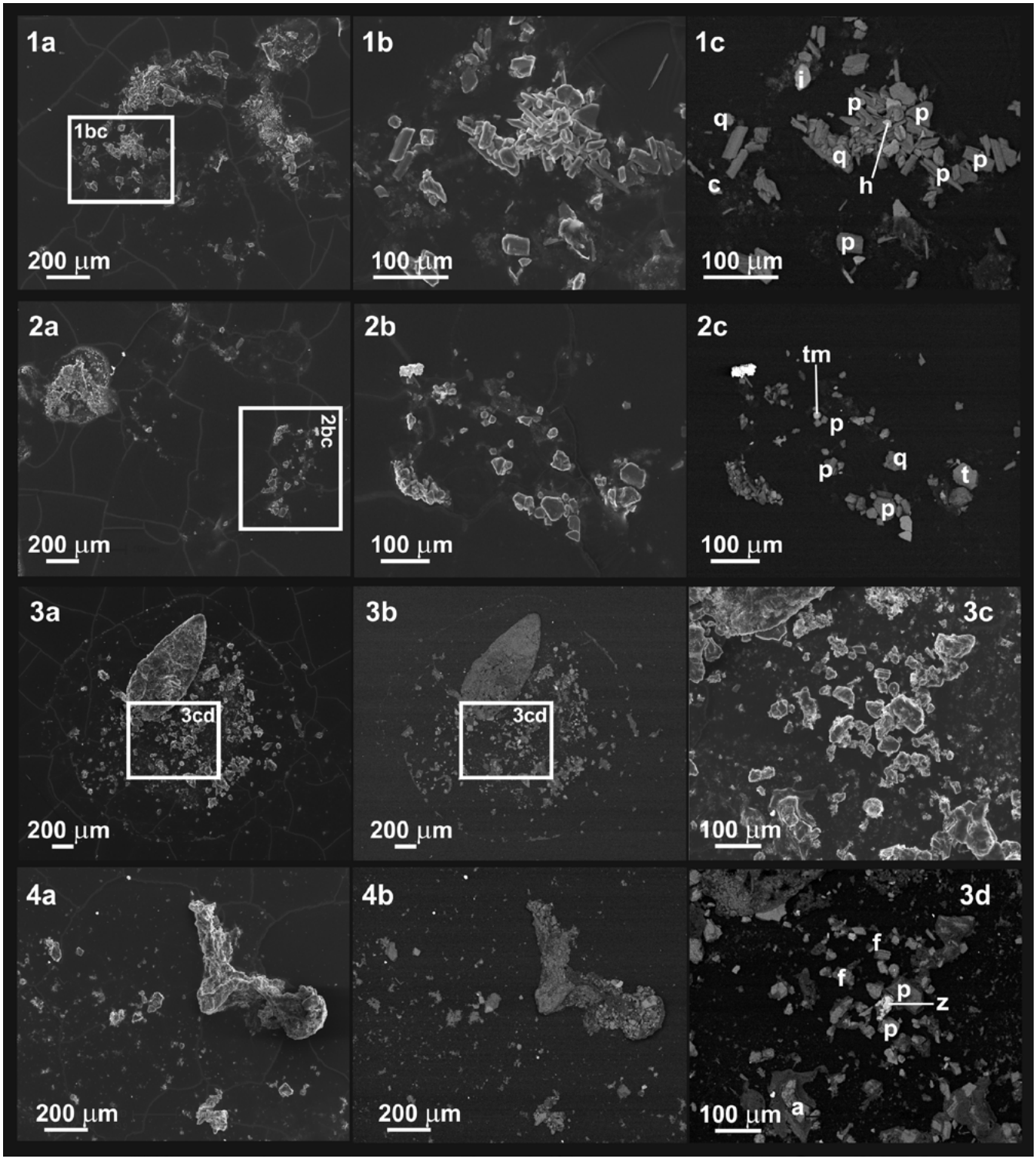

Fig. 5. SEM images of mineral grains found within *Psammophaga fuegia* specimens from sites 17 (**1** and **2**) and 56 (**3** and **4**). Images **1c**, **2c**, **3b**, **3d**, and **4b** are in BSE mode highlighting density differences. All remaining images are in SE mode. Note different scales of various images and insets on images of larger scale showing the position of images in smaller scale. Mineral grains analysed for their chemical composition are marked as follows: **a** – amphibole, **c** – cordierite, **h** – hematite, **i** – ilmenite, **f** – ferrigehlenite, **p** – pyroxene, **q** – quartz, **t** – titanite, **tm** – titanoferous magnetite, and **z** – zircon.

ficult to interpret or can be misleading (Stoeck *et al*. 2014).

Selective incorporation of intracellular mineral particles

The fact that quartz grains are less frequently found in the cytoplasm of *P. fuegia* compared to the sediment indicates the ability of the organism to select different mineral grains. However, the selection is not as spectacular as in *P. magnetica* from Admiralty Bay in South Shetlands (Pawlowski and Majewski 2011), in which almost all grains within its cytoplasm were either magnetite or titanoferous magnetite, which have the highest specific gravity among minerals in the sediment. The reason for the much more diverse mineral composition of grains incorporated into the cytoplasm of *P. fuegia* could be that the complex Patagonian geology results in a more diverse mineralogical composition of marine sediment compared to Admiralty Bay.

Microscopy compared to HTS

In our case, the HTS record corresponds relatively well to the visual detection of *Psammophaga* specimens (Table 1). Both approaches gave similar results in samples with a high *Psammophaga* abundance despite the microscope studies being not designed to gain quantitative data on monothalamous foraminifera abundances but for collecting diverse material for genetic studies. When the species was rare in microscopic samples, it was not detected by HTS. Similarly, specimens were not detected in the microscopic survey in cases where the OTU frequency was 15.5% or lower.

A slight mismatch between OTU frequencies and results from the microscope survey can be caused by several reasons: 1) the samples from the same station come from the same Van Veen Grab but are not identical due to natural patchiness; 2) more material per sample is surveyed in the microscopic study; 3) DNA of rare species can be stochastically lost during the processing of samples for HTS study, where we repeatedly take aliquots of bigger volumes; 4) the highly stringent filtering of HTS data can result in losing rare components of the sample diversity; 5) short fragments of DNA of dead and broken organisms can be preserved in the sediments. It has been demonstrated that the degraded DNA of foraminifera can be pre-

Table 1. Sampling stations, where *Psammophaga fuegia* was detected visually or by HTS.

Station	Location	Coordinates		Depth [m]	Number of specimens in guanidine (isolated)	RNAlater (together)	formalin (together)	extractions made	number of sequences obtained	HTS [relative abundance[1]]
2	Fiordo Pia	54°S 44.25’	69°W 41.01’	220	–	–	–	–	–	7.16%
3	Fiordo Pia	54°S 45.23’	69°W 40.99’	220	–	–	–	–	–	1.86%
4	Fiordo Pia	54°S 46.68’	69°W 40.18’	120	1	–	–	–	–	–
11	Caleta Voilier	N.A.	N.A.	N.A.	3	–	2	3	2*2	–
14	Bahia Romanche	54°S 56.19’	69°W 28.41’	85	1	–	–	–	–	–
15	Bahia Romanche	54°S 57.46’	69°W 29.28’	23	4	–	–	1	1*4	20.6%
16	Bahia Romanche	54°S 57.97’	69°W 31.36’	80	3	90+100	20+20	–	–	80.0%
17	Bahia Romanche	54°S 57.91’	69°W 31.97’	44	5*1+3+3	40		2	1*2	55.5%
19	Beagle Channel	54°S 55.98’	69°W 15.36’	220	1	–	–	1	–	–
31	Seno Ventisquero	54°S 44.65’	70°W 16.38’	82	2	–	–	–	–	N.A.
47	Bahia Yendegaia	54°S 51.82’	68°W 45.98’	92	–	–	–	–	–	2.91%
51	Beagle Channel	54°S 56.76’	67°W 14.3’	28	–	–	–	–	–	15.5%
54	Beagle Channel	55°S 0.65’	67°W 5.9’	128	–	–	–	–	–	0.05%
56	harbour of Puerto Williams	54°S 56.02’	67°W 37.08’	8	5	200+30	–	5	1*3	85.3%
USH–5	Ushuaia	N.A.	N.A.	4	9*1+2*3+5	–	–	?	1*2	N.A.

[1] *Psammophaga fuegia* reads/Foraminifera reads; read numbers are the sum of RNA and DNA reads and they are only considered if reads occur in both RNA and DNA. N.A. – not available.

served even for several thousands of years in deep-sea and cold environments (Lejzerowicz *et al*. 2013, Pawlowska *et al*. 2014).

Global *Psammophaga* diversity and phylogeny

This study highlights, once more, that much work remains to be done in the description of monothalamid diversity. Despite the facts that Clade E and *Psammophaga* are among the best established groups of monothalamids, that *Psammophaga* species are cosmopolitan in easily accessible shallow water marine habitats, and that they are amongst the most abundant members of monothalamid communities, there are only five *Psammophaga* species that have been described until now, including the present description.

Numerous reports and sequence data from different parts of the globe are available and we can discern eight well established phylotypes in addition to the five described species (Fig. 4). Most phylotypes show a restricted distribution; only one phylotype from the Black Sea and *P. crystallifera* were found in very distant locations. The former was recovered mostly in the Black Sea but also once in Southampton, the latter was found in Admiralty Bay and in Sweden but nowhere else until now. Furthermore, one to three phylotypes were detected per sampling location. From New Zealand, Svalbard, Panama, the Beagle Channel we know only a single type per region, from Antarctica we know two distinct species, and from Denmark and the Black Sea we have records of three different phylotypes. The relationships among the different phylotypes remain unclear, and the sampling locations, although distributed over the globe, remain scarce compared to a global scale. If *Psammophaga* is a cosmopolitan genus, which is suggested by the current data, and as the types show restricted distribution patterns, we are missing major parts of its species diversity. No sequences or morphological data is available from Africa, Asia, Australia or polar regions of North America. Moreover, no sequence data is available for *P. simplora*, which is the type species for the genus. Like many other monothalamids, *Psammophaga* remains undersampled on a global scale and the poor phylogeny may reflect this state of knowledge.

High abundances of *Psammophaga* species observed in this and other studies (Majewski *et al*. 2005, 2007; Pawlowski and Majewski 2011) indicate that they have an important ecological role at least on a microbial scale. Moreover, it suggests that they are highly competitive in some environments. The reasons for this would be interesting and it may be possible that the ingestion of mineral particles is part of the answer. Our data and the study of *P. magnetica* suggest that the organisms are capable to select which mineral particles they ingest. The comparison between *P. magnetica* and *P. fuegia* indicates that the selection depends on the species and/or mineral composition of the sediment. However, we do not understand the relative importance of these factors. How did the ability to ingest mineral particles evolve? Do different species prefer different mineral compositions? What are the advantages of the mineral ingestion? Altin-Ballero *et al*. (2013) mention two reasons why mineral ingestion could be beneficial: it could act as ballast and stabilize the *Psammophaga* cells or the species could feed on bacteria growing on the particles.

In summary, we can state that the species diversity of *Psammophaga* is poorly known, but due to their high abundance and diversity, their cosmopolitan distribution in shallow water settings, and their special characteristic features, species of this genus should be targeted for studies focusing on biogeographical, ecological, and evolutionary questions concerning the monothalamid foraminifera.

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