Journal of *Marine Science and Engineering*

MDPI

Article

An Integrative Taxonomic Survey of Benthic Foraminiferal Species (Protista, Rhizaria) from the Eastern Clarion-Clipperton Zone

Oceanne E. Himmighofen [1], Maria Holzmann [1], Inés Barrenechea-Angeles [1,2], Jan Pawlowski [3,4] and Andrew J. Gooday [5,6,*]

[1] Department of Genetics and Evolution, University of Geneva, Quai Ernest Ansermet 30, 1211 Geneva 4, Switzerland; oceanne.himmighofen@gmail.com (O.E.H.); maria.holzmann@unige.ch (M.H.)
[2] Department of Geosciences, UiT the Arctic University of Norway, 9007 Tromsø, Norway; ines.a.angeles@uit.no
[3] Institute of Oceanology, Polish Academy of Sciences, 81-712 Sopot, Poland; janpawlowski@iopan.pl
[4] ID-Gene Ecodiagnostics, Campus Biotech Innovation Park, 1202 Geneva, Switzerland
[5] National Oceanography Centre, European Way, Southampton SO14 3ZH, UK
[6] Life Sciences Department, Natural History Museum, Cromwell Road, London SW7 5BD, UK
* Correspondence: ang@noc.ac.uk

Abstract: The abyssal Pacific Clarion Clipperton Zone (CCZ) hosts vast, commercially valuable seafloor deposits of polymetallic nodules. Foraminifera (testate protists) dominate benthic communities in this region. Here, we present a taxonomic survey, combining morphological and genetic data and focussing on mainly meiofauna-sized Foraminifera from the eastern CCZ. Sequences obtained from >100 specimens, the majority photographically documented, were analysed phylogenetically. Most were single-chambered Monothalamea ('monothalamids'), a high percentage of them squatters inhabiting empty tests of mainly multi-chambered Foraminifera. The first sequences for the monothalamid genus *Storthosphaera* were obtained, while specimens assigned to *Gloiogullmia*, *Hippocrepinella* and *Vanhoeffenella* yielded new sequences. Among multichambered taxa, high-throughput Illumina sequencing (HTS) revealed a second haplotype of the calcareous rotaliid *Oridorsalis umbonatus*, possibly representing a distinct species. Additional HTS sequences were obtained from the rotaliids *Nuttallides umbonifer* and *Globocassidulina subglobosa*, confirming their wide distributions. We also obtained the first sequences for *Cribrostomoides subglobosa*, showing that it branches separately from other members of this genus. The fact that many sequences did not correspond to known morphospecies reflects the scarcity of reference barcodes for deep-sea Foraminifera, particularly the poorly known but highly diverse monothalamids. We recommend using HTS of single specimens to reveal further unknown species. Despite extensive research, much remains to be learnt about the true scale of foraminiferal biodiversity in the CCZ.

Keywords: benthic foraminifera; monothalamids; biodiversity; single-cell barcoding; Clarion-Clipperton Zone; seabed mining

Citation: Himmighofen, O.E.; Holzmann, M.; Barrenechea-Angeles, I.; Pawlowski, J.; Gooday, A.J. An Integrative Taxonomic Survey of Benthic Foraminiferal Species (Protista, Rhizaria) from the Eastern Clarion-Clipperton Zone. *J. Mar. Sci. Eng.* **2023**, *11*, 2038. https://doi.org/10.3390/jmse11112038

Academic Editor: Petra Heinz

Received: 17 September 2023
Revised: 13 October 2023
Accepted: 18 October 2023
Published: 24 October 2023

1. Introduction

The Clarion-Clipperton Zone (CCZ) occupies a vast tract of abyssal seafloor extending across approximately 6 million km^2 of the eastern equatorial Pacific from roughly 5° to 20° N and 115 to 160° W [1,2]. Water depths generally increase from ~4000 m in the east to ~6000 m in the west. The area hosts rich seafloor deposits of polymetallic nodules, which are of considerable commercial importance. Since much of the CCZ lies outside territorial waters, the region is administered by a United Nations body, the International Seabed Authority (ISA), which issues licences for exploration and prospecting to companies and other entities sponsored by national governments that are interested exploiting these

resources [3]. The ISA has also designated a series of Areas of Particular Environmental Interest (APEIs), protected areas that are largely situated outside the license areas [4,5]. The nodules themselves generate considerable small-scale environmental heterogeneity that can be exploited by sessile organisms. At larger spatial scales, low ridges, intervening troughs, abyssal hills, seamounts, and other topographic features create heterogeneity by their presence and by their influence on nodule densities [6]. Together with the organic-matter flux to the seafloor, which generally decreases from east to west, these features exert an important ecological influence on benthic communities [7].

Because of the need to better understand the biodiversity and functioning of benthic communities before seabed mining commences, this area has become the focus of a large body of research by scientists in many countries. This effort has been concentrated in the eastern part of the CCZ [8,9], the western part and the APEIs still being relatively understudied [1]. Although new species and some new higher taxa have been described, the benthic fauna remains largely undescribed, particularly among the smaller size fractions (meiofauna). In a recent synthesis, Rabone et al. [2] recognised a total of 5578 recorded metazoan species, of which only 436 (7.8%) had been scientifically described. Based on this dataset, they estimated the total number of metazoan species across all size classes in the CCZ to be 6233 and 7620, depending on the species richness estimator used.

The Rabone et al. [2] synthesis represents an important step in our understanding of the scale of unknown benthic diversity in the CCZ. However, it excluded the protists, which represent an important component of benthic biodiversity in the CCZ and across deep-sea habitats generally [10]. Among deep-sea protists, the Foraminifera are the best documented group, reflecting their relatively large size, possession of a test ('shell'), and resulting visual prominence in deep-sea samples. Abyssal Pacific faunas have been studied since the 19th Century (e.g., [11,12]) but until recently, there were only a few records from within the boundaries of the CCZ. Most of what we know about Foraminifera in this region has come from studies published during the last 10 years (reviewed in [13]; see also [14,15]). The CCZ Foraminifera span a wide size range from the meiofauna to the megafauna. They are highly diverse, and dominated in all size classes by single-chambered monothalamids, most of them undescribed. Metabarcoding data strongly reinforce the important contribution that monothalamids make to CCZ foraminiferal communities but also reveal that more than half of the obtained sequence clusters recognised cannot be assigned to any foraminiferal taxon. They are therefore designated as Operational Taxonomic Units (OTUs), groupings of highly similar 18s rRNA gene sequences corresponding to unknown foraminiferal groups [13,16].

The present paper provides an overview of genetic data obtained from small (meiofaunal) Foraminifera using Sanger sequencing and high-throughput sequencing (HTS), combined with morphological (photographic) information, where available. Phylogenetic analyses are based on the barcoding fragment of the foraminiferal 18S rRNA gene. The material was collected during several recent cruises to the eastern end of the CCZ. The species from which we obtained sequences represent only a small fraction of the morphospecies known from this part of the CCZ. Moreover, many of the sequences are inconsistent with the specimen they were derived from (usually monothalamid sequences from multichambered Foraminifera) and, therefore, regarded as 'squatters' occupying empty tests. Nevertheless, the new sequences add to our knowledge of foraminiferal diversity in the CCZ and those that correspond to morphospecies enhance the relatively sparse foraminiferal barcode database for this region. In addition, the hypothesis of intragenomic polymorphism was explored in *Oridorsalis umbonatus*, a common rotaliid species, using HTS.

2. Materials and Methods

2.1. Sample Collection and Ship-Board Processing

The samples on which this study was based were collected during four cruises to the Clarion-Clipperton Zone, within an area bounded by the approximate coordinates 11°48′ N to 19°28′ N and from 116°18′ W to 120°11′ W (Figure 1). The BIONOD cruise (March–May 2012) sampled in the German exploration licence area, the first ABYSSLINE cruise (AB01,

October 2013) [17] in 'Stratum 1′ of the UK-1 area, and the second ABYSSLINE cruise (AB02, February–March 2015) [18] in UK-1 'Stratum 2′ as well as the OMS (Singapore) area. Finally, Resource Cruise-01 (RC01; February–March 2020) was also sampled in the UK-1 and OMS areas. Station details are summarised in Table A1.

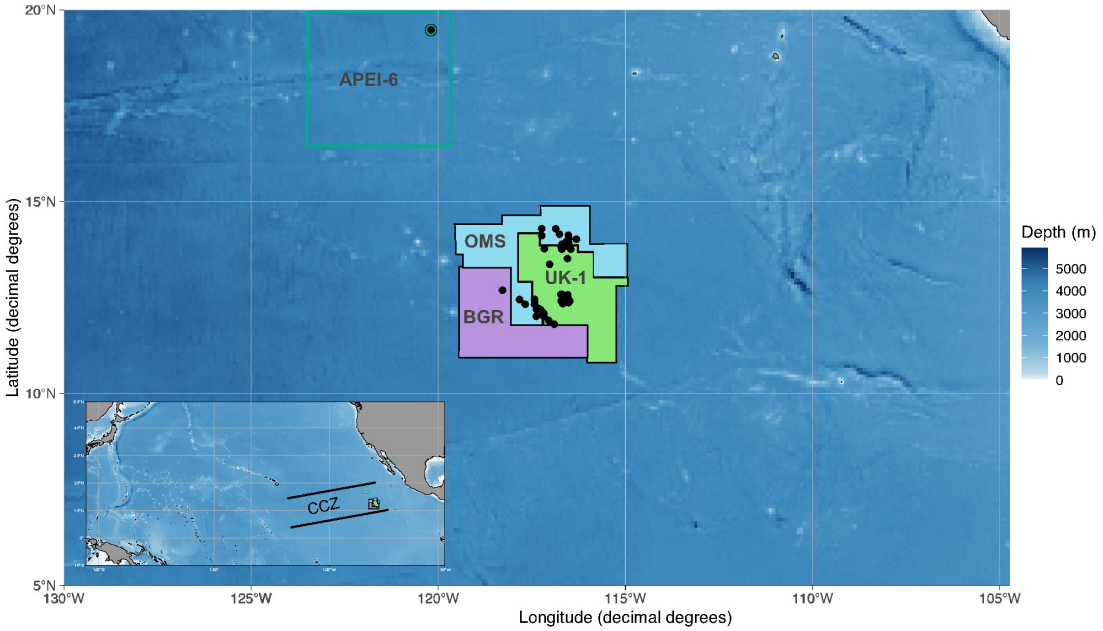

Figure 1. Map showing the exploration license areas, and APEI-6, in which samples were taken. Black dots indicate sampling stations. Precise station positions are given in Table A1.

Most of the material originated from core samples. During the BIONOD and RC01 cruises, the specimens came mainly from samples collected using an USNEL-type box corer with a cross-sectional area of 0.25 m^2. During the two ABYSSLINE cruises, they came mainly from multicores ('megacores') equipped with 12 core tubes, each with a diameter of 10 cm (cross-sectional area of ~78 cm^2). Qualitative samples collected using an epibenthic sledge sample (Brencke-type EBS; [19]) provided some additional material during the AB01 cruise. As soon as possible after collection, subsamples of the top few millimetres of sediment were removed from the core surfaces using sterile spoons and sieved on screens with various mesh sizes, 350 µm, 300 µm, 250 µm, 125 µm and 63 µm, depending on the cruise, using cooled seawater. Some small EBS subsamples were also taken using a spoon. The residues in cooled seawater were transferred into Petri dishes resting on a bed of ice or a freezer pack and Foraminifera that appeared alive (generally based on the presence of cytoplasm) picked out using a pipette or fine brush and sorted into known species, or in most cases morphotypes. They were first photographed using a stereomicroscope fitted with a digital camera, before being transferred into RNAlater® in small 1.25 mL Nalgene vials and stored in a −20 °C or 80 °C freezer.

This study is based on a selection of 516 foraminiferal specimens picked from 46 samples and preserved specifically for genetic analysis. Of these, 39.1% originated from the AB02 cruise, 37.0% from RC01, 17.4% from AB01, and 6.5% from BIONOD.

2.2. Preparation for Analyses

Back in the laboratory, each sample was pre-checked under a Leica S8 AP0 stereomicroscope and if necessary, photographed with a Leica M205 C stereomicroscope fitted with a Leica DFC-450 C camera (Leica Microsystems, Wetzlar, Germany). Crystals of RNAlater were carefully removed from the specimen by adding more RNAlater or with the help of a brush and needle.

2.3. DNA Extraction, PCR Amplification and Sanger Sequencing

Eighty-six foraminiferal specimens were extracted individually using either Guanidin buffer solution [20] or the DNeasy Plant Mini Kit (Qiagen) in case of larger-sized Foraminifera. Isolate numbers are given in Tables A2 and A3. A semi-nested PCR amplification was carried out for the 3′ end of the SSU rRNA gene using primer pairs s14F3 (5′acgcamgtgtgaaacttg-3)—s20R (5′gacgggcggtgtgtacaa-3) for the first amplification and s14F1 (5′aagggcaccacaagaacgc-3)—s20r or s14F1—sB (5′tgatccttctgcaggttcacctac-3) for the second amplification. This fragment represents the standard barcoding fragment in Foraminifera [21]. Thirty-five and 25 cycles were performed for the initial and the semi-nested PCR, respectively, with an annealing temperature of 50 °C for the first and 52 °C for the second amplification.

The amplified PCR products were purified using the High Pure PCR Cleanup Micro Kit (Roche Diagnostics). Twenty-six PCR products were cloned prior to sequencing (Tables A2 and A3) using the TOPO TA Cloning Kit (Invitrogen) following the manufacturer's instructions and transformed into competent *E. coli*. One to three clones were sequenced. Sequencing reactions were performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and analysed on a 3130XL Genetic Analyzer (Applied Biosystems). In total, 55 and 56 sequences were obtained for Globothalamea and Monothalamea, respectively. The newly acquired sequences were deposited in the EMBL/GenBank database (Tables A2 and A3).

2.4. High-Throughput Sequencing (HTS) and Analysis

HTS was used to determine the interindividual diversity of specimens. From the first amplification (s14F3-s20R), the reamplification was performed using 14F1(5′-aagggcaccacaagaacgc-3′) and s17 (5′-cggtcacgttcgttgc-3′) primers and 25 cycles. Each of the samples had a unique combination of individual tags of 8 nucleotides that were attached at each 5′extremities of primers. These primers amplified two hypervariable regions of 18S rRNA gene (37f and 41f) specific to Foraminifera.

The amplified PCR products were quantified using the QIAxcel Advanced system (Qiagen) and pooled equimolarly and using the High Pure PCR Product Purification Kit (Roche). The library was prepared using Illumina's TruSeq free PCR preparation kit. This library was then quantified by qPCR using the KAPA library quantification kit (Roche). It was sequenced on a Miseq instrument using pair-end sequencing for 500 cycles with the standard v3 kit.

The raw data were analysed using the web application SLIM [22]. Briefly, the samples were first demultiplexed and the primers removed. Then, to identify the individual variants, the denoising method [23] producing Amplicon Sequence Variants (ASVs) was used with a pseudo-pool approach. ASVs with a total number of reads below 100 were removed before comparison with the local and NCBI databases. The remaining sequences were annotated using Vsearch at 95% of similarity and carefully manually checked. Sequences not belonging to Foraminifera were removed.

2.5. Phylogenetic Analysis

2.5.1. Sanger Sequences

For Globothalamea, the 36 new sequences obtained were added to 21 sequences belonging to rotaliids, robertinids and textulariids that are part of the publicly available 18S database of Foraminifera (NCBI/Nucleotide; https://www.ncbi.nlm.nih.gov/nucleotide/, accessed on 18 September 2023). The globothalamid alignment contains 57 sequences, and 1233 sites were used for analysis. The monothalamid alignment contains 90 sequences, of which 52 were newly obtained and added to 38 sequences available at the NCBI Nucleotide database. A total of 1383 sites were used for analysis. Globothalamid and monothalamid sequences were aligned separately using the default parameters of the Muscle automatic alignment option as implemented in SeaView vs. 4.3.3. [24].

Phylogenetic trees (Figures 2 and 3) were constructed using maximum likelihood phylogeny (PhyML 3.0) as implemented in ATGC: PhyML [25]. An automatic model selection by SMS [26] based on Akaike Information Criterion (AIC) was used, resulting in a HKY85+R substitution model being selected for the globothalamid and a GTR+R substitution model being chosen for the monothalamid analysis. The initial trees are based on BioNJ. Bootstrap values (BV) are based on 100 replicates.

2.5.2. HTS Sequences

Four ASVs were obtained for two textulariid specimens that were added to 18 sequences belonging to *Cribrostomoides*, *Liebusella*, *Srinivasania*, *Spiroplectammina*, *Trochammina* and *Reophax*. A total of 1008 sites were used for analysis. For monothalamids, 55 ASV were obtained from 18 specimens that were added to 90 monothalamid sequences belonging to 17 Clades. A total of 1675 sites was used for analysis.

Textulariid and monothalamid sequences were aligned separately using the default parameters of the Muscle automatic alignment option as implemented in SeaView vs. 4.3.3. [24].

Phylogenetic trees (Figures 4–6) were constructed using maximum likelihood phylogeny (PhyML 3.0) as implemented in ATGC: PhyML [25]. An automatic model selection by SMS [26] based on Akaike Information Criterion (AIC) was used, resulting in a GTR+G+I substitution model being selected for the textulariid and a GTR+R substitution model being chosen for the monothalamid analysis. The initial trees are based on BioNJ. Bootstrap values (BV) are based on 100 replicates.

The barcoding gap analyses were implemented in R (Version 4.3.1) using the packages seqinr (version 4.2-30) for the distance alignments and ggplot2 (version 3.4.2) for the histograms.

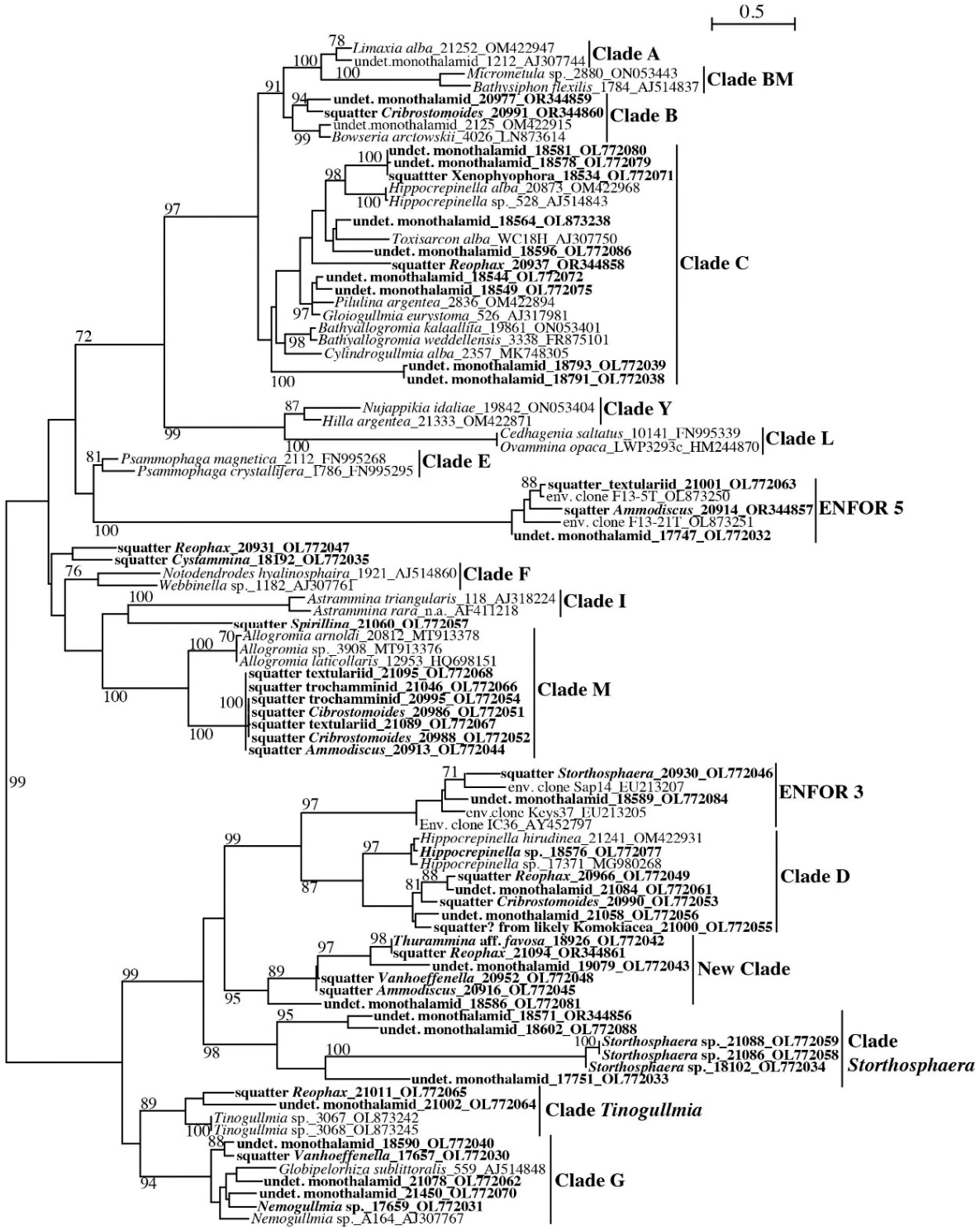

Figure 2. PhyML phylogenetic tree based on the 3′end fragment of the SSU rRNA gene, showing the evolutionary relationships of 90 foraminiferal sequences belonging to monothalamids. Specimens marked in bold indicate those for which sequences were acquired for the present study. The tree is unrooted. Specimens are identified by their isolate (1st) and accession numbers (2nd). Numbers at nodes indicate bootstrap values (BV). Only BV > 70% are shown.

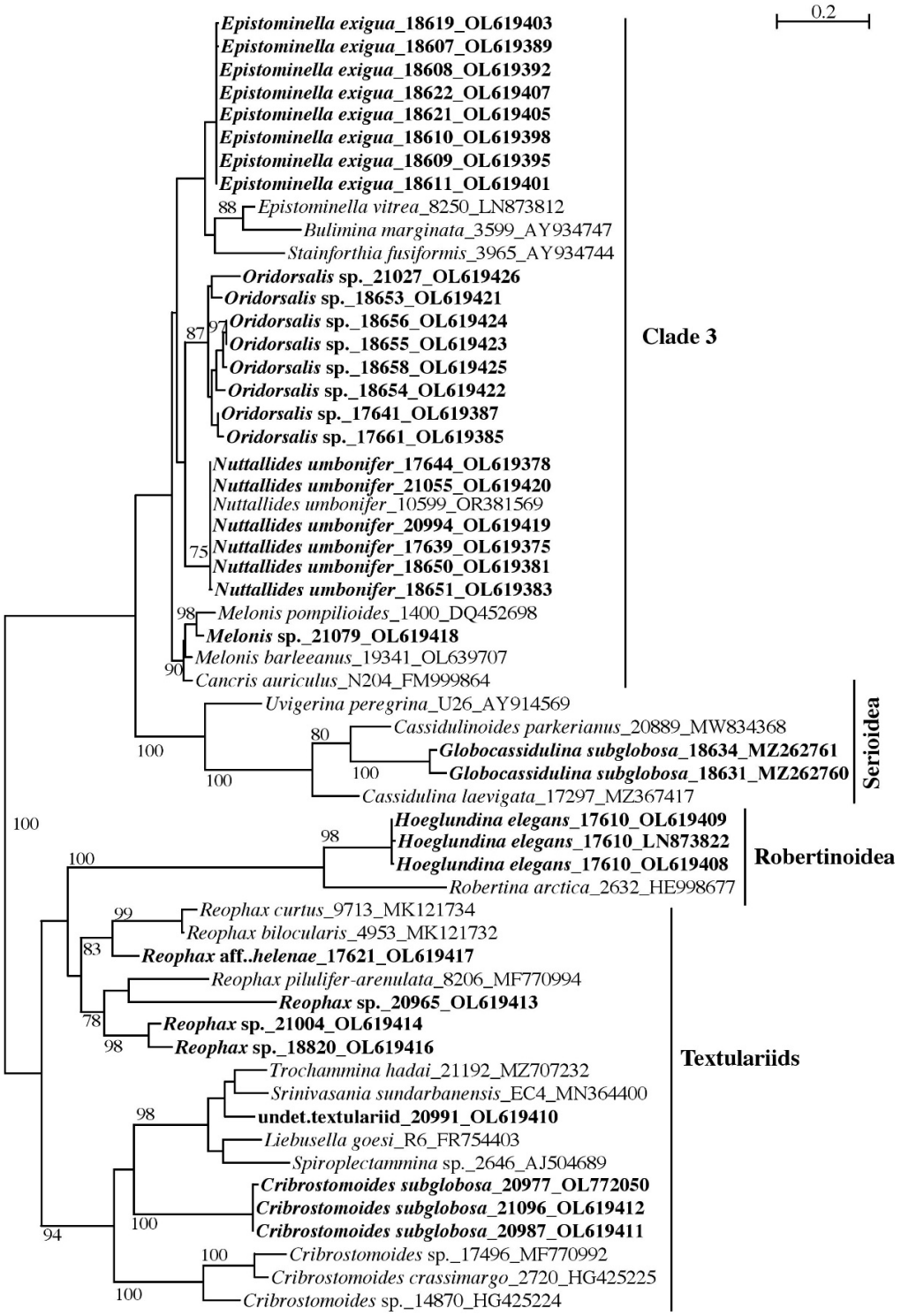

Figure 3. PhyML phylogenetic tree based on the 3′end fragment of the SSU rRNA gene, showing the evolutionary relationships of 57 foraminiferal sequences belonging to Globothalamea. Specimens marked in bold indicate those for which sequences were acquired for the present study. The tree is unrooted. Specimens are identified by their isolate (1st) and accession numbers (2nd). Numbers at nodes indicate bootstrap values (BV). Only BV > 70% are shown.

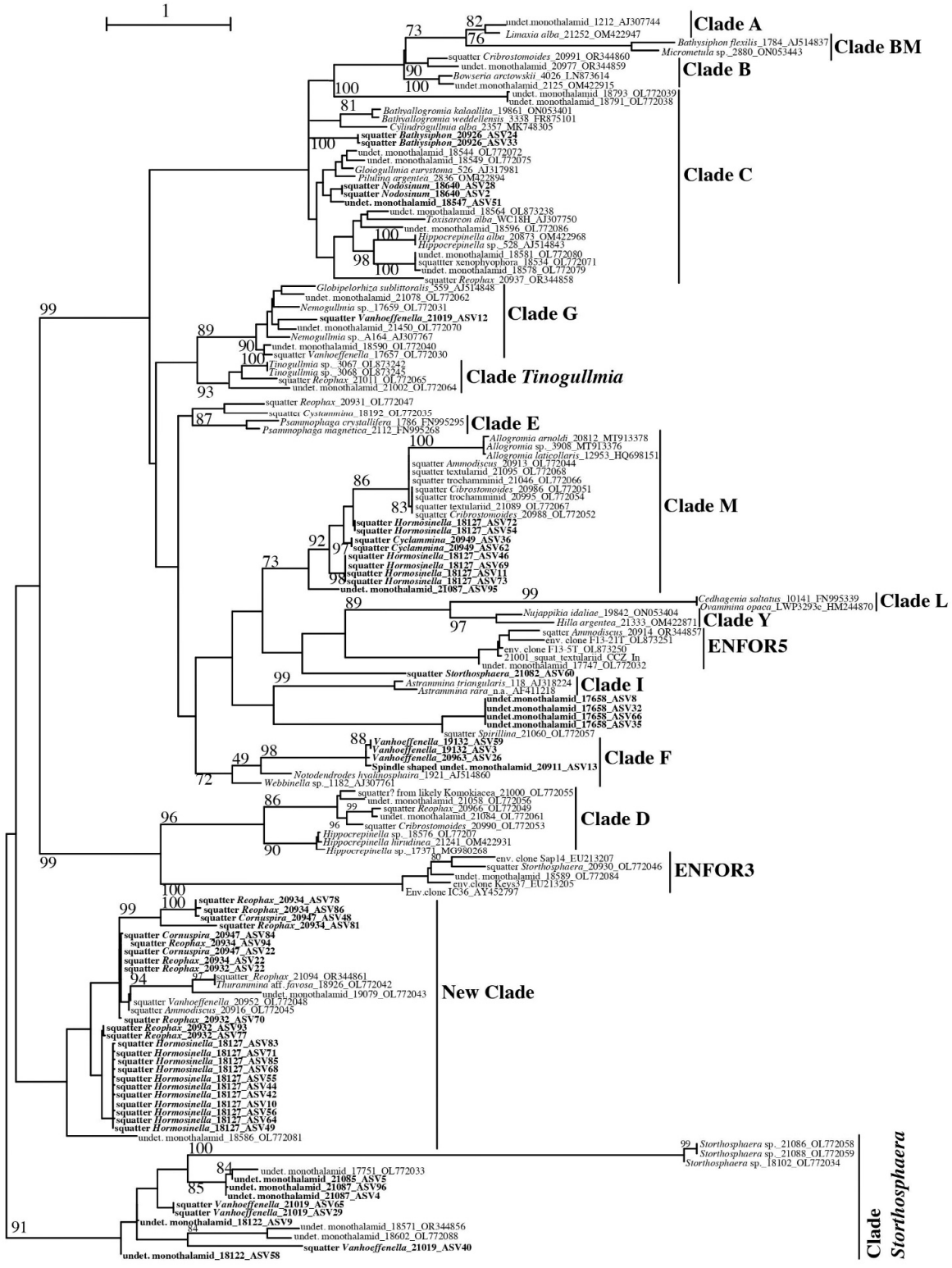

Figure 4. PhyML phylogenetic tree based on the 3′end fragment of the SSU rRNA gene, showing the evolutionary relationships of 145 foraminiferal sequences belonging to monothalamids. Specimens marked in bold indicate those from which ASVs were acquired for the present study. The tree is unrooted. Specimens are identified by their isolate (1st) and accession or ASV numbers (2nd). Numbers at nodes indicate bootstrap values (BV). Only BV > 70% are shown.

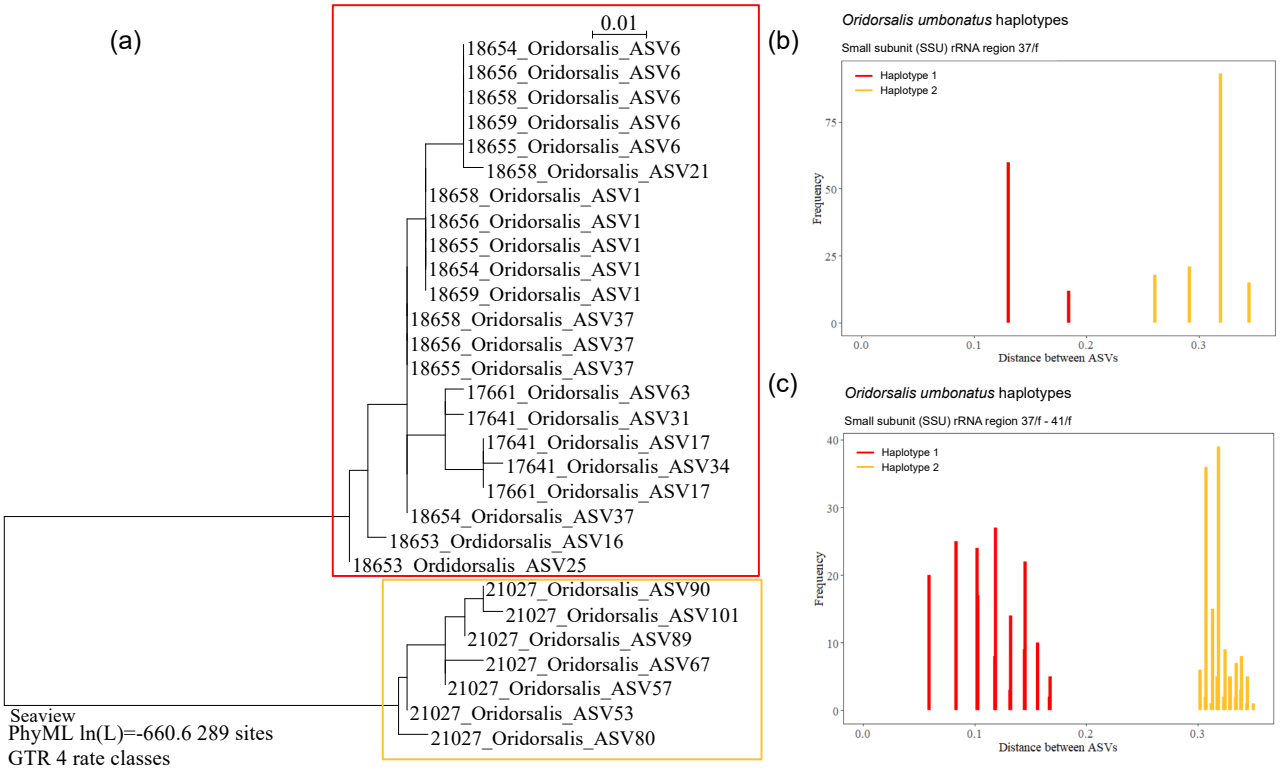

Figure 5. (**a**) Phylogenetic tree of *Oridorsalis umbonatus* based on partial SSU rDNA region 37/f–41/f sequences. The name of each new sequence consists of the foraminiferal DNA extraction number and the amplicon sequence variant (ASV). Major clades are adopted from Pawlowski et al. [27] and Holzmann and Pawlowski [28]. (**b**) Barcoding gaps in *Oridorsalis umbonatus* from partial SSU rDNA region 37f with a distance average of Haplotype 1: 0.157 and of Haplotype 2: 0.304. (**c**) Barcoding gaps in *Oridorsalis umbonatus* from partial SSU rDNA region 37f/41f with a distance average of Haplotype 1: 0.117 and of Haplotype 2: 0.325.

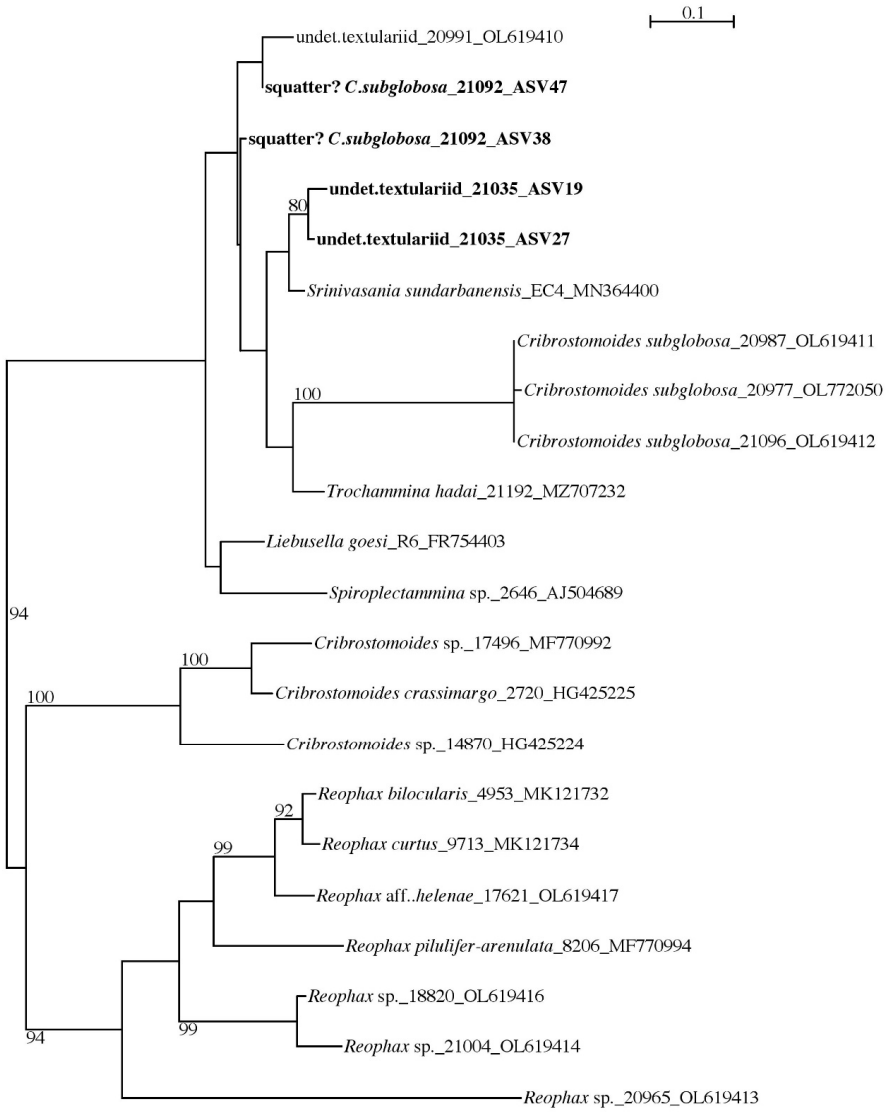

Figure 6. PhyML phylogenetic tree based on the 3′end fragment of the SSU rRNA gene, showing the evolutionary relationships of 22 foraminiferal sequences belonging to textulariids. Specimens marked in bold indicate those from which ASVs were aquired for the present study. The tree is unrooted. Specimens are identified by their isolate (1st) and accession or ASV numbers (2nd). Numbers at nodes indicate bootstrap values (BV). Only BV > 70% are shown.

3. Results

We attempted to obtain sequences from 516 foraminiferal specimens from the German (BGR), Singapore (OMS) and UK-1 exploration license areas, and one specimen from APEI-06. Of these 516 specimens, 112 yielded sequences, 86 of them using Sanger sequencing (Table A4) and 29 using HTS (Table A5). Molecular analyses of the remaining 318 specimens were unsuccessful.

3.1. Sanger Sequencing

Of the 86 specimens that yielded sequences, 52 belonged to the Monothalamea and 34 to the Globothalamea (Table A4). Several sequences were obtained in the case of cloned specimens, resulting in a total of 116 high quality sequences that could be used for analysis. Twenty-five monothalamid sequences, most of them derived from multichambered textulariids and Tubothalamea, are considered to be squatters.

3.1.1. Monothalamea

The 52 new monothalamid isolates analysed encompass two major groups supported by a BV value of 99% (Figure 2). Clades B, C, M and ENFOR5 are part of the first major group, Clade C being the most diverse with 10 new sequences, and Clades B, M and ENFOR 5 containing 2, 7 and 3 new sequences, respectively. Seven other clades (A, BM, E, F, I, L and Y) that do not contain abyssal sequences from the CCZ area branch within the first major group. The remaining five clades (Clades D, G, Clade *Storthosphaera*, Clade *Tinogullmia* and ENFOR5) belong to the second major group, each being represented by between 2 and 6 new sequences.

Nine sequences branch independently, six of them (New Clade) grouping at the base of Clades D and ENFOR 3 and three branching next to Clades I and F.

Monothalamea: Clade C

Clade C includes more new sequences than any other monothalamid clade. Specimens 18793 and 18791 (Figure A1a,b) have approximately spherical, organic-walled tests and resemble *Bathyallogromia* morphologically. They group at the base of Clade C in the tree (Figure 2; Table A2) but without bootstrap support (BV < 70%) and are not closely related genetically to *Bathyallogromia*.

A highly supported (97% BV) group contains the cloned specimen 18544 (Figure A1d), specimen 18549 (Figure A1f), *Pilulina argentea* and *Gloiogullmia eurystoma*. The elongate test of specimen 18544, which had an organic wall with a finely agglutinated veneer and dark contents, resembles that of *Gloiogullmia*, but apparently without the 'sticky' surface typical of this genus. Specimen 18549 resembles *Pilulina argentea* in having a silvery test, although ovoid rather than nearly spherical.

Another indeterminate monothalamid, specimen 18564 (Figure A1e), has a whitish, relatively large, fairly elongate test with tiny apertures at each of the symmetrically rounded ends. It branches as a sister to several *Toxisarcon* species, but the relationship is only weakly supported (BV = 79%).

Two specimens, 18578 and 18581 (Figures A1g and A1c, respectively), cluster with a third monothalamid sequence (18534), derived from a likely squatter associated with a xenophyophore test. These two specimens and the squatter are closely related with a BV value of 100%. This cluster branches as a sister to *Hippocrepinella alba* and *Hippocrepinella* sp. with a BV value of 98%.

Specimens, 18596 (Figure A1h), which has a whitish, finely agglutinated, elongate cylindrical test with a somewhat produced apertural end, branches with *Toxisarcon alba* and the above-mentioned cluster, but without bootstrap support. Finally, a squatter sequence derived from *Reophax* (specimen 20937) branches at the base of the latter two groups but again without support.

Monothalamea: ENFOR5

Specimen 17747 (Figure A2c,d) groups in the ENvironmental FORaminifera 5 (ENFOR5) clade, together with two squatters derived from a textulariid and an *Ammodiscus*. The ENFOR clades were established to accommodate sequences generated by the high-throughput sequencing (HTS) of environmental samples [29]. Specimen 17747 is a sphere composed of fine but discernible agglutinated particles resembling small quartz grains with some scattered dark particles. It seems to be quite friable and resembles some *Crithionina* species, notably *C. granum* and *C. delaci*, although the interior, visible through a crack in the test wall in one of the photographs, appears dark and was possibly filled with stercomata.

Monothalamea: Clade M

New sequences assigned to Clade M comprise squatters, mainly associated with different textulariid tests but in one case with a specimen of *Ammodiscus* (Tubulothalamea). They form a strongly supported (100% BV) monophyletic group that is a sister to allogromiids (*A. laticollaris*, *A. arnoldi*, *Allogromia* sp.). The branching of allogromiids and the squatter group is supported by 100% BV.

The second major monothalamid group encompasses clades D, G, ENFOR 3, clades *Storosphaera* and *Tinogullmia*, although our present data suggests the existence of an additional clade (Figure 2).

Monothalamea: Clade D and ENFOR3

Clade D includes sequences of *Hippocrepinella* sp. (specimen 18576, Figure A3k), clustering together with a published *H. hirudinea* sequence from South Georgia [30] and *Hippocrepinella* sp. from Patagonia, Chile (97% BV). Clustering with these sequences are two squatters, one associated with a *Reophax* test (specimen 20966) and the other with a *Cribrostomoides* (specimen 20990). In addition, Clade D includes sequences derived from three monothalamids. Specimen 21084 (Figure A3e,f) is a relatively large, pale brownish, knobbly sphere (>1 mm diameter) composed of radiolarians, mineral grains, a few micronodules and occasional sponge spicules. Specimen 21000 (Figure A3a,b) is a smaller, whitish structure (~750 µm diameter) that incorporates a large, branched tube. It has some features that resemble those of the komokiacean *Chrondrodapis*, although this cannot be confirmed from the photograph. No genuine sequences exist for komokiaceans and therefore we regard this sequence as that of a possible squatter. Specimen 21058 (Figure A3i,j) is a large, whitish sphere (>1.5 mm diameter) bristling with long sponge spicules, closely resembling *Crithionina hispida*.

Two specimens cluster in the ENvironmental FORaminifera 3 (ENFOR3) clade. Isolate 20930 corresponds to a specimen of *Storthosphaera* (Figure A3g,h), but the fact that it does not cluster with other *Storthosphaera* sequences strongly suggests that the sequence was derived from a squatter. Isolate 18589 corresponds to a smaller (length ~690 µm) broadly ovoid, whitish saccamminid (Figure A3c,d). These two specimens cluster in ENFOR3 where they join earlier sequences from environmental clones.

Monothalamea: Clade *Storthosphaera* and New Clade

The monothalamid isolates 21086, 21088 and 18102 (Figure A4b–d) cluster closely together in a branch of the tree with 100% BV support. They originated from three specimens with pale whitish, irregularly ovoid tests, about 2 cm long and covered in prominent projections and sharp ridges, that conformed well with *Storthosphaera*. In the case of 21086 and 21088, which are closely related in the tree, projections predominate, whereas ridges predominate in the case of 18102, which is slightly separated from the other two sequences. The same clade includes three additional specimens (isolates 18571, 18602 and 17751). Isolate 18602 comes from a small (~700 µm) broadly droplet-shaped monothalamid (Figure A4a) and isolate 17751 comes from a somewhat larger (~1 mm) rounded test (Figure A4e). There is no photograph available of the third specimen. Together, these six sequences constitute a new and well-supported (98% BV) clade, which we propose to call Clade *Storthosphaera*.

A cluster of sequences derived from several monothalamids and squatters may also constitute a new clade, with strong BV support (95%). A yellowish, spherical monothalamid with small-scale surface ornamentation comprising narrow ridges that define pit-like depressions (18926, Figure A4f) resembles *Thurammina favosa* Flint, 1899. However, the reticulations are much finer than those of the original specimens (Figure 2 in [31]) and hence we refer to it as *Th.* aff. *favosa*. The sequence is identical to that of a *Reophax* squatter (21094). Specimen 18586 (Figure A4g) has an elongate greyish, almost transparent test, while another monothalamid (19079, Figure A1i) within this clade has a roughly spherical test with several low bumps. It is somewhat similar to *Thurammina albicans* Brady, 1879 but lacks the well-defined papillae that are typical of this species and of the genus generally.

Finally, two obvious squatters were associated with *Ammodiscus* (20916) and *Vanhoeffenella* (20952) tests. As the monophyletic group is well supported, we propose to establish it as a New Clade.

Monothalamea: Clade G, *Tinogullmia* and ENFOR5

Clade G includes several distinct morphotypes. An elongate thread-like form (17659, Figure A4i–k) is assigned to *Nemogullmia*. Specimen 21078 (Figure A4h) is a white agglutinating *Crithionina*-like dome attached to a nodule fragment with thin, needle-like spicules projecting from the surface. Specimen 21450 (Figure A2e) has a small, elongate, organic-walled test with apertures at both ends. Morphologically, it resembles *Tinogullmia*, despite appearing in Clade G rather than the *Tinogullmia* clade. These three specimens group together with specimen 18590 from the OMS area (said to be a 'saccamminid' but with no photographs available) and a squatter from a *Vanhoeffenella* test.

Specimen 21002 (Figure A2a,b), which appears in clade *Tinogullmia*, corresponds to a relatively small (~1 mm) 'mudlump' attached to a nodule fragment and giving rise to two long, narrow tubes (length ~2.9 mm and 1.9 mm, width ~50 µm) that project away from the 'mudlump'. The nature of the 'mudlump' is unclear, but the photograph shows poorly defined tubule-like features within it, suggesting that it is a komokiacean. Whether the long tubes that extend from it are part of the same organism is also unclear. Because of this, and the fact that there are no confirmed sequences for komokiaceans, we regard the sequence derived from 21002 as that of a squatter. One new undoubted monothalamid squatter sequence derived from a *Reophax* test is also present, together with two previously reported *Tinogullmia* sequences.

3.1.2. Globothalamea

The multichambered Globothalamea was represented by 34 newly sequenced specimens (Table A5). The obtained amplification products of 14 rotaliids and 1 robertinid were cloned prior to sequencing, yielding a total of 55 sequences. The majority of Globothalamea were rotaliids (25 specimens, 44 sequences); the remainder comprised 1 robertinind (3 sequences), 4 *Reophax* specimens (4 sequences) and 4 other textulariids (4 sequences).

Globothalamea—Rotaliida and Robertinida

The 26 specimens that were analysed represent six well-known deep-sea taxa: *Globocassidulina subglobosa*, *Epistominella exigua*, *Oridorsalis umbonatus*, *Nuttallides umbonifer*, *Melonis* sp. (all rotaliids), and *Hoeglundina elegans* (robertinid) (illustrated in Figures A5 and A6). Our new sequences placed them in the same clades to which they had been assigned in previous studies [27,28] (Figure 3).

The new sequences of *G. subglobosa* (Figure A5a–c) cluster with species of *Cassidulina* and *Cassidulinoides*, supported by a bootstrap value (BV) of 100%. They fall within the family Cassidulinidae and superfamily Serioidea, which forms a distinct clade. The remaining rotaliids all group within Clade 3. Eight sequences of *Epistominella exigua* (Figure A5d–f) were obtained that cluster together. Six new sequences were obtained for *Nuttallides umbonifer* (Figure A5g,i). The related species *Oridorsalis umbonatus* (Figure A6a–e) is represented by eight sequences. In contrast to *E. exigua* and *N. umbonifer*, they form two branches, suggesting some degree of intra-individual polymorphism, a possibility that was investigated further using HTS. The last CCZ species belonging to the clade 3 is *Melonis* sp. (Figure A6f–i). The figured specimen grouped next to *M. pompilioides* and *M. barleeanus* and may represent a new species. Finally, the order Robertinida was represented by *Hoeglundina elegans* (Figure A5j–k). Three sequences derived from this species cluster next to *Robertina arctica* (BV 100%).

Globothalamea—Textulariida

The order Textulariida was represented in our genetic dataset by eight sequences derived from several species of the genus *Reophax* (Figure A7a–d) and several specimens of *Cribrostomoides subglobosa* (Figure A7f–h). The latter species is represented by a group of three very similar sequences derived from specimens 20987, 21096 and 20977 (100% BV). *Cribrostomoides subglobosa* branches separately from other, previously obtained *Cribrostomoides* species from littoral and bathyal environments (specimens 2720, 14870, 17496). A fourth sequence (specimen 20991, Figure A7e), obtained from an undetermined textulariid appears somewhat similar to *C. subglobosa*, and branches at the base of *Trochammina hadai* and *Srinivasania sundarbanensis* in a different part of the tree.

We sequenced four specimens of *Reophax* from our CCZ material (21004, 18820, 17621 and 20965). They cluster along with previously obtained *Reophax* sequences from other areas. The specimens illustrated in Figure A7b,c (21004, 18820) cluster together, even though their morphology is somewhat different. The latter is certainly the same as *Reophax* sp. 1 of Goineau and Gooday [32], whereas the former is relatively shorter and wider. The third *Reophax* specimen (17621, Figure A7a) resembles *R.* aff. *helenae* of Goineau and Gooday [32] and groups next to *R. bilocularis* and *R. curtis*, neither of which is particularly similar to it morphologically. The fourth *Reophax* specimen (20965, one of those shown in Figure A7d) branches next to *R. pilulifer-arenulata* but without BV support.

3.2. High-Throughput Sequencing

Thirty-six specimens from the CCZ were sequenced with Illumina High-Throughput sequencing (HTS). Analyses of the metabarcoding data yielded 729,651 reads. After filtering and processing, the remaining 528,079 reads were assigned to 215 ASVs, of which 74 ASVs obtained from 28 specimens were used for analysis. The sequenced specimens listed in Table A5 include 11 members of the Globothalamea and 17 members of the Monothalamea.

3.2.1. Monothalamea

Monothalamids subjected to high-throughput sequencing are included in the tree (Figure 4) and some are illustrated in Figures A8 and A9. Several indeterminate monothalamids appear in clade C. Specimen 18547 (Figure A8a) is a saccamminid with a terminal aperture. The corresponding ASV 51 clusters with two squatter sequences, ASVs 2 and 28, were obtained from *Nodosinum* (specimen 18640).

Sequences derived from three specimens resembling *Crithionina* (18122, 21085, 21087) branch within the Clade *Storthosphaera* (specimens 18102). Specimen 21087 (ASVs 4, 96; Figure A8c) is a large, finely agglutinated sphere without spicules, of ~3 mm diameter, rather similar to *C.* cf. *pisum* from the Andaman Sea [33]. Specimen 21085 (Figure A8d) is a whitish sphere, 1.80 mm diameter, that incorporates some larger particles, including relatively long sponge spicules. Although it clusters close to *Crithionina* cf. *pisum*, this specimen is most similar morphologically to *Crithionina hispida* of Flint (1899). Specimen 18122 (Figure A8e–g) bristles with numerous spicules and resembles typical specimens of *C. hispida*, although it is much smaller (~400 µm) than specimen 21085. It is represented by ASVs 9 and 58 that branch at the base of the clade containing isolates 21085 and 21087. Both *C. hispida*-like specimens are similar to *C.* cf. *hispida* of Cedhagen et al. [33] from the Andaman Sea. All three *Crithionina*-like specimens cluster loosely together but are not supported by strong bootstrap values. They branch close to *Storthosphaera* and do not show any genetic affinity with *Crithionina granum*.

Monothalamea: Squatters

Many of the monothalamid HTS sequences (39 ASVs) come from multi-chambered globothalamiids in the textulariid taxa *Cyclammina*, *Nodosinum*, *Reophax*, and *Hormosinella distans* and the miliolid *Cornuspira*. These squatter ASVs cluster in Clade M (8 ASVs) and the New Clade (23 ASVs). Many ASVs (17) originate from a single specimen of *H. distans* (isolate 18127). These multiple ASVs cluster in Clade M and the New Clade and are likely

the result of several squatters occupying the same host. In addition to these textulariids, a specimen of *Storthosphaera* (21082; Figure A9a,b) yielded an undetermined monothalamid sequence that branches at the base of Clade ENFOR5 but without BV support.

A specimen identified morphologically as *Vanhoeffenella* (isolate 21019; Figure A8b) yielded three unassigned ASVs (29, 40 65) branching close to *Storthosphaera* and the above-mentioned *Crithionina*-like specimens. ASV 12 was obtained from the same specimen (21019) and branches within Clade G. The sequences were most likely either those of squatters, or derived from a narrow tube and an accumulation of detrital material that were entangled with the *Vanhoeffenella*. Finally, two ASVs, 24 and 33, were obtained from isolate 20926, which corresponds to a large *Bathysiphon* species identified as *B.* aff. *flavidus* (Figure A9g). Since neither ASV clusters with other *Bathysiphon* sequences, they also most likely both originate from squatters.

Monothalamea: Clade F and Clade V

Two typical specimens of *Vanhoeffenella*, one about twice the size of the other (19132 and 20963; Figure A9c,d) cluster in Clade F. Previously confirmed *Vanhoeffenella* sequences are also members of Clade F [34]. They are joined by a spindle-shaped, entirely agglutinated morphotype with long terminal tubes but a *Vanhoeffenella*-like cell body (20911, Figure A9e). This resembles *Technitella richardi* of de Folin [35] in overall shape but is constructed from fine mineral grains rather than longitudinally aligned sponge spicules. Another specimen (isolate 17658, Figure A9f) resembles *Vanhoeffenella* but has an entirely organic test and terminal apertural tubes that are unusually long. It clusters at the base of Clade I, together with a squatter sequence obtained from *Spirillina* (21060). A similar, entirely organic *Vanhoeffenella*-like morphotype was illustrated by Cedhagen et al. [33] from much shallower (upper bathyal) depths in the Andaman Sea.

3.2.2. Globothalamea

The globothalamids include nine *Oridorsalis umbonatus* specimens and two textulariids. The *O. umbonatus* alignment comprises 17 ASVs with 285 bp. The alignment of the two textulariids is based on four ASVs with an average of 330 bp. The alignment of the 19 monothalamids contains 55 sequences, with 257 sites used for analysis.

Globothalamea—*Oridorsalis umbonatus*

The phylogenetic analysis of *Oridorsalis umbonatus* divided the nine specimens into two groups, highlighted in red and yellow in the phylogenetic tree (Figure 5a). Eight individuals (17641, 17661, 18653, 18654, 18655, 18656, 18658 and 18659) from the UK and the OMS areas (4070–4198 m depth) cluster with the amplicon sequence variants (ASVs) 1, 6, 16, 17, 21, 25, 31, 34, 37 and 63 in the red branch. The yellow branch includes only ASVs from isolate 21027 from the OMS area (BC005, 4200 m depth). Interestingly, the specimen (Figure A6b,c) has a slightly more ovate shape than those corresponding to isolates 18654–18658 (Figure A6a). However, the 18653 specimen (Figure A6d,e) is again rather different, with a somewhat inflated and protruding final chamber. The differences visible in the tree are also evident in the barcoding gap analyses (Figure 5b,c). The SSU rDNA region 37/f and the SSU rDNA region 37/f to 41/f show distances between the ASVs of 0.147 and 0.208, respectively. A value of 0 corresponds to no differences, a value of 1 corresponds to no similarities. The intraspecific variability corresponds to 14.7% and 20.8% from haplotype I to haplotype II.

Globothalamea—Textulariida

Two textulariid specimens analysed with HTS are shown in the globothalamiid phylogenetic tree (Figure 6). Isolate 21092 (Figure A10a–c), corresponding to ASVs 38 and 47, is morphologically almost identical to the specimen of *Cribrostomoides subglobosa* (21096, Figure A7f–h) that was analysed by Sanger sequencing. However, the ASVs cluster close to specimen 20991, and not with the sequences obtained from *C. subglobosa*, suggesting that

they either belong to a textulariid squatter or that isolate 21092 came from a cryptic species that is morphologically indistinguishable from *C. subglobosa*.

Isolate 21035 is represented by ASVs 19 and 27 in the tree. It is a large triserial textulariid, >3 mm in length and provisionally identified as *Verneuilinulla propinqua* (Figure A10d), a species distributed down to 5000 m in the North Pacific [36]. The ASVs cluster next to *Srinivasania* but without support.

Not much can be learnt from these two textulariids sequenced by Illumina. More sequences are necessary in order to clarify their phylogenetic relationships.

4. Discussion

4.1. Calcareous Globothalamea

The 34 calcareous globothalamid specimens that were sequenced belong to six species (five rotaliids and one robertinid) that group within clades as predicted in previous studies [27,28,37–40].

The new *Globocassidulina subglobosa* sequences cluster close to species of *Cassidulina* and *Cassidulinoides* with 100% bootstrap support within the family Cassidulinidae, a member of the monophyletic superfamily Serioidea, proposed as a distinct clade by Holzmann and Pawlowski [28]. This grouping also includes genera such as *Globobulimina*, *Rectuvigerina* and *Uvigerina*.

The four other rotaliids branch within Clade 3. The eight sequences of *Epistominella exigua* all cluster together within Clade 3. This is a morphologically and genetically well-defined species with a bipolar distribution across the Arctic, Atlantic and Southern Oceans [41] as well as a genetically supported occurrence at relatively shallow depths (1905–1990 m) in the NW Pacific near Japan [42]. Our new sequences from the abyssal eastern equatorial Pacific are therefore consistent with a cosmopolitan distribution for *E. exigua* in different oceans, as also suggested by previous morphology-based studies (e.g., [43,44]). This may be related to its ability to opportunistically exploit inputs of labile organic matter derived from surface production [45,46]. We also obtained sequences from *Nuttallides umbonifer*, the most common rotaliid in the eastern CCZ and another well-known deep-sea species with a cosmopolitan distribution based on morphology. The fact that our sequences are identical to a single *N. umbonifer* sequence (specimen 10599) from the SW Atlantic, collected during the DIVA 3 expedition, support a wide range for this species. Finally, within Clade 3, a specimen assigned to *Melonis* sp. grouped next to *M. pompilioides* and *M. barleeanus*. This and morphologically similar specimens that were not sequenced are less inflated (narrower) in edge view than *M. pompilioides* (which was also present in our samples, although not sequenced), but rather more inflated than *M. barleeanum*. It may represent a new species.

A specimen identified as *Hoeglundina elegans* based on test morphology yielded a sequence closest to *Robertina arctica*, the only member of the order Robertinida for which sequence data were already available [30]. This confirms the existing classification of *Hoeglundina* as a robertinid. It is surprising to find a member of this group, which is characterised by an aragonitic test, at abyssal depths close to the CCD. Cushman [47], however, recorded it from just over 4000 m from the NW Pacific and Smith [48] from even deeper water (5000 m) in the northern North Pacific.

4.2. Polymorphism in Oridorsalis umbonatus

Oridorsalis umbonatus is represented by Sanger sequences 17661, 18654 and 21027 (Figure A6a–e). In contrast to *E. exigua* and *N. umbonifer*, they form two branches, suggesting some degree of intraindividual polymorphism. This was confirmed by our HTS analyses, which revealed a divergence between Amplicon Sequence Variants (ASVs) derived from eight of the analysed specimens (Haplotype 1), originating from both the UK-1 and OMS areas, and ASVs from the ninth specimen (21027, Haplotype 2), originating from the OMS area (Figure 5a). The differences visible in the tree are mirrored by a plot of the distances between ASVs versus their frequencies (Figure 5b,c). This suggest that the two haplotypes

represent distinct species, an interpretation supported by the fact that the haplotypes are associated with different specimens.

Variations of 3–5% between sequences have been used to differentiate eukaryotic haplotypes [49,50]. High intraspecific variability of up to 5.15% has been observed in *Ammonia* and 4.6% in *O. umbonatus* [51]. Earlier, Pawlowski et al. [41] reported a greater degree of sequence divergence in *O. umbonatus* than in two other species that they studied (*Epistominella exigua* and *Cibicidoides wuellerstorfi*). Our data show that molecular differences between Haplotype 1 and Haplotype 2 are 14.7% in the 37f region (0.147 distance between ASVs) and to 20.8% in the 37f-41f regions (0.208 distance between ASVs). These differences are consistent with the two haplotypes being distinct species. A second species of *Oridorsalis*, *O. tener*, was described by Brady [11]. Recent authors [52,53] have synonymised *O. tener* with *O. umbonatus*. However, Lohmann [54] had earlier distinguished the two species based on the presence of straight sutures on the spiral side and chambers of roughly equal size in the final whorl in *O. umbonatus*, and curved sutures on the spiral side and chambers increasing in size in the final whorl in *O. tener*. The specimens corresponding to isolates 18654–18658 (Figure A6a) conform to Lohmann's description of *O. umbonatus* while the specimen corresponding to isolate 21027 (Figure A6b,c) is closer to his description of *O. tener*.

Our results support the existence of two *Oridorsalis* species, as suggested by Lohmann. However, previous studies [41,51] used cloning and Sanger sequencing methods and are, therefore, not entirely comparable with our HTS results, which were based on only eight specimens, too few to draw reliable conclusions. Furthermore, the specimen illustrated in Figure A6d,e is unusual in having a distinctly inflated final chamber, unlike the typical form of *O. umbonatus*, although it groups in the same clade as isolates 18654–18658, albeit somewhat separately. Clearly, the status of the *Oridorsalis* haplotypes revealed by HTS requires some further investigation based on additional specimens that are well documented morphologically.

4.3. New Storthosphaera Clade

We obtained the first sequences for the monothalamid *Storthosphaera*. This is a morphologically distinctive species characterised a highly irregular, globular, or more elongate test covered in variably developed protuberances and ridges. The type species, *S. albida* Schultze 1875, was originally described from a depth of 668 m in Bukenfjord (Norway) [55]. Our specimens closely resemble those illustrated by Brady in Pl. 25, Figure 15 and Figure 16 of ref. [11], one of which was from the type locality and the other from another western Norwegian fjord (Korsfjord). Given the geographical and bathymetric separation between our abyssal Pacific specimens and those of Brady, they probably represent different species, although they certainly belong to the same genus. Three sequences for *Storthosphaera* form a well-supported clade (100% BV) with three unidentified monothalamids (Figure 3).

4.4. Cribrostomoides subglobosa (Cushman, 1910)

This species, which has a complicated taxonomic history [56], is one of the most common textulariids in samples from the eastern CCZ [14,36]. We obtained genetic data from seven specimens, six using Sanger sequencing and one using HTS. In the Sanger tree (Figure 2), three specimens (20977, 20987, 21096; Figure A7f–h) grouped together while the others (20986, 20988, 20990) yielded sequences of monothalamid squatters. A possible seventh specimen (20911, Figure A7e) branched separately to *C. subglobosa* in the Sanger tree. Only a side view of the test was available, and so its morphological similarity to *C. subglobosa* could not be confirmed. It was, therefore, regarded as an indeterminate textulariid.

The specimen analysed by HTS (21092, Figure A10a–c) appeared morphologically identical to specimen 21096, one of the three that grouped together in the Sanger tree. However, in the HTS tree, 21092 branched separately from this group, but close to the indeterminate textulariid referred to above (20911). This puzzling result implies either

that the ASVs originated from a textulariid squatter or that this specimen belonged to a genetically distinct cryptic species resembling *C. subglobosa*. The present data cannot resolve this question. Saidova [57] records another species, *C. profundum* Saidova 1961, occurring at depths of 2380–6240 m in the tropical Pacific. However, her photograph (Pl. XXI, Figure 1 in ref. [57]) shows a specimen looking very similar to our *C. subglobosa*.

4.5. Squatters

A number of authors have found monothalamids inside the empty tests of other Foraminifera. Rhumbler [58] described five new species and genera of organic-walled monothalamiids inhabiting the agglutinated tests of *Saccamminia sphaerica;* Christiansen [59] also reported that this large spherical species acted as a host for an undescribed carnivorous Foraminifera. Moodley [60] coined the term 'squatter' for an undescribed organic-walled species that occupied a *Quinqueloculina* shell during culture experiments. Hughes and Gooday [61] found numerous Foraminifera associated with dead xenophyophore tests on the Scottish margin. Many squatters are probably opportunistic or perhaps even accidental inhabitants, but some unusual benthic Foraminifera have an intimate, likely obligate association with the shells of planktonic Foraminifera in the NE Atlantic [62,63]. In the CCZ, radiolarian shells perform a similar function [64].

In the present study, monothalamid sequences were found to include a large proportion of squatters. These can be distinguished because they were derived from multichambered tests, or in a few cases from those of monothalamids with known sequences. The squatters represented a third (33.3%) of the Sanger sequences, while for HTS, they accounted for two-thirds (66.7%) of the ASVs. They are widely scattered across many of the clades in which our sequences occur, in both the Sanger and HTS monothalamid trees (Figures 2 and 4).

A variety of foraminiferal taxa acted as squatter hosts. Among those recognised by Sanger sequencing, the hosts were predominately textulariids (agglutinated globothalamids). Many of these were unspecified, but they also included trochamminid, *Reophax*, and *Cystammina* specimens, as well as the tubulothalamids *Ammodiscus* and *Spirillina*. Monothalamiid hosts included two specimens of *Vanhoeffenella*, indeterminate xenophyophore fragments, one of the *Storthosphaera* specimens (isolate 20930, Figure A3g,h) and very likely the komokiacean illustrated in Figure A2a,b. The specimen shown in Figure A3a,b (isolate 21000) may also be a komokiacean (it somewhat resembles *Chondrodapis*), in which case the sequence derived from it is probably that of a squatter. Among squatters revealed by HTS, most of the hosts were again textulariids, notably *Hormosinella distans* but also *Reophax, Nodosinum* and *Cyclammina*, in addition to single examples of *Vanhoeffenella* and the miliolid *Cornuspira*.

It is not clear whether all the squatters that we recognised through HTS were present within the host tests as adult individuals, as in the published examples mentioned above. There would be space for fully grown monothalamiids, particularly elongate forms, to hide within the opaque tests of larger agglutinated species (e.g., the hormosinids), but there would be little space inside a komokiacean such as *Septuma*. Monothalamids were not seen inside *Vanhoeffenella* tests, although they would be easily visible through the transparent sides. A genetic study by Lecroq et al. [65] revealed an extraordinary variety of eukaryotes, metazoans, fungi, plants and many protistan groups including Foraminifera associated with the tests of two komokiaceans. Presumably, many of these organisms were represented by propagules or free DNA. This could also apply to at least some of the numerous squatters recognised in our data.

5. Conclusions

Although sequences were obtained from only ~21% of the 516 specimens analysed, the results are consistent with the high levels of foraminiferal diversity recorded in previous studies of morphospecies diversity in the eastern CCZ, and particularly with the strong predominance of undescribed monothalamids [13–15,32,66,67]. The new genetic data for undescribed monothalamids, which are linked by photographs to the corresponding test morphology, will help to improve the interpretation of HTS metabarcoding data derived from environmental samples. Together with the numerous squatter sequences, these data emphasise the major knowledge gap that exists when considering benthic foraminiferal diversity. This applies to the larger macrofaunal and megafaunal Foraminifera described in previous studies, as well as to the predominantly meiofaunal taxa considered here. It is important not to lose sight of these major protistan components of deep-sea benthic communities when evaluating the scale of unknown eukaryotic diversity in the Clarion-Clipperton Zone and other deep-sea settings.

Author Contributions: Conceptualisation, J.P. and M.H.; methodology, O.E.H. and M.H.; validation, M.H. and I.B.-A.; formal analysis, O.E.H., M.H. and I.B.-A.; investigation, O.E.H., M.H. and I.B.-A.; resources, J.P.; data curation, M.H. and I.B.-A.; writing—original draft preparation, A.J.G.; writing—review and editing, A.J.G., M.H., O.E.H. and I.B.-A.; visualization, A.J.G., O.E.H., M.H. and I.B.-A.; supervision, J.P.; project administration, J.P.; funding acquisition, J.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Swiss National Science Foundation, grant no. 31003A_179125 (IBA, MH, JP), the Norwegian Research Council, through AKMA project number 287869 and the Norwegian Petroleum Directorate (IBA), and OEH was supported for this project by the Paul Brönnimann Foundation.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: HTS data are deposited and publicly available in the Sequence Read Archive (SRA) public database under the accession PRJNA1015544. Sanger sequences are available under the accession numbers shown in Table A3 of the appendix.

Acknowledgments: We thank UK Seabed Resources Ltd., who funded ABYSSLINE Cruises AB01 and AB02 through a commercial arrangement. The authors are also grateful to Ocean Mineral Singapore, Keppel Corporation—National University of Singapore Corporate Laboratory, and the National Research Foundation (Prime Minister's Office, Singapore) for supporting sampling in the OMS area. The conclusions put forward reflect the views of the authors alone, and not necessarily those of the institutions within the Corporate Laboratory. We also thank the Federal Institute for Geosciences and Natural Resources of Germany (BGR) for supporting the BIONOD cruise and sampling in the BGR area.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Appendix A

Table A1. Station details. There are no station numbers for the RC01 cruise, but sampling locations can be identified from the deployment numbers.

Cruise	Year	Area	Station	Deployment	Latitude (°N)	Longitude (°W)	Depth (m)
AB01	2013	UK-1	B-K-E	EB01	13°50′13.9″	−116°33′30.4″	4182
AB01	2013	UK-1	C	EB02	13°45′30.0″	−116°41′54.7″	4070
AB01	2013	UK-1	D	MC04	13°57′47.8″	−116°34′05.7″	4084
AB01	2013	UK-1	F	MC06	13°48′42.1″	−116°42′36.1″	4076
AB01	2013	UK-1	H	MC09	13°53′18.0″	−116°41′23.9″	4150
AB01	2015	UK-1	I	MC08	13°45′41.9″	−116°27′36.0″	4111
AB01	2013	UK-1	J	MC11	13°54′06.2″	−116°35′24.1″	4166
AB01	2013	UK-1	K	MC10	13°51.801	−116°32.799	4053
AB02	2015	UK-1	U01	MC01	12°24′58.5″	−116°42′53.4″	4126
AB02	2015	UK-1	U02	MC02	12°22′01.3″	−116°31′01.3″	4166
AB02	2016	UK-1	U03	MC03	12°24′24.5″	−116°29′05.1″	4148
AB02	2015	UK-1	U04	MC05	12°34′44.3″	−116°43′25.5″	4235
AB02	2015	UK-1	U05	MC04	12°22′15.8″	−116°36′49.1″	4163
AB02	2015	UK-1	U06	MC06	12°34′44.6″	−116°41′16.9″	4234
AB02	2015	UK-1	U07	MC13	12°27′03.4″	−116°35′40.1″	4129
AB02	2015	UK-1	U09	MC14	12°27′07.4″	−116°30′44.3″	4198
AB02	2015	UK-1	U10	MC15	12°34′11.1″	−116°32′19.8″	4226
AB02	2015	UK-1	U12	MC16	12°25′11.4″	−116°37′28.8″	4160
AB02	2015	OMS	S02	MC10	12°04′54.4″	−117°10′41.8″	4072
AB02	2015	OMS	S03	MC08	12°10′52.0″	−117°15′39.4″	4114
AB02	2015	OMS	S04	MC09	12°00′33.7″	−117°10′41.5″	4148
AB02	2015	OMS	S05	MC11	12°13′02.2″	−117°19′31.6″	4106
AB02	2015	OMS	S08	MC22	12°11′24.5″	−117°22′17.0″	4179
AB02	2015	OMS	S09	MC19	12°05′59.9″	−117°11′47.9″	4082
AB02	2015	OMS	S11	MC23	12°00′33.1″	−117°22′49.3″	4152
AB02	2015	APEI-06	APEI	BC29	19°28′20.5″	−120°11′29.7″	4115
RC01	2020	OMS		BC001	14°1′7.979″	−116°18′22.4″	4111
RC01	2020	OMS		BC002	14°17′31.6″	−116°51′37.3″	4144
RC01	2020	OMS		BC004	14°17′31.1″	−117°13′58.9″	4155
RC01	2020	OMS		BC005	14°6′38.2″	−117°13′54.2″	4200
RC01	2020	OMS		BC007	14°9′1.13″	−116°45′38.9″	4206
RC01	2020	OMS		BC009	14°2′13.1″	−116°30′28.9″	4138
RC01	2020	OMS		BC015	14°7′34.917″	−116°31′20.4″	4116
RC01	2020	UK-1		BC017	13°55′56.7″	−116°30′39.9″	4144
RC01	2020	UK-1		BC020	13°46′18.3″	−117°9′41.9″	4031
RC01	2020	UK-1		BC028	12°20′19.6″	−116°40′8.6″	4158
RC01	2020	UK-1		BC033	12°23′47.06″	−116°33′28.249″	4183
RC01	2020	OMS		BC035	12°27′12.3″	−117°25′30.9″	4149
RC01	2020	OMS		BC036	12°26′45.5″	−117°49′41.0″	4196
RC01	2020	OMS		BC041	12°19′31.136″	−117°40′34.605″	4137
RC01	2020	OMS		BC043	12°19′34.9″	−117°25′29.7″	4157
RC01	2020	OMS		BC045	12°23′6.3348	−117°25′13.9	4131
RC01	2020	OMS		BC036	13°21′54.0″	−117°1′22.0″	4200
BIONOD	2016	BGR	17KG	BC17	11°48′10.2″	−116°53′51.6″	4142
BIONOD	2016	BGR	24KG	BC24	11°53′58″	−117°02′44″	4144
BIONOD	2016	BGR	56KG	BC56	12°41′22″	−118°16′52″	4234

Table A2. Isolate, accession numbers, and sampling localities for monothalamid foraminifera, arranged according to the clades to which they belong. In column 5, MC = Multiple corer; BC = Box corer; EB = Epibenthic sledge, Depl = Deployment, Sp. = specimen, St = station. Entries in bold are those acquired during the present study.

Isolate	Taxon	Accession Numbers	Cruise	Locality Information	Photograph
		Clade A			
1212	Indet. monothalamid	AJ307744		Antarctica, New Harbour	
21252	*Limaxia alba*	OM422947		GBR, In column South Georgia	
		Clade B			
2125	Indet. monothalamid	OM422915		Antarctica, New Harbour	
4026	*Bowseria arctowskii*	LN873614		Antarctica, Ross Ice Shelf	
20977	**Indet. monothalamid**	**OR344859**	**RC01**	**Depl. BC36, Sp. RC1661**	**No photo**
20991	**Textulariid squatter**	**OR344860**	**RC01**	**Depl. BC002, Sp. RC0136**	
		Clade BM			
1784	*Bathysiphon flexilis*	AJ514837		Sweden, Gullmarsfjord	
2880	*Micrometula* sp.	ON053443		Norway, Svalbard	
		Clade C			
526	*Gloiogullmia eurystoma*	AJ317981		Sweden, Tjaerno	
528	*Hippocrepinella* sp.	AJ514843		Sweden, Tjaerno	
20873	*Hippocrepinella alba*	OM422968		GBR, South Georgia	
2357	*Cylindrogullmia alba*	MK748305		Sweden, Tjaerno	
2836	*Pilulina argentea*	OM422894		Norway, Svalbard	
3338	*Bathyallogromia weddellensis*	FR875101		Weddell Sea, abyssal	
19861	*Bathyallogromia kalaallita*	ON053401		Greenland, Nuuk Fjord	
18534 *	**xenophyophore squatter**	**OL772071**	**AB01**	**St. I, Depl. MC08**	
18544 *	**Indet. monothalamid**	**OL772072, OL772073, OL772074**	**AB02**	**St. U02, Depl. MC02**	**Figure A1d**
18549 *	**Indet. monothalamid**	**OL772075, OL772076**	**AB02**	**St. U05, Depl. MC04**	**Figure A1f**
18564 *	**Indet. monothalamid**	**OL873238**	**AB02**	**St. U05, Depl. MC04**	**Figure A1e**
18578 *	**Indet. monothalamid**	**OL772079**	**AB02**	**St. S09, Depl. MC19**	**Figure A1g**
18581 *	**Indet. monothalamid**	**OL772080**	**AB02**	**St. S09, Depl. MC19**	**Figure A1c**
18596 *	**Indet. monothalamid**	**OL772086, OL772087**	**AB02**	**St. U12, Depl. MC16**	**Figure A1h**
18791	**Indet. monothalamid**	**OL772038**	**AB02**	**St. U04, Depl. MC05**	**Figure A1b**
18793	**Indet. monothalamid**	**OL772039**	**AB02**	**St. U04, Depl. MC06**	**Figure A1a**
20937	***Reophax* squatter**	**OR344858**	**RC01**	**Depl. BC028, Sp. RC1260**	
WC18H	*Toxisarcon alba*	AJ307750		Scotland, Loch Linnhe	
		Clade D			
17371	*Hippocrepinella* sp.	MG980268		Chile, Patagonia	
18576 *	***Hippocrepinella* sp.**	**OL772077, OL772078**	**AB02**	**St. U12, Depl. MC16**	**Figure A3k**
21241	*Hippocrepinella hirudinea*	OM422931		GBR, South Georgia	
20966	***Reophax* squatter**	**OL772049**	**RC01**	**Depl. BC36, Sp. RC1661**	
20990	***Cribrostomoides* squatter**	**OL772053**	**RC01**	**Depl. BC002, Sp. RC0136**	
21000	**Komoki squatter**	**OL772055**	**RC01**	**Depl. BC007, Sp. RC0317**	**Figure A3a,b**
21058	**Indet. monothalamid**	**OL772056**	**RC01**	**Depl. BC020, Sp. RC0755**	**Figure A3i,j**
21084	**Indet. monothalamid**	**OL772061**	**RC01**	**Depl. BC043, Sp. RC1773**	**Figure A3e,f**

Table A2. *Cont.*

Isolate	Taxon	Accession Numbers	Cruise	Locality Information	Photograph
		Clade E			
1786	*Psammophaga crystallifera*	FN995295		Sweden, Gullmarsfjord	
2112	*Psammophaga magnetica*	FN995268		Antarctica, New Harbour	
		Clade F			
1182	*Webbinella* sp.	AJ307761		Antarctica, New Harbour	
1921	*Notodendrodes hyalinosphaira*	AJ514860		Antarctica, New Harbour	
		Clade G			
559	*Globipelorhiza sublittoralis*	AJ514848		Sweden, Tjaerno	
17657	***Vanhoeffenella* squatter**	**OL772030**	**AB01**	**St. C, Depl. EB02**	
17659	***Nemogullmia* sp.**	**OL772031**	**AB01**	**St. B, Depl. EB01**	**Figure A4i–k**
18590	**Indet. Monothalamid**	**OL772040**	**AB02**	**St. S02, Depl. MC10**	**No photo**
21078	**Indet. Monothalamid**	**OL772062**	**RC01**	**RC1211, Depl. BC028**	**Figure A6h**
21450	**Indet. Monothalamid**	**OL772070**	**RC01**	**Depl. BC004, Sp. RC1211**	**Figure A2e**
A164	*Nemogullmia* sp.	AJ307767		Antarctica, New Harbour	
		Clade I			
118	*Astrammina triangularis*	AJ318224		Antarctica, New Harbour	
n.a.	*Astrammina rara*	AF411218		Antarctica, Explorers Cove	
		Clade L			
10141	*Cedhagenia saltatus*	FN995339		Ukraine, Balaklava Bay	
LWP3_29_3c	*Ovammina opaca*	HM244870		USA, Sapelo Island	
		Clade M			
12953	*Allogromia laticollaris*	HQ698151		USA, Cold Spring Harbour, strain CSH	
3908	*Allogromia* sp.	MT913376		Antarctica, Ross Ice Shelf	
20812	*Allogromia arnoldi*	MT913378		Cyprus	
20913	***Ammodiscus* squatter**	**OL772044**	**RC01**	**Depl. BC004, Sp. RC0234**	
20986	***Cribrostomoides* squatter**	**OL772051**	**RC01**	**Depl. BC002, Sp. RC0136**	
20988	***Cribrostomoides* squatter**	**OL772052**	**RC01**	**Depl. BC002, Sp. RC0136**	
20995	**Trochamminid squatter**	**OL772054**	**RC01**	**Depl. BC002, Sp. RC0143**	
21046	**Trochamminid squatter**	**OL772066**	**RC01**	**Depl. BC009, Sp. RC0471**	
21089	**Textulariid squatter**	**OL772067**	**RC01**	**Depl. BC015, Sp. RC0903**	
21095	**Textulariid squatter**	**OL772068**	**RC01**	**Depl. BC015, Sp. RC0903**	
		Clade Y			
19842	*Nujappikia idaliae*	ON053404		Greenland, Nuuk Fjord	
21333	*Hilla argentea*	OM422871		GBR, South Georgia	
		Clade *Storthosphaera*			
17751	**Indet. Monothalamid**	**OL772033**	**AB01**	**St. C, Depl. EB02**	**Figure A4a**
18102	***Storthosphaera* sp.**	**OL772034**	**AB01**	**St. F, Depl. MC06**	**Figure A4d**
21086	***Storthosphaera* sp.**	**OL772058**	**RC01**	**Depl. BC045, Sp. RC1876**	**Figure A4b**
21088	***Storthosphaera* sp.**	**OL772059**	**RC01**	**Depl. BC045, Sp. RC1888**	**Figure A4c**
18571	**Indet. Monothalamid**	**OR344856**	**AB02**		**no photo**
18602 *	**Indet. Monothalamid**	**OL772088, OL772089**	**AB02**	**St. SO8, Depl. MC22**	**Figure A4a**

Table A2. *Cont.*

Isolate	Taxon	Accession Numbers	Cruise	Locality Information	Photograph
		New Clade			
18586 *	**Indet. Monothalamid**	**OL772081, OL772082, OL772083**	**AB02**	**St. APEI-06, Depl. BC29**	**Figure A4g**
18926	***Thurammina* aff. *favosa***	**OL772042**	**Bionod**	**Bionod, 126**	**Figure A4f**
19079	**Indet. Monothalamid**	**OL772043**	**AB02**	**St. U03, Depl. MC03**	**no photo**
20916	***Ammodiscus* squatter**	**OL772045**	**RC01**	**Depl. BC028, Sp. RC1265**	
20952	***Vanhoeffenella* squatter**	**OL772048**	**RC01**	**Depl. BC028, Sp. RC1272**	
21094	***Th.* aff. *favosa*—*Reophax* squatter**	**OR344861**	**RC01**	**Depl. BC015, Sp. RC0903**	
		Clade *Tinogullmia*			
3067	*Tinogullmia* sp.	OL873242		Antarctica, New Harbour	
3068	*Tinogullmia* sp.	OL873245		Antarctica, New Harbour	
21002	**likely komoki squatter**	**OL772064**	**RC01**	**Depl. BC001, Sp. RC0401**	**Figure A2a,b**
21011	***Reophax* squatter**	**OL772065**	**RC01**	**Depl. BC007, Sp. RC0328**	
		ENFOR3			
18589 *	**Indet. Monothalamid**	**OL772084, OL772085**	**AB02**	**St. S03, Depl. MC08**	**Figure A3c,d**
20930	***Storthosphaera* squatter**	**OL772046**	**RC01**	**Depl. BC028, Sp. RC0674**	**Figure A3g,h**
env.clone Sap14	Indet. Monothalamid	EU213207		USA, Sapelo Island	
env.clone Keys37	Indet. Monothalamid	EU213205		USA, Florida, Tennessee Reef	
env.clone IC36	Indet. Monothalamid	AY452797		Antarctica, Explorers Cove	
		ENFOR5			
17747	**Indet. Monothalamid**	**OL772032**	**AB01**	**St. C, Depl. EB02**	**Figure A2c,d**
20914	***Ammodiscus* squatter**	**OR344857**	**RC01**	**Depl. BC004, Sp. RC0234**	
21001	**Textulariid squatter**	**OL772063**	**RC01**	**Depl. BC007, Sp. RC0319**	
env. clone F13-5T	Indet. Monothalamid	OL873250		Southern Ocean, 2997m	
env. clone F13-21T	Indet. Monothalamid	OL873251		Southern Ocean, 2997m	
		Separate lineages			
18192	***Cy pauciloculata* squatter**	**OL772035**	**AB02**	**St. U06, Depl. MC06**	
20931	***Reophax* squatter**	**OL772047**	**RC01**	**Depl. BC028, Sp. RC1260**	
21060	***Spirillina* squatter**	**OL772057**	**RC01**	**Depl. BC020, Sp. RC0804**	

* PCR products cloned prior to sequencing.

Table A3. Isolate, accession numbers, and sampling localities for Globothalamea. Taxa marked in bold are those for which sequences were acquired for the present study. In column 5, MC = Multiple corer; BC = Box corer; EB = Epibenthic sledge, Depl = Deployment, Sp. = specimen, St = station.

Species	Isolate	Accession Numbers	Cruise	Locality Information	Figures
Bulimina marginata	3599	AY934747		Norway, Oslofjord	
Cancris auriculus	N204	FM999864		Namibia, Atlantic Ocean	
Cassidulina laevigata	17297	MZ367417		Chile, Beagle Channel	
Cassidulinoides parkerianus	20889	MW834368		GBR, South Georgia	
Cribrostomoides crassimargo	2720	HG425225		Svalbard	
Cribrostomoides sp.	17496	MF770992		New Zealand	
Cribrostomoides sp.	14870	HG425224		Southern Ocean, 2779 m	
Cribrostomoides subglobosa	**20977**	**OL772050**	**RC01**	**Depl. BC036, sp. RC1661**	
Cribrostomoides subglobosa	**20987**	**OL619411**	**RC01**	**Depl. BC002, sp. RC0136**	
Cribrostomoides subglobosa	**21096**	**OL619412**	**RC01**	**Depl. BC015, sp. RC0903**	**Figure A7f–h**
***Epistominella exigua* ***	**18607**	**OL619389, OL619390, OL619391**	**AB02**	**St. U01, Depl. MC01**	**Figure A5d,e**
***Epistominella exigua* ***	**18608**	**OL619392, OL619393, OL619394**	**AB02**	**St. U04, Depl. MC05**	
***Epistominella exigua* ***	**18609**	**OL619395, OL619396, OL619397**	**AB02**	**St. S04, Depl. MC09**	
***Epistominella exigua* ***	**18610**	**OL619398, OL619399, OL619400**	**AB02**	**St. S09, Depl. MC12**	
***Epistominella exigua* ***	**18611**	**OL619401, OL619402**	**AB02**	**St. S05, Depl. MC11**	**Figure A5f**
***Epistominella exigua* ***	**18619**	**OL619403, OL619404**	**AB02**	**St. S09, Depl. MC19**	
***Epistominella exigua* ***	**18621**	**OL619405, OL619406**	**AB02**	**St. S11, Depl. MC23**	
***Epistominella exigua* ***	**18622**	**OL619407**	**AB02**	**St. U07, Depl. MC13**	
Epistominella vitrea	8250	LN873812		Argentinia, Ushuaia	
Globocassidulina subglobosa	**18631**	**MZ262760**	**AB02**	**St. U10, Depl. MC15**	**Figure A5c**
Globocassidulina subglobosa	**18634**	**MZ262761**	**AB02**	**St. U07, Depl. MC13**	**Figure A5a,b**
***Hoeglundina elegans* ***	**17610**	**LN873822, OL619408, OL619409**	**AB01**	**St. C, Depl. EB02**	**Figure A5j,k**
Liebusella goesi	R6	FR754401		Norway, Oslo Fjord	
Melonis barleeanus	19341	OL639707		Svalbard	
Melonis pompilioides	1400	DQ452697		Sweden, Tjaerno	
***Melonis* sp.**	**21079**	**OL619418**	**RC01**	**Depl. BC035, sp. RC1542**	**Figure A6e,f**
Nuttallides umbonifer	10599	OR381569		Argentinian basin of Atlantic Ocean, 4605 m	
***Nuttallides umbonifer* ***	**17639**	**OL619375, OL619376, OL619377**	**AB01**	**St. D, Depl. MC04**	**Figure A5i**

Table A3. *Cont.*

Species	Isolate	Accession Numbers	Cruise	Locality Information	Figures
Nuttallides umbonifer *	**17644**	**OL619378, OL619379, OL619380**	**AB01**	**St. J, Depl. MC11**	
Nuttallides umbonifer *	**18650**	**OL619381, OL619382**	**AB02**	**St. U12, Depl. MC16**	
Nuttallides umbonifer *	**18651**	**OL619383, OL619384**	**AB02**	**St. S02, Depl. MC19**	
Nuttallides umbonifer	**21055**	**OL619420**	**RC01**	**Depl. BC009, sp. RC0481**	**Figure A5g,h**
Nuttallides umbonifer	**20994**	**OL619419**	**RC01**	**Depl. BC002, sp. RC0137**	
***Oridorsalis* sp.** *	**17641**	**OL619387, OL619388**	**AB01**	**St. F, Depl. MC06**	
***Oridorsalis* sp.** *	**17661**	**OL619385, OL619386**	**AB01**	**St. C, Depl. EB02**	
***Oridorsalis* sp.** *	**18653**	**OL619421**	**AB02**	**St. U09, Depl. MC14**	**Figure A6d,e**
***Oridorsalis* sp.** *	**18654**	**OL619422**	**AB02**	**St. S08, Depl. MC22**	**Figure A6a**
***Oridorsalis* sp.** *	**18655**	**OL619423**	**AB02**	**St. S08, Depl. MC22**	**Figure A6a**
***Oridorsalis* sp.** *	**18656**	**OL619424**	**AB02**	**St. S08, Depl. MC22**	**Figure A6a**
***Oridorsalis* sp.** *	**18658**	**OL619425**	**AB02**	**St. S08, Depl. MC22**	**Figure A6a**
***Oridorsalis* sp.** *	**21027**	**OL619426**	**RC01**	**Depl. BC005, sp. RC0416**	**Figure A6b,c**
Reophax* aff. *helenae	**17621**	**OL619417**	**AB01**	**St. H, Depl. MC09**	**Figure A7a similar sp.**
Reophax bilocularis	4953	MK121732		Antarctica	
Reophax curtus	9713	MK121734		Russia, White Sea	
Reophax pilulifer-arenulata	8206	MF770994		Antarctica, Arctowski	
***Reophax* sp.**	**18820**	**OL619416**	**BIONOD**	**St. US10**	**Figure A7b**
***Reophax* sp.**	**20965**	**OL619413**	**RC01**	**Depl. BC001, sp. RC0401**	**Figure A7d**
***Reophax* sp.**	**21004**	**OL619414**	**RC01**	**Depl. BC005, sp. RC0417**	**Figure A7c**
Robertina arctica	2632	HE998677		Svalbard	
Spiroplectammina sp.	2646	AJ504689		Svalbard	
Srinivasania sundarbanensis	EC_4	MN364400		India, Sundarbans	
Stainforthia fusiformis	3965	AY934744		Norway, Skagerrak	
Trochammina hadai	21192	MZ707232		West Australia	
indet. Textulariid	**20991**	**OL619410**	**RC01**	**Depl. BC002, sp. RC0136**	**Figure A7e**
Uvigerina peregrina	U26	AY914569		Norway, Oslo Fjord	

* PCR products cloned prior to sequencing.

Table A4. Species analysed using Sanger sequencing.

Species	Isolate	Seq. Length	GP Content	Cruise	Area	Station	Deployment	Figure
Hoeglundina elegans *	17610	1020	40.1	AB01	UK-1	C	EB02	Figure A5j,k
Reophax aff. *helenae*	17621	846	41.7	AB01	UK-1	H	MC09	Figure A7a
Nuttallides sp. *	17639	1025	42.4	AB01	UK-1	D	MC04	Figure A5i
Oridorsalis umbonatus *	17641	958	46.3	AB01	UK-1	F	MC06	
Nuttallides sp. *	17644	1033	42.5	AB01	UK-1	J	MC11	
Vanhoeffenella squatter	17657	936	56.7	AB01	UK-1	C	EB02	
Nemogullmia sp.	17659	958	55.7	AB01	UK-1	B-K-E	EB01	Figure A4i–k
Oridorsalis umbonatus *	17661	958	46.3	AB01	UK-1	C	EB02	
indet. monothalamid	17747	947	48.6	AB01	UK-1	C	EB02	Figure A2c,d
indet. monothalamid	17751	708	43.0	AB01	UK-1	C	EB02	Figure A4e
Stortosphaera sp.	18102	753	40.3	AB01	UK-1	F	MC06	Figure A4d
Cystammina squatter	18192	817	42.6	AB02	UK-1	U06	MC06	
Xenophyophore squatter	18534	1167	49.7	AB01	UK-1	I	MC08	
indet. monothalamid *	18544	924	40.3	AB02	UK-1	U02	MC02	Figure A1d
indet. monothalamid *	18549	997	40.5	AB02	UK-1	U05	MC04	Figure A1f
indet. monothalamid	18564	1013	41.0	AB02	UK-1	U05	MC04	Figure A1e
Hippocrepinella sp. *	18576	756	41.8	AB02	UK-1	U12	MC16	Figure A3k
indet. monothalamid	18578	1163	49.7	AB02	OMS	S09	MC19	Figure A1g
indet. monothalamid	18581	1167	49.7	AB02	OMS	S09	MC19	Figure A1c
indet. monothalamid *	18586	702	45.8	AB02	APEI-06	APEI-06	BC29	Figure A4g
indet. monothalamid *	18589	831	47.5	AB02	OMS	S03	MC08	Figure A3c,d
indet. monothalamid	18590	941	57.0	AB02	OMS	S02	MC10	No photo
indet. monothalamid *	18596	1062	45.3	AB02	UK-1	U12	MC16	Figure A1h
indet. monothalamid *	18602	777	44.1	AB02	OMS	S08	MC22	Figure A4a
Epistominella exigua *	18607	984	44.1	AB02	UK-1	U01	MC01	Figure A5d,e
Epistominella exigua *	18608	984	43.9	AB02	UK-1	U04	MC05	
Epistominella exigua *	18609	1021	44.2	AB02	OMS	S04	MC09	
Epistominella exigua *	18610	1021	44.1	AB02	OMS	S09	MC12	
Epistominella exigua *	18611	1021	44.2	AB02	OMS	S05	MC11	Figure A5f
Epitominella exigua *	18619	1021	44.3	AB02	OMS	SO9	MC19	
Epistominella exigua *	18621	1020	44.0	AB02	OMS	S11	MC23	
Epistominella exigua *	18622	1021	44.1	AB02	OMS	S11	MC23	
Globocassidulina	18631	1023	38.3	AB02	UK-1	U10	MC15	Figure A5c
Globocassidulina	18634	1022	37.9	AB02	UK-1	U07	MC13	Figure A5a,b
Nuttallides sp. *	18650	1026	42.7	AB02	UK-1	U12	MC16	
Nuttallides sp. *	18651	1062	42.9	AB02	OMS	S02	MC19	
Oridorsalis umbonatus	18653	848	46.7	AB02	UK-1	U09	MC14	Figure A6d,e
Oridorsalis umbonatus	18654	849	46.9	AB02	OMS	S08	MC22	Figure A6a
Oridorsalis umbonatus	18655	851	46.5	AB02	OMS	S08	MC22	Figure A6a
Oridorsalis umbonatus	18656	851	46.5	AB02	OMS	S08	MC22	Figure A6a
Oridorsalis umbonatus	18658	851	46.4	AB02	OMS	S08	MC22	Figure A6a
indet. monothalamid	18791	976	44.9	AB02	UK-1	U04	MC05	Figure A1b
indet. monothalamid	18793	976	43.7	AB02	OMS	S03	MC08	Figure A1a
Reophax sp.	18820	912	40.4	BIO	BGR	17KG	BC17	Figure A7b
Septuma squatter	18905	716	43.3	BIO	BGR	24KG	BC24	
Thurammina aff. *favosa*	18926	722	44.7	BIO	BGR	56KG	BC56	Figure A4f
indet. monothalamid	19079	765	42.5	AB02	UK-1	U03	MC03	Figure A1i
Ammodiscus squatter	20913	912	40.6	RC01	OMS		BC004	
Ammodiscus squatter	20916	735	43.9	RC01	UK-1		BC028	
Storthosphaera squatter	20930	906	46.4	RC01	UK-1		BC017	Figure A3g,h
Reophax squatter	20931	843	47.6	RC01	UK-1		BC028	
Vanhoeffenella squatter	20952	735	44.0	RC01	UK-1		BC028	

Table A4. *Cont.*

Species	Isolate	Seq. Length	GP Content	Cruise	Area	Station	Deployment	Figure
Reophax sp.	20965	863	43.4	RC01	OMS		BC036	Figure A7d
Reophax squatter	20966	805	43.7	RC01	OMS		BC036	
	20977	901	42.0	RC01	OMS		BC036	
Cribrostomoides squatter	20986	861	39.8	RC01	OMS		BC002	
	20987	903	41.4	RC01	OMS		BC002	
Cribrostomoides squatter	20988	875	39.9	RC01	OMS		BC002	
Cribrostomoides squatter	20990	820	41.4	RC01	OMS		BC002	
Textulariid	20991	904	40.6	RC01	OMS		BC002	Figure A7e
Nuttallides	20994	908	42.5	RC01	OMS		BC002	
Trochamminid squatter	20995	856	39.7	RC01	OMS		BC002	
indet. Monothalamid	21000	759	43.4	RC01	OMS		BC007	Figure A3a,b
Textulariid squatter	21001	914	49.1	RC01	OMS		BC007	
Likely komoki squatter	21002	868	47.3	RC01	OMS		BC007	Figure A2a,b
Reophax sp.	21004	918	40.4	RC01	OMS		BC001	Figure A7c
Reophax squatter	21011	1023	43.0	RC01	OMS		BC001	
Oridoralis umbonatus	21027	848	45.6	RC01	OMS		BC005	Figure A6b,c
Trochamminid squatter	21046	876	39.8	RC01	OMS		BC009	
Nuttallides sp.	21055	909	42.6	RC01	OMS		BC009	Figure A5g,h
indet. monothalamid	21058	738	43.9	RC01	UK-1		BC020	Figure A3i,j
Spirillina squatter	21060	836	43.0	RC01	UK-1		BC020	
indet. Monothalamid	21078	1008	55.4	RC01	UK-1		BC028	Figure A4h
Melonis sp.	21079	865	43.5	RC01	OMS		BC035	Figure A6e,f
indet. Monothalamid	21084	759	42.6	RC01	OMS		BC043	Figure A3e,f
Storthosphaera sp.	21086	791	41.0	RC01	OMS		BC045	Figure A4b
Storthosphaera sp.	21088	719	40.9	RC01	OMS		BC045	Figure A4c
Textulariid squatter	21089	872	39.8	RC01	OMS		BC015	
Textulariid squatter	21095	900	40.0	RC01	OMS		BC015	
Cribro. subglobosa	21096	903	41.4	RC01	OMS		BC015	Figure A7f–h
Indet monothalamid	21450	948	55.7	RC01	OMS		BC036	Figure A2e

* PCR products cloned prior to sequencing.

Table A5. Specimens analysed using HTS. B = *Bathysiphon*, C. = *Cribrostomoides*, Horm. = *Hormosinella*, Nodo = *Nodosinum*, V. = *Verneuilinulla*.

Species	ASVs	Isolate	Seq. Length	GC Content	Cruise	Area	Station	Deployment	Figures
Oridorsalis umbonatus	17, 31, 34	17641	288	45.5	AB01	UK-1	F	MC06	
Vanhoeffenella-like	8, 32, 35, 66	17658	260	41.3	AB01	UK-1	C	EB02	Figure A9f
Oridorsalis umbonatus	17, 63	17661	288	45.9	AB01	UK-1	F	MC06	
Crithionina hispida	9, 58	18122	249	41.1	AB01	UK-1	J	MC22	Figure A8e–g
Horm. distans squatters	10, 11, 42, 44, 46, 49, 54, 55, 56, 64, 68, 69, 71, 72, 73, 83, 85	18127	255	41.7	AB01	UK-1	K	MC10	
Saccamminid	51	18547	367	37.8	AB02	UK-1	U03	MC02	Figure A8a
N. gaussicum squatter	2, 28	18640	293	40.7	AB01	UK-1	C	EB02	
Oridorsalis umbonatus	16, 25	18653	288	46.9	AB02	UK			Figure A6d,e
Oridorsalis umbonatus	1, 6, 37	18654	287	46.3	AB02	OMS			Figure A6a
Oridorsalis umbonatus	1, 6, 37	18655	287	46.3	AB02	OMS			Figure A6a
Oridorsalis umbonatus	1, 6, 37	18656	287	46.3	AB02	OMS			Figure A6a
Oridorsalis umbonatus	1, 6, 21, 37	18658	282/287	46.2	AB02	OMS			Figure A6a
Oridorsalis umbonatus	1, 6	18659	287	46.4	AB02	OMS			
Vanhoeffenella sp.	3, 59	19132	361	40	AB02	OMS			Figure A9c
Spindle-like	13	20911	373	40	RC01	OMS			Figure A9e
B. aff. *flavidus*	24, 33	20926	389	36.7	RC01	OMS			Figure A9g
Reophax squatter	22, 70, 77, 93	20932	255	43.9	RC01	OMS			
Reophax squatter	22, 78, 81, 86, 94	20934	224	41.5	RC01	UK-1			
Cornuspira squatter	22, 48, 84	20947	245	42.3	RC01	UK-1			
Cyclammina squatter	36, 62	20949	333	38.6	RC01	UK-1			
Vanhoeffenella sp.	26	20963	376	38.9	RC01	UK-1			Figure A9d
Vanhoeffenella squatter	12, 29, 40, 65	21019	242	46.1	RC01	OMS			Figure A8b
Oridorsalis umbonatus	53, 57, 67, 80, 89, 90, 101	21027	284	44.6	RC01	OMS			Figure A6b,c
V. propinqua	19, 27	21035	328	38.8	RC01	OMS			Figure A10d
Storthosphaera squatter?	60	21082	306	44.4	RC01	OMS			Figure A9a,b
Indet. monothalamid	5	21085	261	44.8	RC01	OMS			Figure A8d
Indet. monothalamid	4, 95, 96	21087	238	43.2	RC01	OMS			Figure A8c
C. subglobosa squatter	38, 47	21092	333	37.7	RC01	OMS			Figure A10a–c

Table A6. Isolate and ASV numbers for monothalamid and textulariid foraminifera arranged by clade.

Isolate	Taxon	ASV Numbers
	Clade C	
20926	squatter *Bathysiphon*	24, 33
18640	squatter *Nodosinum*	2, 28
18547	undet. Monothalamid	51
	Clade G	
21019	squatter *Vanhoeffenella*	12
	Clade M	
18127	squatter *Hormosinella*	11, 46, 54, 69, 72, 73
20949	squatter *Cyclammina*	36, 62
21087	undet. Monothalamid	95
	Clade F	
19132	*Vanhoeffenella*	3, 59
20963	*Vanhoeffenella*	26
20911	spindle shaped undet. Monothalamid	13
	Clade *Storthosphaera*	
21085	undet. Monothalamid	5
21087	undet. Monothalamid	4, 96
21019	squatter *Vanhoeffenella*	29, 40, 65
18122	undet. monothalamid	9, 58
	New Clade	
20934	squatter *Reophax*	22, 78, 81, 86, 94
20947	squatter *Cornuspira*	22, 48, 84
20932	squatter *Reophax*	22, 70, 77, 93
18127	squatter *Hormosinella*	10, 42, 44, 49, 55, 56, 64, 68, 71, 83, 85
	monothalamids branching independently	
21082	squatter *Storthosphaera*	60
17658	undet. monothalamid	8, 32, 35, 66
	Textulariids	
21092	squatter? *Cribrostomoides*	38, 47
21035	undet. textulariid	19, 27

Appendix B

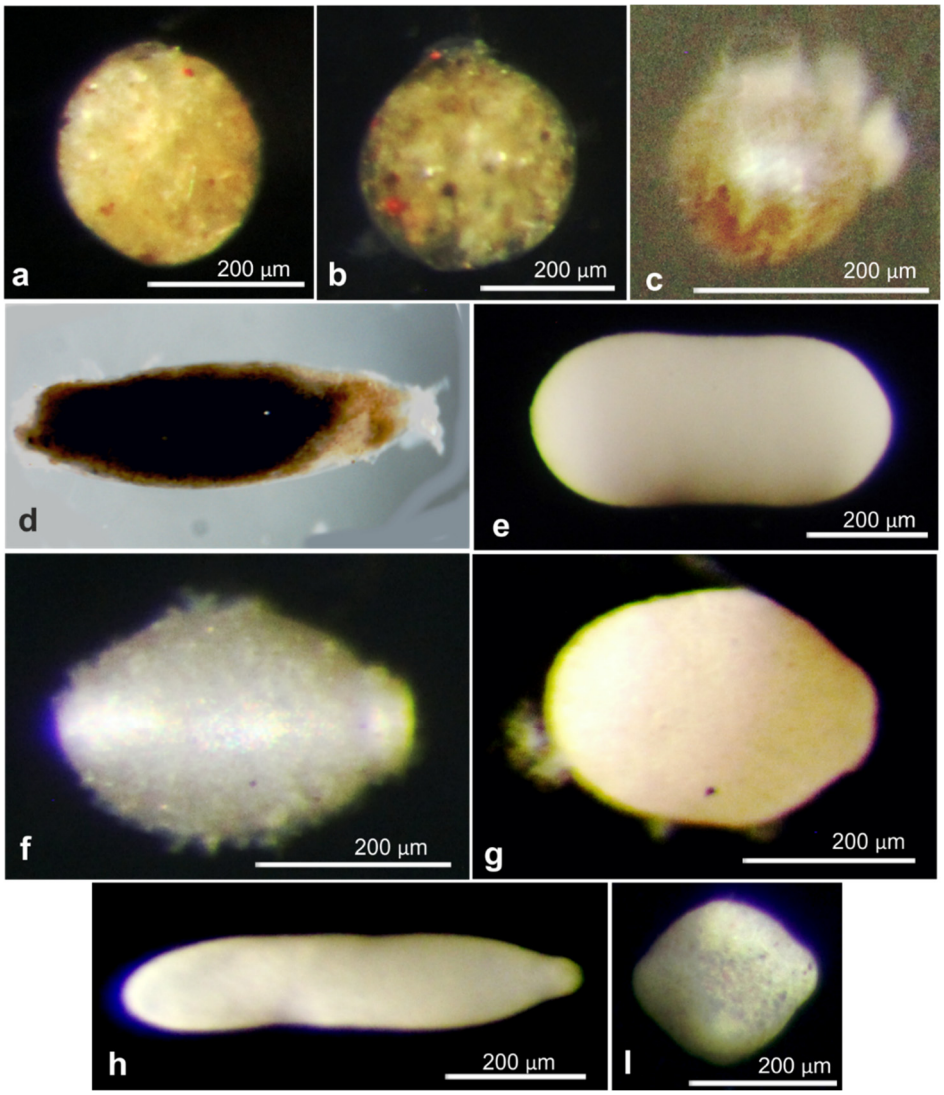

Figure A1. Monothalamids used for Sanger sequencing. (**a**) Spherical organic-walled 'allogromiid' morphologically similar to *Bathyallogromia*. Isolate 18793, AB02 cruise, Station S03, deployment MC08. (**b**) Similar specimen. Isolate 18791, AB02 cruise, Station U04, deployment MC05. (**c**) Tiny, finely agglutinated monothalamid (possible saccamminid) with reflective surface. Isolate 18581, AB02 cruise, Station SO9, deployment MC19. (**d**) Elongate, finely agglutinated monothalamid (saccamminid). Isolate 18544, AB02 cruise, Station U02, deployment MC02. (**e**) Broad, cylindrical saccamminid with two tiny terminal apertures and very smooth, fine-grained surface. Isolate 18564, AB02 cruise, Station U05, deployment MC04. (**f**) Silver saccamminid. Isolate 18549, AB02 cruise, Station U05, deployment MC04. (**g**) White saccamminid. Isolate 18578, AB02 cruise, Station SO9, deployment MC19. (**h**) Elongate, cylindrical saccamminid with fine-grained surface. Isolate 18596, AB02 cruise, Station U12, deployment MC16. (**i**) White sphere with low bumps, somewhat similar to *Thurammina albicans* Brady 1879. Isolate 19079, AB02 cruise, Station U03, MC03. Scale bars = 200 µm. No scale bar is available for (**d**).

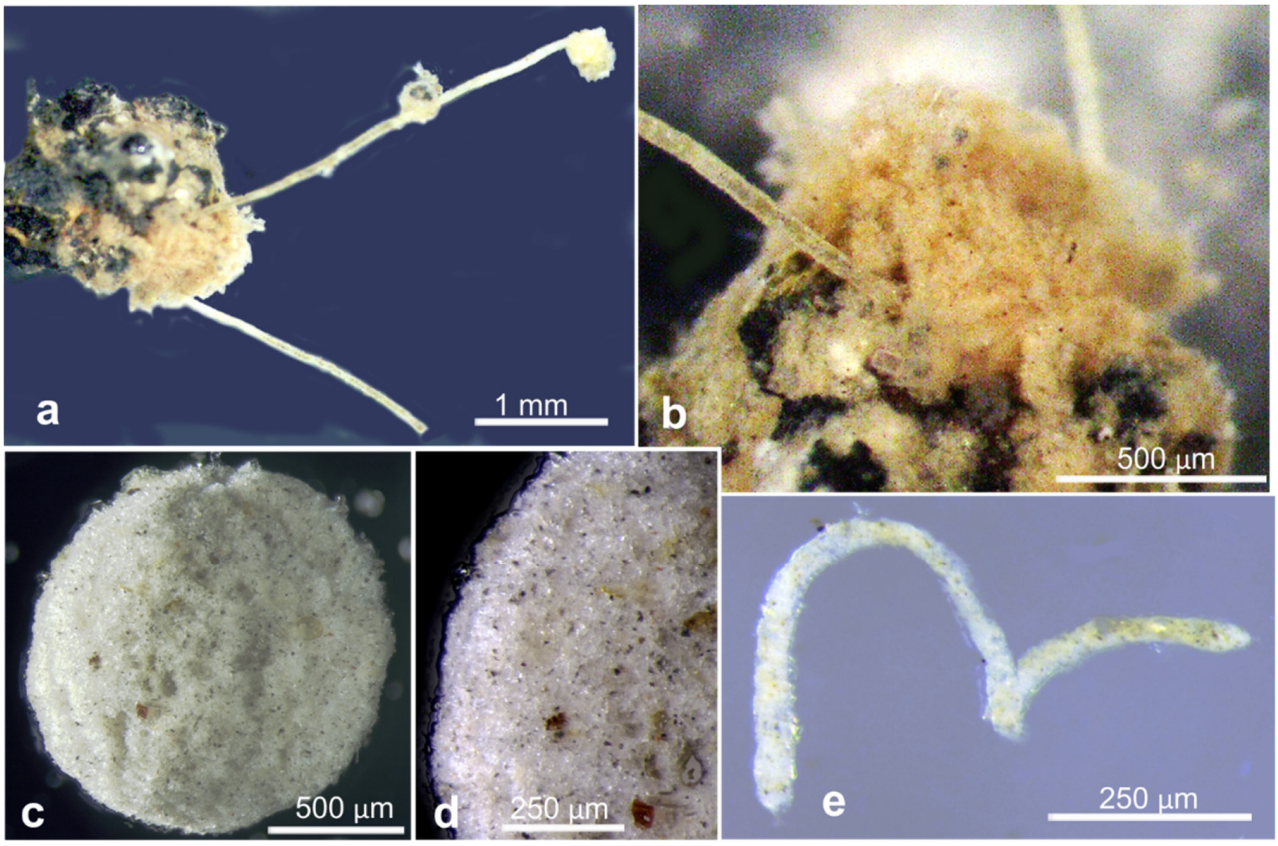

Figure A2. Monothalamids used for Sanger sequencing. (**a**,**b**) Komokiacean with associated tubes on a small nodule. It is not clear whether the two long tubes are an integral part of the komoki or a separate organism. The corresponding sequence is almost certainly that of a squatter. Isolate 21002, RC01 cruise, OMS area, deployment BC007. (**c**,**d**) Soft agglutinated sphere with finely granular test wall. Isolate 17747, AB01 cruise, Station C, deployment EB02. (**e**) Elongate, tubular organic-walled monothalamid with terminal apertures found inside a xenophyophore. Isolate 21450, RC01 cruise, OMS area, deployment BC036. Scale bars = 1 mm (**a**), 500 µm (**b**,**c**), 250 µm (**d**,**e**).

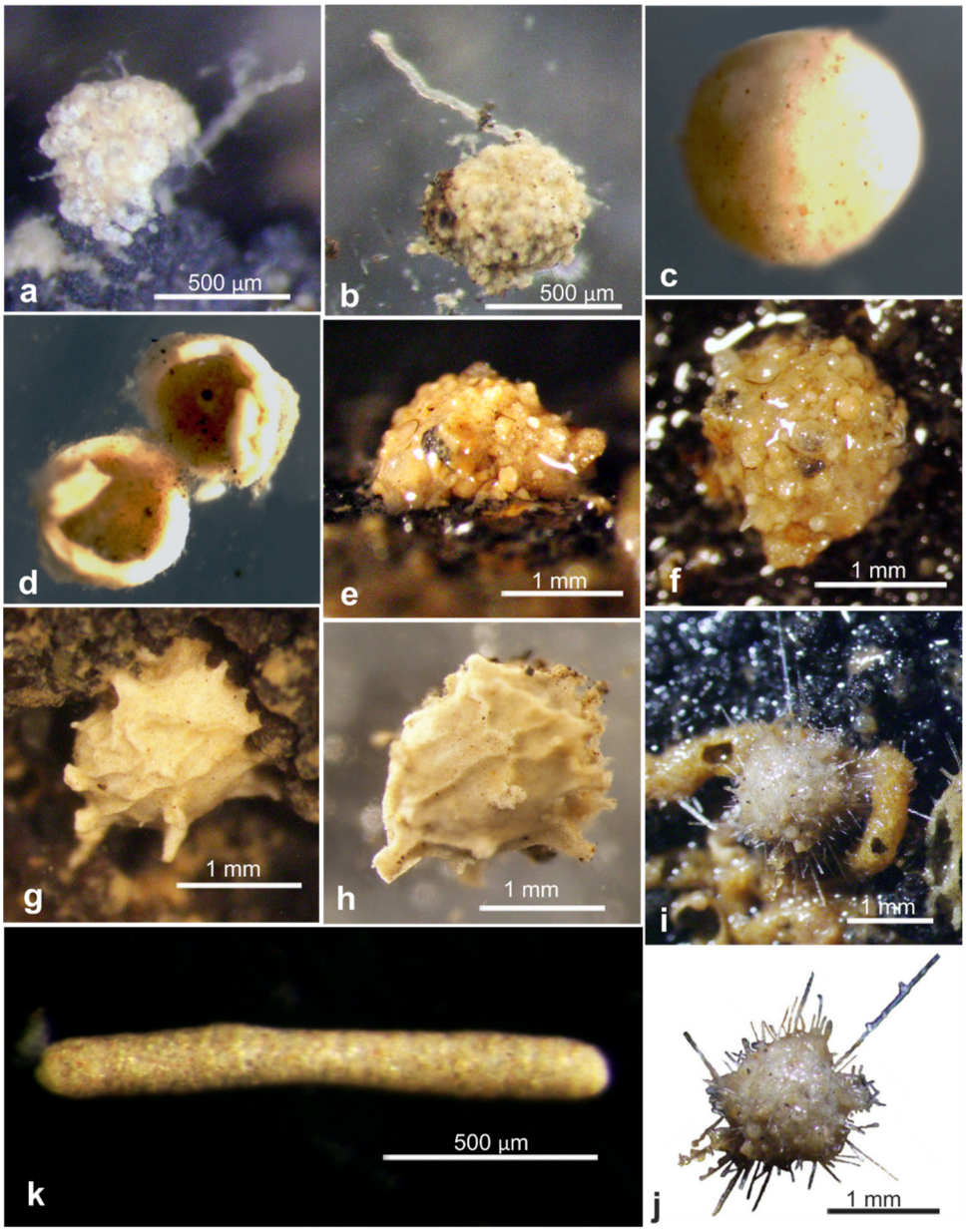

Figure A3. Monothalamids used for Sanger sequencing. (**a**,**b**) Spherical test partly attached to nodule with bumpy surface and giving rise to a long tube; this may be a komokiacean and the corresponding sequence possibly that of a squatter. Isolate 21000, OMS area, deployment BC007. (**c**,**d**) Spherical monothalamid. Isolate 18589, AB02 cruise, Station S03, deployment MC08. (**c**) Intact test with no evidence of an aperture. (**d**) Test broken open to show relatively thick wall. (**e**,**f**) Dome attached to nodule with knobbly test covered in radiolarians. Isolate 21084, RC01 cruise, OMS area, deployment BC43. (**g**,**h**) *Storthosphaera* sp. Isolate 20930, RTC01 cruise, UK-1 area, deployment BC20. The sequence groups separately from other *Storthosphaera* sequences and is, therefore, presumed to be that of a squatter. (**g**) Specimen as originally found attached to a nodule. (**h**) Specimen detached from nodule. (**i**,**j**) White dome bristling with spicules, resembling *Crithionina hispida* Flint, 1899. Isolate 21058, RC01 cruise, UK-1 area, deployment BC20. (**i**) Specimen as originally seen on nodule. (**j**) Detached from nodule. (**k**) *Hippocrepinella* sp. Isolate 18576, AB02 cruise, UK-1 area, Station U12, deployment MC16. Scale bars = 500 µm (**a**,**b**,**k**), 1 mm (**e**–**j**). No scale bars are available for (**c**,**d**).

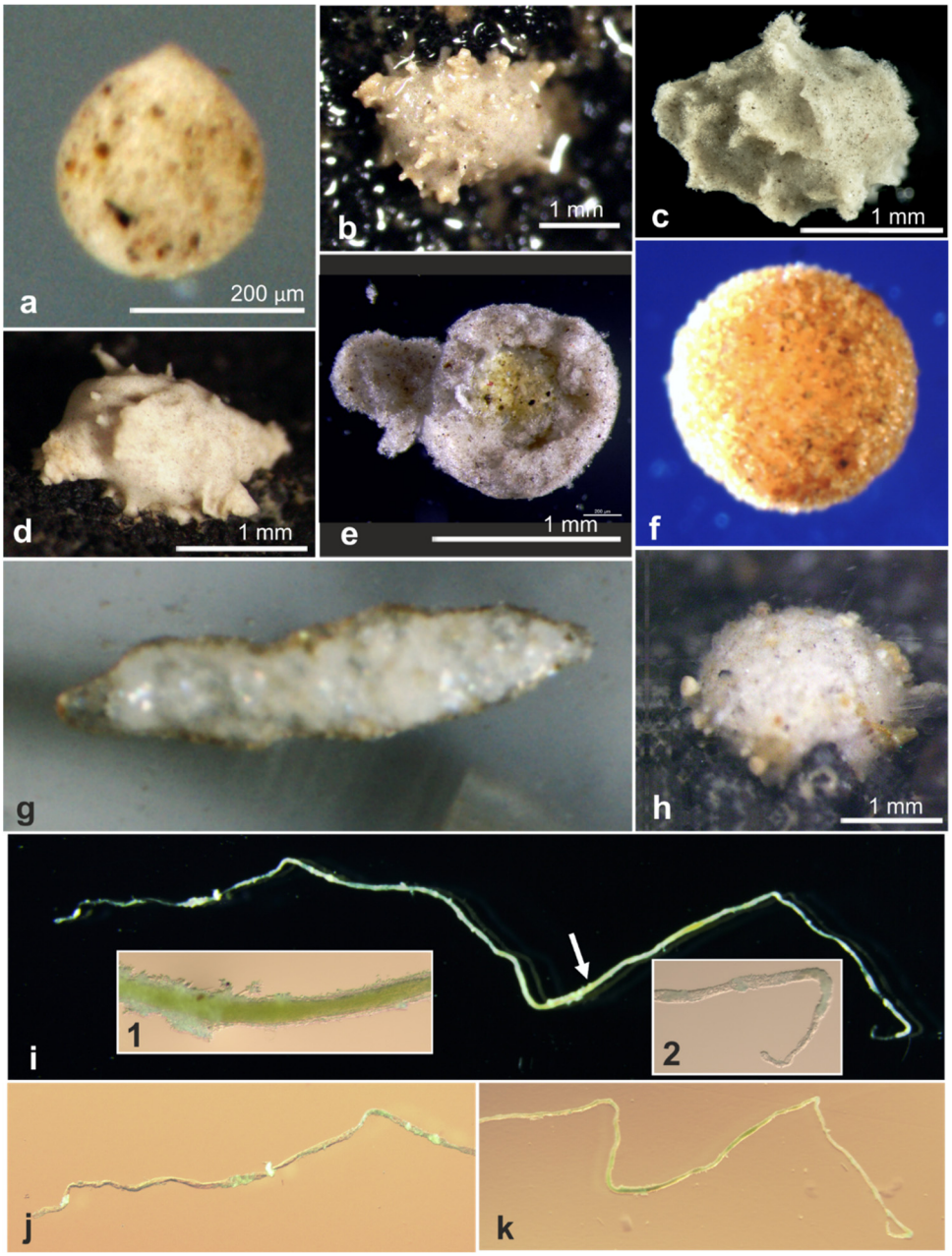

Figure A4. Monothalamids used for Sanger sequencing. (**a**) Small ovate saccamminid; isolate 18602, AB02 cruise, Station SO8, deployment MC22. (**b**–**d**) *Storthosphaera* sp. (**b**) Specimen attached to nodule. Isolate 21086, RC01 cruise, OMS area, deployment BC45. (**c**) Unattached specimen. Isolate 21088, RC01 cruise, OMS area, deployment BC45. (**d**) Specimen attached to a nodule. Isolate 18102, AB01 cruise, Station F, deployment MC06. (**e**) Agglutinated sphere—the test is broken open to show thick, soft test wall and granular test contents. Isolate 17751, cruise A01, Station C, deployment EB02. (**f**) Spherical test resembling *Thurammina favosa* Flint, 1899. Isolate 18926, BIONOD cruise, Station US36, deployment BC56. (**g**) Elongate monothalamid with organic wall. Isolate 18586, AB02 cruise, APEI-6 area, deployment BC29. (**h**) Whitish dome with spicules attached to nodule. Isolate 21078, RC01 cruise, deployment BC28. (**i**–**k**) *Nemogullmia* sp. Isolate 17659, AB01 cruise, Station B, deployment EB01. (**i**) Complete specimen. The two insets show details, viewed with transmitted light, of parts of the test: (1) section indicated by arrow in the main image (note the green cytoplasm in this part of the test and the partial agglutinated sheath); (2) the end of the test. (**j**,**k**) Transmitted-light photographs. Scale bars = 200 µm (**a**), 1 mm (**b**–**h**). No scale bars are available for (**f**,**g**,**i**).

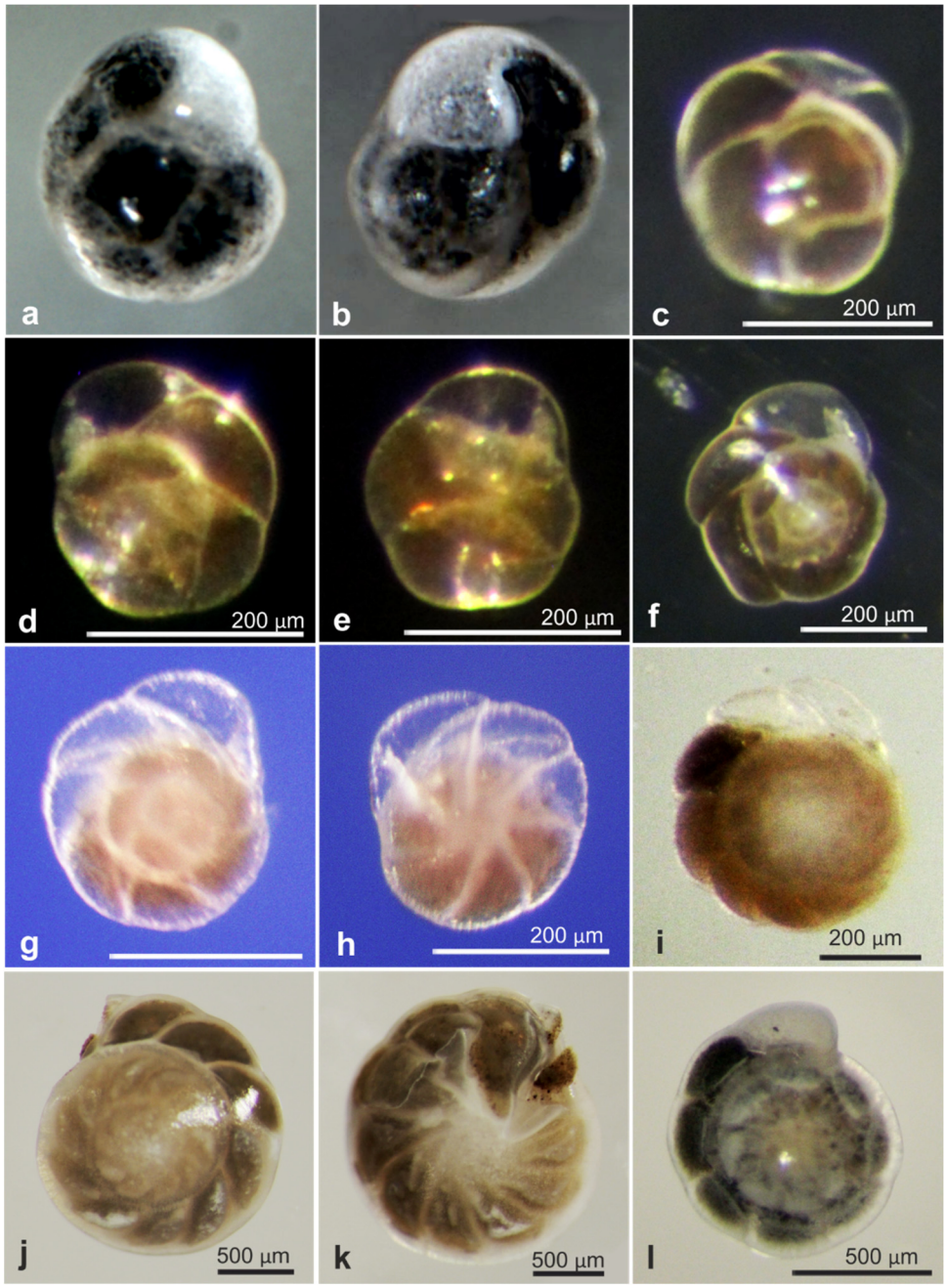

Figure A5. Rotaliids. (**a**–**k**) Specimens used for Sanger sequencing. (**a**–**c**) *Globocassidulina subglobosa* (Brady, 1881). (**a**,**b**) Different views of same test. Isolate 18634, AB02 cruise, UK-1 area, Station U07, deployment MC13. (**c**) Different specimen. Isolate 18631, AB02 cruise, UK 1 area, Station U10, deployment MC15. (**d**–**f**) *Epistominella exigua* (Brady, 1884). (**d**,**e**) Different sides of same test. Isolate 18607, AB02 cruise, UK-1 area, Station U01, deployment MC01. (**f**) Isolate 18611, AB02 cruise, OMS area, Station S05, deployment MC11. (**g**–**i**) *Nuttallides umbonifer* (Cushman, 1933). (**g**,**h**) Different sides of the same test. Isolate 21055, RC01 cruise, OMS area, deployment BC009. (**i**) Isolate 17639, AB01 cruise, Station D, deployment MC04. (**j**–**l**) *Hoeglundina elegans* (d'Orbigny, 1826). (**j**,**k**) Different sides of same specimen. Isolate 17610, AB01 cruise, Station C, deployment EB02. (**l**) Additional specimen not used for sequencing; AB01 cruise, Station C, deployment EB02. Scale bars = 200 µm (**c**–**i**), 500 µm (**j**–**l**). Scale bars were not available for (**a**,**b**).

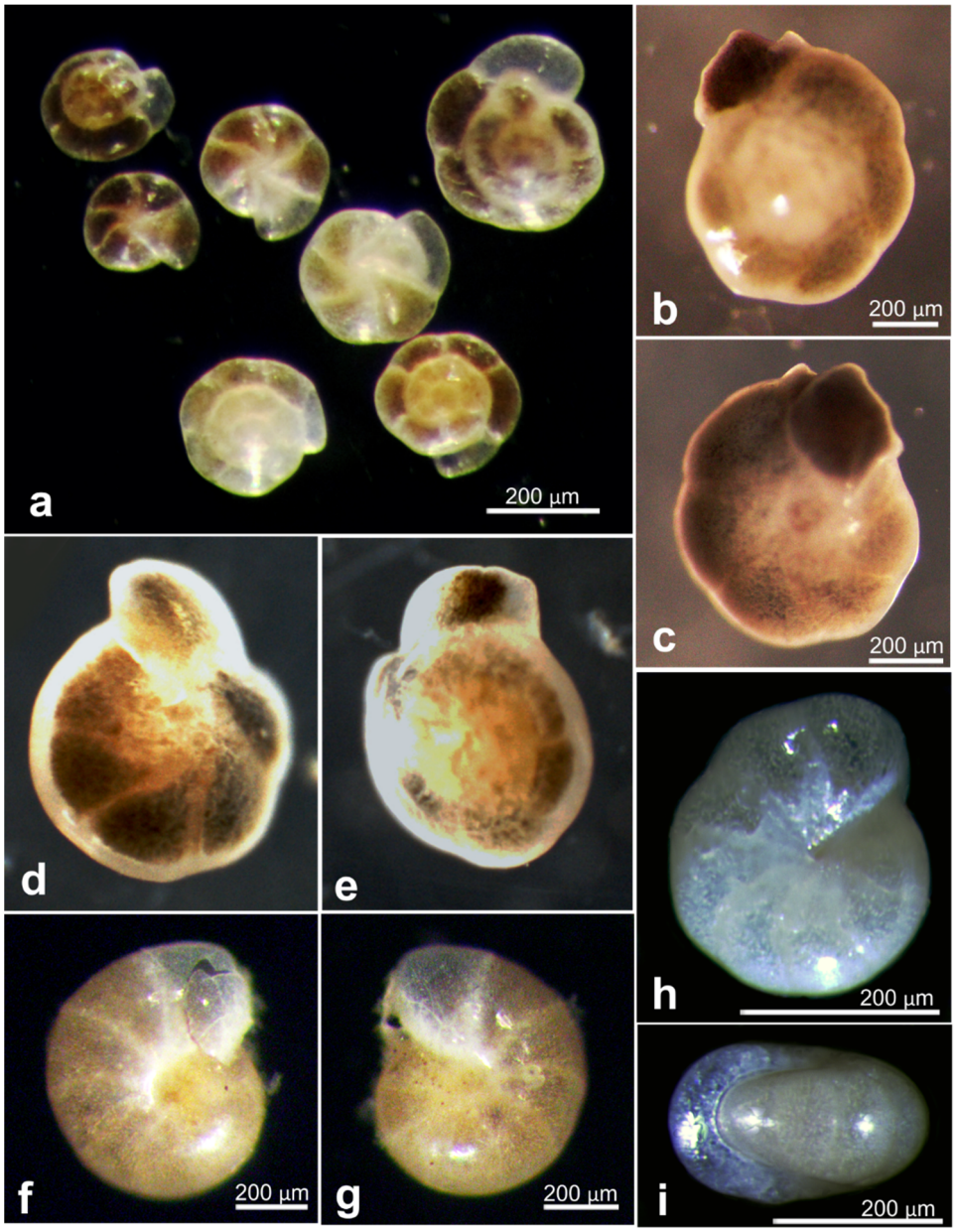

Figure A6. Rotaliids. (**a**–**e**) *Oridorsalis umbonatus* (Reuss, 1851) sequenced using both Sanger and HTS methods. (**a**) Seven specimens, of which four were sequenced. Isolates 18654–18658, but it is not possible to assign numbers to particular specimens; AB02 cruise, Station SO8, deployment MC22. F. (**b**,**c**) Different sides of specimen that branches separately from others in HTS tree. Isolate 21027, RC01 cruise, deployment BC005. (**d**,**e**) Different sides of same specimen. Isolate 18653, AB02 cruise, Station U09, deployment MC14. (**f**–**i**) *Melonis* sp. (**f**,**g**) Different sides of sequenced specimen. Isolate 21079, RC01 cruise, OMS area, deployment BC035. (**h**,**i**) Additional specimen not used for sequencing. AB01 cruise, Station D, deployment MC04. Scale bars = 200 µm.

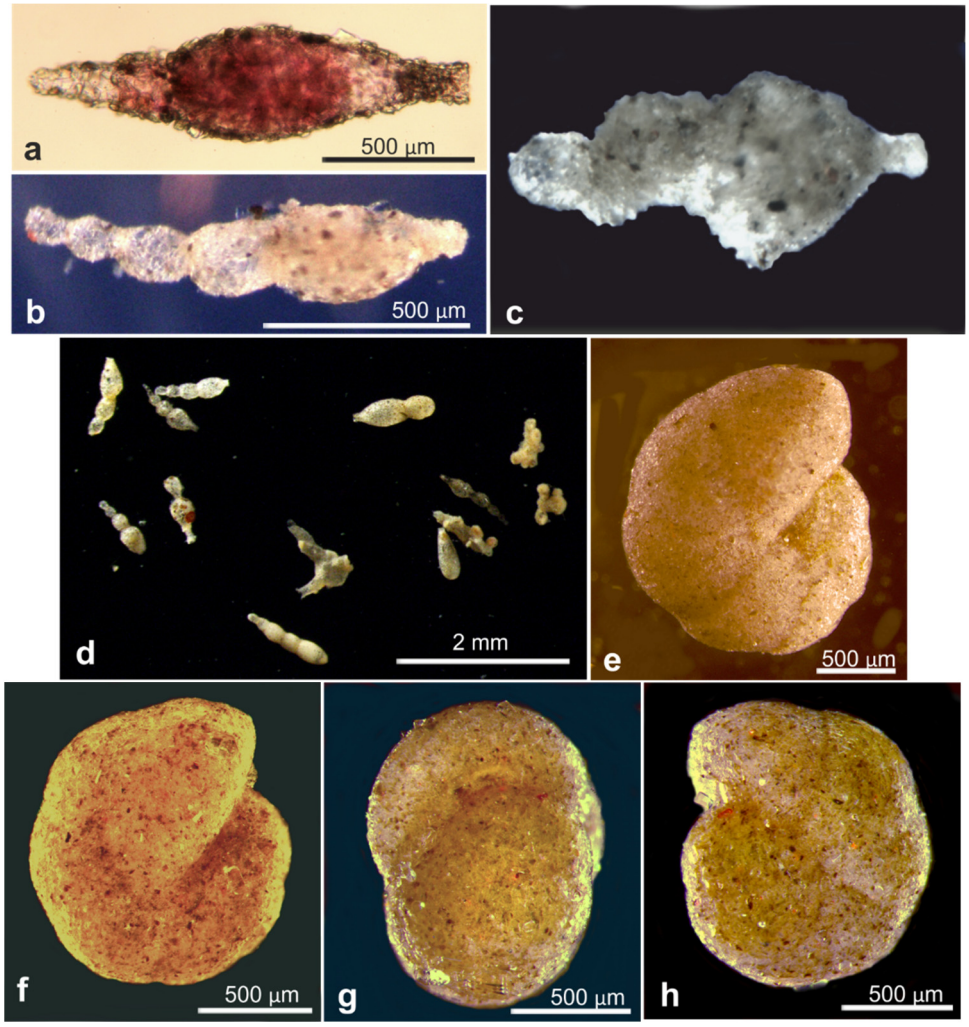

Figure A7. Textulariids used for Sanger sequencing, unless stated otherwise. (**a**) *Reophax* aff. *helenae* Rhumbler, 1931. AB01 cruise, Station D, deployment MC04. This specimen was not used for Sanger sequencing, but it is similar to one that was. (**b**) *Reophax* sp. 1 + 4 sensu Goineau and Gooday (2017). Isolate 21004, RC01 cruise, OMS area, deployment BC001. (**c**) Indeterminate *Reophax*. Isolate 18820, BIONOD cruise, Station US10, deployment BC17. (**d**) *Reophax* spp., including isolate 20968, RC01 cruise, deployment BC001. The collection includes several species but it is not possible to determine which corresponds to the isolate. (**e**) Indeterminate textulariid. Isolate 20991, RC01 cruise, deployment BC002. The specimen appears similar to *Cribrostomoides subglobosa* but is genetically distinct. It is impossible to identify from this single available image. (**f**–**h**) *Cribrostomoides subglobosa*. Isolate 21096, RC01 cruise, OMS area, deployment BC015. Scale bars = 500 µm (**a**,**b**,**e**–**h**). 2 mm (**d**). No scale bar available for (**c**).

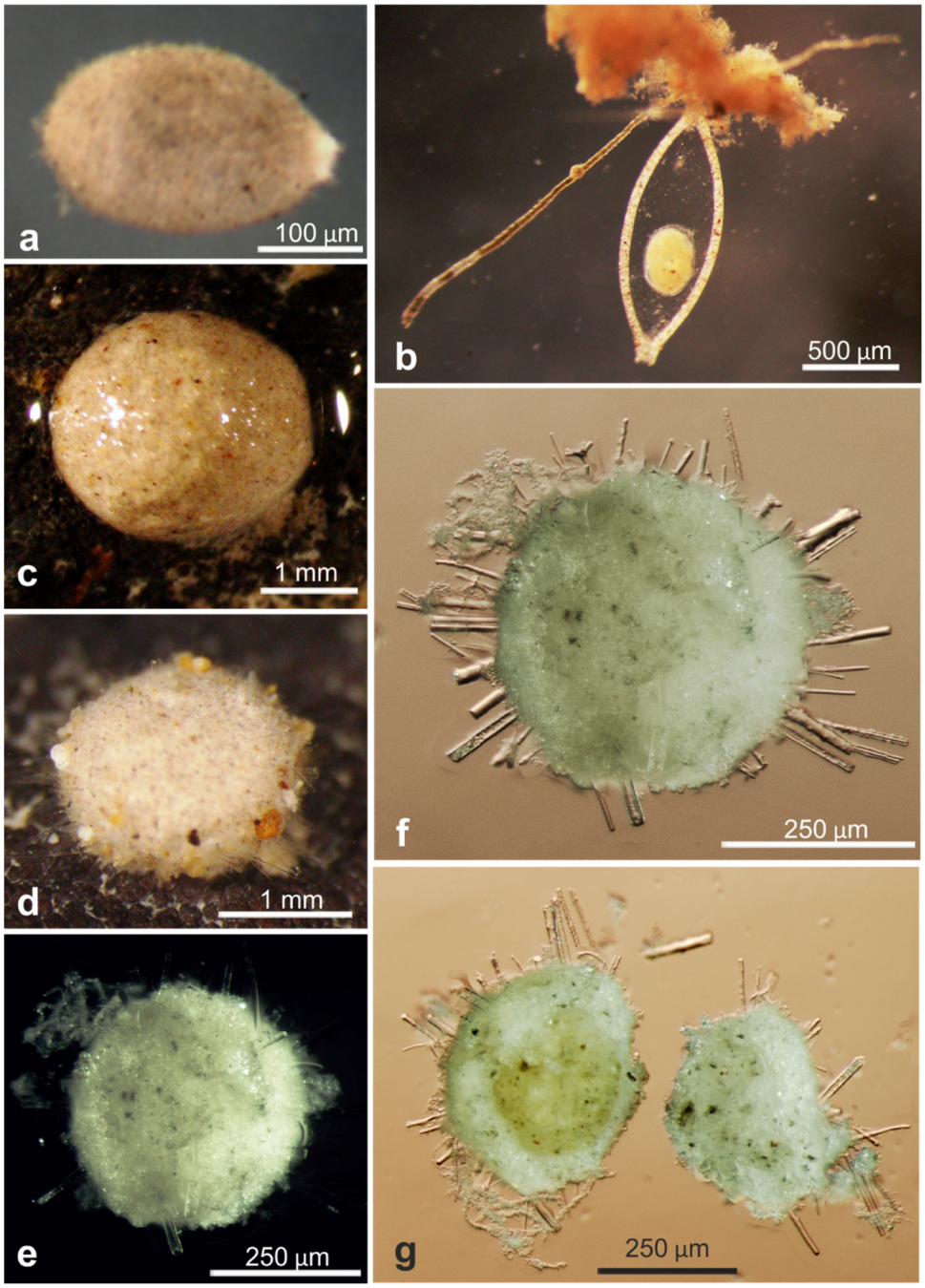

Figure A8. Monothalamids used for HTS. (**a**) Flask-shaped saccamminid. Isolate 18547, AB02 cruise, Station U03, deployment BC004. (**b**) *Vanhoeffenella* sp., specimen with one end entangled with some detritus and a tube. Isolate 21019, RC01 cruise, deployment BC005. (**c**) Finely agglutinated dome on nodule. Isolate 21087, RC01 cruise, OMS area, deployment BC045. (**d**) Whitish dome with spicules on nodule. Isolate 21085, RC01 cruise, deployment BC043. (**e**–**g**) *Crithionina hispida*. Isolate 18122, AB01 cruise, Station J, deployment MC22. (**e**) As originally found attached to a nodule. (**f**) Detached and viewed with transmitted light. (**g**) Broken to reveal thick wall and test contents. Scale bars = 1 mm (**c**,**d**), 500 µm (**b**), 250 µm (**f**,**g**), 100 µm (**a**).

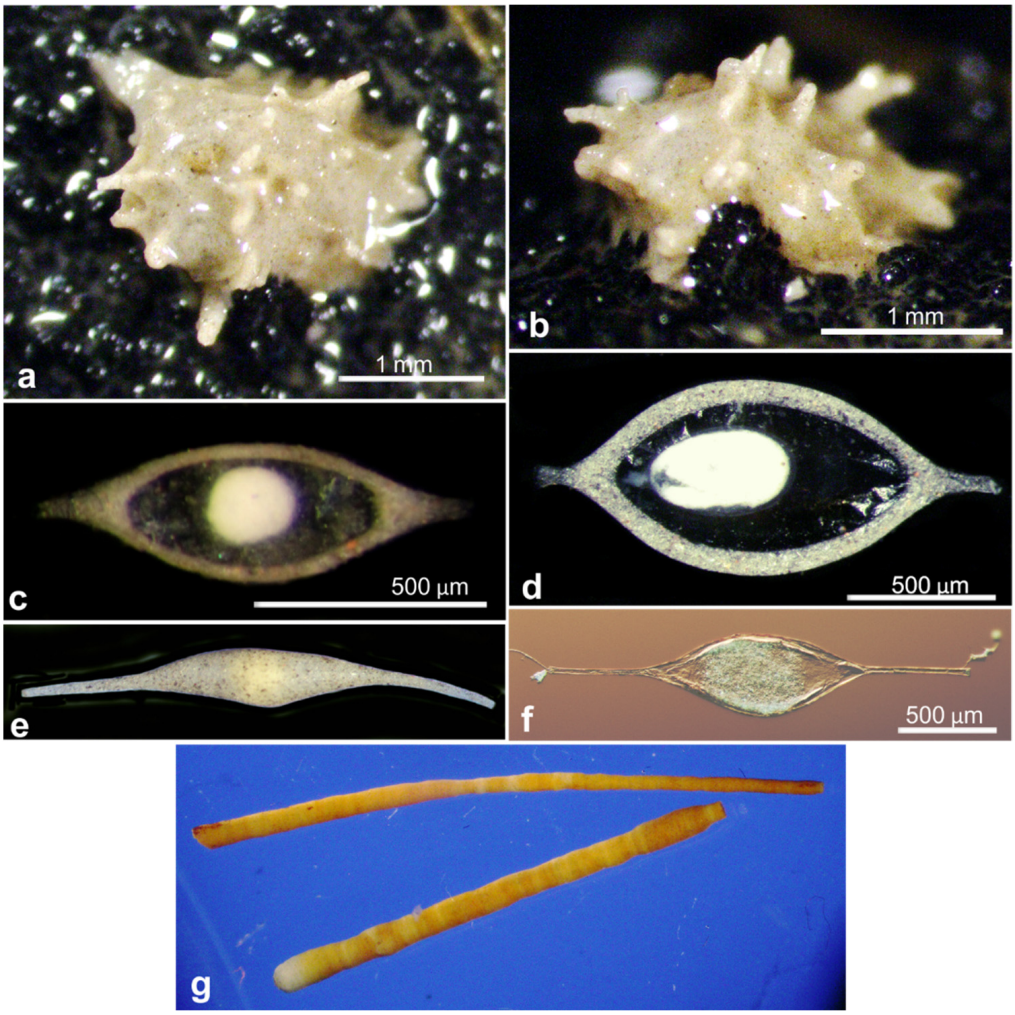

Figure A9. Monothalamids used for HTS. (**a**,**b**) Two views of *Storthosphaera* sp. attached to nodule. Isolate 21082, RC01 cruise, OMS area, deployment BC041. (**c**) *Vanhoeffenella* sp. Isolate 19132, AB02 cruise, Station SO5, deployment MC11. (**d**) *Vanhoeffenella* sp. Isolate 20963, RC01 cruise, deployment BC033. (**e**) Elongate, spindle-shaped test. Isolate 20911, RC01 cruise, OMS area, deployment BC004. (**f**) *Vanhoeffenella*-like specimen with entirely organic-walled test. Isolate 17658, AB01 cruise, Station C, deployment EB02. (**g**) *Bathysiphon* aff. *flavidus* de Folin, 1886. Isolate 20926, RC01 cruise, OMS area, deployment BC004. Scale bars = 1 mm (**a**,**b**), 500 µm (**c**–**f**). There is no scale available for (**g**).

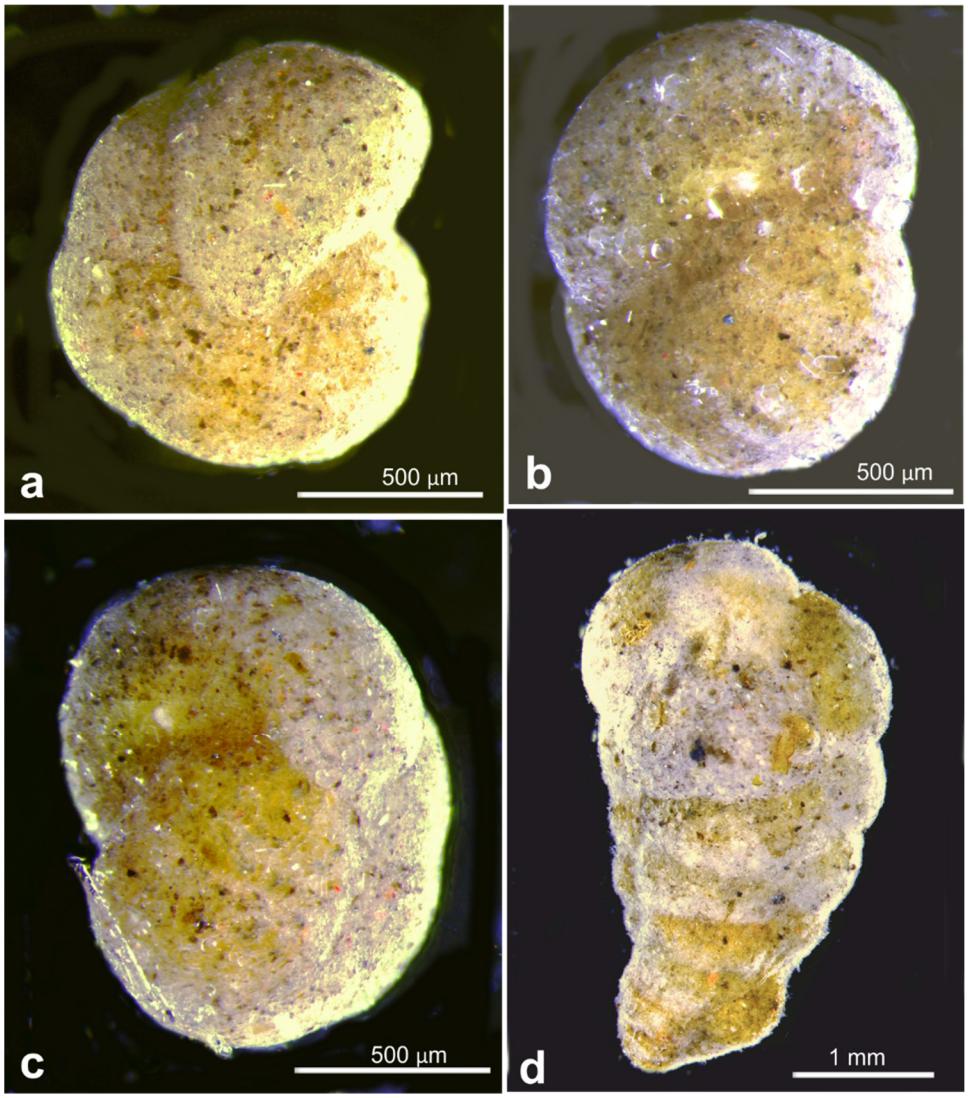

Figure A10. Textulariids used for HTS. (**a**–**c**) *Cribrostomoides subglobosa*. Isolate 21092, RC01 cruise, OMS area, deployment BC15. (**d**) *Verneuilinulla propinqua* (Brady, 1884). Isolate 21035, RC01 cruise, OMS area, deployment BC005. Scale bars = 500 µm (**a**–**c**), 1 mm (**d**).

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