

ORIGINAL PAPER

# Xenophyophores (Rhizaria, Foraminifera) from the Eastern Clarion-Clipperton Zone (equatorial Pacific): the Genus *Psammina*



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**Xenophyophores are important megafaunal organisms in the abyssal Clarion-Clipperton Zone (CCZ; equatorial Pacific), a region hosting commercially significant deposits of polymetallic nodules. Previous studies assigned those with attached, fan-like tests to *Psammina limbata*, a species described from the central CCZ based on morphology. Here, we redescribe the holotype of *P. limbata* and then show that *limbata*-like morphotypes collected in the eastern CCZ include three genetically distinct species. *Psammina* aff. *limbata* is closest morphologically to *P. limbata*. The others are described as *P. microgranulata* sp. nov. and *P. rotunda* sp. nov. These fan-shaped species form a well-supported clade with *P. tortilis* sp. nov., a morphologically variable species exhibiting features typical of both *Psammina* and *Semipsammina*. A second clade containing *Psammina* sp. 3, and two species questionably assigned to *Galatheammima* branches at the base of this group. The genus *Psammina* includes another 9 described species for which there are no genetic data, leaving open the question of whether *Psammina* as a whole is monophyletic. Our study increases the number of xenophyophore species described from the eastern CCZ from 8 to 11, with a further 25 morphotypes currently undescribed. Many additional species of these giant foraminifera undoubtedly await discovery in abyssal settings.**

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**Key words:** Agglutinated foraminifera; monothalamids; new species; SSU rDNA sequences; deep-sea mining; polymetallic nodules.

## Introduction

The Clarion-Clipperton Zone (CCZ) in the eastern equatorial Pacific has long been known as

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an area where xenophyophores (large agglutinated foraminifera) are common and relatively diverse (Schulze 1907; Tendam 1972, 1996). In recent years, a considerable research effort has focused on this region, which hosts vast, commercially important deposits of polymetallic nodules. Sampling in several of the areas licensed for nodule prospecting by the International Seabed Authority has revealed the presence of many additional xenophyophore species (Gooday et al. 2017a). A number of these have been formally described, some from the Russian license area in the central CCZ (Kamenskaya 2005; Kamenskaya et al. 2015, 2017), others from the United Kingdom 1 (UK-1) and Ocean Mineral Singapore (OMS) license areas at the eastern end of the CCZ (Gooday et al. 2017b,c). One such species is *Psammmina limbata* Kamenskaya, Gooday & Tendam 2015, established by Kamenskaya et al. (2015) based on a single specimen from the Russian area, for which no genetic data are available. The specimen had a semicircular test with a distinctive pale rim and was found attached to a nodule by a short basal stalk. According to Gooday et al. (2017a) this species is fairly common in the UK-1 and OMS areas. However, a subsequent reassessment of the genetic data from these two areas has revealed that material assigned to *P. limbata* encompasses at least three distinct species. The one that is most similar to *P. limbata* is assigned tentatively to this species as *P. aff. limbata*, the other two were undescribed. They cluster with a fourth *Psammmina* species, also undescribed, that is morphologically distinct.

The present study has the following main objectives. 1) To redescribe *P. limbata* based on photographs of the freshly-collected holotype and a re-examination of its remaining fragment. 2) To describe (as *P. aff. limbata*) sequenced and unsequenced specimens from the UK-1 and OMS areas that we consider most similar to *P. limbata*. 3) To formally describe the three new *Psammmina* species (*P. microgranulata* sp. nov., *P. rotunda* sp. nov., *P. tortilis* sp. nov.). Two unsequenced specimens of *P. aff. limbata* were included in a recent study of the internal structure of several xenophyophores using Micro-CT 3D imaging (Gooday et al. 2018).

## Results

### Systematics

See Tendam (1972) and Gooday et al. (2017b) for definitions of the special morphological terms

applied to xenophyophores.

### Supergroup Rhizaria Cavalier-Smith 2002

### Foraminifera D'Orbigny 1826

### 'Monothalamids'

### Clade C

### Xenophyophoroidea Tendam 1972

### Genus *Psammmina* Haeckel 1889

*Type species: Psammmina nummulina* Haeckel 1889

**Diagnosis:** Test free or attached, fragile, brittle, basically plate-like, and fan-shaped or rounded with basal stalk attached to substrate, or discoidal without stalk, or folded into more complex shape. Circular apertures ('pores') or lattice-like mesh of spicules developed along margin in some species. External xenophyae firmly cemented to form upper and lower plates; internal xenophyae relatively sparse, sometimes forming pillar-like, bar-like, or partition-like structures between plates. Pale rim often present in fan-shaped species. Granellare branches and well-developed stercomare strings run between plates (Modified after Gooday and Tendam 2000).

**Remarks:** Following Gooday and Tendam (1988, 2000), Tendam (1996) and Kamenskaya et al. (2015, 2017), *Psammmina* is interpreted to encompass plate-like xenophyophores in which the test is folded, undulating or stalked, in addition to the typical, more or less discoidal forms originally described by Haeckel (1889) and included in this genus by Tendam (1972). As discussed below, it is likely that this heterogeneous assemblage of species is polyphyletic.

### *Psammmina limbata* Kamenskaya, Gooday & Tendam 2015

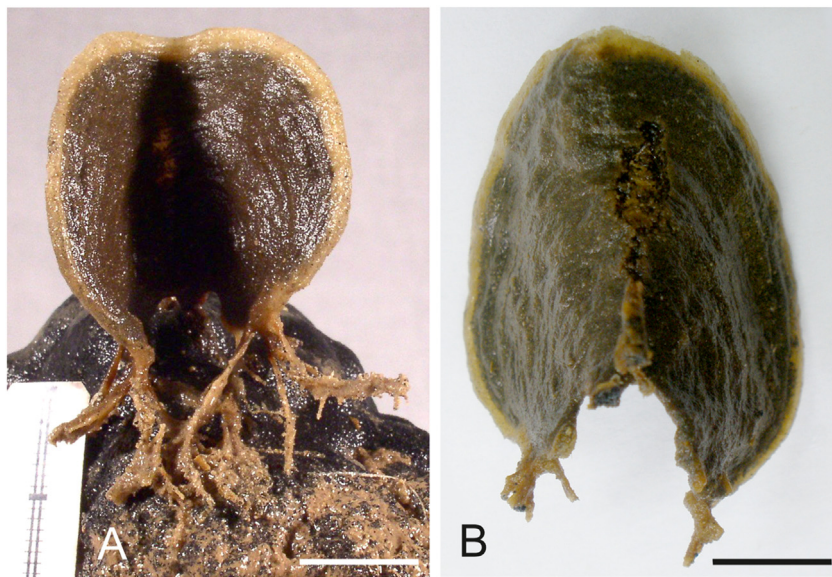
Figures 1, 2; Supplementary Material Figure S1A,B

*Psammmina* sp. Kamenskaya, Melnik, Gooday, 2013, pp. 391–392, Fig. 6b

*Psammmina limbata* Kamenskaya, Gooday, Tendam, 2015, pp. 585–588, Fig. 4

**Material.** Unique holotype collected in the Russian exploration claim area: 13.28°N, 134.45°W; depth 4,724 m (Kamenskaya et al., 2015).

**Diagnosis:** Flattened, plate-like, rigid, semi-circular test attached to nodule surface by basal stalk and root-like structures. Outer test layer relatively



**Figure 1.** *Psammmina limbata* Kamenskaya, Gooday, Tendal 2015; holotype from Russian license area. **A:** Attached to a nodule, as originally collected; view of concave side. **B:** Detached from nodule, convex side. Scale bars: 1 cm.

thin and fragile, composed of radiolarian skeletons and sponge spicule fragments, with some mineral grains. Weakly developed concentric zonation sometimes visible. Curved outer margin distinctly lighter than other parts of test and consisting mainly of sponge spicules. Interior with prominent strings of granellare and masses of stercomare interwoven with loosely agglutinated spicule fragments; stercomare and granellare absent from pale margin (modified slightly from Kamenskaya et al. 2015).

#### *Additional observations on the holotype*

The original description was based on the holotype (housed in the Zoological Museum of the Moscow State University, registration number F-18), the only specimen available to Kamenskaya et al. (2015). This was originally attached to a poly-metallic nodule. It now consists of one half of the plate-like test, the other half having been used for scanning electron microscopy (SEM). The following points supplement the original description. They are based on photographs of the freshly collected specimen (Fig. 1; Supplementary Material Fig. S1), and a re-examination of the remaining part of the holotype (Fig. 2).

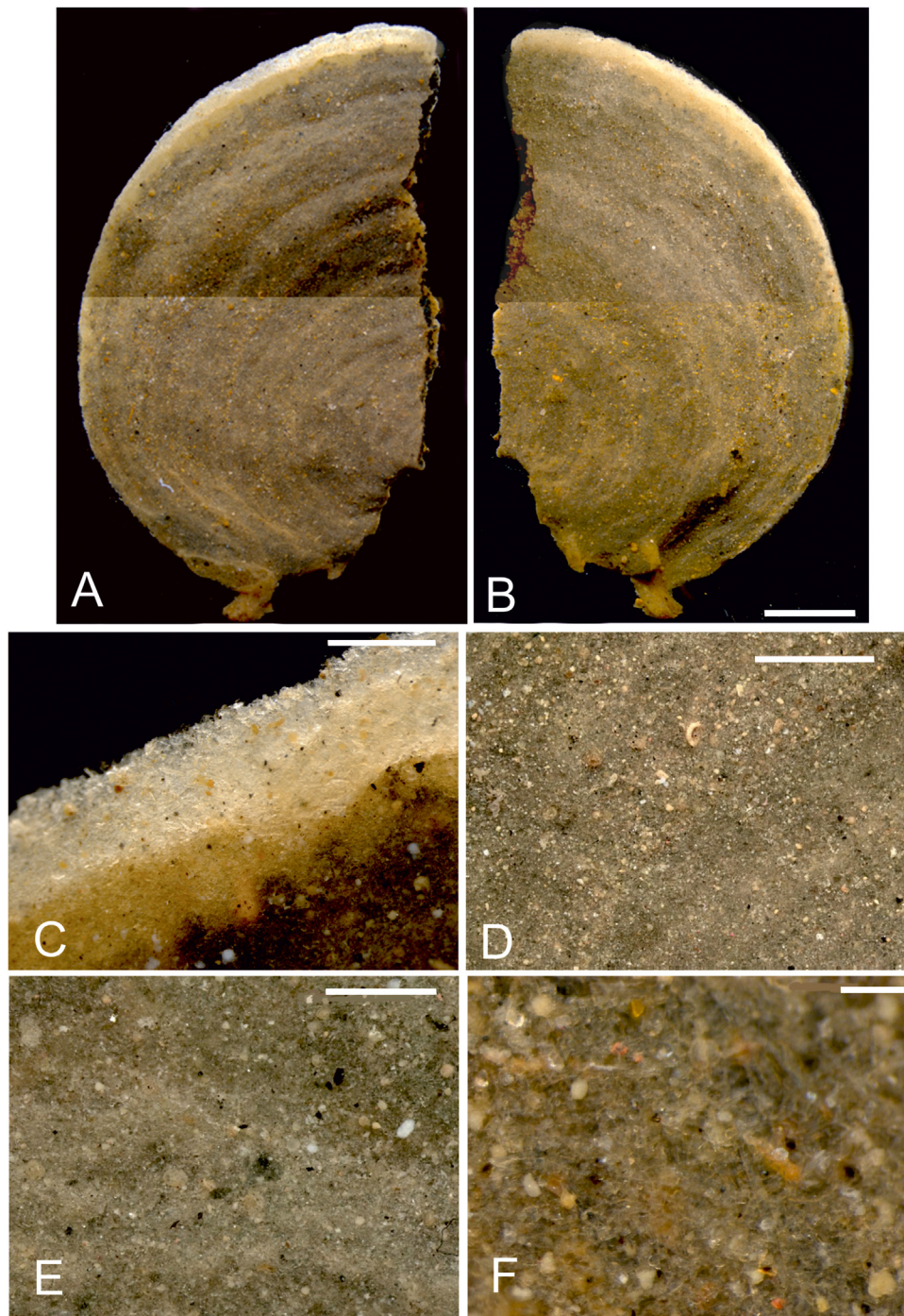
When complete, the test was strongly curved around a vertical axis as well as being curved to some extent towards the summit of the nodule (Supplementary Material Fig. S1A). It was attached to the nodule by a short main stem near the mid line with two additional points of contact on either side. The main stem was directed slightly back-

wards (i.e., away from the concave face of the test) and arose from the base of an abraded ridge-like feature that extended down most of the vertical axis on the convex side of the plate (Fig. 1B). An extensive system of more or less twisted roots-like bars was developed on either side of the base of the test (Fig. 1A). Most of them extended downwards and outwards towards the sloping nodule surface, sometimes splitting into short branches at their extremities. However, these 'roots' terminated just above the nodule surface, which was covered with a thin layer of sediment.

In Figure 1B, which shows the convex side of the test after removal from the nodule, the height exceeds the width resulting in a somewhat elongated shape, and the lower margin of the test forms a concave arc. Based on the remaining part of the holotype (Fig. 2A, B), the test measured 26.5 mm high from the top of this arc to the upper margin of the test and 30.0 mm from the base of the plate to the upper margin. The width of the test measured in a straight line from side to side of the plate was ~29 mm (Fig. 1B). However, when measured around the curvature of the plate it was considerably wider, around 40 mm according to Kamenskaya et al. (2015).

The test wall is thin and consists of sponge spicule fragments, radiolarian tests and mineral grains, with very little intervening fine-grained material (Fig. 2C–F). Many of the mineral grains are yellowish or orange in colour, but others are whitish,





**Figure 2.** *Psammmina limbata* Kamenskaya, Gooday, Tendal 2015; holotype, remaining fragment. **A, B:** Opposite sides. **C:** Detail of the pale rim. **D, E, F:** Progressively closer views of the outer test surface. Scale bars: 5 mm (**A, B**), 500  $\mu\text{m}$  (**C–E**), 250  $\mu\text{m}$  (**F**).

black or reddish. They generally measure between 30 and 100  $\mu\text{m}$  (typically 40–80  $\mu\text{m}$ ) in size. The wall also incorporates occasional complete and fragmentary agglutinated foraminiferal tests. A pale rim is clearly developed and mainly consists of

sponge spicules (Fig. 2C); there is no evidence for circular apertures around the margin. The specimen was stained with Rose Bengal and the original colour of the granellare branches is therefore unknown.



*Psammmina* aff. *limbata* form 1

Figures 3–5; Supplementary Material Figure S1C–F

*Psammmina* aff. *limbata*. Gooday et al. 2018, figures 1, 2a–d, 3, 4 and S1

*New material from the UK-1 and OMS areas*

DNA sequences and morphology. AB02 cruise: Station U12 (MC16), DNA isolate number 18230, accession numbers MF441523 – 441525; Station S07 (BC21), DNA isolate number 18235, accession numbers MF441526 – 441528; Station S07 (MC20), DNA isolate numbers 18281, 18282, accession numbers MF441529 – 441534.

Morphology only. AB02 cruise: Sites U11 (BC16), S02 (BC09), S05 (BC11), S07 (MC20), S10 (BC23), S11 (BC25). Fragment S01 (EB04). The specimens from S10 and S11 are deposited under registration numbers NHMUK PM ZF 7798 and 7799, respectively.

*Description of sequenced specimens* (Figs 3, 4; Supplementary Material Figs S1C–F)

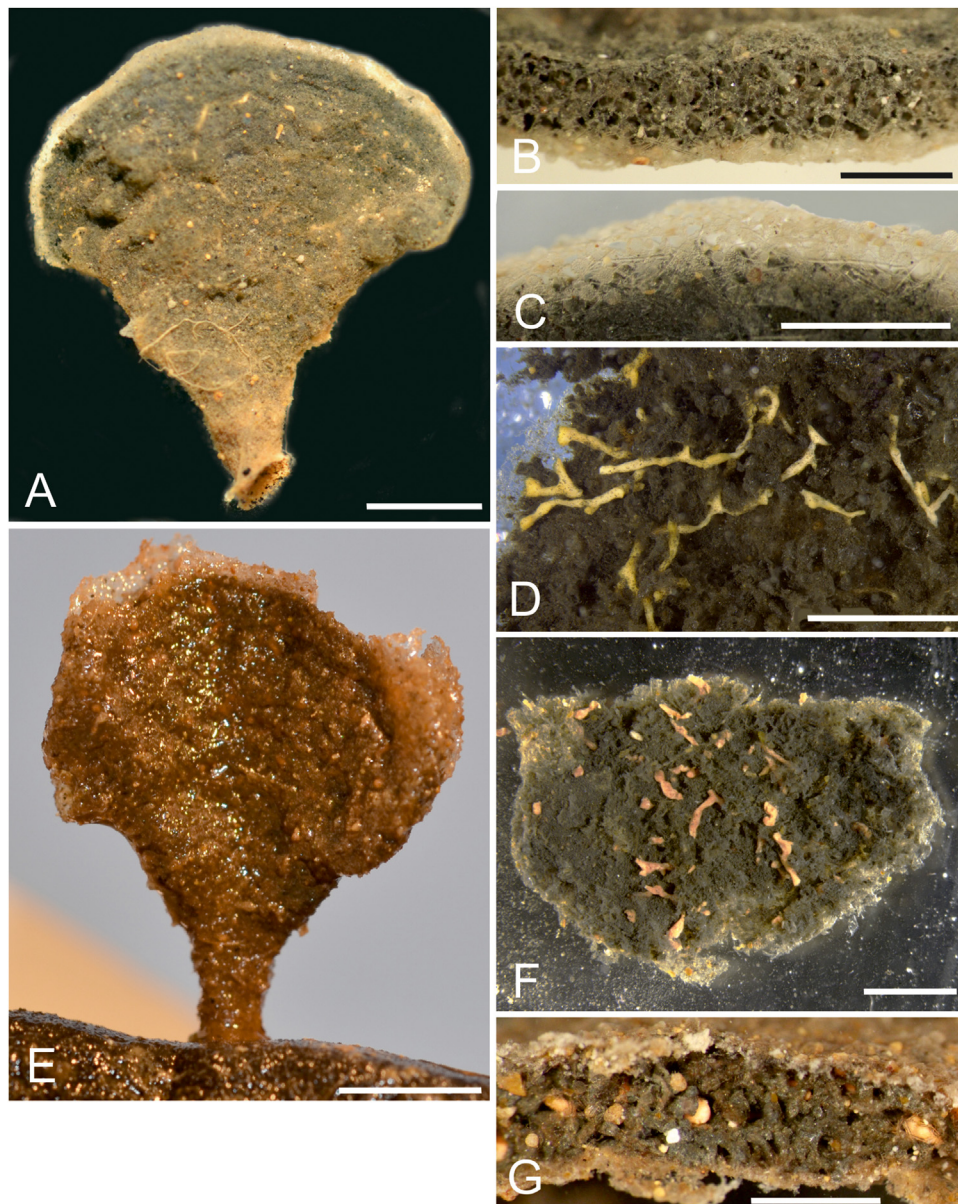
*Overall test morphology.* When found, both tests were attached to nodules (Supplementary Material Fig. S1C–F). They are greyish brown with a slight yellowish tinge, which is particularly evident in the stalk of the specimen from Station S07. The overall shape of the test is approximately subtriangular, with an arcuate (S07) or more or less semi-circular (U12) rim that occupies about 50% of the height and tapers down into the stalk (Fig. 3A, E). The upper part of the specimen from Station U07 was somewhat damaged when recovered. It is ~17 mm high, 14.5 mm maximum width and 2.0–2.6 mm thick; the stalk tapers from ~7.5 mm to 2.2 mm near the base. The specimen from S07 measures 18.9 mm high and 17.5 mm maximum width and is 2.1–2.3 mm thick; the stalk tapers from ~11.5 mm to 2.7 mm near the base. The pale rim is confined to the upper, arcuate part of the test. In both cases, a vague pattern of concentric surface depressions is visible under low-angled illumination.

*Wall structure.* The test wall is thin (170–230 µm) with a fairly rough outer surface and semi-transparent so that short sections of the granellare system embedded in the dark matrix of the stercomare are visible in places (Fig. 3A). It is composed almost entirely of radiolarian shells, sponge spicules and small mineral grains (mostly 100 µm or less in maximum dimension) (Fig. 4). A few agglutinated foraminiferal tests are present

in both specimens (Fig. 4F). The underside of the wall is similar in appearance to the outer surface but somewhat rougher as a result of projecting spicules. The margin of the arcuate part of the test comprises a framework of spicules filled to varying degrees by radiolarians (Fig. 3C). Where spicules are dominant, they create an open, three-dimensional lattice; where radiolarians occupy more of the space between the spicules, numerous chinks in the framework serve as irregular openings (Fig. 3B).

*Stercomare and granellare (light microscope and SEM observations).* Apart from the pale rim, the test interior is largely devoid of internal xenophyae and occupied mainly by dark grey stercomare forming a closely anastomosing system of branches (180–300 µm or more in diameter) and more irregularly-shaped formations, the details of which are often difficult to differentiate (Fig. 3D, F, G). The masses of stercomata that make up the stercomare are enclosed within a thin (<1 µm) organic envelope that is not visually obvious except where it gives rise to reflective highlights. Individual stercomata range from 6.0 to 15.4 µm (mean =  $10.8 \pm 2.23$  µm,  $n = 25$ ) in diameter and have a rather rough surface. They appear to be composed of flake-like particles and yield strong Al peaks, suggesting a clay mineral composition.

The granellare strands are either pale yellowish or orange and weave between the stercomare branches (Fig. 3D, F, G). Although they occupy a smaller volume of the test interior, the strands stand out prominently against the dark stercomare. They are of variable and undulating width, generally 75–130 µm but sometimes with bulbous sections at least 230 µm wide and much narrower necks (~100–120 µm). The strands branch frequently and individual strands sometimes include inflated sections or end blindly with a somewhat bulbous termination. The organic sheath that encloses the cytoplasm to form the granellare system is not obvious and presumably very thin. The cytoplasm itself is packed with crystals (granellae), ranging in size from ~1 to >4 µm (typically 1.5–3.0 µm) in length. Many of them have rounded, pebble-like shapes but a few are regular and faceted. The rounded crystals occasionally have deep, clearly-defined depressions, sometimes with angular shapes. Strong peaks for Ba and S confirm that the crystals are barium sulphate, presumably in the form of barite. In addition to the stercomata and granellae crystals, particle rich in calcium were observed during EDAX surveys (T. Góral and A. Gooday, unpublished).



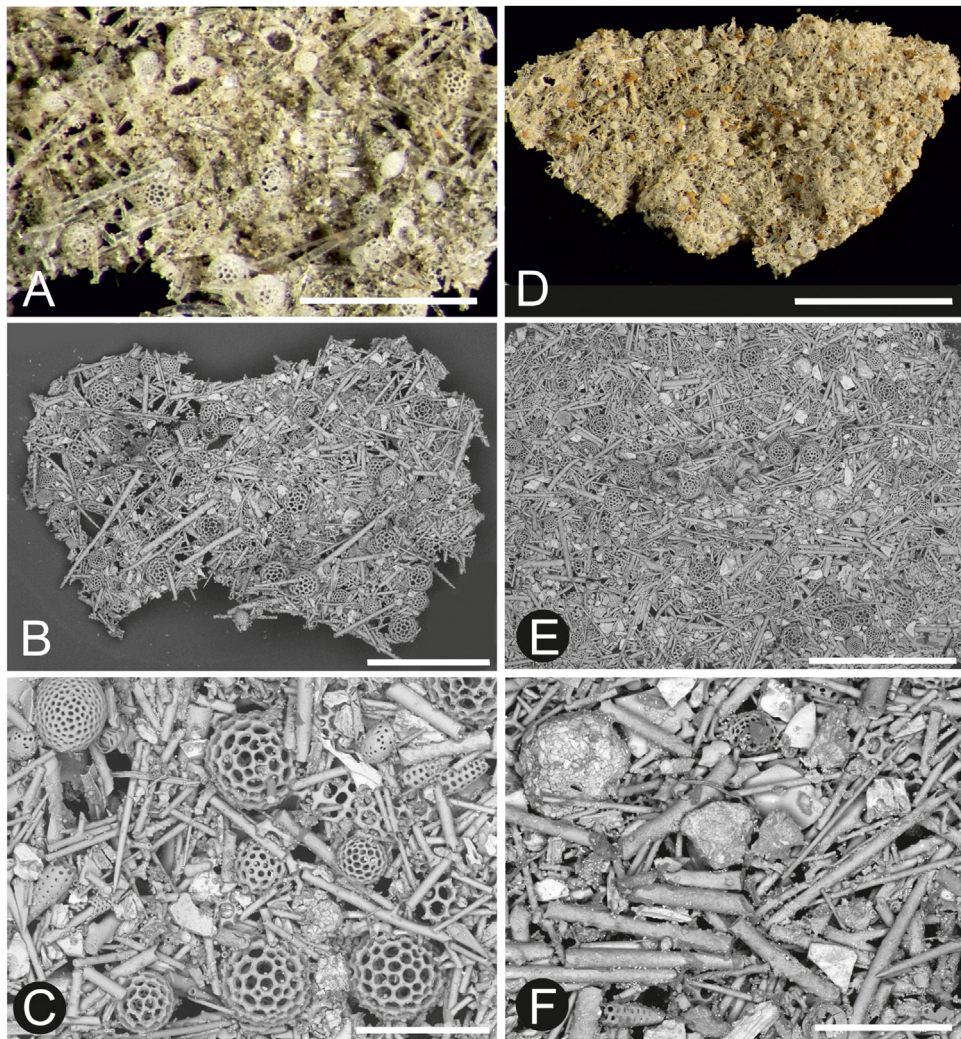
**Figure 3.** *Psammmina* aff. *limbata* form 1, sequenced specimens from AB02 cruise, Stations S07 (A–D) and U12 (E–G). **A:** Complete test detached from nodule. **B:** Detail showing edge of test with sponge spicules forming mesh with many irregular openings. **C:** Detail of pale rim viewed from the side. **D:** Test wall removed to show dark masses of stercomare and pale granellare strands. **E:** Complete test attached to nodule. **F:** Fragment with wall removed showing stercomare and granellare. **G:** Broken edge of test with stercomare and granellare. Scale bars: 5 mm (A, E), 2 mm (B–D, F, G).

*Description of other specimens (morphology only)* (Fig. 5)

Two specimens are assigned to *P. limbata* based on test morphology and wall structure. Both were attached to nodules and were dried soon after collection without being fixed. The tests are rigid, and greyish-brown when dried.

The specimen from Station S10 (Fig. 5A) measures about 34 mm high (including the stalk), 30 mm wide, and 1.3–1.5 mm wide at the edge. The upper part of the test is 24 mm high with a rounded, somewhat trapezoidal shape, and is slightly sinuous when viewed from above. It merges into the basal stalk, which is about 10 mm in length, tapering from ~13 mm where it joins the





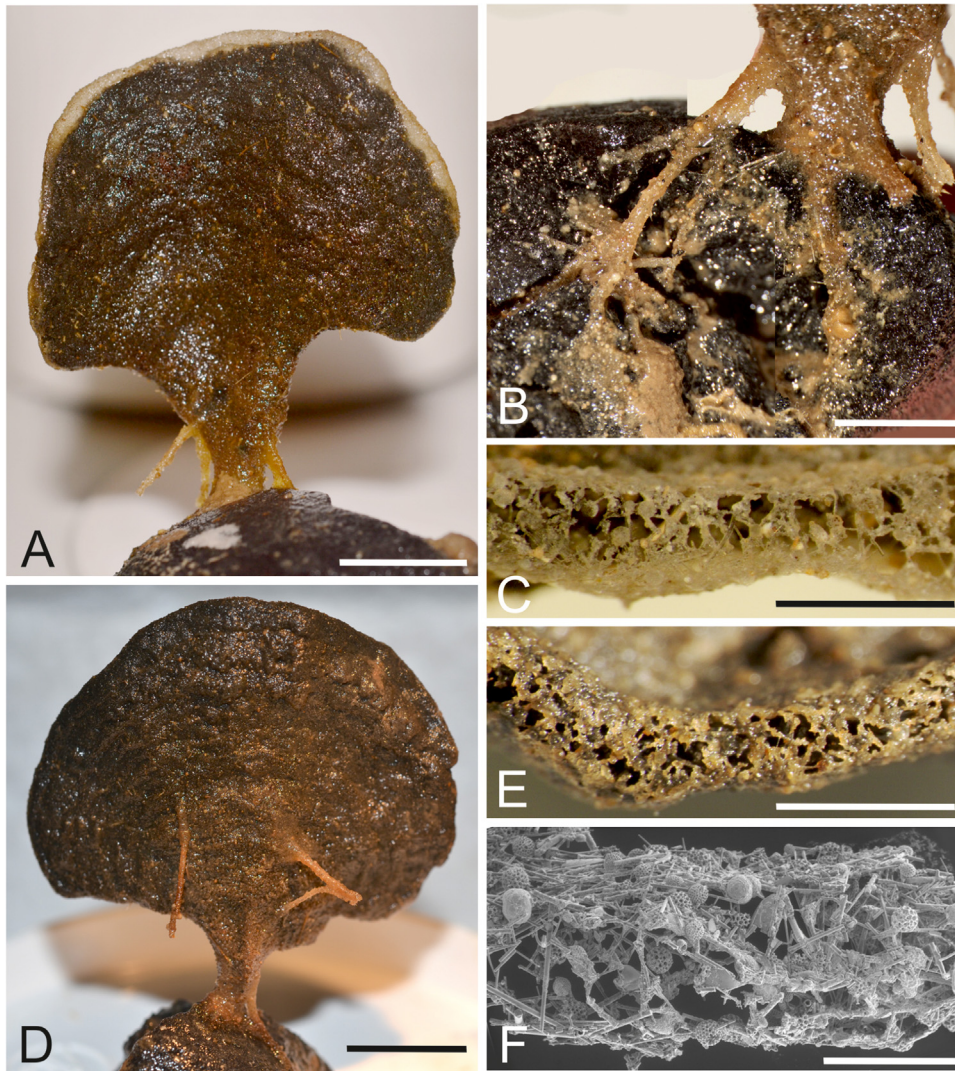
**Figure 4.** *Psammmina* aff. *limbata* form 1, sequenced specimens from AB02 cruise, Stations S07 (**A–C**) and U12 (**D–F**). **A**: Outer surface of test fragment. **B**, **C**: SEM micrographs of same fragment showing construction of wall from sponge spicule fragments, radiolarian tests, and mineral grains. **D**: Outer surface of test fragment. **E**, **F**: SEM micrographs of same fragment showing construction from spicule fragments, radiolarian tests, mineral grains and (in **F**) an agglutinated foraminiferan test; radiolarians are rather less common than in the Station S07 specimen. Scale bars: 1 mm (**A**, **B**, **E**), 2.5 mm (**D**), 250 µm (**C**, **F**).

upper part of the test to 3 mm near the base. Three fairly straight narrow bar-like structures project from the stalk (**Fig. 5A**). One does not extend as far as the nodule but the other two reach the nodule, in one case branching into a root-like structure on the surface (**Fig. 5B**). Micro-CT scan images (Gooday et al. 2018) suggest that the bars are hollow but there is no evidence that the granellare or stercomare extend into them.

The specimen from Station S11 (**Fig. 5D**) measures about 32 mm high (including the stalk), 35 mm wide, and 0.85–1.30 mm (in one place

1.75 mm) wide at the edge. The upper part of the test is ~28 mm high with a semi-circular shape, and is gently curved when viewed from above. The lower margin of the upper part is fairly straight and joins the short stalk rather abruptly. The stalk itself is ~5 mm long and tapers from ~4.5 mm to 3 mm in width. Two short, laterally-directed bars, similar to those seen in the S10 specimen, project from the convex side of the test above the top of the stem. A single long, root-like process extends from the base of the stem down the surface of the nodule.





**Figure 5.** *Psammmina* aff. *limbata* form 1, unsequenced specimens. AB02 cruise, Stations S10 (A–C), S11 (D, E), S07 (F). The specimens from S10 and S11 are deposited under registration numbers UKNHM PM ZF 7778 and 7799, respectively. **A:** Complete test; shipboard photograph. **B:** Base of test and associated root-like structures attached to nodule surface; laboratory photograph. **C:** Edge of test with mesh of sponge spicules; shipboard photograph in water. **D:** Complete test, shipboard photograph. **E:** Edge of test with spicule mesh; laboratory photograph of dried specimen. **F:** SEM micrograph of edge showing spicule mesh dotted with radiolarians. Scale bars: 10 mm (A, D), 5 mm (B), 2 mm (C, E), 1 mm (F).

Both tests displays vague, irregular concentric undulations and furrows. The wall is similar to that of the sequenced specimens, consisting of spicules, radiolarians and small mineral grains. Agglutinated foraminiferal tests are generally absent, except for several narrow tube fragments in the case of the S10 specimen. As in the sequenced specimens, the edge of the upper part of the test has a lattice-like framework with many irregular openings (Fig. 5C, E, F).

#### Remarks

The specimens from the UK-1 and OMS samples closely resemble the holotype of *P. limbata* from the Russian license in the structure and composition of the test wall. In both cases the wall is thin and comprises a felted mass of spicule fragments, radiolarians and mineral grains. On the other hand, the two sequenced specimens have a flat, roughly subtriangular test (Fig. 3A, E), in contrast to the holotype, which is more rounded and strongly curved around a vertical axis. They

also lack the root-like structures that are a prominent feature of the holotype. The two specimens that were not sequenced have an approximately circular upper part (Fig. 5A, D) and are therefore more similar in shape to the holotype; both also have root-like bars (Fig. 5B). However, like the sequenced specimens, they are not strongly curved about a vertical axis. These morphological differences cast doubt on the identification of our specimens as *P. limbata*, but at the same time are not sufficient to justify the establishment of a new species. Moreover, the rather different shapes of the sequenced and unsequenced specimens (subtriangular with the upper part tapering into the stalk in the former, approximately semicircular and more clearly delimited from the stalk in the latter) suggest that they could be distinct species. Genetic data are required to resolve these problems.

There is a striking resemblance in test morphology between some specimens of *Psammmina* aff. *limbata* form 1 and several attached, fan-shaped species from the eastern Pacific for which Haeckel (1889) established the genus *Psammophyllum*. In particular, *Psammophyllum annectens* (illustrated in Pl. IV, fig. 1 of Haeckel 1889) is very similar in shape to the specimen from Station S11 (Fig. 5D). However, in contrast to *P.* aff. *limbata* form 1, the test of Haeckel's species is soft and flexible with felt-like surfaces and a more or less well-developed system of linellae (proteinaceous fibres). There is also no evidence for a pale rim in Haeckel's description or illustrations. Tendal (1972) examined the type specimen of *P. annectens* and judged it to be conspecific with the stannomid xenophyophore *Stannophyllum zonarium*.

### *Psammmina* aff. *limbata* form 2

Figure 6; Supplementary Material Figure S2

?*Psammmina limbata* Kamenskaya, Gooday and Tendal 2015. Gooday et al. 2017a, fig. 2a

#### Material

A single dried specimen from AB02 Station S11 (MC23), registration number NHMUK PM ZF 7807. A second possible specimen of this form was collected at Station F during the AB01 cruise. Morphology only.

#### Description

The test was attached to a nodule (Fig. 6A). It was originally 25 mm long and 24 mm wide, reddish-brown when damp and a lighter, greyish-brown

colour when dried. The upper part of the test is about 19 mm long, plate-like, roughly triangular in overall shape, merging smoothly with the stalk and with a gently curved upper margin. It is rather thick (~1.5 to 2.5 mm) and irregular. On one side the margin of the plate is curved backwards through almost 90 degrees. On the other side the lower edge is distorted so that it projects outwards at right angles to the main plane of the test (Fig. 6B). The stalk measures ~7 mm in length and tapers from ~5 mm to 2.5 mm at the base. The wall is continuous around the margin of the test, except where interrupted by regularly spaced openings, 0.50–0.75 mm diameter (Fig. 6C). Where undamaged, the edges of these openings are well-defined (Fig. 6D), indicating that they are original features. They are only developed around part of the margin.

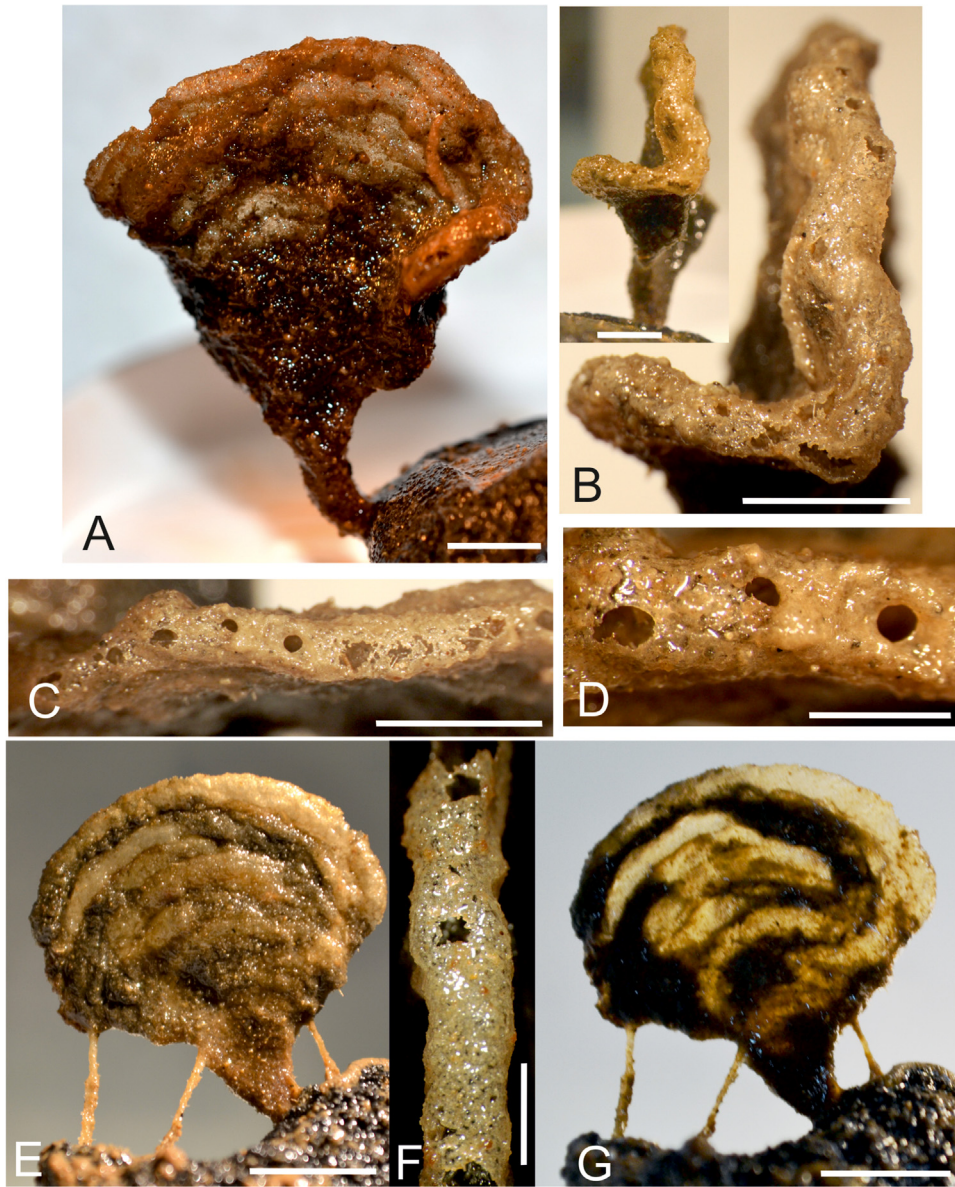
The wall resembles that of typical specimens of *Psammmina* aff. *limbata*, comprising a mesh of sponge-spicules dotted with numerous radiolarians and a subordinate proportion of mineral grains and scattered agglutinated foraminiferal tests (Supplementary Material Fig. S2A–D). There is little or no fine-grained matrix so that light can shine through the chinks in the spicule mesh. The specimen is dead and the test interior empty, but interrupted by thin, well-defined concentric partitions that run parallel to the margin with a spacing of ~50 to 200 µm (Supplementary Material Fig. S2E, F). These correspond to rather poorly-defined undulations on the test surface.

#### Remarks

This specimen, which has a somewhat deformed morphology, is of particular importance because it displays circular openings, presumably apertures, around part of the test margin. Clear marginal openings are not present in any other specimens of *Psammmina* in our collections, although they are typical of the genus, as defined by Tendal (1972). Also of interest are the concentric partitions of the test interior, which correspond to undulations on the outer surface. These features are also typical of the genus.

The test wall forms a continuous layer around the margin of a dead *Psammmina* test collected during the AB01 cruise (Fig. 6E, F). This specimen also displays concentric surface features that appear to correspond to internal partitions (Fig. 6G). The margin has several holes that superficially resemble apertures, but they have irregular margins and are possibly the result of damage. The test is more similar in overall shape to our





**Figure 6.** *Psammmina* aff. *limbata* form 2, dead specimens, shipboard photographs. AB02 cruise, Station S11 (A–D); registration number UKNHM PM ZF 7807. AB01 cruise, Station F (E–G). **A:** Complete test. **B:** Side view showing part of test projecting at right angles to the main plate; the inset shows the complete side view including the basal stalk. **C:** Edge of test showing wall with row of openings, some of which appear to be undamaged apertures. **D:** Detail of intact apertures. **E:** Complete test. **F:** Edge of test showing wall with openings, possibly artefacts caused by damage. **G:** Complete test back-lighted to show interior with possible partitions and dark, decayed stercomare. Scale bars: 5 mm (A–C), 2 mm (D), 10 mm (E, G), 2.5 mm (F).

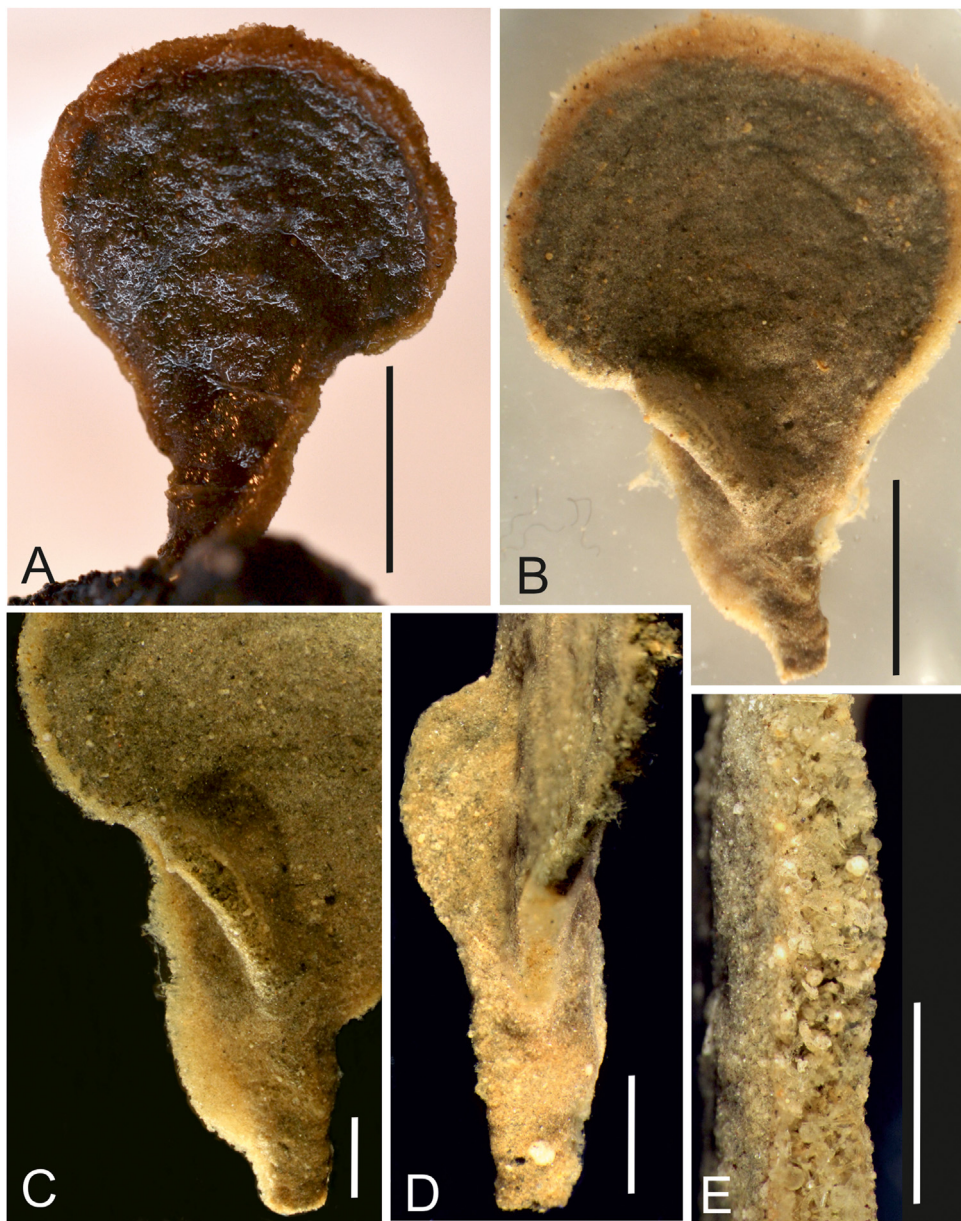
two sequenced specimens of *P.* aff. *limbata* form 1, while the lower part gives rise to three more or less straight, narrow bar-shaped structures that resemble those present in the two unsequenced specimens of form 1. It therefore combines features that are typical of *P.* aff. *limbata* forms 1 and 2.

*Psammmina microgranulata* Gooday and Holzmann sp. nov.

#### Figures 7–9

**Diagnosis:** Attached species of *Psammmina*. Test subtriangular with semicircular upper margin tapering into fairly broad stalk attached at its base to





**Figure 7.** *Psammmina microgranulata* Gooday and Holtzmann sp. nov. AB02 cruise, Station S02; holotype, registration number NHMUK PM ZF 7802. Shipboard photograph showing test attached to a nodule (**A**). Laboratory photographs of test detached from nodule (**B–E**). **B**: Opposite side of test. **C, D**: Lower part of test from different angles showing projecting flange. **E**: Edge of test. Scale bars: 5 mm (**A, B**), 1 mm (**C–E**).

nodule substrate. Test wall largely comprised of small (<50  $\mu\text{m}$ ) mineral grains. Granellare strands strongly developed, pale whitish in colour.

**Etymology.** The name refers to the construction of the test wall from fine-grained particles.

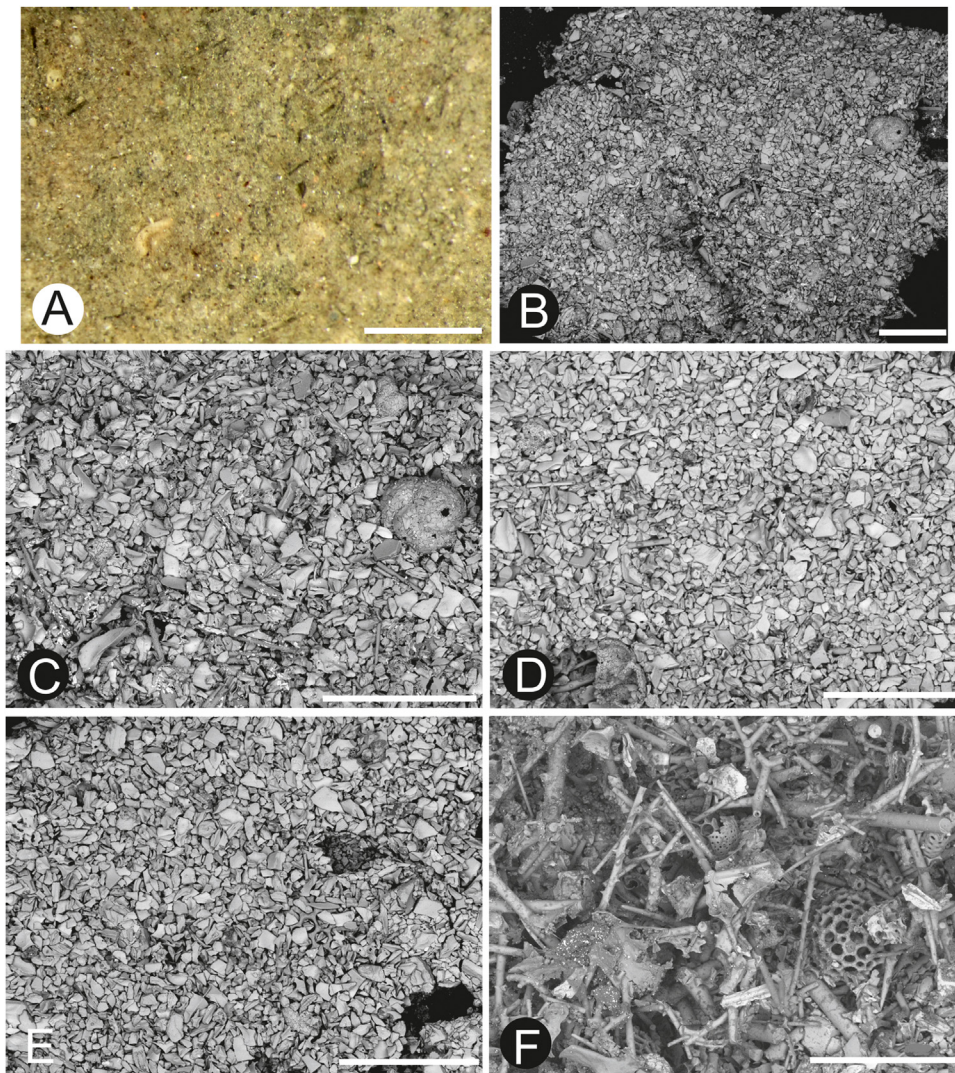
**Holotype.** The unique specimen was collected in a box core (BC09) from Station S02 (12°04.914'N, 117°10.691'W, 4,070 m) during the AB02 cruise.

Registration number NHMUK PM ZF 7802. DNA isolate number 18234 (sample of cytoplasm from holotype), accession numbers MF441521, MF441522, LT576129.

#### *Description*

**Test morphology.** The test was attached to a nodule. It is dark brownish when damp, with a yellowish tinge when immersed in water, and has a distinct



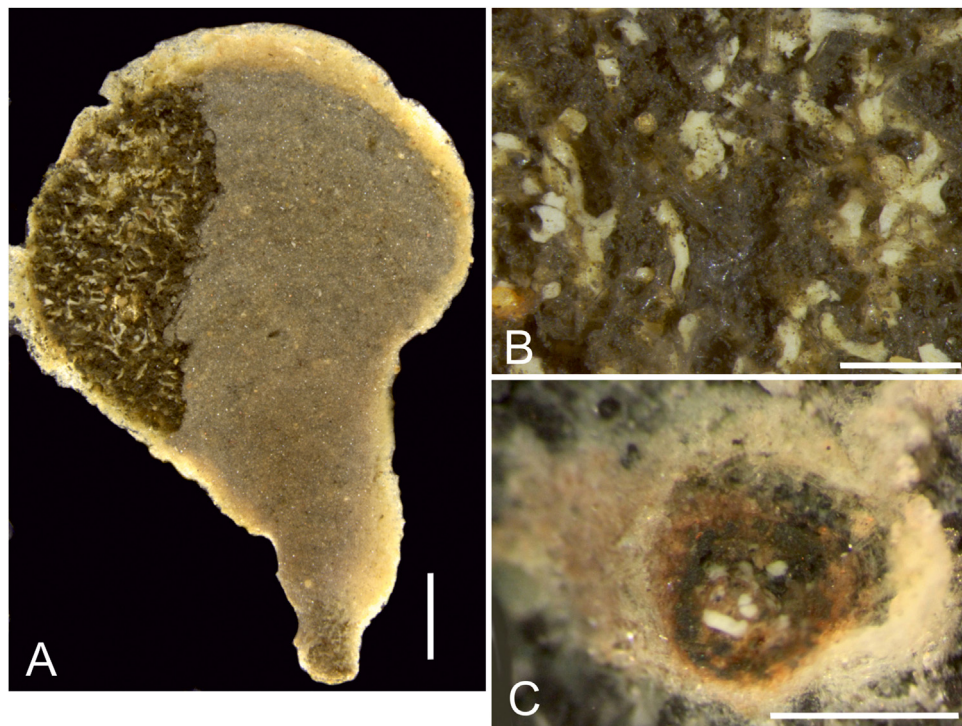


**Figure 8.** *Psammmina microgranulata* Gooday and Holtzmann sp. nov., AB02 cruise, Station S02; test wall of holotype. **A:** Light micrograph of part of outer surface. **B–E:** SEM micrographs of the same fragment; note the agglutinated foraminiferan test in C. **F:** Inner surface of wall; note the higher proportion of spicule fragments compared to the outer surface. Scale bars: 1 mm (**A**), 250  $\mu\text{m}$  (**B–F**).

pale rim (Fig. 7A, B). The test is plate-like, 16.6 mm long, with a maximum width of 12.2 mm and about 0.8 to 1.2 mm thick. The upper part is approximately semi-circular and merges into the lower part, which forms a stalk-like structure, tapering from about 6 mm to 1.2 mm just above the base, before splaying to 1.7–1.9 mm where it is in contact with the substrate. The lower part of the test has a somewhat lumpy appearance. On one side, a raised flange, in effect a weakly developed secondary plate, is developed (Fig. 7C, D). This feature, which follows a slightly curved course, decreases in height from just below the upper fan-shaped part of the test to near the base of the stem. The surface of the test

exhibits vague, concentric undulations.

**Wall structure.** The test wall is relatively thin (115–150  $\mu\text{m}$ ) and predominantly fine-grained with a smooth outer surface consisting mainly of small mineral grains, most of them angular and <50  $\mu\text{m}$  in maximum dimension (Fig. 8A–E). The wall also incorporates some relatively large spicule fragments, as well as scattered agglutinated foraminiferan tests (Fig. 8C). The rim comprises mainly spicule fragments but also includes some agglutinated tests and radiolarians, while the edge of the test is characterised by a mesh of spicule fragments. On one side, the rim, with its distinctive structure, extends down the side of the stem to



**Figure 9.** *Psammmina microgranulata* Gooday and Holtzmann sp. nov., AB02 cruise, Station S02; internal features of holotype, registration number NHMUK PM ZF 7802. **A:** Entire test with part of wall removed, revealing dark stercomare and whitish granellare strands. **B:** Closer views of stercomare and granellare. **C:** Broken base of test with granellare. Scale bars: 2 mm (**A**), 500  $\mu\text{m}$  (**B**), 1 mm (**C**).

the base of the test (Fig. 7C), enhancing the asymmetrical appearance of the lower part of the test. The inner surface of the wall is much rougher and coarser grained than the exterior, consisting largely of spicules and scattered radiolarians that project into the interior (Fig. 8F).

**Test interior.** The interior is occupied by stercomare, granellare and sparse internal xenophyae (Fig. 9). The granellare forms pale whitish branching strands, generally 45–80  $\mu\text{m}$  in diameter with some sections up to 100  $\mu\text{m}$ . The strands pervade the entire test, including the base of the stem (Fig. 9C). The stercomare forms irregular branching masses, bounded by a thin transparent reflective sheath, that occupy much of the space between the granellare. The internal xenophyae are scattered throughout the test interior; most are spicules but radiolarian and agglutinated foraminiferan tests are also present.

#### Remarks

The test of *Psammmina microgranulata* resembles those of our sequenced specimens of *P. aff. limbata* form 1 in having a semicircular upper part, merging

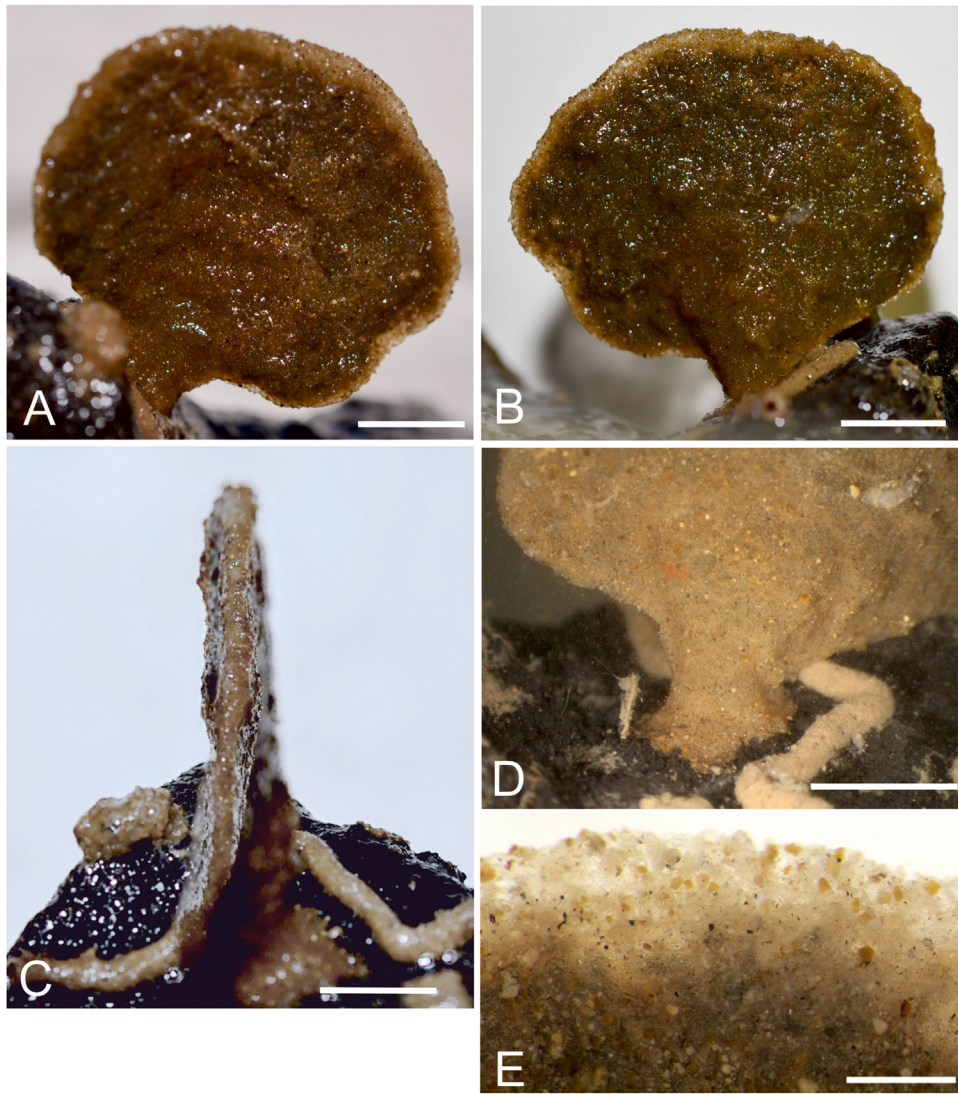
into a stalk that is attached at its base to a nodule. The stalk is complicated by the presence on one side of a low flange, as well as the extension of the pale rim to the base of the structure, features not present in *P. aff. limbata* form 1. However, with only one specimen available, it is impossible to know whether these are characteristics of the species or peculiarities of this individual. The most distinctive morphological feature of the new species is the fine-grained test wall, composed largely of small mineral grains, with subordinate numbers of sponge spicules and almost no radiolarians. This is quite different from the much coarser-grained test wall in *P. limbata* and *P. aff. limbata*, forms 1 and 2, which is constructed largely from spicules and radiolarians.

*Psammmina rotunda* Gooday and Holtzmann sp. nov.

Figures 10–11; Supplementary Material Figure S3

*Psammmina limbata* Kamenskaya, Gooday and Tendal 2015. Gooday et al. 2017a, Supplementary fig. S4b





**Figure 10.** *Psammmina rotunda* Gooday and Holtzmann sp. nov., AB02 cruise, Station U11; shipboard photographs of holotype, registration number NHMUK PM ZF 7803. **A, B:** Opposite sides of test attached to nodule. **C:** Side view of test. **D:** Base of test with short, relatively wide stalk; the pale sinuous feature is a worm tube attached to nodule (also visible in **A** and **C**). **E:** Detail of pale rim. Scale bars: 5 mm (**A–D**), 1 mm (**E**).

?*Psammmina* sp. nov. 1 [Gooday et al. 2018](#) figs 2e,f; 5a–e

**Diagnosis.** Attached species of *Psammmina* with flat, rounded, plate-like test joined to substrate by short, relatively wide stalk. Test wall continuous around margin, which lacks obvious openings. Wall comprises mixture of mineral grains and sponge spicule fragments with scattered radiolarians and occasional agglutinated foraminifera.

**Etymology.** Latin *rotunda*, referring to the circular outline of the upper part of the test.

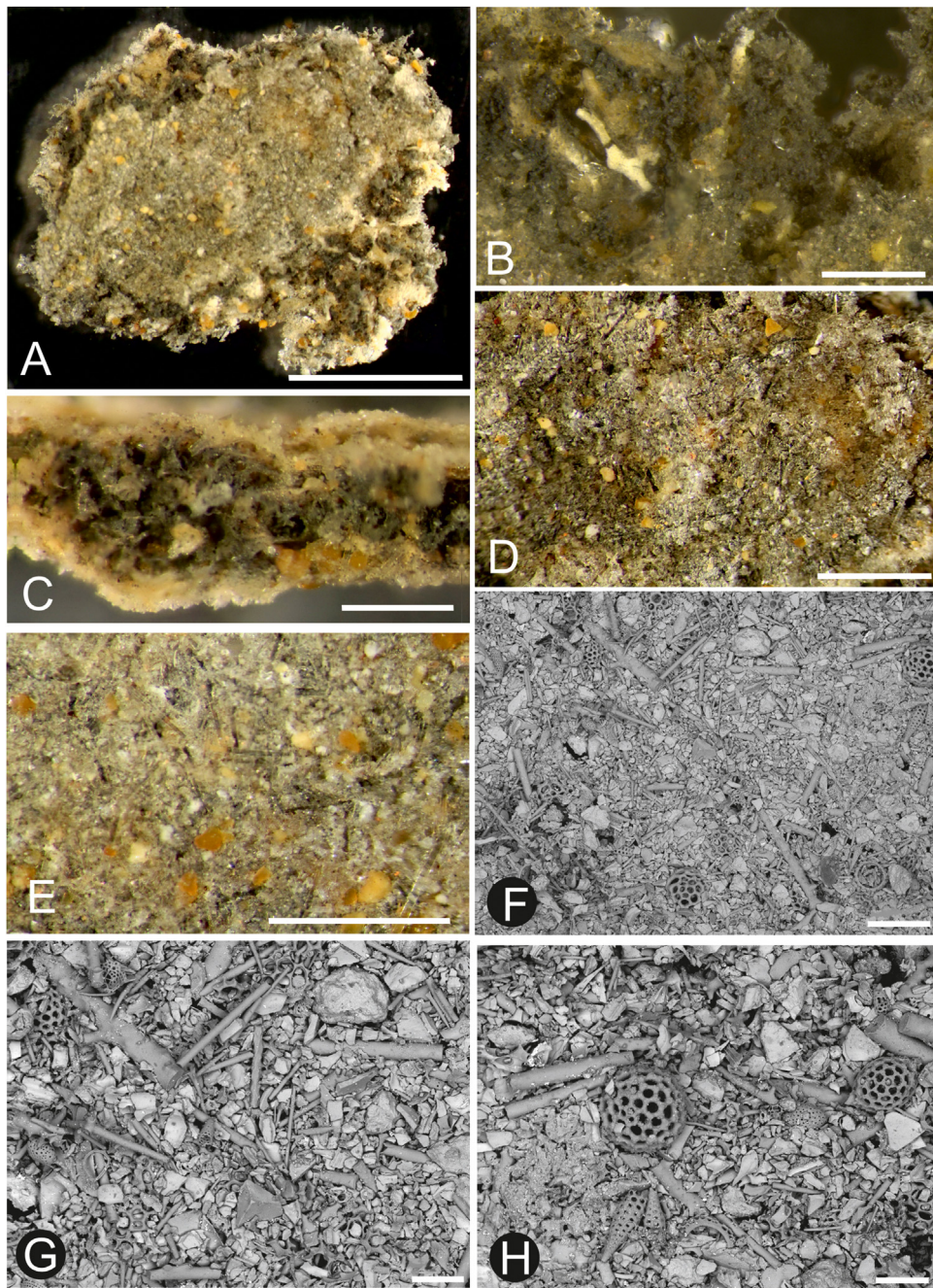
**Holotype.** The unique specimen was collected in a

box core (BC16) from Station U11 (12°30.382'N, 116°29.073'W, 4,244 m) during the AB02 cruise. Registration number NHMUK PM ZF 7803. DNA isolate numbers 18267–18269 (separate samples of cytoplasm from the holotype), accession numbers MF441541–MF441547.

#### *Description of holotype*

**Test morphology.** The test was attached to a nodule, and is brownish, 17.5 mm in total height, 20 mm in width and ~0.90–1.20 mm thick. It comprises a flat, rounded, approximately oval plate, 15.3 mm high, merging at its base with a short stalk, about 3 mm long and 3.6 mm wide at its narrowest





**Figure 11.** *Psammmina rotunda* Gooday and Holtzmann sp. nov., AB02 cruise, Station U11; test wall of holotype. Light micrographs (A–E); scanning electron micrographs (F–H). **A:** Test fragment, external surface partly removed to show interior. **B:** Test interior with granellare strands and stercomare. **C:** Broken edge showing test walls with dark test interior. **D–H:** Details of surface. Scale bars: 2.5 mm (A), 500 µm (B), 1 mm (C–E), 250 µm (F), 100 µm (G, H).

point widening to 4.6 mm where it joins the nodule substrate (Fig. 10A, B, D). Root- and bar-like processes are not developed. A pale rim (Fig. 10E) is present around most of the plate, becoming narrower towards the base. Concentric undulations are

weakly developed on the face of the plate.

*Wall structure.* The test wall is thin (~50–100 µm) and, where still present in the incomplete holotype, is continuous around the rim of the test

(Fig. 10C) rather than forming a lattice-like mesh. It consists mainly of spicule fragments and mineral grains (Fig. 11D–H). The latter include larger particles, yellowish, brownish or orange in colour and 100–200  $\mu\text{m}$  in size, as well as smaller grains (15–50  $\mu\text{m}$ ) that fill the interstices between the larger particles. Some radiolarian tests and a few smaller agglutinated foraminiferal tests are also present.

**Test interior.** The dark stercomare, and in places the white granellare strands, are dimly visible through the test wall. The granellare strands are 40–100  $\mu\text{m}$  diameter (Fig. 11B).

#### Remarks

Genetic data clearly distinguish *Psammmina rotunda* from both *P. aff. limbata* and *P. microgranulata*. In terms of test morphology, *P. rotunda* has a more rounded test with a shorter and relatively wider basal stalk than either of these two species. In addition, the test wall incorporates a higher proportion of mineral grains than *P. limbata* and a higher proportion of spicules than *P. microgranulata*. However, since *P. rotunda* and *P. microgranulata* are each based on a single specimen, we cannot be certain that these differences are consistent.

A *Psammmina* specimen from Station S07 with a rounded test and a short, wide, basal stalk may belong to *P. rotunda* (Supplementary Material Fig. S3). Unfortunately, since the test was preserved in formalin, no sequence data are available and we therefore cannot confirm the identification. This specimen was included (as *Psammmina* sp. nov. 1) in a recent study of xenophyophores based on Micro-CT imaging (Gooday et al. 2018). It is deposited under registration number NHMUK PM ZF 7800.

#### *Psammmina tortilis* Gooday and Holzmann sp. nov.

Figures 12–14; Supplementary Material Figures S4, S5

*Semipsammmina* sp. 4. Gooday et al. 2017a, Supplementary figs 1g, 5e,f, therein

**Diagnosis.** Test typically encrusting nodule surface but also found free. Basically plate-like but often branching into several elongate or lobate sections that may be orientated in different planes. Obvious apertures absent. Encrusting specimens usually give rise to plate-like extensions that stand up more or less vertically, away from substrate. Test wall comprises mainly small mineral grains mixed with variable numbers of spicules and radi-

olarians; larger spicule fragments are noticeable components under the stereo microscope.

**Etymology.** Latin *tortilis* meaning twisted, referring to the appearance of free-growing parts of the test, notably in the holotype.

**Type specimens (unattached).** The holotype was collected in a megacore (MC07) from Station S01 (12°07.074'N, 117°20.604'W, 4,185 m depth) during the AB02 cruise; registration number NHMUK PM ZF 7804. Part of the specimen was used for molecular analysis. DNA isolate numbers 18242, 18243 (separate samples of cytoplasm from the holotype), accession numbers MF441535–MF441540. The paratype, which was not sequenced, originated from the same core; registration number NHMUK PM ZF 7805.

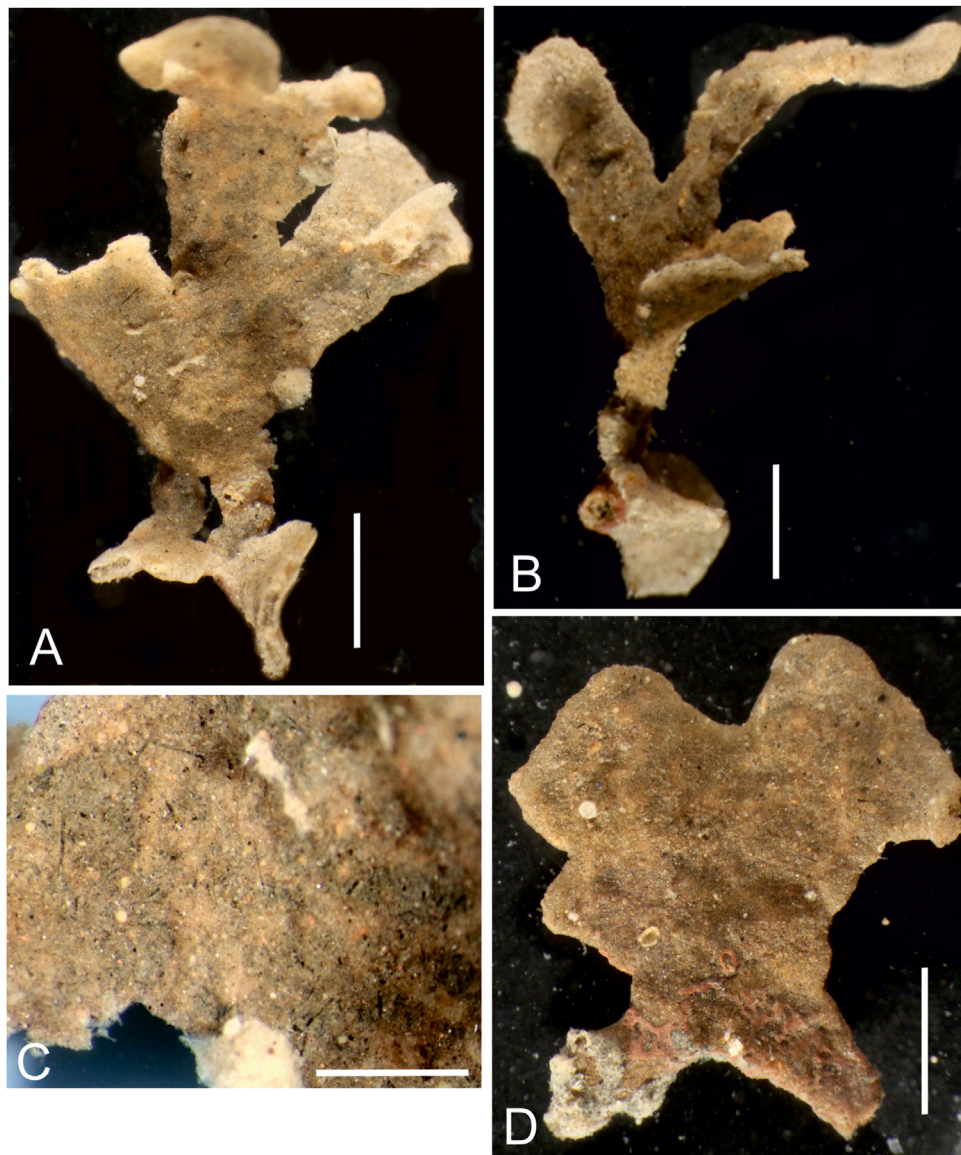
**Other material (attached).** Four main specimens encrusted three nodules collected during the AB02 cruise at Stations U12 (BC18), S01 (MC07) and S09 (BC22). The U12 nodule hosted 2 main formations. The nodule from U12 is deposited under registration number NHMUK PM ZF 7806.

#### Description of type specimens

**Test morphology.** The test was not attached to a nodule when found. It is a fairly light brownish colour with a yellowish–orange tinge. The holotype measured 14.1 mm long and 9.9 mm wide when complete, with a thickness of ~700–760  $\mu\text{m}$ . It has a complex, somewhat twisted, plate-like morphology and is elongated along a main growth axis (Fig. 12A, B). Laterally directed side plates with somewhat curved distal margins and at least one tubular extension are orientated in different planes. When collected, the edges of two processes at the proximal (narrower) end of the test were broken. The paratype has a flat test, measuring 10.3 × 9.6 mm; unlike the holotype all parts lie in the same plane (Fig. 12D). What appears to be the more proximal part comprises two short, flat tubular projections, both about 2.9 mm long and with broken ends. The more distal part features two broad, somewhat rectangular lobes, with a smaller triangular projection also developed laterally. In both specimens the test wall is continuous around the undamaged margins and not interrupted by openings. The orange tube-like structure that encrusts part of the surface of the paratype (Fig. 12D) is probably a sessile foraminiferan.

**Wall structure.** The test wall is thin (~30–40  $\mu\text{m}$ ), generally one grain thick and somewhat translucent with a relatively smooth and finely granular surface. It is composed mainly of small, usually angular



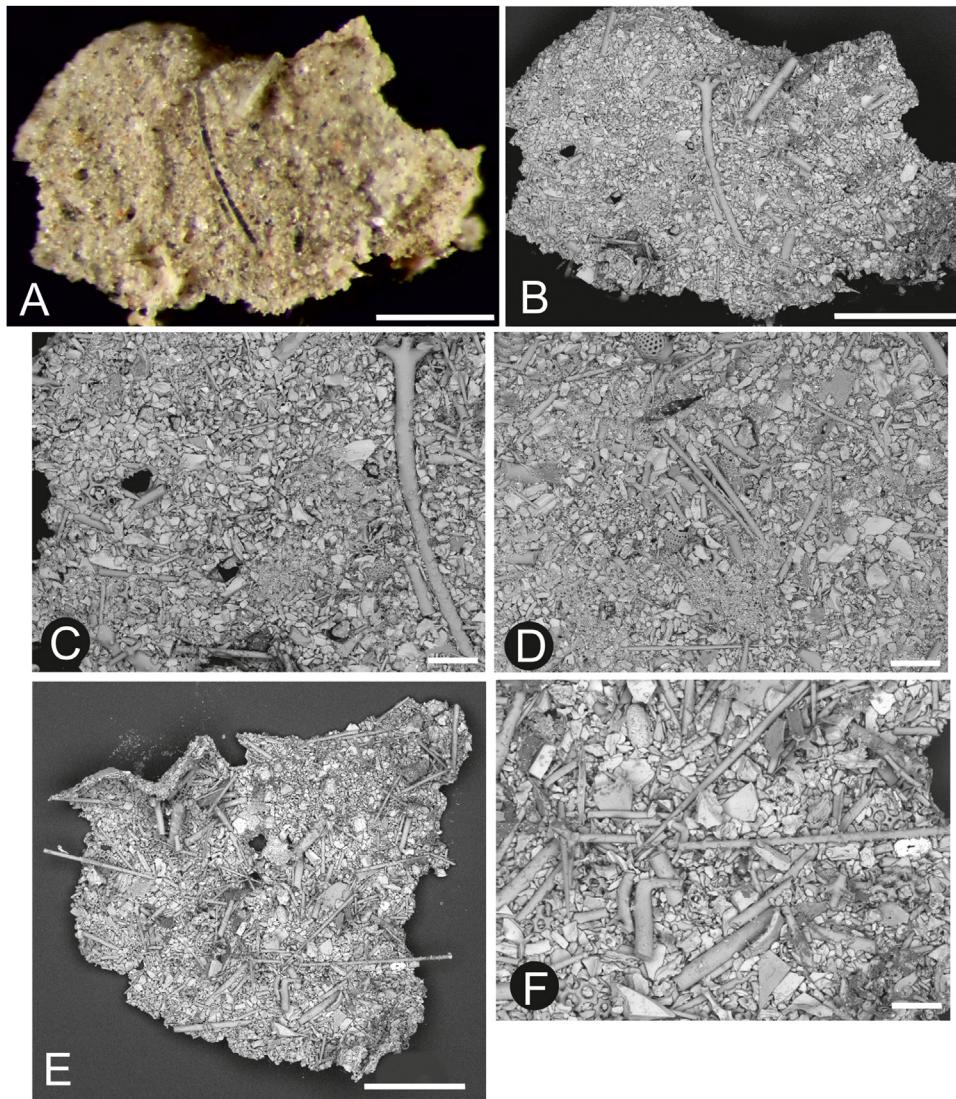


**Figure 12.** *Psammmina tortilis* Gooday and Holtzmann sp. nov., AB02 cruise; shipboard photographs of unattached specimens. Holotype from Station S01, registration number NHMUK PM ZF 7804. (A–C). A, B: Different views of intact specimen. C: Detail showing wall with long spicules. D: Paratype, from Station S01 registration number NHMUK PM ZF 7805. Scale bars: 2.5 mm (A, B, D), 1 mm (C).

mineral grains,  $<100\ \mu\text{m}$  (typically  $<50\ \mu\text{m}$ ) in maximum dimension, together with a variable proportion of sponge spicules and complete and fragmentary radiolarians (Fig. 13A–D). The spicules and radiolarians are more common in the paratype, where the wall also includes scattered agglutinated foraminiferal tests. Some of the spicules are relatively long ( $250\text{--}800\ \mu\text{m}$ ) and stand out prominently when the test is viewed under a stereo-microscope (Fig. 13A). The inner surface of the wall is more uneven with a few particles projecting into the test

interior (Fig. 13E, F).

**Test interior.** The dark stercomare, and in places the pale granellare strands, are dimly visible through the test wall (Fig. 12C), particularly in transmitted light (Supplementary Material Fig. S4C). The stercomare forms irregular masses, generally up to  $500\ \mu\text{m}$  or more in extent. They appear as dark patches and narrower sections, forming a kind of network without any regular pattern but with a mottled appearance overall when seen through the test wall. The granellare strands are reddish-orange,



**Figure 13.** *Psammmina tortilis* Gooday and Holtzmann sp. nov., AB02 cruise, Station U12; test wall of holotype. **A:** Light micrograph of part of outer surface. **B–D:** SEM micrographs of the same fragment. **E, F:** Inner surface of wall. Scale bars: 500  $\mu\text{m}$  (**A, B, E**), 100  $\mu\text{m}$  (**C, D, F**).

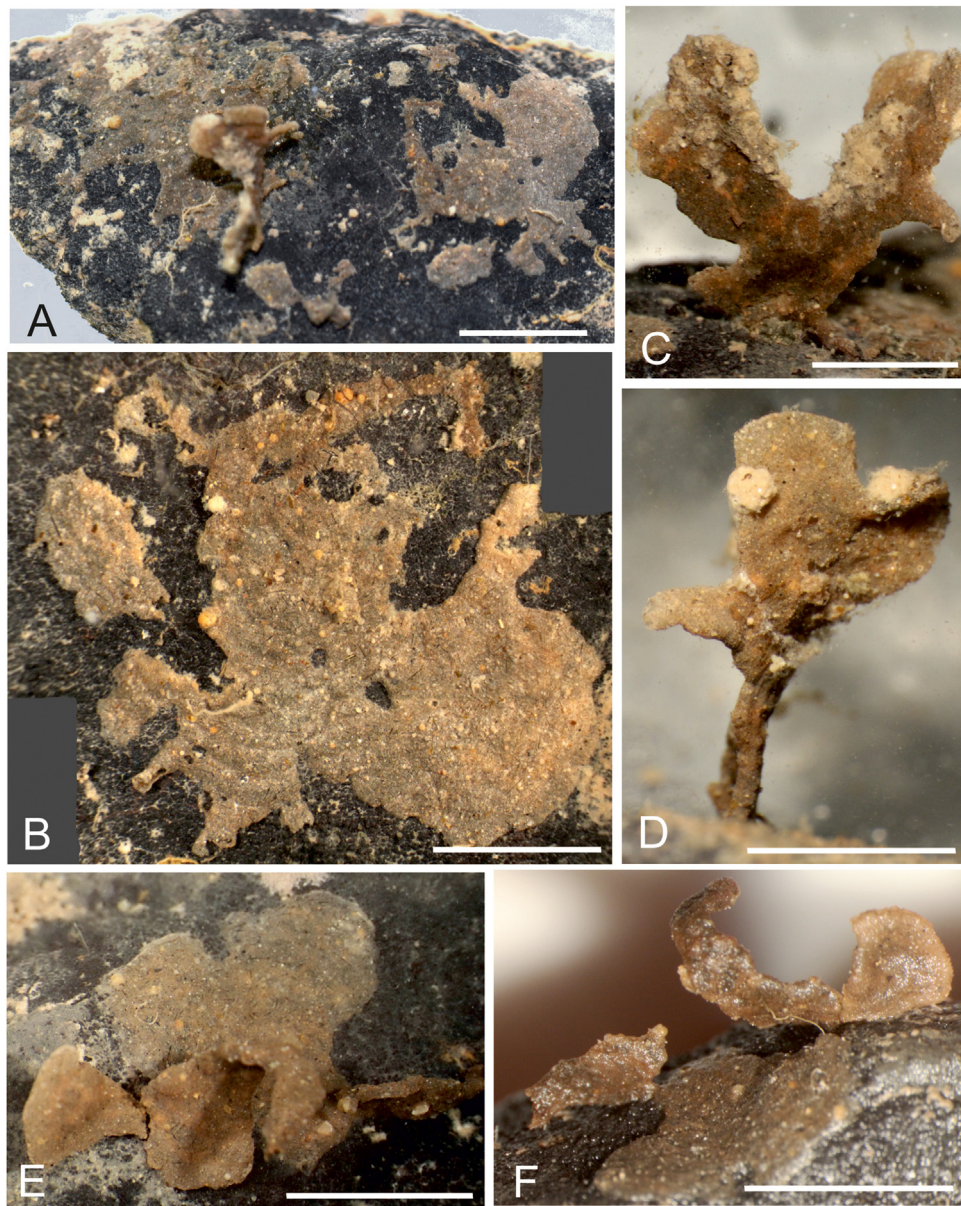
rather diffuse and poorly defined, and of variable width (generally 150–350  $\mu\text{m}$ ).

#### *Encrusting specimens*

Three nodules host 4 main encrusting specimens that we assign to this species (Fig. 14; Supplementary Material Fig. S5). The test forms a crust that spreads across the surface of the substrate, covering areas measuring 13.0  $\times$  6.6 mm (Station S01 specimen), 18  $\times$  10 mm (Station S09 specimen), 17  $\times$  14 mm and  $\sim$ 21  $\times$  20 mm (both on a nodule from Station U12). Some parts have somewhat lobate or indented margins (Fig. 14B, E; Supplementary Material Fig. S5A), but the gen-

eral appearance is more or less irregular. The two most extensive formations (from Stations U12 and S09) have one or more smaller isolated patches located close to the main test (Fig. 14B; Supplementary Material Fig. S5A). Concentric furrows ('growth lines') trending parallel to the margin are sometimes developed (Fig. 14B; Supplementary Material Fig. S5A, C). In three of the four main specimens, part of the edge of the test rises up from the surface of the nodule as an upstanding section. These elevated features are highly variable in form but often branched and/or lobate (Fig. 14C, D, F; Supplementary Material Fig. S5D, E), in one case with a distinct resemblance to the type speci-





**Figure 14.** *Psammia tortilis* Gooday and Holtzmann sp. nov., AB02 cruise, shipboard photographs of encrusting forms attached to polymetallic nodules. Station U12 (**A–D**); Station S01 (**E, F**). The nodule from U12 is deposited under registration numbers UKNHM PM ZF 7806. **A**: Several tests encrusting surface of nodule. **B**: Detail showing one of the larger tests and a smaller patch. **C, D**: Upstanding part of another test on the same nodule. **E**: Complete test. **F**: Detail of upstanding parts. Scale bars: 1 cm (**A**), 5 mm (**B–F**).

mens (Fig. 14D). Another forms a fairly long tubular structure with a terminal bifurcation (Supplementary Material Fig. S5B). The fourth specimen (one of two large formations on the nodule from Station U12) has a lumpy surface with a number of small tubular or irregular excrescences that rise above the general level of the test surface.

#### Remarks

The holotype, which was sequenced, and the formalin-fixed paratype, which was not sequenced, were both unattached when found. Unfortunately, we did not obtain sequences from any of the specimens that encrust nodule surfaces. In the absence of molecular data, these are assigned to *Psam-*

*mina tortilis* based on morphological criteria. First, the composition of the test wall is very similar, particularly the presence in all specimens of long, visually-prominent spicules. Second, some of the upstanding sections that arise from the attached parts of the test closely resemble the type specimens. In fact, the proximal parts of both the paratype and holotype had broken surfaces near the base, suggesting that they may have been detached from encrusting tests.

The holotype and paratype of *Psammmina tortilis* are distinguished from *P. limbata*, *P. microgranulata* and *P. rotunda* by the more complex test, which has wide, branched and lobate sections that develop in different directions and sometimes different planes. The encrusting specimens, on the other hand, comprise a single test layer that forms a low canopy over the stercomare and granellare. The lack of a lower agglutinated plate separating the cellular structures from the substrate would be consistent with a placement in the genus *Semipsammmina* (Tendal 1975), the genus to which these specimens were assigned by Gooday et al. (2017a). In the upstanding sections, however, the stercomare and granellare are sandwiched between two agglutinated plates, consistent with a placement in *Psammmina*. These specimens therefore challenge the distinction between the genera *Psammmina* and *Semipsammmina*. Two species of the genus *Semipsammmina* recently described from the CCZ (*S. licheniformis* Kamenskaya, Gooday and Tendal 2015 and *S. mattaeformis* Gooday and Holzmann 2017) are easily distinguished from encrusting specimens of *P. tortilis* by the composition of the test wall, as well as the absence of elevated sections of the test.

### *Psammmina* sp. 3

Supplementary Material Figure S6

*Psammmina* sp. 3 Gooday et al. 2017a, Supplementary fig. 4d,e

#### Material

The single specimen, from Station U14 (box core BC19), was largely destroyed in order to extract cellular material for genetic analysis. DNA isolate number 18270, accession numbers MF441548, 441549, LT576130.

**Description of test** (based on Gooday et al. 2017a; Supplementary Material)

The test was attached to a nodule. The basic structure is plate-like, but the plate curves around

to form an upright, almost tubular, roughly conical structure, 8.5 mm long and increasing in width from 2.5 mm near the base to 5.5 mm at the top. The thickness of the plate varies from 0.70 to 0.85 mm. The test wall is thin, somewhat transparent, and contains a relatively high proportion of sponge spicules together with radiolarians and mineral grains. The test interior appears to be divided into compartments that are occupied by stercomare masses of irregular width (typically 200–300 µm) and granellare strands 65–80 µm wide.

#### Remarks

The single specimen is distinguished from all other species of *Psammmina*, including those described here, by its strongly curved test morphology (Table 1).

### *Psammmina* sp. 6

Supplementary Figure S7

*Psammmina* aff. *limbata*. Gooday et al. 2017a, Supplementary fig. 4a

**Terminology.** We have designated this species as *Psammmina* sp. 6 in order to avoid confusion with the undescribed *Psammmina* spp. 1–5, recognised by Gooday et al. (2017a).

**Material.** Two complete unattached specimens from Stations S08 (BC24) and S11 (EB12); registration numbers NHMUK PM ZF 7808, and NHMUK PM ZF 7809, respectively. Morphology only.

#### Description of test

These were the only *Psammmina* specimens in our collection that were either not attached to a nodule or showed no evidence of having been attached to one. The test is fairly light brownish when dried. The upper part of the test is rounded and merges either smoothly or in 2–3 steps with the long, slender, tapering stalk that gives rise to several short, lateral branches at the base (Supplementary Material Fig. S7A, B). The specimen from Station S08 measures 35 mm in total length; the upper part is 20 mm long and 22 mm wide and the stem tapers from ~5 mm to 1 mm. The specimen from Station S11 is 30 mm in total length; the upper part is 14 mm long and 17 mm wide and the stem tapers from ~2.3 mm to 1.1–1.2 mm diameter at the lower end. The wall has a generally smooth outer surface that is dominated by small mineral particles (Supplementary Fig. S7C–E), with radiolarian tests becoming common only close to the edge of the test. The margins of both specimens are intact only in a few places



**Table 1.** Main characteristics of *Psammmina* species.

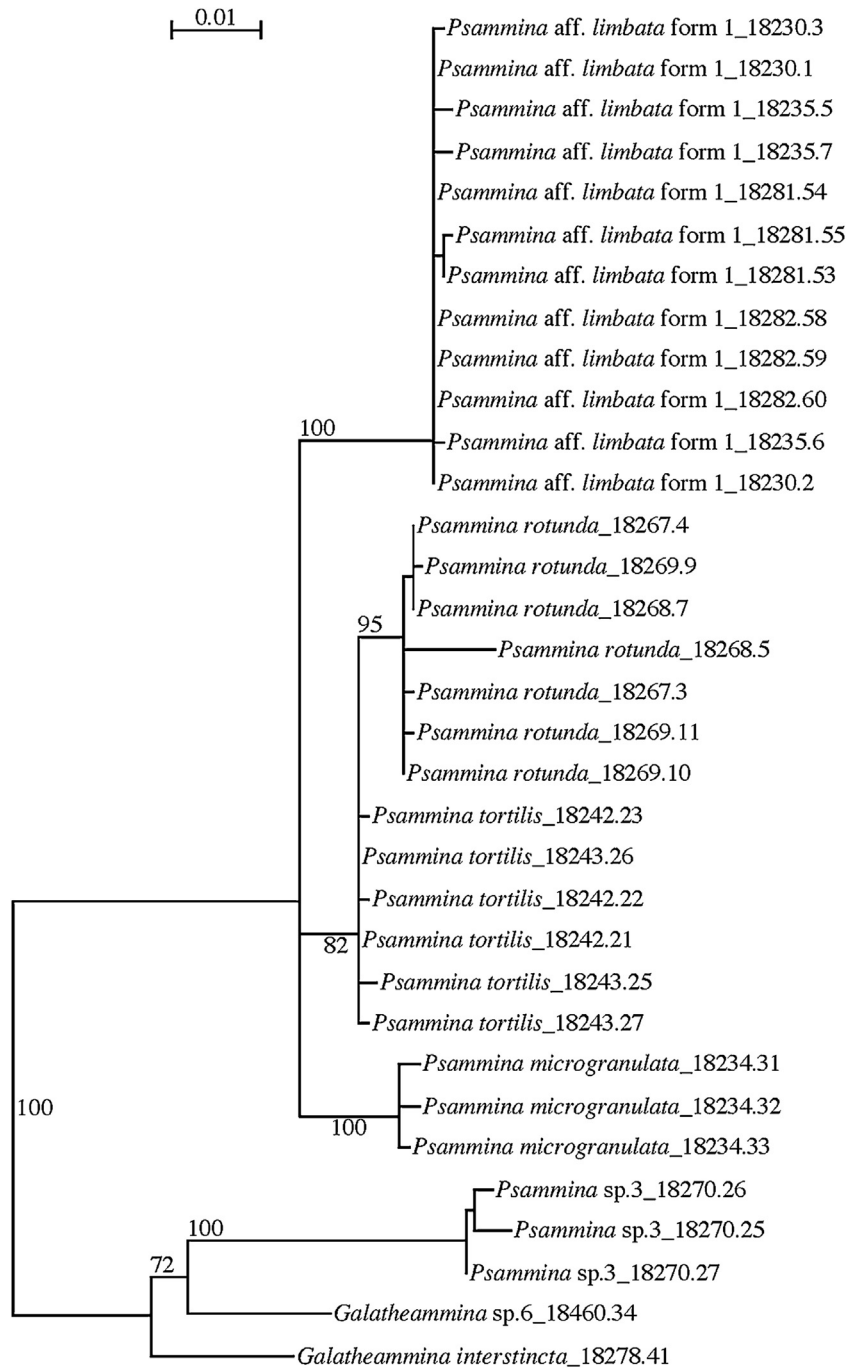
Species	Test shape	Margin	Wall composition	Wall consistency	Internal structure	Reference
<i>P. nummulina</i>	Free, irregular circular	Swollen or tapered edge perforated by pores	Radiolarian tests	Firmly cemented	'A few connecting pillars'	<a href="#">Tendal (1972)</a>
<i>P. globigerina</i>	Free, nearly circular or somewhat irregular	Rounded and tapered with row of pores 0.5–1.0 mm diameter, 1–2 mm spacing	Mainly foraminiferan tests	'Fragile'	'Strong pillars' that can 'form longer or shorter walls'	<a href="#">Tendal (1972)</a>
<i>P. plakina</i>	Free, 'subcircular, faintly convex-concave'	10–12 pores around margin	Foraminiferan tests and test fragments	No information	Numerous internal xenophyae; interior 'partly subdivided into chambers'	<a href="#">Tendal (1972)</a> . Possibly = <i>P. globigerina</i>
<i>P. planata</i>	Plate-like fragments	Natural openings of different shapes, ranging from round to narrow chinks.	Fragments of diatoms, foraminiferal tests, sponge spicules and rare mineral grains	Fragile	Internal xenophyae forming widely-spaced pillars, more loosely agglutinated than outer test layer	<a href="#">Kamenskaya and Saidova (1998)</a>
<i>P. delicata</i>	Free; fragments plate-like with bar-shaped sections and occasional open spaces	Wall continuous around edge without large natural openings	'Large, mainly fragmentary planktonic foraminiferan shells' in finer-grained matrix	Very fragile and weakly cemented	'Pillars consisting of one or more large (planktonic foraminiferan) shells'	<a href="#">Gooday and Tendal (1988)</a>
<i>P. fusca</i>	Free; fragments usually plate-like, occasionally bar-shaped or including base of bar	Wall continuous around edge without large natural openings	Large mineral (mainly quartz) grains and occasional foraminiferan shells in fine-grained matrix	Weakly cemented, fragile and friable	Internal xenophyae mainly large loose quartz grains not organised into obvious pillars	<a href="#">Gooday and Tendal (1988)</a>

<i>P. sabulosa</i>	Free; plate-like fragments, brownish-orange in colour	'Rounded and perforated by well defined, approximately circular apertures'	Well-sorted sand grains	Friable and fragile	'Plates united by bars running parallel to each other and to the plane of the plates.' Bars 'perforated by a single row of evenly spaced and more or less circular holes	<a href="#">Gooday and Tendam (1988)</a>
<i>P. zonaria</i>	'Flat, elongate, spatulate', width increasing from proximal to distal. Probably attached	'Numerous small openings, <0.1 mm in diameter'	Planktonic foraminiferan tests	Hard and brittle	Well-developed bars extending across width of test, dividing interior into compartments	<a href="#">Tendam (1994)</a>
<i>P. multiloculata</i>	Semicircular plate, sometimes with side plate; attached with or without stalk	Numerous small openings around test margin	Spicules, radiolarians and small mineral grains fragments	Hard but not brittle	Divided into small compartments	<a href="#">Kamenskaya et al. (2015, 2017)</a>
<i>P. limbata</i>	Semicircular plate, strongly curved with extensive 'root' system; attached by short stalk	Pale rim. Lattice-like meshwork of sponge spicules and radiolarians	Spicules, radiolarians and small mineral grains	Delicate and brittle	Internal xenophyae loosely organised spicules; no pillars or compartments	<a href="#">Kamenskaya et al. (2015)</a> ; this study. Single specimen
<i>P. aff. limbata</i> form 1	Flat subtriangular to semicircular plate; attached by well-developed stalk	Pale rim. Lattice-like meshwork of sponge spicules and radiolarians	Spicules, radiolarians and small mineral grains	Delicate and brittle	A few spicules and radiolarians; no pillars or compartments	This study
<i>P. aff. limbata</i> form 2	Flat subtriangular plate; attached by well-developed stalk	Rim not clearly pale. Wall continuous around margin with more or less circular openings in places	Spicules, radiolarians and small mineral grains	Delicate and brittle	Thin concentric internal partitions	This study



Table 1 (Continued)

Species	Test shape	Margin	Wall composition	Wall consistency	Internal structure	Reference
<i>P. microgranulata</i> sp. nov.	Flat subtriangular plate; semicircular upper margin tapering into stalk	Pale rim. Lattice-like meshwork of sponge spicules	Mainly small (<50 µm) mineral grains	Delicate and fairly soft	A few spicules and radiolarians; no pillars or compartments	This study. Single specimen
<i>P. rotunda</i> sp. nov.	Flat, rounded, plate; attached by short stalk	Pale rim. Lattice-like meshwork of sponge spicules	Mainly sponge fragments and mineral grains	Delicate and fairly soft	A few spicules and radiolarians; no pillars or compartments	This study. Single specimen
<i>P. tortilis</i> sp. nov.	Plate-like, often with lobate or elongated section, sometimes in different planes; typically with large parts of test forming crust on nodule surface	Wall continuous around rim; no openings	Small mineral grains with variable proportions of spicules and radiolarians	Delicate and brittle	No obvious internal xenophyae	This study.
<i>Psammina</i> sp. 3	Test attached, upright, strongly curved about vertical axis	Wall continuous around rim	Sponge spicules, radiolarians, mineral grains	Delicate and brittle	Some evidence for internal divisions	<a href="#">Gooday et al. (2017a)</a> ; This study
<i>Psammina</i> sp. 6	Test free. Rounded upper part merging into long slender stalk ending in short lateral branches	Wall continuous around rim	Mainly small mineral grains	Delicate and brittle	Internal xenophyae loosely organised spicules; no pillars or compartments	This study
<i>Psammina</i> sp. A	Damaged tests plate-like, more or less rounded, with growth zones	Wall continuous around rim; no openings	Complete and fragmentary planktonic foraminiferan tests in fine-grained matrix	Delicate and brittle	Planktonic foraminiferan tests; no pillars or compartments	<a href="#">Gooday (1996)</a>



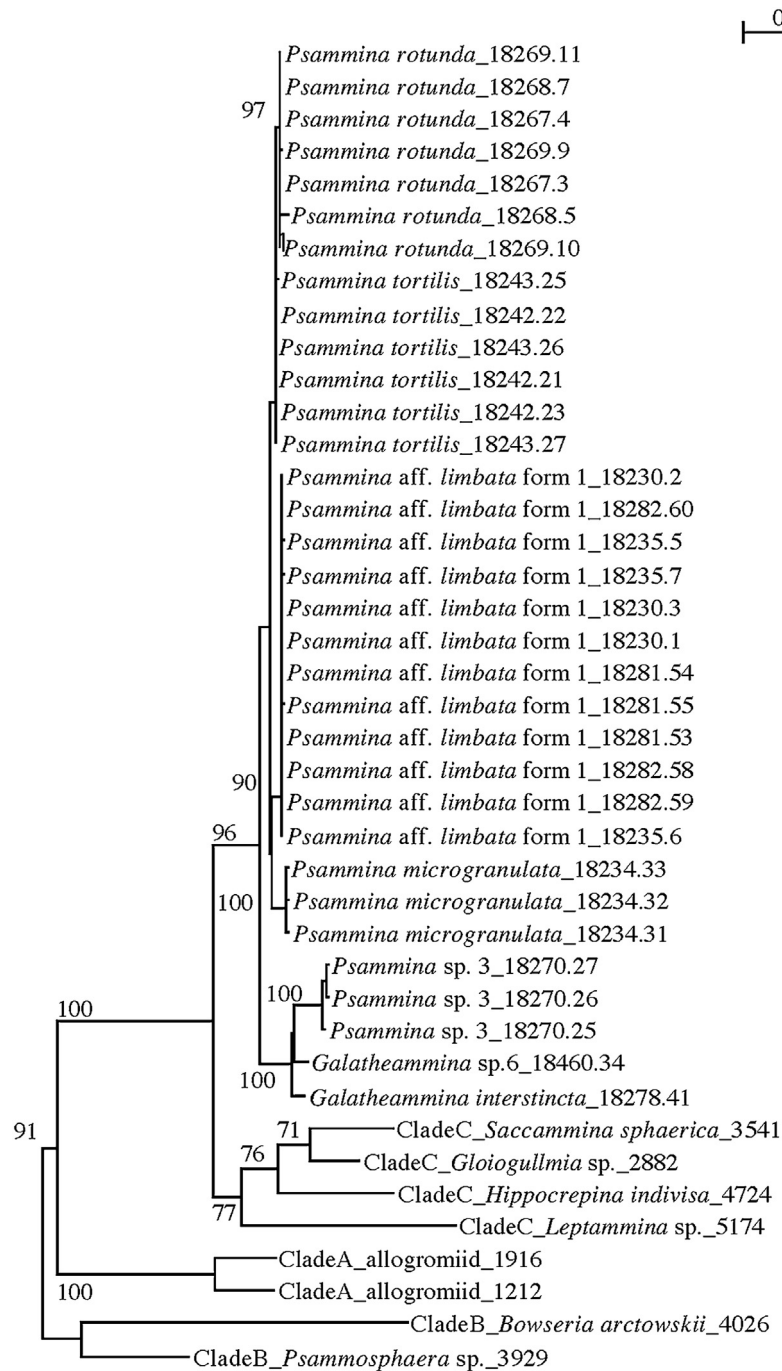
**Figure 15.** PhyML phylogenetic tree showing evolutionary relationships of *Psammmina* species and two similar plate-like species currently assigned to *Galatheammina*. Numbers at nodes indicate bootstrap values (BVs) >70%.

near the base of the upper part, where the wall forms a continuous layer around the edge of the test. The abraded margin reveals that the wall is thin and well-defined; the test interior near the margin is occupied by a meshwork of spicules and radiolarians (Supplementary Material Fig. S7F, G).

#### Remarks

The differences in the shape of the test, particularly the development of a long, apparently unattached stalk, together with the composition of the wall, suggest that this is a distinct species.





**Figure 16.** PhyML phylogenetic tree showing evolutionary relationships of *Psammmina* spp. and *Galatheammina* spp. with eight specimens of monothalamid clades A, B and C. The tree is rooted in Clade A. Numbers at nodes indicate bootstrap values (BVs) >70%.

### Agglutinated mineral grains

A combination of spot analyses of individual grains and element mapping of areas ( $\sim 2400 \mu\text{m}^2$ ) of the outer test surface revealed no strong differences

between species in the elemental composition of the non-biogenic agglutinated grains (data not shown), although the biogenic component, mainly sponge spicules and radiolarians, predominated to a much greater extent in the case of *Psammmina* aff.

*limbata* form 1. The majority of mineral grains are rich in Si, Al, K, Ca, Na, and in some cases Fe. Some are probably Ca-rich feldspar. Others contain a mixture of Si, Al, K, Na and Fe, but no Ca, and display evidence of a vesicular morphology, suggesting that they are fragments of volcanic glass. Several relatively large F-rich grains are present within the scanned areas of *P. rotunda* and *P. aff. limbata*. A minority of grains yielded spectra dominated by Si and O and are presumably quartz. All specimens have a scattering, and in some cases local concentrations, of tiny Ba-rich particles, presumed to be barite granellae.

## Molecular Characterisation

The newly described *Psammmina* species build a clade that also contains two species that were assigned by Gooday et al. (2017a) to *Galatheammina* based on aspects of the test structure (Fig. 15). Within this clade, five *Psammmina* species can be distinguished. *Psammmina* sp. 3 (100% BV) branches at the base and builds a sister to *Galatheammina* sp. 6 and *G. interstincta* (72% BV). The other four species build a clade, with *Psammmina microgranulata* (100% BV) and *Psammmina aff. limbata* form 1 (100% BV) branching next to the sister group of *P. tortilis* (82% BV) and *P. rotunda* (95% BV).

Figure 16 shows that *Psammmina rotunda* (97% BV) and *P. tortilis* branch as sister next to *P. aff. limbata* (90% BV) and *P. microgranulata* (BV 100%). *Psammmina* sp. 3 (100% BV) is a sister to *Galatheammina* sp. 6 and *G. interstincta* (100% BV), these three species forming a group that branches at the base of the four *Psammmina* species (96% BV). The monophyly of the xenophyophores and Clade C is firmly supported (100% BV). The monothalamid clades A and B are the closest relatives to Clade C and branch at its base.

## Discussion

### *Psammmina*: a Polyphyletic Genus?

The genus *Psammmina* was originally established to accommodate three species (the type species *P. nummulina* Haeckel 1889, *P. globigerina* Haeckel 1889 and *P. plakina* Haeckel 1889) with thin, more or less flat, rounded tests comprising well-cemented upper and lower plates with openings around the margin and a pillar-like arrangement of internal xenophyae (Haeckel 1889; Tendal 1972).

There is no evidence that these species were attached to a substrate. Many of the later species assigned to this genus extended its definition in a number of respects (Table 1). *Psammmina delicata* Gooday and Tendal 1988 and *P. fusca* Gooday and Tendal 1988 have a weakly cemented test with no marginal openings and without clearly-defined internal pillars. *Psammmina zonaria* Tendal 1994 has an elongate rather than rounded test that increases in width towards the distal end, lacks well-defined marginal openings, and is strongly compartmentalised internally by transverse bars. Tendal (1994) suggested that *P. zonaria* lives attached to a hard substrate. The first unequivocally attached species, *P. limbata* and *P. multiloculata*, were described by Kamenskaya et al. (2015) from the CCZ. Both have an upright semicircular plate-like test attached to a polymetallic nodule by a short stalk; *P. multiloculata* sometimes has an additional side plate and the interior is divided into numerous internal compartments (see also Kamenskaya et al. 2017). The present study adds three new species, two of them stalked, to this list, making a total of 13 described species, together with six open nomenclature forms (three included here, one recognised by Gooday 1996 and three by Gooday et al. 2017a) that are placed in *Psammmina* based on test characteristics.

In terms of test morphology and structure, these described and undescribed species constitute a heterogeneous assemblage (Table 1). Gooday et al. (2017a) obtained DNA sequences from three of the undescribed species (*Psammmina* sp. 1, *P. sp. 2*, *P. sp. 3*), in addition to '*P. limbata*' (= *P. aff. limbata* form 1 of the present study). These sequences do not group together but are scattered across the xenophyophore tree (fig. 3 in Gooday et al. 2017a), indicating that *Psammmina*-like morphotypes are polyphyletic. Resolving relationships within this group will depend on obtaining DNA sequences from additional species, including the three (or two if *P. plakina* is synonymous with *P. globigerina*) original species of Haeckel (1889).

### The '*Psammmina limbata* Complex'

Apart from the morphologically distinctive *Psammmina tortilis* and *Psammmina* sp. 3, all of the species described here were identified by Gooday et al. (2017a) as *P. limbata*, based on the rounded or fan-shaped test with a basal stalk. Most specimens have a pale rim, as in the holotype of *P. limbata*. However, molecular analyses showed high sequence divergence for *P. limbata* (in the sense of Gooday et al. 2017a), suggesting that it



might encompass several species. Our sequence data confirm that the stalked psamminid xenophyophores in our collections include at least 3 species, with another one or possibly two species recognised based on test morphology. The dead test from Station S11, described here as *P. aff. limbata* form 2 (Fig. 6), is the most *Psammmina*-like xenophyophore in our collection. It has several well-defined marginal apertures ('pores'), features typical of *Psammmina* as defined by Tendal (1972), as well as concentric internal partitions, perhaps comparable to the transverse bars that subdivide the test interior in *P. zonaria* (Tendal 1994). The general test morphology, however, is similar to that of other stalked *limbata*-like morphotypes.

Several outstanding issues remain to be resolved. First, there are no sequence data for *P. limbata* from the Russian license area in the central CCZ. The unique specimen has a much more extensive system of 'roots' than any of ours and is quite strongly folded around a vertical axis, a feature not evident in the AB02 material. We have therefore identified our specimens as *P. aff. limbata* form 1. Resolving the taxonomic status of *P. aff. limbata* form 1 will require sequence data from the Russian area as well as additional data from the UK-1 and OMS areas. As already mentioned, the sequenced and unsequenced specimens of this species have rather different test shapes and could possibly be distinct. Second, the encrusting xenophyophores are assigned to *P. tortilis* based on the composition of the wall and the morphology of the upstanding sections of the test. We feel confident that these represent the same species as the holotype and paratype, but without sequence data we cannot be certain.

Genetic evidence suggests that test morphology is often a rather poor guide to phylogenetic relationships in monothalamous foraminifera (Bowser et al. 2002; Pawlowski et al. 2003), a conclusion that certainly applies to xenophyophores. For example, the recently described *Shinkaiya contorta* resembles *Reticulammina cerebriformis* morphologically but is genetically much closer to *Shinkaiya lindsayi* (Gooday et al. 2017c). An extreme case is the large plate-like *Stannophyllum zonarium* that groups with an undescribed tubular species in the phylogenetic tree published by Gooday et al. (2017a). The species described in the present paper, however, provide a counter-example of a well-supported clade of xenophyophores united by a similar, basically plate-like, test morphology. In three cases (*Psammmina aff. limbata* form 1, *P. microgranulata* and *P. rotunda*), the test resembles a fan with a pale rim, and is attached by a basal stalk to a nodule

surface. There are some morphological differences between them, mainly in the shape of the test and the composition of the test wall, although we cannot be sure that these differences are consistent since two of the species (*P. microgranulata* and *P. rotunda*) are represented by single specimens. Nevertheless, the genetic data clearly support the recognition of three distinct species.

Two species of *Galatheammmina* branch with an undescribed species, *Psammmina* sp. 3. They were assigned to *Galatheammmina* by Gooday et al. (2017a), based mainly on the presence of numerous xenophyae packing the test interior. Internal xenophyae are not present to the same extent in the genus *Psammmina*. On the other hand, both of these *Galatheammmina* species have plate-like tests, as in *Psammmina*. Although a placement in *Psammmina* is clearly supported by genetic data, we prefer to retain them for the present in *Galatheammmina* for consistency with Gooday et al. (2017a). The morphologically most divergent species within this clade is *P. tortilis*, which has a more complex morphology than any of the others, often forming a crust that spreads across the nodule substrate.

## Wider Implications

These new analyses increase the number of formally described xenophyophore species recorded in the UK-1 and OMS license areas from 8 (*Aschemonella aspera*, *A. monile*, *Bizarria bryiformis*, *Galatheammmina interstincta*, *Semipsammmina mattaeformis*, *Shinkaiya contorta*, *Tendalia reteformis*, *Stannophyllum zonarium*; Gooday et al., 2017b,c) to 11, with a larger number (~25) of putative species currently undescribed (Gooday et al. 2017a). Together with previous studies in the Clarion-Clipperton Zone (Kamenskaya 2005; Kamenskaya et al. 2015, 2017) our results emphasise the high xenophyophore diversity that characterises this part of the abyssal equatorial Pacific, as well as demonstrating that similar xenophyophore morphotypes may encompass several distinct species. We anticipate that further sampling across the CCZ will yield additional species. Although the species diversity of xenophyophores (megafauna-sized foraminifera) is undoubtedly less than that of macrofaunal and particularly meiofaunal foraminifera (Goineau and Gooday 2017; Gooday et al. 1998), there is clearly much that remains to be learnt about benthic foraminiferal diversity in abyssal settings across all three size categories.

DNA sequences have been obtained from all of the described xenophyophore species from the UK-1 and OMS areas. Such data are clearly crucial for distinguishing morphologically similar species within a group of protists that offers relatively few taxonomic characters (compared to, for example, crustaceans) and may display considerable intraspecific variability in terms of test morphology. As well as taxonomy, this has implications for the recognition of xenophyophore species in seafloor images. Pale-rimmed, plate-like morphotypes, some of them tentatively assigned to *Psammmina limbata*, are quite common in photographs obtained during the AB01 cruise from the UK-1 Stratum A (Amon et al. 2016) and elsewhere in the CCZ (Gooday and Kamenskaya, 2013 [www.ccfzatlant.com](http://www.ccfzatlant.com)). Our results suggest that these forms may encompass several species that would be difficult or impossible to distinguish in photographs and video records. *In situ* imaging using Remote Operated Vehicles, followed by the collection of the photographed specimens (Gooday et al. 2011), may help to alleviate this problem. In the case of *Aschemonella monile* Gooday and Holzmann 2017, a very common species in the eastern CCZ in which the test is constructed from a series of globular segments, specimens are much easier to recognise in seafloor images (Gooday et al. 2017b). The same may apply to branched morphotypes such as *Spiculammmina delicata* Kamenskaya 2005 (Fig. 5d in Kamenskaya et al. 2013), the most commonly reported species in samples from the central CCZ (Gooday et al. 2017a).

Genetic data are important for confirming identifications as well as for discriminating between species. Confirmation that specimens from different areas represent the same species is necessary in order to establish biogeographic patterns. Information on species distributions is crucial, in turn, for understanding the vulnerability of abyssal species to extinction resulting from the disturbance of their benthic habitat by human activities, notably seabed mining (Miller et al. 2018; Wedding et al. 2015). This applies particularly to sessile organisms such as foraminifera and sponges that depend on polymetallic nodules for an attachment substrate (Gooday et al. 2015, 2017a; Lim et al. 2017; Vanreusel et al. 2016). Based on limited morphological evidence, Gooday et al. (2017a) suggested that some xenophyophore species may have limited ranges in the CCZ nodule fields, but that two species, *Aschemonella* sp. nov. 1 (now described as *Aschemonella monile*) and *P. limbata*, may be distributed more widely, from the central (Russian

area) to the eastern (UK-1 and OMS areas) parts of the CCZ. However, in the absence of molecular data, distributions such as these should be regarded as unconfirmed.

## Methods

**Sample collection and treatment:** Sample collection and treatment followed the methods detailed by Gooday et al. (2017b,c). Briefly, samples were collected as part of the ABYSSLINE (ABYSSal baseLINE) project, using an USNEL box core, an OSIL Bowers & Connolly Megacorer equipped with 10 cm diameter core tubes, or in a few cases a Brenke epibenthic sledge, at 15 stations during the AB02 cruise (R/V *Thomas G Thompson* cruise TN319; February 12 to March 25, 2015). Four were located in the 30 × 30 km 'Stratum B' of the UK-1 licence area and 11 in the comparable OMS Stratum. One additional specimen was obtained during the earlier AB01 cruise (R/V *Melville* cruise MV1313; October 3 to 27, 2013) in UK-1 Stratum A. Station details are summarised in Table 2. Photographs were taken immediately after collection using either a Canon 60D SRL digital camera attached to an Olympus SZX7 microscope, or a hand-held Nikon D3100 SLR digital camera fitted with Nikon 62 mm macro lens. Complete specimens or fragments (including dissected strands of cytoplasm) were preserved for molecular analysis in RNAlater solution (Qiagen). Others were fixed in 4% borax buffered formalin for morphological study. Additional photographs were taken in land-based laboratories using either the same system that was used on the ship (Southampton) or a Leica M205C motorized stereomicroscope equipped with a Leica DFC 450C camera (Geneva).

**Scanning electron microscopy and X-ray microanalysis:** Uncoated xenophyophore test fragments were examined using a Carl Zeiss LEO 1450VP scanning electron microscope (SEM), operated in the variable pressure (VP) mode. Images were obtained with a backscatter detector, operating at 10 kV or 15 kV, nominal probe current 1 nA, working distance (WD) 19 mm. X-ray microanalysis of agglutinated particles in test fragments was undertaken in the SEM using an Oxford Instruments X-Act Silicon Drift Detector (operating conditions, 10 kV or 15 kV, probe current 1 nA, 19 mm WD), using fitted-standards standardless analysis. Although analyses were acquired under reduced vacuum conditions, most X-rays would have been collected from a relatively well-focused beam and beam scattering should not have adversely affected the data.

**DNA extraction, PCR amplification, cloning and sequencing:** Specimens and fragments of xenophyophores preserved in RNAlater solution (Qiagen) were dissected and pieces of cytoplasm removed for analysis. DNA was extracted using the DNeasy Plant Mini Kit (Qiagen). DNA isolate numbers and collection sites are given in Table 3. Semi-nested PCR amplification was carried out with the foraminiferal SSU-specific forward primer s14F3 (5'-ACGCAMGTGTGAACTTG) at the first amplification step, s14F1 (5'-AAGGGCACCACAAGAACGC) for the reamplification, and the 20r eukaryotic SSU reverse primer (5'-GACGGCGGTGTGTACAA) for both amplification steps.

The amplified PCR products were purified using the High pure PCR Purification Kit (Roche Diagnostics) cloned with the TOPO TA Cloning Kit (Invitrogen) following the manufacturer's instructions and transformed into competent *E. coli*. Sequencing reactions were performed using the BigDye Ter-



**Table 2.** Station data for samples collected during the ABYSSLINE cruises.

Cruise	Area	Station	Deployment	Latitude N	Longitude W	Depth (m)	Species
AB01	UK-1 Stratum A	F	BC08	13°48.700'	116°42.600'	4076	<i>Psammina</i> aff. <i>limbata</i> form 2?
AB02	UK-1 Stratum B	U11	BC16	12°30.382'	116°29.073'	4244	<i>Psammina</i> aff. <i>limbata</i> form 1
AB02	UK-1 Stratum B	U12	MC16	12°25.196'	116°37.474'	4137	<i>Psammina</i> <i>rotunda</i> sp. nov. <i>Psammina</i> aff. <i>limbata</i> form 1
AB02	UK-1 Stratum B	U12	BC18	12°25.195'	116°37.477'	4136	<i>Psammina</i> <i>tortilis</i> sp. nov.
AB02	UK-1 Stratum B	U14	BC19	12°31.273'	116°41.889'	4,237	<i>Psammina</i> sp. 3
AB02	OMS Stratum	S01	EB04	12°07.83'–12°08.02'	117°18.67'–117°17.52'	4111–4122	<i>Psammina</i> aff. <i>limbata</i> form 1
AB02	OMS Stratum	S01	MC07	12°07.074'	117°20.604'	4185	<i>Psammina</i> <i>tortilis</i> sp. nov.
AB02	OMS Stratum	S02	BC09	12°04.914'	117°10.691'	4070	<i>Psammina</i> aff. <i>limbata</i> form 1 <i>Psammina</i> <i>microgranulata</i> sp. nov.
AB02	OMS Stratum	S05	BC11	12°13.0425'	117°19.5229'	4090	<i>Psammina</i> aff. <i>limbata</i> form 1
AB02	OMS Stratum	S07	MC20	12°08.163'	117°12.899'	4110	<i>Psammina</i> aff. <i>limbata</i> form 1
AB02	OMS Stratum	S07	BC21	12°08.156'	117°12.900'	4054	<i>Psammina</i> aff. <i>limbata</i> form 1
AB02	OMS Stratum	S08	BC24	12°11.406'	117°22.282'	4182	<i>Psammina</i> sp. 6
AB02	OMS Stratum	S09	BC22	12°05.994'	117°11.796'	4051	<i>Psammina</i> <i>tortilis</i> sp. nov.
AB02	OMS Stratum	S10	BC23	12°03.278'	117°15.103'	4095	<i>Psammina</i> aff. <i>limbata</i> form 1
AB02	OMS Stratum	S11	MC23	12°00.554'	117°22.821'	4185	<i>Psammina</i> aff. <i>limbata</i> form 2
AB02	OMS Stratum	S11	BC25	12°00.559'	117°22.818'	4141	<i>Psammina</i> aff. <i>limbata</i> form 1
AB02	OMS Stratum	S11	EB12	12°02.72'–12°03.03'	117°25.43'–117°24.28'	4223–4235	<i>Psammina</i> sp. 6

**Table 3.** Sequencing details.

Species	Isolate number	Accession numbers	Sampling area
<i>Psammmina</i> aff. <i>limbata</i> form 1	18230	MF441523, MF441524, MF441525	Eastern CCZ <sup>a</sup>
<i>Psammmina</i> aff. <i>limbata</i> form 1	18235	MF441526, MF441527, MF441528	Eastern CCZ
<i>Psammmina</i> aff. <i>limbata</i> form 1	18281	MF441529, MF441530, MF441531	Eastern CCZ
<i>Psammmina</i> aff. <i>limbata</i> form 1	18282	MF441532, MF441533, MF441534	Eastern CCZ
<i>Psammmina microgranulata</i> sp.nov.	18234	MF441521, MF441522, LT576129	Eastern CCZ
<i>Psammmina tortilis</i> sp. nov.	18242	MF441535, MF441536, MF441537	Eastern CCZ
<i>Psammmina tortilis</i> sp. nov.	18243	MF441538, MF441539, MF441540	Eastern CCZ
<i>Psammmina rotunda</i> sp. nov.	18269	MF441541, MF441542, MF441543	Eastern CCZ
<i>Psammmina rotunda</i> sp. nov.	18267	MF441544, MF441545	Eastern CCZ
<i>Psammmina rotunda</i> sp. nov.	18268	MF441546, MF441547	Eastern CCZ
<i>Psammmina</i> sp. 3	18270	MF441548, MF441549, LT576130	Eastern CCZ
<i>Galatheammina interstincta</i>	18278	LT576131	Eastern CCZ
<i>Galatheammina</i> sp. 6	18460	LT576137	Eastern CCZ
Allogromiid	1916	AJ307745	Antarctica
Allogromiid	1212	AJ307744	Antarctica
<i>Bowseria arctowski</i>	4026	FR875094	Antarctica
<i>Gloiogullmia</i> sp.	2882	LT796823	Svalbard
<i>Hippocrepina indivisa</i>	4724	LT796825	Svalbard
<i>Leptammmina</i> sp.	5174	LT796826	Weddell Sea
<i>Psammospaera</i> sp.	3929	LT796822	Antarctica
<i>Saccammmina sphaerica</i>	3541	LT796824	Weddell Sea

<sup>a</sup>CCZ = Clarion-Clipperton Zone.

minator v3.1 Cycle Sequencing Kit (Applied Biosystems) and analysed on a 3130XL Genetic Analyzer (Applied Biosystems). The newly obtained xenophyophore sequences were deposited in the EMBL/GenBank database (accession numbers MF441521–MF441549, LT576129, LT576130).

**Phylogenetic analysis:** The new sequences were added to an existing database using the Muscle automatic alignment option as implemented in Seaview vs. 4.3.3. (Gouy et al. 2010). Sequence length varied from 966 to 1026 base pairs (Bp) (*Psammmina* aff. *limbata* form 1 and *Psammmina* sp.3, respectively), 33 taxa were used for the analysis. The GC content ranges from 37.4% (*Psammmina rotunda*) to 39.3% (*Psammmina microgranulata*). Additionally, an alignment of xenophyophores plus members of Clades A, B and C containing 41 taxa was used for analysis. Sequence length varied from 828 to 1026 Bp (*Psammospaera* sp. and *Psammmina* sp. 3 respectively). The GC content ranges from 35.9% (*Saccammmina sphaerica*) to 43.1% (Clade A allogromiids 1916 and 1212).

Phylogenetic trees were constructed using maximum likelihood phylogeny (PhyML 3.0) as implemented in ATGC: PhyML (Guindon et al. 2010). An automatic model selection based on Akaike Information Criterion (AIC) was used yielding in a GTR substitution model being selected for both analyses. Bootstrap values (BV) are based on 100 replicates (Fig. 15 and Supplementary Material Fig. S8).

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## Appendix A. Supplementary Data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.protis.2018.09.003>.

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