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Vellaria zucchellii sp. nov. a new monothalamous foraminifer from Terra Nova Bay, Antarctica

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Abstract: *Vellaria zucchellii* sp. nov. is described from coastal sediment samples from Terra Nova Bay (Ross Sea, Antarctica, 74°40'28.1"S, 164°04'11.6"E, Tethys Bay, 25 m depth). This organic-walled monothalamous (single chambered) foraminifer is characterized by a wide, prominent aperture that facilitates attachment to larger particles (small sand grains or other foraminiferal shells). It shares this feature with the two other known species of *Vellaria,* both of which were described from an Indian estuary. Phylogenetic analysis of small subunit rRNA gene sequences suggest that *V. zucchellii* is related to the genus *Psammophaga*. However, the new species lacks the mineral grain inclusions that are characteristic of *Psammophaga*. The description of this new organic-walled monothalamous foraminiferal species further documents the high taxonomic diversity of these delicate and abundant protists in the polar benthic communities.

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Key words: allogromiids, polar benthic communities, molecular systematics, taxonomic diversity

Introduction

The foraminiferal order Allogromiida includes species in which the test wall is membranaceous or pseudochitinous, sometimes with an agglutinated veneer of foreign material (Rhumbler 1904, Brönnimann *et al*. 1979, Loeblich & Tappan 1987, Sen Gupta 1999). Allogromiids in this sense form part of a diverse array of monothalamous (i.e. single-chambered) foraminifera that also include forms with agglutinated test walls. Many of these monothalamous genera ('allogromiids *sensu lato*') remain poorly known because their simple tests possess few distinctive morphological features useful for proper species identification. Most allogromiids *sensu lato* occur in marine and brackish water habitats, although freshwater and even terrestrial species are also known (Meisterfeld *et al*. 2001, Holzmann & Pawlowski 2002). In particular, they constitute a major proportion of foraminiferal assemblages in some deep sea and polar settings (Gooday 1990, Korsun & Hald 1998, Schewe & Soltwedel 1998).

Allogromiids are the dominant group of foraminifera in Explorers Cove, McMurdo Sound (Gooday *et al*. 1996, Pawlowski *et al*. 2002) and probably represent a conspicuous element of the fauna in other Antarctic habitats ranging from subtidal glaciomarine settings to the deep sea.

In the present study, we describe a marine organic-walled foraminifer, isolated from sediment samples taken in Terra Nova Bay. The new species was investigated by using morphological and molecular data and assigned to *Vellaria*, a genus described by Gooday & Fernando (1992) from the Vellar Estuary, south-east India. The original Indian material includes two species that live in brackish water, estuarine settings, where they attach to sand grains with a broad, flared apertural structure. Similarities between our new species from polar latitudes and species from tropical latitudes suggest that similar allogromiid morphotypes exist in strikingly dissimilar environmental settings.

Material and methods

Specimen collection

Individuals were collected near the Italian station at Terra Nova Bay (Ross Sea, Southern Ocean; 74°40'28.1"S, 164°04'11.6"E, Tethys Bay). This site was characterized by a steep slope covered in rocks between which were small patches of sediment. One litre sediment samples were taken by scuba divers at 25 m depth, and stored at ambient temperature (-1.8°C) until processed in the laboratory a few days later. Sediments were sieved into > 500 and 125–500 µm size fractions. With the aid of a binocular microscope, living foraminifera were sorted from the 500–125 µm size fraction using a Petri dish of seawater kept cool in a dish of ice. Specimens for molecular analyses were transferred to microtubes containing 60 µl of guanidine DNA extraction buffer. Specimens for morphological study

were fixed in buffered 4% formalin and stored in plastic centrifuge vials.

DNA extraction, amplification and sequencing

DNA was extracted from single or several cells using the guanidine method as described in Tkach & Pawlowski (1999). PCR amplifications were performed in a total volume of 50 µl with an amplification profile consisting of 40 cycles of 30 s at 94°C, 30 s at 50°C and 120 s at 72°C, followed by 5 min at 72°C for final extension. A fragment of the SSU rRNA gene was amplified by PCR with the primer pair s14F3 (5'ACG CA(AC) GTG TGA AAC TTG) and sB (5' TGA TCC TTC TGC AGG TTC ACC TAC). When the first PCR was unsuccessful, the PCR products were re-amplified using the nested primer s14F1 (5' AAG GGC ACC ACA AGA ACG C), with an amplification profile consisting of 20 cycles and 52°C for annealing time. The amplified PCR products were purified using a High Pure PCR Purification Kit (Roche Diagnostics), and then either sequenced directly or ligated into pGEM-T Vector system (Promega) and cloned in XL-2 Ultracompetent Cells (Stratagene). Sequencing reactions were prepared by using ABI-PRISM Big Dye Terminator Cycle Sequencing Kit and analysed with an ABI-377 DNA sequencer (Perkin-Elmer), all according to the manufacturer's instructions.

Sequence analysis

Sequences were aligned manually to the large database of foraminiferal sequences, using the Seaview software of Galtier & Gouy (1996). A number of 668 sites were selected for analysis, including 224 variable and 173 informative sites. Phylogenetic analyses were performed with the maximum likelihood (ML) method using a tree-building algorithm of FASTDNAML (Olsen *et al*. 1994). All characters were equally weighted. The reliability of internal branches was assessed by bootstrapping (Felsenstein 1988) with 100 re-sampling. The PHYLO_WIN_program (Galtier & Gouy 1996) was used for ML tree-building and bootstrapping.

Morphological study

Specimens for molecular study were photographed in seawater using a Nikon Coolpix 990 digital camera mounted on a Wild stereoscopic microscope. For more detailed morphological examination, the allogromiids were placed in ethanol in a cavity slide and examined and photographed using a polarizing Microscope (Nikon, Eclipse E600 POL).

For scanning electron microscopy, a representative specimen stored in ethanol was rehydrated, fixed with 3% glutaraldehyde, dehydrated with ethanol, and then dried using hexamethyldisilazane (Nation 1983). After mounting on a stub, the specimen was sputter-coated with gold and viewed using a LEO 1550VP FESEM.

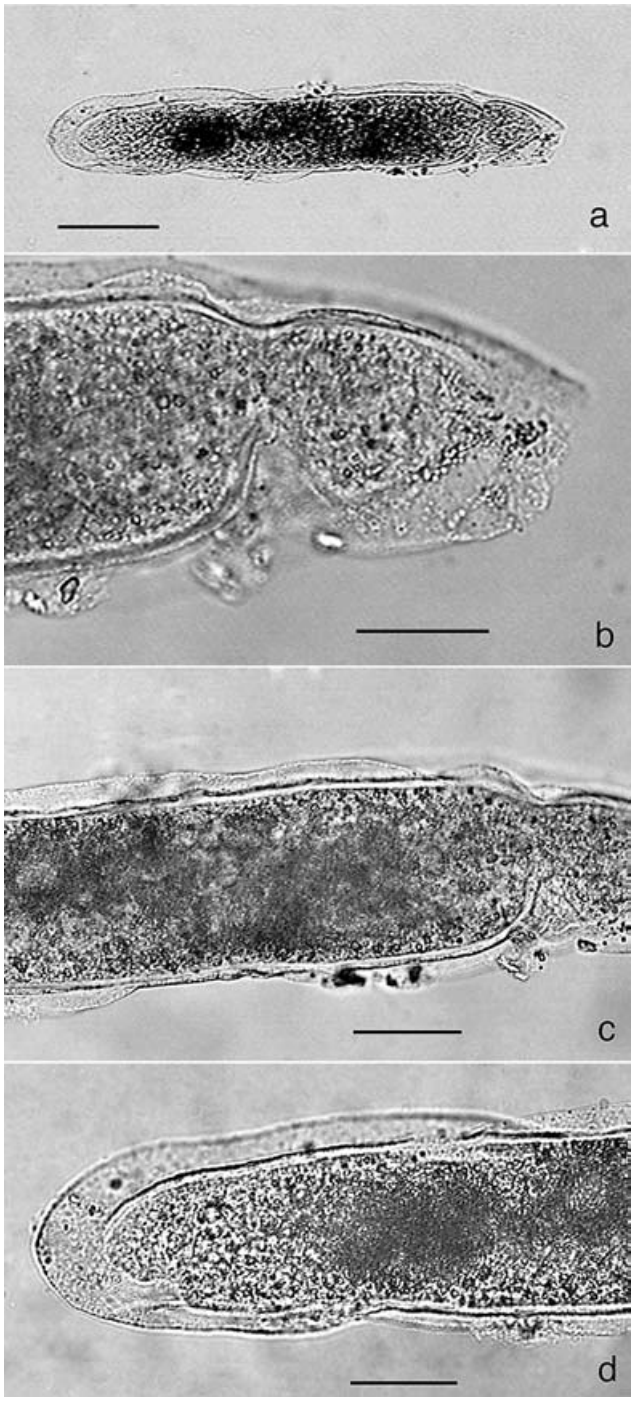

Fig. 1. *Vellaria zucchellii* sp. nov. Specimen of 130 µm length from Station 5 photographed using transmitted light. **a.** General view, **b.** focus on apertural structure, **c.** focus on cytoplasmic contents, **d.** focus on cytoplasmic contents and nucleus. Scale bar: a = 25 µm, b–d = 10 µm

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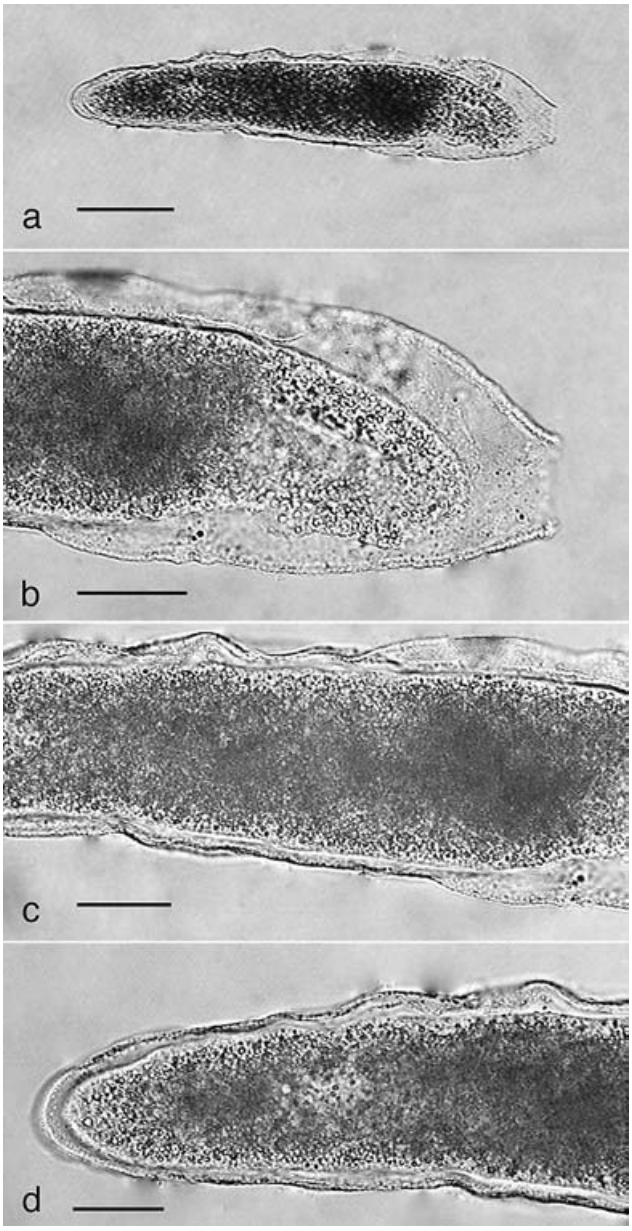

Fig. 2. *Vellaria zucchellii* sp. nov. Specimen of 125 µm length from Station 5 photographed using transmitted light. **a.** General view, **b.** focus on apertural structure, **c.** focus on cytoplasmic contents, **d.** focus on cytoplasmic contents and nucleus. Scale bar: a = 25 µm, b–d = 10 µm.

Results

Systematic account

Class Foraminifera Lee, 1990
Order Allogromiida Loeblich & Tappan, 1961
Family Allogromiidae Rhumbler, 1904
Subfamily Allogromiinae Rhumbler, 1904
Genus *Vellaria* Gooday & Fernando, 1992
Vellaria zucchellii Sabbatini, Pawlowski, Gooday, Bowser, sp. nov.

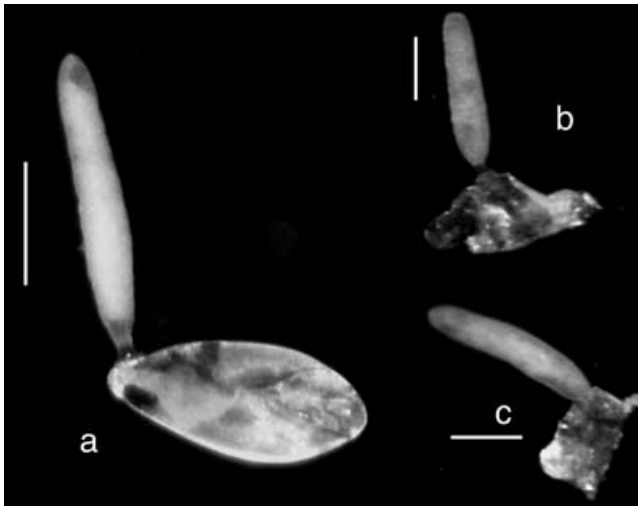

Fig. 3. *Vellaria zucchellii* sp. nov. Specimens are attached to **b & c.** mineral grains, or **a.** to another foraminiferal shell. Scale bars: a –c = 50 µm.

Derivation of specific name. The specific name is after the late Mario Zucchelli, who was in charge of the coordination of the Italian Research Programme in Antarctica between 1986 and 2003.

Diagnosis. Species of *Vellaria* with elongate test, 125 µm in length, L/H ratio ≅ 5, apertural structure broad and flared. Test wall consists of two semitransparent membranes with veneer of fine, plate-like particles present on outer surface.

Type material. The *Vellaria zucchellii* holotype, mounted on an SEM stub, is deposited at the Department of Paleobiology, US National Museum, Washington, DC, under registry number USNM 526088.

Type Locality. Terra Nova Bay, Ross Sea, Antarctica (Tethys Bay, 74°40'28.1"S, 164°04'11.6"E; 25 m depth, January 2003)

Description

Test morphology. The test is elongate, widest near the proximal end and tapering towards the narrowly rounded distal end (Figs 1 & 2). The length ranges from 125 to 130 µm, the width from 24 to 25 µm, and the L/H ratio is around 5. The apertural end is produced into a short tube, giving rise to a broad, expanded, trumpet-shaped distal structure. Small mineral grains or other foraminiferal tests may be attached to its outer margin and specimens are occasionally attached to larger grains (Fig. 3). However, this distal feature is only visible when specimens are immersed in water. When the test is placed in ethanol, the expanded, trumpet-shaped extension contracts and is not clearly visible. Glycerol also causes specimens to shrink and become distorted.

The test wall is transparent, smooth, and flexible with a

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Fig. 4. Test surface of *Vellaria zucchellii* sp. nov. as seen by SEM, showing a veneer of plate-like, agglutinated particles overlying the organic wall. Scale bar = 1 µm.

reflective outer surface. It consists of two distinct, semitransparent organic layers that in some parts of the test are very closely united but in other areas are separated by a distinct space (Figs 1d & 2c–d). When viewed by SEM, the organic wall is overlain by a single layer of fine, plate-like particles (Fig. 4). The outer envelope is partially separated from the cytoplasm by a distinct space (Figs 1a & 2a).

Cell content. The cytoplasm, viewed using a compound microscope, appears finely granular with only a few indeterminate inclusions, although one specimen contained a diatom frustule (Fig. 2b). The cytoplasm is red-brownish in colour, possibly due to the presence of numerous small, rounded particles. In one specimen, the cytoplasm is constricted near the apertural end. A single nucleus was observed in both specimens (Figs 1d & 2c–d).

Molecular analysis

The length of the amplified SSU rDNA fragment in *Vellaria zucchellii* was 941 nucleotides. The GC content was 42%. The sequence divergence between three isolates of *V. zucchellii* was less than 0.2%.

The sequences differ from a sequence (HG3215) derived from a similar morphotype (possibly another *Vellaria* species) collected near Herbertson Glacier (McMurdo Sound, Antarctica) by 9.2% and from the various *Psammophaga* species by 14–16%.

In the phylogenetic tree, *V. zucchellii* branches together with the sequence HG3215 as a sister group to the clade comprising *Psammophaga* sp. from McMurdo Sound, Antarctica, *Psammophaga simplora* from Sapelo Island, Georgia (USA), and *Allogromia crystallifera* from Kosterfjord (Sweden). The grouping of these species is supported by 91% bootstrap value. Interestingly, the sequence HG3215 significantly differs from those of *V. zucchellii* from Terra Nova Bay, and their grouping is supported by only 88% bootstrap values (Fig. 5).

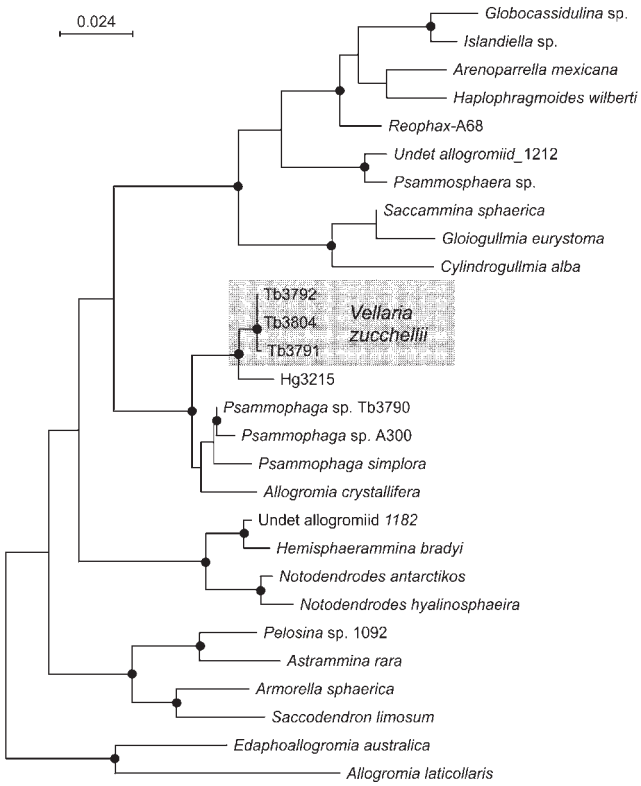

Fig. 5. Phylogenetic position of *Vellaria zucchellii* sp. nov. based on partial SSU rRNA gene sequences. The tree was inferred using maximum likelihood method (lnL=-3898.128). Three sequences of *V. zucchellii* from Terra Nova Bay and one sequence from near Herbertson Glacier are labelled Tb and Hg, respectively. The black dots mark the internal nodes supported in more than 80% bootstrap replicates.

Discussion

Recent developments in molecular systematics are providing new ways to recognise and define species and to understand genetic diversity among marine foraminifera. These studies challenge the traditional, morphology-based classification of foraminifera and have prompted a reassessment of higher-level taxonomy (Pawlowski 2000, Pawlowski & Holzmann 2002). Phylogenetic analysis based on molecular data have revealed that foraminifera include naked freshwater ameboid protists in addition to testate marine species (Pawloswki *et al*. 1999). Molecular studies also show that there is no clear boundary between allogromiids in the "traditional" sense (i.e. organic-walled species) and single-chambered agglutinated foraminifera, traditionally considered as astrorhiziids, suggesting that all of them constitute a paraphyletic group of monothalamous foraminifera (Pawlowski *et al.* 2002).

Our description of this Antarctic species, which is based on a combination of molecular and morphological

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characteristics, adds to our growing knowledge of these primitive foraminifera.

The new species resembles *Vellaria pellucidus* and *V. sacculus* Gooday & Fernando (1992) from the Vellar estuary, India, in general test shape and size, the presence of a flared apertural structure which it uses to attach to sand grains and other larger objects, the finely granular appearance of the cytoplasm, and in the case of *V. sacculus,* the presence of a surface veneer of agglutinated particles. However, the test is distinctly more elongate than in either of the Indian species and the flared structure is not always clearly developed. A funnel-like enlargement of the test is also present in the organic-walled, monothalamous foraminiferan *Psammolagynis atlantica* Golemansky. This tiny species is much smaller (~50 µm) than *Vellaria* and lives in sandy supralittoral sediments (Golemansky 2000).

Soft-walled monothalamous foraminifera that attach to hard substrates by means of apertural extensions are not well documented. In deeper water, small, tubular allogromiiids resembling the genus *Nodellum,* and a tiny two-chambered species resembling the genus *Resigella*, sometimes fix themselves to globigerinacean shells or other firm substrates by means of a tubular apertural extension which may be branched (Gooday *et al.* 2004). The discovery of this new Antarctic species of *Vellaria* suggests that soft-walled monothalamous foraminifera that attach to mineral grains and other hard particles may be widespread in shallow-water, coastal settings.

The flared apertures of these forms are somewhat reminiscent of the apertural neck and sucker-like funnel of psammonobiotid testate amoebae from supralittoral habitats. As in *Vellaria*, this structure is used to attach the organism to sand grains (e.g. Golemansky 1991). A similar adaptation is seen in another testate amoeba, *Paramphitrema pontica* Valkanov (Golemansky 1999). *Sudzukiella marina* Golemansky, a testate rhizopod of uncertain affinity which also inhabits subittoral sands, fixes itself to sand grains by means of a short, curved apertural tube (Golemansky 1991, 2000).

The molecular evidence indicates that *Vellaria zucchellii* is closely related to the clade *Psammophaga* spp. + *Allogromia cristallifera*, which formed an independent lineage of allogromiid foraminifera in the SSU rDNA phylogenetic tree (lineage E of Pawlowski *et al.* 2002). This lineage was characterized by mineral inclusions within cytoplasm (Pawlowski *et al.* 2002). The lack of such inclusions in *V. zucchellii*, however, suggests that this character has a relatively limited taxonomic value. Interestingly, morphologically similar specimens found near Herbertson Glacier, McMurdo Sound, are 9.2% genetically different. In the examined SSU gene fragment such sequence divergence is observed between well distinguished species, for example *Notodendrodes antarctikos* and *Notodendrodes hyalinosphaeira*. This suggests that the genus *Vellaria* is probably represented in Antarctic coastal waters by at least two species. Unfortunately, the Herbertson Glacier specimens were rare and none was preserved, preventing us from making morphological comparisons.

The finding of *V. zucchellii* and of an additional *Vellaria* sp. recognised by molecular analysis within a short geographical range confirms the diversity of allogromiids and the need for taxonomical investigations integrating molecular as well as morphological traits. We anticipate that the exploration of biotopes characterised by different ecological conditions (e.g. energy inputs, hydrodynamic activity) will rapidly increase our knowledge of protistan diversity in Antarctic marine sediments.

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