Electronic supplementary material to

Fossilized spermatozoa preserved in a 50-my-old annelid cocoon from Antarctica

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Geological and palaeontological background information

The La Meseta Formation (Seymour Island Group; Seymour/Marambio Island, Weddell Sea, Antarctic Peninsula; figure S1) consists of shallow-marine to estuarine, poorly consolidated siliciclastic deposits that represent the filling of an incised-valley system [26,27]. It contains rich and very diverse fossil assemblages dominated by marine invertebrates and vertebrates (see e.g. [12,26,27]. The fossil annelid cocoons were collected from localities IAA 1/90 (also known as the 'Ungulate site'; 64°14'04.67"S; 56°39'56.38"W) and IAA 2/95 (known as the 'Marsupial site'; 64°13'58"S; 56°39'06"W) from the northern part of the island; both sites expose lenses of a distinctive conglomerate with abundant shells of naticid gastropods ('*Natica* horizon'), and occur at the same stratigraphic level in the central portion of the Cucullaea I Allomember of the La Meseta Formation (figure S1). In addition to abundant marine fossils, the '*Natica* horizon' in particular has yielded a broad range of fossils of non-marine organisms, including remains of terrestrial mammals and plants [26,27]. Of special interest is the high abundance of dispersed seeds of aquatic angiosperms, such as *Nuphar* ('water lilies') and *Nelumbo* ('lotus') [28], which documents the existence of permanent freshwater bodies as suitable potential habitats for the cocoon-producing clitellates described here. Overall, palaeoclimatic reconstructions indicate temperate and generally ice-free conditions for the Antarctic Peninsula Region during the early Eocene [29].

Although the precise age of the La Meseta Formation is still under debate, a recent chronostratigraphic synthesis [30] placed deposition of the '*Natica* horizon' at ~50 Ma (Ypresian, early Eocene), because it is bracketed by beds that have been dated via Strontium data to 49.4 Ma and 50.8 Ma, respectively [11,26]. This age assignment agrees very well with biostratigraphic assessments based on correlation of mammal assemblages [31].

Methods

Because the host deposit is only poorly consolidated, sediment samples could be dry-sieved immediately on site over 2 cm mesh screens in order to remove larger pebbles, shells, and shell fragments. The collected residues were then dry-sieved again in the laboratory over >4.0, >2.0, and >0.5 mm mesh screens. Cocoon fossils were picked from the residues of the 2.0–4.0-mm-size fraction under a stereomicroscope using an entomology forceps.

Selected specimens were mounted on stubs, sputter-coated with gold, and examined using a Hitachi S-4300 field emission scanning electron microscope at the Swedish Museum of Natural History. In addition, two cocoon fragments were analysed using Synchrotron-radiation-based X-ray tomographic microscopy (SRXTM) at the Tomography Station of the Materials Science Beamline (TOMCAT) at the Swiss Light Source, Paul Scherrer Institute (Villigen, Switzerland) [32,33]. The specimens were analysed using a beam energy of 10 keV and a 20× objective, resulting in voxel dimensions of 0.325 µm. Projections were acquired over 1501 stepwise increments through a rotation of 180°, and processed and arranged following the methods detailed in [34]. Tomographic reconstructions were made using Avizo®. All material is housed in the palaeobiology collections of the Swedish Museum of Natural History (Stockholm, Sweden), under accession numbers NRM-S089727–089730.

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Figure S1

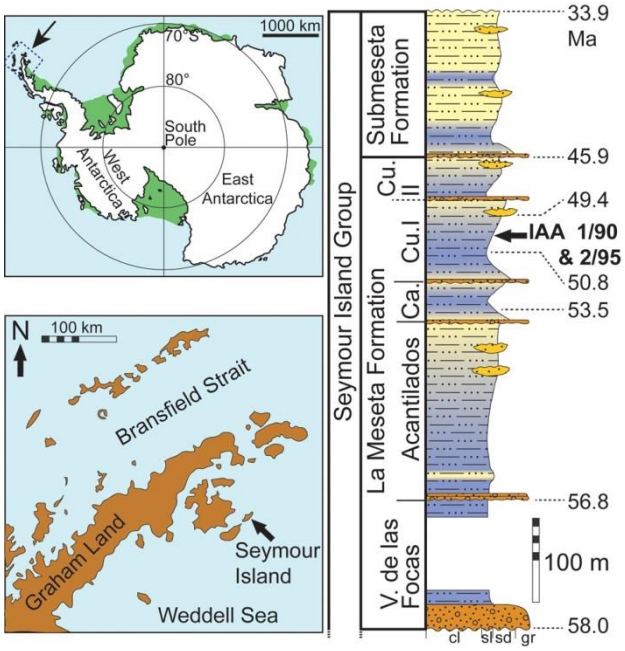

Figure S1. Locality details for the studied fossils: (*a*) map of Antarctica showing the position of the Antarctic Peninsula; (*b*) map of the Antarctic Peninsula showing Seymour Island; (*c*) composite measured section through the La Meseta Formation showing the stratigraphic position of the sampled bed.

Figure S2

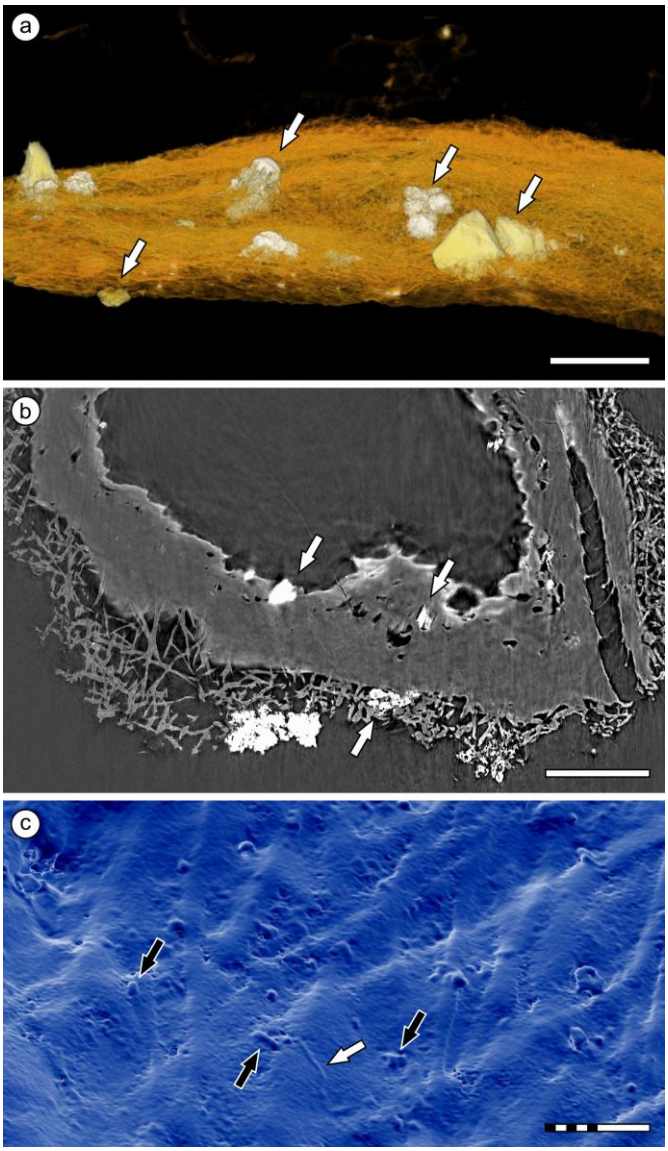

Figure S2. SRXTM reconstructions of an annelid-cocoon wall fragment from the Eocene La Meseta Formation, Seymour/Marambio Island, Antarctica, showing variously sized cocoon-wall inclusions. (*a*) Transparent voltex rendering showing adhering and embedded mineral particles (arrows). (*b*) Orthoslice in oblique tangential section through the clitellate cocoon showing inner solid and outer spongy layer with embedded, highly reflecting mineral particles (arrows). (*c*) Detail of surface rendering of the inner cocoon-wall surface showing embedded structures consistent in overall shape and dimension with an interpretation as included rod-shaped bacilli (black arrows) and a possible spermatozoan fragment (white arrow). Scale bars: (*a*,*b*) = 100 µm; (*c*) = 10 µm.