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MICROSTRUCTURE AND BIOGEOCHEMISTRY OF THE ORGANICALLY PRESERVED EDIACARAN METAZOAN *SABELLIDITES*

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ABSTRACT—Metazoans (multicellular animals) evolved during the Ediacaran Period as shown by the record of their imprints, carbonaceous compressions, trace fossils, and organic bodies and skeletal fossils. Initial evolutionary experiments produced unusual bodies that are poorly understood or conceived of as non-metazoan. It is accepted that sponges, ctenophorans, cnidarians, placozoans, and bilaterians were members of the Ediacaran fauna, many of which have uncertain affinities. The fossil *Sabellidites cambriensis* Yanishevsky, 1926, derived from the terminal Ediacaran strata, is the earliest known organically preserved animal that belonged to a newly evolving fauna, which replaced the Ediacara-type metazoans. Morphologically simple soft-bodied tubular fossils, such as *S. cambriensis*, and biomineralized, as contemporaneous *Sinotubulites* sp., are not easy to recognize phylogenetically because many unrelated organisms developed encasing tubes independently. Therefore, in addition to morphologic information, evidence derived from the microstructure of the organic wall and its biochemistry may be vital to resolving fossil origins and phylogenetic relationships. Here we present morphological, microstructural and biogeochemical studies on *S. cambriensis* using various microscopic and spectroscopic techniques, which provide new evidence that supports its siboglinid, annelidan affinity. The late Ediacaran age of *Sabellidites* fossil constrains the minimum age of siboglinids and the timing of the divergence of including them annelids by fossil record and this could be tested using molecular clock estimates. The fine microstructure of the organic tube in *Sabellidites* is multi-layered and has discrete layers composed of differently orientated and perfectly shaped fibers embedded in an amorphous matrix. The highly ordered and specific pattern of fiber alignment (i.e., the texture of organic matter) is similar to that of representatives of the family Siboglinidae. The biogeochemistry of the organic matter that comprised the tube, which was inferred from its properties, composition, and microstructure, is consistent with chitin and proteins as in siboglinids.

INTRODUCTION

THE CURRENT concept of animal emergence implies that the Ediacaran biota (Narbonne and Gehling, 2003; Narbonne, 2005; Fedonkin et al., 2007; Seilacher, 2007; Valentine, 2007; Pecoits et al., 2012) comprised sponges, ctenophorans, cnidarians, placozoans, and bilaterians and also the stem-and crown-group Metazoa (Budd, 2008; Droser and Gehling, 2008; Liu et al., 2008; Xiao and Laflamme, 2009; Sperling and Vinther, 2010; Erwin et al., 2011; Tang et al., 2011; Vinther et al., 2011; Laflamme et al., 2012; Erwin and Valentine, 2013). Some bizarre organisms became extinct and are known exclusively by their imprints (Brasier and Antcliffe, 2004), and several represent unresolved clades. However, certain Ediacaran fossils once thought to be crown-group metazoans are currently interpreted in other terms (Narbonne, 2005; Antcliffe and Brasier, 2008; Huldtgren et al., 2011; Sappenfield et al., 2011). Only a few generally accepted metazoan fossils of Neoproterozoic age are known. Some very early tubular fossils, such as *Parmia, Protoarenicola, Pararenicola,* and *Sinosabellidites*, which were preserved as organic compressions and claimed to be metazoans, have been reinterpreted as macroalgae (Xiao and Dong, 2006; Dong et al., 2008).

Sabellidites cambriensis Yanishevsky, 1926, represents a fauna of organic tube-dwelling organisms that appeared in the late Ediacaran Period and continued into the Cambrian. Its appearance coincides with that of biomineralizing forms of diverse morphologies, such as *Cloudina*, *Namacalathus, Namapoikia*, and *Sinotubulites*, and with a diversification of cyanobacteria, uni- and multicellular algae, and some biota of unknown origins (Grant, 1990; Moczydłowska, 1991, 2008a, 2008b; Grotzinger et al., 2000; Hua et al., 2003; Xiao and Dong, 2006; Chen et al., 2008; Cortijo et al., 2010). Contemporaneous to *Sabellidites* and probably non-biomineralized or only weakly biomineralized is the conotubular *Conotubus hemiannulatus*, which consists of a series of nested funnel-shaped cylinders with transverse annulations (Hua et al., 2007; Cai et al., 2011). Morphologically, it differs from *Sabellidites*, which organic tube is uniform and without any segmentation or portioning, and its wrinkles on the tube surface are not an expression of annulation (Fig. 1). *Conotubulus* derives from the upper Ediacaran Gaojiashan Member of the Dengying Formation in the Shaanxi Province, South China, and is preserved by strong pyritization with the tube wall totally replaced by pyrite or replication by clay minerals (Cai et al., 2011). Only a few carbonaceous compressions are known and assumed to belong to the same taxon. *Conotubulus* is recognized as metazoan and being related to *Cloudina* (Hua et al., 2007), but its phylogenetic position is uncertain although the fossil morphology broadly resembles that of the extant polychaete family Siboglinidae (Cai et al., 2011).

The characteristically wrinkled tubes of *S. cambriensis* are 0.2–3.0 millimeters wide and up to 16 cm long (Fig. 1.1, 1.4–1.6, 1.8). They occur on bedding planes as either compressed or three-dimensionally preserved specimens without evidence of holdfast structure (Fig. 1.2, 1.3, 1.9). The fossil was discovered in 1921 by M. E. Yanishevsky in the so-called "blue clays" of the lowermost Cambrian Lontova Formation in the area of St. Petersburg. He regarded it as the remains of a sessile polychaete

worm comparable with the extant *Sabellides* (Yanishevsky, 1926). The species name, *cambriensis*, is partly misleading because the stratigraphic range has now been extended into the terminal Ediacaran Period and is estimated to ca. 550–530 Ma (Fedonkin et al., 2007). The first appearance datum of *S. cambriensis* is beyond any doubt of the late Ediacaran age, as is the age of the studied specimens, and is established in the western part of the East European Platform (EEP) by bio- and chronostratigraphic means. In this area, the succession comprises the Ediacara-type biota, vendotaenids, organic-walled microfossils, *Sabellidites,* and trace fossils that occur below the Cambrian fauna and *Trichophycus pedum* trace-fossil (Sokolov and Fedonkin, 1990; Moczydłowska, 1991; Felitsyn et al., 1998). The volcanic ash bed dated to 551±4 Ma (Compston et al., 1995) underlies the Ediacaran sediments. The Ediacaran strata in the White Sea area at the northern extension of the EEP contain two layers of volcanic ashes dated to 555.3±0.3 and 558.3±1 Ma (Martin et al., 2000) and comprise the Ediacaran soft-bodied fossils and also *Sabellidites*-like tubes (Fedonkin and Vickers-Rich, 2007).

The biological affinity and phylogenetic relationships of *Sabellidites* have not yet been fully resolved. Sokolov (1965; *in* Sokolov and Iwanowski, 1990) proposed a pogonophoran (=siboglinid in modern terms: the former phyla Pogonophora and Vestimentifera are now united in the polychaete family Siboglinidae; Rouse and Fauchald, 1997) affinity based on the morphology alone and presumed chitinous composition of the tubes. His proposal was tested by Urbanek and Mierzejewska (1977, 1983) through comparative microstructural studies of *Sabellidites* and two modern siboglinids (*Zenkevitchiana* and *Siboglinum*), but the result was inconclusive because the lamination pattern in the fossils was not satisfactorily preserved to compare with the Recent tubes. Whereas Urbanek and Mierzejewska (1977, 1983) were not able to discern a fibrous structure in the fossils, Ivantsov (1990) unambiguously demonstrated the presence of fibers in *Sabellidites*. He could not, however, observe their alignment and relationship within the layers and therefore declined to speculate on the affinity. Similarly, subsequent authors have regarded *Sabellidites* to be of uncertain affinity (Sepkoski, 1992; Valentine, 2004; Fedonkin et al., 2007).

We studied *Sabellidites* with the goal of identifying the microstructure and biogeochemistry of the organic material and its possible relevance for the biological affiliation. We applied an integrated approach for examining the morphological, microstructural and biogeochemical features of the fossils and the comparative histology and biochemistry of the tube wall of the extant siboglinid *Zenkevitchiana*.

The study reveals that the structural and biogeochemical nature of the organic tubes in *Sabellidites* is consistent with the chitinous-proteinaceous fibrous composition of extant siboglinid tubes.

MATERIALS AND METHODS

This study is based on new materials and specimens of *S. cambriensis* Yanishevsky, 1926, of late Ediacaran and earliest Cambrian ages. The specimens were extracted from siltstone from the Gavrilov-Yam borehole at a depth of 1928.0 m, Nekrasovo Formation, Rovno Stage; Borehole 942-F1, at 1918.0 m, base of the Rovno Formation; Borehole 942-F3 at 1886.0 m, Buiskaya Formation (Rovno Stage), in Russia (the Ediacaran System; Felitsyn et al., 1998, and unpublished data). The specimens from the thin-bedded claystone of the Lontova Formation in the Tverecius-336 borehole at depths of 397.6 m and 409.0 m in Lithuania are from the lowermost Cambrian System (Korkutis, 1981; Paskeviciene, 1986). Abundant specimens, both isolated and embedded in the sediment, were studied using a reflected-light microscope (RLM) Olympus BX 51, and a digital microscope (DM) Keyence at the University of Orléans. Several isolated specimens (n=9) were examined using a scanning electron microscope (SEM Philips XL30) and an environmental scanning transmission electron microscope (STEM Zeiss Supra 35-VP), which is a field emission SEM equipped with a SEM detector for transmission microscopy, a VPE detector for low vacuum conditions, and a Robinson BSD for back-scattered detection. One specimen was studied using a transmission electron microscope (STEM Zeiss Supra 35-VP) in a series of transverse and longitudinal thin sections 60 nanometer (nm) in thickness cut by LKB Ultramicrotome at the Uppsala University. Thin sections were stained by uranite acetite and lead citrate and embedded with TAAB 812 epoxy.

Elemental and molecular microanalyses using a SEM equipped with energy-dispersive X-ray detector (EDX), a laser-confocal Raman-spectrometer and pyrolysis-gas chromatography-mass spectrometry (py-GC-MS) were performed on specimens from the same samples as those studied microscopically. Additionally to the *S. cambriensis* specimens, molecular composition for possible biomarkers was analyzed by py-GC-MS on the kerogen extract from the containing sediment, and the extant siboglinid *Zenkevitchiana* species. Three zooidal tubes of *Zenkevitchiana longissima* Ivanov, 1963, were recovered from the Kamchatka Trench at a depth of 8800–9200 m.

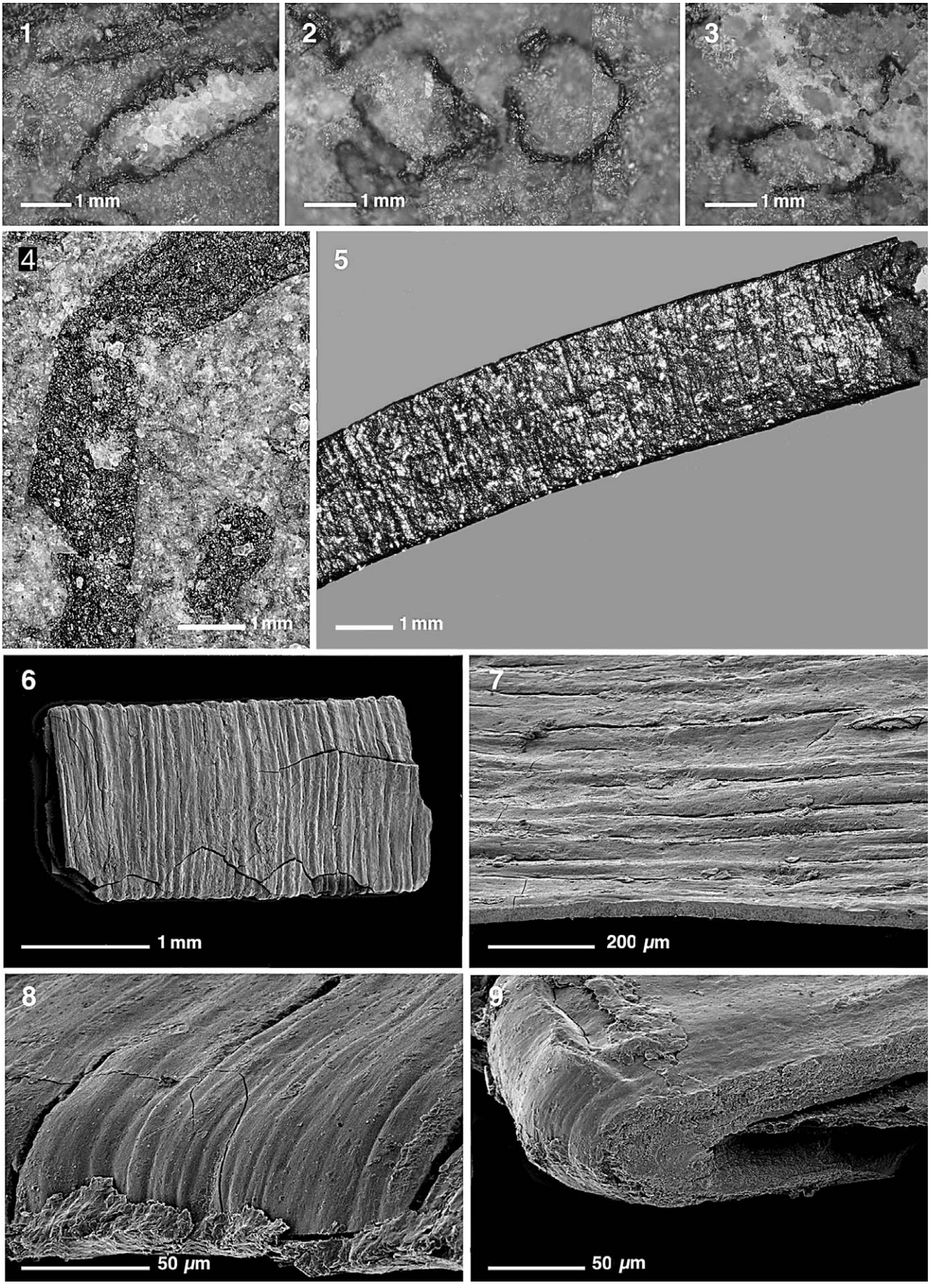
Electron microscopy and EDX analyses were performed at Uppsala University, Evolutionary Biology Centre, and with a WITec alpha 500R confocal Raman spectrometer at Centre de Biophysique Moléculaire CNRS, Orléans. Gas-chromatography-mass-spectrometry was performed at Stanford University, Molecular Organic Geochemistry Program, using a VG Micromass Autospec Q hybrid (magnetic sector-quadrupole) gas chromatography-mass spectrometry system. For this, samples were prepared by pyrolysis at 310°C for 72 hours in sealed Pyrex tubes. The pyrolysates were extracted by sonication using methylene chloride, filtered through a Sep-Pak using hexane solvent to remove polar materials, and analyzed by GC-MS-MS. All analyses followed the standard analytical procedures at the respective laboratories (Peters and Moldowan, 1993, p. 54–58; Peters et al., 2005; Westall et al., 2006).

The specimens illustrated are housed in the collections of the Museum of Evolution, Palaeontological Section, at Uppsala University (ME-Pl-1-30).

PRESERVATION AND BURIAL CONDITIONS

The organic matter of the *Sabellidites* tube is resistant to water, mineral solutions penetrating the host sediment during burial, processes of lithogenesis, and inorganic acids subsequently used in laboratory preparation. *Sabellidites* specimens are preserved three-dimensionally (Fig. 1.2), partially flattened with the tube lumen open (Figs. 1.3, 1.9, 2.1), or compressed (Fig. 2.3). They were originally soft and plastic as has been demonstrated by their post-mortem ductile deformation and the occasional preservation of twisted tubes without breakage. The tubes were sufficiently robust and thick-walled (Fig. 1.7) to be preserved in such a way and then extracted from the sediment without disintegrating.

The host pyrite-bearing argillitic shale and siltstone belong to the Ediacaran–Cambrian succession on the EEP, which was deposited in the epicontinental basin that extends over a large area between the Baltic, White, and Black seas. The strata lie almost horizontally and are free of any tectonic deformation, with the exception of block faulting and gentle tilting, or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metamorphism. The Ediacaran sediments are exclusively detrital: arkosic at the base (unfossiliferous) and then fine-grained siliciclastic. The latter comprises a variety of fossils, notably Ediacaran soft-bodied and trace fossils, and organically preserved *Sabellidites* and microfossils (Sokolov and Iwanowski, 1990). Diverse microfossils include microalgae (acritarchs), cyanobacteria and vendotaenids, and some of uncertain affinities, such as *Valkyria* and *Ceratophyton* (Gnilovskaya, 1990; Moczydłowska, 1991, 2008b). The last taxon could be a fragment of metazoan cuticle and superficially resembles teeth of scalidophoran priapulids (Harvey et al., 2012).

The Ediacaran sediments in the western part of the EEP, including the area studied, are in general richly organic, with TOC values 1.0–2.5 mg/g, and H/C ratio ranging from 0.24–0.57 (Strauss et al., 1997). The microfossils are translucent and light in the coloration of their organic matter (TAI 1–3, below 100°C), indicating a lack of any significant thermal alteration at the stage of diagenesis (Moczydłowska, 1991). The thermal burial gradient of the area has not exceeded the thermal alteration of 70°C (Felitsyn and Pshenitchnova, 1992). Indeed, this is also demonstrated by the Raman spectra taken at different places on the *S. cambriensis* wall (Fig. 6), in which the index of preservation of 9 indicates little metamorphic alteration (cf. Schopf et al., 2005). Fossils accumulated and were preserved in natural taphocoenoses, planktic settled from the water column and benthic in situ, or without any recognizable transport by bottom currents.

The succession studied consists of dark, richly organic, finely laminated shale that is soft and weakly lithified, and mudstone and siltstone, the diagenetic alteration of which is insignificant. They accumulated on the marine shelf below the wave-base level, in a restricted basin with low energy and low circulation (Rozanov and Łydka, 1987; Sokolov and Fedonkin, 1990; Felitsyn et al., 1998). The processes leading to lithification and the early stage of diagenesis of mudrock, from which the *Sabellidites* specimens were isolated, involved migration of silica and phosphate solutions, emplacement of detrital illite, and closed-system authigenic illite crystallisation (lath-like particles) at ca. 533±8 Ma. Among clay minerals, illite dominates in the matrix of sediments. The sedimentary organic matter (extracted microfossils, *Sabellidtes* tubes, and particulate organic matter) and the host shale matrix have a high metallic content, i.e., Co, Au, Ni, Zn, and negative Ce anomaly (Felitsyn et al., 1998). The enrichment by metals has been linked to the excessive blooms of cyanobacteria, vendotaenids, and other microbial organisms, and the bonding of metals in the sedimentary organic matter as a result of bacterial decay. Microbiota thrived in these stagnant, shallow marine environments, as is evident from the fossil record. In some instances, *Sabellidites* tubes were embedded in sapropelic films and occurred on bedding planes with an accumulation of carbonaceous ribbons of vendotaenids. The positive Ce anomaly in the benthic *Sabellidites* tubes, together with their exceptional preservation and abundance of sapropelic films, indicate anaerobic conditions during their accumulation and burial (Felitsyn et al., 1998).

The *Sabellidites* organic body is preserved without permineralization. Minerals have not replicated any part of the soft tissue and the carbonaceous material of the wall is primary, preserving the original layering of the wall, its texture, and fabrics. Moreover, the fabrics, observed in STEM/SEM/TEM (see below), are fibers composed of resilient organic matter embedded in a less resilient organic matrix (Fig. 2.2) that may be dissolved thereby exposing fibers on fractured surfaces (Fig. 2.3–2.6). The organic matter in the tube lumen or on the fractured surfaces is occasionally encrusted by pyrite microcrystals as well as some authigenic clay particles (illite) and trapped allochthonous detrital quartz micro-grains.

Pyrite is embedded in the *Sabellidites* wall, concurrent organic-walled microfossils, and is present in the sediment, but not excessively. Authigenic pyrite is in the form of euhedral crystals (~2–4 μm) and framboids (~10 μm), which sporadically line the fractures in the *Sabellidites* wall or occur in small pockets on the surface. Biogenically mediated pyrite precipitation is a common occurrence in the process of organic matter decomposition, when anaerobic, sulphate-reducing bacteria digest the organic matter and release hydrogen sulphide (H_2S), which combines with iron ions to produce pyrite (Cohen, 1984; Briggs, 2003). Pyrite, as a by-product of bacterial decay, provides evidence of anoxic conditions and facilitates the preservation of soft-bodied organisms by producing a microbial mat "death mask" of the specimen (Gehling, 1999; Gehling et al., 2005), as is the case with many Ediacaran biotas (Laflamme et al., 2011). However, neither pyrite nor phosphate, which occasionally encrusts the *Sabellidites* tube walls, has replicated fossil morphology. Emplacement of authigenic illite lathes along the taphonomically delaminated walls form linings in some poorly preserved specimens.

In the case of *Sabellidites*, the mode of preservation as a three-dimensional, robust organic body differs from Ediacara- or Doushantuo-type fossils, which are preserved as imprints, compressions, or are phosphatized, silicified or both, or from Cambrian Burgess-Shale-type (BST) fossils, preserved as carbonaceous compressions or biofilms ("shadow fossils"), which have no possibility being released from the sediment. Moreover, biofilms of organic matter mimicking organisms shape cannot even be distinguished from the matrix by EDX analyses or SEM imaging.

The quality of Ediacaran fossil preservation resulted from exceptional conditions, although these varied regionally (Laflamme et al., 2012). In the Flinders Ranges of South Australia and in Central Australia, impressions of soft-bodied organisms were protected by microbial mats ("death masks"; Gehling, 1999; Gehling et al., 2005), and their preservation was enhanced by adhesion of clay and silt to those mats (Mapstone and McIlroy, 2006). In Newfoundland such fossils were entombed in volcanic ashes (Narbonne, 2005; Laflamme et al., 2007; Gehling and Narbonne, 2007), and also associated with bacterial biofilms or microbial mats with adhered clay-mineral elemental consortia (Gehling et al., 2000; Laflamme et al., 2011). In Siberia, the Ediacaran biota were preserved through authigenic carbonate cementation and occasionally as carbonaceous compressions (Grazhdankin, 2004; Grazhdankin et al., 2008), whereas in

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FIGURE 1—Photomicrographs of the *Sabellidites* tubular body wall. *1–3*, RLM images of specimens embedded in the sediment and exposed on the bedding planes, showing longitudinal section of the tube in *1*, transverse circular section of the three-dimensionally preserved tube in *2*, and transverse section of a flattened specimen in *3*; *4*, DM image of specimen preserved on rock surface, showing body wall with characteristic wrinkles and softly bended; *5*, RLM image of isolated specimen (color background is artificial); *6*, *7*, STEM images of the specimen surface with regular ridges, which are not the segmentation feature, and cracks on the rigid wall in *6*, and its body wall section seen on the tube end in *7*; *8*, *9*, STEM images of a flattened specimen seen from the side and having sediment particles attached to the organic wall in *8*, and the tube end showing the wall thickness and the internal lumen filled in by host sediment in *9*.

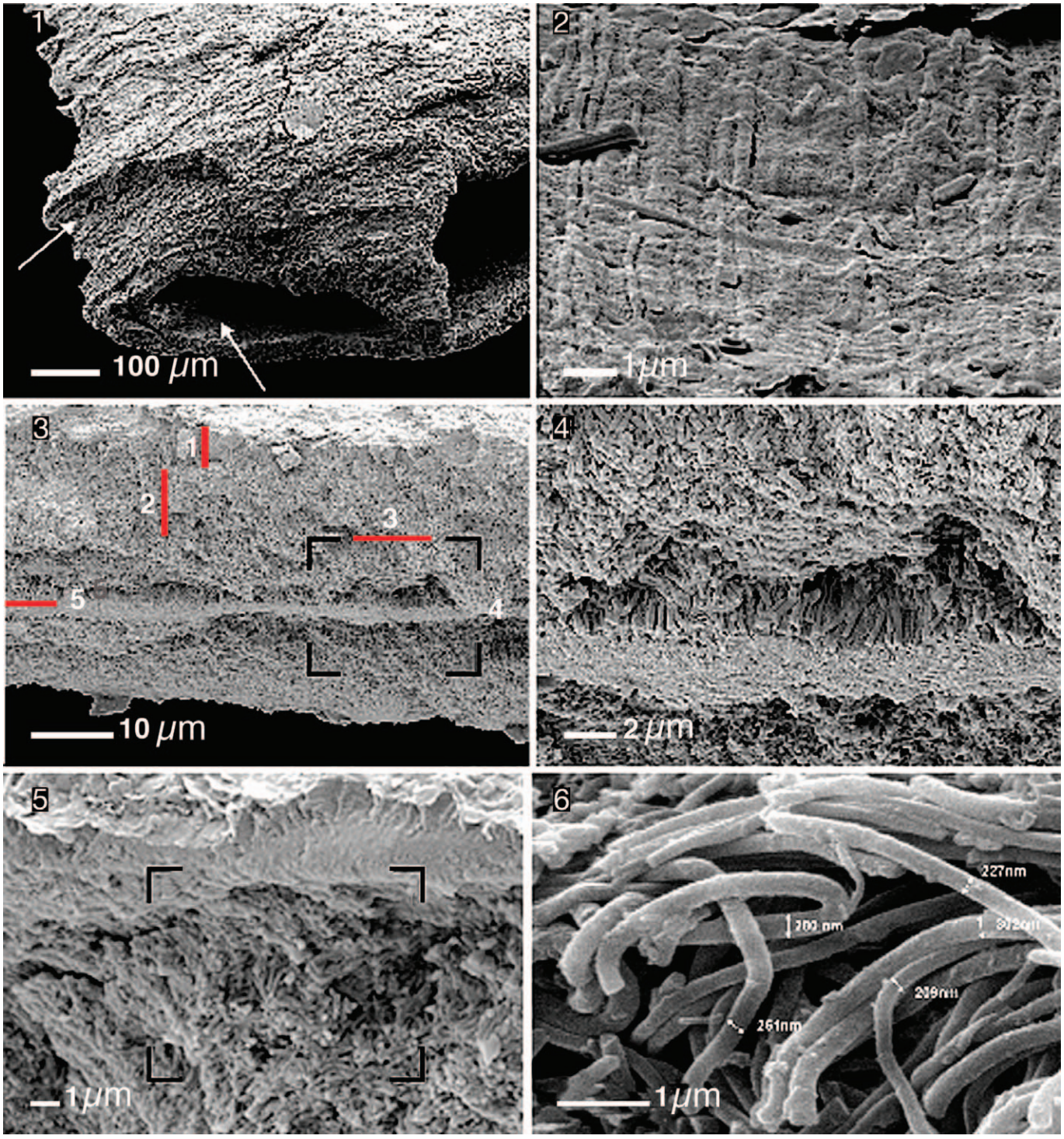


FIGURE 2—Microstructure of the multi-layered body wall with fibrous texture in STEM images. *1*, flattened specimen with empty lumen (right arrow) and partly exfoliated outer layer 2 along the layer 3 (boundary zone; left arrow); the surface of the specimen is formed by the outermost layer 1, and the innermost layer 5 lines the tube lumen; *2*, in situ preserved parallel fibers embedded in amorphous matrix of the internal layer 4 in the same specimen; *3*, cross section of compressed tubular body with layers seen in a sequence from the surface (image upper edge) down to compressed lumen in the middle of the image and marked by red lines and numerals; the outermost layer 1 is homogeneous and thin; outer layer 2 has fibrous texture; layer 3 (or boundary zone) is thin and defines the change in fibers orientation between layers 2 and 4; inner layer 4 has fibrous texture and extends within the framed area on both sides of the compressed lumen; layer 5 is compact and homogeneous, lining the tube lumen; *4*, enlargement of inner layer 4 (framed in *3*) showing parallel fibers aligned along the long tube axis; *5*, the textured outer layer 2 (position in wall is framed) underlies the surface homogeneous layer 1 at the top of image; *6*, individual fibers in another fragment of layer 2 released from the matrix by etching and distorted, and their width is measured at marked positions.

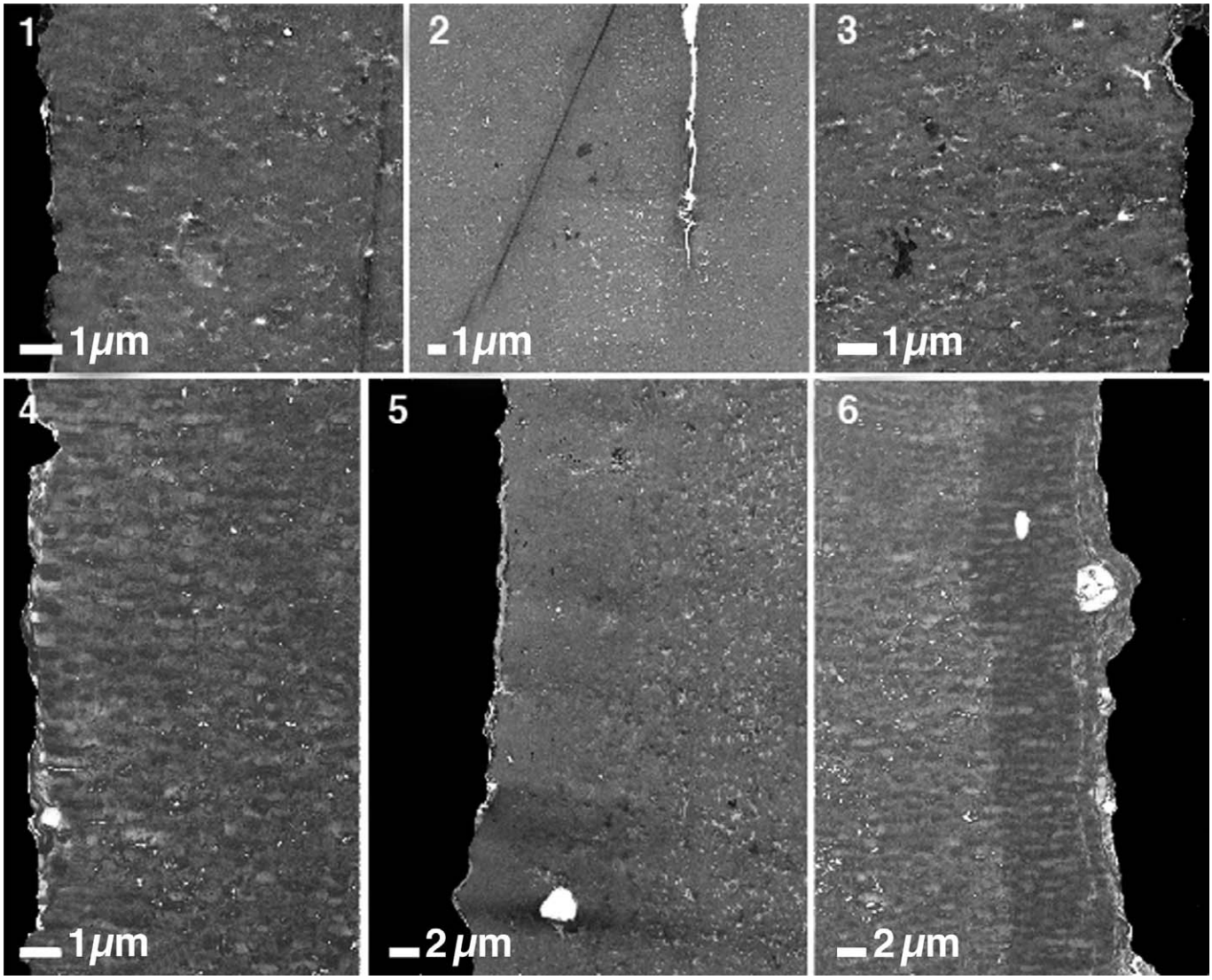


FIGURE 3—TEM images showing the vesicular texture of the organic body wall in transverse thin sections in *1–3* of a flattened tube, and longitudinal thin sections in *4–6* through the outer textured layer 2 and inner layer 4 of the same specimen. Lumen of the tubular body is seen as a white slit in *2*. *1–3*, sectioned fibers appear as vesicles that are aligned in a linear pattern in parallel rows across the wall thickness and are elongated in the outer layer 2 in *1* and *3*, and circular in the inner layer 4 in *2*; *4–6*, sectioned fibers of the outer layer 2 are elongated on the sides of the ultramicrotome thin sections in *4* and *6*, and are circular in the central part of the ultramicrotome thin section in 5. The orientation of the ultramicrotome thin sections is indicated in Figure *4*.

China it was brought about by phosphate and silica permineralization (Xiao and Knoll, 2000) and as carbonaceous compressions and biofilms (Xiao et al., 2002; Zhu et al., 2008; Tang et al., 2011). Exceptional preservation includes also organic tubular fossils (*Conotubus*) preserved as carbonaceous or glauconite impressions or by pyritization (Hua et al., 2007). In Namibia and the White Sea coast of the EEP, siliciclastic sediments preserved the shape of fossils by early mineralization and cementation (Narbonne et al., 1997; Noffke et al., 2002; Grazhdankin and Seilacher, 2002; Grazhdankin, 2004; Narbonne et al., 2009). However, organic preservation of *Sabellidites*-like tubes has been also reported in the White Sea area (Grazhdankin, 2004). Both types of preservation, by imprints and as organic-bodies, are known in the western part of the EEP, where siliciclastic sediments comprise imprints of the Ediacara-type fossils (e.g., *Dickinsonia* and *Tribrachidium* in the Ukraine) and organically preserved *Sabellidites*, studied here from Lithuania and Russia, and also recorded in Poland (Fedonkin, 1990, 1994; Moczydłowska, 1991; Felitsyn et al., 1998; Fedonkin et al., 2007). The other site of the Ediacaran biota in the Baltica paleocontinent regionally closest to the White Sea area is in Finnmark, northern Norway, which has an assemblage of discoid imprints within the sub-tidal siliciclastic succession that is taxonomically similar to the Avalon, NW Canada, and northern Siberia assemblages (Farmer et al., 1992; Narbonne, 2005). No evidence of microbial association or coating has been observed at this site, which may be due to the subsequent Caledonian tectonic activity causing thermal alteration and strong cleavage. Nevertheless, higher up in the succession in the basal Cambrian, the record of organic fossils includes vendotaenids, acritarchs, and *Sabellidites* sp. (Farmer et al., 1992).

For comparison, the best studied Cambrian BST of preservation required inhibition of the normal processes of decay and oxidation, and yet the organic matter is altered (Butterfield, 1995; Butterfield et al., 2007; Page et al., 2008; Gaines et al., 2008; Butterfield and Harvey, 2012). BST organic preservation is due to the authigenic mineralization by phyllosilicates related

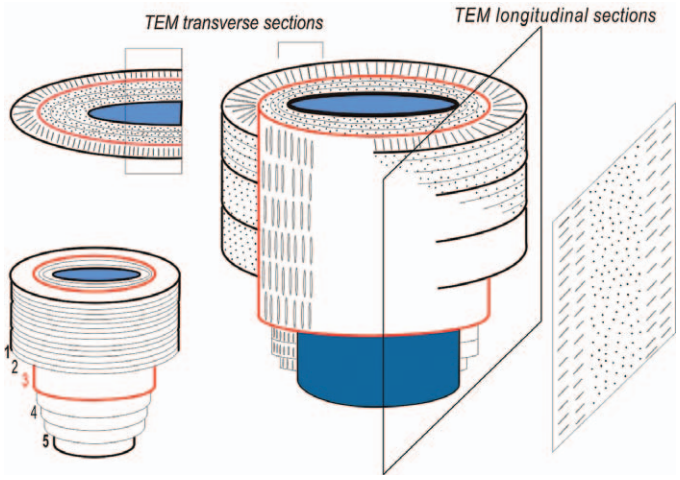


FIGURE 4—The three-dimensional reconstruction of the microstructure of the tube wall in *Sabellidites cambriensis* based on STEM/TEM observations. Orientation of the ultramicrotome thin sections for TEM is shown. The wall is inferred to be composed of the chitin fibers, which are parallel aligned in an amorphous proteinaceous matrix within sheets, and then sheets within layers. Different orientation of the fibers in relation to the long axis of the tube in layers 2 and 4 is demonstrated by a change of the vesicular texture. The numerals 1–5 indicate layers; blue color marks the tube lumen, and red color a change in the fiber orientation in layer 3 (or boundary zone).

to metamorphism (forming "clay templates") (Orr, 1998). The clay-organic interactions (Butterfield, 1990; Butterfield et al., 2007), adsorption of Fe^{+3} onto biopolymers (Petrovich, 2001), or reduced flux of oxidants into sediment after deposition prevented microbial decomposition (Gaines et al., 2008). Although the fossils are primarily organic, their preservation is facilitated by authigenic mineralization. The exceptional preservation of the Chengjiang and the Kaili biotas is either by carbonaceous films alone or with early authigenic mineralization (Zhao et al., 1996, 2008; Chen and Zhou, 1997; Zhang et al., 2008; Hou et al., 2004; Harvey et al., 2012).

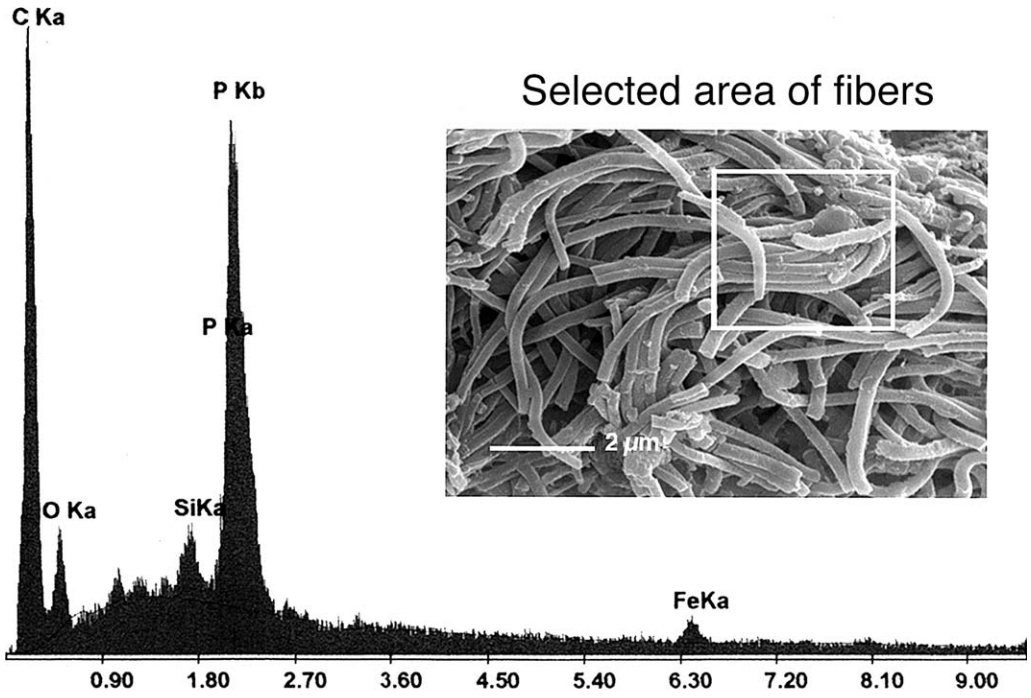
In contrast to macrofossils, the unprecedented record of microscopic metazoan embryos in different developmental stages derives from the middle Cambrian Gaotai Formation. The detailed preservation of cells and soft fertilization envelopes occurred by authigenic apatite permineralization under specific environmental conditions, including elevated phosphorus content in the seawater, poor circulation of currents, and restricted bioturbation (Zhang and Pratt, 1994; Zhang et al., 2011). Subsequently recorded Ediacaran metazoan egg cysts and embryos from the Doushantuo Formation are phosphatized and silicified (Xiao et al., 1998; Xiao and Knoll, 2000; Yin, C.Y. et al., 2004; Yin, L. et al., 2007; Xiao et al., 2014). Although extracted from the rock matrix as microfossils by the palynological method (Xiao et al., 2014), their mode of preservation does not provide any information about the properties of the organic matter with respect to its resistance because they have been replicated by phosphate. In all cases of embryos studied, both Ediacaran and Cambrian, the phosphatic preservation by amorphous and finely crystalline apatite may have involved microbial processes (Zhang et al., 2011).

Primarily, the morphologic features and organic matter properties of organisms, and their ecologic adaptations and life habitats, were decisive factors in their preservation potential. The environmental and burial conditions caused the selective preservation of the Ediacaran biotas in various settings. Taphonomic windows of entombment in volcanic ashes, authigenic carbonate cementation, permineralization, microbial mat "death mask", and biofilms associated with clay mineral templates, were distinguished and thought to facilitate the preservation of soft-bodied metazoans. The ecologically and environmentally-biased preservation is observed more clearly in the taxonomic composition of the assemblages than the biostratigraphic control. However, studies of a great variety of Ediacaran fossil sites convincingly show that bacteria and other microbial mat-builders (cyanobacteria; Steiner and Reitner, 2001) were vital in sealing and preserving the organic bodies in first instance, even though might have been subsequently mineralized. Bacteria contributed to creating the anaerobic conditions at the water–sediment interface by causing the organic matter to decay and releasing H_2S. Preservation by carbonaceous compressions or biofilms occurs in all regional Ediacaran assemblages (with the exception of the Nama assemblage; Narbonne, 2005), regardless of whether they are the major components or a few additions to the assemblages. The Nama-type preservation does not show evidence of microbial coating, yet a microbial texture of sediment is present (Gehling et al., 2000; Noffke et al., 2002). Because they were trapped within a covering biofilms and microbial mats, which additionally limited oxygen influx, produced H_2S, and precipitated iron sulphides (Gehling et al., 2005; Droser et al., 2006), the soft-bodied Ediacaran organisms were more readily preserved (Laflamme et al., 2011).

The role of bacteria in preserving the signs of biogenicity and microbial life in a deep geologic history (Westall et al., 2000, 2006; Noffke et al., 2003; Westall and Southham, 2006; Noffke, 2009), as well as multicellular soft-bodied organisms later on (Gehling, 1999; Toporski et al., 2002; Briggs, 2003; Raff et al., 2008) is recognized. Biologically induced diagenesis within bacterial mats (Jørgensen and Cohen, 1977) leads to their lithification (Des Marais et al., 1992). Bacteria secrete extracellular polymeric substances (EPS), which form slime sheets and biofilms that coat microbial colonies and the substrate. EPS and biofilms are preservable because of the binding of metals and functional groups in EPS and mineral particles thus inducing mineralization (Pierson et al., 1992; Westall et al., 2000). Biofilms may extend over large surfaces. They store nutrients, protect and maintain ecologic microcosm, stabilize the substrate thus allowing buildups of microbial mats, and biogenically mediate permineralization and fossilization (Westall et al., 2000). Bacteria and microorganisms reproduce within hours, and thus biofilms are formed rapidly on the sediment surface in natural environments. The result is, as laboratory experiments have shown, that the process of fossilization of biofilms begins equally rapidly, also taking place within hours (Toporski et al., 2002; Westall and Southham, 2006).

There is a paradox that EPS can be fossilized more easily than individual microorganisms, largely due to the presence of adsorbed metals and an abundance of functional groups. This explains the abundance of stromatolites, which are mineralized by the associated EPS, and the corresponding rarity of preserving the microorganisms that comprised the biofilm (Westall and Southham, 2006). By the same mechanism, EPS and the microbial "death mask" conserved the shape and carbonaceous compressions of large Ediacaran organisms, but not their bodies in full. The exception is *Sabellidites*.

Thus we see that the three-dimensional organic preservation of *Sabellidites* is due to its robustness and its property as an organic material of biochemical resistance, as well as burial under anaerobic conditions at the water-sediment interface that was created by the bacterial decay of mass growth and the



Element	Wt%	At%
C K	78.58	85.86
O K	13.86	11.37
SiK	1.64	0.77
P K	3.27	1.39
FeK	2.65	0.62
Total	100.00	100.00

Element	Net Inte	Bkgd Inte	Inte Error	Pb
C K	137.87	1.52	0.93	90.48
O k	20.79	7.32	2.78	2.84
SiK	17.68	16.19	3.59	1.09
P K	32.31	16.18	2.35	2.00
FeK	8.27	5.17	4.83	1.60

FIGURE 5—SEM/EDX elemental composition of the organic fibers in the *Sabellidites* body wall.

accumulation of microbial organisms and biofilms in a restricted, shallow marine basin below the wave-base level.

ORGANIC WALL MICROSTRUCTURE

The wall microstructure, as revealed by the SEM/STEM/TEM observations, consists of five layers, which are differentiated based on the texture of the organic matter and the thickness of the layers (Figs. 1–3). The texture is due to the spatial arrangement and orientation of the fibers (fabric) and infilling amorphous matter (matrix). The orientation of the nanometer-sized fibers changes between the layers (Fig. 2). The wall is delimited by two thin (1.6–7.7 micrometer, μm) and sharply defined layers, an outermost layer 1 and an innermost layer 5, which are made of compact, homogeneous and electron-dense organic matter (Figs. 1.9, 2.3, 2.5). A thick portion of the wall (20.0–38.0 μm) made of a less electron-dense organic matter with a clear texture extends between these layers (Fig. 2.3–2.5). This portion is divided into two layers, an outer layer 2 and an inner layer 4, with fibrous textures that are separated by a 2.0–3.0 μm thick layer 3 (or boundary zone) marking the change of the fabric orientation between layers 2 and 4 (Fig. 2.1, 2.3, 2.5). Layer 2 is composed of a fibrous fabric embedded in an amorphous matrix and is ~10.0–23.0 μm thick. Individual fibers are exposed on sections of fractured walls (Fig. 2.5, 2.6). The in situ orientation of the fibers is seen in TEM thin sections (Fig. 3.1, 3.3, 3.4, 3.6). The fibers are long, homomorphic, and perfectly shaped (meaning without any defects in shape or change in size of individual fibers), with a consistent diameter of a fiber ranging between 219 and 304 nm (n=7). Although the diameter of only a few fibers has been measured, their constant size amongst a mass of fibers is apparent (Fig. 2.2, 2.4, 2.6). Layer 4 is 10.0–15.0 μm thick and composed of tightly packed parallel fibers, which are orientated along the tube long axis of the tube (Fig. 2.3, 2.4). The fibers range in diameter from 209 to 324 nm (n=14). Observed in a few sites to be preserved intact, they are embedded in an amorphous organic matrix and appear to form sheets (planes) (Fig. 2.2).

For TEM observations, the ultramicrotome thin sections were cut transverse and longitudinal to the long axis of the tube wall (Figs. 3, 4). The fibers in layers 2 and 4 produce in two-dimensional section an apparent vesicular texture, interpreted to be cross sections of individual fibers, which were seen using SEM/STEM. The shapes of the elongated to circular vesicles embedded in an amorphous matrix (Fig. 3) change depending on the orientation of the fibers within the wall. The vesicles are densely packed and aligned in a linear pattern in parallel rows. The vesicular texture and alignment patterns indicate that the fibers are orientated radially to the long axis of the tube in the

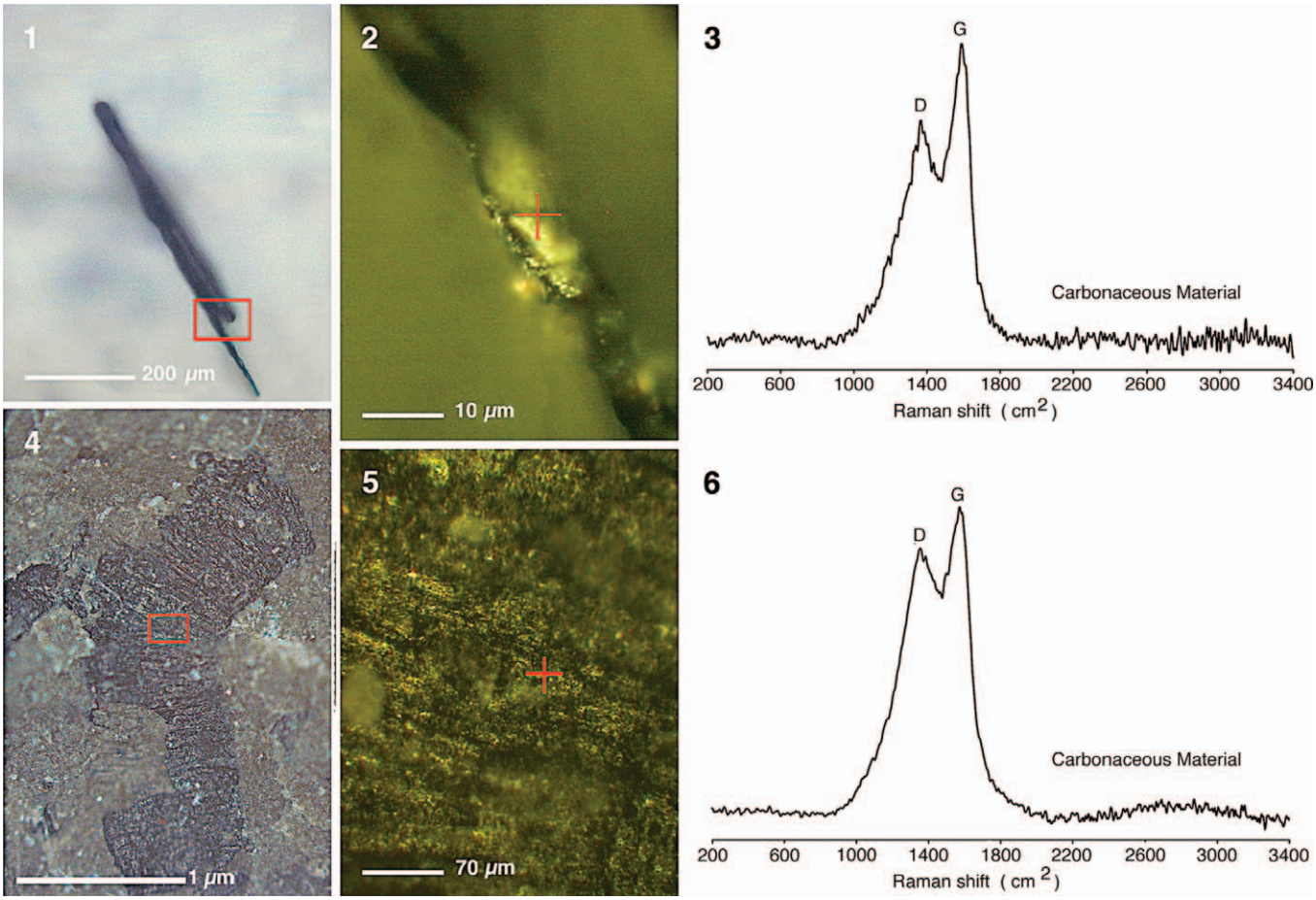


FIGURE 6—The laser-confocal Raman spectra of the *Sabellidites* body wall. Single spectra were probed on the cross section on the wall in *1–3*, and on the surface in *4–6*, identifying the wall material as carbonaceous. Positions of the probed sites are marked by red boxes and their enlargements by red crosses in *1*, *2*, and respectively in *4*, *5*. In the spectra, the prominent vibrational peaks at ~1360 cm^{-1} (the D disordered band) and ~1580 cm^{-1} (the G graphitic band) indicate a very low degree of maturation of organic matter.

layer 2 and vertically in layer 4. The fibers are embedded in amorphous matter and are inferred to form thin, ring-shaped sheets stacked horizontally upon one another in layer 2 (Fig. 4). In layer 4, the sheets are cylindrical and arranged concentrically (tube-in-tube) (Fig. 4).

The fibers and their spatial arrangement patterns in *S. cambriensis* are comparable to those observed in the fibrous walls of extant siboglinids (pogonophoran and vestimentiferan annelids). The fibers of the latter are chitinous in composition and embedded in an amorphous proteinaceous matrix (Urbanek and Mierzejewska, 1983; Gaill et al., 1992a, 1992b; Shillito et al., 1993, 1995). The set of microstructure features, i.e., the shape of the fibers, their alignment within the sheets and layers, and the arrangement of the layers, is characteristic of siboglinids and similar architecture is interpreted here in *S. cambriensis*.

Apart from the microstructure, there is a striking similarity between biogeochemical properties of the organic material and preservation mode in the tube of *S. cambriensis* and that of extant siboglinids. Although textural pattern in the chitin fibers and their layering in a proteinaceous matrix is a generalized feature in metazoans and it is well recognized in arthropod cuticles (Briggs, 2003; Raabe et al., 2005a, 2005b), differences in these proportions may be used to distinguish certain groups. This pattern reflects the habit of the chitin crystallites, the process of their synthesis and assembling into fibers, and their microstructure in the amorphous organic material embedding these fibers, which differs between taxa (Shillito et al. 1995; Raabe et al., 2005a). Knowing the pattern may be helpful for recognizing, in the first instance, the presence of chitin in fossils and perhaps even the affinity of the organisms.

ORGANIC MATTER PROPERTIES AND BIOGEOCHEMISTRY

The organic matter of the *Sabellidites cambriensis* tube is resistant to water, mineral solutions, processes of burial and diagenesis, and to the inorganic acids and solvents applied during preparation. The specimens survived the hydrochloric (HCl), hydrofluoric (HF) and nitric acid (HNO_3) baths, and brief oxidation by the Schultze solution and hydrogen peroxide (H_2O_2), whereas burning of the isolated specimens resulted in "ashes" remaining in the shape of the tube (Yanishevsky, 1926; Ivantsov, 1990; this study). The laboratory chemical treatment indicated that the fibers, which were partly etched from the embedding amorphous matrix, were made of resistant biopolymers.

The substance of the *S. cambriensis* wall is recognized as predominantly carbonaceous using SEM/EDX elemental composition analyses (Fig. 5). The EDX spectra showed the following elemental contents in percentage of the total weight (wt%): C, 70.33–78.58 percent; O, 13.86–24.67 percent; S, 2.78 percent; Fe, 1.40–2.65 percent; Si, 1.24–1.64 percent; in addition, subsidiary amounts of Na (0.68–1.53%), Mg (0.81%), Al (0.92%), K (0.42%), and Ca (0.32%) (n=2). The

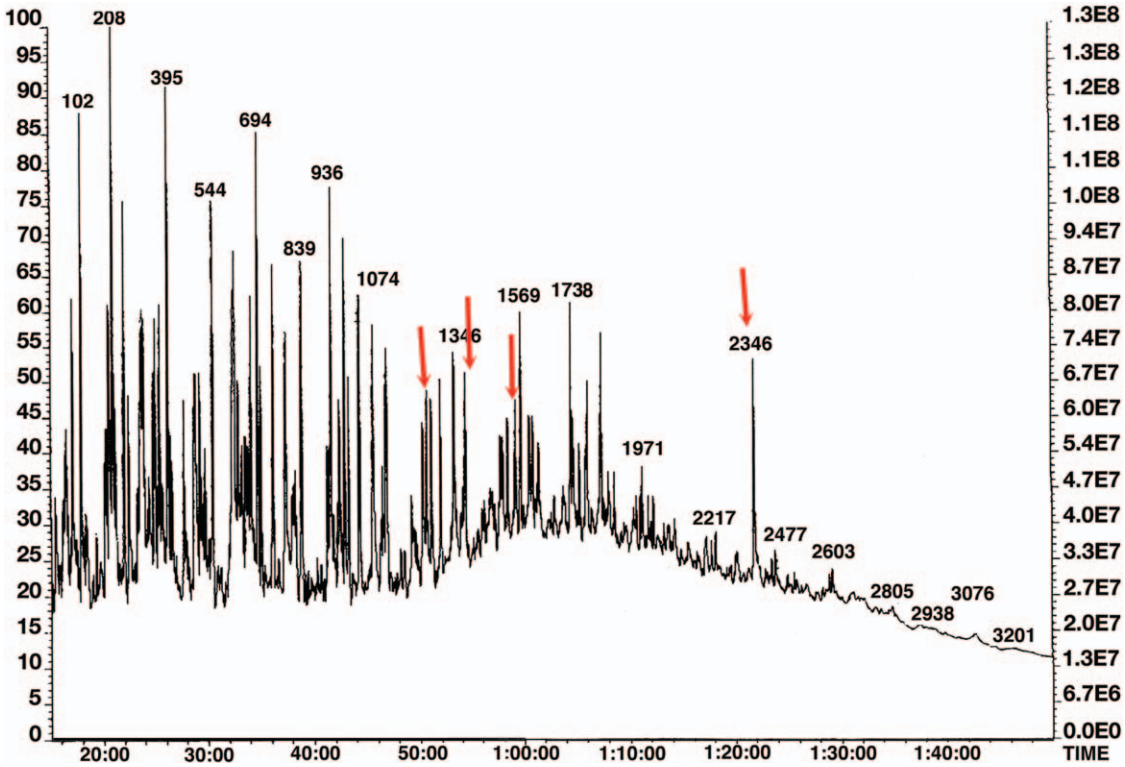


FIGURE 7—Total ion current (TIC) chromatogram and mass spectra of four compounds (4 peaks in rock kerogen marked by red arrows) indicative of the molecules containing a normal or branched acyclic saturated hydrocarbon chain.

carbonaceous material is defined by high concentration of carbon. The Raman single spectra of the *S. cambriensis* wall, which were probed in five spot sites on the specimen surface and thirteen spot sites on a cross-section of the wall, identified the wall material as carbonaceous (Fig. 6). The spectrum curves were very consistent and almost identical with the disordered band (D) peak at ~1360 cm^{-1} and the graphitic band (G) peak at ~1580 cm^{-1}, which indicate a very low degree of alteration. Raman spectrum of carbonaceous matter can be used to estimate the maturation stage in particular conditions. Schopf et al. (2005) defined the Raman Index of Preservation (RIP) based on studies of a series of samples containing fossilized carbonaceous matter of biological origin that have undergone thermal alteration at different metamorphic grades. They calibrated the RIP values corresponding to the ratio of the areas under the D peak in the 1100–1300 cm^{-1} region and the 1300–1370 cm^{-1} region on a scale ranging from 1 for the most altered to 9 for the least altered kerogen studied. Based on this method, we were able to estimate a RIP of about 9 in accordance with a very low grade of alteration.

Molecular composition was analyzed by py-GC-MS on the *S. cambriensis* specimens, the kerogen extract from the containing sediment, and the extant siboglinid *Zenkevitchiana* species. The total ion current chromatogram of all the samples (Fig. 7) indicates a possibly homologous series, or branched chain compounds, with a possibility that some of them may contain nitrogen. This series is either the dominant series, or in the case of the *Sabellidites* kerogen, sub-dominant to a series of *n*-alkanes. Mass spectra of all compounds are indicative of molecules containing a normal or branched acyclic saturated hydrocarbon chain. This compound series has rarely been reported and may derive from the fossils themselves or from some dietary component or symbiont. Biomarker fingerprints of terpanes (m/z 191) were similar between the extant siboglinid worm and the fossil *Sabellidites* showing a display of 17*a*-hopanes dominated by C_{29}, C_{30} and extending up to C_{35}. C_{27} hopanes were also present. Substantial 17, 21*a*(H)-hopanes (moretanes) were present for the C_{29} and C_{30} homologues and 17-trisnorhopane (C_{27}) was of similar importance to 17*a*-hopane (C_{27}, Tm). The kerogen pyrolysate in the sedimentary matrix showed a more substantial relative abundance of C_{27} and C_{29} homologues of 17*a*- hopanes and moretanes.

Hopanes and moretanes undoubtedly have a bacterial origin. The bacterial biomarkers incorporated both in the extant siboglinid and in the fossil *S. cambriensis* are explained by a common presence of chemoautotrophic symbiotic bacteria within the internal tissue and the organic walls. Such bacteria are obligatory symbionts in siboglinids (Felbeck, 1981; Dando et al., 1992) while the animals are alive and additionally post-mortem as saprophytes. The studied *Zenkenvitchiana* was not decayed.

Thus far the py-GC-MS analyses have not identified any conclusive biomarkers neither for *Sabellidites* nor for the extant *Zenkenvitchiana*. More work is needed to isolate and identify these compounds and to determine their significance. However, the demonstration that the same bacterial biomarkers are incorporated into the organic tube of the fossil *Sabellidites* as in the tissue of *Zenkenvitchiana* is consistent with the interpretation of *Sabellidites* as a siboglinid. Siboglinids live in symbiosis with chemoautotrophic sulfide-oxidizing bacteria, which are a source of their nutrition. Such bacteria are shown, by biomarkers, to be present in the examined siboglinid organic body wall, as they were in the animal tissue and tentacles.

The biogeochemical property of resistance alone is meaningful considering the organically preserved metazoan fossils because it points to one particular group of biopolymers. The

resistance of organic matter to acids, and destructive hydrofluoric acid in particular, is typical of various groups of biopolymers, including algaenans, sporopollenin, dinosporin, cellulose, lignin and chitin (Briggs, 1999; Allard and Templier, 2000; Versteegh and Blokker, 2004; Lee, 2008). With the exception of chitin, all of these biomolecules are synthesized by photosynthetic bacteria, algae and vascular plants (Moczydłowska, 2010). Chitin is present in all major eukaryote clades except plants (Ruiz-Herrera et al., 2002) and is commonly produced in both marine and terrestrial animals and certain fungi (Saito et al., 1995; Shillito et al., 1995; Stankiewicz et al., 1996, 1998, 2000; Ehrlich et al., 2007; Webster and Weber, 2007), but it was considered non-preservable under prolonged burial conditions (Briggs, 1999, 2003). True chitin with the longest burial history has been preserved in fossil insects in Tertiary and Pleistocene deposits (Stankiewicz et al., 1997a, 1997b; Briggs, 1999). Mesozoic and Paleozoic arthropods have not revealed any evidence of chitin (Stankiewicz et al., 1998), and other observations have led to the conclusion that chitin is absent from older fossil cuticles because it could not survive degradation beyond early diagenesis (Stankiewicz et al., 2000; Briggs, 2003; Gupta and Briggs, 2011). However, the molecular signature of a relict chitin-protein complex has been detected in Pennsylvanian (310 Ma) and Silurian (417 Ma) arthropod cuticles using X-ray absorption near edge structure (XANES) spectromicroscopy (Cody et al., 2011). New recoveries of organically preserved late Ediacaran and Cambrian metazoan cuticles and small carbonaceous fossils clearly demonstrate biochemical resistance of their organic compounds and preservation potential (Butterfield and Harvey, 2012; Harvey et al., 2012). Among metazoans, we may expect to detect chitin.

Although the biopolymers in the body wall of *S. cambriensis* are difficult to identify beyond their general properties and composition, the presence of chitin is indirectly indicated by positive staining reactions to uracyl acetate, which is characteristic of chitin, during the preparation of thin sections for TEM examination. The microstructure and texture further suggest that the organic fabrics of *S. cambriensis* were derived from a chitin-protein complex.

Chitin is a sugar molecule, an insoluble linear poly-β-(1Õ4)-N-acetylglucosamine biopolymer that occurs in nature in different crystallographic structures. The form α chitin is most abundant and is found in fungal cell walls and arthropods, while β chitin is rare and occurs in lophotrochozoans including siboglinids (both pogonophoran and vestimentiferan). The α chitin is composed of antiparallel arrays of polymer chains, whereas β chitin is a parallel array of chains (crystallites). The chitin chains are aggregated into nanofibrils a few nanometers in diameter and wrapped by proteins, forming complex fibers 50–300 nm in diameter (Blackwell, 1973; Gaill et al., 1992b; Saito et al., 1995; Shillito at al., 1995; Raabe et al., 2005a). The fibers are aggregated crystalline chitin strands and are observable fabrics in organic materials.

In the α chitin arthropod cuticles, the fibers are parallel arranged or woven in sheets (called also planes or thin layers), which are helicoidally stacked one upon the other. Depending on the direction of sectioning of the cuticles, the fibrous sheets will appear in a complex pattern resembling "peacock feather structure", helicoidal sequence, twisted plywood or Bouligand pattern (Raabe et al., 2005a, 2005b). The basic ultrastructural unit is, however, the sheet of fibers encoated by proteins and embedded in protein matrix. The spatial, three-dimensional arrangement of sheets, combined in bundles, thick layers or arches, differs between taxa and is seen in the thin sections in various patterns, reflecting the topological orientation of fibers.

The β chitin molecules are monoclinic (one chain) and synthesized unidirectionally and their concomitant crystallization results in a parallel arrangement of crystalline nanofibrils. The nanofibrils are of high perfection and crystallinity. The β chitin parallelism is a mode of perfect alignment between chains, including even a shared crystal polarity (Gaill et al., 1992b; Saito et al., 1995; Shillito at al., 1995).

The uniformity in shape and size of the rod-like fibers and their perfect parallel alignment inferred to form thin sheets in *S. cambriensis* are comparable to that of β chitin body cuticles and tubes of extant vestimentiferans and pogonophorans (Gaill et al., 1992b; Shillito et al., 1993, 1995). Relying on the specific wall microstructure in details of the nanometer scale, the material is consistent with β chitin.

The properties of the chitin-protein composite material, rigid or flexible, depend on the topological and crystallographic arrangement of the structural constituents, and on possible additional biomineralization (Raabe et al., 2005a). The flexible tubes of siboglinids are constructed of β chitin polar-parallel chains with parallel fiber arrangement. In contrast, the rigid cuticles in arthropods are made of α chitin with polar-antiparallel chains and more complicated, hierarchical structural arrangement of fibers. The tube of *S. cambriensis* was flexible, as shown by its soft deformation and preservation (Figs. 1.4, 6.4), and composed of fibers perfect in habit and parallel arranged in sheets (Fig. 2.2), and then sheets in layers (Fig. 2.3–2.5). The different orientation of fibers in relation to the long axis of the tube is seen in two differentiated layers (Figs. 2–4). These characteristics are consistent with the β chitin tubes of siboglinid animals.

BIOLOGICAL AFFINITY

Comparative morphology and histological and microstructural observations on the *Sabellidites cambriensis* fossil support the interpretation of a siboglinid affinity. The family Siboglinidae belongs to the phylum Annelida (Halanych, 2005; Struck et al., 2011).

Two extant siboglinid taxa, *Zenkevitchiana longissima* and *Siboglinum* sp., alongside with the fossil *Sabellidites* sp., were previously studied using TEM by Urbanek and Mierzejewska (1983). The zooidal tube of *Z. longissima* is made of semi-transparent and flexible organic material; it is thick-walled and segmented with smooth annular portions separated by wrinkled interspaces (Urbanek and Mierzejewska, 1983). TEM images of longitudinal and transverse thin sections of the *Zenkevitchiana* tube wall showed a layered structure of the wall and a texture produced by fibers that were variably orientated and embedded in an amorphous matrix. The fibers and the matrix differed in composition as indicated by chemical reactions with the stains used. The staining by uranyl acetate indicated the crystalline nature of the chitin fibers, which remained un-stained, whereas the reaction of the matrix with lead citrate was indicative of a mucopolysaccharide-protein complex. The dense lines defining the fibers were inferred to contain proteins and carbohydrates in the surface coats of fibers (Urbanek and Mierzejewska, 1983). The same chemical reactions with stains and electron conductivity have been observed in the organic wall fabrics of *Sabellidites cambriensis* studied here.

Individual fibers in *Zenkevitchiana* could not be observed in TEM images as isolated fabric elements by Urbanek and Mierzejewska (1983) but were seen in sections as elongated "scattered vesicles". The "vesicles" were consistently aligned in lines or within thin sheets. However, the three-dimensional

reconstruction of the available sections and the orientation of fibers re-analyzed here suggest that the wall was constructed of thin cylindrical layers containing fibers variously orientated, the arrangement seen in cross section as vertical chevron-like patterns. The aligned vesicular texture in the species of *Zenkevitchiana* was similar to that seen here in *Sabellidites cambriensis*.

In the zooidal tube of *Siboglinum* sp., the wall is differentiated between an inner and a medial part as shown by the texture, which appears as a pattern of "disordered electron dense and lucent spots and lines" (Urbanek and Mierzejewska, 1983). To us, it seems to show a different texture between the wall layers. The two textured layers with variably orientated fabrics within thin sheets in *Siboglinum* sp. were similar to those recognized in *Sabellidites cambriensis* herein.

In his research on the microstructure of the *S. cambriensis* wall by SEM, Ivantsov (1990) provided convincing evidence of the nanometer-scale filamentous texture of organic matter in multi-layered walls. Because his observations were exclusively on the surface of the wall, he could not compare them with TEM observations by Urbanek and Mierzejewska (1983), and thus he stated that the new microstructural evidence was not conclusive of the biological affinity of the fossil taxon. However, in agreement with our new evidence, the thin compact outmost and innermost layers distinguished in sections of Ivantsov were equivalent to homogeneous layers 1 and 5 in the present study (Fig. 4). The two filamentous layers were equivalent to layers 2 and 4, and the "weak connection" between them is referred here as layer 3 or a boundary zone, which indicated a discontinuity of the texture.

The extant vestimentiferan taxa, species of *Riftia* and *Tevnia*, were examined by SEM, TEM and Fourier Transform Infrared Spectroscopy by Gaill et al. (1992a, 1992b) and Shillito et al. (1993, 1995). Their detail insights into microstructure of the tube wall allow us to recognize similar features in the fossil and to propose a common affinity.

The vestimentiferan tube (species of *Riftia* and *Tevnia*) is multi-layered and consists of concentric sheets of organic material composed of β chitin fibers embedded in a proteinaceous matrix (Gaill and Hunt, 1986; Gaill et al., 1992a, b; Gardiner and Jones, 1993; Shillito et al., 1993, 1995). The tube layers are composed of a criss-crossed (herring-bone or plywood structure) arrangement of thin flat sheets, which are made of parallel chitin fibers embedded in a protein matrix and the fibers are single β chitin crystals (Gaill et al., 1992b). The fibers appear as dense rods and are assembled by individual chitin polymer chains (crystallites) (Shillito et al., 1995). In β chitin form, the arrays of polymer chains are parallel to the longitudinal axis of the fibers and could be single crystals (Gaill et al., 1992a; Roberts, 1992; Shillito et al., 1993, 1995). The individual fibers seen in a transverse section are circular, in a longitudinal section are long rods, and in oblique section are long vesicles (Shillito et al., 1995). This is similarly observed in TEM sections of the *Sabellidites* tube wall.

In sum, it is a characteristic feature of the extant siboglinid (both pogonophoran and vestimentiferan) tube to have multi-layered structure of the wall, in which the layers are made of superimposed sheets of chitin fibers embedded in a protein matrix. The microstructure is organized in highly hierarchical system. The fibers are parallel in sheets but sheets are stacked upon one another in a parallel or rotated way resulting either in parallel or plywood pattern of fiber appearance in various sections of the wall.

Based on the above comparative studies, the fossil *S. cambriensis* is interpreted to be a siboglinid, and perhaps a vestimentiferan, annelid worm.

TIMING PHYLOGENETIC DIVERGENCE

Implications of the siboglinid affinity of *Sabellidites*, if accepting the present inference, and its first appearance datum in the terminal Ediacaran are significant for metazoan origins and timing of their divergences. The current interpretation of metazoan phylogeny is that almost all metazoan phyla appeared rapidly in a relatively short time interval at the base of Cambrian, although it may be that this is simply the record of clades that acquired a skeleton (Budd, 2008; Erwin et al., 2011). Molecular clock estimates suggest that the earliest diversifications of animals occurred during the Cryogenian Period (ca. 850–635 Ma) (Blair, 2009) and that the stem lineages of most extant phyla and crown-groups of demosponges and cnidarians arose by the end of the Ediacaran, whereas the crown-groups of other phyla appeared only at the beginning of Cambrian or, in case of Annelida, at the base of Ordovician (Erwin et al., 2011). Proposed late divergence coincides with the Cambrian Explosion (Erwin and Valentine, 2013), whereas the alternative hypothesis posits a long cryptic evolution undocumented by fossils, and a molecular estimate of Annelida divergence at ca. 698 Ma (Blair, 2009). The latter, even broad, estimate would be more feasible because allows a substantial time interval for early annelid evolution to produce the crown-group siboglinids by ca. 550 Ma, if the present interpretation of *Sabellidites* affinity is confirmed.

Sabellidites is not the only late Ediacaran taxon of such inferred affinity. *Conotubus hemiannulatus* (ca. 551–541 Ma) from southern Shaanxi, China, is a tubular non-biomineralized metazoan of unresolved phylogenetic affinity, but similar to modern annelids and members of the family Siboglinidae (Cai et al., 2011). *Conotubus* is an organic relative of the biomineralized *Cloudina* and is probably its phylogenetic precursor, suggesting that an organic skeleton preceded the rise of a mineralized skeleton (Hua et al., 2007).

The supposed origin of the vestimentiferan (=siboglinid) tube worms based on molecular divergence is estimated at 126 Ma (Chevaldonné et al., 2002), but this calibration is inconsistent with a previous fossil record ranging in age from the Devonian to the Miocene (Himmler et al., 2008).

Extant vestimentiferan tubes (Siboglinidae) of *Lamellibrachia luymesi* (from the Gulf of Mexico methane seeps associated with carbonates) and *Escarpia southwardae* (West African Coast), studied in petrographic thin sections by field emission scanning microscope and EDX demonstrate an organic-wall structure of densely packed laminae (diameter 3–7 mm, wall thickness vary from 0.17–0.37 mm) (Haas et al., 2009). The modern tubes can be replaced by aragonite in post-mortem mineralization to produce tubular structures of fine-scale concentric lamination, which are identical to diagenetic features observed in Devonian, Carboniferous and Oligocene fossils that were composed originally of organic material (Goedert et al., 2000), and are comparable in size and shape (Hass et al., 2009). The Paleozoic fossils attributed to vestimentiferans (preserved by calcite mineralization and clearly distinguishable from serpulids) show distinctive, concentrically laminated tube walls with organic material in remnants of the original laminae as well as characteristic taphonomic delamination structures (Peckmann et al., 2005; Haas et al., 2009).

We conclude that because features of the organic tube-wall structure and layering, as well as the overall morphology and paleoecology, both in extant vestimentiferans and Paleozoic

representative specimens, are similar to those in *Sabellidites*, it belongs to siboglinids. If our interpretation of the here presented fossils as siboglinids is correct, they would range from the later Ediacaran. Consequently, we argue that the timing of the origin of the Siboglinidae family considerably pre-dates the molecular clock estimate of 126 Ma for the group, making it a living fossil (see also Little and Vrijehoek, 2003, for discussion).

Unresolved controversy over the Ediacaran macrofauna exists whether some organisms resembling modern animal groups represent early bilaterians, including mollusks, annelids and arthropods and those producing horizontal trace fossils (Narbonne, 2005; Fedonkin et al., 2007; Vickers-Rich and Komarower, 2007; Xiao and Laflamme, 2009; Pecoit et al., 2012; Erwin and Valentine, 2013). Phylogenetic reconstructions of metazoans place alternatively origins of bilaterians in Cryogenian and major divergences of placozoans, priapulids, molluscs, nemerteans and annelids, and arthropods in early Ediacaran (Sperling and Vinther, 2010; Erwin et al., 2013; Sperling et al., 2013). The here inferred presence of annelids in the Ediacaran would indicate the presence of early representatives or close relatives of other spiralian groups (=lophotrochozoans, which are monophyletic) such as mollusks, but also cycloneuralians (including priapulids), arthropods, and deuterostomes. The existence of these groups has been suggested repeatedly. Interpretation of *Kimberella*, which occurs below the 555 Ma volcanic ash bed (Martin et al., 2000), as the stem group mollusc (Fedonkin et al., 2007) is in agreement with the divergence of derived molluscan and annelid-nemertean sister groups in the phylogeny of spiralians (Erwin and Valentine, 2013). Nemertea, a sister group of Mollusca diverged in early Ediacaran times (prior to 580 Ma Gaskiers glaciation), and the nemertean crown group existed in the terminal Ediacaran (Sperling et al., 2013). It is suggested that Priapulida diverged from the sister group Arthropoda in early Ediacaran (Sperling et al, 2013). The presence of truly bilaterians like *Spriggina* (possible arthropod), *Parvancorina* (arthropod), *Kimberella* (mollusk) and *Yorgia* (cephalozoan) (Fedonkin et al., 2007), and annelids (herein) in the Ediacaran Period may alternatively suggest that many organisms have affinity to crown clades.

CONCLUSIONS

Together with the detailed morphological resemblance the new data allows inference of a siboglinid affinity of *Sabellidites* as plausible. Our histological and microstructural observations of the *S. cambriensis* fossil are comparable with extant both pogonophoran (*Zenkevitchiana longissima* and *Siboglinum* sp.) and vestimentiferan (*Riftia* and *Tevnia*) taxa. Morphological, microstructural and biochemical features suggest that the fossil genus *Sabellidites* is an extinct member of the family Siboglinidae within the phylum Annelida (following the systematics of Struck et al., 2011). Accepting the siboglinid affinity of S. *cambriensis* would imply the extension of the temporal range of the oldest body-preserved annelids hitherto from the Sirius Passet Lagerstätte (Conway Morris and Peel, 2008) of the early Cambrian age to the late Ediacaran succession on the East European Platform. We consider the consequences of this for our understanding of early annelid evolution and phylogeny, i.e., the appearance and the presence of its crown-group representative of the phylum in the Ediacaran Period.

The body wall of *S. cambriensis* comprises a chitin-structural protein composite, thus indicating that the synthesis of chitin developed early in metazoan evolution and functioned in body construction and protection in early annelids, if the fossil represents the latter clade. The organic material of the *S. cambriensis* tube is recalcitrant and robust in structure and resisted early biodegradation, prolonged burial and early diagenesis for ca. 550 Ma due to its particular chitin-scleroprotein crystallite scaffolding. The *S. cambriensis* example extends the record of chitin preservation (Cody et al., 2011) by some 130 Ma.

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REFERENCES

Allard, B. and J. Templier. 2000. Comparison of neutral lipid profile of various trilaminar outer cell wall (TLS)-containing microalgae with emphasis on algaenan occurrence. Phytochemistry, 54:369–380.

Antcliffe, J. B. and M. D. Brasier. 2008. *Charnia* at 50: Developmental models for Ediacaran fronds. Palaeontology, 51:11–26.

Blair, J. E. 2009. Animals (Metazoa), p. 223–230. *In* S. B. Hedges and S. Kumar (eds.), The Timetere of Life. Oxford University Press, Oxford.

Briggs, D. E. G. 1999. Molecular taphonomy of animal and plant cuticles: Selective preservation and diagenesis. Philosophical Transactions of the Royal Society, London, Ser. B, 354:7–17.

Briggs, D. E. G. 2003. The role of decay and mineralization in the preservation of soft-bodied fossils. Annual Review of Earth Planetary Sciences, 31:275–301.

Blackwell, J. 1973. The polysaccharides, p. 464–513. *In* A. G. Walton and J. Blackwell (eds.), Biopolymers. Academic Press, New York/London.

Brasier, M. and J. Antcliffe. 2004. Decoding the Ediacaran enigma. Science, 305:1115–1117.

Budd, G. E. 2008. The earliest fossil record of the animals and its significance. Philosophical Transactions of the Royal Society, London, Ser. B, 363: 1425–1434.

Butterfield, N. J. 1990. Organic preservation of non-mineralizing organisms and the taphonomy of the Burgess Shale. Paleobiology, 16:272–286.

Butterfield, N. J. 1995. Secular distribution of Burgess-Shale-type preservation. Lethaia, 28:1–13.

Butterfield, N. J., U. Balthasar, and L. A. Wilson. 2007. Fossil diagenesis in the Burgess Shale. Palaeontology, 50:537–543.

Butterfield, N. J. and T. H. P. Harvey. 2012. Small carbonaceous fossils (SCFs): A new measure of early Paleozoic paleobiology. Geology, 40:71–74.

Cai, Y., J. D. Schiffbauer, H. Hua, and S. Xiao. 2011. Morphology and palaeoecology of the late Ediacaran tubular fossil Conotubus hemiannulatus from the Gaojiashan Lagerstätte of southern Shaanxi Province, South China. Precambrian Research, 191:46–57.

Chen, J. I. and G. Q. Zhou. 1997. Biology of the Chengjiang fauna. Bulletin of the National Museum of Natural History, 10:11–105.

Chen, Z., S. Bengtson, C-M. Zhou, H. Hua, and Z. Yue. 2008. Tube structure and original composition of *Sinotubulites*: Shelly fossils from the late Neoproterozoic in southern Shaanxi, China. Lethaia, 41:37–45.

Chevaldonné, P., D. Jollivert, D. Desruyères, R. A. Lutz, and R. C. Vrijenhoek. 2002. Sister-species of eastern Pacific hydrothermal vent worms (Ampharetidae, Alvinellidae, Vestimentifera) provide new mitochondrial COI clock calibration. Cahiers de Biologie Marine, Brest, 43: 367–370.

Cody G. D., N. S. Gupta, D. G. Briggs, A. L. D. Kilcoyne, R. E. Summons, F. Kenig, R. E. Plotnick, and A. Scott. 2011. Molecular signature of chitin-protein complex in Paleozoic. Geology, 39:255–258.

Cohen, Y. 1984. Oxygenic photosynthesis, anoxygenic photosynthesis and sulfate reduction in cyanobacterial mats, p. 435–441. *In* M. J. Klug and C. A. Reddy (eds.), Current Perspective in Microbial Ecology. American Society for Microbiology, Washington, D. C.

Compston, W., M. S. Sambridge, R. F. Reinfrank, M. Moczydłowska, G. Vidal, and S. Claesson. 1995. Numerical ages of volcanic rocks and the

earliest faunal zone within the late Precambrian of east Poland. Journal of the Geological Society London, 152:599–611.

Conway Morris, S. and J. S. Peel. 2008. The earliest annelids: Lower Cambrian polychaetes from the Sirius Passet Lagerstätte, Peary Land, North Greenland. Acta Palaeontological Polonica, 53:137–148.

Cortijo, I., M. Martí Mus, S. Jensen, and T. Palacios. 2010. A new species of *Cloudina* from the terminal Ediacaran of Spain. Precambrian Research, 176:1–10.

Dando, P. R., A. J. Southward, and E. C. Southward. 1992. Shipwrecked tube worms. Nature, 356:667.

Des Marais, D. J., E. D'Amilio, J. D. Farmer, B. B. Jørgensen, A. C. Palmisano, and B. K. Pierson. 1992. Case study of a modern microbial mat-building community: The submerged cyanobacterial mats of Gurrero Negro, Baja California Sur, Mexico, p. 325–333. *In* J. W. Schopf and C. Klein (eds.), The Proterozoic Biosphere: A Multidisciplinary Study. Cambridge University Press.

Dong, L., S. Xiao, B. Shen, X. Yuan, X. Yan, and Y. Peng. 2008. Restudy of the worm-like carbonaceous compression fossils *Protoarenicola*, *Pararenicola*, and *Sinosabellidites* from early Neoproterozoic successions in North China. Palaeogeography, Palaeoclimatology, Palaeoecology, 258: 138–161.

Droser, M., J. G. Gehling, and S. R. Jensen. 2006. Assemblage palaeoecology of the Ediacara biota: The unabridged edition. Palaeogeography, Palaeoclimatology, Palaeoecology, 232:131–147.

Ehrlich, H., M. Maldonado, K.-D. Spindler, C. Eckert, T. Hanke, R. Born, C. Goebel, P. Simon, S. Heinemann, and H. Worch. 2007. First evidence of chitin as a component of the skeletal fibers of marine sponges. Part I. Verongidae (Demospongia: Porifera). Journal of Experimental Zoology Part B: Molecular and Developmental Evolution, 308B: 347–356.

Erwin, D. H., M. Laflamme, S. M. Tweedt, E. A. Sperling, D. Pisani, and K. J. Peterson. 2011. The Cambrian conundrum: Early divergence and late ecological success in the early history of animals. Science, 334:1091–1097.

Erwin, D. H. and J. W. Valentine. 2013. The Cambrian Explosion: The Construction of Animal Biodiversity. Roberts and Company, Greenwood Village, Colorado, 406 p.

Farmer, J., G. Vidal, M. Moczydłowska, H. Strauss, P. Ahlberg, and A. Siedlecka. 1992. Ediacaran fossils from the Innerelv Member (late Proterozoic) of the Tanafjorden area, northeastern Finnmark. Geological Magazine, 129:181–195.

Fedonkin M. A. 1990. Systematic Description of Vendian Metazoa, p. 71–120. *In* B. S. Sokolov and A. B. Iwanowski (eds.), The Vendian System. Vol. 1. Paleontology, Springer-Verlag.

Fedonkin M. A. 1994. Vendian body fossils and trace fossils, p. 370–388. *In* S. Bengtson (ed.), Early Life on Earth. Nobel Symposium No. 84. Columbia University Press, New York.

Fedonkin M. A., J. G. Gehling, K. Grey, G. M. Narbonne, and P. Vickers-Rich. 2007. The Rise of Animals Evolution and Diversification of the Kingdom Animalia. The Johns Hopkins University Press, 326 p.

Fedonkin, M. A. and P. Vickers-Rich. 2007. The White Sea's windswept coasts, p. 115–148. *In* M. A. Fedonkin, J. G. Gehling, K. Grey, G. M. Narbonne, and P. Vickers-Rich. The Rise of Animals Evolution and Diversification of the Kingdom Animalia. The Johns Hopkins University Press, 326 p.

Felbeck, H., 1981. Chemoautotrophic potential of the hydrothermal vent tube worm, *Riftia pachyptila* Jones (Vestimentifera). Science, 213:336–338.

Felitsyn, S. B. and T. G. Pshenitchnova. 1992. Gold content and thermal history of late Vendian sedimentary organic matter. Transactions of the U.S.S.R. Academy of Sciences, 325:374–377.

Felitsyn, S. B., G. Vidal, and M. Moczydłowska. 1998. Trace elements and Sr and C isotopic signatures in late Neoproterozoic and earliest Cambrian sedimentary organic matter from siliciclastic successions in the Eat European Platform. Geological Magazine, 135:537–551.

Gail, F. and S. Hunt. 1986. Tubes of deep sea hydrothermal vent worms *Riftia pachyptila* (Vestimentifera) and *Alvinella pompejana* (Annelida). Marine Ecology Progress Series, 34:267–274.

Gaill, F., F. J. Persson, J. Sugiyama, R. Vuong, and H. Chanzy. 1992a. The chitin system in the tubes of deep sea hydrothermal vent worms. Journal of Structural Biology, 109:116–128.

Gaill, F., M.-F. Voss-Foucart, C. Gerday, P. Compere, and G. Goffinet. 1992b. Chitin and protein contents in the tubes of vestimentiferans from hydrothermal vents, p. 232–236. *In* C. J. Brinne, P. A. Sanford, and J. P. Zizakis (eds.), Chitin and Chitosan. Elsevier, Advances in Applied Science.

Gaines, R. R., D. E. G. Briggs, and Y. Zhao. 2008. Cambrian Burgess Shale-type deposits share a common mode of fossilization. Geology, 36:755–758.

Gardiner, S. L. and M. L. Jones. 1993. Vestimentifera. Chapter 9, p. 371–460. *In* F. W. Harrison and M. E. Rice (eds.), Microscopic Anatomy of Invertebrates, Volume 12: Onychophora, Chilipoda, and Lesser Protostomata, Wiley-Liss, Inc., New York.

Gehling, J. G. 1999. Microbial mats in terminal Proterozoic siliciclastics: Ediacaran death masks. Palaios, 14:40–57.

Gehling, J. G., M. L. Droser, S. Jensen, and B. N. Runnegar. 2005. Ediacaran organisms: Relating form to function, p. 43–66. *In* D. E. G. Briggs (ed.), Evolving Form and Function: Fossils and Development. Proceedings of a symposium honoring Adolph Seilacher for his contributions to paleontology in celebration of his 80th birthday. Peabody Museum of Natural History, Yale University, New Haven.

Gehling, J. G., G. M. Narbonne, and M. M. Anderson. 2000. The first named Ediacaran body fossil, *Aspidella terranovica*. Palaeontology, 43:427–456.

Gnilovskaya, M. B. 1990. Vendotaenids—Vendian Metaphytes, p. 138–147. *In* B. S. Sokolov and A. B. Iwanowski (eds.), The Vendian System. Vol. 1. Paleontology, Springer-Verlag.

Goedert, J. L., J. Peckmann, and J. Reitner. 2000. Worm tubes in an allochthonous cold-seep carbonate from lower Oligocene rocks of Western Washington. Journal of Paleontology, 74:992–999.

Grant, S. W. F. 1990. Shell structure and distribution of *Cloudina*, a potential index fossil for the terminal Proterozoic. American Journal of Science, 290-A:261–294.

Grazhdankin, D. 2004. Patterns of distribution in the Ediacaran biotas: Facies versus biogeography and evolution. Paleobiology, 30:203–221.

Grazhdankin, D. V., U. Balthasar, K. E. Nagovitsin, and B. B. Kochnev. 2008. Carbonate-hosted Avalon-type fossils in arctic Siberia. Geology, 36:803–806.

Grazhdankin, D. V. and A. Seilacher. 2002. Underground Vendobionta from Namibia. Palaeontology, 45:57–78.

Grotzinger, J. P., W. A. Watters, and A. H. Knoll. 2000. Calcified metazoans in thrombolite-stromatolite reefs of the terminal Proterozoic Nama Group, Namibia. Paleobiology, 26:334–359.

Gupta, N. S. and D. E. G. Briggs. 2011. Taphonomy of animal organic skeletons through time, p. 199–221. *In* P. A. Allison and D. J. Bottjer (eds.), Taphonomy, Process and Bias Through Time. Springer.

Halanych, K. M. 2005. Molecular phylogeny of siboglinid annelids (a.k.a. pogonophorans): A review, p. 297–307. *In* T. Bartolomaeus and G. Pursche (eds.), Morphology, Molecules, Evolution and Phylogeny in Polychaeta and Related Taxa. Hydrobiologi 535–536, Springer, 388 p.

Harvey, T. H. P., J. Ortega-Hernández, J.-P., Lin, Y. Zhao, and N. J. Butterfield. 2012. Burgess Shale-type microfossils from the middle Cambrian Kaili Formation, Guizhou Province, China. Acta Palaeontologica Polonica, 57:423–436.

Hass, A., C. T. S. Little, H. Sahling, G. Bohrmann, T. Himmler, and J. Peckmann. 2009. Mineralization of vestimentiferan tubes at methane seeps on the Congo deep-sea fan. Deep-Sea Research, 56:283–293.

Himmler, T., A. Freiwald, H. Stollhofen, and J. Peckmann. 2008. Late Carboniferous hydrocarbon-seep carbonates from the glaciomarine Dwyka Group, southern Namibia. Palaeogeography, Palaeoclimatology, Palaeoecology, 257:185–197.

Hou, X., R. J. Aldridge, J. Bergström, D. J. Siveter, and X. Feng. 2004. The Cambrian Fossils of Chengjiang, China—the Flowering of Early Animal Life. Blackwell Science Ltd., Oxford, 233 p.

Hua, H., Z. Chen, and X. Yuan. 2007. The advent of mineralized skeletons in Neoproterozoic Metazoa—new fossil evidence from the Gaojiashan Fauna. Geological Journal, 42:263–279.

Hua, H., B. R. Pratt, and L. Zhang. 2003. Borings in *Cloudina* shells: Complex predator-prey dynamics in the terminal Neoproterozoic. Palaios, 18:454–459.

Huldtgren, T., J. Cunningham, C. Yin, M. Stampanoni, F. Marone, P. C. J. Donoghue, and S. Bengtson. 2011. Fossilized nuclei and germination structures identify Ediacaran "animal embryos" as encysting protists. Science, 334:1696–1699.

Ivantsov, A. Yu. 1990. Novye dannye po ultrastructure sabelliditov (Pogonophora?). (New data on the ultrastructure of sabelliditids (Pogonophora?), Paleontologitcheskiy Zhurnal 4, 125–128. (In Russian)

Jørgensen, B. B. and Y. Cohen. 1977. Solar Lake (Sinai). 5. The sulfur cycle of the benthic cyanobacterial mts. Limnology and Oceanography, 22:657–666.

Korkutis, V. 1981. Late Precambrian and early Cambrian in the East European Platform. Precambrian Research, 15:75–94.

Laflamme, M., L. I. Flude, and G. M. Narbonne. 2012. Ecologic tiering and the evolution of a stem: The oldest stemmed frond from the Ediacaran of Newfoundland, Canada. Journal of Paleontology, 86:193–200.

Laflamme, M., J. D. Schiffbauer, G. M. Narbonne, and D. E. G. Briggs. 2011. Microbial biofilms and the preservation of the Ediacara biota. Lethaia, 44:203–213.

Lee, R. E. 2008. Phycology. Cambridge University Press, Cambridge, 547 p.

Little, C. T. S. and R. C. Vrijenhoek. 2008. Are hydrothermal vent animals living fossils? Trends in Ecology and Evolution, 18:582–588.

Liu, P., S. Xiao, C. Yin, C. Zhou, L. Gao, and F. Tang. 2008. Systematic description and phylogenetic affinity of tubular microfossils from the Ediacaran Doushantuo Formation at Weng'an, South China. Palaeontology, 51:339–366.

Mapstone, N. B. and D. McIlroy. 2006. Ediacaran fossil preservation: Taphonomy and diagenesis of a discoid biota from the Amadeus Basin, Central Australia. Precambrian Research, 149:126–148.

MARTIN, M. W., D. V. GRAZHDANKIN, S. A. BOWRING, D. A. D. EVANS, M. A. FEDONKIN, AND J. L. KIRSCHVINK. 2000. Age of Neoproterozoic bilaterian body and trace fossils, White Sea, Russia: Implications for metazoan evolution. Science, 288:841–845.

MOCZYDŁOWSKA, M. 1991 Acritarch biostratigraphy of the lower Cambrian and the Precambrian–Cambrian boundary in southeastern Poland. Fossils and Strata 29, 126 p.

MOCZYDŁOWSKA, M. 2008a. The Ediacaran microbiota and the survival of Snowball Earth Conditions, Precambrian Research, 167:1–15.

MOCZYDŁOWSKA, M. 2008b. New records of late Ediacaran microbiota from Poland. Precambrian Research, 167:71–92.

MOCZYDŁOWSKA, M. 2010. Life cycle of early Cambrian microalgae from the *Skiagia*-plexus acritarchs. Journal of Paleontology, 84:216–230.

NARBONNE, G. M. 2005. The Ediacara Biota: Neoproterozoic origin of animals and their ecosystems. Annual Review of Earth and Planetary Sciences, 33: 421–442.

NARBONNE, G. M. AND J. G. GEHLING. 2003. Life after snowball: The oldest complex Ediacaran fossils. Geology, 31:27–30.

NARBONNE, G. M, M. LAFLAMME, C. GREENTREE, AND P. TRUSLER. 2009. Reconstructing a lost world: Ediacaran rangeomorphs from Spaniard's Bay, Newfoundland. Journal of Paleontology, 83:503–523.

NARBONNE, G. M., B. Z. SAYLOR, AND J. P. GROTZINGER. 1997. The youngest Ediacaran fossils from South Africa. Journal of Paleontology, 71:953–967.

NOFFKE, N. 2009. The criteria for the biogeneicity of microbially induced sedimentary structures (MISS) in Archean and younger, sandy deposits. Earth-Science Reviews, 96:173–180.

NOFFKE, N., R. HAZEN, AND N. NHLEKO. 2003. Earth's earliest microbial mats in a siliciclastic marine environment (2.9 GA Mozaan Group, South Africa). Geology, 31:673–676.

NOFFKE, N., A. H. KNOLL, AND J. P. GROTZINGER. 2002. Sedimentary controls on the formation and preservation of microbial mats in siliciclastic deposits: A case study from the upper Neoproterozoic Nama Group, Namibia. Palaios, 17:533–544.

PAGE, A., S. E. GABBOTT, P. R. WILBY, AND J. A. ZALASIEWICZ. 2008. Ubiquitous Burgess Shale-style "clay templates" in low-grade metamorphic mudrocks. Geology 36:855–858.

PASKEVICIENE, L. T. 1986. On the stratigraphy of the Rovno Stage in northwestern part of the East European Platform, p. 127–137. *In* E. A. Pirrus (ed.), Vendian and Cambrian Facies and Stratigraphy of the Western Part of the East-European Platform, Academy of Sciences of the Estonian S.S.R., Institute of Geology, Tallin. (In Russian)

PECOITS, E., K. O. KONHAUSER, N. R. AUBET, L. M. HEAMAN, G. VEROSLAVSKY, R. A. STERN, AND M. K. GINGRAS. 2012. Bilaterian burrows and grazing behavior at >585 million years ago. Science, 336:1693–1696.

PECKMANN, J., C. T. S. LITTLE, F. GILL, AND J. REITNER. 2005. Worm tube fossils from the Hollard Mound hydrocarbon-seep deposit, Middle Devonian, Morocco: Palaeozoic seep-related vestimentiferans? Palaeogeography, Palaeoclimatoloy, Palaeoecology, 227:242–257.

PETERS, K. E. AND J. M. MOLDOWAN. 1993. The Biomarker Guide Interpreting Molecular Fossils in Petroleum and Ancient Sediments. Prentice Hall, Englewood Cliffs, p. 54–58.

PETERS, K. E., C. C. WALTERS, AND J. M. MOLDOWAN. 2005. The Biomarker Guide. Volume 1. Biomarkers and Isotopes in the Environment and Human History. Cambridge University Press, 471 p.

PETROVICH, R. 2001. Mechanisms of fossilization of the soft-bodied and lightly armored faunas of the Burgess Shale and of some other classical localities. American Journal of Sciences, 301:683–726.

PIERSON, B. K., J. BAULD, R. W. CASTENHOLTZ, E. D'AMELIO, D. J. DES MARAIS, J. D. FARMER, J. P. GROTZINGER, B. B. JØRGENSEN, D. C. NELSON, A. C. PALMISANO, J. W. SCHOPF, R. E. SUMMONS, M. R. WALTER, AND D. M. WARD. 1992. Modern mat-building microbial communities: A key to the interpretation of Proterozoic stromatolitic communities, p. 247–342. *In* J. W. Schopf and C. Klein (eds.), The Proterozoic Biosphere: A Multidisciplinary Study. Cambridge University Press.

RAABE, D., P. ROMANO, A. AL-SAWALMIH, C. SACHS, G. SERVOS, AND H. G. HARTWIG. 2005a. Mesostructure of the exoskeleton of the lobster *Homarus americanus*. Materials Research Society Symposium Proceedings 874, L5.2.1–5.2.6.

RAABE, D., P. ROMANO, C. SACHS, A. AL-SAWALMIH, H.-G. BROKMEIR, S.-B. YI, G. SERVOS, AND H. G. HARTWIG. 2005b. Discovery of a honeycomb structure in the twisted plywood patterns of fibrous biological nanocomposite tissue. Journal of Crystal Growth, 283:1–7.

RAFF, E. C., K. SCHOLLAERT, D. E. NELSON, P. C. J. DONOGHUE, C-W. THOMAS, F. R. TURNER, B. D. STEIN, X. DONG, S. BENGTSON, T. HULDTGREN, M. STAMPANONI, AND Y. CHONGYU. 2008. Embryo fossilization is a biological process mediated by microbial biofilms. Proceedings of the National Academy of Sciences U.S.A., 105:19360–19365.

ROBERTS, G. A. F. 1992. Chitin Chemistry. MacMillan and Co., London, 368 p.

ROUSE, G. AND K. FAUCHALD. 1997. Cladistics and the polychaetes. Zoologica Scripta, 26:139–204.

ROZANOV, A. YU. AND K. ŁYDKA. 1987. Palaeogeography and Lithology of the Vendian and Cambrian of the Western East European Platform. Publishing House Wydawnictwa Geologiczne Warsaw, 114 p.

RUIZ-HERRERA, J., J. M. GONZÁLEZ-PRIETO, AND R. RUIZ-MEDRANO. 2002. Evolution and phylogenetic relationships of chitin synthases from yeasts and fungi. FEMS Yeast Research, 1:247–256.

SAITO, Y., T. OKANO, H. CHAZY, AND J. SUGIYAMA. 1995. Structural study of α chitin from grasping spines of the arrow worm (*Sagitta* spp.). Journal of Structural Biology, 114:218–228.

SAPPENFIELD, A., M. L. DROSER, AND J. G. GEHLING. 2011. Problematica, trace fossils, and tubes within the Ediacaran Member (South Australia): Redefining the Ediacaran trace fossils record one tube at a time. Journal of Paleontology, 85:256–265.

SCHOPF, J. W., A. B. KUDRYAVTSEV, D. G. AGRESTI, A. D. CZAJA, AND T. J. WDOWIAK. 2005. Raman imagery: A new approach to assess the geochemical maturity and biogenicity of permineralized Precambrian fossils. Astrobiology, 5:333–371.

SEILACHER, A. 2007. The nature of vendobionts, p. 387–397. *In* P. Vickers-Rich and P. Komarower (eds.), The Rise and Fall of the Ediacaran Biota. Geological Society of London, Special Publication 286.

SEPKOSKI, J. J. JR. 1992. Proterozoic–early Cambrian diversification of metazoans and Metaphytes, p. 553–561. *In* J. W. Schopf and C. Klein (eds.), The Proterozoic Biosphere. A Multidisciplinary Approach. Cambridge University Press, Cambridge.

SHILLITO, B., J. P. LECHAIRE, AND F. GAILL. 1993. Microvilli-like structures secreting chitin Crystallites. Journal of Structural Biology, 111:59–67.

SHILLITO, B., B. LÜBBERING, J. P. LECHAIRE, J. J. CHILDRESS, AND F. GAILL. 1995. Chitin localization in the tube secretion system of a repressurized deep-sea tube worm. Journal of Structural Biology, 114:67–75.

SOKOLOV, B. S. 1965. Drevnejshie otlozheniya rannego kembriya i sabelliditidy. [The oldest early Cambrian deposits and sabelliditids]. In Vsesoyuznyj simposium po paleontologii dokembriya i rannego kembriya 25–30 oktyabrya 1965 (tezisy dokladov), 78–92. Institut Geologiya i Geofizika SO AN SSSR, Novosibirsk.

SOKOLOV, B. S. AND M. A. FEDONKIN. 1990. The Vendian System. Vol. 2. Regional Geology. Springer-Verlag, 273 p.

SOKOLOV, B. S. AND A. B. IWANOWSKI. 1990. The Vendian System. Vol. 1. Paleontology. Springer-Verlag, 383 p.

SPERLING, E. A., C. A. FRIEDER, A. V. RAMAN, P. R. GIRGUIS, L. A. LAEVIN, AND A. H. KNOLL. 2013. Oxygen, ecology, and the Cambrian readiation of animals. Proceedings of the National Academy of Sciences, U.S.A., 110: 13446–13451.

STANKIEWICZ, B. A., P. F. VAN BERGEN, I. J. DUNCAN, J. F. CARTER, D. E. G. BRIGGS, AND R. P. EVERSHED. 1996. Recognition of chitin and proteins in invertebrate cuticles using analytical pyrolysis-gas chromatography and pyrolysis-gas chromatography/mass spectrometry. Rapid Communications in Mass Spectrometry, 10:1747–1757.

STANKIEWICZ, B. A., D. E. G. BRIGGS, R. P. EVERSHED, M. B. FLANNERY, AND M. WUTTKE. 1997a. Preservation of chitin in 25-million-years-old fossils. Science, 276:1541–1543.

STANKIEWICZ, B. A., D. E. G. BRIGGS, R. P. EVERSHED, AND I. J. DUNCAN. 1997b. Chemical preservation of insect cuticle from the Pleistocene asphalt deposits of California, U.S.A. Geochimica et Cosmochimica Acta, 61:2247–2252.

STANKIEWICZ, B. A., M. MASTALERZ, C. H. J. HOF, A. BIERSTEDT, M. B. FLANNERY, D. E. G. BRIGGS, AND R. P. EVERSHED. 1998. Biodegradation of the chitin-protein complex in crustacean cuticle. Organic Geochemistry, 28: 67–76.

STANKIEWICZ, B. A., D. E. G. BRIGGS, R. MICHELS, M. E. COLLINSON, M. B. FLANNERY, AND R. P. EVERSHED. 2000. Alternative origin of aliphatic polymer in kerogen. Geology, 28:559–562.

STRAUSS, H., G. VIDAL, M. MOCZYDŁOWSKA, AND J. PACZESNA. 1997. Carbon isotope geochemistry and palaeontology of Neoproterozoic to early Cambrian siliciclastic successions in the East European Platform, Poland. Geological Magazine, 134:1–16.

STEINER, M. AND J. REITNER. 2001. Evidence of organic structures in Ediacara-type fossils and associated microbial mats. Geology, 29:1119–1122.

STRUCK, T. H., C. PAUL, N. HILL, S. HARTMANN, C. HÖSEL, M. KUBE, B. LIEB, A. MEYER, R. TIEDEMANN, G. PURSCHE, AND C. BLEIDORN. 2011. Phylogenetic analyses unravel annelid evolution. Nature, 471:95–98.

TANG, F., S. BENGTSON, Y. WANG, X-L. WANG, AND C. YIN. 2011. *Eandromeda* and the origin of Ctenophora. Evolution and Development, 13:408–414.

TOPORSKI, J. K. W., A. STEELE, F. WESTALL, R. AVCI, D. M. MARTILL, AND D. S. MCKAY. 2002. Morphologic and spectral investigation of exceptionally well-preserved bacterial biofilms from the Oligocene Enspel Formation, Germany. Geochimica et Cosmochimica Acta, 66:1773–1791.

URBANEK, A. AND G. MIERZEJEWSKA. 1977. The fine structure of zooidal tubes in Sabelliditida and Pogonophora with reference to their affinity. Acta Palaeontologica Polonica, 22:223–240.

URBANEK, A. AND G. MIERZEJEWSKA. 1983. The fine structure of zooidal tubes in Sabelliditida and Pogonophora, p. 100–111. *In* A. Urbanek and A. Yu. Rozanov (eds.), Upper Precambrian and Cambrian Palaeontology of the East-European Platform. Publishing House Wydawnictwa Geologiczne, Warszawa.

VALENTINE, J. W. 2004. On the Origin of Phyla. Chicago, University of Chicago Press, 614 p.

VALENTINE, J. W. 2007. Seeing ghosts: Neoproterozoic bilaterian body plan, p. 369–375. *In* P. Vickers-Rich and P. Komarower (eds.), The Rise and Fall of the Ediacaran Biota. Geological Society of London, Special Publication 286.

VERSTEEGH, G. J. M. AND P. BLOKKER. 2004. Resistant macromolecules of extant and fossil Microalgae. Phycological Research, 52:325–339.

VINTHER, J., E. A. SPERLING, D. E. G. BRIGGS, AND K. J. PETERSON. 2011. A molecular palaeobiological hypthesis for the origin of aplacophoran molluscs and their derivation from chiton-like ancestors. Proceedings of the Royal Society B, doi.:.

WEBSTER, J. AND R. W. S. WEBER. 2007. Introduction to Fungi. Cambridge University Press, Cambridge, 841 p.

WESTALL, F., S. T. DE VRIES, W. NIJMAN, V. ROUCHON, B. ORBERGER, V. PEARSON, J. WATSON, A. VERCHOVSKY, I. WRIGHT, J.-N. ROUZAUD, D. MARCHESINI, AND A. SEVERINE. 2006. The 3.466 Ga "Kitty Gap Chert", an early Archean microbial Ecosystem. Geological Society of America Special Paper, 405:105–131.

WESTALL, F. AND G. SOUTHAM. 2006. The early record of life. Archean Geodynamics and Environments Geophysical Monograph Series, 164:283–304.

WESTALL, F., A. STEELE, J. TOPORSKI, M. WALSH, C. ALLEN, S. GUIRDY, D. MCKAY, E. GIBSON, AND H. CHAFETZ. 2000. Polymeric substances and biofilms as biomarkers in terrestrial materials: Implications for extraterrestrial samples. Journal of Geophysical Research, 105:24, 511–24, 527.

XIAO, S. AND L. DONG. 2006. On the morphological and ecological history of Proterozoic Macroalgae, p. 57–90. *In* S. Xiao and A. J. Kaufman (eds.), Neoproterozoic Geobiology and Paleobiology. Topics in Geobiology 27, Springer.

XIAO, S. AND A. H. KNOLL. 2000. Phosphatized animal embryos from the NeoproterozoicDoushantuo Formation at Weng'an, Guizhou South China. Journal of Paleontology, 74:767–788.

XIAO, S. AND M. LAFLAMME. 2009. On the eve of animal radiation: Phylogeny, ecology and evolution of the Ediacara biota. Trends in Ecology and Evolution, 24:31–40.

XIAO, S., X. YUAN, M. STEINER, AND A. H. KNOLL. 2002. Macroscopic carbonaceous compressions in a terminal Proterozoic shale: A systematic reassessment of the Miaoehe biota, South China. Journal of Paleontology, 76:345–374.

XIAO, S., C. ZHOU, P. LIU, X. YUAN, AND D. WANG. 2014. Phosphatized acanthomorphic acritarchs and related microfossils from the Ediacaran Doushantuo Formation at Weng'an (South China) and their implications for biostratigraphic correlation. Journal of Paleontology Memoir 72, Supplement to Volume 88, Issue 1, 139 p.

YANISHEVSKY, M. 1926. Ob ostatkah trubchatyh chervei iz kembriyskoy siney gliny. [On the remains of the tubular worms from the Cambrian blue clays]. Ezhegodnik Russkogo Paleontologicheskogo Obchestva 4, p. 99–112.

YIN, C. Y., S. BENGTSON, AND Z. YUE. 2004. Silicified and phosphatised *Tianshushania*, spheroidal microfossil of possible animal origin from the Neoproterozoic of South China. Acta Palaeontologica Polonica, 49:1–12.

YIN, L.-M., M.-Y. ZHU, A. H. KNOLL, X.-L. YUAN, J. M. ZHANG, AND J. HU. 2007. Doushantuo embryos preserved inside diapause egg cysts. Nature, 446:661–663.

ZHANG, X., W. LIU, AND Y. ZHAO. 2008. Cambrian Burgess Shale-type Lagerstätten in south China: Distribution and significance. Gondwana Research, 14:275–279.

ZHANG, X.-G. AND B. R. PRATT. 1994. Middle Cambrian arthropod embryos with blastomeres. Science, 266:637–639.

ZHANG, X.-G., B. R. PRATT, AND C. SHEN. 2011. Embryonic development of a middle Cambrian (500 MYR old) scalidophoran worm. Journal of Paleontology, 85:898–903.

ZHAO, Y., Y. HUANG, X. CHEN, J. YUAN, Z. ZHANG AND Z. ZHOU. 1996. Composition and significance of the middle Cambrian Kaili Lagerstätten in Taijiang county, Guizhou Province, China—a new Burgess Type Lagerstätten. Guizhou Geology, 13:7–14.

ZHU, M., J. G. GEHLING, S. XIAO, Y. ZHAO, AND M. L. DROSER. 2008. Eight–armed Ediacaran fossil preserved in contrasting taphonomic windows from China and Australia. Geology, 36:867–870.

ZHAO, Y., R. L. PARSLEY, AND J. PENG. 2008. Basal middle Cambrian short-stalked eocrinoids from the Kaili Biota: Guizhou Province, China. Journal of Paleontology, 82:415–422.

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