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Triassic leech cocoon from Antarctica contains fossil bell animal

Benjamin Bomfleur^a, Hans Kerp^b, Thomas N. Taylor^{a,1}, Øjvind Moestrup^c, and Edith L. Taylor^a

^aDepartment of Ecology and Evolutionary Biology and Biodiversity Institute, University of Kansas, Lawrence, KS 66044; ^bForschungsstelle für Paläobotanik am Institut für Geologie und Paläontologie, University of Münster, D-48143 Münster, Germany; and ^cBiologisk Institut, Københavns Universitet, 1353 Copenhagen, Denmark

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Our understanding of the evolution of life on Earth is limited by the imperfection of the fossil record. One reason for this imperfect record is that organisms without hard parts, such as bones, shells, and wood, have a very low potential to enter the fossil record. Occasionally, however, exceptional fossil deposits that preserve soft-bodied organisms provide a rare glimpse of the true biodiversity during past periods of Earth history. We here present an extraordinary find of a fossil ciliate that is encased inside the wall layer of a more than 200 Ma leech cocoon from Antarctica. The microfossil consists of a helically contractile stalk that attaches to a main body with a peristomial feeding apparatus and a large C-shaped macronucleus. It agrees in every aspect with the living bell animals, such as *Vorticella*. Vorticellids and similar peritrichs are vital constituents of aquatic ecosystems worldwide, but so far have lacked any fossil record. This discovery offers a glimpse of ancient soft-bodied protozoan biotas, and also highlights the potential of clitellate cocoons as microscopic “conservation traps” comparable to amber.

ciliate protozoan | Late Triassic | *Vorticella*-like fossil | preservation

Ciliates are single-celled eukaryotes with dimorphic nuclei and a unique type of cilia (1). Historically, the earliest-described ciliate is the bell-animal *Vorticella*, first observed by van Leeuwenhoek in 1674 and later formally named by Linnaeus in 1767 (2, 3). The Ciliophora is today known to be a large and remarkably diverse group, comprising 11 classes with more than 8,000 species (1, 4). Ciliates occur worldwide in a variety of habitats, and play a vital role in the food webs of many aquatic and moist-terrestrial ecosystems (1, 4, 5). The evolutionary history of the Ciliophora remains poorly understood, however (1, 6), largely because most ciliates are soft-bodied organisms, which—like any other organisms that lack hard parts such as bones, shells, or wood—can become fossilized only under exceptional circumstances (7, 8).

Perhaps the most iconic paleontological treasure troves that can preserve soft-bodied organisms are so-called “conservation traps” (8–10), in which organisms become rapidly entombed in a fossilization medium that prevents further decay. Famous types of conservation traps include amber (fossil resin) and asphalt pits (8, 9). Another peculiar kind of fossilization medium has so far been largely ignored by paleontologists, however. Clitellate annelids (earthworms and leeches) produce characteristic cocoons into which the eggs are laid (11). The cocoon walls are secreted from the clitellum initially in the form of a mucous substance composed mainly of polysaccharides and fibrous proteins; depending on habitat conditions, this substance may then cure for up to several days into a solid, protective egg capsule that can be highly resilient toward thermal, chemical, and proteolytic decay (11). As a result, clitellate cocoons have a rather high fossilization potential and are not uncommon in the terrestrial fossil record since the Triassic (12, 13). Fossil leech cocoons were identified in Miocene lake deposits more than 150 y ago (14). Similar fossils were later variously interpreted as remains of algae (15), fungi (16), lichens (17, 18), or various

plant parts (16, 19), until a detailed comparative study corroborated their affinity with the Clitellata (12). Morphology and structural features indicate that most cocoon fossils likely were produced by leeches (Hirudinea) (12, 13).

After initial secretion, the solidifying cocoon wall has the potential to trap and preserve microorganisms in a way comparable to plant resin, as demonstrated by the description of a pristine fossil nematode encased in a Cretaceous clitellate cocoon (20). We searched for microinclusions in clitellate cocoons collected from an exposure of the Section Peak Formation (Victoria Group, Beacon Supergroup) at Timber Peak in the Eisenhower Range, north Victoria Land, East Antarctica (Fig. 1). The sedimentary section at this site is intercalated between two dolerite sills and consists of a ~80-m-thick succession of coal-bearing fluvio-lacustrine deposits (21, 22) (Fig. 1). Palynological analyses indicate a Late Triassic age for these strata (23, 24).

Description

Abundant and well-preserved plant fossils occur in a mudstone bed overlying a coal seam in the lower part of the section (22) (Fig. 1). Fossil clitellate cocoons were found among organic residues of bulk-macerated rock samples from this plant-bearing layer. The cocoons and cocoon fragments belong to the *Burejosperrum* type (12), which is characterized by a thick, solid inner wall layer (alytine) and an outer layer composed of a loose meshwork of filaments (hapsine) (Fig. 2). This cocoon type is particularly similar to cocoons produced by extant leeches, such as the medicinal leech *Hirudo medicinalis* (12) (Fig. 2).

Encased within the alytine layer of one cocoon is a fossil microorganism that can be identified as a ciliate protozoan of the family Vorticellidae *sensu lato* (Fig. 3). The fossil consists of an approximately 25-μm-long, teardrop-shaped main body (zooid) attached at the end of a helically contracted stalk (Fig. 3). A pronounced furrow-like rim on the anterior region of the zooid indicates the position of the retracted feeding apparatus (peristome). A large, horseshoe-shaped macronucleus is discernible inside the zooid. The stalk with its spasmoneme, a molecular “spring fiber,” is contracted into a tight helix approximately 50 μm long. It attaches to a narrow basal projection (scopular rim) of the zooid and terminates in a jagged region of the outer cocoon wall surface, where the organism was probably anchored.

Discussion

The Triassic microfossil agrees in every observable detail with the living bell animal *Vorticella*, including the solitary habit, the helically contractile stalk, and the large C-shaped macronucleus (2, 3). The pronounced peristomial bulges make it particularly similar to the species *Vorticella campanula* (2, 3). The exact affinities of the fossil cannot be determined, however, because

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¹To whom correspondence should be addressed. E-mail: tntaylor@ku.edu.

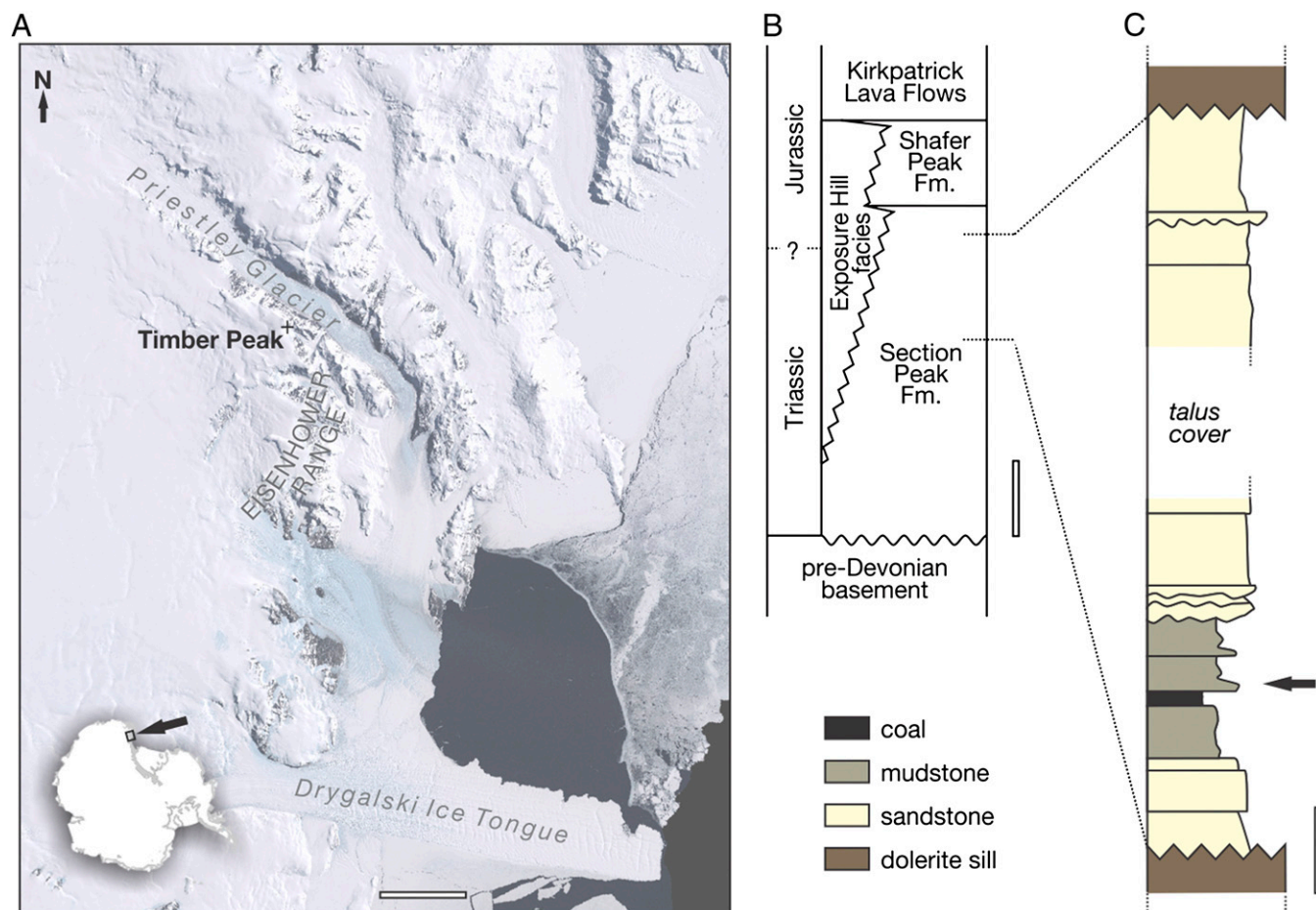


Fig. 1. Locality information for the Timber Peak section where the cocoon fossils were found. (A) Geographic location. (B) Stratigraphic context. (C) Simplified lithological column. (Scale bars: A, 25 km; B, ~50 m; C, 10 m.)

superficially similar taxa, such as *Pseudovorticella*, differ only in micromorphological and molecular features (25, 26) that cannot be observed in the fossil. Moreover, recent molecular studies

indicate that the genus *Vorticella* and the family Vorticellidae are probably paraphyletic, and a taxonomic revision has yet to be completed (26).

Vorticellids are among the most common and widely distributed ciliates, occurring in marine, brackish, freshwater, and moist terrestrial environments worldwide (1). They are hemisessile filter feeders anchored by their retractable stalks to plant matter, metazoan shells, and other substrates. Under unfavorable conditions, the zooid can detach from the stalk and become a free-swimming swarmer (telotroch) to relocate itself; once the swarmer has settled on a suitable new substrate, a new stalk is produced within a few hours (27). It appears that the Triassic *Vorticella*-like organism from Antarctica must have anchored itself to a freshly deposited clitellate cocoon, and then became trapped in the solidifying cocoon wall. The plant fossil assemblage from the cocoon-bearing deposit has been interpreted as a parautochthonous association buried in crevasse splay deposits (22); thus, the original location of cocoon deposition, and hence the habitat of the microorganism, was probably a fluvial aquatic or moist terrestrial flood basin environment.

Fossils of soft-bodied protozoans are overall exceedingly rare (28). The Antarctic microinclusion forms the first fossil record of a *Vorticella*-like ciliate, and only the second fossil record of the large and diverse ciliate subclass Peritrichia; the only other occurrence is an assemblage of phosphatized peritrichs preserved inside ostracod shells from the Lower Triassic of Svalbard (29). In addition, fossils of soft-bodied, *Paramecium*-like ciliates have been sporadically reported from chert and amber deposits (30, 31), some of which date back to the Late Triassic (32). These rather

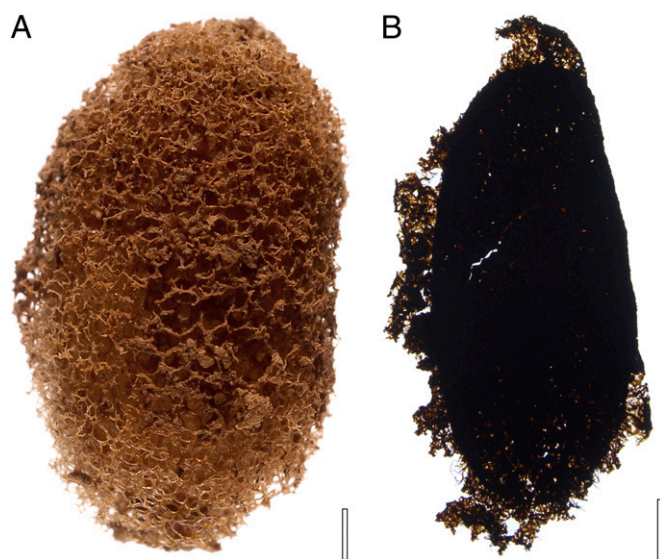


Fig. 2. Comparison of extant and fossil clitellate cocoons. (A) Cocoon of the extant medicinal leech (*H. medicinalis*). (B) Nearly complete *Burejospermum*-type cocoon fossil from the Upper Triassic of Timber Peak. (Scale bars: 1 mm.)

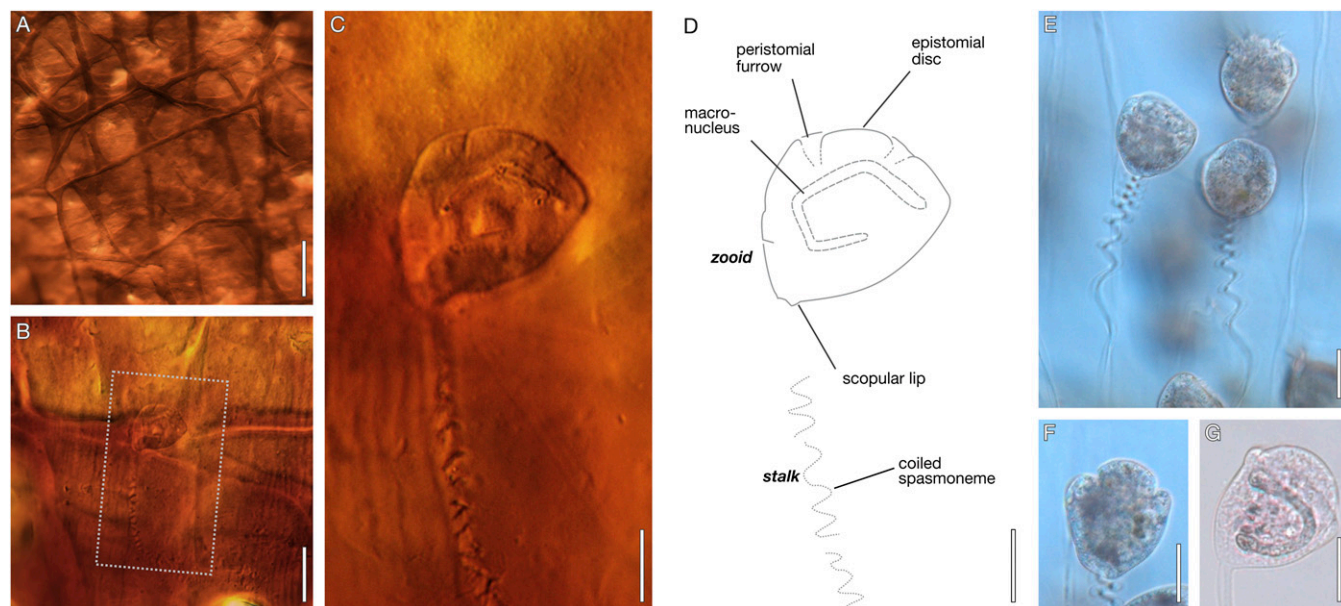


Fig. 3. Triassic *Vorticella*-like fossil inside a clitellate cocoon wall compared with extant *V. campanula*. (A) Solid wall layer of Triassic clitellate cocoon from Antarctica. (B) Encased *Vorticella*-like fossil. (C) Detail of animal in B showing coiled stalk and macronucleus. (D) Traced line drawing of the microfossil indicating anatomic features. (E) Group of extant *V. campanula* with partially contracted stalks. (F) Extant *Vorticella* with partially retracted peristome. Note the resemblance of the resulting peristomial furrow and protruding epistomial disk to structures in the microfossil. (G) Horseshoe-shaped macronucleus in stained extant *Vorticella*. (Scale bars: A, 100 μ m; B and E–G, 25 μ m; C and D, 10 μ m.)

isolated finds are of utmost importance for a better understanding of the evolutionary history of the Protozoa, providing sorely needed minimum age constraints for phylogenetic analyses that up to now had to rely on rather vague age estimations based on molecular divergence rates (6). In this respect, the remarkable similarity of the more than 200 Ma fossil bell animal to living species provides an example for the antiquity of protozoan bauplans.

The preservation of microorganisms inside fossil clitellate cocoons appears to be surprisingly common; in addition to the Triassic bell animal and the Cretaceous nematode *Captivonema*, trilete spores and other unicellular organisms also have been reported encased in these microscopic conservation traps (20). We anticipate that a deliberate search for microinclusions in fossil clitellate cocoons may open up an invaluable source of information on ancient soft-bodied microorganisms and the past ecosystems in which they lived.

Materials and Methods

Fossil Material. Fossils were collected during the Ninth German Antarctic North Victoria Land Expedition (GANOVEX IX, 2005/2006). Cocoon fossils were found among dispersed plant cuticles and other organic residues of bulk macerations prepared following slightly modified standard procedures. Samples of the fossiliferous mudstone bed were treated with 48% (vol/vol) hydrofluoric acid for up to several months until organic contents were sufficiently isolated. The organic residues were then macerated using Schulze's reagent (35% nitric acid with a few crystals of potassium chlorate) for 5 d,

and finally cleaned and bleached using a 4% potassium hydroxide solution for several seconds. After each step, the material was neutralized by thorough rinses with distilled water. Fragments of clitellate cocoons were picked manually from the obtained bulk of organic residues, dehydrated in glycerol, and mounted on microscope slides with glycerol jelly. The cocoon fossil containing the microinclusion was analyzed using a Leitz Diaplan compound microscope with Nomarski interference contrast; photos were taken with a Nikon DS-5M digital microscope camera.

Extant Material. Extant *Vorticella* was collected from Potter Lake on the University of Kansas campus in Lawrence, KS. Living specimens were placed on a concavity microscope slide and then analyzed and photographed using a Leica DM5000 compound microscope equipped with a Leica DC500 digital microscope camera. To demonstrate the macronuclei, selected specimens were stained *in vivo* by adding a drop of acidulated methyl green (in 1% acetic acid) near the margin of the cover glass. Overviews of recent clitellate cocoons were photographed with a Canon EOS 5D camera with a Canon MP-E 65-mm f/2.8 1–5 \times macrophoto lens.

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