Phylogenetic position of Loricifera inferred from nearly complete 18S and 28S rRNA gene sequences
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Abstract
Background: Loricifera is an enigmatic metazoan phylum; its morphology appeared to place it with Priapulida and Kinorhyncha in the group Scalidophora which, along with Nematoidea (Nematoda and Nematomorpha), comprised the group Cycloneuralia. Scarce molecular data have suggested an alternative phylogenetic hypothesis, that the phylum Loricifera is a sister taxon to Nematomorpha, although the actual phylogenetic position of the phylum remains unclear.

Methods: Ecdysozoan phylogeny was reconstructed through maximum-likelihood (ML) and Bayesian inference (BI) analyses of nuclear 18S and 28S rRNA gene sequences from 60 species representing all eight ecdysozoan phyla, and including a newly collected loriciferan species.

Results: Ecdysozoa comprised two clades with high support values in both the ML and BI trees. One consisted of Priapulida and Kinorhyncha, and the other of Loricifera, Nematoidea, and Panarthropoda (Tardigrada, Onychophora, and Arthropoda). The relationships between Loricifera, Nematoidea, and Panarthropoda were not well resolved.

Conclusions: Loricifera appears to be closely related to Nematoidea and Panarthropoda, rather than grouping with Priapulida and Kinorhyncha, as had been suggested by previous studies. Thus, both Scalidophora and Cycloneuralia are a polyphyletic or paraphyletic groups. In addition, Loricifera and Nematomorpha did not emerge as sister groups.

Keywords: Molecular phylogeny, Ecdysozoa, Scalidophora, Cycloneuralia, Nematoidea, Panarthropoda

Introduction
Since its first description as a new phylum [1], Loricifera has been one of the most enigmatic metazoan phyla. Although only 35 loriciferan species have been described worldwide, the actual species diversity is higher, as many new species await description [2–6]. All known loriciferan species are microscopic (80–800 μm) and occur in marine sediments, such as mud, sand, and shell gravel. The most extreme habitat for Loricifera is the hypersaline anoxic deep basin in the Mediterranean Sea, where members of this phylum are metabolically active [6, 7]. Our knowledge of loriciferan life cycles is also only fragmentary, given the recent findings of new life cycles and larval types [3–5, 8].

There are two alternative hypotheses on the position of Loricifera within Ecdysozoa, both based on morphological data. One is the ‘Scalidophora hypothesis’ [9–11], in which Loricifera, Kinorhyncha, and Priapulida together comprise a clade, Scalidophora. Morphological similarities between Scalidophora and Nematomorpha [12–15] and between Scalidophora and Nematoidea (Nematomorpha and Nematoda) [9, 11, 16–21] have indicated that these five phyla in turn comprise a clade, Cycloneuralia [20, 21].

The alternative is the ‘Loricifera + Nematomorpha hypothesis’ [22]. While the first molecular phylogenetic study that included a loriciferan sequence (18S rRNA) failed to establish the phylogenetic position of Loricifera [23], Sørensen et al. [22] detected a sister group relationship...
between Loricifera and Nematomorpha based on 18S rRNA and histone-3 sequences, although with low nodal support (posterior probability = 0.83). The latter study also detected a sister group relationship between Priapulida and Kinorhyncha, but not monophyly for Cycloneuralia, which several previous molecular studies that lacked loriciferan sequences had indicated [24–29].

The present study investigated the phylogenetic position of phylum Loricifera within Ecdysozoa using nearly complete 18S and 28S rRNA sequences. Also of interest was the phylogenetic status of the taxa Scalidophora and Cycloneuralia.

Materials and methods

Sampling and DNA sequencing

The loriciferan specimen used in this study was collected from Ise Bay, Japan, northwestern Pacific (34°9.77′N, 136°51.40′E, 161–174 m depth) during a cruise of the TR/V Seisui-maru (Mie University) on 21 November 2013. A sediment sample was collected with a biological dredge, subsequently frozen to prevent DNA degradation, and sent to the laboratory. In the laboratory, meiofaunal specimens were extracted by floatation [30] with Ludox® HS 40. The extracted sample was sorted under a stereomicroscope, and a single adult loriciferan specimen (Fig. 1a) was obtained and preserved in 99% EtOH for DNA extraction.

Total genomic DNA was extracted [31] from the specimen with a DNeasy Tissue Kit (Qiagen, Tokyo). After DNA extraction, the exoskeleton was mounted in Fluoromount G® as a hologenophore (Fig. 1b). The loriciferan specimen was identified as Rugiloricus sp. based on the morphology of the hologenophore.

Nearly complete 18S rRNA (18S) and 28S rRNA (28S) genes sequences were amplified by PCR using previously published primer sets and conditions [31]. All nucleotide sequences were determined by direct sequencing with a BigDye Terminator Kit ver. 3.1 (Life Technologies, Co., USA) and a 3730 DNA Analyzer (Life Technologies, Co., USA). Sequence fragments were assembled by using MEGA 5 [32]. After assembly, 18S (1872 bp) and 28S (3450 bp) sequences were deposited in GenBank under accession numbers LC032019 and LC032020.

Phylogenetic analyses

18S and/or 28S sequences for 66 taxa were obtained from GenBank. We prepared the following five datasets for analyses (Table 1): “18S + 28S (50OTU)” including 18S and 28S sequences for all 50 taxa which both 18S and 28S are available (note that we treated the 18S sequence from Milnesium tardigradum and the 28S sequence from Milnesium sp. as a single OTU, because nearly complete 18S and 28S sequences were unavailable from a single tardigrade species); “18S (50OTU)” including 18S sequences for the same taxa of “18S +28S (50 OTU)”; “28S (50OTU)” including 28S sequences for the same taxa of “18S +28S (50 OTU)”; “18S (65 OTU)” including 18S sequences for more comprehensive taxon sampling especially in Tardigrada, Nematoda, Nematomorpha, Priapulida, and Kinorhyncha than the former three datasets; “18S (63 OTU)” including 18S sequences for same OTU to “18S (65 OTU)” except for Nanaloricus sp. due to its short sequence and Meiopriapulus fijiensis to avoid long branch attraction [22]. Sequences from each gene were pre-aligned separately with MAFFT software [33] using the FFT-NS-2 option and

Fig. 1 Rugiloricus sp., an undescribed loriciferan. Nomarski photomicrographs of the hologenophore of the specimen of Rugiloricus sp. used in this study. a, Entire animal before DNA extraction; b, Exoskeleton of the specimen after DNA extraction
Table 1 List of taxa included in each dataset

| Taxa                        | Species                  | 18S + 28S (50OTU) | 18S (50OTU) | 28S (50OTU) | 18S (65OTU) | 18S (63OTU) | Accession number |
|-----------------------------|--------------------------|-------------------|-------------|-------------|-------------|-------------|-----------------|
| Loricifera                  | Rugiloricus sp.          | ○ ○ ○ ○ ○          |             |             |             |             | LC032019 LC032020 |
|                             | Nanaloricus sp.          | ○ ○ ○ ○             |             |             |             |             | EU669461        |
|                             | Pliciloricus sp.         | ○ ○ ○ ○             |             |             |             |             | AY746986 -      |
| Arthropoda                  | Euchelicerata            | ○ ○ ○ ○ ○           |             |             |             |             |                 |
|                             | Limulus polyphemus       | ○ ○ ○ ○ ○           |             |             |             |             | U91490 AF212167 |
|                             | Calocheiridae cf. termithophilus | ○ ○ ○ ○ ○ |             |             |             |             | AY859559 AY859558 |
|                             | Siro rubens              | ○ ○ ○ ○ ○           |             |             |             |             | U36998 AY859602 |
|                             | Eremobates sp.           | ○ ○ ○ ○ ○           |             |             |             |             | AY859573 AY859572 |
|                             | Pandinus imperator       | ○ ○ ○ ○ ○           |             |             |             |             | AY210831 AY210830 |
|                             | Magnostroctus giganteus  | ○ ○ ○ ○ ○           |             |             |             |             | AFO00546 AY859587 |
|                             | Misumensops asperatus    | ○ ○ ○ ○ ○           |             |             |             |             | AY210445 AY210461 |
| Pycnogonida                 | Anoplodactylus portus    | ○ ○ ○ ○ ○           |             |             |             |             | AY859551 AY859550 |
|                             | Calipallene sp.          | ○ ○ ○ ○ ○           |             |             |             |             | AY210808 AY210807 |
| Myriapoda                   | Polyxenidae sp.         | ○ ○ ○ ○ ○           |             |             |             |             | AY859596 AY859595 |
|                             | Orthoporus sp.           | ○ ○ ○ ○ ○           |             |             |             |             | AY210829 AY210828 |
|                             | Cherokia georgiana       | ○ ○ ○ ○ ○           |             |             |             |             | AY859563 AY859562 |
|                             | Scutigera coleoptrata    | ○ ○ ○ ○ ○           |             |             |             |             | AF173238 AY859601 |
|                             | Craterostigmus tasmanianus | ○ ○ ○ ○ ○ |             |             |             |             | AFO00774 AY859569 |
| Crustacea                   | Cyprididae sp.          | ○ ○ ○ ○ ○           |             |             |             |             | AY210816 AY210815 |
|                             | Anaspides tasmaniae      | ○ ○ ○ ○ ○           |             |             |             |             | L81948 AY859549 |
|                             | Squilla empusa           | ○ ○ ○ ○ ○           |             |             |             |             | L81946 AY210842 |
|                             | Heteromysis tasmaniae    | ○ ○ ○ ○ ○           |             |             |             |             | AY859580 AY859578–79 |
|                             | Gaeticus depressus       | ○ ○ ○ ○ ○           |             |             |             |             | AY859577 AY859575–76 |
|                             | Panulirus argus          | ○ ○ ○ ○ ○           |             |             |             |             | U19182 AY210833–35 |
|                             | Homarus americanus       | ○ ○ ○ ○ ○           |             |             |             |             | AF235971 AY859581 |
| Hexapoda                    | Eulimnadia texana       | ○ ○ ○ ○ ○           |             |             |             |             | AF144211 AY859574 |
|                             | Triops longicaudatus     | ○ ○ ○ ○ ○           |             |             |             |             | AY210838 AY157606 |
|                             | Podura aquatica         | ○ ○ ○ ○ ○           |             |             |             |             | AFO00542 AY210838 |
|                             | Smiththrus viridus       | ○ ○ ○ ○ ○           |             |             |             |             | AY859604 AY859603 |
|                             | Dilta littoralis         | ○ ○ ○ ○ ○           |             |             |             |             | AFO005457 AY859570–71 |
|                             | Callibaetis ferrugineus  | ○ ○ ○ ○ ○           |             |             |             |             | AF370791 AY859557 |
|                             | Mantis religiosa         | ○ ○ ○ ○ ○           |             |             |             |             | AY859586 AY859585 |
|                             | Zootermopsis angusticolis | ○ ○ ○ ○ ○ |             |             |             |             | AY859615 AY859614 |
|                             | Gramphodarthis laevigata | ○ ○ ○ ○ ○ |             |             |             |             | AY210820 AY210819 |
|                             | Gomphoherinae sp.       | ○ ○ ○ ○ ○           |             |             |             |             | AY859547 AY859546 |
|                             | Vespula pensylvanica     | ○ ○ ○ ○ ○           |             |             |             |             | AY859613 AY859612 |
|                             | Merope tuber             | ○ ○ ○ ○ ○           |             |             |             |             | AF286287 DQ20351 |
| Onychophora                 | Peripatoides novaezealandiae | ○ ○ ○ ○ ○ |             |             |             |             | AF342794 AF342791–93 |
| Tardigrada                  | Milnesium tardigradum    | ○ ○ ○ ○ ○           |             |             |             |             | U49909 -         |
|                             | Milnesium sp.           | ○ ○ ○ ○ ○           |             |             |             |             | - AY210826      |
were subsequently divided into domains by eye. Domain sequences were realigned individually with MAFFT software using the L-INS-i option (Additional files 1, 2, 3 and 4). Alignment-ambiguous positions were removed with TrimAl software [34] in "strict setting," and all positions bearing gaps were also removed. The trimmed domain sequences were recombined to form the final dataset for analysis (Additional files 5, 6, 7 and 8), which was 1426 bp long for 18S and 2189 bp long for 28S in "18S + 28S (50OTU)," 18S (50OTU), and 28S (50OTU), 1277 bp long for 18S in 18S (65 OTU), and 1302 bp long for 18S in 18S (63 OTU). The chi-square test in Kakusan4 [35] indicated that the base composition of each dataset was significantly homogeneous.

Before the analyses, the optimal substitution model was determined with Kakusan4 to be the general time-reversible model with the gamma distribution (GTR + Γ). Phylogenetic trees were constructed by maximum likelihood (ML) implemented in raxmlGUI 1.2 [36, 37], and Bayesian inference (BI) implemented in MrBayes 3.2.1 [38, 39]. Nodal support for the ML tree was assessed through analyses of 1000 bootstrap pseudoreplicates. For BI, Markov-chain Monte-Carlo searches were performed with four chains, each of which was run for 1,000,000 generations, with trees sampled every 100 generations. Stationarity was evaluated by monitoring likelihood values graphically. The initial 20 % of trees from each run were discarded as burn-in, and the remaining trees were used to construct majority-rule consensus trees.

### Table 1 List of taxa included in each dataset (Continued)

| Domain       | Taxon                          | GenBank Accession Numbers |
|--------------|--------------------------------|----------------------------|
| Nematoda     | Spiurina *Ascaris lumbricoides* | o o o o o o U94366 AY210806 |
|              | Dorylaimia *Trichinella spiralis* | o o o o o o U60231 AF342803 |
|              | Enoplia *Pantonema vulgare*   | o o o o o o AY210845       |
|              | Desmodorida *Spinacia elongata* | o o o EFS27426             |
|              | Monhysterida *Theristus agilis* | o o AY284095              |
| Nematomorpha | *Chordodes morgani*           | o o o o o o AF362787       |
|              | *Gordius aquaticus*           | o o o o o o X80233 AY210817 |
|              | *Nectonema agile*             | o o o AF421767             |
| Priapulida   | *Priapulus caudatus*          | o o o o o o Z80009 AY210840 |
|              | *Halicyptus spinulosus*       | o o o o o o AF342790 AF342789 |
|              | *Tubiluchus carliclicola*     | o o o AF119086             |
|              | *Metapiapulus fijensis*       | o o o JN211192             |
| Kinorhyncha  | *Pycnophyes sp.*              | o o o o o o AY210808       |
|              | *Dracoderes abei*             | o o o AB738350 AB738351    |
|              | *Echinoderes dujardinii*      | o o o LC070044 LC070065    |
|              | *Centroderes spinosus*        | o o o KF27858              |
|              | *Campyloderes cf. vanhoefeni* | o o o LC07037              |
| Lophotrochoza (Outgroup) | *Amphiporus sp.* | o o o o o o AF119077 AF342786 |
| Mollusca     | *Placomecten magellanicus*    | o o o o o o X53099 AF342798 |
|              | *Platychelmintes*             | o o o o o o AF342801 AF342800 |
|              | *Echiura*                     | o o o o o o AF342805 AF342804 |
| Deuterostomes (Outgroup) | *Psychroderes fava* | o o o o o o AF278681 AF212176 |
| Chordata     | *Ciona intestinalis*          | o o o o o o AB013017 AF212177 |

*Taxa included in each data set, with GenBank accession numbers for sequences*
and determine the Bayesian posterior probability for each clade [39].

Results and discussion

Overall topology in Ecdysozoa

None of the trees conflicted with the others in their overall topology; however, supporting values were lower in datasets with more OTU and shorter sequences (Table 2; Additional files 9, 10, 11 and 12). In our results, increasing the available sequence length with slightly limited taxa generated a better-resolved tree than using more taxa with markedly shortening the sequence length. Thus, we present and mainly discuss the result of 18S + 28S (50 OTU) dataset (Fig. 2). Both the ML and BI trees showed monophyly for the Ecdysozoa (nodal support ML/PP = 99/1.00) as well as for the phyla Priapulida (100/1.00), Nematoda (99/1.00), Nematomorpha (100/1.00), and Arthropoda (89/1.00). Although the monophyly of each phyla were not tested for Kinorhyncha, Loricifera, and Tardigrada in 18S + 28S (50 OTU) dataset, they were supported in 18S (65 OTU) and 18S (63 OTU) with the maximum supporting values (Table 2). Monophyly for Onychophora was not tested due to the inclusion of a single representative of the phylum in all datasets.

Within the Ecdysozoa, two basal clades were detected with high nodal support: Priapulida + Kinorhyncha (Scalidophora, excluding Loricifera; nodal support 100/1.00) and Nematoda + Nematomorpha + Loricifera + Tardigrada + Onychophora + Arthropoda (99/1.00). The latter basal clade in turn comprised the clades Nematoda + Nematomorpha clade (= Nematoida), and Loricifera + Tardigrada + Onychophora + Arthropoda clade (= Loricifera + Panarthropoda) in both the ML and BI trees. Support for the Nematoida clade was only moderate (71/0.90), and that for Loricifera + Panarthropoda clade was low (63/0.66). Support for the monophyly of Tardigrada + Onychophora + Arthropoda (= Panarthropoda) was also low (54/0.76). Tardigrada, Onychophora, and Arthropoda formed an unresolved trichotomy.

Phylogenetic evaluation of loricifera, scalidophora, and cycloneuralia

The clade we detected consisting of Loricifera, Nematoida, and Panarthropoda received high nodal support (96/1.00), but the phylogenetic position of Loricifera within this clade remains unclear, as support for the node grouping Loricifera with Panarthropoda was quite low (63/0.66). However, the scalidophoran phyla Priapulida and Kinorhyncha together comprised a clade with high nodal support (100/1.00) to the exclusion of Loricifera, which instead grouped in a highly supported (96/1.00) clade with Nematoida and Panarthropoda. Our results thus do not support both the ‘Scalidophora hypothesis,’ in which Loricifera comprises a clade with Kinorhyncha and Priapulida, and the ‘Loricifera + Nematomorpha

| Table 2 Summary of the results of each dataset |
|-----------------------------------------------|
| **Clade** | **supporting value (ML/BI)** |
|          | 18S + 28S (50 OTU) | 28S (50 OTU) | 18S (50 OTU) | 18S (65 OTU) | 18S (63 OTU) |
|---------|---------------------|-------------|-------------|-------------|-------------|
| Ecdysozoa | 99/1.00            | 71/0.99     | 94/1.00     | 89/1.00     | 88/1.00     |
| Priapulida + Kinorhyncha | 100/1.00             | 96/1.00     | 89/1.00     | -/0.93      | 76/0.99     |
| Nematoida + Loricifera + Panarthropoda | 96/1.00             | 72/0.99     | -/0.90      | -/-         | -/-         |
| Nematoida | 71/0.91            | 50/-        | -/0.91      | -/-         | -/-         |
| Loricifera + Panarthropoda | 63/-                | 75/0.95     | -/-         | -/-         | -/-         |
| Panarthropoda | 54/-               | 54/-        | -/-         | -/-         | -/-         |
| Priapulida | 100/1.00            | 100/1.00    | 96/1.00     | -/-         | 98/1.00     |
| Kinorhyncha | -/-                | -/-         | -/-         | 100/1.00    | 100/1.00    |
| Nematod | 99/1.00            | 85/1.00     | 91/1.00     | 77/1.00     | 61/0.99     |
| Nematomorpha | 100/1.00            | 100/1.00    | 100/1.00    | 96/1.00     | 95/1.00     |
| Loricifera | -/-                 | -/-         | -/-         | 100/1.00    | 100/1.00    |
| Tardigrada | -/-                | -/-         | -/-         | 100/1.00    | 100/1.00    |
| Arthropoda | 89/1.00            | 82/1.00     | -/-         | -/-         | -/-         |
| Nematoda + Tardigrada + Arthropoda | -/-            | -/-         | -/-         | -/0.98      | -/0.91      |
| Tardigrada + Arthropoda | -/-                | -/-         | -/0.90      | -/-         | -/-         |
| Nematoda + Tardigrada | -/-            | -/-         | -/-         | -/-         | -/0.93      |

Summary of the results of analyses based on each dataset. Reconstructed clades with supporting values (maximum-likelihood bootstrap/Bayesian posterior probability) in each dataset are listed. Supporting values lower than 50 % (bootstrap values) or 0.90 (posterior probability) are considered as nonsignificant and indicated by dashes. Dark highlighted clades are supported only in Bayesian tree of short-sequence datasets, 18S (50 OTU), 18S (65 OTU), and 18S (63 OTU), thus these clades are not regarded as actual clades.
hypothesis’. Our trees also indicated non-monophyly for Cycloneuralia, as Loricerida and Nematoida showed closer relationships to Panarthropoda than to other cycloneuralian phyla (Priapulida and Kinorhyncha).

Evaluation of synapomorphies for scalidophora and cycloneuralia
Morphological synapomorphies have previously been proposed that uniting the scalidophoran phyla (Loricifera, Priapulida and Kinorhyncha) and the cycloneuralian phyla (Scalidophora plus Nematoda and Nematomorpha). Putative synapomorphies [11] among Loricerida, Priapulida, and Kinorhyncha include (1) an introvert that has short, spinose scalids that are staggered in arrangement and triradiate in cross-section, and that has (2) inner and outer retractor muscles; (3) a compound filter of protonephridia consisting of two or more terminal cells; (4) basally thickened cuspidate spines; and (5) sensory organs (flosculi) with external cuticular micropapillae and a central pore. The most important synapomorphy proposed for cycloneuralians is the collar-shaped circumoral brain consisting of a ring neuropil [20, 21]. Our results failed to support the monophyly of either Scalidophora or Cycloneuralia, and the putative synapomorphies supporting these groups thus need to be reevaluated.

With regard to the monophyly of Loricerida + Nematoida + Panarthropoda that we detected, three possible topologies among these groups (Fig. 3) in turn suggest two possible
Conclusions
We reconstructed the phylogeny of ecdysozoan phyla using nearly complete 18S and 28S rRNA gene sequences, and our results suggested a new hypothesis for the phylogenetic position of Loricifera. These results did not support the previously proposed ‘Scalidophora’ or the ‘Loricifera + Nematomorpha’ clades, but detected a ‘Loricifera + Nematoida + Panarthropoda’ clade with rather high nodal support. Cycloneuralia emerged as paraphyletic, with high nodal support. Relationships among phyla in the ‘Loricifera + Nematoida + Panarthropoda’ clade were not well resolved, and phylogenetic analysis using transcriptomic or genomic data will be necessary to reconstruct the relationships within this clade, and to elucidate evolutionary transitions within Ecdysozoa.

Availability of supporting data
The data sets supporting the results of this article are included within the article and its additional files.

Additional files

Additional file 1: Raw 18S sequence alignment for 18S + 28S (50 OTU) and 18S (50 OTU) datasets. Aligned 18S sequences from 50 species (44 ecdysozoan and six outgroup species) before the removal of alignment-ambiguous positions and gaps.

Additional file 2: Raw 28S sequence alignment for 18S + 28S (50 OTU) and 28S (50 OTU) datasets. Aligned 28S sequences from 50 species (44 ecdysozoan and six outgroup species) before the removal of alignment-ambiguous positions and gaps.

Additional file 3: Raw 18S sequence alignment for 18S (65 OTU) dataset. Aligned 18S sequences from 65 species (59 ecdysozoan and six outgroup species) before the removal of alignment-ambiguous positions and gaps.

Additional file 4: Raw 18S sequence alignment for 18S (63 OTU) dataset. Aligned 18S sequences from 63 species (57 ecdysozoan and six outgroup species) before the removal of alignment-ambiguous positions and gaps.

Additional file 5: Final 18S sequences for 18S + 28S (50 OTU) and 18S (50 OTU) datasets. Aligned 18S sequences of 50 species after the removal of alignment-ambiguous positions and gaps.

Additional file 6: Final 28S sequences for 18S + 28S (50 OTU) and 18S (50 OTU) datasets. Aligned 28S sequences of 50 species after the removal of alignment-ambiguous positions and gaps.

Additional file 7: Final 18S sequences for 18S (65 OTU) dataset. Aligned 18S sequences of 65 species after the removal of alignment-ambiguous positions and gaps.

Additional file 8: Final 18S sequences for 18S (63 OTU) dataset. Aligned 18S sequences of 63 species after the removal of alignment-ambiguous positions and gaps.

Additional file 9: Maximum-likelihood tree of 18S (50 OTU) dataset. The tree is based on 18S (50 OTU) dataset. Labelling of values is as in Figure 2.

Additional file 10: Maximum-likelihood tree of 28S (50 OTU) dataset. The tree is based on 28S (50 OTU) dataset. Labelling of values is as in Figure 2.

Additional file 11: Maximum-likelihood tree of 18S (65 OTU) dataset. The tree is based on 18S (65 OTU) dataset. Labelling of values is as in Figure 2.

The most parsimonious scenario is that ‘scalidophoran’ characters arose independently in Loricifera and in the common ancestor of Priapulida + Kinorhyncha and represent convergent characters. Alternatively, if Loricifera is basal in the Loricifera + Nematoida + Panarthropoda clade (Fig. 3c), the most parsimonious interpretation is that the common ancestor of Ecdysozoa possessed ‘scalidophoran’ characters, which the common ancestor of Nematoida and Panarthropoda subsequently lost.

In all three topologies (Fig. 3), the most parsimonious evolutionary scenario for ‘cycloneuralian’ characters is that they originated once in the common ancestor of Ecdysozoa and were lost once in the common ancestor of Panarthropoda. In other words, the ‘cycloneuralian’ characters are plesiomorphic in ecdysozoans.
The tree is based on 18S (63 OTU) dataset. Labelling of values is as in Figure 2.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
H.Y. extracted the specimen, did the molecular laboratory work, and analyzed the data. S. F. identified the specimen to genus. H.Y., S. F., and K. M. discussed the results and wrote the manuscript. All authors read and approved the final manuscript.

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