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Supplemental Information

Aplacophoran Mollusks Evolved from Ancestors with Polyplacophoran-like Features

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Inventory of Supplemental Information

Supplemental Experimental Procedures: This provides a detailed description of the methods applied in this study.

Supplemental References: This includes the one reference cited in the Supplemental Experimental Procedures.

Supplemental Information

Supplemental Experimental Procedures

Animal Cultures

Adult *Wirenia argentea* Odhner, 1921 were extracted from sediment samples collected with a hyperbenthic sled at 180-220m water depth on muddy seafloor in Hauglandsosen (Bergen, Norway). Adult *Leptochiton asellus* (Gmelin, 1791) were collected from coarse gravel, cobbles and boulders dredged at 80m water depth at Liholmsrenna (Bergen, Norway). Adult *W. argentea* produced clutches of fertilized eggs which were reared at 7° C in Millipore-filtered and UV-treated seawater at the wet lab facilities of Bergen University (see also [S1]). Embryos and larvae of *L. asellus* were treated equally.

Fixation, Muscle Labeling, Microscopy and Imaging

Larvae were relaxed for 20 minutes at 4°C by adding 3.2 % $MgCl_2$ and subsequently fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (PB; pH = 7.3) for 2 hours at room temperature (RT). Thereafter, the specimens were washed three times in 0.1 M PB and then stored in 0.1 M PB containing 0.1% NaN_3. The specimens were decalcified in 50 mM EGTA for 2 hours prior to staining. For fluorescence labeling of the musculature, Alexa Fluor 488 phalloidin (Invitrogen, Molecular Probes, Eugene, OR, USA) was applied to the samples overnight at RT as a 1:40 dilution in 0.1 M PB with 2% Triton X-100 (PBT). The specimens were then rinsed 3 times in PB and mounted in Fluoromount G (Southern Biotech, Birmingham, AL, USA) between two coverslips. Myogenesis was investigated in detail from early larval stages through metamorphosis for both *Leptochiton asellus* and *Wirenia argentea*,

but only crucial stages are depicted herein. Of these, 10 individuals of *L. asellus* late-stage larvae and eight specimens of early-stage as well as 15 specimens of late-stage *W. argentea* larvae were scanned with a Leica TCS SP5 II confocal microscope (Leica, Wetzlar, Germany) with bidirectional mode, scan speed 200 Hz and system-optimized step size of 0.13 µm (*W. argentea*) or 0.17 µm (*L. asellus*) along the Z-axis. All specimens were scanned both from dorsal and from ventral to allow for detailed comparisons and rendered consistent results for each stage per species. The number of optical sections used for the 3D reconstructions presented herein ranged from 328 to 479 for either side, depending on the thickness of the specimens. All confocal image stacks were compiled with a resolution of 1024 x 1024 dpi. 3D reconstructions were generated from the confocal stacks using the isosurface rendering mode of the Imaris Imaging Software (Bitplane, Switzerland). For better visualization of the musculature, unspecific signal (from, e.g., spicule-secreting cells) was removed prior to the 3D reconstructions of Fig. 2. In order to distinguish individual muscle systems from each other, they were color-coded with 70% opacity by using Adobe Photoshop CS5 (Adobe Systems, San José, CA, USA).

Supplemental Reference

S1. Todt, C. and Wanninger, A. (2010). Of tests, trochs, shells, and spicules: Development of the basal mollusk *Wirenia argentea* (Solenogastres) and its bearing on the evolution of trochozoan larval key features. Front. Zool. *7*, 6.