A new piece in the puzzle for the riverine slugs of the Acochlidiidae (Panpulmonata: Acochlidia)

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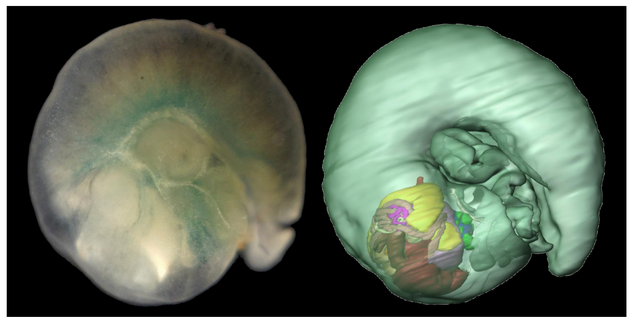
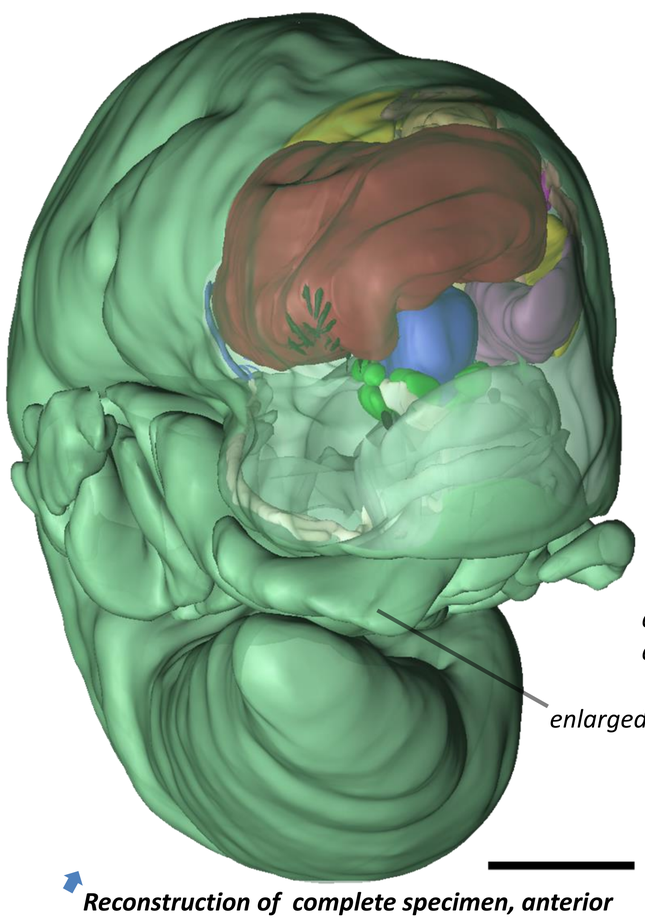
Introduction The Acochlidiidae are a family of unique freshwater slugs found in coastal streams of islands in the Indo-West Pacific. They are amphidromous, and specialized predators on the calcareous egg capsules of neritid snails. Compared to other mostly marine, meiofaunal Acochlidia, their body-size is huge[1,2].

A hitherto unknown species of blue-green acochlidiid was discovered in 2010 on the island of Ambon, Indonesia. Externally, it falls between two morphological clades of Acochlidiidae: the slender, uniformly reddish colored *Strubellia* and a second lineage with seemingly more derived, flattened and pigmented, brown or greenish *Acochlidium* and *Palliohedyle*. This intermediate position is confirmed by a phylogeny based on multilocus markers.

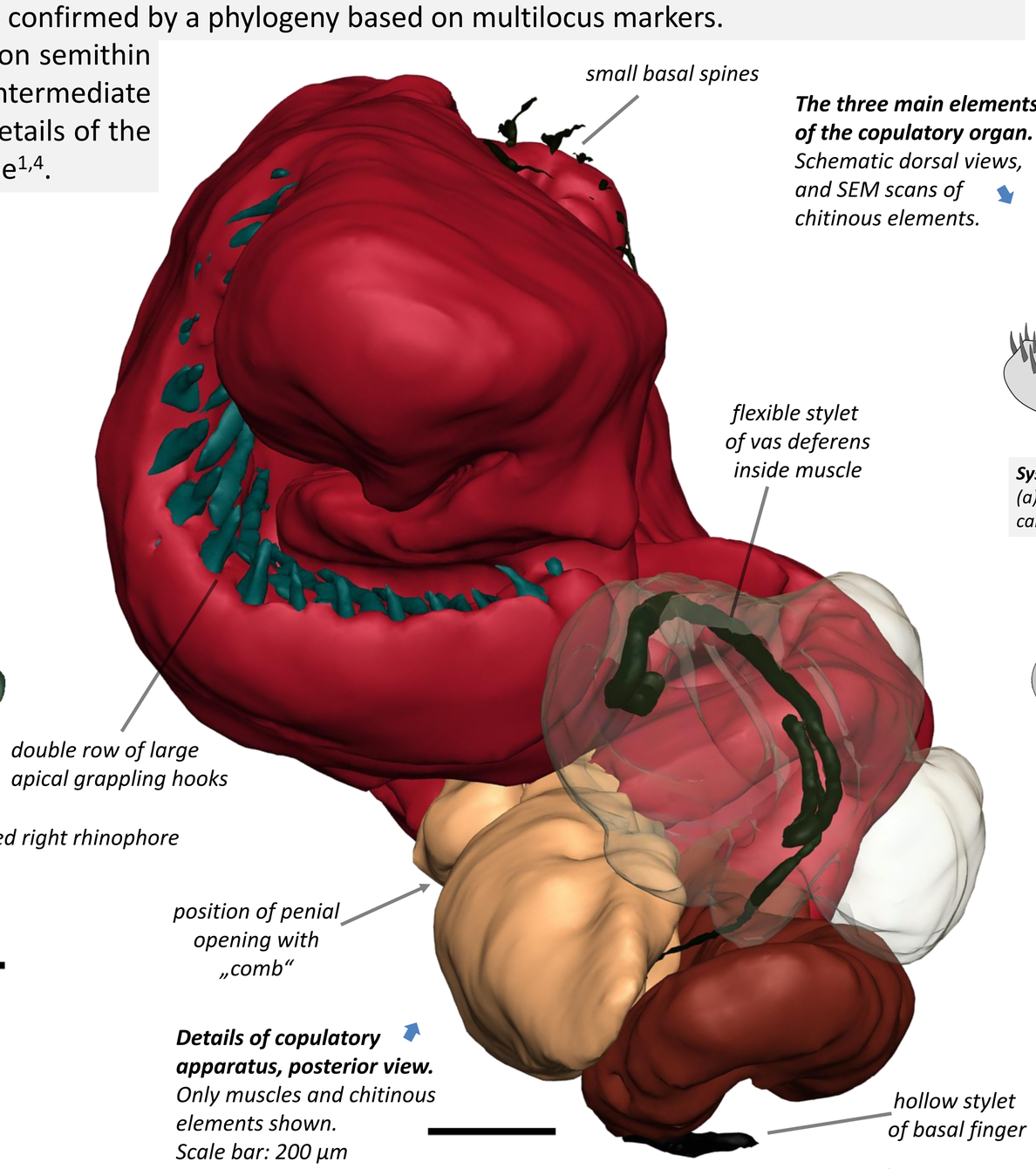
Three-dimensional reconstruction based on semithin histological sections shows further intermediate characters, and reveals so far unknown details of the complicated "rapto-penis" of Acochlidiidae[1,4].



Preserved 7-mm specimen *showing remnants of the original blue-green coloration. 3D reconstruction of same specimen at right.*

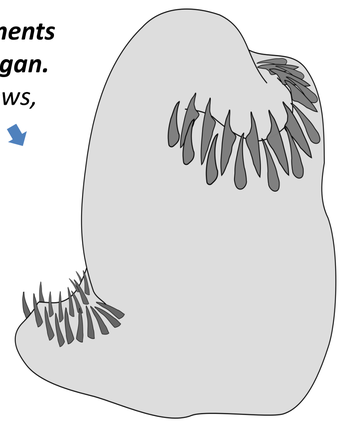


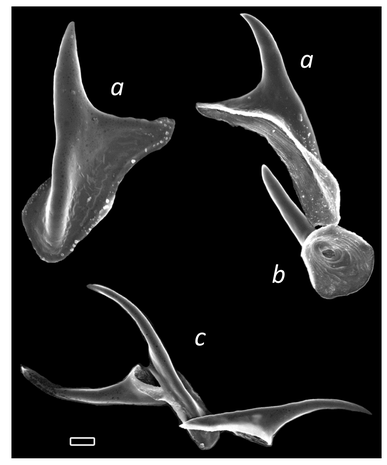
Reconstruction of complete specimen, anterior right view. *The copulatory organ with associated glands fills much of the anterior body. Scale bar: 500 µm.*



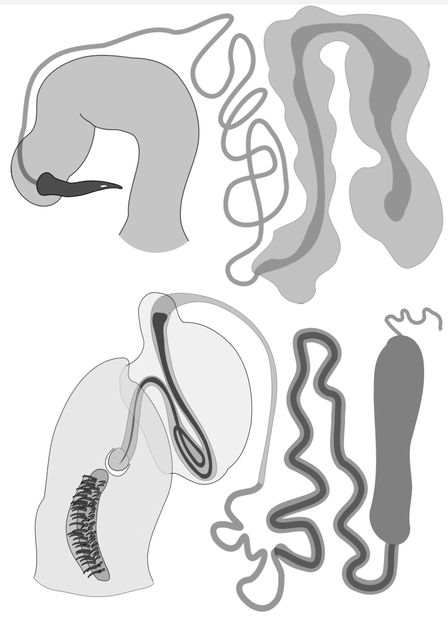
Details of copulatory apparatus, posterior view. *Only muscles and chitinous elements shown. Scale bar: 200 µm*

The three main elements of the copulatory organ. *Schematic dorsal views, and SEM scans of chitinous elements.*

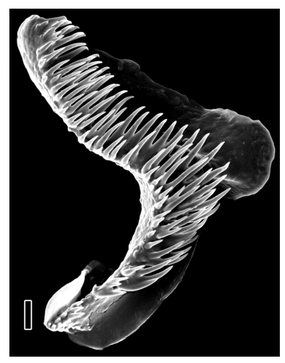




System 1: Grappling organ. *Likely the primary coupling device. Large hooks (a) form the inner, small ones (b) the outer apical row. The smaller spines (c) can be found at the base of the grappling organ. Bar: 20 µm.*

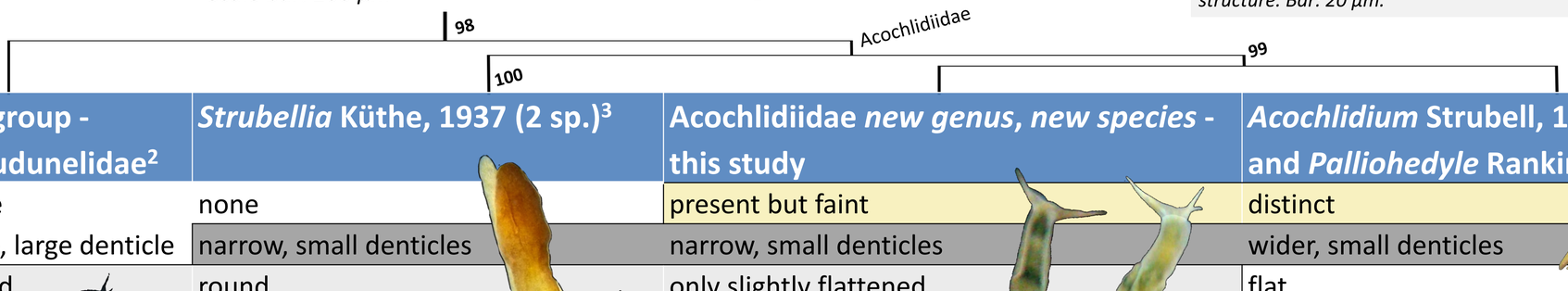


System 2: Basal finger. *Hollow apical stylet with associated paraprostate. Injection of fluid possibly precedes sperm transfer.*

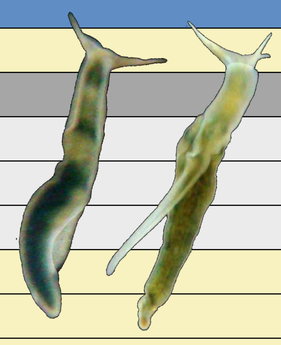


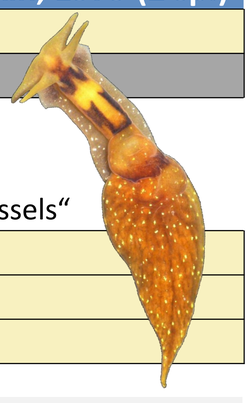
System 3: Penis. *Vas deferens with prostatic region and flexible terminal stylet embedded in muscle. The* ***penial comb*** *may act as a secondary coupling device, but also forms a gutter-like structure. Bar: 20 µm.*

Phylogeny of Acochlidiidae *(nodes show bootstrap values).* ***Comparison of anatomical features*** *among genera of freshwater Acochlidiidae and the marine, mostly mesopsammic outgroup. Size of specimens shown: 3-9 mm.*



	outgroup - Pseudunelidae[2]	*Strubellia* Küthe, 1937 (2 sp.)[3]	Acochlidiidae *new genus, new species* - this study	*Acochlidium* Strubell, 1892 (3 sp.)[1,4] and *Palliohedyle* Rankin, 1979 (2 sp.)
Pigment patterns on head	none	none	present but faint	distinct
Radula – rhachidian tooth	wide, large denticle	narrow, small denticles	narrow, small denticles	wider, small denticles
Visceral sac, cross-section	round	round	only slightly flattened	flat
Gonad and digestive gland	tubular	tubular	tubular	branched
Kidney, renopericardioducts *rpd*	single rpd	single rpd	single rpd	multiple rpd's, „dorsal vessels“
Bursa and vas deferens	bursa, vd closed	bursa, vas deferens an open groove	no bursa?, vd closed?	no bursa, vd closed
Grappling organ	not present	not present	present	present
Penial stylet	present	not present	present	present





Interpretation The new species possesses a copulatory organ similar to that of *Acochlidium*[4]. During copulation, the large grappling organ with two fields of chitinous hooks and spines probably works as primary attachment, after which the basal finger injects paraprostatic fluid by its stylet. Sperm transfer is via a long and flexible penial stylet.

The new species shows that in freshwater acochlidiids, the copulatory organ with additional grappling structure evolved before the flattened habitus with multiplied visceral structures (renopericardioduct funnels, dorsal vessels, digestive gland and ovotestis lobes). Therefore, sexual selection preceded ecological selection in the invasion of freshwater habitats by acochlidiids.

Material and Methods Two specimens were collected in 2010 in a stream close to the coast on the island of Ambon, Maluku Utara province, Indonesia. Specimens were fixed in ethanol (ZMB Mol. 193944, 193966). One specimen was embedded in epoxy resin, sectioned serially and reconstructed in 3D using the software Amira. The second specimen was used for SEM study of hard parts and DNA extraction. Phylogeny: a maximum-likelihood tree based on concatenated COI, 16S, 28S and 18S datasets (conducted with RAxML).

References [1]Schrödl M & Neusser TP. 2010. **Towards a phylogeny and evolution of Acochlidia (Mollusca: Gastropoda: Opisthobranchia).** *Zool J Linn Soc* 158:124-154. [2]Neusser TP, Jörger KM & Schrödl M. 2012. **Cryptic species in tropic sands - interactive 3D anatomy, molecular phylogeny and evolution of meiofaunal Pseudunelidae (Gastropoda, Acochlidia).** *PLoS ONE* 6(8): e23313. doi:10.1371/journal.pone.0023313. [3]Brenzinger B, Neusser TP, Jörger KM & Schrödl M. 2011. **Integrating 3D microanatomy and molecules: natural history of the Pacific freshwater slug Strubellia Odhner, 1937 (Heterobranchia: Acochlidia), with description of a new species.** *J Moll Stud* 77: 351–374. [4]Haase M & Wawra E. 1996. **The genital system of *Acochlidium fijiense* (Opisthobranchia: Acochlidioidea) and its inferred function.** *Malacologia* 38(1-2):143-151.