

Under (re-) construction: Evolution in Acochlidia (Gastropoda, Opisthobranchia)

Jörger, Katharina and Schrödl, Michael

Zoologische Staatssammlung München, Münchhausenstr. 21, 81247 München, Germany
Email: Katharina.Joerger@zsm.mwn.de, schroedl@zi.biologie.uni-muenchen.de



INTRODUCTION

The Acochlidia are a small group of opisthobranch gastropods in which a large variety of morphological and biological characters is combined. They are distributed worldwide and inhabit mainly the marine interstitial. A robust phylogenetic hypothesis will be the basis to explain the major events in acochlidian evolution such as the conquering of true limnic systems and the development of special reproductive features (i.e. secondary gonochorism – uniquely within the usually hermaphroditic opisthobranchs – and different methods of sperm transfer). Morphological analysis faces the problem of a high degree of convergent development due to adaptations to the similar (interstitial) habitat (such as i.e. the worm-shaped body and reduction of pigmentation and eyes). Thus, molecular markers – independent from ecological pressures – are needed to critically evaluate morphologically based phylogenetic hypothesis and enlighten the complex evolution of the Acochlidia. The present study aims to reconstruct the phylogeny of Acochlidia based on the nuclear 18S-sequence (complete) and the mitochondrial 16S and Col-sequences (partial) and presents a first preliminary molecular phylogeny.

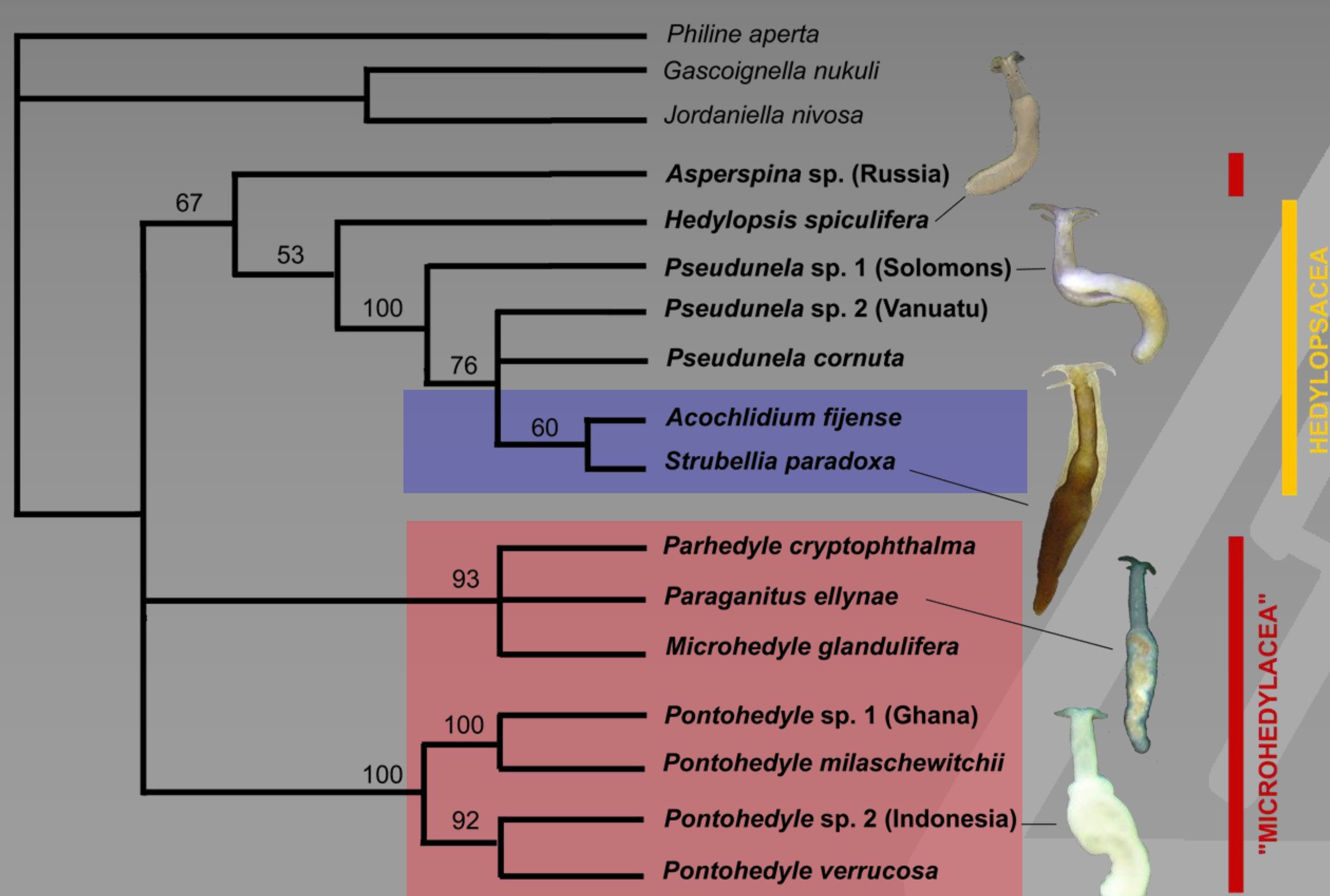


Figure 1: Strict consensus tree of the 10 most-parsimony trees based on the complete 18S sequence, bootstrap indices (50% majority rule) are given; showing the different acochlidian subgroups. Occurrence of secondary gonochorism highlighted in red; freshwater species shown in blue. 18S results confirm with the morphology-based hypothesis. But *Asperspina* is the sister clade to Hedylopsacea, rendering the basally unresolved Microhedylacea paraphyletic.

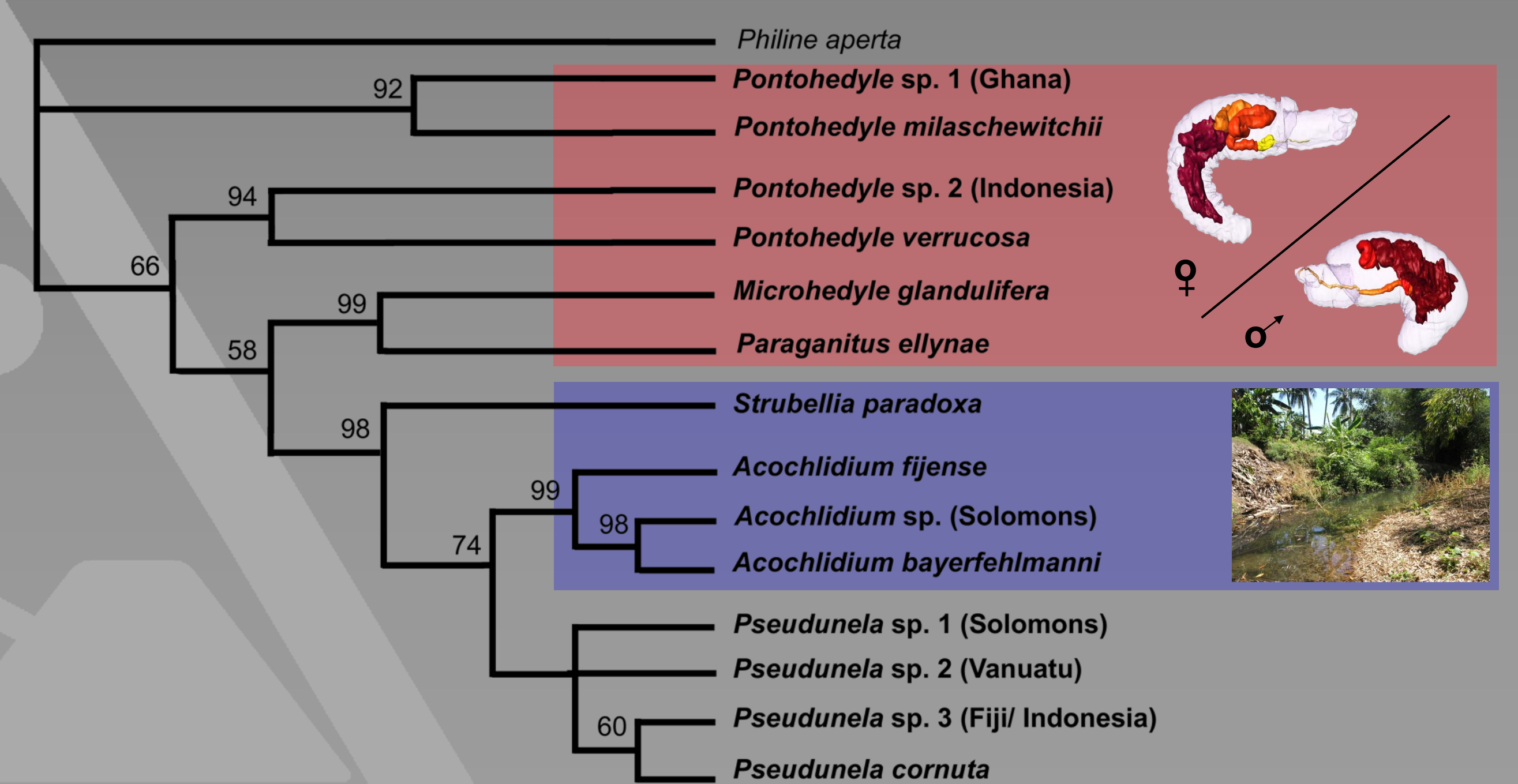


Figure 2: Strict consensus tree of the most-parsimony trees based on the combined 16S (partially) and Col-sequence (partially), bootstrap indices (50% majority rule) are given. Occurrence of secondary gonochorism highlighted in red; freshwater species shown in blue. The basal topology of this tree is incongruent with 18S and morphology-based phylogenies. Due to their high variability these markers seem to be inappropriate for the reconstruction of basal events in acochlidian evolution.

Conquering of limnic systems

In accordance with the morphology-based phylogeny the 18S results suggest that the invasion of freshwater occurred once in the Indopacific Acochliidae (see Fig. 1). Contradicting results based on 16S and Col markers (see Fig. 2) might be misleading due to the high variability of the markers. Results from morphology suggest a second independent colonisation of limnic systems in the enigmatic Caribbean *Tantulum elegans* (see Schrödl & Neusser, in press), not yet included in the present study.

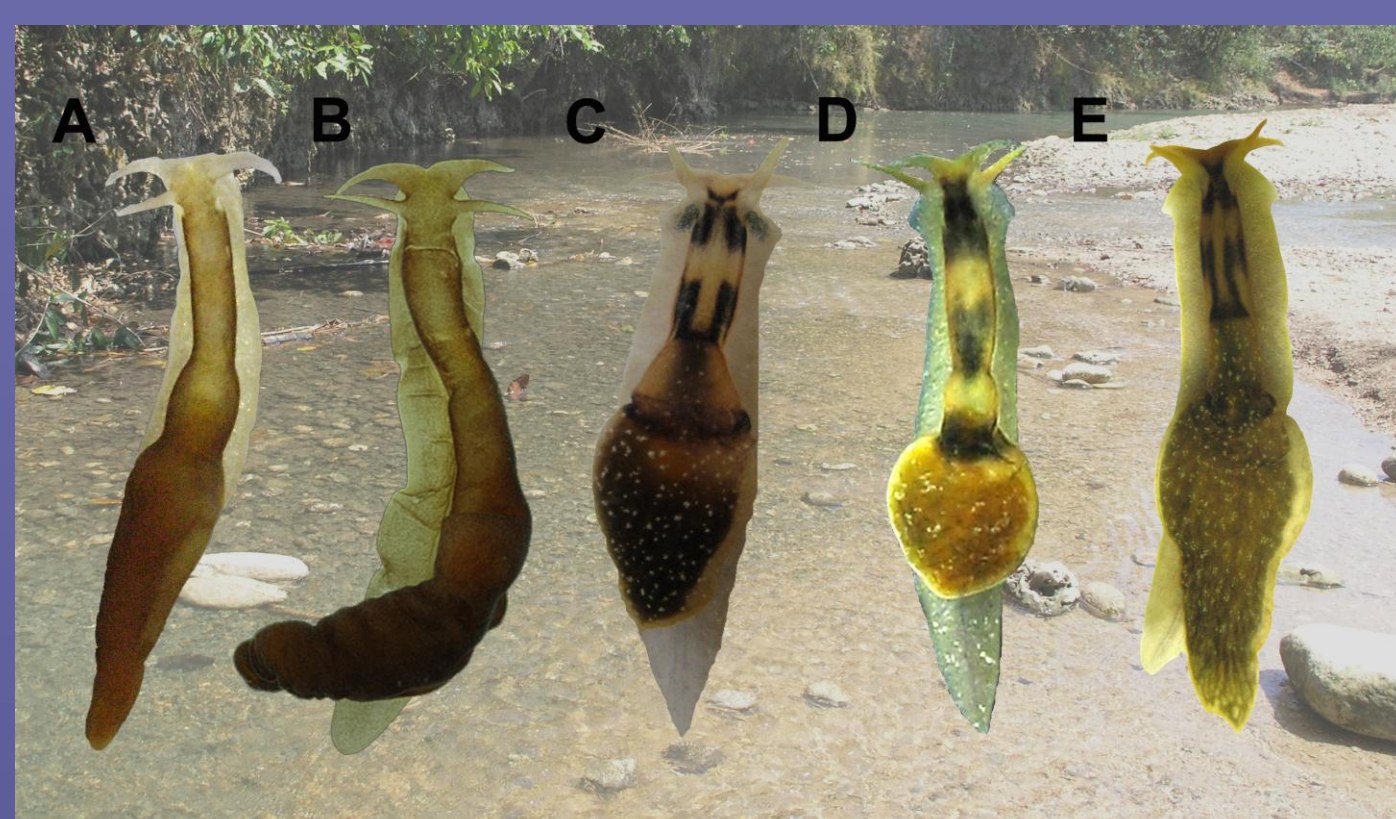


Figure 3: Radiation in limnic Acochliidae.
A *Strubellia paradoxa*
B *Strubellia* sp. (Vanuatu)
C *Acochlidium* sp. (Solomons)
D *A. fijense*
E *Palliohedyle sutteri*

The strong similarity in Col and 16S sequences of different populations of *Strubellia paradoxa* and *Palliohedyle sutteri* from different rivers and even neighbouring islands indicate a genetic exchange between populations, i.e. via marine larvae or a very recent radiation in the limnic systems.

DISCUSSION and OUTLOOK

- The presented mitochondrial markers reveal recent radiations and help to uncover cryptic species such as *Pseudunela* sp. 1 from the Solomons and *Pseudunela* sp. 3 from Fiji and Indonesia or *Pontohedyle milaschewitchii* and *Pontohedyle* sp. 2 from Indonesia, being identical concerning their external morphology. However, these markers – like in other Opisthobranchs – seem to be too variable to solve the basal nodes.
- The 18S results largely confirm morphological data (see Schrödl and Neusser, in press). Current genera such as *Pontohedyle* and even basal hedylopsacean nodes show at least some to very good statistical support, indicating that the 18S is a suitable marker for reconstructing acochlidian phylogeny from its likely origin in the mesozoic to quite recent radiations. Accomplishing a better outgroup and ingroup taxon sampling, complete 18S, together with the, in parts, similarly conservative 28S, are expected to uncover the evolutionary history of this group.

Evolution of reproductive features

Based on the preliminary 18S phylogeny secondary gonochorism might have evolved two times independently in two different Microhedylacean lineages. The seldom occurrence of secondary gonochorism in the animal kingdom, however, makes a unique evolution more likely. A combined analysis of the different genetic markers might lead to a better resolution in the basally unresolved Microhedylacea.

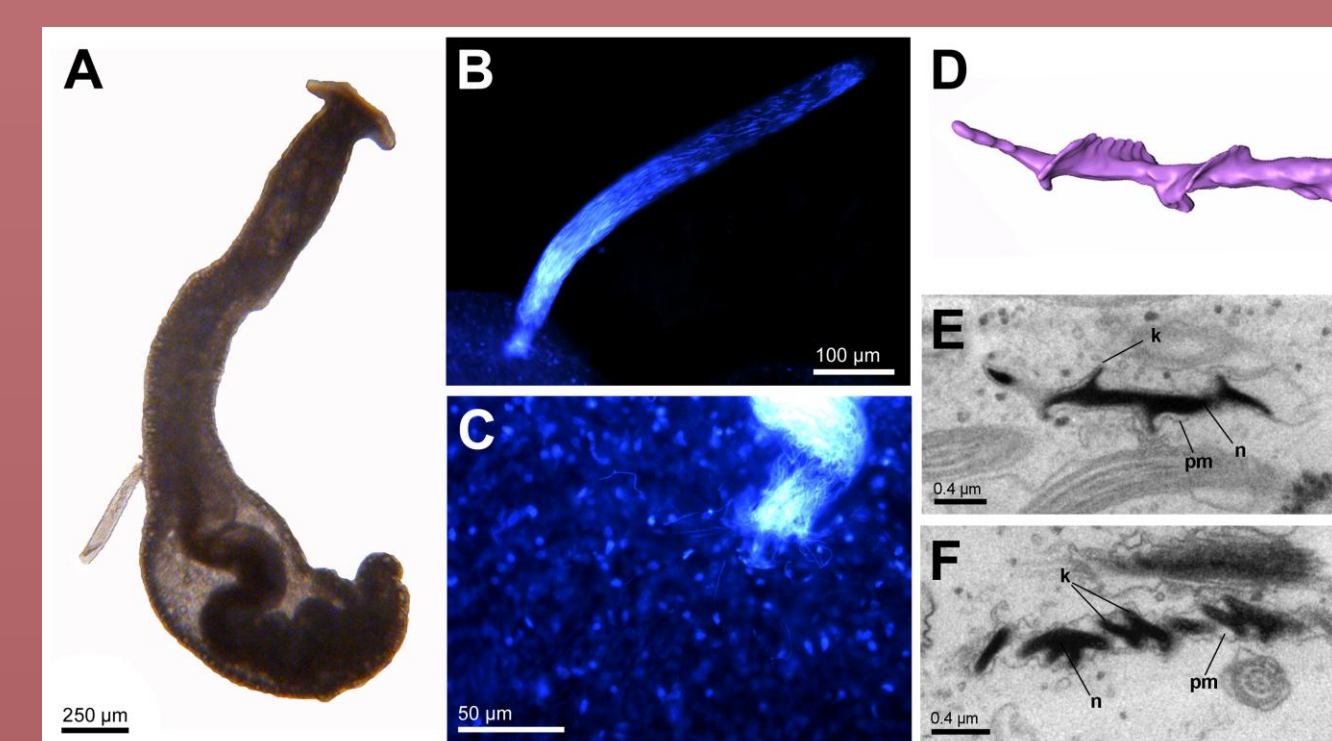


Figure 4: Sperm transfer via spermatophores in *Pontohedyle milaschewitchii*. A Specimen with attached spermatophore. B DAPI-staining of emptying spermatophore. C DAPI-staining of attachment site showing intruding sperm. D 3D reconstruction of the tip of the corkscrew-like sperm nucleus. E, F TEM-micrographs of sperm nucleus.

Microhedylacean species and supposedly the hedylopsacean *Hedylopsis ballantinei* are aphallic and transfer sperm via spermatophores combined with dermal fertilisation. The aphallic condition and this mode of sperm transfer might have evolved convergently as an adaptation to the, sparsely restricted, interstitial environment (see Jörger et al., in press).

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