



BYE BYE “OPISTHOBRANCHIA”!

A REVIEW ON THE CONTRIBUTION OF MESOPSAMMIC SEA SLUGS TO EUTHYNEURAN SYSTEMATICS

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ABSTRACT

During the last decades, textbook concepts of “Opisthobranchia” have been challenged by morphology-based and, more recently, molecular studies. It is no longer clear if any precise distinctions can be made between major opisthobranch and pulmonate clades. Worm-shaped, mesopsammic taxa such as Acochlidia, Platyhedylidae, Philinoglossidae and Rhodopemorpha were especially problematic in any morphology-based system. Previous molecular phylogenetic studies contained a very limited sampling of minute and elusive meiofaunal slugs. Our recent multi-locus approaches of mitochondrial COI and 16S rRNA genes and nuclear 18S and 28S rRNA genes (“standard markers”) thus included representatives

of most mesopsammic “opisthobranchs” within a comprehensive euthyneuran taxon set.

The present study combines our published and unpublished topologies, and indicates that monophyletic Rhodopemorpha cluster outside of Euthyneura among shelled basal heterobranchs, acteonids are the sister to rissoellids, and Nudipleura are the basal offshoot of Euthyneura. Furthermore, Pyramidellidae, Sacoglossa and Acochlidia cluster within paraphyletic Pulmonata, as sister to remaining “opisthobranchs”. Worm-like mesopsammic heterobranch taxa have clear independent origins and thus their similarities are the result of convergent evolution. Classificatory and evolutionary implications from our tree hypothesis are quite dramatic, as shown by some examples, and need to be explored in more detail in future studies.

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We do not claim that these concatenated “standard marker” gene trees reflect the true phylogeny of all groups; exploring additional, suitable markers is required. We do claim, however, that improved taxon sampling and improved data quality (such as sequences, alignments) were beneficial towards

revealing relationships of higher euthyneuran taxa, and that phylogenetic hypotheses based on this data set are converging. The traditional taxon concept of Opisthobranchia is clearly artificial and thus obsolete. Novel phylogenetic hypotheses, as disturbing they may be at first glance, give us the opportunity and perhaps the obligation to refine our approaches and rethink older paradigms. Most importantly, we see no more way to explore morphology, systematics and evolution of “opisthobranchs” separately from “lower heterobranchs” and “pulmonates”.

INTRODUCTION

Milne Edwards (1848) split the gastropods into Prosobranchia, Pulmonata and Opisthobranchia. The latter two taxa are usually combined as Euthyneura. Both researchers and amateurs easily associate opisthobranchs as marine slugs or snails, with a more or less reduced or internalized shell, having an almost bilaterally symmetrical body and either a head shield or head tentacles, whereas pulmonates appear almost exclusively related to limnic and terrestrial habitats. Unconventional taxa such as interstitial worm-like forms, limnic opisthobranchs and marine pulmonates occur, but are obviously too exceptional to challenge the practical value of the traditional Opisthobranchia-Pulmonata concept. The often beautifully coloured and bizarrely shaped approx. 6000 opisthobranch species thus are treated as belonging to a clade in virtually all older field guides and zoological textbooks (e.g. Westheide & Rieger, 2007), current molluscan classifications (e.g. Bouchet & Rocroi, 2005), and reviews (e.g. Schmekel & Portmann, 1982, Schmekel, 1985, Rudman & Willan, 1998), including the most recent one by Wägele *et al.* (2008) that was published within a compendium on molluscan phylogeny and evolution (Ponder & Lindberg, 2008). Recent comprehensive field guides on Caribbean and Indo-Pacific opisthobranchs, however, left monophyly open (Valdés *et al.*, 2006, Gosliner *et al.*, 2008).

There has always been a certain disagreement with regards to which major subtaxa should be included

into Opisthobranchia (Gosliner, 1981). Commonly accepted “core groups” are Cephalaspidea, Anaspidea, Thecosomata, Gymnosomata, Sacoglossa, Acochlidia, Tylodinoidea (=Umbraculida) and Nudipleura, the latter consisting of side-gilled Pleurobranchomorpha and Nudibranchia, which are the sea slugs in a strict sense. Some taxa with more or less well-developed helicoidal shells such as Acteonoidea (see Mikkelsen, 1996 vs. 2002) and Pyramidelloidea (e.g. Fretter & Graham, 1949) and the limpet-like Siphonarioidea have also occasionally been discussed as part of Opisthobranchia (see review by Wägele *et al.*, 2008). While the worm-like Rhodopemorpha were either seen as turbellarians or transitional forms between worms and gastropods in early approaches, most modern authors treated them as euthyneurans or integral part of opisthobranchs (e.g. Haszprunar & Heß, 2005).

Establishing the Heterobranchia concept, Haszprunar (1985, 1988) reconstructed an apomorphy-based phylogeny implying a progressive evolution from simple “allogastropod” (=“lower heterobranch”) taxa such as Valvatoidea, Architectonicoidea and Pyramidelloidea towards Pentaganglionata (=Euthyneura). Haszprunar’s phylogeny showed Acteonoidea (Architectibranchia) as the sister to monophyletic Pulmonata (including pentaganglionate Rhodopemorpha), which was itself the sister to remaining opisthobranchs (including vermiform Smeagolidae), rendering “Opisthobranchia” paraphyletic. Haszprunar thus was the first to phylogenetically infer and discuss the artificial nature of Opisthobranchia rather than comparing similarities and modifying the inclusiveness of the concept. Using cladistic analyses on a morphological dataset, Salvini-Plawén & Steiner (1996) recovered monophyletic Euthyneura, and Pulmonata plus Thecosomata as sister to remaining Opisthobranchia including Rhodopemorpha (as Rhodopida) as sister to equally shell-less and small-sized Acochlidia and Gymnosomata. Dayrat & Tillier (2002) found Pyramidelloidea within euthyneuran taxa and summarized an unresolved euthyneuran topology with

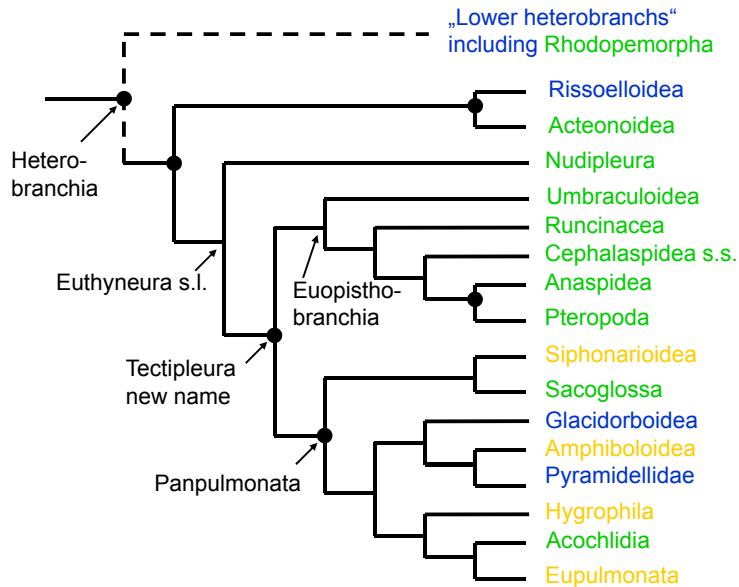


Figure 1:

“Opisthobranch” phylogeny as inferred from “standard genes” analyses, combining results by Jörger et al. (2010) and Wilson et al. (2010); robustly supported nodes (bootstrap support >75 and posterior probability >0.95) indicated by black dots. Taxa formerly regarded as opisthobranchs in green, pulmonate taxa in yellow, “lower heterobranch” taxa in blue. Note that the assemblage of “Lower heterobranchs including Rhodopemorpha” is paraphyletic but collapsed for illustrative purposes.

monophyletic Pulmonata arising as one of many clades from an opisthobranch grade of organization. An even more comprehensive morphology-based parsimony analysis by Wägele & Klussmann-Kolb (2005) showed Pteropoda (Gymnosomata plus Thecosomata) as sister to Pulmonata plus remaining Opisthobranchia, but this is contradicted by a more focused molecular study (Klussmann-Kolb & Dinapoli, 2006). In the study by Wägele & Klussmann-Kolb (2005) the remaining Opisthobranchia included a clade of exclusively interstitial (and/or small sized) cephalaspidean subtaxa, Rhodopemorpha and Acochlidia as sister to Sacoclossa, rendering Cephalaspidea polyphyletic. In the light of the latest morphology-based cladistic analysis focussing on Acochlidia (Schrödl & Neusser, 2010), such results are in doubt. While resolving inner relationships of Acochlidia quite nicely, other mesopsammic euthyneurans included, regardless their supposed affiliation, had a tendency to cluster

with Acochlidia; Schrödl & Neusser (2010) explained that by parallel concerted reductions of body-size and organs, but also by convergent evolution of vermiform bodies having a set of special organs as adaptations to a special habitat. Summarizing, 1) the Heterobranchia concept has always conflicted with a monophyletic Opisthobranchia, 2) no morphology-based analyses have recovered a monophyletic Opisthobranchia, 3) morphology-based analyses are misled by problems of interpreting morphological similarities and a generally high degree of parallelism (Gosliner, 1981, 1991); in particular, convergences displayed by small-sized slugs that occur in many subgroups may outnumber characters showing true phylogenetic signal, and thus lead to unreliable or completely wrong topologies.

Molecular markers, in contrast, offer an extremely large number of characters (via nucleotide sequences)

and many genes such as rRNA genes may not be directly influenced by habitat-specific ecological selective pressures. Early molecular approaches on opisthobranch phylogeny counted with single genes (partial 16S rDNA, Tholleson, 1999a,b, Wägele *et al.*, 2003; 18S rDNA, Wollscheid & Wägele 1999; partial 28S rDNA, Dayrat *et al.*, 2001), for relatively small sets of taxa. Whenever pulmonates were included in such analyses, opisthobranchs were not recovered as monophyletic unless the taxon definitions were extraordinarily modified. The same happened to the mitochondrial genome-based data sets of Grande *et al.* (2004a,b, 2008) and Medina *et al.* (2011). Vonnemann *et al.* (2005) were the first to combine the more conservatively evolving nuclear 18S and 28S rRNA gene fragments sequenced from a larger and more representative euthyneuran taxon set (including 3 different acochlidian species), recovering monophyletic Opisthobranchia as sister to potentially paraphyletic Pulmonata, but only in Maximum Parsimony analysis of the combined data set. Successively extending the taxon sampling to further pulmonate subgroups and especially to lower heterobranchs, using a combined set of mitochondrial CO1, 16S rRNA gene fragments, and nuclear 18S rRNA (complete) plus 28S rRNA genes (D1-3), and applying Maximum Likelihood algorithms became the standard for further analyses. None of the studies increasing in sophistication (e.g. Klussmann-Kolb *et al.*, 2008, Dinapoli & Klussmann-Kolb, 2010) recovered a monophyletic Opisthobranchia, usually due to acochlidian, but also sacoglossan and pyramidelloidean taxa clustering among pulmonates.

Since we failed to trace the origin of Acochlidia in morphology-based frameworks (Schrödl & Neusser, 2010), we carefully designed molecular studies including representatives of all the hard-to-find groups with interstitial slugs and all but one acochlidian families, plus all taxa that were mentioned to be potentially related to some of them (Jörger *et al.*, 2010, Wilson *et al.*, 2010). Special attention was paid to alignments and to the potential effect of ambiguous alignment portions, which were

masked and more or less rigorously removed by the programs Aliscore and Gblocks (see Jörger *et al.*, 2010 for details). The topology showing best likelihood resulting from Jörger *et al.* (2010) rejected all traditional hypotheses on the origin of Acochlidia, but indicate a pulmonate relationship of Acochlidia. In particular, tree hypotheses were considered as robust and reliable enough to propose a reclassification of Euthyneura, abandoning the taxon name and concept of Opisthobranchia.

The present paper combines results of Jörger *et al.* (2010), Dinapoli & Klussmann-Kolb (2010), Dinapoli *et al.* (2011) and some preliminary data on the origin of Rhodopomorpha (see Wilson *et al.*, 2010), and reviews and discusses the status of Opisthobranchia in the light of improving data sets and analytical methods. Finally, it gives some examples how new phylogenetic hypotheses affect old paradigms on opisthobranch evolution, and recommends facing the consequences of changing concepts.

Challenging the Opisthobranchia concept

Combining the results on the origin of Acochlidia by Jörger *et al.* (2010) with a preliminary analysis on the origin of Rhodopomorpha by Wilson *et al.* (2010) by hand shows a consensus topology (Fig. 1) that radically differs from traditional heterobranch classifications. Monophyletic Rhodopomorpha cluster among basal, shelled lower heterobranchs with high support; thus, based on molecular data, Rhodopomorpha are preliminary not related to any of the euthyneuran taxa or even to dorid nudibranchs as was suspected based on morphological data before. The Opisthobranchia are polyphyletic: Acteonoidea plus Rissoelloidea is the sister to Euthyneura, with Nudipleura as first euthyneuran offshoot. Pulmonates in a traditional sense are paraphyletic, including the “opisthobranch” clades Sacoglossa and Acochlidia, and the potential lower heterobranchs *Glacidorbis* and Pyramidellidae, and thus were called Panpulmonata by Jörger *et al.* (2010). The remaining opisthobranchs form a clade called Euopisthobranchia by Jörger *et*

al. (2010). Several, but not all of these nodes (Fig 1; see Jörger *et al.*, 2010) are robustly supported and the topologies slightly vary according to different analyses and parameters. Nevertheless, Opisthobranchia are not recovered monophyletic under any circumstances. In particular, worm-like sluggish opisthobranch taxa undoubtedly have independent origins and thus structural, functional and biological similarities evolved convergently due to selective pressure in an extreme habitat (Jörger *et al.*, 2010, Schrödl & Neusser, 2010, Wilson *et al.*, 2010). Bootstrap support and posterior probability values are high for most of the morphologically well-defined opisthobranch and pulmonate subclades usually treated as superfamilies or (sub)orders (collapsed to terminal taxa in Fig 1). Excluding the historically enigmatic Rhodopemorpha and Acteonoidea conceptually still results in paraphyletic Opisthobranchia at best, with Nudipleura as sister to all other euthyneurans, and both Sacoglossa and Acochlidia clustering among pulmonate taxa. Constraining the analyses of Jörger *et al.* (2010) towards monophyletic Opisthobranchia was highly significantly rejected based on their data. Excluding Acochlidia or Sacoglossa or both from an Opisthobranchia concept still does not render them monophyletic. Standard molecular markers clearly reject the monophyly of Opisthobranchia under any historic or reasonable taxon definition, and the topology (Fig. 1) differs from any morphology-based classifications, apomorphy-based reconstructions and, in particular, cladistic analyses that, thus, all were misled.

New trees, new truths?

By showing the non-monophyly of Euthyneura, Opisthobranchia and Pulmonata in a traditional sense, our standard marker based tree hypothesis (Fig. 1) is consistent to most previous molecular analyses available, regardless of using single genes, combinations of nuclear and mitochondrial genes or mitochondrial genomic data. More problematic than showing the deficiency of traditional classifications, however, is to present a convincing alternative: data

sets, methods used and resulting topologies may greatly differ depending on the data used and there is no way of a direct numerical evaluation of how reliably these trees reflect evolutionary history.

However, there is some evidence that the design and performance of molecular studies on heterobranchs evolved over time, and thus there is hope that some of the latest topologies are superior to previous ones. Early single gene analyses (e.g. Thollesson, 1999a,b) were limited by still poor taxon and character sampling, simplistic alignment tools and parsimony as a single optimization criterion. Studies using mitochondrial genes (Grande *et al.* 2004a,b) or mitochondrial genomes (Grande *et al.*, 2002, 2008, Medina *et al.*, 2011) also were based on inadequate and unrepresentative heterobranch taxon sampling, the signal to noise ratio of markers remains untested, and topologies still differ. Supplementing the landmark studies on combined 18S and 28S rRNA genes by Vonnemann *et al.* (2005) by further taxa and using the whole set of what we now call "standard" genes of Klussmann-Kolb *et al.* (2008) and Dinapoli & Klussmann-Kolb (2010), our current approaches (Jörger *et al.*, 2010, Wilson *et al.*, 2010) use a multi-locus set of a truly representative taxon sampling i.e. several lineages of lower heterobranchs, all previously recognized or suspected euthyneuran clades, and all the enigmatic interstitial target taxa in question are included, plus assumed relatives of Rhodopemorpha such as dorid nudibranchs and several runcinids. In addition, the few European acochlidian taxa used in previous analyses (e.g. Vonnemann *et al.* 2005, Dinapoli & Klussmann-Kolb, 2010) were shown to be highly derived ones; especially *Hedylopsis spiculifera*, but also the microhedylacean species *Pontohedyle milaschewitschii* and *Microhedyle glandulifera* showed long branches due to aberrantly evolved loci in comparison to other, more slowly evolving acochlidian species from other parts of the worlds oceans (Jörger *et al.*, 2010). Selecting a sufficient number of basal and slow-evolving taxa from old groups is clearly beneficial for minimizing branch

lengths and the effects of signal erosion (e.g. Wägele & Mayer, 2007). On the data quality side, state of the art procedures have been applied to minimize errors and noise, i.e. sequences were checked by BLAST searches and hypervariable regions of the alignments removed by masking programs, and only the most recent studies (e.g. Dinapoli & Klussmann-Kolb, 2010, Holznagel *et al.*, 2010, Jörger *et al.*, 2010, Dayrat *et al.*, 2011, Dinapoli *et al.*, 2011,) used both ML and Bayesian analyses, which is beneficial to reveal and control for effects of differential evolutionary rates among lineages (e.g. Paps *et al.*, 2009). While Holznagel *et al.* (2010) limited their study on partial 28S of an incomplete panpulmonate sampling, i.e. lacking Sacoglossa and Acochlidia, the more representative and comprehensive standard gene studies by Dinapoli & Klussmann-Kolb (2010) and Jörger *et al.* (2010) seem to converge towards a topology that is largely congruent to Fig. 1. We thus assume that this topology will be fairly robust to taxon addition. In particular, adding several more species of Pyramidellidae to the standard gene set, Dinapoli *et al.* (2011) already confirmed the Pyramidellidae as part of a common clade with *Glacidorbis* and Amphiboloidea. Göbbeler & Klussmann-Kolb (2010) showed that the node of Rissoelloidea and Acteonoidea is robust to adding representatives of all acteonoidean families.

Despite all these efforts to optimize taxon sampling, data quality, and alignment procedures, neighbor-net analyses by Dinapoli & Klussmann-Kolb (2010) and Jörger *et al.* (2010) show a still high level of conflict in the data, with split support for some groups only. Since none of the well-supported nodes in the tree is contradicted by the split analyses, we do not interpret this as general evidence against our tree but as a warning that the power of our standard marker set for resolving heterobranch evolution has its limitations. The topology shown herein (Fig. 1) needs to be tested and refined by a truly independent set of molecular markers showing a high signal to noise ratio and minimizing the risk of alignment artefacts, i.e. conservative, protein coding nuclear genes.

Violating morphology?

Our phylogenetic consensus hypothesis (Fig. 1) is based on a large and representative taxon sampling, and on alignments of several thousands of nucleotides; its major weakness is due to just 4 - and always the same - “standard genes” involved. However, most of the traditionally accepted heterobranch taxa on order or family level such as Nudipleura, Acochlidia, Sacoglossa, Eupulmonata and Ellobioidea were recovered as robustly supported lineages. These molecular results are congruent with morphology-based ideas, and thus are likely to represent the evolutionary history. This also implies that both morphology-based inference and standard genes are informative at least at these levels. What remains problematic are the interrelationships between such major clades that have just poorly supported and sometimes incongruent trees based on standard markers. There is no doubt that much of the conflict with previous morphology-based hypotheses (e.g. Wägele & Klussmann-Kolb, 2005 as the most comprehensive one) is due to misconceptions that based on misinterpretations of homology and on extreme levels of homoplasy in the latter, as already suspected by Gosliner (1981) and Gosliner & Ghiselin (1984). The best examples refer to mesopsammic, convergently evolved worm-like taxa (Fig. 1) all showing a similar set of reductions and innovations (e.g. Jörger *et al.*, 2010, Schrödl & Neusser, 2010), that are obviously adaptive to their special habitat. Moreover, at present, we are not able to present any conspicuous apomorphies for the recently established clades, except for Euopisthobranchia having evolved an oesophageal/gizzard cuticle (Jörger *et al.*, 2010). Morphology thus has to be re-examined carefully and *a priori* homology assumptions might have to be changed according to *a posteriori* relationships unravelled. On the other hand, even some of the most intriguing relationships proposed by recent molecular analyses (Dinapoli & Klussmann-Kolb, 2010; 2011; Jörger *et al.*, 2010) may fit within a morphological framework. *Glacidorbis* clusters within pulmonates,

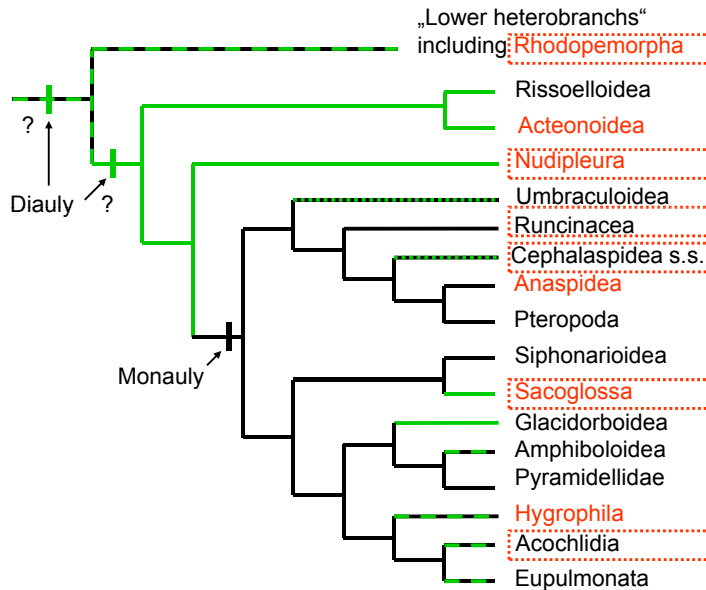


Figure 2:

Evolution of “opisthobranchs”. Taxa with interstitial members are framed in red; mesopsammic habitat is basal in Acochlidia and possibly Rhodopemorpha only; meiofaunal subclades, all more or less vermiform and showing an array of further adaptations, thus evolved many times independently among Heterobranchia. Taxa with at least one secure pentaganglionate stage known in at least a single species (see Dayrat & Tillie, 2000) are marked red; the only regularly pentaganglionate higher taxa may be Rhodopemorpha (juveniles only of *Rhodope*, adults of *Helminthope*) and Acteonoidea (adult). Stem lineages of taxa showing monaulic reproductive systems are colored black, those having androdialytic (including triaulic) conditions are green; clades with mixed states are broken black/green, and clades with just exceptional and/or non-basal androdialytic taxa are dotted black/green. Note that it is parsimonious to assume that monaulic evolved in the common, tectipleuran ancestor of Euopisthobranchia and Panpulmonata. If so, true androdialy (gonoducts split into oviduct and vas deferens proximal to the female gland mass) re-evolved in the stem lineages of Sacoglossa, Glacidorboidea and within several other panpulmonate subclades. Also note that a variety of structurally differing monaulic and dialytic conditions occur and that different authors use different terms; e.g. the special androdialytic condition occurring in some acochlidians is called monaulic by Valdés *et al.* (2010). In the light of novel phylogenetic hypotheses, the characters and evolution of heterobranchs need to be re-examined in much greater depth.

i.e. as sister of Amphiboloidea, as suggested by Ponder (1986), rather than being related with lower heterobranchs as proposed by Haszprunar (1985, 1988). The Pyramidelloidea *sensu stricto*, (i.e. all those Pyramidelloidea having a buccal stylet rather than a complex jaw apparatus as in Murchisonelloidea) is an integral part of Euthyneura even when comparing mitochondrial genomes (Grande *et al.*, 2008). This placement is supported by central nervous features such as the possession of giant nerve cells and a rhinophoral ganglion (see Huber, 1993). Siphonarian intertidal (or even fully marine) limpets were suggested to be opisthobranchs (Haller, 1892) or most basal pulmonates based on

their morphology (Hyman, 1967), which fits with their position as early panpulmonate descendants of an opisthobranch grade. As discussed by Jörger *et al.* (2010), morphological features usually suggested to be synapomorphic for pulmonates are either plesiomorphic, poorly explored, or of limited significance. Even more straightforward, accepting the proposed homology of the pulmonate procerebrum and opisthobranch rhinophoral ganglia (Haszprunar, 1988) that has received increasing evidence from results of several microanatomical studies (e.g. Huber, 1993, Neusser *et al.* 2007), there is not a single putative synapomorphy left for Opisthobranchia (Jörger *et al.*, 2010).

Summing up, it is the absence of contradiction, rather than unambiguous support, which makes the novel euthyneuran phylogenetic hypothesis presented by Jörger *et al.* (2010) and herein alluring. Still, the monophyly of Pulmonata and Opisthobranchia are clearly rejected by current knowledge (Fig. 1) and this fact cannot be longer ignored.

Consequences

Accepting the core topology presented here (Fig. 1), or just parts of it, has dramatic consequences for opisthobranch (and pulmonate) research.

First, neither “Opisthobranchia” nor “Pulmonata” can be retained as monophyletic taxa and thus have to be abandoned from our thinking and the literature. A reclassification has been proposed by Jörger *et al.* (2010) recently, modifying old names according to new concepts, i.e. Nudipleura as sister to a clade composed of Euopisthobranchia plus Panpulmonata; the latter, well-supported clade (Fig. 1) is named Tectipleura herein. In particular, polyphyletic “Opisthobranchia” do not even form a grade that can be characterized by any conspicuous set of plesiomorphies. Traditional “Opisthobranchia” thus are nothing else than an artificial assemblage of usually marine slugs or snails with limpet-like, bivalved or bubble shells showing tendencies of reduction or internalization, having a more or less detorted and externally bilateral symmetrical body with usually at least one pair of head tentacles or a head shield, including many exceptions. Rather than having a phylogenetic or evolutionary or even merely descriptive value, the “Opisthobranchia” concept is of historical and –to many of us– emotional value “only”.

Second, hypotheses on structures, functions or any other features, homology, character polarity, and evolution of opisthobranchs have to be reassessed in the light of new phylogenetic evidence. Some of the rampant parallelism assigned to Opisthobranchia is actually attributable to a taxon misconception, while

even higher levels of homoplasy are indicated e.g. by the independent origins of meiofaunal groups showing an array of independently derived features (e.g. Jörger *et al.*, 2010, Schrödl & Neusser, 2010). Intriguingly, basal Rhodopemorpha are one of the few taxa supposedly showing a pentaganglionate condition (in juveniles and/or adults), but, according to Figs. 1 and 2, are not part of the Pentaganglionata (=Euthyneura) *sensu* Haszprunar, a concept that has been criticized before (Dayrat & Tillier, 2000). Additionally, rhodopemorpha are euthyneurous slugs that are not part of Euthyneura (Fig. 2). The simple, monaulic condition of the reproductive system was taken for granted to be plesiomorphic for Opisthobranchia (e.g. Ghiselin, 1966, Gosliner, 1981, Valdés *et al.*, 2010). Structurally more complex diaulic conditions with separate male and female gonoducts were thought to have evolved from such a “primitive” level of organization, either as a single event or in multiple convergence (Valdés *et al.*, 2010), with the condition in pulmonates unclear (Wägele *et al.*, 2008). Widening the taxonomic focus and mapping monaulic and diaulic conditions on our novel topology (Fig. 2) may question these paradigms at least. It appears that (andro)diaulic evolved at least once already in the heterobranch stemline and was plesiomorphically retained in Nudipleura. Opisthobranch monaulic thus evolved at least once from a diaulic condition, possibly already in the common ancestor of Euopisthobranchia and Panpulmonata; monaulic may be a synapomorphy of Tectipleura. While basal clades of Euopisthobranchia are monaulic, a few androdialic taxa exist (“triaulic” *Anidolyta*, certain *Ringicula* spp; Valdés *et al.*, 2010), indicating secondary androdialic. Also, some secondary, more or less incomplete structural and functional subdivisions of gonoducts may occur in certain subtaxa, e.g. leading to a sometimes called “oodialic” system in Anaspidea (Gosliner, 1994) or some cephalaspidean genera (Rudman & Willan, 1998, Valdés *et al.*, 2010). The situation within panpulmonates is very complex showing a mosaic of (primary or secondary) monaulic and diaulic conditions in many major subgroups (Fig. 2),

implying much homoplasy involved. Androdiauly in panpulmonates is structurally heterogeneous, e.g. the vas deferens may split off the hermaphroditic duct in a proximal ("true androdiauly") or in more distal position ("special androdiauly", e.g. of some Acochlidia), and may run freely in the body cavity or in association to the body wall (as a "sunken" or "closed" sperm groove) (e.g., see Hubendick, 1978; Golding *et al.*, 2008; Schrödl & Neusser, 2010). Complex evolutionary scenarios proposed by Visser (1977, 1988) trusted on a direct descent of pulmonates from prosobranch ancestors that is, however, rejected by all modern phylogenetic results. The actual variation, homology and evolution of heterobranch genital systems clearly merit detailed comparative and integrative exploration. Even more fundamentally changing our view, rather than being a "crown group" the opisthobranchs including the diverse Nudipleura and Euopisthobranchia now may be considered as just moderately species rich and successful early offshoots of the panpulmonate stem line, leading to much higher ecological and species diversity therein (Fig. 1).

Third, and of practical importance, in future studies on traditional opisthobranch (or pulmonate) taxa it is no longer tenable to just define and use "Opisthobranchia" (or "Pulmonata") as an ingroup, as a taxon concept, or just as a point of reference, without proving its monophyly by using an adequate heterobranch taxon sampling. In simple words, there is no more way to study opisthobranchs without considering lower heterobranchs and pulmonates, and vice versa. Instead, the traditionally isolated research communities on basal heterobranch, opisthobranch or pulmonate taxa have to recognize that barriers are perceived rather than of a systematic nature; the earlier we combine our knowledge and efforts the better it is for furthering our branch of science.

Fourth: Yes, we now advocate for renaming the International Opisthobranch workshops as Heterobranch workshops, to bring people together!

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REFERENCES

- Bouchet P., Rocroi J.-P. (2005). Classification and nomenclator of gastropod families. *Malacologia*, 47: 1-397.
- Dayrat B, Conrad M, Balayan S, White TR, Albrecht C, Golding R, Gomes SR, Harasewych MG, de Frias Martins AM (2011). Phylogenetic relationships and evolution of pulmonate gastropods (Mollusca): New insights from increased taxon sampling. *Molecular Phylogenetics and Evolution*. doi: 10.1016/j.ympev.2011.02.014
- Dayrat B, Tillier S (2000). Taxon sampling, character sampling and systematics: how gradist presuppositions created additional ganglia in gastropod euthyneuran taxa. *Zoological Journal of the Linnean Society*, 129: 403–418.
- Dayrat B, Tillier S (2002). Evolutionary relationships of euthyneuran gastropods (Mollusca): a cladistic re-evaluation of morphological characters. *Zoological Journal of the Linnean Society*, 135: 403-470.
- Dayrat B, Tillier A, Lecointre G, Tillier S (2001). New clades of euthyneuran gastropods (Mollusca) from 28S rRNA sequences. *Molecular Phylogenetics and Evolution*, 19: 225-235.
- Dinapoli A, Klussmann-Kolb A (2010). The long way to diversity - phylogeny and evolution of the Heterobranchia (Mollusca: Gastropoda). *Molecular*

- Phylogenetics and Evolution, 55: 60-76.
- Dinapoli A, Zinssmeister C, Klussmann-Kolb A (2011). New insights into the phylogeny of the Pyramidellidae (Gastropoda). *Journal of Molluscan Studies*, 77: 1-7.
- Fretter V, Graham A (1949). The structure and mode of life of the Pyramidellidae, parasitic opisthobranchs. *Journal of the Marine Biological Association, United Kingdom*, 28: 493-532.
- Ghiselin MT (1996). Reproductive function and the phylogeny of opisthobranch gastropods. *Malacologia*, 3: 327-378.
- Göbbeler K & Klussmann-Kolb A. (2010). The phylogeny of the Acteonoidea (Gastropoda): Molecular systematics and first detailed morphological study of *Rictaxis punctocaelatus* (Carpenter, 1864). *Journal of Molluscan Studies* 76: 303-316.
- Golding RE, Ponder WF, Byrne M (2008). Novel copulatory structures and reproductive functions in Amphiboloidea (Gastropoda, Heterobranchia, Pulmonata). *Invertebrate Biology*, 127: 168-180.
- Gosliner TM (1981). Origins and relationships of primitive members of the Opisthobranchia (Mollusca: Gastropoda). *Biological Journal of the Linnean Society*, 16: 197-225.
- Gosliner TM (1991). Morphological parallelism in opisthobranch gastropods. *Malacologia*, 32: 313-327.
- Gosliner TM (1994). Gastropoda: Opisthobranchia. In *Microscopic Anatomy of Invertebrates, Mollusca I.*: Volume 5. Edited by: Harrison FW, Kohn AJ. Wiley-Liss, Inc.; pp 253-355.
- Gosliner TM, Behrens DW, Valdés Á (2008). Indo-Pacific Nudibranchs and Sea Slugs. A field guide to the World's most diverse fauna. *Sea Challengers Natural History Books & The California Academy of Sciences*. 426pp.
- Gosliner TM, Ghiselin MT (1984). Parallel evolution in opisthobranch gastropods and its implications for phylogenetic methodology. *Systematic Zoology* 33: 255-274.
- Grande C, Templado J, Cervera JL, Zardoya R (2002). The complete mitochondrial genome of the nudibranch *Roboastra europaea* (Mollusca: Gastropoda) supports the monophyly of opisthobranchs. *Molecular Biology and Evolution* 19: 1672-1685.
- Grande C, Templado J, Cervera JL, Zardoya R (2004a). Molecular phylogeny of the Euthyneura (Mollusca: Gastropoda). *Molecular Biology and Evolution*, 21: 303-313.
- Grande C, Templado J, Cervera JL, Zardoya R (2004b). Phylogenetic relationships among Opisthobranchia (Mollusca: Gastropoda) based on mitochondrial *cox 1*, *trnV*, and *rrnL* genes. *Molecular Phylogenetics and Evolution*, 33: 378-388.
- Grande C, Templado J, Zardoya R (2008). Evolution of gastropod mitochondrial genome arrangements. *BMC Evolutionary Biology*, 8: 61.
- Haller B (1892) Die Anatomie von *Siphonaria gigas* Less., eines opisthobranchiaten Gastropoden. Arbeiten aus den Zoologischen Instituten der Universität Wien und der Zoologischen Station in Triest, 10: 71-100.
- Haszprunar G (1985). The Heterobranchia - a new concept of the phylogeny of the higher Gastropoda. *Zeitschrift für zoologische Systematik und Evolutionsforschung*, 23: 15-37.
- Haszprunar G (1988). On the origin and evolution of major gastropod groups, with special reference to the Streptoneura. *Journal of Molluscan Studies*, 54: 367-441.
- Haszprunar G, Heß M (2005). A new *Rhodope* (Gastropoda: Nudibranchia?) from the Roscoff area (Bretagne), with a review of *Rhodope* species. *Spixiana*, 28: 193-197.
- Holznagel WE, Colgan DJ, Lydeard C (2010). Pulmonate phylogeny based on 28S rRNA gene sequences: A framework for discussing habitat transitions and character transformation. *Molecular Phylogenetics and Evolution*, 57: 1017-1025.
- Hubendick B (1978). Systematics and comparative morphology of the Basommatophora. In *Pulmonates*. 2A. V. Fretter and J.V. Peake, eds. J. London.; Academic Press, pp. 1-47.
- Huber G (1993). On the cerebral nervous system of marine Heterobranchia (Gastropoda). *Journal of Molluscan Studies*, 59: 381-420.
- Hyman LH (1967). *The Invertebrates. VI. Mollusca I.* New York: McGraw-Hill Book Co., New York.
- Jörger KM, Stöger I, Kano Y, Fukuda H, Knebelberger T, Schrödl M (2010). On the origin of Acochlidia and other enigmatic euthyneuran gastropods, with implications for the systematics of Heterobranchia.

- BMC Evolutionary Biology, 10: 323.
- Klussmann-Kolb A, Dinapoli A (2006). Systematic position of the pelagic Thecosomata and Gymnosomata within Opisthobranchia (Mollusca, Gastropoda) - revival of the Pteropoda. *Journal of Zoological Systematics and Evolutionary Research*, 44: 118-129.
- Klussmann-Kolb A, Dinapoli A, Kuhn K, Streit B, Albrecht C (2008). From sea to land and beyond—new insights into the evolution of euthyneuran Gastropoda (Mollusca). *BMC Evolutionary Biology*, 8: 57.
- Medina M, Lal S, Vallès Y, Takaoka TL, Dayrat BA, Boore JL, Gosliner TM (2011). Crawling through time: Transition of snails to slugs dating back to the Paleozoic, based on mitochondrial phylogenomics. *Marine Genomics*, 4: 51-59.
- Mikkelsen PM (1996). The evolutionary relationships of Cephalaspidea s.l. (Gastropoda: Opisthobranchia): a phylogenetic analysis. *Malacologia*, 37: 375-442.
- Mikkelsen PM (2002). Shelled opisthobranchs. *Advances in Marine Biology* 42: 67–136.
- Milne-Edwards H (1848). Note sur la classification naturelle chez Mollusques Gasteropodes. *Annales des Sciences Naturelles*, series 3, 9: 102-112.
- Neusser TP, Jörgen KM, Schrödl M (2007). Exploring cerebral features in Acochlidia (Gastropoda: Opisthobranchia). *Bonner Zoologische Beiträge*, 55: 301-310.
- Paps J, Baguña J, Riutort M (2009). Lophotrochozoa internal phylogeny: new insights from an up-to-date analysis of nuclear ribosomal genes. *Proceedings of the Royal Society B*, 276: 1245-1254.
- Ponder WF (1986). Glacidorbidae (Glacidorbacea: Basommatophora), a new family and superfamily of operculate freshwater gastropods. *Zoological Journal of the Linnean Society*, 87: 53–83.
- Ponder WF, Lindberg D (2008). *Phylogeny and Evolution of the Mollusca*. University of California Press, USA, 488 pp.
- Rudman WB, Willan RC (1998). Opisthobranchia, Introduction. In *Mollusca: The Southern Synthesis*. Vol. 5, Fauna of Australia. P. L. Beesley, G. J. B. Ross, and A. Wells, eds. Melbourne, CSIRO Publishing, pp. 915–942.
- Salvini-Plawen Lv, Steiner G (1996). Synapomorphies and plesiomorphies in higher classification of Mollusca. In: *Origin and evolutionary radiation of the Mollusca*. Taylor J., ed. Oxford, Oxford University Press, pp 29-51.
- Schmekel L (1985). Aspects of evolution within the opisthobranchs. In: *The Mollusca*. KM Wilbur, ed. Academic Press, London; pp. 221-267.
- Schmekel L, Portmann A (1982). *Opisthobranchia des Mittelmeeres, Nudibranchia und Saccoglossa*. Springer-Verlag, Berlin, 410 pp.
- Schrödl M, Neusser TP (2010). Towards a phylogeny and evolution of Acochlidia (Mollusca: Gastropoda: Opisthobranchia). *Zoological Journal of the Linnean Society*, 158: 124-154.
- Thollessen M (1999a). Phylogenetic analysis of dorid nudibranchs (Gastropoda, Doridoidea) using the mitochondrial 16S rRNA gene. *Journal of Molluscan Studies*, 65: 335-353.
- Thollessen M (1999b). Phylogenetic analysis of Euthyneura (Gastropoda) by means of the 16S rRNA gene: use of a 'fast' gene for 'higher-level' phylogenies. *Proceedings of the Royal Society of London Series B Biological Sciences*, 266: 75-83.
- Valdés Á., Hamann J, Behrens DW, DuPont A (2006). *Caribbean Sea Slugs. A field guide to the opisthobranch mollusks from the tropical northwestern Atlantic*. Sea Challengers, Gig Harbour, 289 pp.
- Valdés Á, Gosliner TM, Ghiselin MT (2010). Chapter 8. Opisthobranchs. In: *The Evolution of Primary Sexual Characters in Animals*. Leonard JL, Córdoba-Aguilar A, eds. Oxford University Press, pp. 148–172.
- Visser MHC (1977). The morphology and significance of the spermiduct and prostate in the evolution of the reproductive system of the Pulmonata. *Zoologica Scripta*, 6: 43–54.
- Visser MHC (1988). The significance of terminal duct structures and the role of neoteny in the evolution of the reproductive system of Pulmonata. *Zoologica Scripta*, 17: 239–252.
- Vonnemann V, Schrödl M, Klussmann-Kolb A, Wägele H (2005). Reconstruction of the phylogeny of the Opisthobranchia (Mollusca: Gastropoda) by means of 18S and 28S rRNA gene sequences. *Journal of Molluscan Studies*, 71: 113-125.
- Wägele H, Klussmann-Kolb A (2005). Opisthobranchia

(Mollusca, Gastropoda) - more than just slimy slugs. Shell reduction and its implications on defence and foraging. *Frontiers in Zoology*, 2: 1-18.

Wägele H, Klusmann-Kolb A, Vonnemann V, Medina M (2008). Heterobranchia I: The Opisthobranchia. In: *Phylogeny and Evolution of the Mollusca*. Ponder WF, Lindberg D, eds. University of California Press, Berkeley, pp. 385-408.

Wägele H, Vonnemann V & Wägele JW (2003). Toward a phylogeny of the Opisthobranchia. In: *Molecular systematics and phylogeography of mollusks*. C Lydeard, D Lindberg, eds. Smithsonian Institution Press, Washington, pp. 185-228.

Wägele JW, Mayer C (2007). Visualizing differences in phylogenetic information content of alignments and distinction of three classes of long-branch effects. *BMC Evolutionary Biology*, 7: 147.

Westheide W, Rieger R (2007). *Spezielle Zoologie. Teil 1: Einzeller und Wirbellose Tiere*. 2nd Ed. *Elsevier, Munich*, 982 pp.

Wilson NG, Jörger KM, Schrödl M (2010). Reducing an enigma: placing the vermiform Rhodopemorpha (Gastropoda) in a phylogeny [abstract]. *Tropical Natural History*, Suppl 3: 37.

Wollscheid E, Wägele H (1999). Initial results on the molecular phylogeny of the Nudibranchia (Gastropoda, Opisthobranchia) based on 18S rDNA data. *Molecular Phylogenetics and Evolution*, 13: 215-226.