

## Reflections

### Molluscan higher classification: why is molecular technology not resolving?

Luiz Ricardo L. Simone

Museu de Zoologia da Universidade de São Paulo  
[lrsimone@usp.br](mailto:lrsimone@usp.br); [lrsimone@gmail.com](mailto:lrsimone@gmail.com)  
ORCID: 0000-0002-1397-9823

#### Argumentation

As a teenager, full of ideologies and a desire to learn, I was enrolled in Medical School at the prestigious Faculdade de Medicina de Ribeirão Preto, University of São Paulo. It was 1982, and instead of studying human specimens, I had collected some local landsnails, bringing them to the Biochemistry Department in an attempt to do something highly technological and revolutionary. I had heard about comparing amino acids in proteins, isozymes, electrophoresis, and other advanced techniques. I wanted to apply these medical techniques to mollusks. When I shared my ideas with a professor, he advised me to "forget that; the easiest approach should be DNA sequencing – quicker, cheaper, easier, and the best for comparisons." Following his advice, we took the first steps towards DNA extraction from some local bulimulids. It was exciting. However, the demands of medical school were intense, preventing me from pursuing extra activities, and the malacological project was ultimately abandoned. Nevertheless, by the 1980s, DNA sequencing was already feasible and being used for comparisons, at least in non-mollusks.

Decades later, around 2001, with the conclusion of the Human Genome Project, I had already switched to Biology. I attended a lecture by one of the best Brazilian geneticists, Prof. Warwick Kerr. I remember his excitement about the project's completion. He said something like, "I don't understand the reluctance of physicians to get excited about this project, something that will make their diplomas obsolete." That assertion has stayed with me, especially during the COVID-19 pandemic when the entire world was confined by a simple virus, two decades later. Indeed, knowing human DNA sequencing has provided limited help, if any, in medicine.

Sequencing projects are often accompanied by grandiose scenarios and promises. This is understandable, as they must justify the significant resources invested in advanced technology. On one hand, DNA sequencing conveys an impression of precision—nothing is more detailed than breaking down an organism at the molecular level to demonstrate the meticulous nature of the research. On the other hand, DNA sequencing eliminates subjectivity, a factor that biologists have

often been embarrassed about. These factors practically elevate Biology to the level of the Exact Sciences, like Mathematics and Physics, shedding its reputation as a "second-class science," as some have accused it of being.

All these factors lead us to the question: why, after more than four decades, is the high-level classification of mollusks still a mess?

This does not imply that the lower-level classification of mollusks is any better, but that is not the focus of this paper.

By the end of the 20<sup>th</sup> century, the higher classification of mollusks was relatively stable. At that time, phylogenetic methodology, though late compared to other zoological branches, began to be practiced in the phylum. In the early 21<sup>st</sup> century, molecular approaches started to be applied with greater emphasis, and the confusion began.

An example of this confusion is the classification of Scaphopoda. Its clear relationship with Bivalvia, forming the Diasoma branch, was initially supported (e.g., Runnegar & Pojeta, 1974; Haszprunar, 2000). Gradually, other phylogenetic arrangements emerged as molecular studies progressed. One such arrangement grouped scaphopods with Cephalopoda (e.g., Steiner & Dreyer, 2003). Another placed scaphopods as a sister branch to a Cephalopoda+Gastropoda branch (e.g., Haszprunar, 2000). Additionally, some studies found scaphopods to be the sister group of Gastropoda (e.g., Smith et al., 2011; Kocot et al., 2020). More recently, the 'old' vision was revived (Song et al., 2023), placing Scaphopoda and Bivalvia as sister groups, i.e., the Diasoma. Interestingly, Song et al. (2023) devoted almost half of their paper to explaining why other molecular approaches yielded such disparate results. This is understandable, as the methodology itself cannot be discredited. In an attempt to provide a morphological perspective on this conceptual instability, Simone (2009) published a phenotypic-based analysis. That study included basal protobranch bivalves and representatives of both main branches of Scaphopoda (Gadilida and Dentaliida). The analysis supported a monophyletic Diasoma with 14 synapomorphies. Strangely, subsequent molecular studies have strategically overlooked that paper.

Beyond the instability of taxa, as illustrated by the examples above, there are taxa defined by molecular databases that lack morphological or other supporting evidence. Examples include Serialia, which groups Polyplacophora and Monoplacophora (e.g., Giribet et al., 2006), and Psilogastropoda, which combines Vetigastropoda and Patellogastropoda (e.g., Aktipis & Giribet, 2010; Cunha & Giribet, 2019).

Thus, the question remains: why has such a detailed, expensive, and objective method, which generates huge databases, not resolved taxonomy unequivocally?

Currently, no one has a definitive answer. Perhaps the vast databases are full of homoplasies, creating noise in the final results. Alternatively, the algorithms used, such as Bayesian or maximum-likelihood analysis, might not accurately translate biological phenomena. The uncertainties persist.

What we can presently do is compare mollusk classification with other zoological branches. The high-level classification of other groups, such as vertebrates and arthropods, is much more stable than that of mollusks. Any incongruence or inconsistency obtained with molecular approaches in these groups is treated as noise and is given little or no consideration.

What distinguishes mollusks from vertebrates and arthropods in this context? Simply put, it is the robust morphological framework underpinning their classifications.

Vertebrates and arthropods have a far more developed morphological foundation in their basic taxonomy compared to mollusks. This topic warrants a comprehensive exploration in another issue of *Malacopedia*. In essence, the vast majority of molluscan species are known primarily by their shells or, if shell-less, by external features alone. While the shell is an important structure, it does not provide insights into food capture, locomotion, respiration, reproduction, perception, and other traits crucial for phylogenetic inference. These characteristics are often documented in only a few species, typically described in 19<sup>th</sup>-century papers, and then extrapolated to entire higher taxa. Consequently, the morpho-anatomical blueprint of these higher taxa resembles a Frankenstein-like assembly, pieced together from disparate organisms without proper prior phylogenetic analysis.

The end result is intriguing yet fragile, relying on a weak foundation and riddled with exceptions. This inconsistency often leads to frustration and the prevailing notion that morpho-anatomy is of limited use in mollusks—deemed sometimes "conservative" and other times highly variable, adaptable, or subject to change due to factors like preservation. Consequently, it is seen as uninteresting to study. This scenario has steered Malacology away from focusing solely on shells or external features to a direct emphasis on molecules.

Between the study of shells and molecules, there are numerous aspects that have been overlooked throughout much of the 20<sup>th</sup> and early 21<sup>st</sup> centuries. However, exploring these aspects requires years of learning, training, and mastering various techniques for interpreting anatomical structures. These factors, crucial in morphological approaches, are abbreviated when using molecular methods.

Observing the scenario described above, I have come to realize the fundamental importance of improving the morphological framework for mollusks. This has been my focus throughout my entire biological career. Of course, it has not been easy. Publishing phylogenies based on morphology, for instance, has been incredibly challenging due to the reluctance of referees and editors. This resistance seems partly rooted in prejudice against morphology, but also because demonstrating the utility of morphology challenges the prevailing reliance on molecular approaches. Some colleagues have openly expressed this view, pointing out that morphological projects are considerably cheaper than molecular ones.

The phrase "someone must do that" resonates strongly with me. Therefore, for nearly four decades, I have dedicated myself to conducting detailed morphological studies and meticulously documenting their findings. My research spans representatives from virtually all branches of mollusks, exploring organs that have seldom been studied comparatively before. I have published extensively on these studies, including monographs on gastropod phylogeny (e.g., Simone, 2011), and more recently on Bivalvia (e.g., Simone et al., 2015; Simone & Amaral, 2021), with ongoing work in other branches.

On the other hand, another answer to the question of why mollusk taxonomy at higher levels remains chaotic is simple: because most decision-makers overlook papers based on morphology. This is a mistake that colleagues in other zoological branches do not typically make.

I have proposed a more stable alternative: a classification primarily based on morpho-anatomy, which has been published in high-level international, peer-reviewed journals

(<https://www.researchgate.net/profile/Luiz-Simone>; [www.moluscos.org](http://www.moluscos.org)). This classification is always grounded in phylogenetic analyses, with all data, methodologies, explanations, matrices, and lists fully disclosed and available for nearly three decades. None of these phylogenetic results have faced direct criticism, only indirect molecular results that yield different, unexplained outcomes not based on synapomorphies. Despite this, the morphological-based taxonomic arrangement has rarely been applied in current literature and databases, which tend to favor controversial, incongruent results instead. Some of these issues will be explored in future Malacopedia publications.

## References

- Aktipis, SW & Giribet, G, 2010. A phylogeny of Vetigastropoda and other “archaeogastropods”: reorganizing old gastropod clades. *Invertebrate Biology* 129(3): 220–140. DOI: 10.1111/j.1744-7410.2010.00198.x
- Cunha, TJ & Giribet, G, 2019. A congruent topology for deep gastropod relationships. *Proceedings of the Royal Society B* 286(1898): e20182776.
- Giribet, G; Okusu, A; Lindgren, A; Huff, S; Schrödl, M & Nishiguchi, MK, 2006. Evidence for a clade composed of molluscs with serially repeated structures: Monoplacophorans are related to chitons. *Proceeding of the Natural Academy of Sciences USA* 103: 7723–7728.
- Haszprunar, G, 2000. Is the Aplacophora monophyletic? A cladistic point of view. *American Malacological Bulletin* 15: 115–130.
- Kocot, KM; Poustka, AJ; Stöger, I; Halanych, KM & Schrödl, M, 2020. New data from Monoplacophora and a carefully-curated dataset resolve molluscan relationships. *Scientific Reports* 10: 1–8.
- Runnegar, B & Pojeta Jr, J, 1974. Molluscan phylogeny: the paleontological viewpoint: The early Paleozoic fossil record shows how living and extinct molluscan classes originated and diversified. *Science* 186: 311–317.
- Simone, LRL, 2009. Comparative morphology among representatives of main taxa of Scaphopoda and basal protobranch Bivalvia (Mollusca). *Papéis Avulsos de Zoologia* 49(32): 405–457. <http://www.moluscos.org/trabalhos/2009/Simone%202009%20Diasoma.pdf>
- Simone, LRL, 2011. Phylogeny of the Caenogastropoda (Mollusca), based on comparative morphology. *Arquivos de Zoologia* 42(4): 161–323. <http://www.moluscos.org/trabalhos/Caenogastro/Simone%202011a%20Caenogastropoda%20Phylogeny%20LIGHT.pdf>
- Simone, LRL & Amaral, VS, 2021. Phenotypic features of *Dimya* cf. *japonica* (Bivalvia, Dimyidae) from the Niue Island (south Pacific) with accounts on its phylogeny and taxonomic relationships. *Malacologia* 64(1): 121–136.
- Simone, LRL; Mikkelsen, PM & Bieler, R, 2015. Comparative anatomy of selected marine bivalves from the Florida Keys, with notes on Brazilian congeners (Mollusca: Bivalvia). *Malacologia* 58(1–2): 1–127.
- Smith, SA; Wilson, NG; Goets, FE; Feehery, C; Andrade, SCS; Rouse, GW; Giribet, G & Dunn, CW, 2011. Resolving the evolutionary relationships of molluscs with phylogenomic tools. *Nature* 480: 364–367.

Song, H; Wang, Y; Shao, H; Li, Z; Hu, P, Yap-Chiongco, MK; Shi, P; Zhang, T; Li, C; Wan, Y; Ma, P; Vinther, J; Wang, H & Kocot, KM, 2023. Scaphopoda is the sister taxon to Bivalvia: Evidence of ancient incomplete lineage sorting. PNAS 120(40): e2302361120 Doi: 10.1073/pnas.2302361120.

Steiner, G & Dreyer, H, 2003. Molecular phylogeny of Scaphopoda (Mollusca) inferred from 18S rDNA sequences: support for a Scaphopoda–Cephalopoda clade. Zoologica Scripta 32(4): 343–356.

More of *Malacopedia* in [http://www.moluscos.org/malacopedia\\_previous.html](http://www.moluscos.org/malacopedia_previous.html)

*Malacopedia* serves as both a popular scientific **journal** and a comprehensive **book** that has been publishing topics in the field of Malacology, based on experience for over 40 years.