

## DOCTORAL THESIS

### Molecular evolution of egg perivitelline fluid proteins in apple snails (ampullariidae, caenogastropoda)

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## Abstract

Gastropods are a highly diverse clade of molluscs that includes limpets, snails and slugs, and the only molluscan lineage with members that have successfully colonized land. Amphibious operculate freshwater gastropods, Ampullariidae, exhibit various morphological and physiological adaptations to their respective habitats, which make them ideal candidates for studying the mechanisms underlying adaptive evolution in gastropods. In the last two decades, there have been interests in the diverse reproductive behaviors of ampullariids: while most genera of apple snails deposit their eggs in a jelly mass underwater, two genera (i.e., *Pomacea* and *Pila*) deposit calcareous egg clutches above the waterline. The shift from aquatic to aerial oviposition required the concerted evolution of the adult snail's egg laying behavior as well as the composition of the egg. Today, only limited genomic information is available for this ecologically important family, except in the genus *Pomacea*, which hinders the investigation of its evolutionary history and mechanism. I have, therefore, developed the publicly accessible transcriptome database, AmpuBase, as molecular resources to support various transcriptomic and proteomic analyses. The database comprise *de novo* transcriptome assembly of eight species that belong to five representative genera of Ampullariidae. For each species there were 20,730 to 35,828 unigenes with predicted open reading frames, with an N50 ranging from 1,320 to 1,803 bp and 69.7% to 80.2% with functional annotation.

Since the ability of laying out-of-water eggs is one of the key steps of terrestrialisation for aquatic snails, I analysed the proteome of perivitelline fluid (PVF) for the eggs of *Marisa cornuarietis* that were laid underwater. The PVF proteome of *P. diffusa* also investigated to reveal and compare the biochemical adaptation between *P. diffusa* clade and *P. canaliculata* clade. Using LC-MS/MS coupled with transcriptome database, I identified a total of 36 and 32 PVF proteins from *M. cornuarietis* and *P. diffusa*, respectively. Comparison of PVF proteomes among the aquatic (*M. cornuarietis*) and three aerial ovipositing ampullariids (*P. diffusa*, *P. canaliculata* and *P. maculata*) showed that the four species all contain several major perivitellins that are important for the nutrition of the embryos. *M. cornuarietis* invests heavier in protection against pathogens, whereas the unique presence of calcium binding protein and their high albumen gland expression in three *Pomacea*, implying the importance of the novel acquisition of this protein for forming the calcareous eggshell of terrestrial eggs. I also found the absence of a neurotoxin (PV2) in *M. cornuarietis* and *P. diffusa* PVF, indicating this protein was acquired after the divergence between the ancestors of *P. diffusa* clade and the *P.*

*canaliculata* clade, and protects *P. canaliculata* and *P. maculata* eggs against terrestrial predators. Evolutionary analyses detected the involvement of gene duplication, positive selection and neofunctionalisation in the formation of several major PVF proteins.

With the availability of ampullariid genomes and transcriptomes, I conducted an integrated evolutionary analysis of *Pomacea* PVF with an aim to understand the molecular adaptations underlying the transition from underwater to terrestrial egg deposition. There were many expanded gene families in the New World ampullariids; some of them may be important for their evolution towards terrestrial egg laying. The PV1 oligomer protein was a novel protein acquired by the common ancestor of New World species to nourishing their terrestrial eggs. Meanwhile, gene duplication followed by neofunctionalisation of MACPF-tachylectin paralogs has contributed to the novel acquisition of the toxic PV2 protein in the *P. canaliculata* species complex. Overall, using Ampullariidae as an example, my study has demonstrated the power of integrative genomics to address various fundamental questions in evolutionary biology. The findings have enhanced our mechanistic understanding of invasion of terrestrial habitats by aquatic gastropods.

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