Molecular insights into the role of perivitellins in the evolution of terrestrial oviposition in Pomacea Perry, 1811 (Gastropoda: Ampullariidae)

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Among New World Ampullariidae, out-of-water egg laying is unique to the *Pomacea* genus<sup>1</sup>. This transition from aquatic to terrestrial oviposition involved major structural and biochemical changes in the egg<sup>1,2</sup>. Perivitellines, the main egg fluid proteins, provide nourishment and antipredation defense. Among these, the MA-CPF-tachylectin complex, known as perivitellin-2 (PV2) (Fig. 1) is a potent neurotoxin exclusive from *P. canaliculata* and P. maculata<sup>3,4</sup>. Here, we investigate the PV2 coding sequence (CDS) gene duplication

in three endemic Pomacea species (P. reevei, P. aulanieri, P. nobilis).

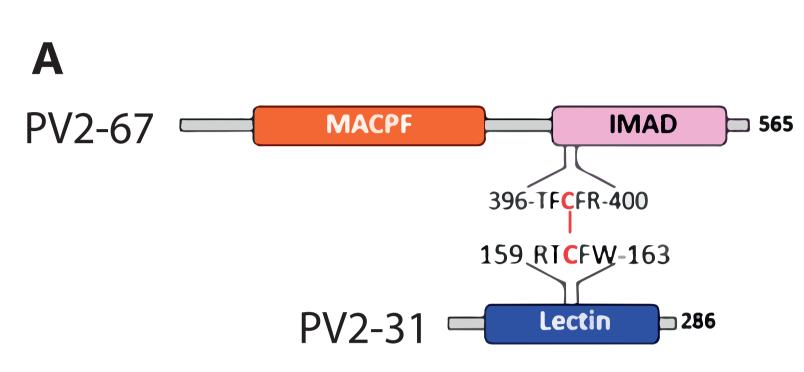
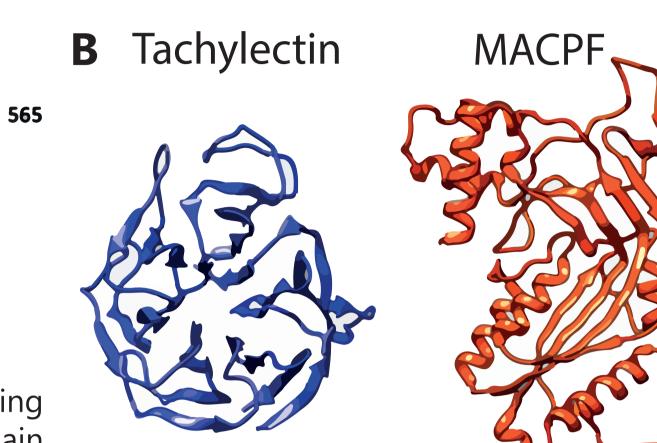
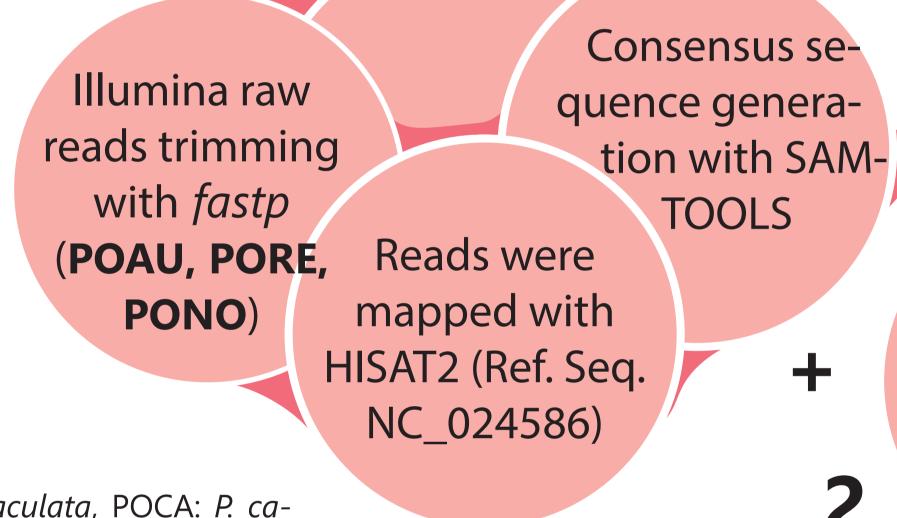


Figure 1. (A) Tertiary and (B) quaternary 3D modelling of PV2 from P. maculata, with the tachylectin-like chain domain in PV2-31 kDa subunit (blue), the and MACPF domain in PV2-67 kDa subunit (red), reproduced from Giglio et. al. (2020)<sup>5</sup>.



Methodology

# **PV2 Gene Extraction**



POMA: P. maculata, POCA: P. canaliculata, PORE: P. reevei, POAU: P. aulanieri, PONO: P. nobilis

Sequences flanks

PV2 CDS retrieval from Xiong et. al. 2025 with Blastn (POCA y POMA)

were trimmed with TrimAl Maximum Likeli-Multiple sequenhood phylogenetic analysis with IQce alignment for 67 kDa and 31 TREE2 kDa CDS subunits

## Results

The PV2-67 kDa and PV2-31 kDa subunits exhibit approximately two and four gene duplications, respectively (**Fig. 2**). Both PV2 domains appear to be present not only in the P. canaliculata group but also across other congeners, suggesting that this genomic arrangement is conserved throughout the *Pomacea* genus.

Pomacea Cladogram

POMA

POCA

PORE (

POAU O

(Group P. canaliculata)

(Group P. canaliculata)

### What does this mean?

The presence of multiple PV2 gene copies in *P. reevei, P. aula*nieri, and P. nobilis suggest that duplication events within the PV2 complex occurred prior to the divergence of the genus Pomacea (Fig. 3). These duplications, followed by functional diversification, may have contributed to the ecological success and radiation of *Pomacea* compared to other Ampullariids<sup>1</sup>, and could have facilitated resilience and a competitive advantage in invasive contexts<sup>2</sup>. PV2 gene complex duplications may have been subject to selective pressure optimizing defensive, nourishment and developmental functions within this group.



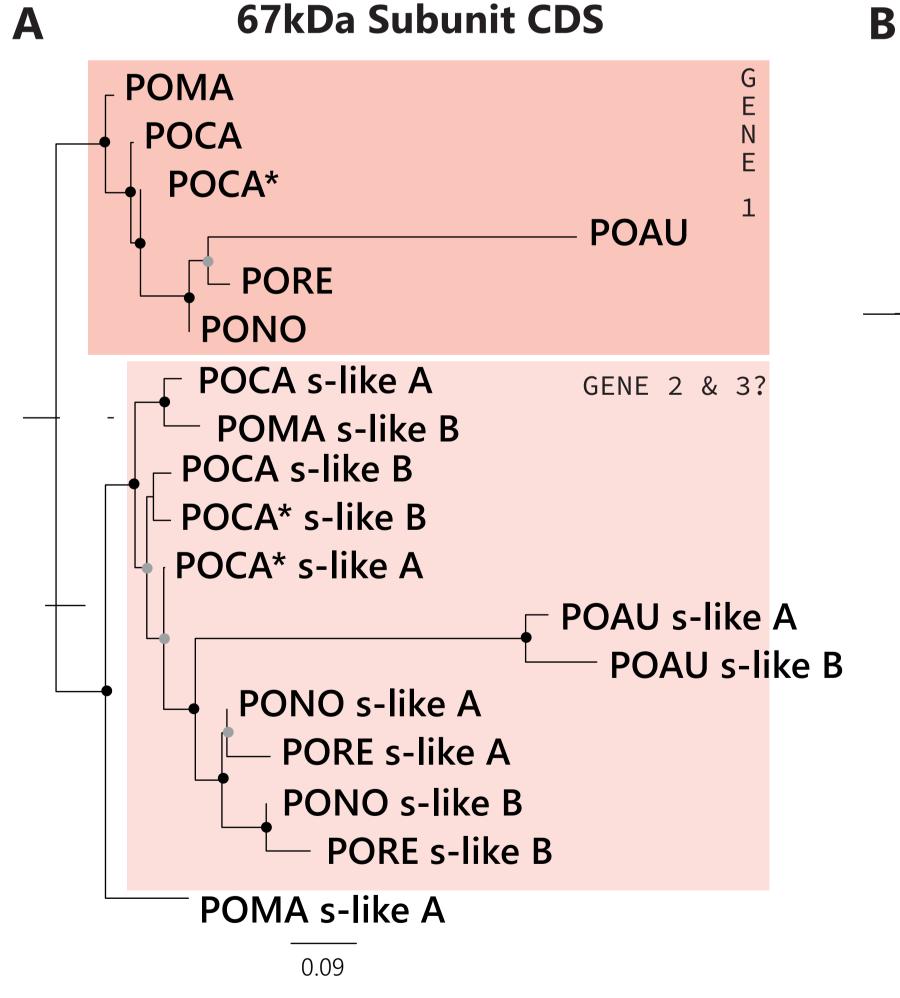


Figure 2. Maximum-likelihood phylogenetic analysis of PV2-67kDa and PV2-31kDa coding sequence (CDS) with the endemic *Pomacea* species included in this study. \*Annotated sequences in the POCA reference genome.

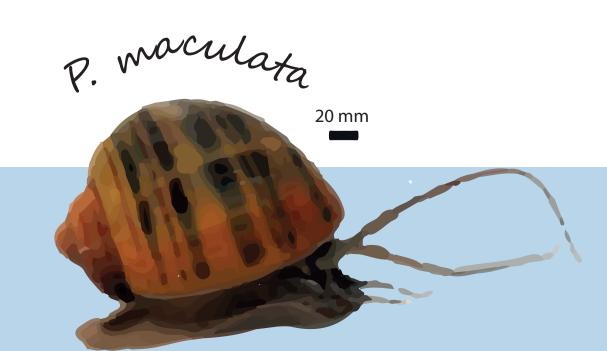
Detecting positive selection in PV2 gene lineages may reveal adaptive evolution linked to aerial egg-laying via neofunctionalisation or subfunctionalisation.

POCA\* PORE PONO POAU POCA s-like C POCA\* s-like C PONO s-like C PORE s-like C POAU s-like C POMA s-like C **POMA** POCA s-like A POCA\* s-like A PONO s-like A PORE s-like A POAU s-like A POMA s-like A POCA\* s-like B 0.04 PORE s-like B PONO s-like B POAU s-like B **POCA** POCA s-like B POMA s-like B

31kDa Subunit CDS

Studying other proteins related to egg defense mechanisms, such as the calcium-binding protein, would allow us to complement our understanding of the aerial oviposition adaptation.

(Group P. bridgessi) PONO (Group P. glauca) Figure 3. The cladogram of *Pomacea* species with its egg coloration is next to each one. Adapted from Hayes et al.  $(2009)^1$ , Mendivil et. al. (2023)<sup>6</sup>, Ramírez et. al. (2020)<sup>8</sup>. Despite their ecological relevance, the systematics and phylogenetic relationships within this group is still in debate<sup>1,7</sup>. Collection sites for POAU<sup>7</sup>, PORE<sup>9</sup> y PONO<sup>8</sup> are annotated in the map.



#### KEY REFERENCES

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