**Supplementary Figures**

* **Supplementary Figure 1:** Effect of sample inclusion in number of predicted orthologs
* **Supplementary Figure 2:** Overlap of PSGs between the four genomic samples
* **Supplementary Figure 3:** Summary of *RELAX* results
* **Supplementary Figure 4:** Overlap between orthologs from this study and marine-associated genes obtained from the literature

Chart, bar chart

Description automatically generated

***Supplementary Figure 1:*** The effect of including additional samples when identifying shared orthologs. The y-axis scale is the same between each row to emphasize the difference in the number of retained orthologs between the four-sample and nine-sample conditions. The coloured bars within each facet-pane represent different p-value thresholds.

Chart, box and whisker chart

Description automatically generated

***Supplementary Figure 2:*** An Upset plot visualising the shared positively selected genes between all combinations of samples. Each column represents a different sample combination, as illustrated by the filled in black dots. The top bar-plot illustrates the number of intersecting genes belonging to each sample combination, whilst the coloured bar-plot on the right represents the number of positively selected genes reported for each snake sample (non-unique).

Chart, bar chart

Description automatically generated

***Supplementary Figure 3:*** A breakdown of the *RELAX* results with *Aipysurus* *laevis* as the *test* set and the remaining leaves marked as ‘*reference*’. The left facet shows the number of orthologs that reached significance (*p* ≤ 0.001), with the red bar showing the number of orthologs reporting an intensification of selection (*k 1*) and the black bar showing relaxation of selection (k1). The right facet shows the remaining genes in the ortholog set that did not pass the significance threshold and how they separated out based on their insignificant *k*-values.

Chart, bar chart, box and whisker chart

Description automatically generated

**Supplementary Figure 4:** The number of intersecting *Aipysurus laevis* positively selected genes (PSGs) against (A) and non-positively selected orthologs (B) against marine-associated genes in secondarily marine vertebrates from five other studies. The y-axis represents the number of shared genes with each dataset, whilst the number above each column represents the number of genes in each respective study. **A)** Overlap between the 379 *A. laevis* PSGs and the marine-associated genes from the five literature datasets. **B)** Overlap between the remaining 3,215 orthologs and the marine-associated gene-sets from the literature.