

# IDENTIFICATION AND CHARACTERIZATION OF DIGESTIVE ENZYMES IN BLACK FLOUNDER USING BIOINFORMATIC TOOLS



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## INTRODUCTION

- Black flounder (*Paralichthys orbignyanus*) is a flatfish with significant potential as both a fisheries resource and for aquaculture in South America.
- The design of efficient food requires the understanding of digestive enzymes, critical in nutrient processing (e.g., carbohydrate and protein digestion).
- Our primary emphasis is on the two digestive enzymes,  $\alpha$ -amylases (*amy2*) and alanine aminopeptidases (*anpep*), due to their crucial roles in both the initial and final stages of carbohydrate and protein digestion.
- Integrating different bioinformatics approaches together with phylogenetic and comparative genomic analysis is important to study the evolution of digestive enzymes.

## GENE ANNOTATION AND 3D STRUCTURE OF THE PREDICTED AMINOACID SEQUENCES

- Prediction of the genes for these digestive enzymes in *P. orbignyanus* reveals 2 copies of pancreatic  $\alpha$ -amy genes in tandem (Fig. 1 and 3) and one copy of the *anpep* gene (Fig. 2 and 4). In addition, these predictions share a high identity in their protein sequences with other sequences from related organisms with conserved domains and distinctive characteristics.

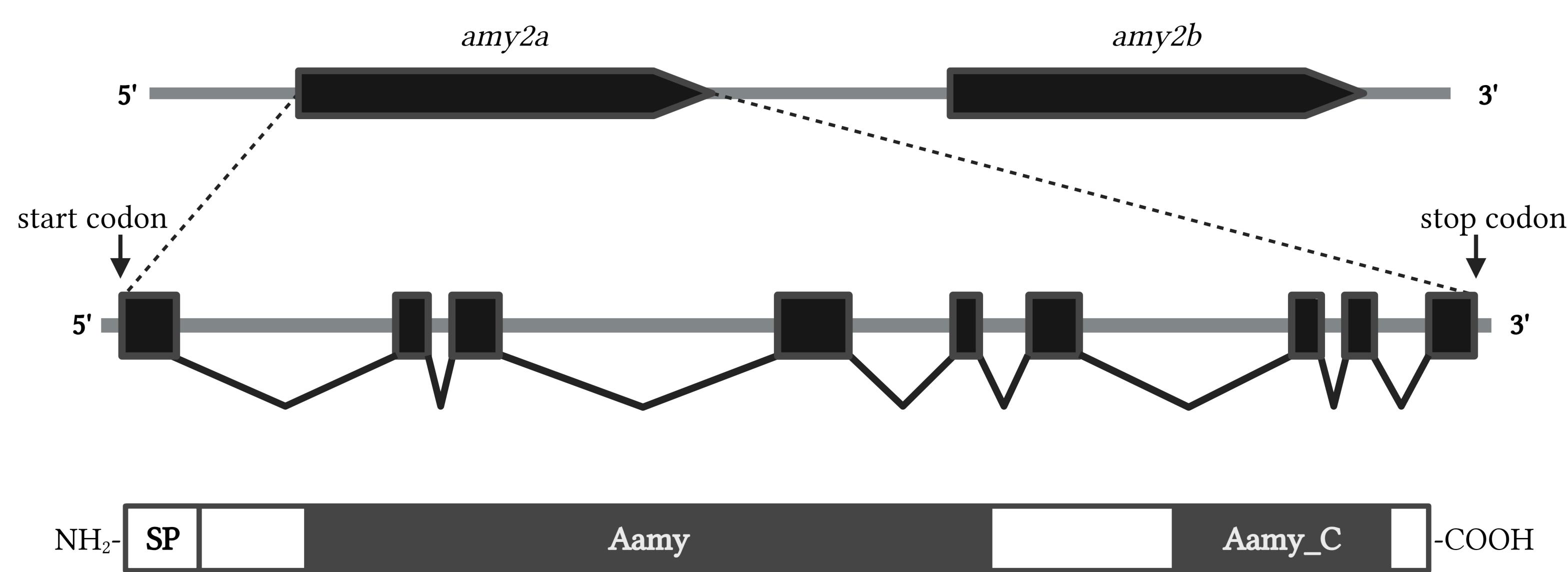


Figure 1. Gene structure and copies of  $\alpha$ -amylases in *P. orbignyanus*.

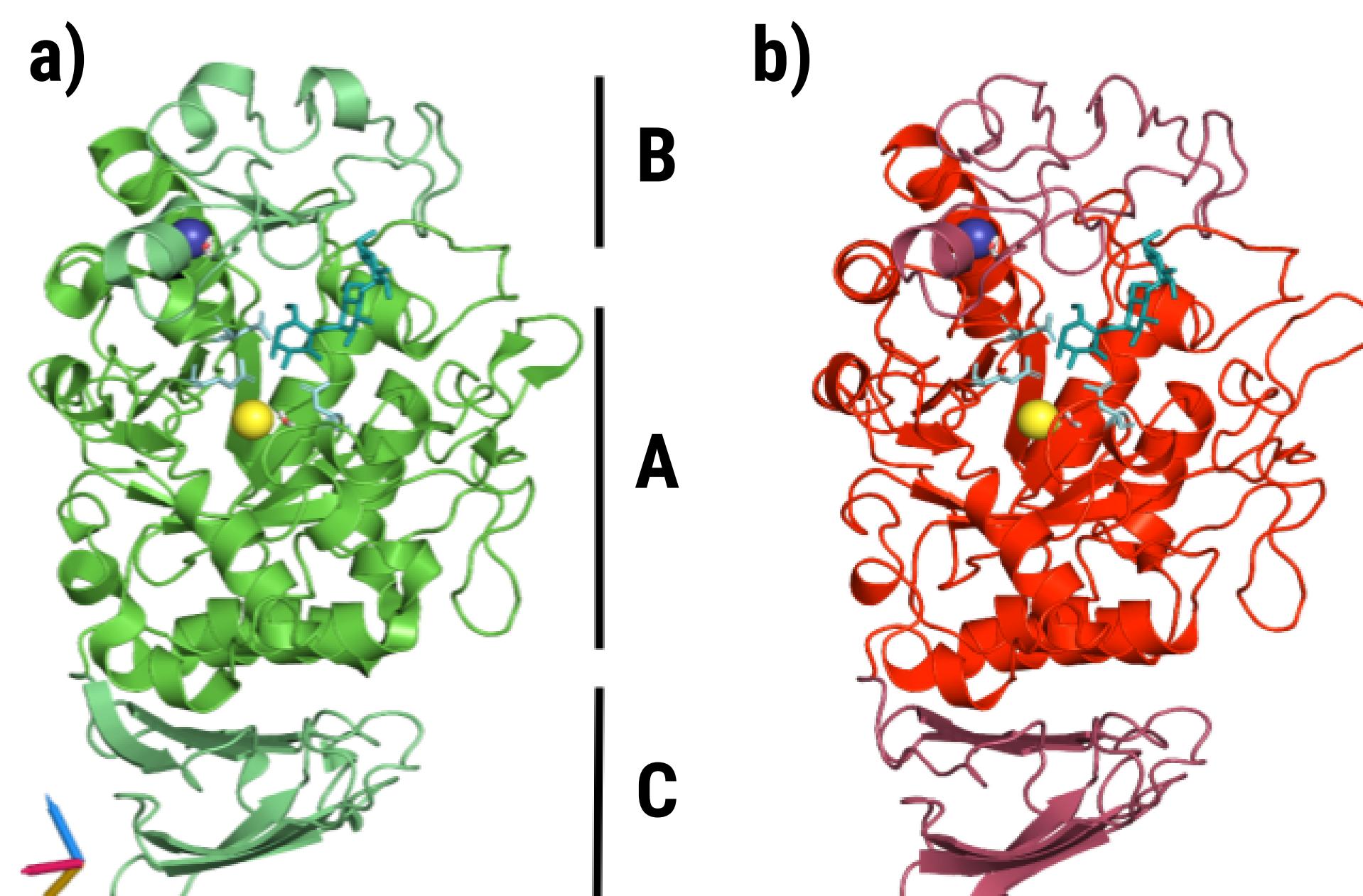


Figure 3. Predicted structure of  $\alpha$ -AMY 2A (b) and 2B (c). Characteristic  $\alpha$ -AMY domains (A, B, and C) are shown for the protein crystallized in pig (*Sus scrofa*, PDB ID 1UA3) (a) and for the two predictions obtained. Active site residues: cyan; Substrate (amylotriose): greenish blue; Cl<sup>-</sup>: yellow sphere; Ca<sup>2+</sup>: blue sphere. In the lower left corner are represented with different colors x-y-z axes (red, green and blue, respectively).

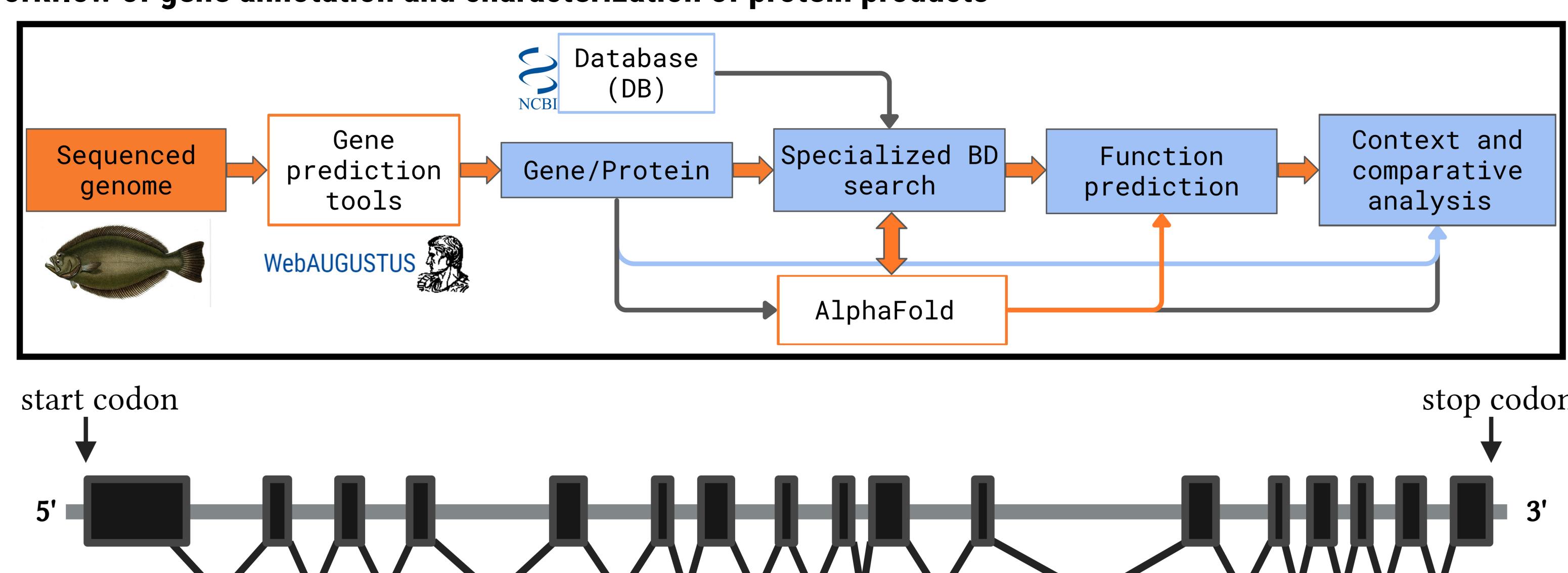
## AIMS

- Perform a curated gene annotation of  $\alpha$ -amylases (*amy2*) and alanine aminopeptidases (*anpep*) in the genome of *P. orbignyanus*.
- Characterize the protein products in *α-amy* and *anpep* using AlphaFold and different databases.
- Conduct a comparative genomics analysis to characterize the *α-amy* and *anpep* gene families in fish with diverse dietary preferences.



Scan for more

### Workflow of gene annotation and characterization of protein products



### Figure 2. Gene structure and copies of alanyl-aminopeptidases in *P. orbignyanus*.

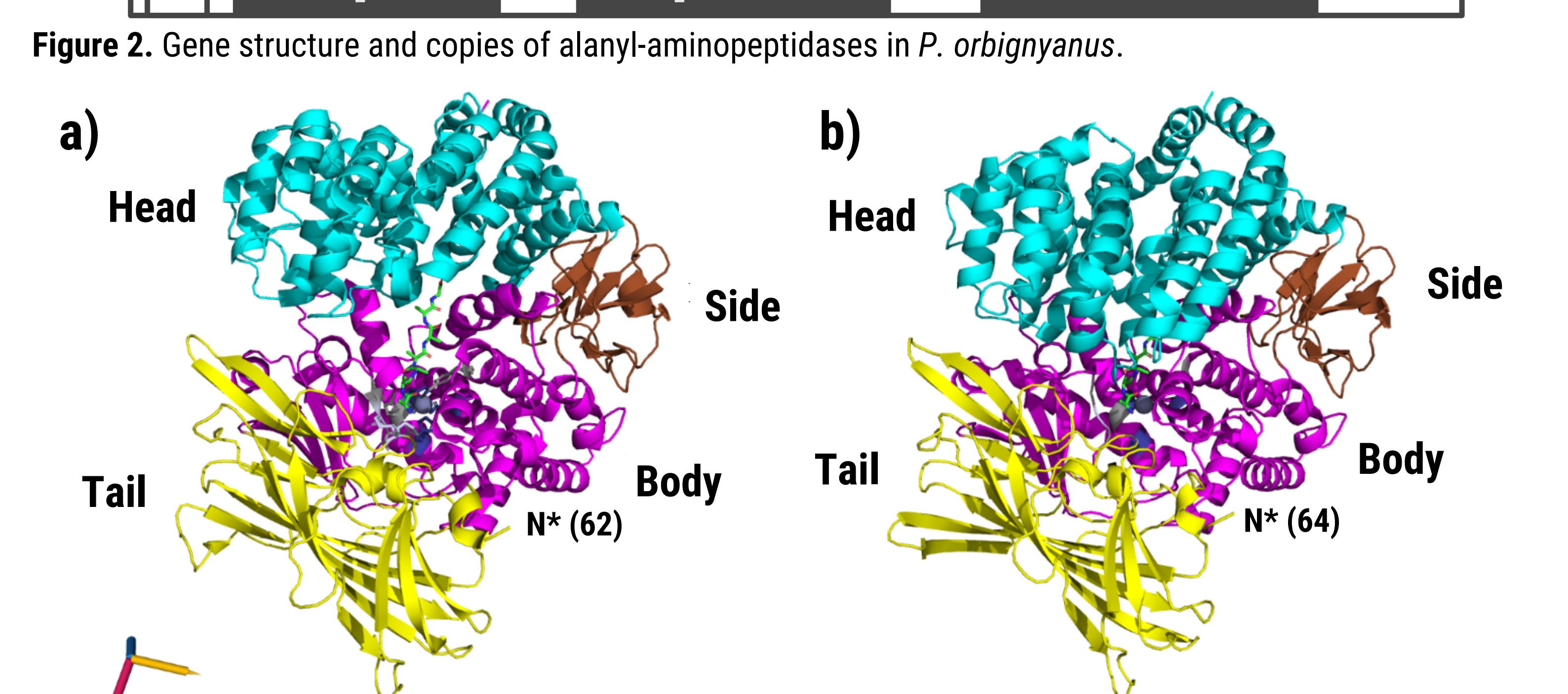


Figure 4. Predicted structure of ANPEP. The ectodomain is shown with its respective parts: tail (yellow), body (purple), side (brown) and head (cyan). (a) presents the crystallized structure of the pig (*S. scrofa*, PDB ID 4NZ8) ANPEP monomer used for structural comparison, with the prediction obtained for the sole ANPEP monomer (b).

## PHYLOGENETIC RECONSTRUCTION OF A-AMYLASE AND ALANYL-AMINOPEPTIDASE SUPERFAMILIES

- No correlation was observed between the number or type of sequences and the feeding habits. In both cases, regardless of diet, sequence clustering consistently aligned with the evolutionary relationships among different orders (Fig. 5 and 6)
- ANPEP sequences aligns with previous findings indicating that fish typically possess five *anpep* genes. The distinct clades in Fig. 6 initially support this observation, suggesting that the evolutionary history of aminopeptidases in fish may be linked to additional genomic duplication events.

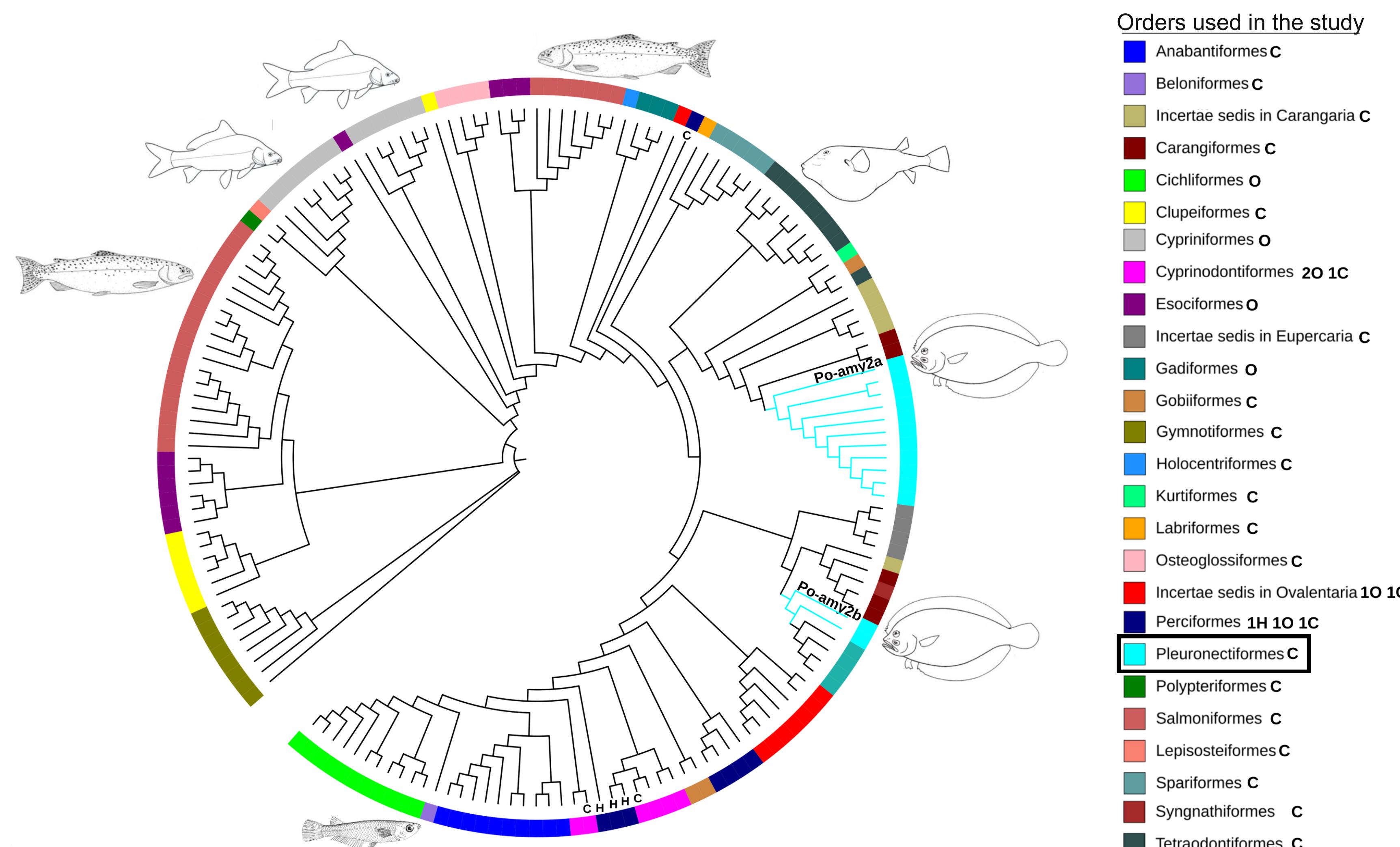


Figure 5. Phylogenetic relationships of  $\alpha$ -AMY in fish. The tree was generated from 168 amino acid sequences belonging to 48 proteomes, distributed in 26 orders. A color system was used to represent the order to which each sequence belongs in the tree. dietary affinities are indicated as: carnivore (C), herbivore (H) and omnivore (O).

## CONCLUSIONS

- P. orbignyanus* has the genetic capacity for initial carbohydrate digestion and final protein digestion.
- There was no correlation between gene copy number and diet in the fish, as shown by phylogenetic and comparative genomic analyses.

### Workflow of phylogenetic analysis in fish

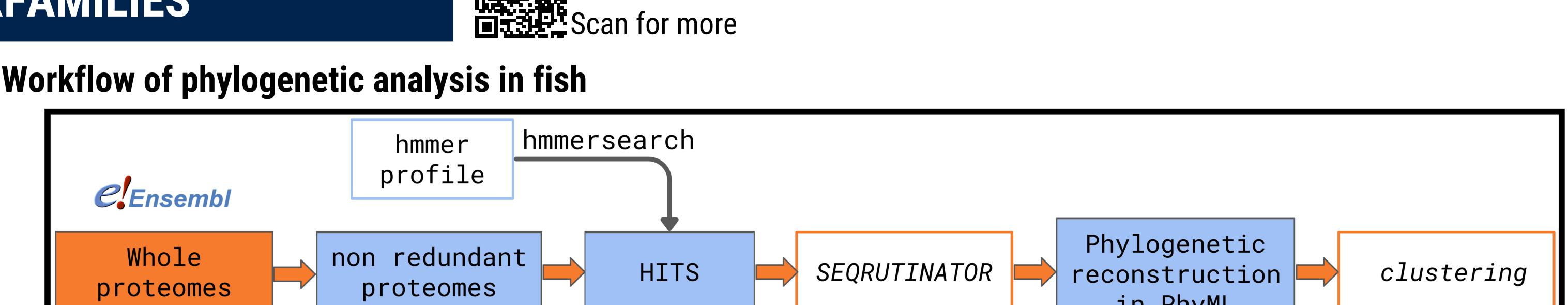


Figure 6. Phylogenetic relationships of ANPEP in fish. The tree was generated from 702 amino acid sequences belonging to 48 proteomes, distributed in 26 orders. Black arrows indicate the beginning of the different clades (A-E).

## ACKNOWLEDGMENTS

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