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Conservation of desaturase and elongases in a Brazilian freshwater carnivorous teleost: The red piranha (*Pygocentrus nattereri*)

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ABSTRACT

The endogenous biosynthesis of long-chain polyunsaturated fatty acids (LC-PUFAs) in fish is species specific and depends on several factors, such as trophic level, feeding habits, environmental characteristics, taxonomic position and mainly the conservation and activity of fatty acid desaturases (Fads) and elongases (Elovl). Therefore, the study of the evolutionary conservation of these genes in different species of fish has become routine in order to understand lipid metabolism and its essentiality in each species studied. Despite its importance to fish, there is a lack of understanding how the environment and trophic level affect the capacity for biosynthesis of LC-PUFAs in freshwater carnivorous teleosts. The red piranha (*Pygocentrus nattereri*) is a carnivorous freshwater fish of interest in Brazilian aquaculture. In this present project, at a bioinformatics level, we identified the genes and characterized the proteins of the Fads2 and Elovls present in the red piranha genome. Sequence comparison and phylogenetic analysis suggested that the Fads2 and Elovls proteins are closely related to previously characterized proteins from freshwater carnivorous and herbivorous fish species. As a conclusion, we suggest that the red piranha has the possible, at least partial, ability to bioconvert C18 PUFA into LC-PUFAs through the activities of Fads2 and Elovls.

Keywords: Long-chain polyunsaturated fatty acids; Endogenous biosynthesis; Native species.

Conservação de dessaturase e elongases em um teleósteo carnívoro brasileiro de água doce: a piranha vermelha (*Pygocentrus nattereri*)

RESUMO

A biossíntese endógena de ácidos graxos poli-insaturados de cadeia longa (LC-PUFAs) em peixes é espécie específica e depende de vários fatores, como nível trófico, hábitos alimentares, características ambientais, posição taxonômica e principalmente conservação e atividade das dessaturases de ácidos graxos (Fads) e elongases (Elovl). Portanto, o estudo da conservação evolutiva desses genes em diferentes espécies de peixes se tornou rotineiro para entender o metabolismo lipídico e sua essencialidade em cada espécie estudada. Apesar de sua importância para os peixes, há a falta de compreensão de como o ambiente e o nível trófico afetam a capacidade de biossíntese de LC-PUFAs em teleósteos carnívoros de água doce. A piranha vermelha (*Pygocentrus nattereri*) é um peixe carnívoro de água doce de interesse para a aquicultura brasileira. No presente projeto, em nível de bioinformática, identificamos os genes e caracterizamos as proteínas das Fads2 e Elovls presentes no genoma da piranha vermelha. A comparação de sequências e a análise filogenética sugeriram que as proteínas Fads2 e Elovls estão intimamente relacionadas a proteínas previamente caracterizadas de espécies de peixes carnívoros e herbívoros de água doce. Como conclusão, sugerimos que a piranha vermelha tem a possível, pelo menos parcial, capacidade de bioconverter C18 PUFA em LC-PUFAs por meio das atividades de Fads2 e Elovls.

Palavras-chave: Ácidos graxos poli-insaturados de cadeia longa; Biossíntese endógena; Espécies nativas.

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INTRODUCTION

Long-chain polyunsaturated fatty acids (LC-PUFA) play an important role in the growth and metabolism of all vertebrates, including fish (Bell & Tocher, 2009; Hashimoto et al., 2008). The biosynthesis of LC-PUFAs, namely eicosapentaenoic acid (EPA; 20:5n-3), docosahexaenoic acid (DHA; 22:6n-3), and arachidonic acid (ARA; 20:4n-6), is already well known in different fish species. It is species specific and depends on several factors, such as trophic level, eating habit, environmental characteristics (Garrido et al., 2019; Trushenski & Rombenso, 2020), and mainly the activities of fatty acid desaturases (Fads) and elongases (Elovl) enzymes, which cause the capacity to biosynthesize LC-PUFAs to differ fish species (Castro et al., 2016).

There are different theories surrounding this biosynthesis ability in fish. Initially, it was considered that the habitat (freshwater or marine) was the main determining factor of this ability. Studies have revealed that freshwater fish and salmonid species can convert the precursors C18 PUFA, α-linolenic acid (18:3n-3; ALA), and linoleic acid (18:2n-6; LA) into LC-PUFAs through a series of desaturation and elongation reactions catalyzed by the enzymes Fads2 and Elovls (Morais et al., 2009; Oboh et al., 2017). In contrast, marine teleost species have a very limited capacity for this conversion (Tocher, 2010). However, it was noticed that this classical understanding is superficial, as it has recently been discovered that two marine herbivorous fish (*Siganus canaliculatus* and *Scatophagus argus*) possess the ability to biosynthesize LC-PUFAs using C18 precursors (Li et al., 2020; Xie et al., 2018). The trophic level to which the species belongs says a lot about the capacity to biosynthesize LC-PUFAs, since more basal species need endogenous biosynthesis of LC-PUFAs, because they have a diet that is poor in LC-PUFAs, and predatory species do not need this biosynthesis capacity, since they already acquire LC-PUFAs via their diet (Galindo et al., 2021; Xie et al., 2020).

In studies to understand the ability of carnivorous fish to biosynthesize LC-PUFAs, recent work has suggested that *Trachinotus ovatus*, a marine carnivorous fish, has the ability to convert α-linolenic acid (18:3n-3) or linoleic acid (18:2n-6) into EPA (20:4n-3) and amino acid (20:3n-6), respectively, while also having some ability to synthesize DHA using EPA in the brain and eyes (Wang et al., 2020). Similarly, the Senegalese flounder (*Solea senegalensis*), a marine carnivorous teleost, also has the Fads2 and Elovl required for EPA and DHA conversion (Morais et al., 2012).

In the same context, to clarify the metabolism of LC-PUFAs in freshwater carnivorous fish, some initial steps have already been taken. According to a study by Kuah et al. (2015), cloning and functional characterization of a desaturase (Δ4 Fads2) and an elongase (Elovl5) from *Channa striata*, a freshwater carnivorous fish species, was reported. The results showed that both genes are expressed at considerable levels in the brain and liver and that they were upregulated by the intake of C18 PUFA. Subsequently, the ability of Δ6 and Δ5 desaturation activities for Fads2 was also demonstrated, with the highest activities focused on the conversion of omega-3 (n-3) PUFAs, with low Δ4 desaturation activity. Thus, studies indicate that *C. striata* has a full range of Fads and Elovl enzymes for the biosynthesis of LC-PUFASfrom C18 PUFA (Kuah et al., 2015; Kuah et al., 2016). More recently, one study focused particularly on the characterization of Elovl5 activity through tridimensional modeling and demonstrated that the European perch (*Perca fluviatilis*), a freshwater carnivorous fish, is capable of metabolizing PUFAs into highly unsaturated fatty acids (Tinti et al., 2019).

Although the capacity for biosynthesis in freshwater carnivorous fish has been already clarified, our objective was to clarify the capacity for LC-PUFAs biosynthesis in the red piranha (*Pygocentrus nattereri*). It is the first study focused on a carnivorous freshwater fish that is native to Brazil, considering it to be a model species that can serve as a comparison for other species of carnivorous fish of importance in aquaculture. The study can contribute to sustainable aquaculture production, in which the use of alternative ingredients to those rich in LC-PUFAs can be suggested at safe levels even for carnivorous species. For this, we identified the biomolecular aspects of desaturation and elongation. From the genome of *P. nattereri* available in GenBank at the National Center for Biotechnology Information (NCBI), we isolated the orthologous copies of *fadas2* and *elovls*. Additionally, we performed a comparative phylogenetic analysis and described the structure of proteins in two dimensions at a bioinformatic level.

MATERIALS AND METHODS

Phylogenetic trees

Two independent phylogenetic trees were elaborated using Fads and Elovls protein sequences from fish that are endemic to the Amazon basin, which included pirarucu (*Arapaima gigas*), tambaqui (*Colossoma macropomum*), and red piranha (*P. nattereri*), as well as other fish (teleosts and chondricts), as detailed in https://widgets.figshare.com/articles/28696142/embed?show_title=1. 1. The sequences were aligned with the MAFFT program using the L-INS-I methods and then exported

to BioEdit, in which the gaps were removed. Subsequently, the alignments were imported into MEGA, by which the phylogenetic trees were generated.

For the construction of phylogenetic trees, the neighbor-joining method was used. In it, the tree is drawn to scale, with the length of the branches representing the evolutionary distances used to infer the phylogenetic tree. Evolutionary distances were calculated using the Poisson correction method and are in the units of the number of amino acid substitutions per site. The proportion of sites where at least one unambiguous base is present in at least one sequence for each descending clade is shown next to each inner node in the tree.

In addition, the Fads tree has 35 amino acid sequences, with all ambiguous positions being removed for each pair of sequences (peer exclusion option). There was a total of 347 positions in the final dataset. As roots (base) of the phylogeny, the invertebrates *Octopus vulgaris* and *Saccoglossus kowalevskii*, cephalopod and hemichordate, respectively, were used. For the Elovls tree, which has 51 amino acid sequences, all the ambiguous positions were removed for each pair of sequences (pairwise deletion option). There was a total of 170 positions in the final dataset. Elovl6 of the invertebrate *Ciona intestinalis* was used as roots of the phylogeny.

Comparison of amino acid sequences deduced from Fads and Elovs proteins

For the alignment of the amino acid sequences deduced from the target fish of this study, we used the sequences of *A. gigas*, *Colossoma macropomum*, and *P. nattereri* (accession numbers: AOO19789.1; AYN59457.1; XP_017562208.2, respectively), in addition to the sequences of Fads2 and Elovs of other species such as *Danio rerio*, *Lepisosteus oculatus*, *Astyanax mexicanus*, and *Scleropages formosus*. *Danio rerio* was chosen because it is a research parameter in protein activity among the fish studied (Bláhová et al., 2022); *L. oculatus*, beucase it is a pre-teleost fish; *A. mexicanus*, because it belongs to the order Characiformes; and *S. Formosus*, because it belongs to the order Osteoglossiformes, with an annotated sequence available in GenBank, thus serving as a basis for comparative analysis.

All sequences were taken from GenBank, aligned in MAFFT (L-INS-i method) and edited in BioEdit with identical residues highlighted in black, and similar residues shaded in gray. In the alignment of Fads sequences, the conserved heme binding motif HPGG and three histidine boxes HXXXH, HXXHH, and QXXHH are recommended (Lopes-Marques et al., 2017). In Elovs, the conserved histidine box motif HXXHH and four conserved motifs (KXXEXXDT, QXXFLHXXHH, NXXXHXXMYXYY, and TXXQXX) are recommended (Sam et al., 2022).

Synteny maps

Synteny maps were made using the annotated genomes available from NCBI and Ensembl for *P. nattereri*, *C. macropomum*, *A. mexicanus*, *D. rerio*, and *L. oculatus* (Elovls only). The synteny maps have *fads2*, *elovl2*, and *elovl5* as the main genes. They also have four neighboring genes on each side (right and left).

Bidimensional topology of Fads protein sequences

The amino acid sequences of Fads2 for tambaqui and red piranha were submitted to the TOPCONS server (http://topcons.net/), which is used for consensus prediction of membrane protein topology (Tsirigos et al., 2015). For the visualization of the topology, the PROTTER website was used (http://wlab.ethz.ch/protter/start/) (Omasits et al., 2014).

RESULTS

Phylogenetic analysis of the family of Fads and Elovls in Amazonian fishes

The representative phylogenetic tree of the Fads family (Fig. 1) revealed two main clades in the vertebrates: one corresponding to Fads1, and the other one to Fads2. In these clades, there are representatives of the main groups of vertebrates: mammals, birds, amphibians and reptiles, including the chondrichthyan fish *Callorhinchus milii* and the coelacanth *Latimeria chalumnae*. Teleost fish have sequences only in the Fads2 family, since Fads1 in this group has been lost evolutionarily (Castro et al., 2012). From this tree, we highlight the fish that are the focus of this study, *P. nattereri*, which only have the copy of Fads2, namely XP_017562208.

The representative phylogenetic tree for Elovl (Fig. 2) revealed two main clades. The first clade includes Elovl3 and Elovl6, while the second clade consists of Elovl5, Elovl2, Elovl4a and Elovl4b, Elovl8a and Elovl8b, and Elovl7a and Elovl7b. From this tree, we can highlight the fish that are the focus of this study, *P. nattereri*, which have all the isolated copies of ELOVs, namely XP_017572456.1 (Elovl 1a), XP_017572096.1 (Elovl 1b), XP_017551678.1 (Elovl 2), XP_017579373.1 (Elovl 3), XP_017565830.1 (Elovl 4a), XP_017574113.1 (Elovl 4b), XP_017549638.1 (Elovl 5), XP_017548874.1(Elovl 6), XP_017578855.1 (Elovl 7a), XP_017549065.1 (Elovl 7b), XP_037386808.1 (Elovl 8), and XP_017577856.1 (Elovl 8b).

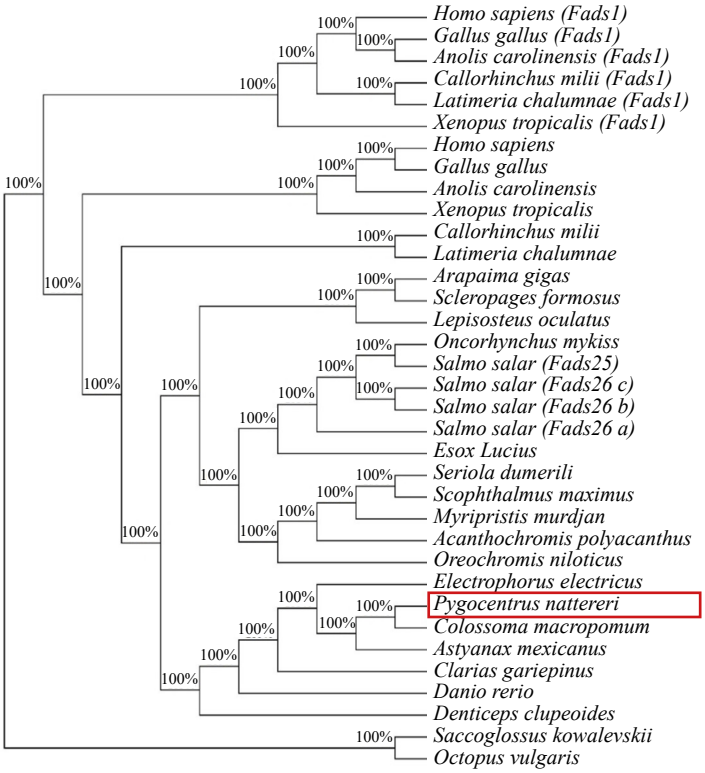

Figure 1. Neighbor-joining phylogenetic analysis of fatty acid desaturases (Fads) amino acid sequences rooted with the invertebrate clade. *Pygocentrus nattereri* Fads2 studied herein is highlighted. Accession numbers for all Fads sequences are available in https://widgets.figshare.com/articles/28696142/embed?show_title=1.

Comparison of Fads and Elovl protein sequences

The alignment of the Fads2 sequences (Fig. 3) shows the conservation of the histidine box (HXXXH, HXXHH, and QXXHH) among the fish studied. Although the cytochrome and conserved residues (highlighted in blue and yellow, respectively) do not show high conservation when compared between species, a 100% identity is observed between *P. nattereri* and *C. macropomum*. Similarly, the sequence alignments of Elovl2 (Fig. 4) and Elovl5 (Fig. 5) show that the histidine box and the conserved motif (highlighted in red) are preserved among the studied species, with a small variation in the Elovl5 of *S. formosus* (HVYHH to HIYHH).

Synteny map

The *fads2* desaturase synteny map (Fig. 6) together with the elongases *elovl2* (Fig. 7) and *elovl5* (Fig. 8) were located among the four neighboring genes on each side, comparing them with five more fish species for *elovls* and four for *fads2* (*A. mexicanus*, *C. macropomum*, *D. rerio, S. formosus*, and *L. oculatus*).

The *elovl2* of *P. nattereri* is located next to the neighboring genes *tmem14ca*, *mak*, *gcm2*, *sycp2l*, *gnaI*, and *mppe1*, in high conservation with the locus of *A. mexicanus* and *C. macropomum*. In addition, the *Elovl2* of the red piranha shows a moderate degree of conservation with the loci of the teleosts *D. rerio* and *S. formosus*. The *elovl5* of *P. nattereri* is located close to the genes *lhb*, *arfgef3*, *fbxo9*, *gclc*, *klhl31*, *eloal*, and *lrrc1* and exhibits a high level of conservation of the locus with *C. macropomum* and *A. mexicanus*, and a moderate level of conservation with the locus of *D. rerio* and *S. formosus*. The *fads2* locus of the red piranha has high conservation with the tambaqui locus (*C. macropomum*) (*slc25a22a*, *syt7a*, *ppp1r32*, *lrrc10b*, *eps8l2*, *tmem80*, *myrf*, and *vwasa*), and a moderate level of conservation with the locus of *A. mexicanus* and *D. rerio*.

Bidimensional structure of Fads2

Analysis of the bidimensional topology revealed a similarity between the Fads2 of *P. nattereri* and *C. macropomum*, exhibiting four domains in the membrane, with the three histidine motifs oriented towards the cytosol (Fig. 9). The conserved residues were located at the base of the third domain that crosses the membrane.

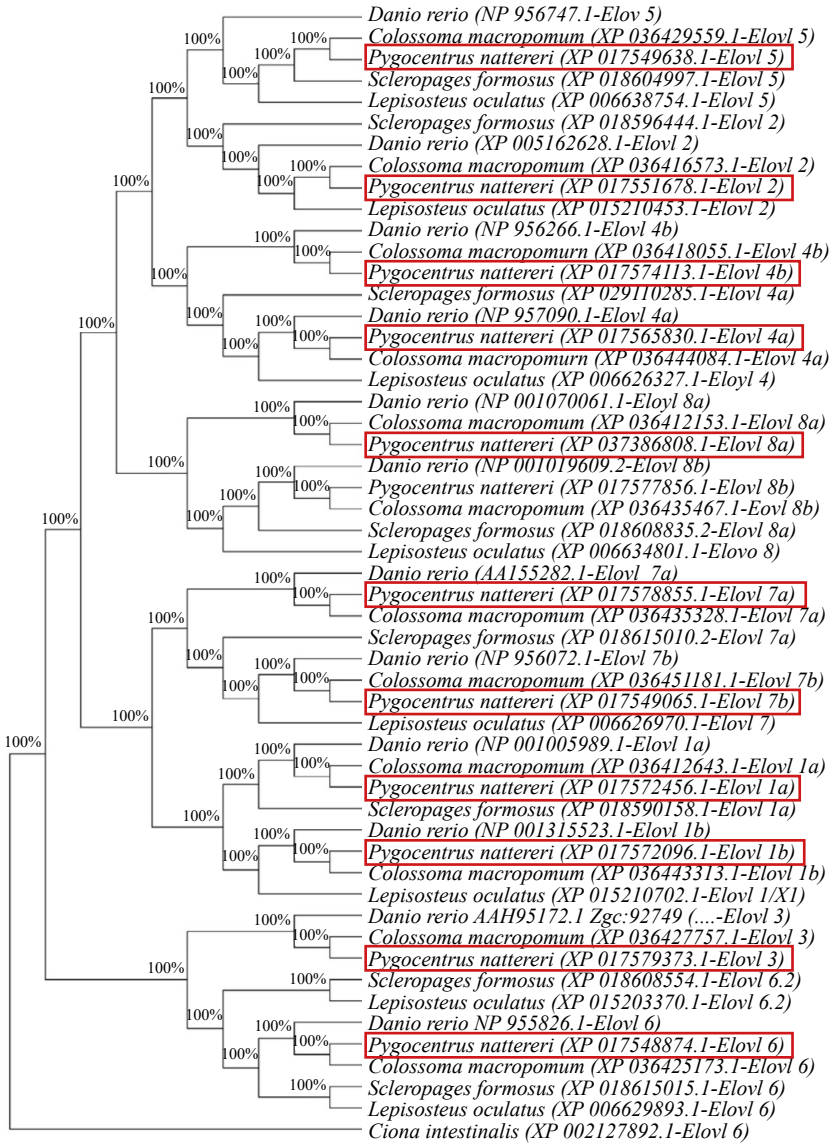

Figure 2. Neighbor-joining phylogenetic analysis of elongases (Elovls) amino acid sequences rooted with the invertebrate clade. *Pygocentrus nattereri* Elovls studied herein is highlighted. Accession numbers for all Elovls sequences are available in https://widgets.figshare.com/articles/28696142/embed?show_title=1.

The bidimensional topology of the tambaqui is consistent with the structural organization proposed in the literature for other desaturases (Lim et al., 2014; Lopes-Marques et al., 2017; Los & Murata, 1998; Meesapyodsuk et al., 2007). The bidimensional topology of the red piranha has 100% similarity with the structure of the tambaqui.

DISCUSSION

The ability to biosynthesize LC-PUFAs in teleosts was associated with the trophic level (Li et al., 2010; Morais et al., 2012) and the taxonomic position of the species (Garrido et al., 2019). Studies of this biosynthesis in carnivorous fish are still initial. The study of the red piranha (*P. nattereri*) is therefore the first study with Brazilian carnivorous fish, which provides an understanding of the conservation of Fads2 and Elovls in the context of freshwater. The identification of these genes in the genome and bidimensional topology of the structure of the protein were done to understand the conservation of these enzymes in the neotropical context.

The independent phylogeny constructed for the sequences of Fads revealed that the *fads2* gene identified in the genome of *P. nattereri* in fact grouped with the corresponding sequences

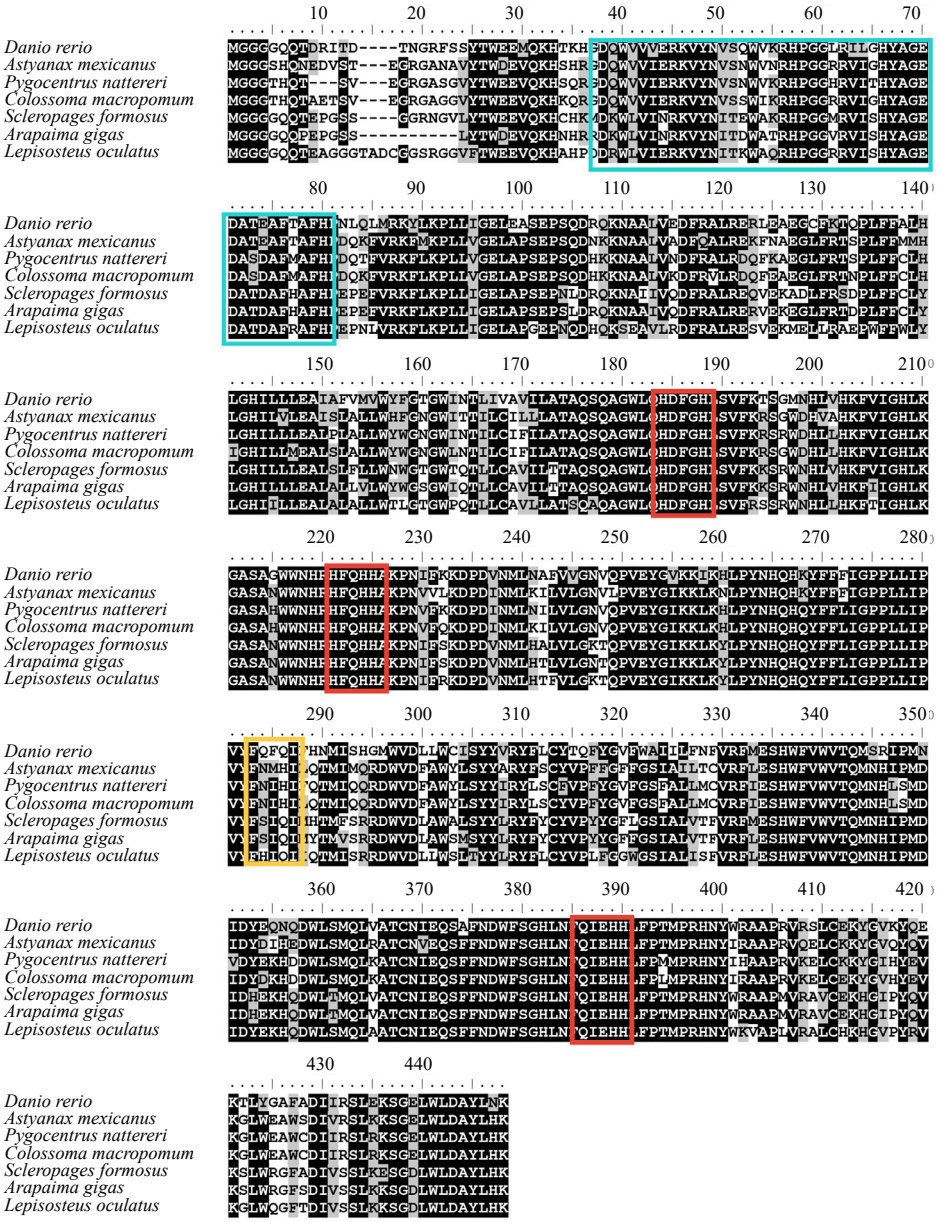

Figure 3. Comparison of the deduced amino acid (AA) sequences of fatty acid desaturases (Fads2). The AA sequences were aligned using BioEdit. Identical residues are shaded black, and similar residues are shaded grey. Indicated are the conserved (HXXXH, HXXHH, and QXXHH) histidine box motifs (highlighted in red). The conserved cytochrome and residue are highlighted in blue and yellow, respectively.

in other fish, as well as main representatives of the groups of the mammals, amphibians, birds, and reptiles. Additionally, phylogenetic analysis showed a proximity between the Fads2 sequence in *P. nattereri* and some fish species, including *C. macropomum*, *A. gigas*, *S. formosus*, *A. mexicanus*, and *D. rerio*. It is relevant to note that the genome of most teleosts underwent a third round of genomic duplication, resulting in the loss of Fads1, and that in a second evolutionary moment there was the occurrence of Fads2 with bifunctional activity (Δ5/Δ6) in many teleosts studied (Castro et al., 2016; Lopes-Marques et al., 2017). In addition, as observed in *C. macropomum* (Ferraz et al., 2019), the red piranha has the cytochrome B5 domain and conserved histidine boxes (HXXXH, HXXXHH, and QXXHH), as well as the substitution of histidine H for glutamine Q in the third histidine box (QXXHH), which is conserved between teleost Fads2 sequences. Such characteristics are described as essential

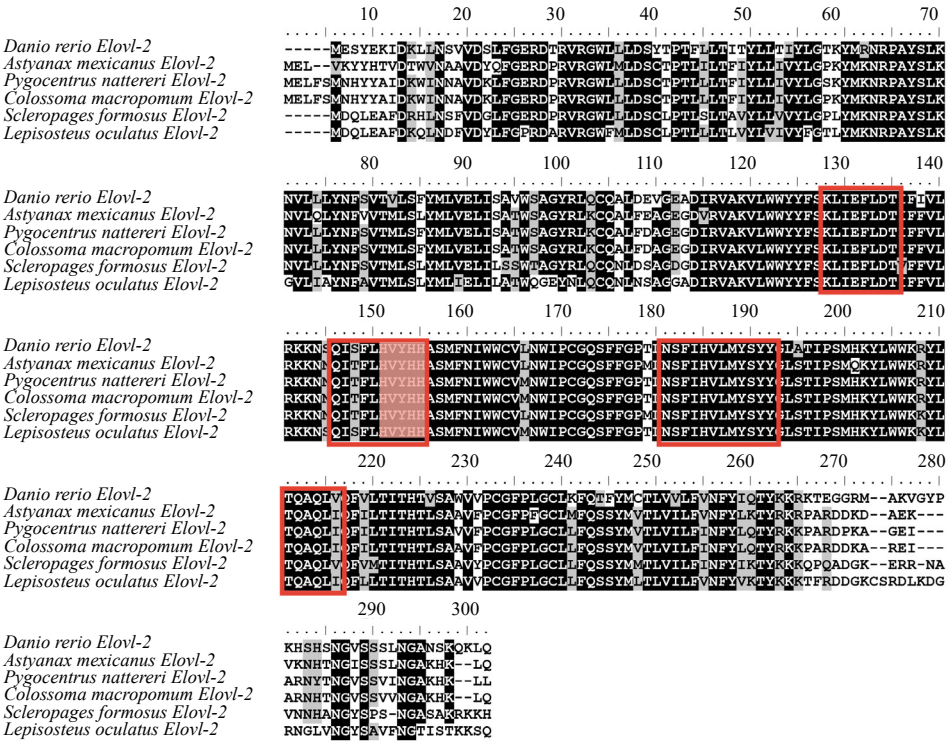

Figure 4. Comparison of the deduced amino acid (AA) sequences of Elovls2. The AA sequences were aligned using BioEdit. Identical residues are shaded black and similar residues are shaded grey. Four conserverted motifs (KXXEXXDT, QXXFLHXXHH, NXXXHXXNYXYY and TXXQXX), highlighted in red, and histidine box motif (HXXHH), shaded in red, are indicated.

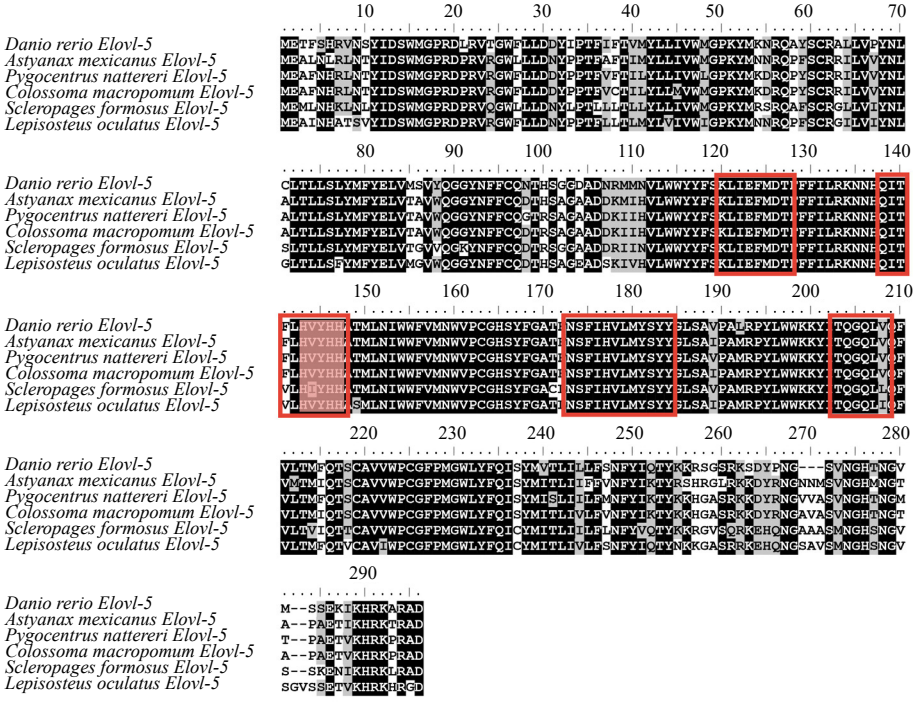

Figure 5. Comparison of the deduced amino acid (AA) sequences of Elov5. The AA sequences were aligned using BioEdit. Identical residues are shaded black, and similar residues are shaded grey. Four conserverted motifs (KXXEXXDT, QXXFLHXXHH, NXXXHXXNYXYY, and TXXQXX), highlighted in red, and histidine box motif (HXXHH), shaded in red, are indicated.

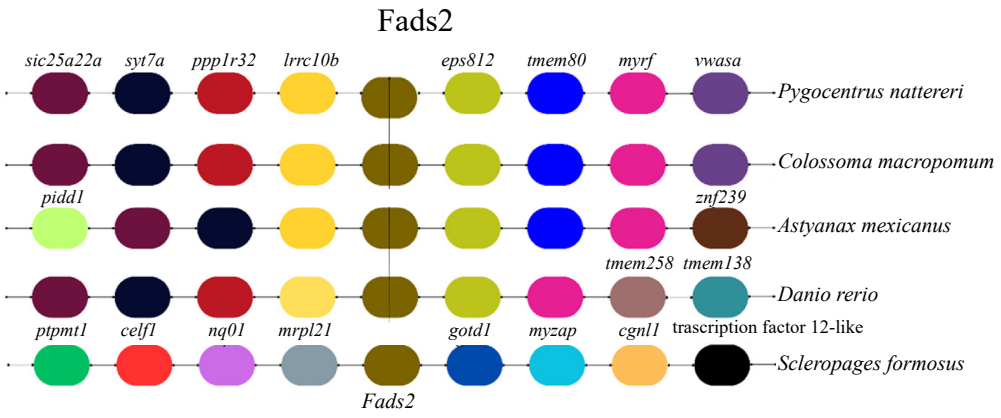

Figure 6. Gene annotation of *Pygocentrus nattereri* genomic scaffold and comparative synteny maps. Synteny maps of gene involved in the biosynthesis of the polyunsaturated fatty acids fatty acid desaturase type 2 (Fads2). The target Fads2 is represented in brown, and neighboring cross-species conserved genes are represented in different colors.

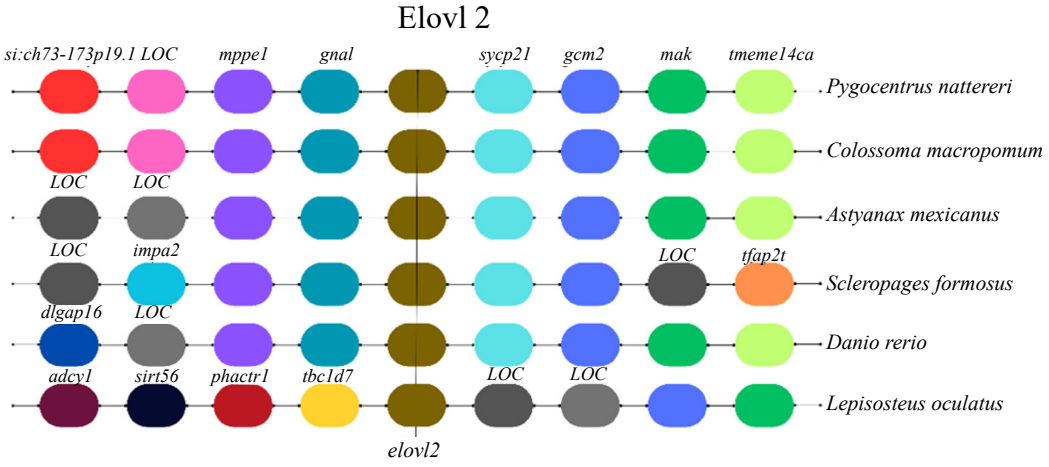

Figure 7. Gene annotation of *Pygocentrus nattereri* genomic scaffold and comparative synteny maps. Synteny maps of the gene involved in the biosynthesis of the polyunsaturated fatty acids fatty acid elongase 2 (Elovl2). The target Elovl2 is represented in brown, and neighboring cross-species conserved genes are represented in different colors.

for the catalytic activity of Fads in teleosts (Castro et al., 2016; Sayanova et al., 2001).

The phylogeny of the Elovls identified all the Elovls present in the genome of *P. nattereri*, totaling eight copies, as reported for teleosts (Ferraz et al., 2022). The analysis confirmed that all the Elovl sequences identified in the red piranha are orthologous to the corresponding Elovls found in other fish species, such as *C. macropomum*. The sequence of these Elovls–Elovl8 (Elovl8a and Elovl8b), Elovl4 (Elovl4a and Elovl4b), Elovl2 and Elovl5–exhibited the conservation of the four motifs (KXXEXXDT, QXXFLHXXHH, NXXXHXXMYXYY, and TXXQXX) and a histidine box (HXXHH). As in Fads, the histidine box is responsible for the electron transfer process during elongation (Leonard et al., 2004; Sam et al., 2022) and is therefore recognized as a region of protein activity, thus suggesting an activity of these proteins.

Comparative analysis of synteny assumes that true orthologous genes are located at identical loci on chromosomes. As a rule, these loci are conserved between species and, when they are identified, this gives a more secure identification of the isolated sequence. Therefore, detailed synteny was performed to confirm the conservation and synteny of the main genes of LC-PUFA metabolism (*fads2*, *elovl2* and *elovl5*) in *P. nattereri* compared with other species already studied, such as *C. macropomum*, which

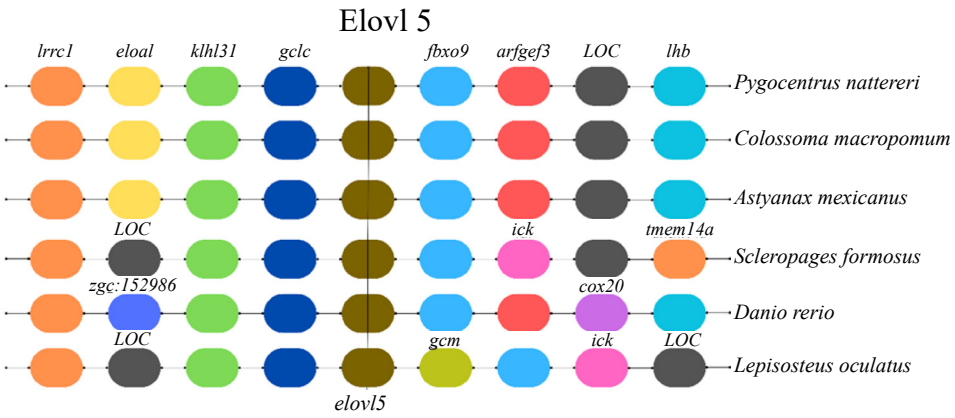

Figure 8. Gene annotation of *Pygocentrus nattereri* genomic scaffold and comparative synteny maps. Synteny maps of gene involved in the biosynthesis of the polyunsaturated fatty acids fatty acid elongase 2 (Elovl5). The target Elovl5 is represented in brown, and neighboring cross-species conserved genes are represented in different colours.

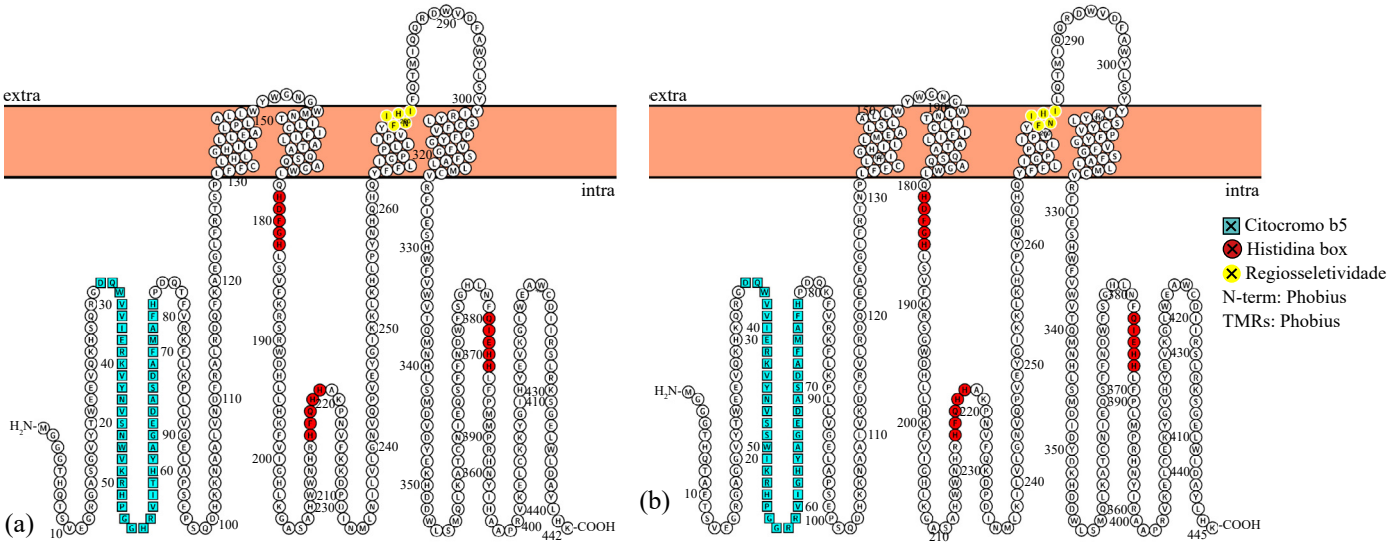

Figure 9. Predicted bidimensional topology of (a) *Pygocentrus nattereri* fatty acid desaturase 2 (Fads2) and (b) *Colossoma macropomum* Fads2. Comparison between the two bidimensional topologies demonstrates the similarity between the two proteins. The histidine box is highlighted in red, the cytochrome in blue, and the conserved residue in yellow.

confirmed the conservation of the position of these genes in the red piranha. Finally, the analysis of the bidimensional topology of Fads2 in the piranha predicted four transmembrane domains, the three histidine boxes, and the domain similar to the cytochrome b5 are located in the cytosol, which is similar to the organization in pirarucu (*A. gigas*) (Lopes-Marques et al., 2017) and tambaqui (*C. macropomum*) (Ferraz et al., 2020; Ferraz et al., 2022).

CONCLUSION

The red piranha has a *fads2* gene with all the typical characteristics of desaturases, as well as Elovl5 and Elovl2, thus having the complete characteristics of biosynthesis of LC-PUFAs. As related by different authors, the functional characterization of different desaturases (Fads) and elongases (Elovl) of different teleosts suggests that the activities of these proteins are influenced by both feeding habits and ecological habits, which created (or did not create) an evolutionary pressure to conserve the activities of these proteins for LC-PUFA biosynthesis between different species. Two examples that go against common sense are *C. striata* and *P. fluviatilis*, despite they being carnivorous freshwater fish that have the ability to synthesize LC-PUFAs from C18 PUFA in the diet (Kuah et al., 2015; Kuah et al., 2016; Morais et al., 2009; Tinti et al., 2019). This approach then generates a broader discussion of the conservation and activity of Fads2 and

Elovls during the evolutionary process, not being based solely on feeding habits and ecological habits, but rather on the set of evolutionary and environmental factors.

Authors argue that there is an essential genetic component involved in LC-PUFA biosynthesis, specifically about the conservation of these genes in ray-finned fish, which may even occur regardless the trophic level. This suggests that the trophic level may not directly drive the activities of these proteins in teleosts, and other factors, such as the phylogeny of the species, seem to be more influential (Garrido et al., 2019; Lopes-Marques et al., 2018). It is believed that the capacity for LC-PUFA biosynthesis is intricately related to feeding habits. However, in the case of the red piranha, despite being a carnivorous species, and already having diet rich in LC-PUFAs, which in theory would not need to have the endogenous biosynthesis of these LC-PUFAs, its phylogenetic proximity to freshwater omnivorous species, such as *C. macropomum*, may be an explanation of the conservation of the gene and the activity of these proteins.

The phylogenetic similarity suggests that all these features, at least structurally, of the proteins related to biosynthesis of LC-PUFAs were retained, possibly due to a shared evolutionary history, in which evolutionary bifurcations, with the loss and conservation of some genes, can perhaps explain this retention and activity of Fads2 and Elovls in the red piranha. Importantly, *in-vivo* and protein activity tests are necessary to confirm this hypothesis, which were conclusive based on in-silico studies.

This study, therefore, offers a new perspective on the conservation of genes involved in the metabolism of LC-PUFAs, suggesting that the understanding of the capacity for endogenous biosynthesis goes beyond the simple analysis of feeding habits or trophic level between species; here, for example, we emphasize the phylogenetic proximity.

Future studies to identify the requirements of LC-PUFAs in some specific tissues of freshwater carnivorous species are still necessary in order to gain a bigger picture of what occurs. For example, studies with levels of safe replacement of ingredients rich in LC-PUFAs by alternatives, thus ensuring more sustainable national productivity, are a suggestion. Furthermore, in this study, we presented evidence for the first time that indicates the conservation of essential genes for biosynthesis of LC-PUFAs in a Brazilian freshwater carnivorous species and evidence of phylogenetic proximity of this conservation with Amazonian species.

CONFLICT OF INTEREST

Nothing to declare.

DATA AVAILABILITY STATEMENT

The data are available in https://widgets.figshare.com/articles/28696142/embed?show_title=1.

AUTHORS' CONTRIBUTIONS

Conceptualization: Ferraz, R.; **Formal Analysis:** Costa, K.V.; **Investigation:** Costa, K.V.; **Resources:** Costa, K.V.; **Writing – original draft:** Costa, K.V., Neto, R., Souza, Y.; **Data curation:** Neto, R., Souza, Y.; **Validation:** Paixão, R.; **Writing – Review and editing:** Paixão, R.; Ferraz, R.; **Supervision:** Ferraz, R.; **Final approval:** Ferraz, R.

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