Isolation, identification, and gene expression analysis of the main digestive enzymes during ontogeny of the Neotropical catfish *Pseudoplatystoma punctifer* (Castelnau, 1855)

Diana Castro-Ruiz[a*], Karl B. Andree[b], Eva Blondeau-Bidet[c], Christian Fernández-Méndez[d], Carmen García-Dávila[a], Enric Gisbert[b] and Maria J. Darias[c*]

[a] Instituto de Investigaciones de la Amazonía Peruana (IIAP), Dirección de Investigación en Ecosistemas Acuáticos Amazónicos (AQUAREC), Laboratorio de Biología y Genética Molecular (LBGM), Carretera Iquitos-Nauta km. 4.5, Iquitos, Peru

[b] Institut de Recerca i Tecnologìa Agroalimentaries (IRTA), Centre de Sant Carles de la Ràpita (IRTA-SCR), Aquaculture Program, Crta. Poble Nou km 5.5, 43540 Sant Carles de la Ràpita, Spain

[c] MARBEC, Univ Montpellier, CNRS, Ifremer, IRD, Montpellier, France

[d] Instituto de Investigaciones de la Amazonía Peruana (IIAP), Dirección de Investigación en Ecosistemas Acuáticos Amazónicos (AQUAREC), Laboratorio de Bromatología, Carretera Iquitos-Nauta km. 4.5, Iquitos, Peru

* Corresponding authors: dcastro@iiap.gob.pe (D.C.-R.); maria.darias@ird.fr (M.J.D.)

Abstract

The ontogeny of the digestive capacities in fish is species-specific and its knowledge is essential for understanding the nutritional requirements of fish larvae. While the Amazon basin contains the world's highest fish biodiversity, the molecular basis of the early digestive

physiology has not yet been reported in any Amazonian fish species. In order to increase basic knowledge on the molecular ontogeny of the digestive system of a commercially important Amazonian fish species, six digestive enzyme genes (α-*amylase*, *phospholipase A2*, *lipoprotein lipase*, *trypsin*, *chymotrypsin* and *pepsin*) were isolated and identified, and their expression analyzed from 3 to 24 days post fertilization (dpf) in *Pseudoplatystoma punctifer*, a catfish species with high potential for aquaculture diversification. The present results, together with those previously obtained on the development of the digestive enzyme activity, showed that gene expression and enzymatic activities are synchronized and that the enzymatic machinery of *P. punctifer* was completely prepared for the onset of exogenous feeding (4 dpf, 6 mm total length, TL) and had reached its maturity between 10 and 13 dpf (11-14 mm TL). This indicated that the transition from the larval to the juvenile mode of digestion had been completed and they were suitable for weaning under culture conditions. Furthermore, the gene expression analyses suggest that this species displays an omnivorous behavior with a preference towards carnivory during early development. The present study provides the first comprehensive ontogenetic analysis of the digestive function from a molecular point of view of a species of the genus *Pseudoplatystoma*, and contributes to the development of feeding strategies in the context of South American aquaculture diversification.

Keywords

Digestive system; ontogeny; enzymatic precursors; catfish; fish larvae; phylogeny

1. Introduction

The early life stages of fish constitute a very sensitive phase during which morphogenesis occurs in a very short time period and optimal feeding and nutrition at mouth opening are key for survival and growth (Yúfera and Darias, 2007); however, very little is known about

the nutritional requirements of fish larvae (Holt, 2011). Studying the natural diets of developing larvae can be difficult, and indirect approaches under culture conditions, such as the characterization of the ontogeny of the digestive system, have been widely used to better understand their developing digestive capacities, and nutritional requirements and conditions (Gisbert et al., 2008; Lazo et al., 2011; Rønnestad et al., 2013; Zambonino Infante et al., 2008; Zambonino Infante and Cahu, 2001). The development of the digestive system is species-specific and is a genetically programmed process that is affected by the general life history and reproductive strategy of each species, and by a variety of abiotic and biotic factors, such as water temperature and food availability and composition (Lazo et al., 2011; Rønnestad et al., 2013; Zambonino Infante et al., 2008). In the context of aquaculture, knowledge of the interspecific differences in the relative timing of the differentiation, development, and functionality of the digestive tract and accessory glands during early life stages is essential to develop feeding protocols adapted to the physiological stages of development of each species. The most common approach to estimate the digestive capacities of fish has been the determination of the activity of digestive enzymes by biochemical analysis. Studies evaluating larval digestive performance are generally focused on the appearance of the activity of pancreatic enzymes before the onset of exogenous feeding, the enzymatic maturation of the brush border of enterocytes, and the appearance of pepsin activity in gastric fish, which mark the transition from the larval to the juvenile mode of digestion (Lazo et al., 2011; Rønnestad et al., 2013; Yúfera et al., 2018; Zambonino Infante et al., 2008). Among the different enzymes involved in digestion, α-amylase (EC 3.2.1.1) is synthesized in the exocrine pancreas and is key for the digestion of complex carbohydrates in fish (Cahu and Zambonino Infante, 1994; Darias et al., 2006; Ma et al., 2005; Moyano et al., 1996). The phospholipases A2 (PLA2, EC 3.1.1.4) are essential lipolytic enzymes that hydrolyze phospholipids to generate free fatty acids and lysophospholipids (Dennis, 1994). The pancreatic phospholipase A2-IB (PLA2-IB) is one of the different types of secretory

PLA2 (sPLA2) and it is considered the most important digestive enzyme in marine fish (Cahu et al., 2003; Rønnestad et al., 2013). The pancreatic enzyme trypsin (EC 3.4.21.4) is considered the most important alkaline proteolytic enzyme in early life stages of fish and it also plays a key role in activating other pancreatic enzymes in the gut lumen (Rønnestad et al., 2013). Chymotrypsin (EC 3.4.21.1) is another important pancreatic proteolytic enzyme, whose activity is complementary to that of trypsin. During the digestive system ontogeny of gastric fish, trypsin, and chymotrypsin are responsible of protein digestion in the alkaline environment of the intestine until the stomach is formed. At that time, a third proteolytic enzyme, pepsin, appears. Among the two main classes identified, pepsin A and C, the first one is the predominant form, and several isoforms exist in gastric fish (Kapoor et al., 1976). They are responsible for the initial and partial hydrolysis of proteins in the stomach in the presence of an acidic environment. Its precursor, *pepsinogen*, is produced and secreted by the gastric glands of the stomach, where it is activated by hydrochloric acid (Darias et al., 2005; Darias et al., 2007a; Douglas et al., 1999; Gawlicka et al., 2001). Lipoprotein lipase (LPL, EC 3.1.1.34) is a key regulator of lipid metabolism that hydrolyzes triglyceride-rich lipoproteins transported in the bloodstream as chylomicrons and very-low-density lipoproteins, and the released fatty acids are taken up by the tissues for oxidation or storage (Mead et al., 2002). Contrary to the activity of the main digestive enzymes, the ontogenetic expression pattern of the genes encoding for these enzymes has been studied in relatively few fish species, even though basic knowledge on the molecular mechanisms underlying the function and modulation of the enzymatic hydrolysis of the various dietary macronutrients is necessary to better understand the process of digestion in fish (Yúfera et al., 2018).

Covering more than 6 000 000 km^2, the Amazon basin is home to the richest fish fauna in the world with 2,406 valid species, 1,402 of which are endemic (Jézéquel et al., 2020); however, to our knowledge, no data has been reported on the molecular basis of the early digestive physiology of any Amazonian fish species. Fish is the main source of proteins, essential fatty

acids, and micronutrients for the local population, especially for low-income families, and per capita fish consumption is one of the highest in the world (Isaac and de Almeida, 2011). Fish populations are increasingly faced with numerous threats such as pollution, deforestation, hydropower dams, invasive species, and overfishing (Carolsfeld et al., 2003; Winemiller et al., 2016). To counter-balance these effects, aquaculture has been developing steadily for the last decades to contribute to the food needs of a fast growing population (FAO, 2020). Among the cultured species, the highly prized species of the genus *Pseudoplatystoma* Bleeker, 1862 (maximum total lengths of up to 140 cm (Buitrago–Suárez and Burr, 2007)) are the most produced catfish species in South America, and Brazil is the largest producer (IBGE, 2020; Valladão et al., 2018). Production mostly relies on interspecific hybrids (e.g., *P. reticulatum* x *P. corruscans*) for their better growth performance, and more recently, on intergeneric hybrids between *Pseudoplatystoma* spp. and omnivorous catfish species such as *Leiarius marmoratus* or *Phractocephalus hemioliopterus*, since they are less cannibalistic during early life stages, readily accept compound diets, and exhibit faster growth rates than the *Pseudoplatystoma* spp. parent species (Hashimoto et al., 2012). However, the production of hybrids entails risks for the environment and the aquaculture industry. Hybrids have been frequently detected in natural environments and, in the case of the interspecific hybrids, are contaminating natural stocks due to their fertility (Hashimoto et al., 2013). Additionally, some genetic monitoring studies have revealed that the production, trade, and management of these hybrids are currently uncontrolled in Brazil, as broodstocks are often mistakenly composed of interspecific hybrids and even post-F1 hybrids, causing economic losses (Hashimoto et al., 2015). In this context, in order to achieve sustainability in *Pseudoplatystoma* spp. aquaculture, genetic improvement programs and culture techniques should be developed for pure species seeking to obtain similar performances as those of hybrids (Alves et al., 2014).

In order to increase basic knowledge on the molecular basis of the ontogeny of the digestive system of commercially important Amazonian fish species, the aim of this study was to understand the molecular phylogeny of the main digestive enzyme precursors and to analyze their ontogenetic expression pattern in *Pseudoplatystoma punctifer* (Castelnau, 1855). This is a carnivorous migratory catfish species widely distributed in the Amazon basin in Bolivia, Brazil, Colombia, Ecuador, Peru, and Venezuela (Buitrago–Suárez and Burr, 2007) with high potential for aquaculture diversification in the region. We previously analyzed the histological development of the digestive system (Gisbert et al., 2014) and the ontogeny of the digestive enzyme activity (Castro-Ruiz et al., 2019) of this species and in this study we focused on the molecular ontogeny. For that purpose, the digestive enzyme precursors of α-*amylase* (*amy*), *phospholipase A2* (*sPLA2-IB*), *lipoprotein lipase* (*lpl*), *trypsinogen* (*try*), *chymotrypsinogen* (*ctr*), and *pepsinogen* (*pga*) of this species were isolated, partially sequenced, and identified, with gene expression patterns characterized from 3 to 24 days post-fertilization (dpf).

2. Materials and Methods

2.1 Fish rearing and feeding protocol

Pseudoplatystoma punctifer larvae were obtained by hormonal-induced spawning of a sexually mature couple of genitors (♀: 4.73 kg; ♂: 1.15 kg) maintained in captivity at the Instituto de Investigaciones de la Amazonia Peruana (IIAP, Iquitos, Peru). The female and male were injected intramuscularly with the synthetic hormone Conceptal® (Intervet, Huixquilucan, México) at 2.6 ml kg^{-1} and 1 ml kg^{-1} BW, respectively. Hormone injections were administered in two doses: a first one at 10% and 50% of the total dose, and a second one 12 h later at 90% and 50% of the total dose for female and male, respectively. After ovulation, the female was stripped and the obtained eggs (400 g, *ca.* 800000 eggs) were distributed in batches of 100 g and gently mixed with 5 ml of physiological serum and 300

153 µl of previously collected sperm for 1 min. Then, 30 ml of distilled water was added with
154 constant gentle stirring for another 1 min. Fertilized eggs were rinsed three times with 100
155 ml of water from the incubators and transferred thereafter to 60 L cylindroconical incubators
156 connected to a recirculation water system at 28 °C. The larvae hatched 18 ± 2 h later (hatching
157 rate = 84%) and were transferred at 3 dpf to three 30 L fiberglass tanks connected to a water
158 recirculation system provided with mechanical and biological filters. The rearing conditions
159 were as follows: temperature, 28.3 ± 0.4 °C; pH, 6.9 ± 0.2; dissolved oxygen, 8.2 ± 0.5 mg
160 l^{-1}; NO_2^-, 0.04 ± 0.02 mg l^{-1}, NH_4^+, 0.14 ± 0.05 mg l^{-1}, and water flow rate of 0.2 l min^{-1}.
161 The larvae were reared in triplicate (initial density 90 larvae l^{-1}) under a photoperiod of 0L:
162 24D and fed six times a day from 4 to 17 dpf with non-enriched *Artemia* spp. nauplii (37%
163 proteins, 14% lipids, and 11% carbohydrates) in slight excess (0.4 to 17 nauplii ml^{-1})
164 considering the larval density, the weight increase of the larvae, and the daily food ration and
165 weaned onto a commercial inert diet (BioMar®, Nersac, France; proximate composition: 58%
166 proteins, 15% lipids, 20% carbohydrates, 11% ash; particle size: 0.5 mm) within 4 days. Once
167 weaned, individuals were fed five times a day at 5% of the larval wet weight until the end of
168 the experiment at 24 dpf.
169 In the absence of an *ad hoc* ethical committee at the IIAP where this trial was conducted, the
170 animal experimental procedures were conducted in compliance with the Guidelines of the
171 European Union Council (2010/63/EU) on the protection of animals used for scientific
172 purposes.
173
174 *2.2 Sampling and growth measurements*
175 Whole larvae and early juveniles were collected at 3, 10, 13, 18, and 24 dpf and euthanized
176 with an overdose of Eugenol (0.05 µl ml^{-1}; Moyco®, Moyco, Lima, Peru). The sampling
177 criterion was based both on the developmental stage and the feeding protocol used (Figure
178 1). Sampling was done in the morning before the first feeding of the day. For total length

(TL) measurements, 15-30 individuals, depending on the size, were placed in a Petri dish, photographed using a scale bar and TL was measured on the pictures using ImageJ software (Schneider et al., 2012). For evaluating the expression of the digestive enzyme genes throughout development, 100 mg of pooled individuals (125 to 6 individuals, depending on size) at each sampling point were preserved in RNA*later* (1:10 dilution) at -20 °C until further analyses. Survival was evaluated by counting the individuals surviving at the end of each feeding period and considering the number of sampled individuals.

2.3 Partial mRNA isolation, identification and phylogenetic analysis

Total RNA was extracted using TRIzol™ (Invitrogen, San Diego, CA, USA) according to manufacturer's protocol. RNA concentration and quality were determined by spectrophotometry (NanoDrop2000, Thermo Fisher Scientific, Madrid, Spain) measuring the absorbance at 260 and 280 nm and a denaturing electrophoresis in TAE agarose gel (1.5 %). For the preparation of cDNA, total RNA was treated with DNAse I Amplification Grade (Invitrogen) according to manufacturer's protocol to remove genomic DNA traces. Total RNA was then reverse transcribed in 10 µl reaction volume containing 3 µg total RNA using the SuperScript™ First-Strand Synthesis System for RT-PCR (Invitrogen) with oligo (dT) $_{(12\text{-}18)}$ (0.5 µg/ul) and random hexamers primers (50 ng μl^{-1}), 10X RT buffer (200mM Tris-HCl (pH 8.4), 500 mM KCL) 25 mM $MgCl_2$, 0.1 M DTT, 10 mM dNTP mix, SuperScript™ II RT (50 U μl^{-1}), RNaseOUT™ (40 U μl^{-1}) followed by RNase H (2 U μl^{-1}) (Invitrogen) treatment. Reverse transcription reactions were carried out in a thermocycler (Mastercycle R nexus GSX1, Eppendorf AG, Hamburg, Germany) and run according to manufacturer's protocol. Samples were diluted 1:20 in molecular biology grade water and stored at -20 °C until further analyses. To obtain the specific sequences of *try*, *ctr*, *amy*, *lpl*, *sPLA2-IB*, *pga*, and Gliceraldehide-3-fosphate deshydrogenase (*gadph*) genes, alignments of teleost homologs for these genes' sequences obtained from GenBank were made using BioEdit

Sequence Alignment Editor ver. 7.0.5.2 (Hall, 1999). Consensus primers designed from conserved regions identified in these alignments were used for amplification of *P. punctifer* specific gene sequences. The fragments amplified were separated in 2% agarose gel electrophoresis and resulting bands of the expected size were excised, isolated, purified (QIAQuick PCR purification kit, Qiagen, Hilden, Germany) and sequenced. The identity of each sequence was verified using the NCBI Blast analysis tool (www.ncbi.nlm.nih.gov/BLAST) and sequences were deposited in Genbank (Table 1).

For the phylogenetic analyses, protein sequences from different species coding for each gene were obtained at NCBI (Tables A1-6). Multiple protein alignments were performed with MAFFT (Katoh et al., 2002) and ambiguous regions were removed with Gblocks V0.91b (Talavera and Castresana, 2007). The phylogenetic trees were inferred using the maximum likelihood (ML) method implemented in the PhyML program (v3.1/3.0 aLRT) (Guindon and Gascuel, 2003). Best model of evolution was selected using Modelgenerator V.85 (Keane et al., 2006) following the corrected Akaike Information Criterion (with four discrete gamma categories) and used to construct a phylogenetic tree. Bayesian posterior probabilities were computed with MrBayes 3.2.1 (Ronquist and Huelsenbeck, 2003). Two different runs with four incrementally heated simultaneous Monte Carlo Markov chains were conducted over one million generations. Trees were sampled every 100 generations to produce 10,000 trees. In order to estimate posterior probabilities, 25% of the trees were discarded as a burn-in stage, observing when average standard deviation of split frequency (ASDSF) values dropped below 0.01. Trees were generated using MEGA 10.1.7 software and robustness of the phylogeny assumption was evaluated by the bootstrapping procedure from 1000 data-set replicates and with posterior probabilities (PP). The nodes supported at or above the 50% level in the bootstrap analysis were emphasized.

2.4 Gene expression analyses of digestive enzymes during development

The transcript sequences were used as templates to design specific primers with the primer 3 software (Table 1). Quantitative PCR analyses for each gene were performed in triplicate in a 7300 Real-Time PCR System (Applied Biosystems, Roche, Barcelona, Spain). The amplification mix contained 1 µl cDNA, 0.5 µl primers (20 µM) and 10 µl SYBR Green Supermix (Life Technologies, Carlsbad, CA, USA) in a total volume of 20 µl. A negative control was included for each set of reactions on each 96-well plate. The amplification conditions were: 10 min at 95 °C, 40 cycles of 20 s at 95 °C and 1 min at 65 °C, followed by 15 s at 95 °C, 1 min at 60 °C, 15 s at 95 °C, and finally 15 s at 60 °C. A standard curve was obtained by amplification of a dilution series of cDNA to calculate the amplification efficiency (E) for each set of primers. Real-time PCR efficiencies were determined for each gene from the slopes obtained with Applied Biosystems software, applying the equation E=10[−1/slope], where E is PCR efficiency. The relative gene expression ratio (R) for each gene was calculated according to Pfaffl's (2001) formula: $R = (E_{\text{target gene}})^{\Delta Cq \text{ target gene (mean sample} - \text{mean reference sample)}} / (E_{\text{reference gene}})^{\Delta Cq \text{ reference gene (mean sample} - \text{mean reference sample)}}$, where ΔCq is the deviation of the target sample minus the reference sample. The initial time point (3 dpf) was chosen as the reference sample and the relative gene expression was normalized using *gadph* as the reference gene since it did not exhibit any significant variation in expression between the samples.

2.5 Statistics

Results of gene expression were expressed as mean ± SD (n=9). All data were checked for normality (Kolmogorov–Smirnov test) and homogeneity of variance (Bartlett's test). One-Way ANOVA was performed to analyze differences in gene expression during development. All Pairwise Multiple Comparisons were performed using the Holm-Sidak method when significant differences were found at $P<0.05$. Statistical analyses were conducted using SigmaStat 3.0 (Systat Software Inc., Richmond, VA, USA).

3. Results

3.1 Growth and survival

Growth during the ontogeny of *P. punctifer* followed an exponential curve TL (mm) = 4.181 $e^{0.23\times T}$ (r^2 = 0.97, P <0.05) (Figure 1). Survival rate was 95 % and 49% at the end of the *Artemia* feeding period (17 dpf) and at the end of the experiment (24 dpf), respectively.

3.2 Sequences and phylogenetic analyses

The size of the partial nucleotide sequences isolated for *P. punctifer* is indicated in Table 1. Since the aim of this work was to analyze the expression patterns of these genes during ontogeny, obtaining the full-length cDNAs was not pursued.

3.2.1 Alpha amylase

Phylogenetic analysis of 14 teleost α-amylase protein sequences revealed a broad congruence among the results of the Maximum Likelihood (ML) and Bayesian analyses (Figure 2). Seven nodes received strong support in each (ML bootstrap supports between 67 and 100%, Bayesian posterior probabilities of 1.0), whereas 6 nodes received no support (ML bootstrap between 28 and 41%, Bayesian posterior probabilities between 0.6 and 0.8). The phylogenetic analysis revealed two main clades. The first one was composed of two clusters, one containing 4 species from the order Siluriformes (*Tachysurus fulvidraco*, *Bagarius yarelli*, *P. punctifer*, *Pangasianodon hypophthalmus*) and the other including two species from the order Cypriniformes (*Ctenopharyngodon idella*, *Labeo rohita*). The second clade was also divided in two other clusters. The first one was represented by Salmoniformes (*Salmo salar*) and the second one included different orders of Eupercaria represented by Tetraodontiformes (*Tetraodon nigroviridis*), Caragiformes (*Seriola lalandi dorsalis*), Perciformes (*Epinephelus coioides*, *Siniperca chuatsi*), Spariformes (*Sparus aurata*, *Pagrus*

283 *pagrus*), and Pleuronectiformes (*Pseudopleuronectes americanus*). *Pseudoplatystoma*
284 *punctifer* was grouped within the catfish (Siluriformes) clade (Bayesian = 1), where the
285 analyzed sequences displayed the highest percentage of identity with *P. punctifer* that ranged
286 between 85 and 87% (Table A1). The following groups with higher identity percentage with
287 *P. punctifer* were species from the order Spariformes, Perciformes, Carangiformes and
288 Pleuronectiformes (78-77%), and the lowest percentage of identity corresponded to species
289 from the orders Cypriniformes, Perciformes and Tetraodontiformes (76%).
290
291 3.2.2 Phospholipase A2
292 Phylogenetic analysis of 18 teleost phospholipase A2 protein sequences revealed a broad
293 consistency among the results of the ML bootstrap and Bayesian posterior analyses (Figure
294 3). Four nodes received strong support in each (ML bootstrap supports between 85 and 96%,
295 Bayesian posterior probabilities of 0.9-1.0), 4 nodes received medium support for ML
296 bootstrap (50-70%) but high support by Bayesian posterior probabilities (0.92-0.99) and 6
297 nodes received no support (ML bootstrap support between 28 and 55%, Bayesian posterior
298 probabilities between 0.6 and 0.9). The phylogenetic analysis revealed two main clades.
299 The first one was represented by *Oncorhynchus mykiss* (Salmoniformes) and the second one
300 was composed of two clusters. One of them contained species from the orders Gobiiformes
301 (*Parambassis ranga*), Cichliformes (*Oreochromis niloticus*, *Neolamprologus brichardi*), and
302 Carangiformes (*Seriola dumerili*), and the other one was divided into two more clusters. The
303 first cluster was represented by a species from the order Osteoglossiformes (*Scleropages*
304 *formosus*) and the second included two clades: one was composed of species from the orders
305 Siluriformes (*Ictalurus punctatus*, *P. hypophthalmus*, *P. punctifer*, *T. fulvidraco*),
306 Gymnotiformes (*Electrophorus electricus*), and Characiformes (*Astyanax mexicanus*,
307 *Colossoma macropomum*); and the other was composed of species belonging to the orders
308 Cyprinodontoformes (*Kryptolebias marmoratus*, *Xiphophorus couchianus*), Beloniformes

(*Oryzias melastigma*, *O. latipes*), and Esociformes (*Esox lucius*). The node of the clade Siluriformes was supported by the Bayesian posterior analysis but not by the ML bootstrap, and the only supported classification within the clade was that grouping *P. punctifer* and *T. fulvidraco* (Figure 3). The phospholipase A2 of *P. punctifer* presented the highest percentage of identity with the sequences of the Siluriformes species (97%, Table A2). The following groups with higher identity percentage with *P. punctifer* corresponded to species from the order Characiformes and Gymnotiformes (89-92%), and the lowest percentage of identity corresponded to the species from the orders Cyprinodontiformes, Beloniformes, and Osteoglossiformes (66-69%, Table A2).

3.2.3 Lipoprotein lipase

Phylogenetic analysis of 19 teleost lipoprotein lipase protein sequences revealed a very broad congruence among the results of the ML bootstrap and Bayesian posterior analyses (Figure 4). Fourteen nodes received strong support in each (ML bootstrap supports between 77 and 100%, Bayesian posterior probabilities of 0.93-1.0), one node received medium support for ML bootstrap (59%) but good support by Bayesian posterior probabilities (0.93) and two nodes received support by bootstrap (60 and 64%) but not by Bayesian analysis (0.6 and 0.8). The phylogenetic analysis revealed two main clades. The first clade was composed of two clusters. The first one included species from the order Salmoniformes (*Oncorhynchus clarkia*, *O. mykiss*) and second one was represented by species from the orders Moroniformes (*Dicentrarchus labrax*), Pleuronectiformes (*Scophthalmus maximus*, *Paralichthys olivaceus*), Perciformes (*S. chuatsi*), and Spariformes (*Pagrus major*, *S. aurata*). The second clade was also divided into two clusters. One grouped species from the orders Characiformes (*A. mexicanus*), Gymnotiformes (*E. electricus*), and Siluriformes (*I. punctatus*, *P. hypophthalmus*, *P. punctifer*, *T. fulvidraco*), and the other was represented by several species of the order Cypriniformes (*C. idella*, *Danio rerio*, *Carassius auratus*, *Cyprinus carpio*). The

node of the clade Siluriformes was supported by both the ML and the Bayesian posterior analyses (Figure 4). The lipoprotein lipase sequence of *P. punctifer* showed the highest percentage of identity with the sequences of omnivorous species of the order Cypriniformes (91-89%), followed by species from the orders Salmoniformes, Characiformes, Siluriformes, and Cypriniformes (88%). The lowest percentage of identity corresponded to species from the orders Pleuronectiformes, Moroniformes, and Spariformes (82-79%, Table A3).

3.2.4 Trypsin

Phylogenetic analysis of 16 teleost trypsin protein sequences revealed three main clusters that were fully supported by the ML (100%) and Bayesian posterior analyses (1.0) (Figure 5). A first cluster was composed of species represented by the orders Pleuronectiformes (*P. americanus*), Tetraodontiformes (*Takifugu flavidus*), Perciformes (*Gymnodraco acuticeps*), Salmoniformes (*Oncorhynchus nerka*, *Salmo trutta*), Esociformes (*E. lucius*), Gobiiformes (*P. ranga*), and Spariformes (*S. aurata*, *P. pagrus*). The only supported node of this clade was that grouping the Salmoniformes species (ML 65%, Bayesian 0.97) and the Spariformes species (ML 79%, Bayesian 0.99) together. A second cluster was represented by the orders Cypriniformes (*C. carpio*) and Clupeiformes (*Denticeps clupeoides*), and a third cluster grouped species from the order Siluriformes (*Clarias magur*, *I. punctatus*, *P. hypophthalmus*, *P. punctifer*, and *T. fulvidraco*). Regarding the latter, two clades were observed, one represented by *T. fulvidraco*, and the other composed by *C. magur* and another group of species including *I. punctatus*, *P. hypophthalmus*, and *P. punctifer*. The only supported nodes were those that grouped the last three species together (ML 59%, Bayesian 0.94; ML 33%, Bayesian 0.92). The sequence of *P. punctifer* showed the highest percentage of identity with that of the species of the order Siluriformes (86-82%) and also with *D. clupeoides* (84%). The lowest percentage of identity corresponded to species from the orders Pleuronectiformes and Spariformes (73%, Table A4).

361

362 3.2.5 Chymotrypsin

363 The phylogenetic tree of 14 teleost chymotrypsin protein sequences is shown in Figure 6.
364 The ML and Bayesian posterior analyses revealed 7 nodes that received strong support in
365 each (ML 69-99%, Bayesian 1.0), one node only supported by the Bayesian posterior
366 probabilities (0.97) and 5 nodes that were not supported (28-48%, 0.5-0.8). The phylogenetic
367 analysis revealed two first clades that were fully supported. One of them was represented by
368 the order Clupeiformes (*D. clupeoides*), whose sequences corresponded to the isoform B, and
369 the other one grouped the isoform A of the sequences and was divided in two clusters. The
370 first one included species from the order of Siluriformes (*P. hypophthalmus*, *P. punctifer*)
371 and the second one contained two more clusters: one included species from the order
372 Cypriniformes (*D. rerio*, *C. auratus*, *L. rohita*) and the other was represented by species from
373 the order Carangiformes (*S. lalandi dorsalis*), Perciformes (*Perca flavescens*, *Lutjanus*
374 *guttatus*), Scombriformes (*Thunus orientalis*), and Clupeiformes (*Clupea harengus*). The
375 node of the clade Siluriformes that grouped together *P. punctifer* and *P. hypophthalmus* was
376 not supported (ML 47%, B 0.6) and their sequences displayed an 81% identity (Table A5).
377 Similar percentage of identity as with the Siluriformes species was observed for species from
378 the order Cypriniformes, Perciformes and Clupleiformes (81%), and the lowest percentage
379 of identity corresponded to species from the order Cypriniformes and Carangiformes (74-
380 76%, Table A5).

381

382 3.2.6 Pepsin

383 Phylogenetic analysis of 22 teleost pepsinogen protein sequences revealed a broad
384 congruence among the results of the ML bootstrap and Bayesian posterior analyses (Figure
385 7). Twelve nodes received strong support by both analyses (ML 73-100%, Bayesian 0.94-
386 1.0), 3 nodes received medium or low support by ML (39-64%) but high support by Bayesian

posterior analysis (0.94-0.99), and 6 nodes were not supported (ML 31-40%, Bayesian 0.58-0.86). The phylogenetic tree of pepsinogen revealed two first clusters. The first one was represented by one species from the order Osteoglossiformes (*S. formosus*) and the second was divided in two main clades. The first clade grouped together species from the order Spariformes (*P. pagrus, S. aurata*), Cichliformes (*Cichlasoma trimaculatum, O. niloticus*), Pleuronectiformes (*P. olivaceus, P. americanus, Solea senegalensis*), and Scombriformes (*T. orientalis*). The second clade was divided into two clusters, one of them was represented by species from the orders Siluriformes (*P. hypophthalmus, T. fulvidraco, Bagarius yarrelli, P. punctifer*) and Gymnotiformes (*E. electricus*), and the other one grouped together species from the orders Perciformes (*S. chuatsi*, *Siniperca scherzeri*, *Micropterus salmoides*), Pleuronectiformes (*P. americanus*), Scombriformes (*T. orientalis*), Gadiformes (*Gadus macrocephalus*), Esociformes (*E. lucius*), and Clupeiformes (*D. clupeoides*). According to the phylogenetic tree, the sequences of the species from the order of Siluriformes, including *P. punctifer*, corresponded to pepsinogen A1. The node of the clade Siluriformes was highly supported and grouped *P. punctifer* and *B. yarrelli* together (ML 89%, Bayesian 0.98). They displayed the highest identity percentage (100%) of all sequences analyzed, followed by *T. fulvidraco* and *P. hypophthalmus* (93%), and then by *E. electricus,* in accordance with the tree classification. The following groups with higher identity percentage with *P. punctifer* were the species from the order Clupeiformes and Perciformes (75%) and the lowest percentage of identity corresponded to the species from the order Osteoglossiformes (65%) and Chicliformes (64%) (Table A6).

3.3 Gene expression analyses of digestive enzymes during early stages of development

The expression profile of the analyzed digestive genes during the early development of *P. punctifer* is shown in Figure 8. The expression of all genes was detected from 3 dpf (6 mm TL at 28 °C). The level of *amy* expression increased 8-fold from 3 dpf (0.17 ± 0.004 relative

expression units, reu) to 10 dpf (11 mm TL at 28 °C) (1.4 ± 0.39 reu, $P < 0.05$), then decreased at 13 dpf (14 mm TL at 28 °C) (1.07 ± 0.133 reu, $P < 0.05$) and remained constant until 18 dpf (18 mm TL at 28 °C) (1.10 ± 0.003 reu, $P > 0.05$) to increase again 3-fold at 24 dpf (26 mm TL at 28 °C) (2.76 ± 0.05 reu, $P < 0.05$). *Try* expression increased from 3 dpf (3.15 ± 0.22 reu) to 10 dpf (5.12 ± 0.43 reu, $P < 0.05$), then decreased 2.5-fold at 13 dpf (2.05 ± 0.07 reu, $P < 0.05$) and progressively increased until the end of the trial (6.65 ± 0.53 reu; $P < 0.05$). *Ctr* expression increased 6-fold from 3 dpf (0.14 ± 0.02 reu) to 10 dpf (0.88 ± 0.14 reu, $P < 0.05$) to abruptly decrease at 13 dpf (0.24 ± 0.04 reu, $P < 0.05$). *Ctr* expression continued to decrease until 18 dpf (0.04 ± 0.0003 reu, $P < 0.05$) to remain constant thereafter (0.02 ± 0.005 reu, $P > 0.05$). The level of *pga* expression significantly increased from 10 dpf (0.71 ± 0.11 reu) to 13 dpf (237.23 ± 59.06 reu, $P < 0.05$) and continued to increase in an exponential manner until the end the trial (1496.7 ± 619.9 reu, $P < 0.05$). The *sPLA2-IB* expression significantly increased from 3 dpf (0.23 ± 0.02 reu) to 10 dpf (0.94 ± 0.15 reu, $P < 0.05$), and progressively decreased until 18 dpf (0.55 ± 0.24 reu, $P < 0.05$) to remain invariable until the end of the study (0.84 ± 0.08 reu, $P > 0.05$). The level of *lpl* expression significantly increased from 3 dpf (0.03 ± 0.01 reu) to 10 dpf (0.15 ± 0.004 reu, $P < 0.05$), remained constant until 13 dpf (0.17 ± 0.05 reu, $P > 0.05$), and increased again from 13 to 18 dpf (0.33 ± 0.12 reu, $P < 0.05$) to remain constant until the end of the study (0.37 ± 0.23 reu, $P > 0.05$).

4. Discussion

This study provides the first comprehensive analysis of the transcriptional ontogeny of some of the most important digestive enzymes of an Amazonian fish species of the genus *Pseudoplatystoma* and gives insights into the molecular phylogeny of the digestive enzymes and the development of the digestive capacities and feeding preferences during the early life stage of *P. punctifer*.

Overall, the phylogenetic relationships of the protein sequences of the studied digestive genes of *P. punctifer* corresponded to the phylogenetic classification of bony fishes (Betancur-R et al., 2017). As expected, the protein sequences of *P. punctifer* were clustered together with those of other catfish species in all the phylogenetic trees, and were mostly grouped with the protein sequences of species belonging to the primarily freshwater clade of the Otophysi (Siluriformes, Cypriniformes, Characiformes, and Gymnotiformes) (Betancur-R et al., 2017; Nelson et al., 2016), except in those trees with a higher number of less supported nodes (*try*, *ctr*). Similarly, the protein sequences of the species contained in the Superorder Acanthopterygii (Beloniformes, Carangiformes, Cichliformes, Cyprinodontiformes, Gobiiformes, Moroniformes, Perciformes, Pleuronectiformes, Scombriformes, Spariformes, Tetraodontiformes (Betancur-R et al., 2017; Nelson et al., 2016)) were grouped together in most trees. In addition, most of the studied protein sequences of *P. punctifer* (*amy*, *plA2*, *try*, and *pga*) also showed maximum homology with those of other catfish species, with the exception of *lpl* and *ctr*, probably due to the use of partial sequences and/or problems in the annotation of the isoforms. Nevertheless, the phylogenetic relationships allowed identifying some isoforms of the protein sequences. The *ctr* and *pga* sequences isolated from *P. punctifer* were located in clades with sequences of the isoforms *ctr*A and *pga*A1, respectively. In contrast, it was difficult to identify the isoform of *try* as the sequences of the other catfish species were all predicted coding sequences.

The expression of the different genes analyzed in *P. punctifer* was detected before the onset of exogenous feeding, denoting that it is a genetically programmed process. Similar results have been found in other reared fish species (Cahu et al., 2004; Darias et al., 2006; Darias et al., 2007a, 2007b; Galaviz et al., 2015; Mata-Sotres et al., 2016; Péres et al., 1998; Zambonino Infante et al., 2008).

In fish, the stomach is one of the last digestive organs to develop during ontogeny. The onset of the acidic digestion shows the switch from the larval to the juvenile mode of digestion,

464 characterized by a notable improvement of the digestion of complex proteins. This moment
465 is usually considered the transition from the larval to the juvenile stage from a digestive
466 physiology perspective and a suitable moment for weaning onto compound feeds under
467 culture conditions (Lazo et al., 2011; Rønnestad et al., 2013; Zambonino Infante et al., 2008).
468 The development of the stomach is generally determined by the appearance of gastric glands,
469 while stomach functionality is characterized by the expression of pepsinogen and proton
470 pump (H^+/K^+-ATPase) genes in the gastric glands, which are responsible for the production
471 of pepsin and hydrochloric acid, respectively (Darias et al., 2005; Darias et al., 2007a, 2007c;
472 Douglas et al., 1999; Gawlicka et al., 2001). However, the onset of acidic digestion may or
473 may not be synchronized with the morphological development of the gastric glands. For
474 instance, stomach functionality has shown to be delayed by 67 degree days (DD) in haddock,
475 *Melanogrammus aeglefinus* (Perez-Casanova et al., 2006), 147 DD in summer flounder,
476 *Paralichthys dentatus* (Huang et al., 1998), 154 DD in Atlantic halibut, *Hippoglossus*
477 *hippoglossus* (Murray et al., 2006), 195 DD in red porgy, *Pagrus pagrus* (Darias et al., 2005),
478 or 233 DD in Atlantic cod, *Gadus morhua* (Perez-Casanova et al., 2006). In *P. punctifer*, the
479 increase of *pga* expression observed in *P. punctifer* between 10 and 13 dpf was synchronized
480 with the appearance of the gastric glands (Gisbert et al., 2014)—as has also been observed in
481 other fish species, such as in winter flounder, *Pleuronectes americanus* (Douglas et al., 1999),
482 orange-spotted grouper, *Epinephelus coioides* (Feng et al., 2008), spotted rose snapper,
483 *Lutjanus guttatus* (Galaviz et al., 2012), or Sobaity sea bream, *Sparidentex hasta*
484 (Nazemroaya et al., 2020)—and indicated the achievement of the functional maturation of
485 the gastric glands, and hence marked the transition from the larval to the juvenile stage. These
486 results coincide with those we observed at enzymatic activity levels in this species (Castro-
487 Ruiz et al., 2019). The level of *pga* expression generally increases with development and
488 larval growth, which is associated with the increasing number of gastric glands in the stomach
489 (Darias et al., 2005; Darias et al., 2007c; Galaviz et al., 2012; Gao et al., 2013; Mir et al.,

2018; Moguel-Hernández et al., 2016; Murray et al., 2006), leading to full acidification capacity and hence a more efficient protein digestion that can be attained several weeks after the onset of acidic digestion (Darias et al., 2005; Hoehne-Reitan et al., 2001; Yúfera et al., 2004). In *P. punctifer*, the exponential increase of *pga* expression after 13 dpf (14 mm TL) is in accordance with the greater development both in number and size of the gastric glands of the stomach observed histologically (Gisbert et al., 2014).

Although the expression profile of *pga* observed in *P. punctifer* is common to carnivorous fish, *amy* expression did not follow the usual ontogenetic pattern observed in carnivorous species, characterized by an initial high level of expression that gradually decreases until the end of the larval stage (Cahu et al., 2004; Darias et al., 2006; Galaviz et al., 2015; Moguel-Hernández et al., 2016; Péres et al., 1998; Srichanun et al., 2013; Zambonino Infante and Cahu, 1994). Instead, *amy* expression in *P. punctifer* gradually increased until the end of the larval development (10 dpf, 11 mm TL) and remained relatively high afterwards. The capacity to synthesize α-amylase at larval stages is not only considered an indicator of the maturation of the exocrine pancreas (Cahu et al., 2004; Cahu and Zambonino Infante, 1994), but is also related to feeding habits (Kuz'mina, 1996). In particular, it has been reported that α-amylase activity is higher in omnivorous than in carnivorous fish (Fernández et al., 2001; Hidalgo et al., 1999; Kim et al., 2014). An increasing pattern of *amy* expression during development has also been observed in the omnivorous thick lipped grey mullet *Chelon labrosus* (Zouiten et al., 2008), and it has been suggested that constitutive expression of *amy* may represent a true dietary specialization for herbivory and omnivory in prickleback fishes (Kim et al., 2014). The feeding habits of *P. punctifer* larvae and early juveniles in the wild are unknown; however, considering its *amy* expression profile, carbohydrates are probably important in their larval diet. During this developmental period, it seems plausible that this species displays an omnivorous feeding behavior with preference to carnivory and that phytoplankton and invertebrates may be a significant component of the diet. Although *P.*

516 *punctifer* also presents histological and biochemical digestive characteristics of a carnivorous
517 species (Castro-Ruiz et al., 2019; Gisbert et al., 2014), this species has the ability to produce
518 high levels of amylase throughout the onset of acidic digestion, as has been observed later on
519 during the early juvenile stage (Castro-Ruiz et al., 2021). Similar findings were reported in
520 *Odax pullus*, an herbivorous fish that consumes starch-rich red algae as a juvenile, but not
521 much starch as an adult, and yet presents an elevated amylase activity in adulthood (Skea et
522 al., 2005). Between 18 and 24 dpf (18 and 26 mm TL, respectively), *P. punctifer* showed a
523 drastic increase in *amy* expression coinciding with the weaning period, in order to adapt the
524 enzymatic activity to the carbohydrate content (11% in *Artemia vs.* 20% in compound diet)
525 of the new diet supplied. This increase in *amy* expression resulted in an increase of the activity
526 of its enzyme (Castro-Ruiz et al., 2019), showing that the dietary modulation of the α-amylase
527 activity occurs at a transcriptional level during the juvenile stage in this species, as has been
528 observed in other fish species such as European sea bass, *Dicentrarchus labrax* (Péres et al.,
529 1998). The ability of *P. punctifer* to digest carbohydrates during the larval stage could
530 represent an advantage from an industrial perspective since this would permit the formulation
531 of larval feeds with lower protein content if the total replacement of live prey by compound
532 diets is achieved for this species (Darias et al., 2015). However, an increase in gene
533 expression does not necessarily mean that the fish is adequately using this source of energy.
534 Indeed, a recent study performed on the nutritional needs of *P. punctifer* during the early
535 juvenile stage (from 13 to 26 dpf) showed that a dietary carbohydrate content higher than
536 25% induced the production of α-amylase at higher levels than diets with lower carbohydrate
537 content, but reduced growth performance and induced fatty livers (Castro-Ruiz et al., 2021).
538 Therefore, results of digestive enzymes gene expression and activity should be considered
539 along with additional physiological responses, including nutrient interaction.
540 The importance of phospholipids in fish larval nutrition is widely recognized (reviewed in
541 Cahu et al., 2009). The secretory sPLA2-IB does not discriminate fatty acid species and needs

542 bile acid for full enzymatic activity in the intestinal lumen (Murakami et al., 2015). As with
543 α-amylase, an increase in *sPLA2-IB* gene expression during larval development is an
544 indicator of the maturation of the exocrine pancreas (Cahu et al., 2003). Similarly to our
545 results, an increase in sPLA2 gene expression and/or activity during development has also
546 been observed in other fish species such as in turbot, *Scophthalmus maximus* (Hoehne-Reitan
547 et al., 2003), Atlantic cod, *Gadus morhua* (Sæle et al., 2011), spotted rose snapper (Moguel-
548 Hernández et al., 2016), gilthead seabream, *Sparus aurata* (Mata-Sotres et al., 2016), large
549 yellow croaker, *Larimichthys crocea* (Cai et al., 2017), and California halibut, *Paralichthys*
550 *californicus* (Fuentes-Quesada and Lazo, 2018). Moreover, phospholipid content can also
551 affect the maturation of both the pancreas and the intestine, and the regulation of PLA2
552 activity mainly occurs at the transcriptional level (Cahu et al., 2003). For instance, the
553 regulation of s*PLA2-IB* expression by the exogenous diet has been observed in large yellow
554 croaker (Cai et al., 2017). The fact that the *PLA2-IB* gene expression in *P. punctifer* remained
555 high and constant after the onset of acidic digestion indicates that the expression levels were
556 adequate to digest the phospholipids present in the compound diet supplied at weaning. The
557 level of s*PLA2-IB* expression could be also considered an indicator of the nutritional
558 condition of fish, as a down-regulation of the expression of *sPLA2* has been observed in
559 fasted specimens (Benedito-Palos et al., 2014).
560 In the present study, *lpl* gene expression increased between 3 and 10 dpf (6 and 11 mm TL,
561 respectively), which coincides with the complete maturation of the intestine (Castro-Ruiz et
562 al., 2019; Gisbert et al., 2014), and continued to increase thereafter in response to the switch
563 of diet at weaning. A transcriptional regulation of this gene by the diet has also been found
564 in other fish species such as orange-spotted grouper (Li et al., 2016), spotted rose snapper
565 (Moguel-Hernández et al., 2016), and large yellow croaker (Cai et al., 2017).
566 *Try* expression in *P. punctifer* followed an expression profile that has been observed in
567 several fish species, which is characterized by an increase of its expression until the complete

formation and functionality of the stomach (Darias et al., 2007b; García-Gasca et al., 2006; Kortner et al., 2011; Kurokawa et al., 2002; Mir et al., 2018; Murray et al., 2004; Parma et al., 2013; Péres et al., 1998; Srichanun et al., 2013; Srivastava et al., 2002). Similar to what has been reported for several fish species (Darias et al., 2005; Darias et al., 2007b; Galaviz et al., 2011; Mir et al., 2018; Suzer et al., 2006), *try* expression in *P. punctifer* decreased from 10 dpf (11mm TL) concomitant with the increase of *pga* expression, indicating the change from the basic and less efficient digestion of proteins in the anterior intestine to the acidic digestion in the stomach that allows a more efficient digestion of complex proteins from this point onward. However, this pattern can be modulated by the nutritional composition of the diet, as the present study showed at weaning, where both *try* and *pga* expressions increased. This is not surprising considering that both enzymes work together: pepsin makes a first hydrolysis of protein molecules by selective cleavage at Phe, Tyr, and other aromatic amino acids, and trypsin completes the process acting on Lys and Arg residues. As occurred with *amy*, the expression profile of *try* coincided with that of the trypsin activity (Castro-Ruiz et al., 2019), showing that the switch from *Artemia* to the compound diet induced a modulation of the activity of this enzyme at the transcriptional level, as has also been observed in other species (Péres et al., 1998).

Ctr followed a similar expression profile to *try* during the larval stage of *P. punctifer*, denoting the complementary action of their corresponding enzymes trypsin and chymotrypsin until the onset of acidic digestion (Rønnestad et al., 2013). However, the opposite pattern observed after 13 dpf (decrease in *ctr* expression) did not coincide with the increase in the activity of chymotrypsin (Castro-Ruiz et al., 2019), which would indicate that the activity of this enzyme was regulated at a post-transcriptional level during the juvenile stage in response to the dietary change. Alternatively, this difference in the expression profile between *try* and *ctr* could be related to the role of trypsin in the activation of chymotrypsin. Indeed, knowledge about the chymotrypsin dynamics and the importance of this enzyme in

the digestive processes during the early life stages of fish is still limited (Rønnestad et al., 2013).

5. Conclusions

The gene expression of the digestive enzymes analyzed during the development of *P. punctifer* followed the typical profile of a carnivorous species with the exception of *amy*, which increased during development. Based on this, it is suggested that *P. punctifer* displays an omnivorous feeding behavior with a preference towards carnivory during the early life stage. The gene expression results, together with those previously obtained at the protein activity level (Castro-Ruiz et al., 2019), showed that the enzymatic machinery of *P. punctifer* is completely prepared before the onset of exogenous feeding at 4 dpf (6 mm TL) and reaches its maturity between 10 and 13 dpf (11-14 mm TL), when individuals present a digestive system with the adult mode of digestion. This indicates, from a digestive physiology perspective, the transition from the larval to the juvenile stage, and the suitable moment for weaning under culture conditions. Overall, these results contribute to the understanding of the molecular basis of the ontogeny of the digestive system of a commercially important Amazonian fish species and to the development of feeding strategies for fish species of interest for aquaculture diversification in the region.

Funding

This work was funded by the International Joint Laboratory 'Evolution and Domestication of the Amazonian Ichthyofauna' (LMI EDIA, IRD-IIAP-UAGRM, France, Peru and Bolivia) and the IRTA. D.C.-R. benefited from a travel grant from the National Fund for Scientific, Technological Development and Technological Innovation (FONDECYT, Peru) and from a Sud-Nord mobilization grant from the IRD (France).

Acknowledgments

This work has been done within the framework of the network LARVAplus 'Strategies for the development and improvement of fish larvae production in Ibero-America' (117RT0521) funded by the Ibero-American Program of Science and Technology for Development (CYTED, Spain).

References

Alves, A.L., Varela, E.S., Moro, G.V., Kirschnik, L.N.G., 2014. Riscos genéticos da produção de híbridos de peixes nativos. Embrapa Pesca e Aquicultura, Palmas, Brazil.

Benedito-Palos, L., Ballester-Lozano, G., Pérez-Sánchez, J., 2014. Wide-gene expression analysis of lipid-relevant genes in nutritionally challenged gilthead sea bream (Sparus aurata). Gene 547, 34–42. https://doi.org/10.1016/j.gene.2014.05.073

Betancur-R, R., Wiley, E.O., Arratia, G., Acero, A., Bailly, N., Miya, M., Lecointre, G., Ortí, G., 2017. Phylogenetic classification of bony fishes. BMC Evol. Biol. 17, 162. https://doi.org/10.1186/s12862-017-0958-3

Buitrago–Suárez, U.A., Burr, B.M., 2007. Taxonomy of the catfish genus Pseudoplatystoma Bleeker (Siluriformes: Pimelodidae) with recognition of eight species. Zootaxa 1512, 1–38. https://doi.org/10.11646/zootaxa.1512.1.1

Cahu, C., Rønnestad, I., Grangier, V., Zambonino Infante, J.L., 2004. Expression and activities of pancreatic enzymes in developing sea bass larvae (Dicentrarchus labrax) in relation to intact and hydrolyzed dietary protein; involvement of cholecystokinin. Aquaculture 238, 295–308. https://doi.org/10.1016/j.aquaculture.2004.04.013

Cahu, C.L., Gisbert, E., Villeneuve, L.A.N., Morais, S., Hamza, N., Wold, P.-A., Zambonino Infante, J.L., 2009. Influence of dietary phospholipids on early ontogenesis of fish. Aquac. Res. 40, 989–999. https://doi.org/10.1111/j.1365-2109.2009.02190.x

Cahu, C.L., Infante, J.L.Z., Barbosa, V., 2003. Effect of dietary phospholipid level and phospholipid:neutral lipid value on the development of sea bass (Dicentrarchus labrax) larvae fed a compound diet. Br. J. Nutr. 90, 21–28. https://doi.org/10.1079/BJN2003880

Cahu, C.L., Zambonino Infante, J.L., 1994. Early weaning of sea bass (Dicentrarchus labrax) larvae with a compound diet: Effect on digestive enzymes. Comp. Biochem. Physiol. A Physiol. 109, 213–222. https://doi.org/10.1016/0300-9629(94)90123-6

Cai, Z., Xie, F., Mai, K., Ai, Q., 2017. Molecular cloning and genetic ontogeny of some key lipolytic enzymes in large yellow croaker larvae (Larimichthys crocea R.). Aquac. Res. 48, 1183–1193. https://doi.org/10.1111/are.12960

Carolsfeld, J., Harvey, B., Ross, C., Baer, A., 2003. Migratory fishes of South America: Biology, fisheries and conservation status. The World Bank, Washington.

Castro-Ruiz, D., Andree, K.B., Solovyev, M.M., Fernández-Méndez, C., García-Dávila, C., Cahu, C., Gisbert, E., Darias, M.J., 2021. The digestive function of Pseudoplatystoma punctifer early juveniles is differentially modulated by dietary protein, lipid and

carbohydrate content and their ratios. Animals 11, 369. https://doi.org/10.3390/ani11020369

Castro-Ruiz, D., Mozanzadeh, M.T., Fernández-Méndez, C., Andree, K.B., García-Dávila, C., Cahu, C., Gisbert, E., Darias, M.J., 2019. Ontogeny of the digestive enzyme activity of the Amazonian pimelodid catfish Pseudoplatystoma punctifer (Castelnau, 1855). Aquaculture 504, 210–218. https://doi.org/10.1016/j.aquaculture.2019.01.059

Darias, M.J., Castro-Ruiz, D., Estivals, G., Quazuguel, P., Fernández-Méndez, C., Núñez-Rodríguez, J., Clota, F., Gilles, S., García-Dávila, C., Gisbert, E., Cahu, C., 2015. Influence of dietary protein and lipid levels on growth performance and the incidence of cannibalism in *Pseudoplatystoma punctifer* (Castelnau, 1855) larvae and early juveniles. J. Appl. Ichthyol. 31, 74–82. https://doi.org/10.1111/jai.12978

Darias, M.J., Murray, H.M., Gallant, J.W., Astola, A., Douglas, S.E., Yúfera, M., Martínez-Rodríguez, G., 2006. Characterization of a partial α-amylase clone from red porgy (Pagrus pagrus): Expression during larval development. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 143, 209–218. https://doi.org/10.1016/j.cbpb.2005.11.010

Darias, M.J., Murray, H.M., Gallant, J.W., Douglas, S.E., Yúfera, M., Martínez-Rodríguez, G., 2007a. Ontogeny of pepsinogen and gastric proton pump expression in red porgy (Pagrus pagrus): Determination of stomach functionality. Aquaculture 270, 369–378. https://doi.org/10.1016/j.aquaculture.2007.04.045

Darias, M.J., Murray, H.M., Gallant, J.W., Douglas, S.E., Yúfera, M., Martínez-Rodríguez, G., 2007b. The spatiotemporal expression pattern of trypsinogen and bile salt-activated lipase during the larval development of red porgy (Pagrus pagrus, Pisces, Sparidae). Mar. Biol. 152, 109–118. https://doi.org/10.1007/s00227-007-0663-7

Darias, M.J., Murray, H.M., Martínez-Rodríguez, G., Cárdenas, S., Yúfera, M., 2005. Gene expression of pepsinogen during the larval development of red porgy (Pagrus pagrus). Aquaculture 248, 245–252. https://doi.org/10.1016/j.aquaculture.2005.04.044

Darias, M.J., Ortiz-Delgado, J.B., Sarasquete, C., Martínez-Rodríguez, G., Yúfera, M., 2007c. Larval organogenesis of Pagrus pagrus L., 1758 with special attention to the digestive system development. Histol. Histopahtology 22, 753–768. https://doi.org/10.14670/HH-22.753

Dennis, E.A., 1994. Diversity of group types, regulation, and function of phospholipase A2. J. Biol. Chem. 269, 13057–13060.

Douglas, S.E., Gawlicka, A., Mandla, S., Gallant, J.W., 1999. Ontogeny of the stomach in winter flounder: characterization and expression of the pepsinogen and proton pump genes and determination of pepsin activity. J. Fish Biol. 55, 897–915. https://doi.org/10.1111/j.1095-8649.1999.tb00729.x

FAO, 2020. The State of World Fisheries and Aquaculture 2020. FAO, Rome, Italy. https://doi.org/10.4060/ca9229en

Feng, S.Z., Li, W.S., Lin, H.R., 2008. Identification and expression characterization of pepsinogen A in orange-spotted grouper, Epinephelus coioides. J. Fish Biol. 73, 1960–1978. https://doi.org/10.1111/j.1095-8649.2008.01999.x

Fernández, I., Moyano, F.J., Díaz, M., Martínez, T., 2001. Characterization of α-amylase activity in five species of Mediterranean sparid fishes (Sparidae, Teleostei). J. Exp. Mar.

Biol. Ecol. 262, 1–12. https://doi.org/10.1016/S0022-0981(01)00228-3
Fuentes-Quesada, J.P., Lazo, J.P., 2018. The effect of lipid type on lipid digestion enzymes during larval development of the California halibut, Paralichthys californicus. Aquaculture 488, 49–60. https://doi.org/10.1016/j.aquaculture.2018.01.018
Galaviz, M.A., García-Gasca, A., Drawbridge, M., Álvarez-González, C.A., López, L.M., 2011. Ontogeny of the digestive tract and enzymatic activity in white seabass, Atractoscion nobilis, larvae. Aquaculture 318, 162–168. https://doi.org/10.1016/j.aquaculture.2011.05.014
Galaviz, M.A., García-Ortega, A., Gisbert, E., López, L.M., Gasca, A.G., 2012. Expression and activity of trypsin and pepsin during larval development of the spotted rose snapper Lutjanus guttatus. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 161, 9–16. https://doi.org/10.1016/j.cbpb.2011.09.001
Galaviz, M.A., López, L.M., García Gasca, A., Álvarez González, C.A., True, C.D., Gisbert, E., 2015. Digestive system development and study of acid and alkaline protease digestive capacities using biochemical and molecular approaches in totoaba (Totoaba macdonaldi) larvae. Fish Physiol. Biochem. 41, 1117–1130. https://doi.org/10.1007/s10695-015-0073-6
Gao, C., Yang, R.B., Hu, W.B., Wang, J., 2013. Ontogeny of the stomach in yellow catfish (Pelteobagrus fulvidraco): detection and quantifictation of pepsinogen and H+/K+ -ATPase gene expression. J. Anim. Physiol. Anim. Nutr. 97, 20–26. https://doi.org/10.1111/j.1439-0396.2011.01238.x
García-Gasca, A., Galaviz, M.A., Gutiérrez, J.N., García-Ortega, A., 2006. Development of the digestive tract, trypsin activity and gene expression in eggs and larvae of the bullseye puffer fish Sphoeroides annulatus. Aquaculture 251, 366–376. https://doi.org/10.1016/j.aquaculture.2005.05.029
Gawlicka, A., Leggiadro, C.T., Gallant, J.W., Douglas, S.E., 2001. Cellular expression of the pepsinogen and gastric proton pump genes in the stomach of winter flounder as determined by in situ hybridization. J. Fish Biol. 58, 529–536. https://doi.org/10.1111/j.1095-8649.2001.tb02271.x
Gisbert, E., Moreira, C., Castro-Ruiz, D., Öztürk, S., Fernández, C., Gilles, S., Nuñez, J., Duponchelle, F., Tello, S., Renno, J.F., García-Dávila, C., Darias, M.J., 2014. Histological development of the digestive system of the Amazonian pimelodid catfish *Pseudoplatystoma punctifer*. Animal 8, 1765–1776. https://doi.org/10.1017/S1751731114001797
Gisbert, E., Ortiz-Delgado, J.B., Sarasquete, C., 2008. Nutritional cellular biomarkers in early life stages of fish. Histol. Histopathol. 23, 1525–1539. https://doi.org/10.14670/HH-23.1525
Guindon, S., Gascuel, O., 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52, 696–704. https://doi.org/10.1080/10635150390235520
Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.
Hashimoto, D.T., Prado, F.D. do, Senhorini, J.A., Foresti, F., Porto-Foresti, F., 2013.

745 Detection of post-F1 fish hybrids in broodstock using molecular markers: approaches for
746 genetic management in aquaculture. Aquac. Res. 44, 876–884.
747 https://doi.org/10.1111/j.1365-2109.2012.03092.x
748 Hashimoto, D.T., Prado, F.D., Senhorini, J.A., Foresti, F., Porto-Foresti, F., 2015.
749 Aquaculture of neotropical catfish hybrids: genetic strategies for conservation and
750 management, in: Regan, B. (Ed.), Carp and Catfish: Biology, Behavior and Conservation
751 Strategies. Nova Science Publishers, New York, pp. 1–30.
752 Hashimoto, D.T., Senhorini, J.A., Foresti, F., Porto-Foresti, F., 2012. Interspecific fish
753 hybrids in Brazil: management of genetic resources for sustainable use. Rev. Aquac. 4,
754 108–118. https://doi.org/10.1111/j.1753-5131.2012.01067.x
755 Hidalgo, M.C., Urea, E., Sanz, A., 1999. Comparative study of digestive enzymes in fish
756 with different nutritional habits. Proteolytic and amylase activities. Aquaculture 170,
757 267–283. https://doi.org/10.1016/S0044-8486(98)00413-X
758 Hoehne-Reitan, K., Kjørsvik, E., Reitan, K., 2001. Development of the pH in the intestinal
759 tract of larval turbot. Mar. Biol. 139, 1159–1164.
760 https://doi.org/10.1007/s002270100653
761 Hoehne-Reitan, K., Kjørsvik, E., Reitan, K.I., 2003. Lipolytic activities in developing turbot
762 larvae as influenced by diet. Aquac. Int. 11, 477–489.
763 https://doi.org/10.1023/B:AQUI.0000004192.36467.0d
764 Holt, G.J., 2011. Larval Fish Nutrition. John Wiley & Sons, Ltd, Ames, Iowa.
765 Huang, L., Schreiber, A.M., Soffientino, B., Bengtson, D.A., Specker, J.L., 1998.
766 Metamorphosis of summer flounder (Paralichthys dentatus): Thyroid status and the
767 timing of gastric gland formation. J. Exp. Zool. 280. https://doi.org/10.1002/(sici)1097-
768 010x(19980415)280:6<413::aid-jez5>3.0.co;2-q
769 IBGE, 2020. Pesquisa Pecuária Municipal: Produção da aquicultura [WWW Document].
770 URL https://sidra.ibge.gov.br/Tabela/3940 (accessed 11.20.20).
771 Isaac, V.J., de Almeida, M.C., 2011. El consumo de pescado en la amazonía brasileña. FAO,
772 Rome.
773 Jézéquel, C., Tedesco, P.A., Bigorne, R., Maldonado-Ocampo, J.A., Ortega, H., Hidalgo, M.,
774 Martens, K., Torrente-Vilara, G., Zuanon, J., Acosta, A., Agudelo, E., Barrera Maure,
775 S., Bastos, D.A., Bogotá Gregory, J., Cabeceira, F.G., Canto, A.L.C., Carvajal-Vallejos,
776 F.M., Carvalho, L.N., Cella-Ribeiro, A., Covain, R., Donascimiento, C., Dória, C.R.C.,
777 Duarte, C., Ferreira, E.J.G., Galuch, A.V., Giarrizzo, T., Leitão, R.P., Lundberg, J.G.,
778 Maldonado, M., Mojica, J.I., Montag, L.F.A., Ohara, W.M., Pires, T.H.S., Pouilly, M.,
779 Prada-Pedreros, S., de Queiroz, L.J., Rapp Py-Daniel, L., Ribeiro, F.R.V., Ríos Herrera,
780 R., Sarmiento, J., Sousa, L.M., Stegmann, L.F., Valdiviezo-Rivera, J., Villa, F., Yunoki,
781 T., Oberdorff, T., 2020. A database of freshwater fish species of the Amazon Basin. Sci.
782 Data 7, 96. https://doi.org/10.1038/s41597-020-0436-4
783 Kapoor, B.G., Smit, H., Verighina, I.A., 1976. The alimentary canal and digestion in teleosts,
784 in: Russell, F.S., Yonge, M. (Eds.), Advances in Marine Biology. Academic Press, pp.
785 109–239. https://doi.org/10.1016/S0065-2881(08)60281-3
786 Katoh, K., Misawa, K., Kuma, K., Miyata, T., 2002. MAFFT: a novel method for rapid
787 multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 30,

3059–3066. https://doi.org/10.1093/nar/gkf436
Keane, T.M., Creevey, C.J., Pentony, M.M., Naughton, T.J., Mclnerney, J.O., 2006. Assessment of methods for amino acid matrix selection and their use on empirical data shows that ad hoc assumptions for choice of matrix are not justified. BMC Evol. Biol. 6, 29. https://doi.org/10.1186/1471-2148-6-29
Kim, K.H., Horn, M.H., Sosa, A.E., German, D.P., 2014. Sequence and expression of an α-amylase gene in four related species of prickleback fishes (Teleostei: Stichaeidae): ontogenetic, dietary, and species-level effects. J. Comp. Physiol. B 184, 221–234. https://doi.org/10.1007/s00360-013-0780-1
Kortner, T.M., Overrein, I., Øie, G., Kjørsvik, E., Bardal, T., Wold, P.-A., Arukwe, A., 2011. Molecular ontogenesis of digestive capability and associated endocrine control in Atlantic cod (Gadus morhua) larvae. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 160, 190–199. https://doi.org/10.1016/j.cbpa.2011.05.033
Kurokawa, T., Suzuki, T., Ohta, H., Kagawa, H., Tanaka, H., Unuma, T., 2002. Expression of pancreatic enzyme genes during the early larval stage of Japanese eel Anguilla japonica. Fish. Sci. 68, 736–744. https://doi.org/10.1046/j.1444-2906.2002.00487.x
Kuz'mina, V.V., 1996. Influence of age on digestive enzyme activity in some freshwater teleosts. Aquaculture 148, 25–37. https://doi.org/10.1016/S0044-8486(96)01370-1
Lazo, J.P., Darias, M.J., Gisbert, E., 2011. Ontogeny of the Digestive Tract, in: Holt, G.J. (Ed.), Larval Fish Nutrition. John Wiley & Sons, Ltd, pp. 3–46. https://doi.org/10.1002/9780470959862.ch1
Li, S., Mai, K., Xu, W., Yuan, Y., Zhang, Y., Zhou, H., Ai, Q., 2016. Effects of dietary lipid level on growth, fatty acid composition, digestive enzymes and expression of some lipid metabolism related genes of orange-spotted grouper larvae (Epinephelus coioides H.). Aquac. Res. 47, 2481–2495. https://doi.org/10.1111/are.12697
Ma, H., Cahu, C., Zambonino, J., Yu, H., Duan, Q., Le Gall, M.-M., Mai, K., 2005. Activities of selected digestive enzymes during larval development of large yellow croaker (Pseudosciaena crocea). Aquaculture 245, 239–248. https://doi.org/10.1016/j.aquaculture.2004.11.032
Mata-Sotres, J.A., Martos-Sitcha, J.A., Astola, A., Yúfera, M., Martínez-Rodríguez, G., 2016. Cloning and molecular ontogeny of digestive enzymes in fed and food-deprived developing gilthead seabream (Sparus aurata) larvae. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 191, 53–65. https://doi.org/10.1016/j.cbpb.2015.09.006
Mead, J.R., Irvine, S.A., Ramji, D.P., 2002. Lipoprotein lipase: structure, function, regulation, and role in disease. J. Mol. Med. Berl. Ger. 80, 753–769. https://doi.org/10.1007/s00109-002-0384-9
Mir, I.N., Srivastava, P.P., Bhat, I.A., Muralidhar, A.P., Varghese, T., Gireesh-Babu, P., Jain, K.K., 2018. Expression and activity of trypsin and pepsin during larval development of Indian walking catfish (Clarias magur). Aquaculture 491, 266–272. https://doi.org/10.1016/j.aquaculture.2018.03.049
Moguel-Hernández, I., Peña, R., Andree, K.B., Tovar-Ramirez, D., Bonacic, K., Dumas, S., Gisbert, E., 2016. Ontogeny changes and weaning effects in gene expression patterns of digestive enzymes and regulatory digestive factors in spotted rose snapper (Lutjanus

831 guttatus) larvae. Fish Physiol. Biochem. 42, 1319–1334. https://doi.org/10.1007/s10695-
832 016-0220-8
833 Moyano, F.J., Díaz, M., Alarcón, F.J., Sarasquete, M.C., 1996. Characterization of digestive
834 enzyme activity during larval development of gilthead seabream (Sparus aurata). Fish
835 Physiol. Biochem. 15, 121–130. https://doi.org/10.1007/BF01875591
836 Murakami, M., Sato, H., Miki, Y., Yamamoto, K., Taketomi, Y., 2015. A new era of secreted
837 phospholipase A2. J. Lipid Res. 56, 1248–1261. https://doi.org/10.1194/jlr.R058123
838 Murray, H.M., Gallant, J.W., Johnson, S.C., Douglas, S.E., 2006. Cloning and expression
839 analysis of three digestive enzymes from Atlantic halibut (Hippoglossus hippoglossus)
840 during early development: Predicting gastrointestinal functionality. Aquaculture 252,
841 394–408. https://doi.org/10.1016/j.aquaculture.2005.03.030
842 Murray, H.M., Perez-Casanova, J.C., Gallant, J.W., Johnson, S.C., Douglas, S.E., 2004.
843 Trypsinogen expression during the development of the exocrine pancreas in winter
844 flounder (Pleuronectes americanus). Comp. Biochem. Physiol. A. Mol. Integr. Physiol.
845 138, 53–59. https://doi.org/10.1016/j.cbpb.2004.02.020
846 Nazemroaya, S., Nematollahi, M.A., Yazdanparast, R., Farahmand, H., Rezaie, A.,
847 Najafabadi, M.Z., 2020. Pepsinogen expression during larval development of a Persian
848 Gulf Sparid, Sobaity. Aquaculture 523, 735131.
849 https://doi.org/10.1016/j.aquaculture.2020.735131
850 Nelson, J.S., Grande, T.C., Wilson, M.V.H., 2016. Fishes of the World, 5th Edition. ed. John
851 Wiley & Sons, Hoboken, New Jersey.
852 Parma, L., Bonaldo, A., Massi, P., Yúfera, M., Martínez-Rodríguez, G., Gatta, P.P., 2013.
853 Different early weaning protocols in common sole (Solea solea L.) larvae: Implications
854 on the performances and molecular ontogeny of digestive enzyme precursors.
855 Aquaculture 414–415, 26–35. https://doi.org/10.1016/j.aquaculture.2013.07.043
856 Péres, A., Zambonino Infante, J.L., Cahu, C., 1998. Dietary regulation of activities and
857 mRNA levels of trypsin and amylase in sea bass (Dicentrarchus labrax) larvae. Fish
858 Physiol. Biochem. 19, 145–152. https://doi.org/10.1023/A:1007775501340
859 Perez-Casanova, J.C., Murray, H.M., Gallant, J.W., Ross, N.W., Douglas, S.E., Johnson,
860 S.C., 2006. Development of the digestive capacity in larvae of haddock
861 (Melanogrammus aeglefinus) and Atlantic cod (Gadus morhua). Aquaculture 251, 377–
862 401. https://doi.org/10.1016/j.aquaculture.2005.06.007
863 Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RT–
864 PCR. Nucleic Acids Res. 29, e45–e45. https://doi.org/10.1093/nar/29.9.e45
865 Rønnestad, I., Yúfera, M., Ueberschär, B., Ribeiro, L., Sæle, Ø., Boglione, C., 2013. Feeding
866 behaviour and digestive physiology in larval fish: current knowledge, and gaps and
867 bottlenecks in research. Rev. Aquac. 5, S59–S98. https://doi.org/10.1111/raq.12010
868 Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under
869 mixed models. Bioinformatics 19, 1572–1574.
870 https://doi.org/10.1093/bioinformatics/btg180
871 Sæle, Ø., Nordgreen, A., Olsvik, P.A., Hamre, K., 2011. Characterisation and expression of
872 secretory phospholipase A2 group IB during ontogeny of Atlantic cod (Gadus morhua).
873 Br. J. Nutr. 105, 228–237. https://doi.org/10.1017/S0007114510003466

874 Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of
875 image analysis. Nat. Methods 9, 671–675. https://doi.org/10.1038/nmeth.2089
876 Skea, G.L., Mountfort, D.O., Clements, K.D., 2005. Gut carbohydrases from the New
877 Zealand marine herbivorous fishes Kyphosus sydneyanus (Kyphosidae), Aplodactylus
878 arctidens (Aplodactylidae) and Odax pullus (Labridae). Comp. Biochem. Physiol. B
879 Biochem. Mol. Biol. 140, 259–269. https://doi.org/10.1016/j.cbpc.2004.10.008
880 Srichanun, M., Tantikitti, C., Utarabhand, P., Kortner, T.M., 2013. Gene expression and
881 activity of digestive enzymes during the larval development of Asian seabass (Lates
882 calcarifer). Comp. Biochem. Physiol. B Biochem. Mol. Biol. 165, 1–9.
883 https://doi.org/10.1016/j.cbpb.2013.02.005
884 Srivastava, A.S., Kurokawa, T., Suzuki, T., 2002. mRNA expression of pancreatic enzyme
885 precursors and estimation of protein digestibility in first feeding larvae of the Japanese
886 flounder, Paralichthys olivaceus. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 132,
887 629–635. https://doi.org/10.1016/s1095-6433(02)00107-1
888 Suzer, C., Fırat, K., Saka, Ş., 2006. Ontogenic development of the digestive enzymes in
889 common pandora, Pagellus erythrinus, L. larvae. Aquac. Res. 37, 1565–1571.
890 https://doi.org/10.1111/j.1365-2109.2006.01598.x
891 Talavera, G., Castresana, J., 2007. Improvement of phylogenies after removing divergent and
892 ambiguously aligned blocks from protein sequence alignments. Syst. Biol. 56, 564–577.
893 https://doi.org/10.1080/10635150701472164
894 Valladão, G.M.R., Gallani, S.U., Pilarski, F., 2018. South American fish for continental
895 aquaculture. Rev. Aquac. 10, 351–369. https://doi.org/10.1111/raq.12164
896 Winemiller, K.O., McIntyre, P.B., Castello, L., Fluet-Chouinard, E., Giarrizzo, T., Nam, S.,
897 Baird, I.G., Darwall, W., Lujan, N.K., Harrison, I., Stiassny, M.L.J., Silvano, R. a. M.,
898 Fitzgerald, D.B., Pelicice, F.M., Agostinho, A.A., Gomes, L.C., Albert, J.S., Baran, E.,
899 Petrere, M., Zarfl, C., Mulligan, M., Sullivan, J.P., Arantes, C.C., Sousa, L.M., Koning,
900 A.A., Hoeinghaus, D.J., Sabaj, M., Lundberg, J.G., Armbruster, J., Thieme, M.L., Petry,
901 P., Zuanon, J., Vilara, G.T., Snoeks, J., Ou, C., Rainboth, W., Pavanelli, C.S., Akama,
902 A., Soesbergen, A. van, Sáenz, L., 2016. Balancing hydropower and biodiversity in the
903 Amazon, Congo, and Mekong. Science 351, 128–129.
904 https://doi.org/10.1126/science.aac7082
905 Yúfera, M., Darias, M.J., 2007. The onset of exogenous feeding in marine fish larvae.
906 Aquaculture 268, 53–63. https://doi.org/10.1016/j.aquaculture.2007.04.050
907 Yúfera, M., Fernández-Díaz, C., Vidaurreta, A., Cara, J.B., Moyano, F.J., 2004.
908 Gastrointestinal pH and development of the acid digestion in larvae and early juveniles
909 of Sparus aurata (Pisces: Teleostei). Mar. Biol. 144, 863–869.
910 https://doi.org/10.1007/s00227-003-1255-9
911 Yúfera, M., Moyano, F.J., Martínez-Rodríguez, G., 2018. The Digestive Function in
912 Developing Fish Larvae and Fry. From Molecular Gene Expression to Enzymatic
913 Activity, in: Yúfera, M. (Ed.), Emerging Issues in Fish Larvae Research. Springer
914 International Publishing, Cham, pp. 51–86. https://doi.org/10.1007/978-3-319-73244-
915 2_6
916 Zambonino Infante, J.L., Cahu, C.L., 2001. Ontogeny of the gastrointestinal tract of marine

fish larvae. Comp. Biochem. Physiol. Part C Toxicol. Pharmacol. 130, 477–487. https://doi.org/10.1016/S1532-0456(01)00274-5

Zambonino Infante, J.L., Cahu, C.L., 1994. Influence of diet on pepsin and some pancreatic enzymes in sea bass (Dicentrarchus labrax) larvae. Comp. Biochem. Physiol. A Physiol. 109, 209–212. https://doi.org/10.1016/0300-9629(94)90122-8

Zambonino Infante, J.L., Gisbert, E., Sarasquete, C., Navarro, I., Gutiérrez, J., Cahu, C., 2008. Ontogeny and physiology of the digestive system of marine fish larvae, in: Cyrino, J.E.P., Bureau, D.P., Kapoor, B.G. (Eds.), Feeding and Digestive Functions in Fishes. Science Publishers, Enfield, New Hampshire, pp. 281–348.

Zouiten, D., Khemis, I.B., Besbes, R., Cahu, C., 2008. Ontogeny of the digestive tract of thick lipped grey mullet (Chelon labrosus) larvae reared in "mesocosms." Aquaculture 279, 166–172. https://doi.org/10.1016/j.aquaculture.2008.03.039

Tables

Table 1. Accession numbers and oligonucleotide primers used for PCR and relative quantification of gene expression of six digestive enzymes during development of *P. punctifer*. *Gadph* was used as reference gene. The amplification efficiency of each gene was close to 100%.

Gene name	Genbank accession number	Primer	Nucleotide sequence (5' – 3')	Tm (°C)	Product size (bp)
Amy	MT006358	PpuAmy F	CAACAACGYTGGGGTCAACATC	56.7	300
		PpuAmy R	GTCRATCAGCTTGTTCADGAAG	54.8	
		qpAMYPf F	CATGTGGCCTGGAGATTTACAGGC	60.3	113
		qpAMYPf R	CCACCCAGATCAATAACCTCCTGG	58.9	
Try	MT006359	Tryp1 F2	TGTGTCTGCTGCTCACTGC	57.9	384
		Tryp1 R2	GTCACCCTGGCAAGAGTCC	57.2	
		qpTRYPf F	TATGACTCCTGGACCATTGACAATG	57.0	190
		qpTRYPf R	CAGACACTGCAGCTTGTTGCCATC	61.4	
Ctr	MT006344	LgChym2F	GCGGTGCCTCACTCCTGGCCC	64.1	250
		LgChym2R	AGSRSGATGTCGTTGTTGATGGTG	59.1	
		qpCHTPf F	TGCCCACTGCAATGTCAACACTTTC	61.0	100
		qpCHTPf R	ACCTTGGCAATCCTCATGACCTGG	61.2	
sPlA2-IB	MT006345	LgPhLipF	RRATGATCCTGTGYGTGATGCC	58.6	230
		LgPhLipR	CTCRCAGATGAACATCTCRCATTC	57.4	
		qpPLPPf F	ATGCAACATGATGCATGCTGGCC	61.6	100
		qpPLPPf R	GTTGTTGCTCTTGCAGGTGATTGTG	59.9	
Lpl	MT006346	LgLip-qF	AAGCTGGTGTCTGCCCTCTACG	61.0	250
		LgLip-qR	AGCCACATGTGCTCCCAGACTG	61.5	
		qpLLPPf F	TGCCAACGTCATAGTGGTGGACTG	59.1	130
		qpLLPPf R	GTAATCGAGTTCCATCATAAGCCAG	56.0	
Pga	MT006343	PEP 2F	GATGCTGACCTGTCCTACTA	52.8	600
		PEP 2R	TTGATGGTAACACTGTCCAT	51.3	
		qpPEPPf F	TGTCTACCTAAGCAGCAACTCTC	56.4	160
		qpPEPPf R	ATGACAGAGGGATCCAGACCAGAG	59.4	
Gadph	MT006341	GAPD2HF	TATCAATGGATTCGGCCGCA	56.9	500
		GAPD2HR	TGGCAGTGATGGCATGAACT	57.0	
		qpGAPDHPf F	GGTCTTGAGGGCCTGCCTGCAG	64.3	160
		qpGADPHPf R	CGATGAGCTTGCCATCCTCGTG	60.6	

Figure legends

Figure 1. Larval and early juvenile growth in total length (mm) of *P. punctifer* from 0 to 24 days post fertilization. Data are represented as means ± S.D. (n = 45). Values with a different letter denote significant differences during development (one-way ANOVA, $P < 0.05$). The ochre dashed lines indicate some key events in the ontogeny of the digestive system. The green area indicates the transition period from the larval to the juvenile mode of digestion, and the gray area indicates the weaning period. The feeding protocol is indicated by horizontal arrows below the growth curve.

Figure 2. Phylogenetic tree for α-amylase protein sequences from several teleosts, inferred from the Maximum Likelihood method (ML) and rooted on Anura species as outgroups. Numbers close to nodes display branch support values for ML (based on 1000 bootstrapping) followed by Bayesian posterior analysis. Full name of the species, GenBank accession numbers and identity percentage of the α-amylase sequences with respect to that of *P. punctifer* are shown in Table A1. The *amy* obtained in this study from *P. punctifer* is marked with a black star. The clade of the order Siluriformes is demarcated by a violet dotted rectangle. The two-colored columns on the right correspond to feeding habits and water environments of the analyzed species. Code of colors is as follows: salmon pink, carnivorous; yellow, omnivorous; green, herbivorous; dark blue, marine water; medium blue, freshwater/brackish; light blue, freshwater. A, anadromous.

Figure 3. Phylogenetic tree for phospholipase A2 (PLA2) protein sequences from several teleosts, inferred from the Maximum Likelihood method (ML) and rooted on Testudines and Crocodilia species as outgroups. Numbers close to nodes display branch support values for ML (based on 1000 bootstrapping) followed by Bayesian posterior analysis. Full name of the species, GenBank accession numbers and identity percentage of the PLA2 sequences with

967 respect to that of *P. punctifer* are shown in Table A2. The *plA2* obtained in this study from
968 *P. punctifer* (*sPLA2-IB)* is marked with a black star. The clade of the order Siluriformes is
969 demarcated by a violet dotted rectangle. The two-colored columns on the right correspond to
970 feeding habits and water environments of the analyzed species. Code of colors is as follows:
971 salmon pink, carnivorous; yellow, omnivorous; dark blue, marine water; medium blue,
972 freshwater/brackish; light blue, freshwater. A, anadromous.
973
974 **Figure 4.** Phylogenetic tree for lipoprotein lipase (LPL) protein sequences from several
975 teleosts, inferred from the Maximum Likelihood method (ML) and rooted on Testudines and
976 Crocodilia species as outgroups. Numbers close to nodes display branch support values for
977 ML (based on 1000 bootstrapping) followed by Bayesian posterior analysis. Full name of the
978 species, GenBank accession numbers and identity percentage of the LPL sequences with
979 respect to that of *P. punctifer* are shown in Table A3. The *lpl* obtained in this study from *P.*
980 *punctifer* is marked with a black star. The clade of the order Siluriformes is demarcated by a
981 violet dotted rectangle. The two-colored columns on the right correspond to feeding habits
982 and water environments of the analyzed species. Code of colors is as follows: salmon pink,
983 carnivorous; yellow, omnivorous; dark blue, marine water; medium blue,
984 freshwater/brackish; light blue, freshwater. A, anadromous.
985
986 **Figure 5.** Phylogenetic tree for trypsin (TRY) protein sequences from several teleosts,
987 inferred from the Maximum Likelihood method (ML) and rooted on Testudines and
988 Crocodilia species as outgroups. Numbers close to nodes display branch support values for
989 ML (based on 1000 bootstrapping) followed by Bayesian posterior analysis. Full name of the
990 species, GenBank accession numbers and identity percentage of the TRY sequences with
991 respect to that of *P. punctifer* are shown in Table A4. The *try* obtained in this study from *P.*
992 *punctifer* is marked with a black star. The clade of the order Siluriformes is demarcated by a

violet dotted rectangle. The two-colored columns on the right correspond to feeding habits and water environments of the analyzed species. Code of colors is as follows: salmon pink, carnivorous; yellow, omnivorous; dark blue, marine water; medium blue, freshwater/brackish; light blue, freshwater. A, anadromous.

Figure 6. Phylogenetic tree for chymotrypsin (CTR) protein sequences from several teleosts, inferred from the Maximum Likelihood method (ML) and rooted on Anura species as outgroups. Numbers close to nodes display branch support values for ML (based on 1000 bootstrapping) followed by Bayesian posterior analysis. Full name of the species, GenBank accession numbers and identity percentage of the CTR sequences with respect to that of *P. punctifer* are shown in Table A5. The *ctr* obtained in this study from *P. punctifer* is marked with a black star. The clade of the order Siluriformes is demarcated by a violet dotted rectangle. The two-colored columns on the right correspond to feeding habits and water environments of the analyzed species. Code of colors is as follows: salmon pink, carnivorous; yellow, omnivorous; green, herbivorous; dark blue, marine water; medium blue, freshwater/brackish; light blue, freshwater.

Figure 7. Phylogenetic tree for pepsin (PGA) protein sequences from several teleosts, inferred from the Maximum Likelihood method (ML) and rooted on two Anura species as outgroups. Numbers close to nodes display branch support values for ML (based on 1000 bootstrapping) followed by Bayesian posterior analysis. Full name of the species, GenBank accession numbers and identity percentage of the PGA sequences with respect to that of *P. punctifer* are shown in Table A6. The *pga* obtained in this study from *P. punctifer* is marked with a black star. The clade of the order Siluriformes is demarcated by a violet dotted rectangle. The two-colored columns on the right correspond to feeding habits and water environments of the analyzed species. Code of colors is as follows: salmon pink, carnivorous;

yellow, omnivorous; dark blue, marine water; medium blue, freshwater/brackish; light blue, freshwater.

Figure 8. Relative expression of α-amylase (*amy*), phospholipase A2 (*sPlA2-IB*), lipoprotein lipase (*lpl*), trypsinogen (*try*), chymotrypsin (*ctr*), and pepsinogen (*pga*) genes during the development of *P. punctifer* reared at 28 °C. The ochre dashed line indicates hatching (*ca*. 18 hours post fertilization). The green area indicates the transition period from the larval to the juvenile mode of digestion, and the gray area indicates the weaning period. Data are represented as means ± S.D. (n = 9). Values with a different letter denote significant differences during development (one-way ANOVA, $P < 0.05$).

Appendix A

Table A1. Sequence ID of the teleost species used for the phylogenetic analysis of α-amylase. Information on the feeding habits of the species as well as on the percentage of identity at amino acid level among *amy* sequences of *P. punctifer* and different species is also provided. Species from the order Anura were used as outgroups.

Sequences ID	Species	Family	Order	Feeding habit	Identity (%)
AAX37668.1	*Tachysurus fulvidraco*	Bagridae	Siluriformes	Omnivorous	86.82
XP_026767155.1	*Pangasianodon hypophthalmus*	Pangasiidae	Siluriformes	Omnivorous	86.13
TST47702.1	*Bagarius yarrellii*	Sisoridae	Siluriformes	Carnivorous	84.54
ALB35087.1	*Sparus aurata*	Sparidae	Spariformes	Carnivorous	78.32
ACJ26844.1	*Epinephelus coioides*	Serranidae	Perciformes	Carnivorous	77.73
AAU93830.1	*Pagrus pagrus*	Sparidae	Spariformes	Carnivorous	77.53
XP_023263181.1	*Seriola lalandi dorsalis*	Carangidae	Carangiformes	Carnivorous	77.34
AAF65827.1	*Pseudopleuronectes americanus*	Pleuronectidae	Pleuronectiformes	Carnivorous	76.95
ACX35465.1	*Ctenopharyngodon idella*	Cyprinidae	Cypriniformes	Herbivorous	76.56
ACJ06746.1	*Siniperca chuatsi*	Sinipercidae	Perciformes	Carnivorous	76.37
AHY00275.1	*Labeo rohita*	Cyprinidae	Cypriniformes	Herbivorous	76.03
CAD20312.1	*Tetraodon nigroviridis*	Tetraodontidae	Tetraodontiformes	Omnivorous	75.83
XP_002938902.1	*Xenopus tropicalis*	Pipidae	Anura	Omnivorous	75.59
AAL87102.1	*Xenopus laevis*	Pipidae	Anura	Omnivorous	74.46
ABD13895.1	*Salmo salar*	Salmonidae	Salmoniformes	Carnivorous	72.07

Table A2. Sequence ID of the teleost species used for the phylogenetic analysis of phospholipase A2. Information on the feeding habits of the species as well as on the percentage of identity at amino acid level among *sPLA2-IB* sequences of *P. punctifer* and different species is also provided. Species from the orders Testudines and Crocodilia were used as outgroups.

Sequences ID	Species	Family	Order	Feeding habit	Identity (%)
XP_017345520	*Ictalurus punctatus*	Ictaluridae	Siluriformes	Omnivorous	97.22
XP_026765864	*Pangasianodon hypophthalmus*	Pangasiidae	Siluriformes	Omnivorous	97.22
XP_036451599	*Colossoma macropomum*	Serrasalmidae	Characiformes	Omnivorous	91.67
XP_026852122	*Electrophorus electricus*	Gymnotidae	Gymnotiformes	Carnivorous	91.43
XP_007241794	*Astyanax mexicanus*	Characidae	Characiformes	Carnivorous	88.89
XP_026988397.1	*Tachysurus fulvidraco*	Bagridae	Siluriformes	Omnivorous	86.11
XP_022609172.1	*Seriola dumerili*	Carangidae	Carangiformes	Carnivorous	80.56
XP_028270170.1	*Parambassis ranga*	Ambassidae	Gobiiformes	Carnivorous	75.00

XP_003445720.1	*Oreochromis niloticus*	Cichlidae	Cichliformes	Omnivorous	72.22
XP_006805482.1	*Neolamprologus brichardi*	Cichlidae	Cichliformes	Omnivorous	72.22
XP_010875989.1	*Esox lucius*	Esocidae	Esociformes	Carnivorous	72.22
XP_021461418.1	*Oncorhynchus mykiss*	Salmonidae	Salmoniformes	Carnivorous	72.22
XP_017280986.1	*Kryptolebias marmoratus*	Rivulidae	Cyprinodontiformes	Carnivorous	69.44
XP_024155431.1	*Oryzias melastigma*	Adrianichthyidae	Beloniformes	Omnivorous	69.44
XP_023816872.1	*Oryzias latipes*	Adrianichthyidae	Beloniformes	Carnivorous	69.44
XP_027881920.1	*Xiphophorus couchianus*	Poeciliidae	Cyprinodontiformes	Omnivorous	66.67
XP_018587154.2	*Scleropages formosus*	Osteoglossidae	Osteoglossiformes	Carnivorous	65.71
XP_005288773.1	*Chrysemys picta bellii*	Emydidae	Testudines		56.25
XP_014373187.1	*Alligator sinensis*	Alligatoridae	Crocodilia		45.16

Table A3. Sequence ID of the teleost species used for the phylogenetic analysis of lipoprotein lipase. Information on the feeding habits of the species as well as on the percentage of identity at amino acid level among *lpl* sequences of *P. punctifer* and different species is also provided. Species from the orders Testudines and Crocodilia were used as outgroups.

Sequences ID	**Species**	**Family**	**Order**	**Feeding habit**	**Identity (%)**
XP_026091567.1	*Carassius auratus*	Cyprinidae	Cypriniformes	Omnivorous	91.07
ACN66300.1	*Ctenopharyngodon idella*	Cyprinidae	Cypriniformes	Herbivorous	89.29
ACN37860.1	*Carassius auratus*	Cyprinidae	Cypriniformes	Omnivorous	89.29
AFL69952.1	*Oncorhynchus clarkii*	Salmonidae	Salmoniformes	Carnivorous	87.50
XP_007240188.2	*Astyanax mexicanus*	Characidae	Characiformes	Carnivorous	87.50
XP_026786482.1	*Pangasianodon hypophthalmus*	Pangasiidae	Siluriformes	Omnivorous	87.50
NP_571202.1	*Danio rerio*	Cyprinidae	Cypriniformes	Carnivorous	87.50
AIU47021.1	*Cyprinus carpio*	Cyprinidae	Cypriniformes	Omnivorous	87.50
CAB40545.1	*Oncorhynchus mykiss*	Salmonidae	Salmoniformes	Carnivorous	85.71
XP_026861273.1	*Electrophorus electricus*	Gymnotidae	Gymnotiformes	Carnivorous	85.71
NP_001316205.1	*Ictalurus punctatus*	Ictaluridae	Siluriformes	Omnivorous	85.71
ACN66301.1	*Cyprinus carpio*	Cyprinidae	Cypriniformes	Omnivorous	85.71
AFH75405.1	*Scophthalmus maximus*	Scophthalmidae	Pleuronectiformes	Carnivorous	82.14
ADY05335.1	*Paralichthys olivaceus*	Paralichthyidae	Pleuronectiformes	Carnivorous	82.14
CAL69901.1	*Dicentrarchus labrax*	Moronidae	Moroniformes	Carnivorous	80.36
ACI32420.1	*Siniperca chuatsi*	Sinipercidae	Perciformes	Carnivorous	80.36
BAE95413.1	*Pagrus major*	Sparidae	Spariformes	Carnivorous	78.57
AAS75120.1	*Sparus aurata*	Sparidae	Spariformes	Carnivorous	78.57
KYO35095.1	*Alligator mississippiensis*	Alligatoridae	Crocodilia		67.86
XP_005278929.1	*Chrysemys picta bellii*	Emydidae	Testudines		66.07

Table A4. Sequence ID of the teleost species used for the phylogenetic analysis of trypsin. Information on the feeding habits of the species as well as on the percentage of identity at amino acid level among *try* sequences of *P. punctifer* and different species is also provided. Species from the orders Testudines and Crocodilia were used as outgroups.

Sequences ID	Species	Family	Order	Feeding habit	Identity (%)
XP_017311586.1	*Ictalurus punctatus*	Ictaluridae	Siluriformes	Omnivorous	86.18
KAF5908450.1	*Clarias magur*	Clariidae	Siluriformes	Omnivorous	85.37
XP_028837466.1	*Denticeps clupeoides*	Denticipitidae	Clupeiformes	Omnivorous	83.74
XP_026779498.1	*Pangasianodon hypophthalmus*	Pangasiidae	Siluriformes	Omnivorous	82.93
XP_027022007.1	*Tachysurus fulvidraco*	Bagridae	Siluriformes	Carnivorous	82.11
TWW54618.1	*Takifugu flavidus*	Tetraodontidae	Tetraodontiformes	Omnivorous	79.67
XP_034077433.1	*Gymnodraco acuticeps*	Bathydraconidae	Perciformes	Carnivorous	79.67
XP_028282177	*Parambassis ranga*	Ambassidae	Gobiiformes	Carnivorous	79.67
XP_029535066.1	*Oncorhynchus nerka*	Salmoninae	Salmoniformes	Carnivorous	78.86
ABE68639.1	*Sparus aurata*	Sparidae	Spariformes	Carnivorous	78.05
XP_029593925.1	*Salmo trutta*	Salmonidae	Salmoniformes	Carnivorous	78.05
XP_034144643	*Esox lucius*	Esocidae	Esociformes	Carnivorous	78.05
BAL04386.1	*Cyprinus carpio*	Cyprinidae	Cypriniformes	Omnivorous	76.42
AAC32752.1	*Pseudopleuronectes americanus*	Pleuronectidae	Pleuronectiformes	Carnivorous	73.17
AAX39390.1	*Pagrus pagrus*	Sparidae	Spariformes	Carnivorous	72.88
XP_023969437	*Chrysemys picta bellii*	Emydidae	Testudines		44.96
XP_025055986	*Alligator sinensis*	Alligatoridae	Crocodilia		41.54

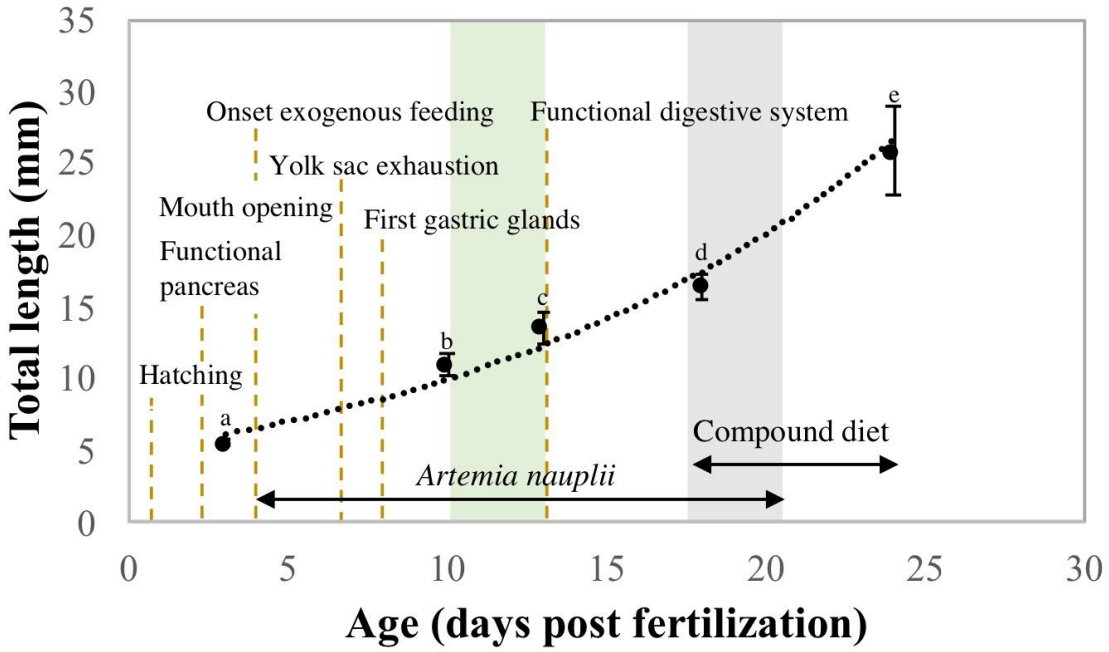
Table A5. Sequence ID of the teleost species used for the phylogenetic analysis of chymotrypsin. Information on the feeding habits of the species as well as on the percentage of identity at amino acid level among *ctr* sequences of *P. punctifer* and different species is also provided. Species from the order Anura were used as outgroups.

Sequences ID	Species	Family	Order	Feeding habit	Identity (%)
BAL72705.1	*Thunnus orientalis*	Scombridae	Scombriformes	Carnivorous	82.09
BAL14137.1	*Thunnus orientalis*	Scombridae	Scombriformes	Carnivorous	82.09
XP_026793160.1	*Pangasianodon hypophthalmus*	Pangasiidae	Siluriformes	Omnivorous	80.88
XP_026793159.2	*Pangasianodon hypophthalmus*	Pangasiidae	Siluriformes	Omnivorous	80.88
RXN21076.1	*Labeo rohita*	Cyprinidae	Cypriniformes	Herbivorous	80.60
XP_028434889.1	*Perca flavescens*	Percidae	Perciformes	Carnivorous	80.60
XP_031424552.1	*Clupea harengus*	Clupeidae	Clupeiformes	Carnivorous	80.60
AAH55574.1	*Danio rerio*	Cyprinidae	Cypriniformes	Carnivorous	76.12
XP_026071043.1	*Carassius auratus*	Cyprinidae	Cypriniformes	Omnivorous	76.12

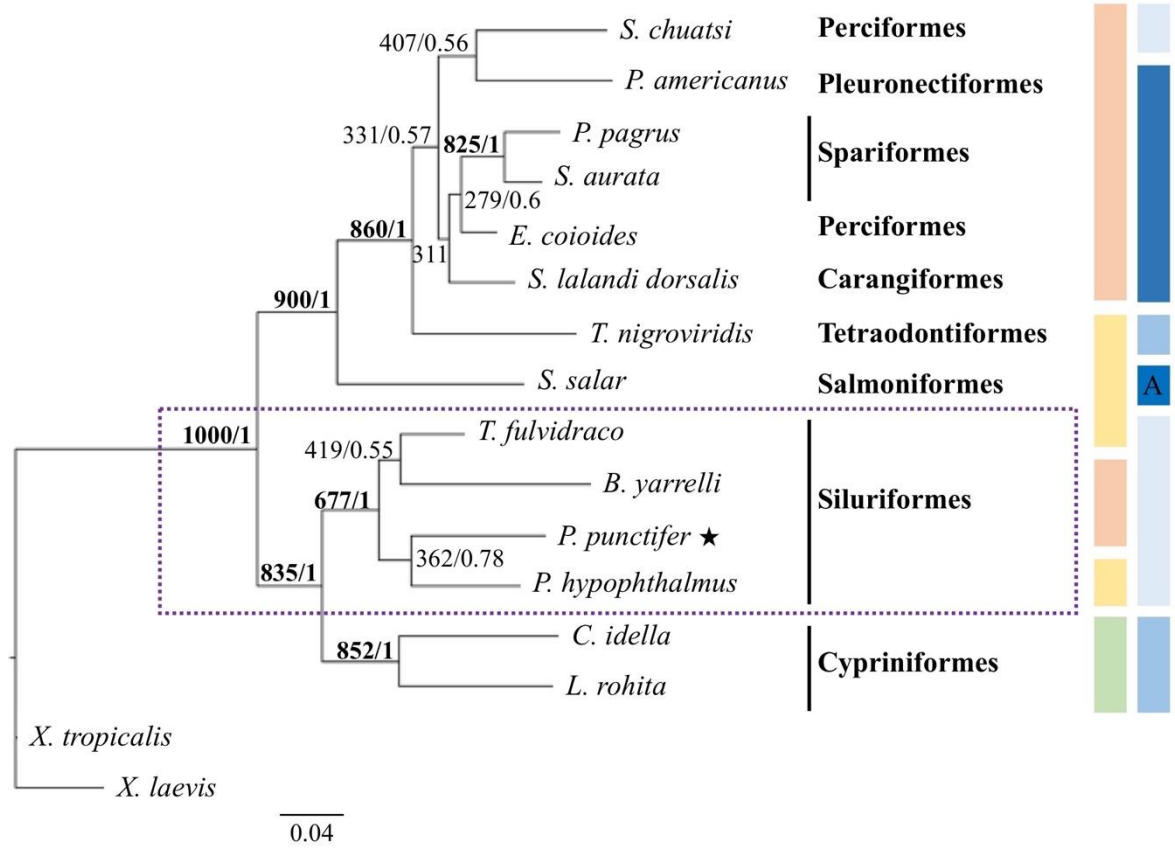
XP_023261815.1	*Seriola lalandi dorsalis*	Carangidae	Carangiformes	Carnivorous	74.60
AAH89075.1	*Xenopus tropicalis*	Pipidae	Anura		73.13
NP_001011477.1	*Xenopus tropicalis*	Pipidae	Anura		73.12
XP_028812135.1	*Denticeps clupeoides*	Denticipitidae	Clupeiformes	Omnivorous	69.12
AIS23637.1	*Lutjanus guttatus*	Lutjanidae	Perciformes	Carnivorous	64.71
XP_028812134.1	*Denticeps clupeoides*	Denticipitidae	Clupeiformes	Omnivorous	63.01

Table A6. Sequence ID of the teleost species used for the phylogenetic analysis of pepsin. Information on the feeding habits of the species as well as on the percentage of identity at amino acid level among *pga* sequences of *P. punctifer* and different species is also provided. Species from the order Anura were used as outgroups.

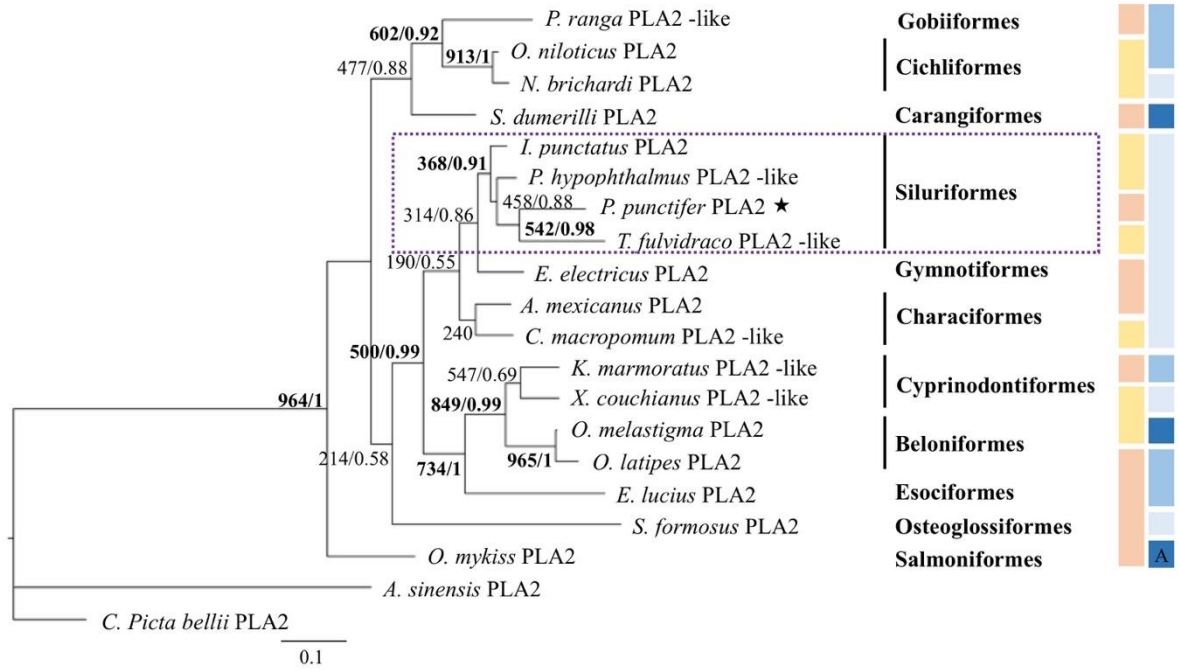
Sequences ID	Species	Family	Order	Feeding habit	Identity (%)
TUC86171.1	*Bagarius yarrelli*	Sisoridae	Siluriformes	Carnivorous	100.00
XP_027011481.1	*Tachysurus fulvidraco*	Bagridae	Siluriformes	Omnivorous	93.18
XP_026771141.1	*Pangasianodon hypophthalmus*	Pangasiidae	Siluriformes	Omnivorous	93.18
XP_026864186.1	*Electrophorus electricus*	Gymnotidae	Gymnotiformes	Carnivorous	88.64
XP_028822570.1	*Denticeps clupeoides*	Denticipitidae	Clupeiformes	Omnivorous	75.00
ACF18588.1	*Siniperca scherzeri*	Sinipercidae	Perciformes	Carnivorous	75.00
ACF18587.1	*Siniperca chuatsi*	Sinipercidae	Perciformes	Carnivorous	75.00
XP_010890229.2	*Esox lucius*	Esocidae	Esociformes	Carnivorous	72.50
AAD56287.1	*Pseudopleuronectes americanus*	Pleuronectidae	Pleuronectiformes	Carnivorous	71.05
BAM76489.1	*Gadus macrocephalus*	Gadidae	Gadiformes	Carnivorous	70.45
BAG48263.1	*Thunnus orientalis*	Scombridae	Scombriformes	Carnivorous	70.45
AAD56288.1	*Pseudopleuronectes americanus*	Pleuronectidae	Pleuronectiformes	Carnivorous	70.27
ABX89618.1	*Sparus aurata*	Sparidae	Spariformes	Carnivorous	68.89
AAZ29603.1	*Pagrus pagrus*	Sparidae	Spariformes	Carnivorous	68.89
BAC87742.1	*Paralichthys olivaceus*	Paralichthyidae	Pleuronectiformes	Carnivorous	68.29
ASW27226.1	*Solea senegalensis*	Soleidae	Pleuronectiformes	Carnivorous	68.29
BAU37037.1	*Micropterus salmoides*	Centrarchidae	Perciformes	Carnivorous	68.18
BAG48264.1	*Thunnus orientalis*	Scombridae	Scombriformes	Carnivorous	66.67
KPP65830.1	*Scleropages formosus*	Osteoglossidae	Osteoglossiformes	Carnivorous	65.12
XP_003444873.1	*Oreochromis niloticus*	Cichlidae	Cichliformes	Omnivorous	64.44
AXB22642.1	*Cichlasoma trimaculatum*	Cichlidae	Cichliformes	Carnivorous	64.44
BAB20798.1	*Xenopus laevis*	Pipidae	Anura		60.00
BAB20092.1	*Lithobates catesbeianus*	Ranidae	Anura		60.00


Total length (mm)
Age (days post fertilization)
Onset exogenous feeding
Functional digestive system
Yolk sac exhaustion
Mouth opening
First gastric glands
Functional pancreas
Hatching
Compound diet
Artemia nauplii
a
b
c
d
e

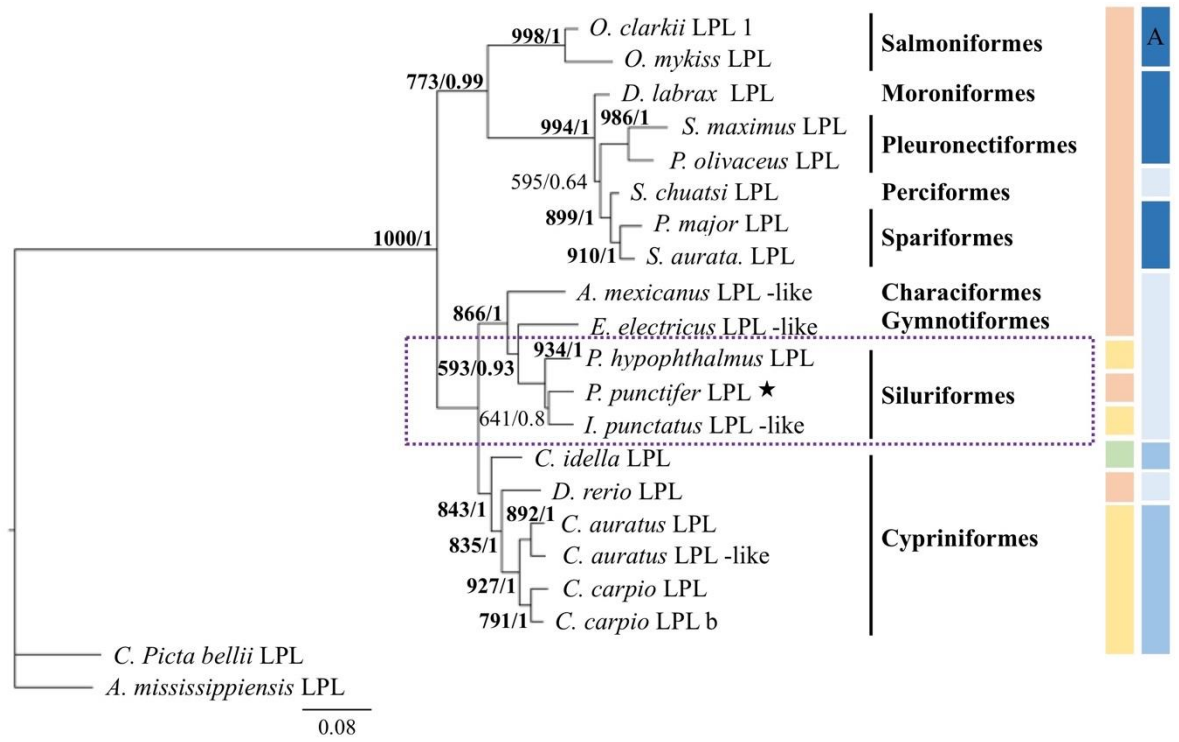
1062


S. chuatsi
P. americanus
P. pagrus
S. aurata
E. coioides
S. lalandi dorsalis
T. nigroviridis
S. salar
T. fulvidraco
B. yarrelli
P. punctifer ★
P. hypophthalmus
C. idella
L. rohita
X. tropicalis
X. laevis
407/0.56
331/0.57
825/1
279/0.6
860/1
311
900/1
1000/1
419/0.55
677/1
362/0.78
835/1
852/1
0.04
Perciformes
Pleuronectiformes
Spariformes
Perciformes
Carangiformes
Tetraodontiformes
Salmoniformes
Siluriformes
Cypriniformes
A

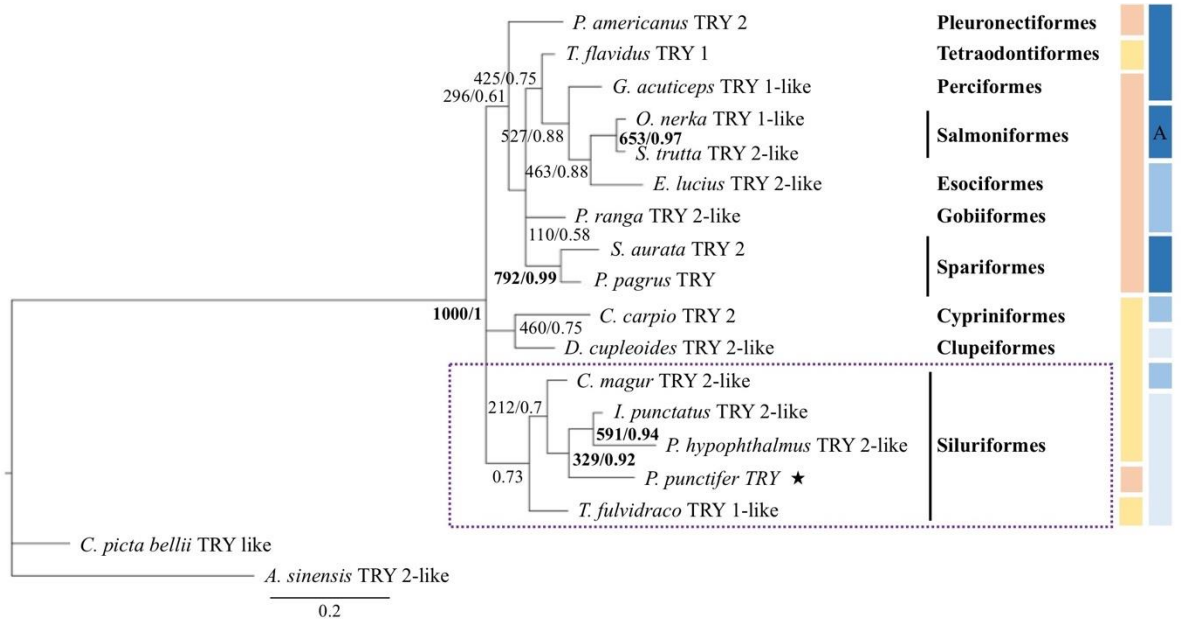
1063


P. ranga PLA2 -like
602/0.92
913/1
O. niloticus PLA2
N. brichardi PLA2
477/0.88
S. dumerilli PLA2
I. punctatus PLA2
368/0.91
P. hypophthalmus PLA2 -like
458/0.88
P. punctifer PLA2 ★
314/0.86
542/0.98
T. fulvidraco PLA2 -like
190/0.55
E. electricus PLA2
A. mexicanus PLA2
240
C. macropomum PLA2 -like
500/0.99
547/0.69
K. marmoratus PLA2 -like
X. couchianus PLA2 -like
964/1
849/0.99
O. melastigma PLA2
214/0.58
734/1
965/1
O. latipes PLA2
E. lucius PLA2
S. formosus PLA2
O. mykiss PLA2
A. sinensis PLA2
C. Picta bellii PLA2
0.1
Gobiiformes
Cichliformes
Carangiformes
Siluriformes
Gymnotiformes
Characiformes
Cyprinodontiformes
Beloniformes
Esociformes
Osteoglossiformes
Salmoniformes
A

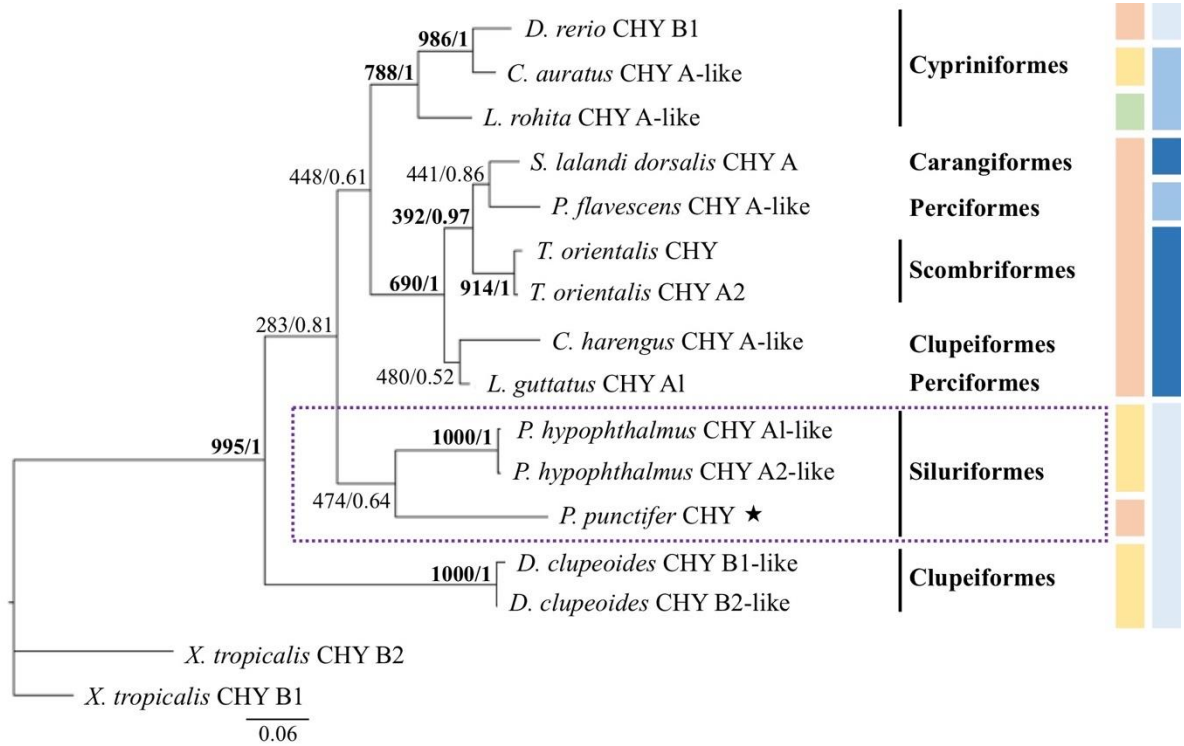
1064


998/1
O. clarkii LPL 1
O. mykiss LPL
773/0.99
D. labrax LPL
994/1
986/1
S. maximus LPL
P. olivaceus LPL
595/0.64
S. chuatsi LPL
899/1
P. major LPL
1000/1
910/1
S. aurata. LPL
A. mexicanus LPL -like
866/1
E. electricus LPL -like
934/1
P. hypophthalmus LPL
593/0.93
P. punctifer LPL ★
641/0.8
I. punctatus LPL -like
C. idella LPL
D. rerio LPL
843/1
892/1
C. auratus LPL
835/1
C. auratus LPL -like
927/1
C. carpio LPL
791/1
C. carpio LPL b
C. Picta bellii LPL
A. mississippiensis LPL
0.08
Salmoniformes
Moroniformes
Pleuronectiformes
Perciformes
Spariformes
Characiformes
Gymnotiformes
Siluriformes
Cypriniformes
A

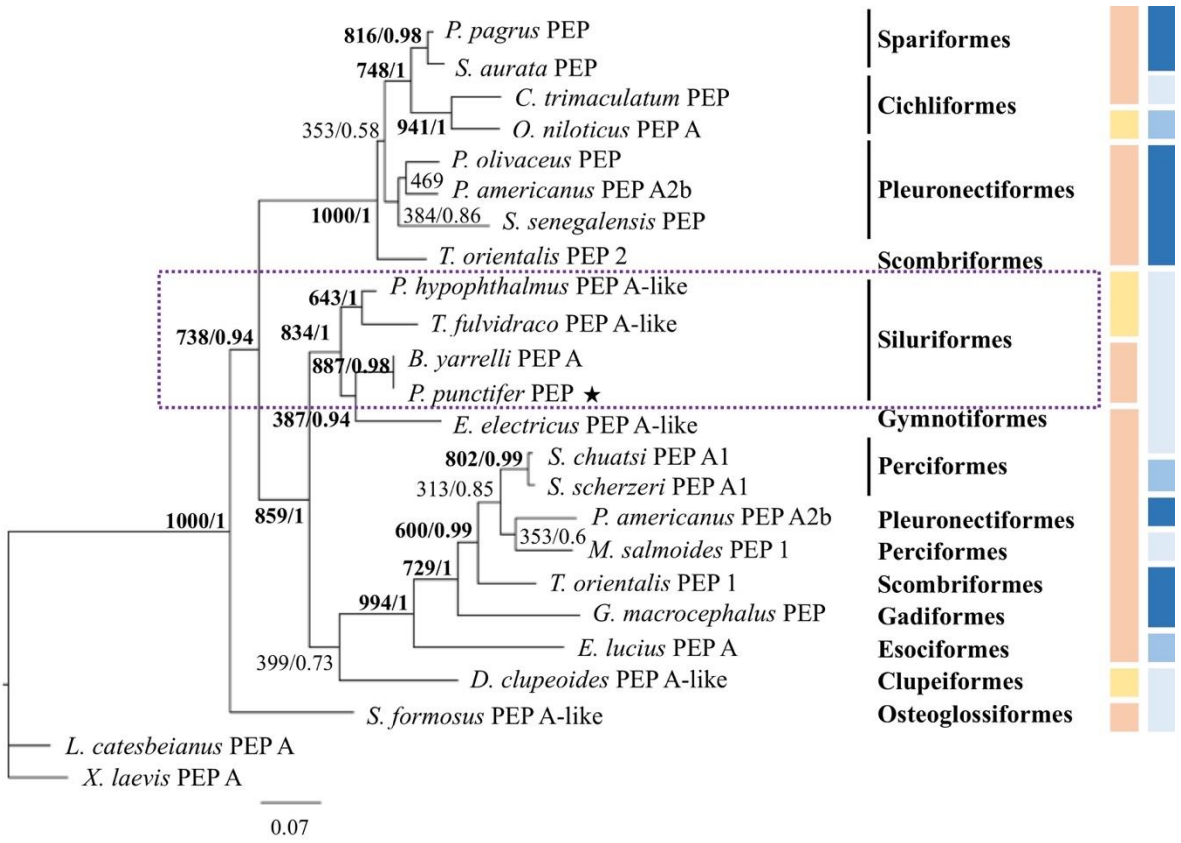
1065


P. americanus TRY 2
T. flavidus TRY 1
G. acuticeps TRY 1-like
O. nerka TRY 1-like
S. trutta TRY 2-like
E. lucius TRY 2-like
P. ranga TRY 2-like
S. aurata TRY 2
P. pagrus TRY
C. carpio TRY 2
D. cupleoides TRY 2-like
C. magur TRY 2-like
I. punctatus TRY 2-like
P. hypophthalmus TRY 2-like
P. punctifer TRY ★
T. fulvidraco TRY 1-like
C. picta bellii TRY like
A. sinensis TRY 2-like
425/0.75
296/0.61
527/0.88
653/0.97
463/0.88
110/0.58
792/0.99
1000/1
460/0.75
212/0.7
591/0.94
329/0.92
0.73
0.2
Pleuronectiformes
Tetraodontiformes
Perciformes
Salmoniformes
Esociformes
Gobiiformes
Spariformes
Cypriniformes
Clupeiformes
Siluriformes
A

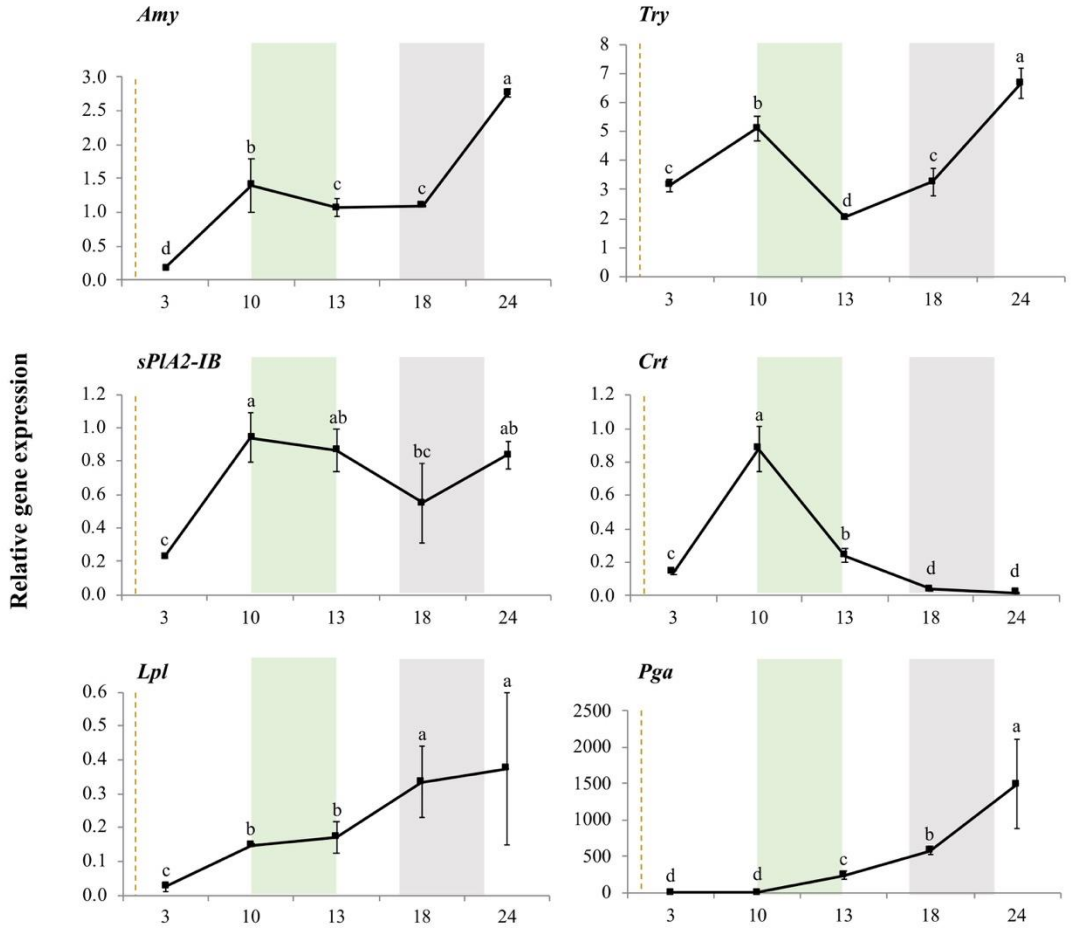
1066


D. rerio CHY B1
C. auratus CHY A-like
L. rohita CHY A-like
S. lalandi dorsalis CHY A
P. flavescens CHY A-like
T. orientalis CHY
T. orientalis CHY A2
C. harengus CHY A-like
L. guttatus CHY Al
P. hypophthalmus CHY Al-like
P. hypophthalmus CHY A2-like
P. punctifer CHY ★
D. clupeoides CHY B1-like
D. clupeoides CHY B2-like
X. tropicalis CHY B2
X. tropicalis CHY B1
986/1
788/1
448/0.61
441/0.86
392/0.97
690/1
914/1
283/0.81
480/0.52
995/1
1000/1
474/0.64
1000/1
0.06
Cypriniformes
Carangiformes
Perciformes
Scombriformes
Clupeiformes
Perciformes
Siluriformes
Clupeiformes

1067


816/0.98
P. pagrus PEP
748/1
S. aurata PEP
C. trimaculatum PEP
353/0.58
941/1
O. niloticus PEP A
P. olivaceus PEP
469
P. americanus PEP A2b
1000/1
384/0.86
S. senegalensis PEP
T. orientalis PEP 2
643/1
P. hypophthalmus PEP A-like
738/0.94
834/1
T. fulvidraco PEP A-like
887/0.98
B. yarrelli PEP A
P. punctifer PEP ★
387/0.94
E. electricus PEP A-like
802/0.99
S. chuatsi PEP A1
313/0.85
S. scherzeri PEP A1
1000/1
859/1
P. americanus PEP A2b
600/0.99
353/0.6
M. salmoides PEP 1
729/1
T. orientalis PEP 1
994/1
G. macrocephalus PEP
E. lucius PEP A
399/0.73
D. clupeoides PEP A-like
S. formosus PEP A-like
L. catesbeianus PEP A
X. laevis PEP A
0.07
Spariformes
Cichliformes
Pleuronectiformes
Scombriformes
Siluriformes
Gymnotiformes
Perciformes
Pleuronectiformes
Perciformes
Scombriformes
Gadiformes
Esociformes
Clupeiformes
Osteoglossiformes

1068


Amy
Try
sPlA2-IB
Crt
Lpl
Pga
Relative gene expression
Days post fertilization

1069