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Prospection of putative genes for digestive enzymes based on functional genome of the hepatopancreas of Amazon river prawn

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ABSTRACT. Over recent years, *Macrobrachium amazonicum* has become a popular species for shrimp farming due to their fast growth, high survival rates, and marketability. Several studies have focused on the development of new technology for the culture of this species, but many aspects of their nutrition and physiology remain unknown. Thus, the goal of the present study was to obtain transcripts of putative genes encoding digestive enzymes, based on a library of the cDNA from the hepatopancreas of *M. amazonicum*, sequenced in the *Ion Torrent™* platform. We identified fragments of nine genes related to digestive enzymes, acting over proteins, carbohydrates and lipids. Endo and exoproteases were also recorded in the hepatopancreas, indicating adaptation to the digestion of protein-rich foods. Nonetheless, the enzymes involved in the carbohydrate metabolism formed the largest functional group in *M. amazonicum*, including enzymes related to the digestion of starch, chitin, and cellulose. These findings indicate that the species has a genetic apparatus of a well-adapted omnivorous animal. This information may provide important insights for the selection of ingredients for the formulation of a more appropriate diet to the enzymatic repertoire of *M. amazonicum*.

Keywords: transcriptome; trypsin; cathepsin; amylase; cellulase.

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Introduction

The Amazon river prawn, *Macrobrachium amazonicum* (Heller, 1862), is widely distributed in South America and Caribbean (Holthuis, 1952; Anger, 2013). This species plays a key ecological role, representing a major item in several trophic webs of both continental and coastal regions (Anger, 2013). Furthermore, this species is largely exploited by local fisheries in northern and northeastern Brazil, providing food and income to thousands of families (Moraes-Valenti & Valenti, 2010; Araújo, Silva, Silva, Ferreira, & Cintra, 2014).

Many studies have been carried out to develop the intensive culture of *M. amazonicum*, since this is an economically important shrimp species for fisheries and with potential to farming (Marques & Moraes-Valenti, 2012). Several aspects focusing on their larval development have been established, such as hatchery management (Moraes-Valenti & Valenti, 2010; Soeiro, Rocha, Maciel, Abrunhosa, & Maciel, 2016), feeding behavior (Moraes-Valenti & Valenti, 2010; Maciel & Valenti, 2014a), coloration of raising tanks (Maciel & Valenti, 2014b), osmotic regulation (Leone et al., 2012; Pinto et al., 2016) and endotrophic potential (Anger & Hayd, 2009). The grow-out phase has also been evaluated in terms of density of enclosures (Marques, Barros, Mallasen, Boock, & Moraes-Valenti, 2012), effluent outlet in grow-out ponds (Kimpara, Rosa, Preto, & Valenti, 2011) and in integrated systems (Henry-Silva, Maia, Moura, Bessa Junior, & Valenti, 2015; David, Proença, & Valenti, 2017; Rodrigues et al., 2019). It was verified that *M. amazonicum* is well-adapted to captive conditions, showing fast growth, high survival rates, and proper cultivation techniques for all developmental stages (Moraes-Valenti & Valenti, 2010).

Despite these advances, no data about nutrition requirements that could be helpful to formulate commercial feed are available for this species, representing a major bottleneck for the commercial culture of Amazon river prawn. A well balanced diet should rely not only on the nutrients requirement of the specie as

also on its feed habit which reflects on its ability in digesting nutrient from different origins. Santos et al. (2014) identified proteases in the hepatopancreas (trypsin, leucine aminopeptidase and chymotrypsin) of adult males of this species by using specific substrates and inhibition tests. These are considered indirect methods to evaluate enzyme activity as previously reported in other congeneric species: *M. tenellun* (Montoya Martinez et al., 2018), *M. rosenbergii* (Wattanakul, Wattanakul, Thongprajukaew, & Muenpo, 2015) and *M. nipponense* (Ding et al., 2016).

Recent studies have also contributed to the characterization of digestive enzymes, including data on amino acid sequences and active sites. This approach allows designing specific markers to determine enzyme expression. The utilization of Next Generation sequencing – NGS system enabled the massive sequencing of genes (functional genome), further improving the knowledge in this area. Nine putative functional sequences have been described in *C. quadricarinatus* (Dammannagoda, Pavasovic, Prentis, Hurwood, & Mather, 2015). This approach has also been used to characterize cellulose-digesting enzymes in *M. rosenbergii* (Ong et al., 2016) and to provide the transcriptome of *M. nipponense* under environmental hypoxia (Sun et al., 2014). Jiang et al. (2009) obtained a comparative transcriptome of the hepatopancreas and testicles of *Eriocheir sinensis* to determine the relationship between nutrition and reproduction.

Nevertheless, no information about the putative genes encoding digestive enzymes in the hepatopancreas was available until now for the Amazon river prawn. The identification of these genes may be potentially useful for the development of aquaculture techniques, particularly related to the formulation of proper diet suitable for the nutrient requirement and feed habit of this species. Therefore, the present study was based on the sequencing of a cDNA library of the hepatopancreas of *M. amazonicum* to obtain transcripts of putative genes that encode digestive enzymes.

Material and methods

Sampling

The prospection of the genes in hepatopancreas of *M. amazonicum* was based on the analysis of a pool of tissue samples from 10 adult males aged ~4.5 months, obtained from the grow-out ponds of the Crustacean Sector at CAUNESP (the UNESP Aquaculture Center in Jaboticabal, São Paulo, Brazil). The broodstock that produced these individuals was captured in the Furo das Marinhas estuary (1°13'25" S, 48°17'40" W) in Pará, Brazil. The prawns were anesthetized by immersion in iced water for 2 minutes. Afterwards, the hepatopancreas was removed and incubated in RNAlater (Sigma) for 12 hours at 4°C. Next, the samples were stored in an ultra-freezer at - 80°C.

Extraction of the RNA and preparation of sequences and libraries in *Ion Torrent™*

The tissue was first homogenized in liquid nitrogen until a powder was obtained. The total RNA was then extracted using 2-Mercapthaethanol and purified using the Pure Link™- RNA Mini kit (Life Technologies), following the manufacturer's instructions. For the purification of the total RNA, DNAse (Invitrogen) was added to remove the remaining fragments of DNA. The integrity and the quality of the samples were assessed by electrophoresis in 1% agarose gel stained with ethidium bromide, under UV light. The amount of total RNA was verified using a Quibit® fluorometer (Life Technologies). The samples were then stored in an ultra-freezer at -80ºC.

Once the total RNA was obtained, a number of additional stages of purification and preparation of the cDNA libraries were carried out prior to sequencing in *Ion Torrent™* by the RNA-Seq method, according to the manufacturer's instructions (Life Technologies). Initially, the ribosomal DNA was removed, isolating only mRNAs using the RiboMinus kit (Cat A10837-02). The integrity and purity of the mRNA was then verified for the preparation of the library. The mRNA was hybridized to attach the adaptors at both extremities of the RNA fragments using components of the Ion Total RNA-Seq kit v2 (Cat. no. 4475936). The cDNA was then obtained by reverse transcription, followed by the ligation of barcodes to the adaptors. The fragments were amplified and libraries were enriched using emulsion PCR (ePCR), following the templated brands preparation guide of the solid emulsion collection tray kit (Cat. no 4415129). The ePCR products were purified and recovered using kits with magnetic beads. The samples were prepared for sequencing in the same chip (318) in *Ion Torrent*™ (PGM) using the Ion PGM 200 Sequencing kit (Life Technologies).

Bioinformatic analyses

The *Ion Torrent*™ system generated a file in FastaQ format containing the sequences selected from the reads, based on a Q ⩾ 20 Phred value. The Trinity software (https://github.com/trinityrnaseq/trinityrnaseq/releases) was used to assemble and reconstruct the transcripts without a reference genome using the *De Novo* approach. Trinity also generated the statistical data on the assembling (the script *TrinityStats.pl*), N50, the GC rate and the mean size of the assemblies.

The BLASTx software was used to record the transcripts from the assemblies produced by Trinity, based on the public Uniprot and Uniref90 databases. An e-value of $1e^{-5}$ was used as cutoff. The BLAST2GO program (Conesa et al., 2005) was used to obtain the functional notation (Gene Ontology - GO). Following the general analysis of the database using BLAST2GO and the available literature, the transcripts with a putative digestive enzyme function in the hepatopancreas of *M. amazonicum* were selected. The similarity between the selected transcripts and those from other species was verified in the online NCBI database, using BLASTx and BLASTn (http://blast.ncbi.nlm.nih.gov/Blast.cgi). In addition, sequences from other organisms that are similar to the genes recorded in the present study were obtained and aligned in BIOEDIT 7.0.5.3 (Hall, 1999).

The online ORF Finder software (http://www.ncbi.nlm.nih.gov/projects/gorf/) was used to obtain the codifying amino acid sequences (CDs) of fragments assigned to digestive enzymes. When these fragments of amino acids (AA) were 100 AA or longer, they were aligned with the sequences of other species. These sequences were obtained based on the results of the BLAST hits, using BLAST2GO.

Results and discussion

We reported amino acid fragments from nine genes related to feed digestion in the functional genome of hepatopancreas from *M. amazonicum*. From this total, three genes were related to the digestion of proteins, four to the carbohydrate digestion, and two for the digestion of lipids. These data of the Amazon river prawn provided evidence this species has a genetic apparatus to feed from a wide range of resources. Furthermore, the mining of amino acid sequences usually provides more accurate results than that obtained from indirect studies, which quantify the enzymatic activity based on their substrates and inhibition tests.

There were two putative genes for cysteine protease (cathepsin L and cathepsin B) and one for serine protease (trypsin) found in the hepatopancreas of *M. amazonicum*. Cathepsin L was the most common type, with 47 ESTs (67-221 AA). A total of 20 hits were listed in BLAST2GO for this gene, with the highest similarity values in closely related marine prawns *Palaemon varians* (89%), *Palaemon carinicauda* (89%), and *Pandalus borealis* (82%).

The multiple alignment produced the longest partial putative fragment of the cathepsin L enzyme, with two conserved residues (C(Cys);H(His)) in the active site of cysteine proteases (Berti & Storer, 1995). It also contained conserved amino acid residues of the NFD motif (Coulombe et al., 1996). A second conserved motif (CNGG) was also recorded, which may have an important structural function (Karrer, Peiffert, & Ditomas, 1993) (Figure 1). This is characteristic of the cysteine proteases from the papain superfamily, being considered an important component of the lysosomal proteolytic system (Hu & Leung, 2007; Jung, Lyons, Hurwood, & Mather, 2013). Its role in food digestion has been identified in *Nephrops norvegicus* (Le Boulay, Van Wormhoudt, & Sellos, 1995), *Matapenaeus ensis* (Hu & Leung, 2004) and *Eriocheir sinensis* (Li et al., 2010). A high expression of this enzyme was reported in B and F cells, and the lumen of the tubules in the hepatopancreas of *M. ensis*, indicating digestion in both intra- and extra-cellular environments (Hu & Leung, 2004; Hu & Leung, 2007).

The cathepsin B-like sequence is another member of the cysteine protease family, observed in the transcriptome of *M. amazonicum*, being represented by six ESTs (63-70 AA). Comparisons in BLAST2GO revealed 20 hits for each partial fragment of this putative enzyme in *M. amazonicum*, which was 85% similar to the cathepsin B protein of *P. borealis*.

The multiple alignment indicated that the partial fragment of the putative cathepsin B enzyme of *M. amazonicum* contains conserved amino acids in the N-terminal region of the mature protease, including residues of the active site, (H)His and (N)Asn, in common with cysteine proteases (Berti & Storer, 1995). Residual amino acids were also shared with other crustacean species (Figure 2). Previous studies indicated that this enzyme is found predominantly in the hepatopancreas and digestive acids in the stomach of *P. borealis* (Aoki, Ahsan, & Watabe, 2003) and *L. vannamei* (Stephens, Rojo, Araujo-Bernal, Garcia-Carreño, & Muhlia-Almazan, 2012), reinforcing its role as a digestive enzyme in crustaceans. Cathepsin B is distinct from the other members of the papain superfamily because it functions as both endopeptidase and exopeptidase

(McGrath, 1999), thus expanding the ability of absorbing amino acids from the dietary proteins.

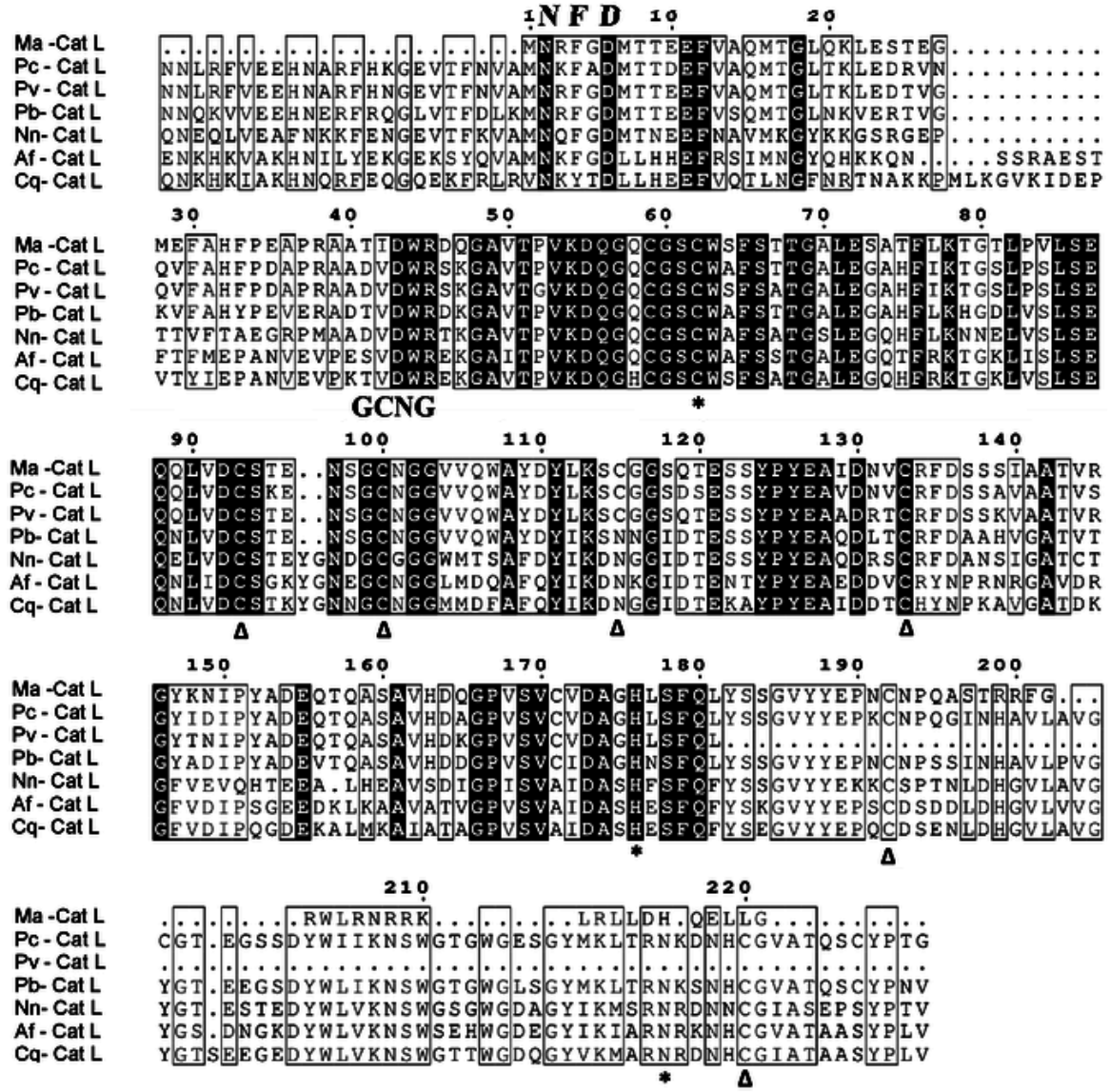

Figure 1. Alignment of the amino acid sequences of the cathepsin L protein obtained from the cDNA of the hepatopancreas in *M. amazonicum*, aligned with the sequences of crustaceans and insects. Conserved and semi-conserved residues are highlighted in black and in boxes, respectively. The conserved amino acid sequences in cathepsin L gene ('FND' and 'GCNGG') are identified at the top of the alignments. The residues of the catalytic triad, Cys(C), His(H) and Asn(N), are marked with an asterisk (*), and the cysteines (Cys(C)) by a delta (Δ). Ma-CatL, Pc-CatL (AGJ03550.1), Pv-CatL (ACR54126.1), Pb-CatL (BAC65418.1), Nn-CatL (CAA56915.1), Af-CatL (AAV63977.1), Cq-CatL(XP_001848344.1).

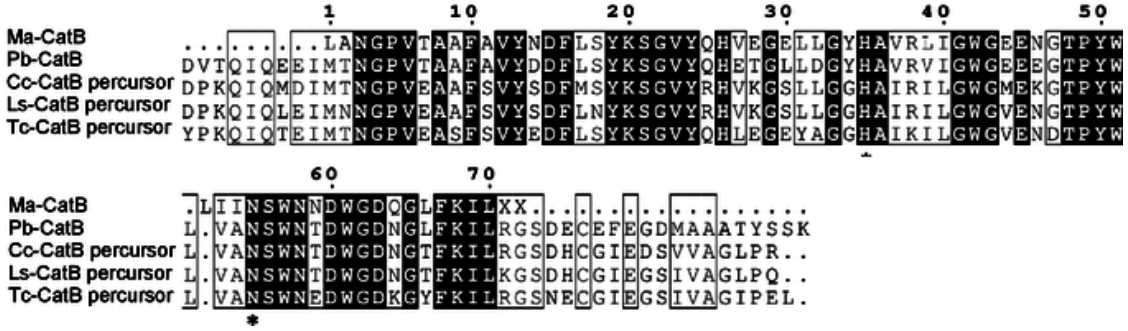

Figure 2. Alignment of the amino acid sequences in the N-terminal region of cathepsin B protein from *M. amazonicum* and related crustaceans. Conserved and semi-conserved residues are highlighted in black and in boxes, respectively. The residues in the active site are marked with an asterisk (*). Ma-catB, Pb-catB(BAC65419.1), Cc-catB(ACO14731.1), Ls-catB (ACO12454.1), Tc-catB(NP_001164205.1).

Trypsin, a member of the serina protease family, was also found in the hepatopancreas of the *M. amazonicum*, which was represented by five ESTs (66-186 AA). The BLAST2GO data indicated 20 hits for the trypsin fragments with high similarities in relation to the trypsin of *Daphnia pulex* (67%) and the trypsin-like serine proteinase 1 of *Fenneropenaeus chinensis* (63%). The multiple alignments indicated that the longest trypsin-like fragment in the Amazon river prawn encompasses conserved regions shared with the trypsin of other crustaceans. These conserved regions included the IVGG sequence, which typifies the N-terminal end of the active enzyme (Wang, Magoulas, & Hickey, 1993), and two residues, His (H) and Asp (D), out of the three amino acids that compose the catalytic triad (His, Asp and Ser). These residues are involved in the catalysis of serine proteases (Hedstrom, Szilagyi, & Rutter, 1992) (Figure 3). This enzyme is regarded as the most abundant and widespread protease in crustaceans (Shi, Ren, Zhao, & Wang, 2009; Jiang et al., 2011; Saborowski, 2015). It plays a key role in the hydrolysis of the carboxylic region of lysine or arginine residues from protein substrates (Hedstrom, 2002). Previous studies indicated the presence of this enzyme in the Amazon river prawn through inhibition tests (Santos et al., 2014). The present data reinforced these data by revealing active sites in the amino acid sequences of this enzyme.

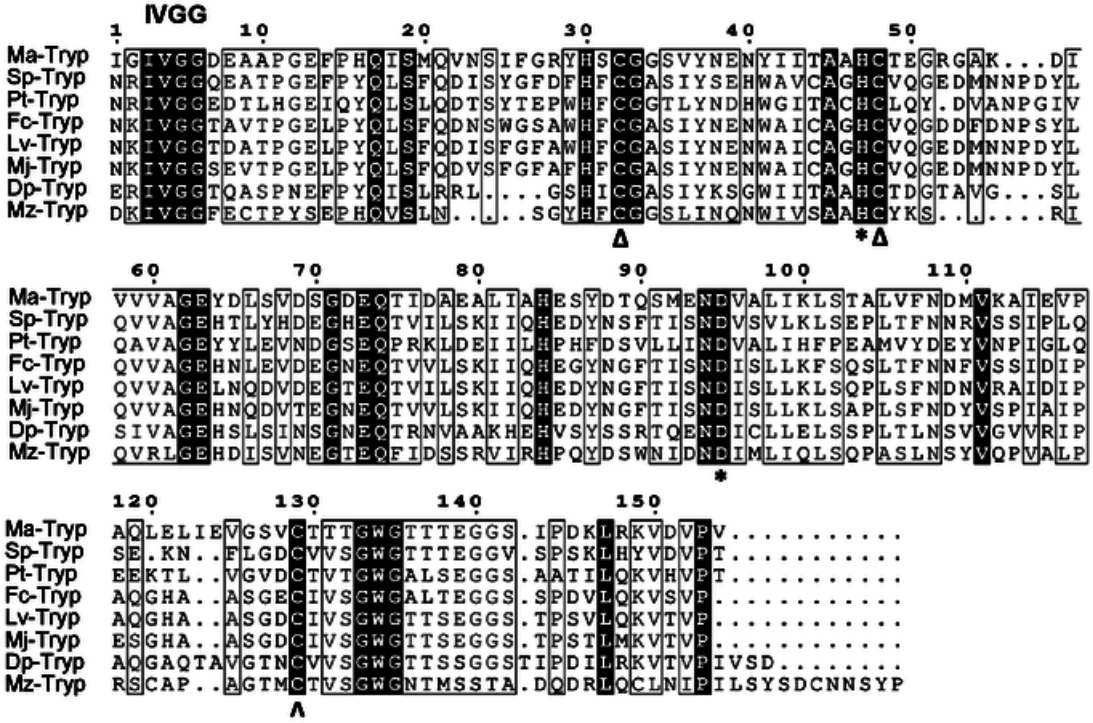

Figure 3. Alignment of the amino acid sequences in the trypsin and trypsin-like proteins of *M. amazonicum*, other crustaceans, and a vertebrate species (cichlid fish *M. zebra*). Conserved and semi-conserved residues are highlighted in black and in boxes, respectively. The conserved amino acid sequences ('IVGG') are identified at the top of the alignment. The residues in the conserved catalytic triad - His (H) and Asp (D) – are marked with an asterisk (*), while cysteines - Cys(C) are marked by a delta (Δ). Ma-tryp, Sp-tryp (AGO02163.1), Pt-tryp(AHJ81099.1), Fc-tryp(ACO45454.1), Lv-tryp (CAA60129.1), Mj-tryp(ACE80257.1), Dp-TRYP(EFX80960.1), Mz-tryp(XP_014263714.1).

Many crustaceans are known to be rich in proteases, using the amino acids for growth and energy source (Saborowski, 2015). This pattern was also detected in adult males of the Amazon river prawn (Santos et al., 2014; Augusto & Valenti, 2016). In the present study, three proteases were sequenced, with high abundance. Both endopeptidases (cathepsin L, cathepsin B and trypsin) and exopeptidases (cathepsines B) were recorded, a promising adaptation for protein-rich diets by this species.

Four putative genes related to the digestion of carbohydrates were identified in *M. amazonicum* – chitinase, alpha-amylase, endo-beta-mannanase and beta-endoglucanase. The digestion of polysaccharides reflecting the possibility of this species in digesting both animal and plant carbohydrates. Chitinase-like gene was the most common enzyme found in the hepatopancreas of *M. amazonicum,* accounted for 56 distinct ESTs (34-185 AA). The BLAST2GO identified hits with 20 species for each fragment. The greatest similarities were found in relation to the decapod crustaceans *M. nipponense* (97%), *Penaeus monodon* (84%), and *Scylla serrata* (82%). The multiple alignment of the longest fragment of the chitinase-like enzyme along with those reported in other species revealed

a major catalytic domain containing three out of the four conserved domains that characterize this gene (FDGXDLDXEYP, MXYDXXG, and GXXXWXXDXD) (Salma et al., 2012) (Figure 4).

Chitinolytic enzymes are essential to crustaceans because of their role in the molt cycle, the digestion of chitin-rich nutrients, and possibly in their defense against pathological agents containing chitin (Proespraiwong, Tassanakajon, & Rimphanitchayakit, 2010). Larger fragments attributed to chitinase in the functional genome of *M. amazonicum* had a great similarity to the family 3A isoform (97%) of *M. nipponense* (MnCh3A) (Zhang et al., 2014). This isoform is mainly expressed in the hepatopancreas and stomach, but not expressed in the cuticle. This same finding was reported in *Penaeus japonicus*, being related to the presence of isoforms to exploit chitin-rood food sources (Watanabe, Kono, Aida, & Nagasawa, 1998). The same pattern herein reported for the Amazon river prawn corroborates with the fact that this species feeds on other arthropods, as previously observed in the stomach content of specimens collected in nature (Kensley & Walker, 1982).

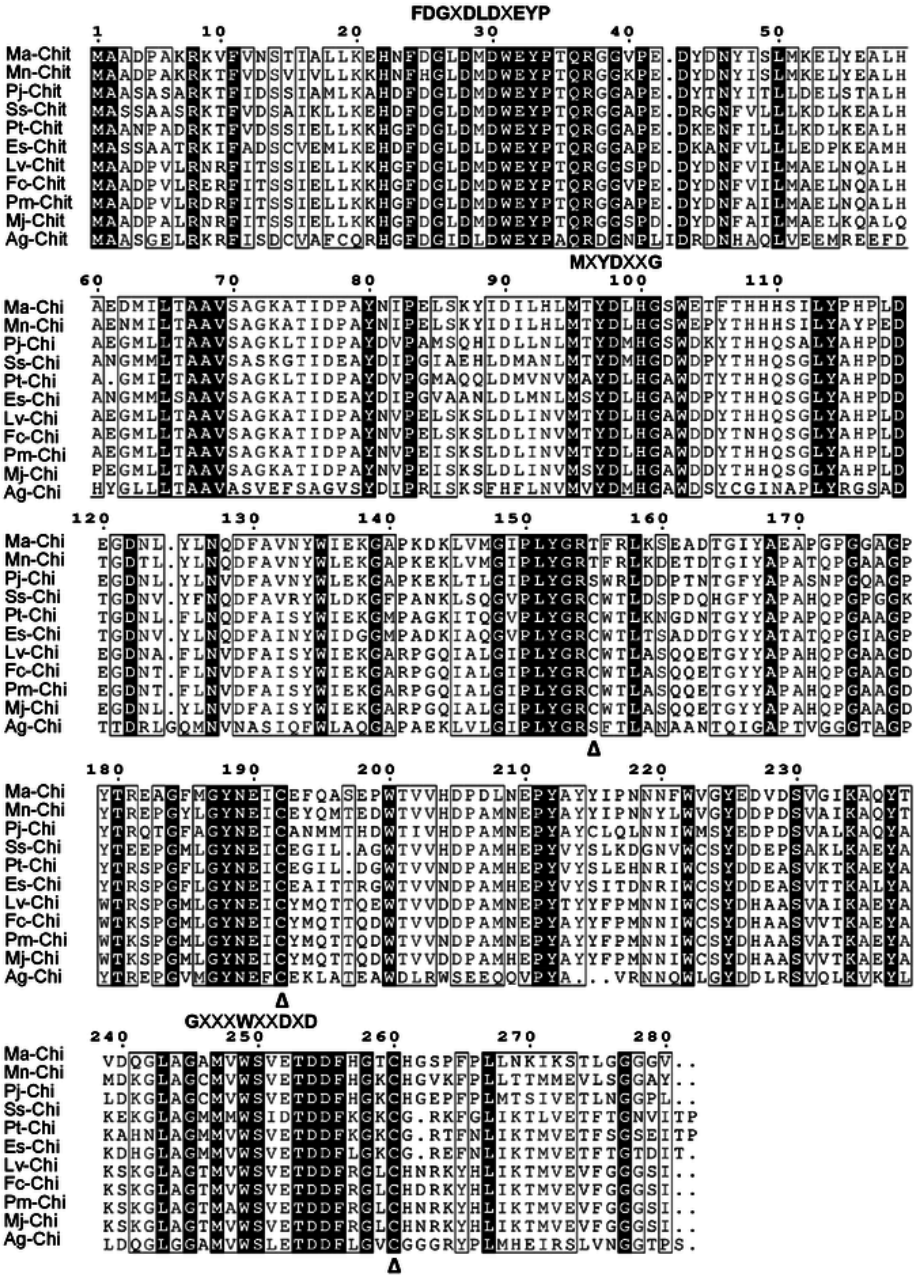

Figure 4. Alignment of the partial amino acid sequence in the N-terminal portion of chitinase protein from *M. amazonicum*, other crustaceans and an insect species (*A. gambiae*). Conserved and semi-conserved residues are highlighted in black and in boxes, respectively. The domains of the catalytic site (FDGXDLDXEYP, MXYDXXG, and GXXXWXXDXD) are identified at the top of the alignment, and the cysteines - Cys(C) are marked by a delta (Δ). Ma-chi, Mn-chi(AHL28106.1), Pj-chi (AFC60660.1), Ss-chi(ABY85409.1), Pt-chi(BAP28983.1), Es-chi(AHZ21076.1, Lv-chi(AAT80625.1),Fc-chi(AAY44300.1), Pm-chi(ADG22163.1), Mj-chi(BAA22854.1), and Ag-chi(XP_316448.2).

The alpha-amylase was the second most important putative enzyme associated with the digestion of carbohydrates identified in *M. amazonicum*, accounted for four ESTs (65-143 AA). The BLAST2GO analysis revealed similarities with crustaceans and mollusks, with 20 hits pairing the fragments of this gene with that from the Amazon river prawn. The greatest similarities were recorded with *Litopenaeus vannamei* (97%) and for a hypothetical protein (DAPPUDRAFT_68162) of *D. pulex* (83%). Two of the longest partial amino acid sequences of the alpha-amylase gene aligned with those from other species (Figure 5), indicating they encompass the partial fragment 1 of the alpha-amylase-like enzyme in *M. amazonicum* (*M. amazonicum* alpha-amylase CGI150101) (Figure 5A), which includes conserved amino acids in the N-terminal region. Three calcium-binding residues were identified (Asn (N); Arg (R); Asp (D)), besides one residue (Arg (R)) of the chloride-binding sites (Arg(R), Asn(N), Arg(R)), and another component (Asp (D)) from the active site (Asp(D), (E), Asp(D)). The partial fragment 2 (*M. amazonicum* alpha-amylase CGI3420101) (Figure 5B) included four cysteine residues typical of the C-terminal region of the alpha-amylase gene found in other species. A glycoside digestive enzyme has already been described in *M. amazonicum* (Santos et al., 2014) and in other decapod crustaceans: *L. vannamei* (Van Wormhoudt & Sellos, 1996; Wei, Zhang, Yu, Li, & Xiang, 2014) and *Saccostrea forskali* (Thongsaiklaing et al., 2014). This enzyme is involved in the hydrolysis of the α-1.4 glycosidic bonds in starch and related compounds (Van der Maarel, Van der Veen, Uitdehaag, Leemhuis, & Dijkhuizen, 2002). The presence and the activity levels of both amylases and maltases determine the capacity of the hepatopancreas in digesting starch and glucose (Johnston, 2003; Asaro, Del Valle, & Mañanes, 2011).

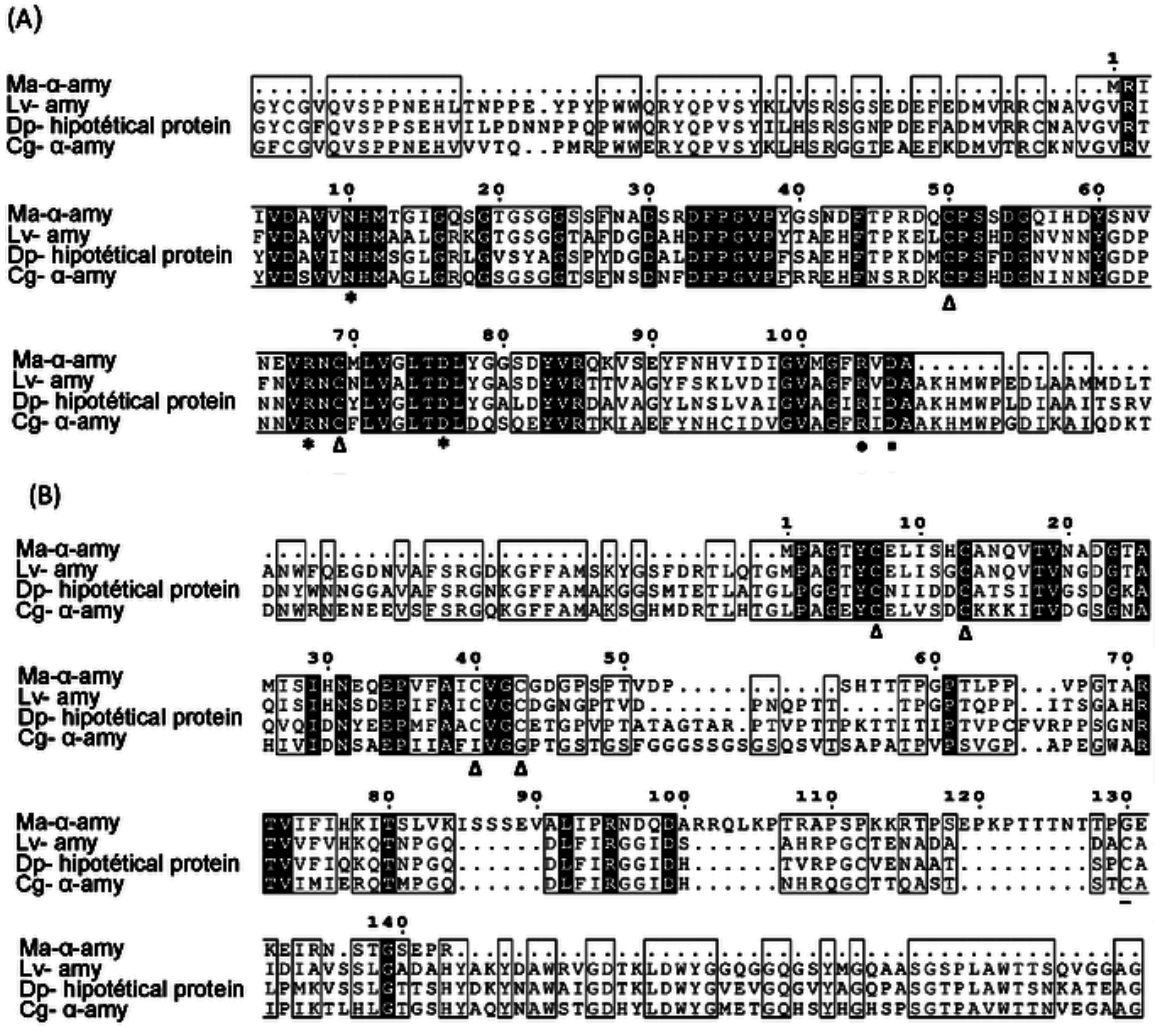

Figure 5. Alignment of the amino acid sequences in alpha-amylase of *M. amazonicum* and similar proteins from crustaceans and mollusks. Conserved and semi-conserved residues are highlighted in black and in boxes, respectively, and the cysteines - Cys(C) are marked by a delta (Δ) in both alignments. (A) alignment of partial fragment of the N-terminal region of the putative alpha-amylase enzyme of *M. amazonicum*. The calcium-binding residues are marked with an asterisk (*).The chloride-binding residue are marked by an circle (•) and the residues of the active site are marked with a square (▪); (B) alignment of the C-terminal region of the partial fragment 2 in the putative alpha-amylase enzyme of *M. amazonicum*.

Non-Starch Polysaccharides (NSP) are hardly digested by most monogastric animals (Choct, 1997). However, two small amino acid fragments associated with the digestion of cellulose were found in the hepatopancreas of *M. amazonicum*, including the putative Beta-endoglucanase and Endo-beta-mannanase enzymes. The amino acid fragments were short (< 100 AA), thus hindering their alignment. The identities of the five beta-endoglucanase (45-96 AA) transcripts were evaluated by comparing hit values with other 20 species. The greatest similarities were found with the endo-beta-1.4-glucanase protein of *Parasesarma erythrodactyla* (85%) and a protein of the GH9 family in *Liminoria quadripunctata* (76%). This enzyme was also detected in a recent study of the hepatopancreas in *M. rosenbergii*, which provided evidence for the active digestion of cellulose into glucose by this species (Ong et al., 2016).

The BLAST2GO comparisons revealed 20 hits for two partial ESTs in the putative endo-beta-mannanase enzyme (53-80 AA). Yet, a search in the NCBI database revealed that this was the first time that an endo-beta-mannanase has been reported for the genus *Macrobrachium*. The enzyme amino acids sequences presented in *M. amazonicum* have high similarities with two registered species of endogenous mannanase production: *Limnoria quadripunctata* isopode 81% (King et al., 2010) and *Cherax quadricarinatus* decapode, 79% (Dammannagoda et al., 2015), and were classified as GH5 family constituent. The enzyme found in the present study was classified as key component for the degradation of structural polysaccharides (mannane) found in plant hemicellulose (Handford et al., 2003). The presence of these enzymatic fragments in *M. amazonicum*, along with previous studies about their feeding habits (Kensley & Walker, 1982; Heldt, Suita, Dutra, Pereira, & Ballester, 2019), indicate that this species may be able to digest plant fibers. Further studies should be performed to improve the characterization of these enzymes associated with digestibility analyses to verify the effective digestibility of NSP by *M. amazonicum*.

Two partial ESTs were identified for a putative pancreatic triacylglycerol lipase enzyme (52-75 AA) related to the digestion of lipids, with 20 hits when compared to those from other species. Digestive lipases are responsible for the hydrolysis of triacylglycerides (Rivera-Pérez & García-Carreño, 2011) in the crustacean hepatopancreas, assuring the digestion of lipid-rich food (Wang, Wu, Liu, Zheng, & Cheng, 2014; Saborowski, 2015). These fragments were mostly similar to the sequences of the triacylglycerol lipase-like protein of *D. pulex* (65%) and a partial intracellular lipase protein of *L. vannamei* (78%) (Wang et al., 2014; Saborowski, 2015). Another transcript related to the absorption and transportation of lipids in the Amazon river prawn was similar to a low-density lipoprotein receptor-related (LDL) enzyme (45 AA). This fragment was similar to sequences of this protein obtained in *Sorex araneus* (53%), totaling 15 hits. Due to the small size of the fragments (< 100 AA), reliable alignments could not be obtained. This enzyme was previously detected in the hepatopancreas of *P. trituberculatus* (Wang et al., 2014), which should play a key role in regulating the cholesterol levels in hemolymph (Ishibashi et al., 1993).

Previous studies on the stomach content of *M. amazonicum* and other palaemonid shrimps from natural habits reported a high frequency of arthropods (insects and crustaceans), oligochaetes, vegetal matter and debris (Kensley & Walker, 1982; Collins, Williner, & Giri, 2007; Lima, Garcia, & Silva, 2014). These data combined with those herein described about the enzymatic repertoire of the Amazon river prawn indicate this species has a genetic apparatus to feed from a wide range of resources, characterizing an omnivore species. Furthermore, the mining of amino acid sequences usually provides more accurate results than that obtained from indirect studies, which quantify the enzymatic activity based on their substrates and inhibition tests.

Conclusion

M. amazonicum have an enzymatic apparatus to digest proteins, lipids and carbohydrates, included Non-Starch Polysaccharides (NSP), corroborating the omnivorous habit. This information may provide important insights for the selection of ingredients for the formulation of a more appropriate diet to the enzymatic repertoire of *M. amazonicum*. In addition, the encoding sequences of digestive enzymes provided markers to be used in further nutrition experiments specific for Amazon river prawn, such as genomic expression ($_{RT}$PCR, qPCR or in situ hybridization).

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