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Phylogenetic Analysis of Vitellogenin Peptides in *Hemibagrus nemurus*

Roshani O. [*1], Harmin S. A.[1] and Ina-Salwany M. Y.[2]

[1]Faculty of Science and Biotechnology, Universiti Selangor, Bestari Jaya Campus, Jalan Timur Tambahan, 45600 Batang Berjuntai, Selangor, Malaysia.
***E-mail: roshani@unisel.edu.my**
[2]Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang Selangor, Malaysia.

ABSTRACT

Vitellogenin is a female-specific glycolipophosphoproteins and it plays a significant role in embryo development and reproduction of *Hemibagrus nemurus*. The vitellogenin have been characterized to have various molecular weights in different fish species and has led to questions over ways to identify the vitellogenin peptides in river catfish that has been evolutionarily conserved in teleost. In this study, the amino acid sequences for the vitellogenin peptides for *H. nemurus* are AYLAGAAADVLEVGVR and AYLAGAAADVLDIGVR and were used to investigate the phylogenetic relationships between vitellogenin in different fish species in the database. The phylogenetic analysis based upon the vitellogenin peptides of *H. nemurus* indicates a close phylogenetic relationship with vitellogenin in *Clarias batrachus*. Vtg is a highly conserved protein and the molecular weight or size is species-specific, thus the similarity value was obtained due to the fact that both species belong to the Asian freshwater catfish family.

Keywords: *vitellogenin, Hemibagrus nemurus, phylogenetic*

INTRODUCTION

Vitellogenin (vtg) is a major protein present abundantly in female fish during ovarian development. It is synthesized in the liver under stimulation of estrogen after repeated induction or exposure of 17-β estradiol and has high molecular weight (200-600 kDa) (Mommsen and Korsgaad, 2008). Its structure and size varies significantly among different types of fish. *Hemibagrus nemurus* is an indigenous freshwater fish and is one of the most important cultured species of the Southeast Asia. Good meat quality makes it a favourite food delicacy among locals in Malaysia and other countries in Southeast Asia. At present, these catfish are cultured in small cages, ponds, cement tanks, canvas tanks and ex-mining pools for local consumption and export. Unfortunately, the seed supply for catfish is seasonal and their inability to reproduce in captivity is a major hindrance to mass production. In natural habitat, this species is threatened by development, industrialisation, deforestation and overfishing. One of the ways to overcome this problem is by determining the spawning period of this species through the identification of vtg, the specific protein related to oocyte growth. The phylogenetic diversity and evolution of vtg in fish was conducted to understand the evolution of vtg. In this study, the main aim is to use bioinformatics approach to characterize vtg peptides from *H. nemurus* as preliminary data for phylogenetic analysis of other fishes.

MATERIALS AND METHODS

Samples collection. River catfish was collected throughout this study. The fish was captured by local fisherman at Sungai Selangor, Bestari Jaya, Malaysia.

Amino acid sequence of vtg. For phylogenetic analyses, the steps begin with the amino acid sequence. The peptide sequence of the *Hemibagrus nemurus* vtg was obtained from the results of MALDI-TOF mass spectrometry (Roshani et al., 2015) and other amino acid sequence is obtained using the blastx analysis. The sequence was then blast using NCBI, BLAST program against protein

database and matching peptides of *Hemibagrus nemurus* vtg were retrieved via the homology peptide masses using available database (Ludwig, Swiss-Prot and MASCOT).

Phylogenetic construction. The amino acid sequences were analysed using MEGA6 (Molecular Evolutionary Genetic Analysis) software. The vtg peptides sequences obtained in previous work was aligned with other fishes' partial vtg sequences retrieved from Genbank, using CLUSTALX set as default parameter, with subsequent visual editing. The phylogenetic tree was constructed using Maximum-Likelihood (ML) and Maximum-Parsimony (MP), with phylogenetic support (bootstrap support values) displayed on the respective majority rule consensus tree.

RESULTS AND DISCUSSION

The vtg peptides sequence (Table 1) was obtained from the *Hemibagrus nemurus* (Roshani et al., 2015). Figure 1 shows the results of Blastx analysis, which was performed against protein database to find the most similar protein with the query sequence.

Table 1. Amino acid sequences of the trypsin digestion of *Hemibagrus nemurus*

	Sequence	Mr (obs)	Mr (calc)	Proteins matching the same set of peptides
Peptide 1	AYLAGAAADVLEVGVR	1573.77	1573.85	Vtg B *Carassius auratus* spp Pengze
Peptide 2	AYLAGAAADVLDIGVR	1573.77	1573.85	Vtg B2 *Cyprinus carpio*

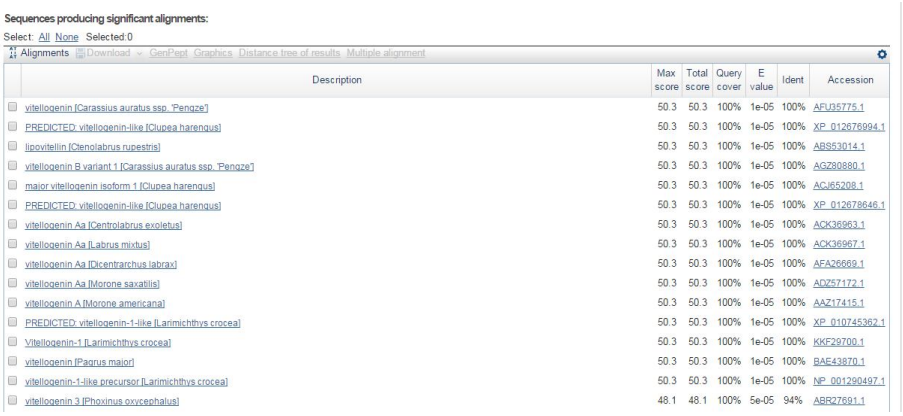

Figure. 1. Blastx result showed the total score and percentage identity of the most similar hit sequence.

Results of phylogenetic analysis using Maximum-Likelihood (ML) and Maximum-Parsimony (MP) methods showed tree analysis generated four and two separated tree topology (Figure 1 and Figure 2), respectively. The topology of the ML tree shows four clades (Figure 2). The first clade (A) consisted of vtg from freshwater carp, freshwater catfish and Atlantic herring, while the fourth clade (D) consisted of vtg similar to cluster A but without Atlantic herring. However, the second clade (B) and third clade (C) in ML tree are slightly different; consisted of vtg from freshwater catfish and saltwater catfish respectively. It is noted that the distribution of vtg phylogenetic was significantly different between freshwater catfish (Cluster B; *Clarias batrachus* and *Hemibagrus nemurus*), saltwater catfish (Cluster C; *Bagre marinus* and *Ariopsis felis*) but not signicantly different between Cluster A and D; *Cyprinus carpio*, *Clarias macrocephalus*, *Clupea harengus*, *Cathorops melanopus* and *Clarias batrachus*. Therefore, *H. nemurus* vtg evolution is more related to *Clarias batrachus* vtg. This classification confirms that vtg is a conserved protein and that both species belong to the Asian freshwater catfish. The findings was supported by a previous study done by Hayward et al. (2010), stated that the origin of vtg is far more ancient than previously claimed, which could possibly date

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back to at least 700 milllion years ago. Furthermore, the constructed phylogenetic tree shows that the vtg from individuals which are clustered together under the same node is highly conserved as compared to indiduals in other clusters.

The evolutionary history via MP method shows that the two peptides of *H. nemurus* were clustered together with other catfish (*Clarias batrachus*, *Bagre marinus* and *Ariopsis felis*) and *Cyprinus carpio*, (common carp) in Cluster B (Figure 3). The Clade A in Figure 3 consisted of vtg from four species (*Cyrinus carpio*, *Clupea harengus*, *Clarias macrocephalus* and *Cathorops malanopus*) with majority of the species are non-catfish.

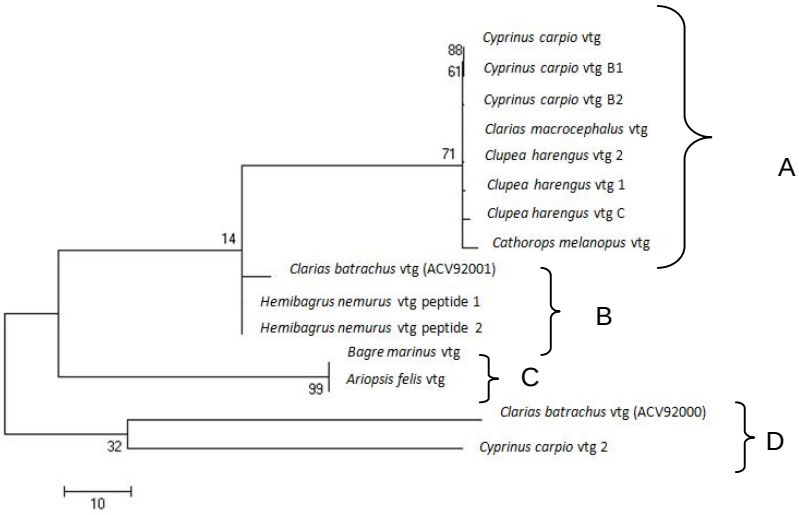

Figure. 2. Phylogenetic tree using Maximum-Likelihood method showing the relationships of vtg. Numbers besides nodes indicate the percent of bootstrap values for each branch of the tree in the 1000 bootstrap trials.
Cluster A=freshwater carp, freshwater catfish and Atlantic herring, Cluster B=freshwater catfish, Cluster C=saltwater catfish, Cluster D=freshwater carp and freshwater catfish.

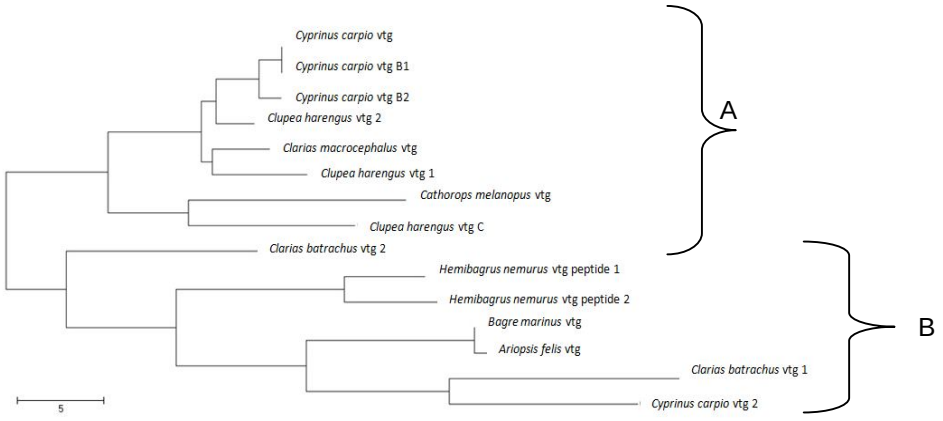

Figure. 3. Phylogenetic tree using Maximum-Parsimony method showing the relationships of vtg. Numbers besides nodes indicate the percent of bootstrap values for each branch of the tree in the 1000 bootstrap trials. Cluster A=majority freshwater catfish, Cluster B=majority saltwater fish.

Arif et al. (2009) reported that the MP method is the only method that posed no difficulty to tackle the insertions/deletion of amino acids, it is efficient in obtaining the correct topology however produced incorrect trees even if the rate of amino acid or nucleotide substitution is fairly constant among the taxa. In addition, Steel and Penny (2000) mentioned that the parsimonious arguments which included MP method which fall short statistically can be justified by ML method.

The comparison between phylogenetic trees using ML and MP methods allows accurate classification of vtg from freshwater and saltwater catfish. However, Wong et al. (2011) stated that GenBank was unable to fully discriminate sequences as a result of existing shortcomings in database for catfish species. Furthermore, the phylogenetic analysis using the vtg peptides has been extensively conducted in fish due to the high-resolution information on the evolutionary relationships between vtg sizes and species-specific. Vtg proteins are well conserved and have been sequenced in various animal ancestries (Hayward, 2010).

CONCLUSION

Classification of the vtg in *Hemibagrus nemurus* and *Clarias batrachus* are more related using Maximum-Likelihood (ML) and Maximum-Parsimony (MP) methods because they belong to the Asian freshwater catfish family. The Asian freshwater catfish has a high evolutionary relationship compared to other fish species as revealed by protein databases.

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