Advances in Biological Research 10 (6): 351-353, 2016
ISSN 1992-0067

DOI: 10.5829/idosi.abr.2016.351.353

Molecular Phylogeny of Four Gouramis Based on Divergent Domain D11 of 28S rRNA Gene

Madhu Awasthi, Ankur Kashyap and Mohammad Serajuddin

Department of Zoology, University of Lucknow, Lucknow-226007, India

Abstract: The phylogenetic relationship among the four gouramis namely blue, dwarf, gold and yellow was studied using D11 domain of 28S rRNA gene. The ribosomal RNA (rRNA) genes were widely used for the detection of phylogenetic relationships and studying the genetic variability and divergence within and between species of many organisms. The study suggested that blue gourami is most closely related to gold gourami and less closely related to dwarf and pearl gouramis. The genetic distance between the dwarf and blue gourami was highest and most distantly related to each other, whereas the genetic distance between the blue and gold gouramis was found to be least and considered to be closely related to each other in phylogenetic tree as they also formed a separate cluster due to minimum genetic distance. The nucleotide sequences and G+C percent of the D11 domain of 28S rRNA gene ranged between 585- 613bp and 53.3 - 54.2% respectively.

Key words: Gourami · Phylogeny · 28S rRNA

INTRODUCTION

The nuclear ribosomal genes and Insilco techniques are used to establish evolutionary relationships among different species of animals. The eukaryotic nuclear rRNA genes have multiple copies that are organized in tandem arrays consist of three rRNA genes (18S, 5.8S and 28S) and two internal and one external transcribed spacers.The28S ribosomal RNA is the structural RNA for the large component of eukaryotic ribosome and thus one of the basic components of all eukaryotic cells. The 28S rRNA gene is formed by several highly conserved cores interrupted by divergent domains evolve rapidly with substitution rates that are at least two orders of magnitude higher than those of core regions which creating possibility for variations in these fast evolving divergent domains [1]. These evolving domains are considered to be best for studying phylogenetic relationship between closely related species. Divergent domains of ribosomal RNA genes and associated spacers regions have been sequenced and studied by many workers in anuran, *Xenopus leavis* [2-5].

The gouramis are inland water teleostean fish. The dwarf gourami, *Trichogaster lalius* having oblique bands of orange colour descending downwards and backwards from back to the abdomen. The pearl gourami, *Trichopodus leerii* originated from Thailand, Malaysia, South East Asia and Borneo. The pearl gourami is an omnivorous fish considered as one of the most attractive aquarium fish. Blue gourami, *Trichopodus trichopterus* is labrynth fish and endemic to Mekong basin in Cambodia, Thailand and Vietnam and Yannan in South East Asia. The fish is marketed for food in fresh or salted and pickled forms and they are also popular in the fish keeping hobby, being commonly kept in aquarium. It also has a gold colour variant known as gold gourami which was also selected along with *T. lalius*, *T. leerii* and *T. trichopterus* for the phylogenetic study.

The objective of this study was to amplify and align the sequences of D11 domain (28S rRNA) for studying the phylogenetic relationship among the four gouramis.

MATERIALS AND METHODS

The specimens of dwarf gourami (*T. lalius*), blue gourami (*T. trichopterus*), gold colour variant of blue gourami and pearl gourami (*T. leerii*) were purchased from the aquarium shop in Lucknow, Uttar Pradesh, India.

Genomic DNA was isolated from muscle tissue using the phenol-chloroform-isoamylalcohol method and it was visualized on 0.8% agarose gel stained with ethidium bromide and quantified using spectrophotometric method.

Corresponding Author: Mohammad Serajuddin, Department of Zoology, University of Lucknow, Lucknow-226007, India.

Primers were used to amplify the divergent domain D11 of 28S rDNA as per Zordoya and Meyer [6]. A standard PCR reaction of 50µl was carried out in a thermal cycler. Reaction comprised of 2.5µl of 10X Taq DNA polymerase buffer, 1µl of 10 mM dNTP, 1.5µl of 10 pmoles primers (Each forward and reverse), 0.25µl of 1U Taq DNA polymerase, 2µl of 50 ng/µl of genomic DNA and 41.25µl double distilled water. A negative control without template DNA was also included. The PCR cycle included the initial denaturation at 94°C for 4 min, 35 cycles of denaturation at 94°C for 30 seconds, primer annealing at 35° C for 35 seconds and 72°C elongation step for 45 seconds. A final extension of 10 min at 72°C was appended after 35 cycles. The amplified DNA fragments were separated on 1.2% agarose gel (Himedia) stained with ethidium bromide. The amplified pattern was visualized on the UV trans-illuminator and photographed by gel documentation system. Products were sequenced from Cistron Biotech Ltd. and then the sequences were submitted to Genbank database for obtaining accession numbers maintained by National Center for Biotechnology Information (NCBI).

The sequences were aligned using ClustalW multiple sequence alignment tool available at the website of NCBI. Phylogenetic tree was constructed using Neighbor-Joining method Saitou and Nei [7] and evolutionary distances were computed using Maximum Composite Likelihood method Tamura *et al*. [8] in MEGA4 software, version 4.0.2. The robustness of tree was checked at bootstrap of 500. All gaps and missing data were eliminated from the dataset (Complete deletion option).

RESULTS AND DISCUSSION

Divergent domain of D11 nucleotide sequences and their G+C content ranged from 585 to 613 bp and 53.3% to 54.2% respectively (Table 1) and composed of multiple sequence alignment scores ranged from 99.67 to 100 (Table 2). Altogether 17 conserved sites, 594 variable sites and 88 parsimony informative sites in multiple sequence alignment of D11 domain were recorded. The Optimal phylogenetic tree constructed on the basis of the sequences was composed of total sum of branch length of 11.95239576 in D11 domain. The phylogeny on the basis of the present findings of D11 domain sequences inferred that the blue gourami is most closely related to gold gourami and less closely related to dwarf and pearl gouramis (Figure 1). Highest genetic distance was found between dwarf and the blue gouramis and lowest between blue and gold gouramis (Table 3).

Table 1: Accession number, base pair length and GC content of 28S rRNA in domain D11 of four gouramis

S. No.	Accession No.	Common Name	Bases	GC content (%)
1	KF846525	Dwarf gourami	606	53.6
2	KF846528	Gold gourami	613	54.2
3	KF846527	Pearl gourami	585	53.3
4	KF846526	Blue gourami	611	54.0

Table 2: Multiple sequence alignment of D11 domain between gouramis

S. No.	Fish 1	Bases	Fish 2	Bases	Alignment score (%)
1	Dwarf gourami	606	Blue gourami	611	99.67
2	Dwarf gourami	606	Gold gourami	613	99.83
3	Dwarf gourami	606	Pearl gourami	585	99.83
4	Blue gourami	611	Gold gourami	613	99.84
5	Blue gourami	611	Pearl gourami	585	100.0
6	Gold gourami	613	Pearl gourami	585	100.0

Table 3: Pair wise genetic distance among four gouramis

	Dwarf gourami	Blue gourami	Gold gourami
Dwarf gourami			
Blue gourami	15.814		
Gold gourami	15.517	0.002	
Pearl gourami	4.676	3.532	3.591

The 28S rRNA genes were used by Zordoya *et al*. to established the evolutionary relationships of lungfishes, coelacanth, rainbow trout, eel, sturgeon and tetrapods and reported the divergent domain of 28S rRNA genes are useful for phylogenetic analysis [6].Divergent domains of 28S rRNA gene have been studied in fishes, mouse and humans where 28S rRNA gene was found to be shorter in fishes as compared to mammals. The variations in 28S rRNA gene are due to some unique sites embedded within the largely conserved secondary structure of the genes [10].

The D11 domain was found to be highly rich in G+C content in all the four species of gourami. Verma *et al*. also reported high G+C content in D11 domain of fish [11]. Highest genetic distance and low alignment score was noted between blue gourami and dwarf gourami as more differences in the sequences lead to less alignment scores as well as high genetic distance. Sequence divergence showing interspecific variations in the present study may be because of the transition, transversion and insertion/deletion in the sequences [1].

This is first report of phylogenetic analysis of four gourami species on the basis of divergent domains of 28S rRNA. However, Verma and Serajuddin also reported the divergent domain D8 of 28S rRNA gene in four species of family siluridae [12]. Clark *et al.* identified nine expansion segment of 28S rDNA that accounted for the major differences in secondary structure between *E. coli* and *Xenopus leavis* [13]. The present study will also

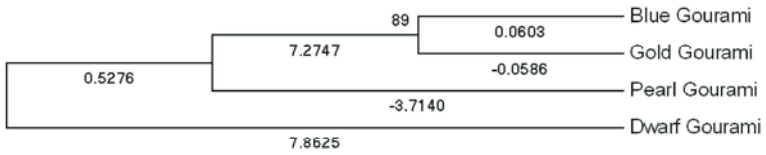

Fig. 1: Phylogenetic tree of four gouramis on the basis of D11 domain of 28S rRNA gene

contribute to the limited amount of sequenced data available in terms of divergent domains of 28S rRNA genes in family Belontidae. Phylogenetic hypothesis of the evolutionary relationships among species provide frameworks for comparative research on mechanism of diversification and speciation.

ACKNOWLEDGEMENTS

The authors thank the Head, Department of Zoology, University of Lucknow, Lucknow for providing facility and administrative support. The first two authors are also thankful to University Grant Commission (UGC) and Department of Science and Technology - Promote University Research for Scientific Excellence (DST-PURSE) respectively for financial support.

REFERENCES

1. Olsen, G.J. and C.R. Woese, 1993. Ribosomal RNA: a key to phylogeny. Federation of American Societies for Experimental Biology, 7: 113-123.
2. Boseley, P., T. Moss, M. Machler, R. Portman and M. Birnstiel, 1979. Sequence organization of the spacer DNA in a ribosomal gene unit of Xenopus Zuevis. Cell, 17: 19-31.
3. Hall, L.M.C. and B.E. Maden, 1980. Nucleotide sequence through the 18S-28s inter gene region of a vertebrate ribosomal transcription unit. Nucleic Acids Research, 8: 5993-6005.
4. Salim, M. and B.E. Maden, 1981. Nucleotide sequence of Xenopus Zaevis 18s ribosomalRNA inferred from gene sequence. Nature, 291: 205-208.
5. Ware, V.C., B.W. Tague, C.G. Clark, R.L. Gourse, R.C. Brand and S.A. Gerbi, 1983. Sequence analysis of 28s ribosomal DNA from the amphibian Xenopus laevis. Nucleic Acids Research, 11: 7795-78 17.
6. Zardoya, R. and A. Meyer, 1996. Evolutionary relationships of the coelacanth, lungfishes and tetrapods based on 28S ribosomal RNA gene. Proceedings of the National Academy of Sciences USA, 93: 5449-5454.
7. Saitou, N. and M. Nei, 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution, 4:406-425.
8. Tamura, K., J. Dudley, M. Nei and S. Kumar, 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution, 24: 1596-1599.
9. Hassouna, N., B. Michot and J.P. Bachelleire, 1984. The complete nucleotide sequence of mouse 28S rRNA gene. Implications for the process of size increase of the large subunit rRNA in higher eukaryotes. Nucleic Acids Research, 12: 3563-3583.
10. Verma J. and M. Serajuddin, 2012. Phylogenetic relationship between four species using divergent domain d8 in family: Siluridae. International Journal of Life Science and Pharma Reviews, 2: 240-244.
11. Verma, J., W.S. Lakra, B. Kushwaha, M. Serajuddin, R. Kumar and N.S. Nagpure, 2011. Phylogenetic relationship between four species using divergent domain D9 and D11 in family: Siluridae (Pisces). International Journal of Innovations in Biological and Chemical Sciences, 1: 12-15.
12. Clark, C.G., B.W. Tague, V.C. Ware and S.A. Gerbi, 1984. Xenopus Zaevis 28s ribosomal RNA: a secondary model and its evolutionary and functional implications. Nucleic Acids Research, 12: 6197-6220.