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Research Article

Molecular variation and phylogenetic status of ponyfish (Perciformes: Leiognathidae) in Karaikal, South India

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Abstract

The family Leiognathidae, commonly known as ponyfish and they are mainly classified by mouth morphology. In this study, genetic variation and phylogenetic relationships among nine genera and 13 species of Leiognathidae, inferred from Cytochrome Oxydase I gene. The Neighbour Joining tree showed the paraphyly of the studied species form genus *Secutor* and monophyly status all the other eight genera. *Leiognathus equulus* is occupying a basal branch of the family NJ tree. The highest genetic distance (0.349) showed between *Eubleekeria* and *Nuchequula*. The lowest genetic distance (0.007) showed between the genus *Nuchequula* and *Leiognathus*. Among the nine genera, very less nucleotide diversity (π= 0.1100) was in *Secutor* and higher value (π = 0.7558) was recorded in *Leiognathus*. The Tajima's test statistic (*D*) value was positive, so, the entire genus is in balancing selection.

Keywords: COI; genetic variation; Leiognathidae; NJ tree; nucleotide diversity

Introduction

Leiognathids (family: Leiognathidae) are food fishes commonly known as slip mouths or ponyfishes. They are represented by about 30 species and distributed over most of the Indo-Pacific area, ranging from South Africa in the west to Tahiti in the east, and from Australia in the south to Japan and the Red Sea in the north (Froese and Pauly, 2017). They characterized by having a highly protractible mouth and a circumesophageal light organ harboured various luminescent bacterium (Kaeding *et al.*, 2007). The family comprises nine genera, namely *Gazza, Leiognathus, Secutor, Photopectoralis, Nuchequula, Eubleekeria, Equulites, Aurigequla* and *Karalla* (Chakrabarty *et al.*, 2008; Eschmeyer, 2010; Abraham *et al.*, 2011).

Leiognathids fishes are difficult to diagnose and identify based on external characteristics like shape, size and colours of body parts because they are morphologically similar among genera, and may form species complex (Sparks *et al.*, 2005). Hebert *et al.* (2003) introduced the concept of DNA based barcoding system for species identification in the animal kingdom. This DNA barcode system has been successfully used in many fishes worldwide (Costa and Carvalho, 2007). It is a helpful tool to resolve some uncertainties of identification and also enhance the taxonomic revision (Chakrabarty and Sparks, 2008). Family-level phylogenetic analysis

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has provided a better understanding of the relationship of Leiognathids at the generic and species levels (Ikejima *et al.*, 2004; Sparks and Dunlap, 2004). Such phylogenetic analysis of Indian Leiognathid fishes is lacking. Therefore, here, the phylogenetic assessment and genetic variation in four ponyfish species on Karaikal coast using COI gene sequences was studied and discussed.

Materials and Methods

Sample collection

Leiognathus equulus, *Nuchequula blochii*, *Karalla dussumieri* and *Photopectoralis bindus* were collected from the Karaikal landing center (10°55'31.58"N and 79°50'16.82"E), in the Southeast coast of India. Ten individuals were taken from each fish species and the tissue samples (caudal fin) were dissected and stored in 95% ethanol for DNA isolation.

DNA isolation and PCR

DNA was isolated by standard Proteinase-K/Phenol- Chloroform- Ethanol method (Sambrook *et al.*, 1989). The quality of the DNA was checked by measuring the ratio of absorbance at 260 nm and 280 nm (260/280). The DNA was diluted in TAE buffer to a final concentration of 100 ng μl^{-1}. The COI gene was amplified in a 50 µl volume PCR mix with 5 µl of 10X Taq polymerase $MgCl_2$ (25 mM) buffer, 1 µl of each dNTP (0.05 mM), 1 µl of each primer (0.01 mM), 0.6 U of Taq polymerase, 2 µl of genomic DNA and 36 µl of double distilled water. The universal primer, *FishF1*-5'TCAACCAACCACAAAGACATTGGCAC3' and *FishR1*- 5'TAGACTTCTGGGTGGCCAAAGAATCA3' (Ward *et al.*, 2005) was used for the amplification of the COI gene. The thermal regime consisted in an initial step of 2 min at 95 °C followed by 35 cycles of 30 sec at 94 °C, 30 sec at 54 °C and 60 sec at 72 °C followed by final extension of 10 min at 72 °C. The PCR products were checked using 1.5% agarose gel and the most representative bands were selected for sequencing. The cleaned-up PCR product was sequenced by a commercial sequencing facility (Macrogen, Korea).

Sequence analysis

The partial COI gene sequences of eight individuals were edited using MEGA 5.0 (Kumar *et al.*, 2011) and aligned with Clustal W 1.6. The haplotype definitions have been submitted to the NCBI GenBank through Bank its portal. To support the hereby studied eight COI sequences of four genus, another 18 sequences of five genus were retrieved from NCBI GenBank. The nucleotide diversity (π) (Lynch and Crease, 1990) and Tajima's *D* (Tajima, 1989) was calculated in Dnasp 4.0 (Rozas *et al.*, 2003). Genetic relationships among individuals were constructed based on the neighbor-joining (NJ) method (Saitou and Nei, 1987).

Results and Discussion

Species confirmation and genetic distance

All the six sequences get assigned the accession number as MT000154, MT000163, MT000164, MT000165, MT000169, MT000310, MT000311, MT000312. During BLAST, all the sequences showed 99% identity of more than 90% query coverage with previously published COI gene sequences of *Leiognathus equulus*, *Nuchequula blochii*, *Karalla dussumieri* and *Photopectoralis bindus* in the NCBI's nucleotide database and confirmed the species (Figure 1).

Among the nine genus the A+T content of the COI gene was ranged from 51.76 to 53.99% the G+C content was from 46.01 to 48.26% (Figure 2). Genetic distance was measured by K2P parameter within and

between genus of Leiognathidae and the result is shown in Table 1. The highest genetic distance (0.349) showed between *Eubleekeria* and *Nuchequula*. The lowest genetic distance (0.007) showed between the genus *Nuchequula* and *Leiognathus*.

Tajima's test for neutrality

Tajima's test was performed using all the COI gene sequences from nine genus of Leiognathidae. Among the nine genera, very less nucleotide diversity (π = 0.1100) was in *Secutor* and higher value (π = 0.7558) was recorded in *Leiognathus*. The Tajima's test statistic (*D*) value was showed positive, so this entire genus is in balancing selection (Table 2).

Genus	*Leiognathus*	*Karalla*	*Nuchequula*	*Photopectoralis*	*Gazza*	*Aurigequula*	*Equulites*	*Eubleekeria*
Karalla	0.289							
Nuchequula	0.007	0.283						
Photopectoralis	0.299	0.227	0.294					
Gazza	0.267	0.284	0.266	0.327				
Aurigequula	0.131	0.310	0.133	0.344	0.252			
Equulites	0.281	0.259	0.278	0.217	0.290	0.281		
Eubleekeria	0.340	0.299	0.349	0.298	0.280	0.327	0.333	
Secutor	0.309	0.294	0.307	0.291	0.193	0.275	0.288	0.313

Table 1. K2P genetic distance between genus of the Leiognathidae family

Table 2. Tajima's Neutrality Test for COI gene in Leiognathidae family

Genus	*M*	*S*	*Ps*	π	*D*
Leiognathus	2	593	0.94426	0.7558	5.0587
Karalla	4	614	0.9839	0.7518	4.2130
Nuchequula	2	561	0.9130	0.7165	4.4693
Photopectoralis	2	460	0.7313	0.4880	2.7431
Gazza	4	464	0.7353	0.4899	2.3275
Aurigequula	2	600	0.9404	0.7439	4.7054
Equulites	2	483	0.7618	0.5320	1.2014
Eubleekeria	2	471	0.7239	0.5163	1.7642
Secutor	4	107	0.1651	0.1100	2.3256

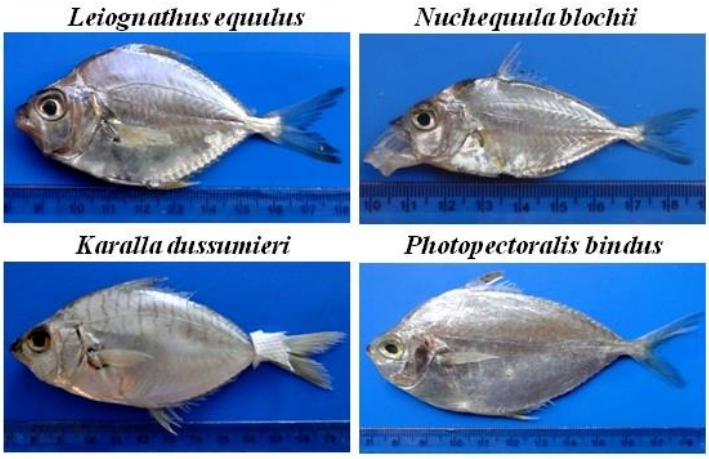

Figure 1. The Leiognathid fishes collected from Karaikal

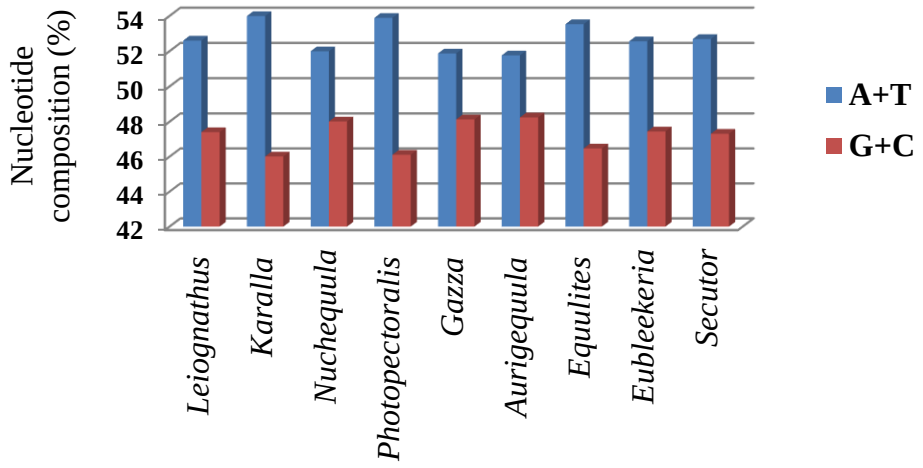

Figure 2. Nucleotide composition of COI gene in different genus of Leiognathidae

Phylogenetic tree

Figure 3 shows the phylogenetic tree constructed by the partial COI gene sequences of Leiognathidae species using neighbour-joining algorithm with distance scale. This tree shows three major clades with high bootstrap values. The first clad includes *Leiognathus*, *Nuchequula*, *Aurigequula*, *Gazza* and *Eubleekeria.* The second clade contains *Karalla*, *Potopectoralis* and *Equulites.* The third minor clad only occupied by *Secutor recoinus.* Interestingly another *Secutor* species, *Secutor insidiator* occupies the first major clade. All the nodes in the tree showed above 50% bootstrap values and indicate that reliable phylogenetic relationship pattern. Similar results were obtained by Ikejima *et al.* (2004), Sparks and Dunlap (2004) and Sparks *et al.* (2005) using a combination of different genetic markers. The clade containing *Photopectoralis* was more closely related to *Equulites* and *Karalla* in the subset tree. Earlier report (Seah *et al.*, 2008) showed *P. bindus* and *L. jonesi* formed the base of the *Gazza*, *Secutor* and *Photoplagios* clades.

In this study the level of intra-species variation was low which may be due to the low number of haplotypes identified in the sample with limited numbers collected for this study. Similarly, Lakra *et al.* (2011) reported very low intra-specific genetic divergence for scombroid fishes. Peris *et al.* (2009) also recorded very low within species genetic distance for Indian carangid fishes. Ward *et al.* (2005) identified that, in COI of fishes the GC content was 42.2 - 47.1%, which reflects in this study. The mean GC content was 46.01 to 48.26% among the nine genera. The mean nucleotide diversity (Pi) among the 13 species was estimated at 0.5671. Earlier studies in Indian marine fishes it was estimated at 0.2029 (Lakra *et al.*, 2011).

The absolute clustering of conspecifics in the present study indicates the diagnostic ability of COI to correctly identify species. Carangoids are hypothesized to be close relatives of pony fishes (Sparks *et al.,* 2005) were used as out group in the present study. Here in neighbour-joining reconstruction, the monophyly of Leiognathidae was strongly supported by a bootstrap value of 100%. Earlier, within Leiognathidae family, six clades of *Nuchequula*, *Photoplagios*, *Photopectoralis*, *Gazza* and *Secutor* with bootstrap support of 94%, <50%, 83%, 100% and 81% by Seah *et al.* (2008). Sparks *et al.* (2005) suggested that *Aurigequula fasciata* and *Leiognathus equulus* each formed independent species complex. Both do not appear to be internally or externally sexually dimorphic with regard to the light organ system. Phylogenetic analysis in this study indicated that *Aurigequula fasciata*, which also do not exhibit sexual dimorphism of the light organ, formed a sister group to *Leiognathus equulus. Secutor insidiator* and *Secutor ruconius* have a small sized body and are probably sister taxa. But molecular data placed these two species in different clade. Sparks *et al.* (2005) showed that *Secutor ruconius* was a sister taxon to *S. hanedai* and *S. megalolepis.*

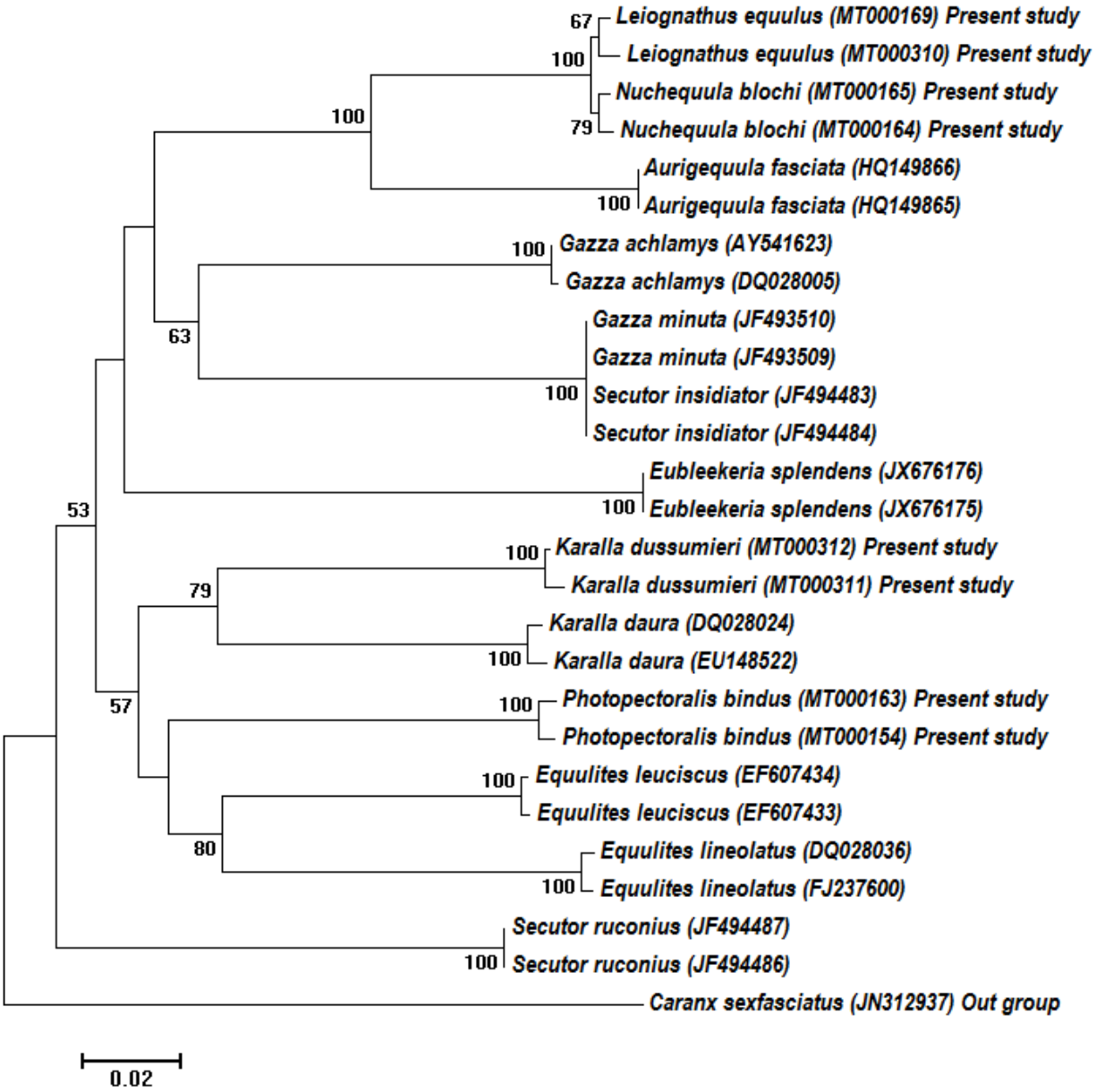

Figure 3. Neighbour-joining phylogenetic tree using COI gene sequences

The molecular phylogenetic positions of the fishes were in congruence with morphological delineation. These results, and those from previous studies, suggested that a more robust morphological criterion, coupled with relevant molecular data should be employed to resolve taxonomic uncertainties among this group of fishes.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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