Full Length Research Paper

Phylogenetic relationship of Sailfin (*Poecilia latipinna*) and Shortfin (*Poecilia sphenops*) mollies (Order: Cyprinodontiformes) as inferred from the mitochondrial cytochrome gene sequence

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Poeciliid fishes are used as model organisms for experimental studies on natural and sexual selection. The present study is aimed at phylogenetic analysis of Poeciliid fishes, focussing on *P. latipinna* (Sailfin) and *P. sphenops* (Shortfin). This paper reports result of phylogenetic analyses based upon nucleotide sequence data from the mitochondrial COI gene of Poeciliids. Briefly, PCR amplification of DNA was carried out using the COI gene primers and sequences were analysed using BioEdit, ExPASy, GeneDoc and Mega 5.0. Partial COI amplicon of 595 bp could be recovered from *P. sphenops* (JF752336) and 645 bp amplicon from *P. latipinna* (JF752337) respectively. Phylogenetic relationship of 13 species of Poeciliid fishes, were constructed employing both neighbor joining and maximum likelihood methods. The results of phylogenetic analysis confirmed the basal placement of mollies in the fish phylogeny. The phylogenetic relationships among the *Poeciliid* fishes in the ML tree were virtually identical to those in the NJ tree. The present study shows that *P. sphenops* and *P. latipinna* are more closely related to each other than to other Poeciliid fishes. Molecular phylogenetic arrangements as inferred from the present study also suggest the presence of single origin of Sailfin species from a Shortfin ancestor.

Key words: Phylogenetic analysis, cytochrome oxidase I, *Poecilia*, cytochrome oxidase I, ornamental fish.

INTRODUCTION

The Poeciliidae is one of the most extensively studied fish families, and its representatives, which include the well-known guppy (*Poecilia reticulata*), mollies (*Mollienesia* spp.), swordtails (*Xiphophorus* spp.), and mosquito fishes (*Gambusia* spp.) are often used as model systems in evolutionary biology theory, especially for experimental studies on natural and sexual selection, and comparative studies of life-history evolution.

Members of this family viz., swordtails, guppies and mollies are also commonly found in the pet trade. The group of Poeciliid fishes commonly known as the mollies (genus *Poecilia*, group *Mollienesia*; Rauchenberger, 1999) are an ideal system for the study of speciation

because of their enormous interspecific diversity in male secondary sex characters, male behaviour and mating system dynamics. One of the reasons why Poeciliids are so appealing to evolutionary biologists is their short generation time, ease of culture and staggering diversity in reproductive adaptations found in this family, which include internal fertilization, oviparity, clonality, viviparity, lecithotrophy, matrotrophy and superfetation (Meffe and

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Snelson, 1989). They are one of four groups of cyprinodontiform fishes that evolved internal fertilization, and one of three groups that evolved live-bearing (Parenti, 1981; Meyer and Lydeard, 1993).

Family Poeciliidae (Rosen and Bailey, 1963) is widespread and includes diverse group of small-sized fishes comprising 220 species in 28 genera (Lucinda, 2003; Lucinda and Reis, 2005). Rosen and Bailey (1963) have placed several well-established genera, including *Mollienesia, Allopoecilia, Limia, Pamphorichthys, Lebistes* and *Micropoecilia* into one genus *Poecilia,* retaining four subgenera. The genus, *Poecilia,* is a complex and widely distributed group found in a wide range of habitats. Mollies have fascinated biologists for decades because of their diversity in habitat, body size, male secondary sex characters and mating behaviour. Species of *Poecilia* have been studied extensively for the effects of natural and sexual selection. The genus *Poecilia,* commonly referred to as mollies, contains 20 named species, which can be divided into two readily distinguishable species complexes the Sailfins of the *P. latipinna* complex and the Shortfins of the *P. sphenops* complex (Hubbs, 1933; Miller, 1983).

The primary goal of the present study is to analyse the molecular phylogeny of the Poeciliid fishes focussing on the group *Mollienesia* especially *P. latipinna* and *P. sphenops.* This paper reports results of phylogenetic analyses based upon nucleotide sequence data from the mitochondrial cytochrome oxidase gene of these fishes. The specific aims in recovering the molecular phylogeny were: to test the classification of Family Poeciliidae especially group *Mollienesia*; to test the taxonomic divisions of Shortfin species that is, *P. sphenops* and Sailfin species that is, *P. latipinna*; and to examine the relationships of Sailfin species of mollies to the Shortfin species.

MATERIALS AND METHODS

Experimental animals and tissue collection

Mitochondorial DNA sequences were obtained from two species of Mollies *viz., Poecilia sphenops* (Shortfin species) and *Poecilia latipinna* (Sailfin species) collected from an ornamental fish aquarium in Kochi, India. The muscle samples were dissected out and transferred to TEN buffers and subjected to DNA extraction.

DNA extraction, PCR amplification, and sequencing

Total genomic DNA was isolated from a piece of muscle tissue taken from fresh specimens. Tissue was digested by proteinase K/SDS solution at 37°C. DNA was purified by standard phenol: chloroform extraction and ethanol precipitation techniques. DNA was quantified and qualified by spectrophotometry and agarose gel electrophoresis. PCR amplification was carried out to

obtain sequences of the partial mitochondrial COI gene, in a total of 25 µl volume containing 1x standard Taq buffer (10 mM Tris-HCl, 50 mM KCl, pH 8.3), 3.5 mM $MgCl_2$, 200 µM dNTPs, 0.4 µM each primer, 1U Taq DNA polymerase (Fermentas, Inc.) and 1µl DNA template (100 ng). The primer pairs used were COI- F (5'-tcaaccaaccacaaagacattggcac-3') and COI- R (5'-tagacttctgggtggccaaagaatca-3'). The thermal profile used was 94°C for 2 min followed by 35 cycles of 94°C for 15 s, 55°C for 30 s and 68°C for 30 s and a final extension at 68°C for 10 min. 10 µl of the amplified PCR product was analyzed by electrophoresis in 1.5 % agarose gel in TBE buffer, stained with ethidium bromide and visualized under UV light. Gel documentation was performed using GelDocXR BioRad unit. Purified PCR products were bidirectionally sequenced using COI primers at Scigenom, Kochi, India.

Sequence analysis

The sequence homology and the deduced amino acid sequence comparisons were carried out using BLAST algorithm at the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/blast). Gene translation and prediction of deduced protein were performed with ExPASy (http://www.au.expasy.org/). The multiple sequence alignments were performed for these sequences using ClustalW and GeneDoc software programs. COI nucleotide sequences were retrieved from the NCBI GenBank and phylogenetic tree was constructed by two independent methods viz., the Maximum Likelihood method and the Neighbor-Joining method based on the nucleotide sequences. Phylogenetic tree was drawn based on the COI sequences using MEGA version 5.0. Confidence in estimated relationships of maximum likelihood and neighbor joining tree topologies were evaluated by a bootstrap analysis with 100 and 1,000 replicates respectively with MEGA version 5.0. The nucleotide and deduced amino acid sequences were submitted to GenBank**.**

RESULTS

Partial COI amplicon of 595 bp could be obtained from the experimental sample, *P. sphenops* (JF752336) and 645 bp amplicon from *P. latipinna* (JF752337) (Figure 1A and B). Analysis of BLAST results obtained for the nucleotide sequences of *P. sphenops* (JF752336) confirmed the organism to be *P. sphenops*. The COI sequence also showed identity to COI sequence of other Poeciliid fishes *viz., P. velifera* (100%), *P. latippinna* (98%), *P. petenensis* and *P. mexicana* (96%), *Pamphorichthys hollandi* (89%), *Limia vittata* and *Heterandria jonesii* (88%), *Xiphophorus variatus* and *Belonesox belizanus* (86%) and *Gambusia panuco* and *Girardinus microdactylus* (84%) respectively (Table 1).

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ggtgcttgagccggcatagtggggacagctctgagtctttttaatccgagccgaactcagt
 G  A  W  A  G  I  V  G  T  A  L  S  L  L  I  R  A  E  L  S
caaccaggatccctcctaggtgatgatcaaatttataatgtaatcgtcacagctcatgcc
 Q  P  G  S  L  L  G  D  D  Q  I  Y  N  V  I  V  T  A  H  A
tttgtaataatctttttttatggtcatgccaattataattggcggctttggtaattgacta
 F  V  I  I  F  F  M  V  M  P  I  I  I  G  G  F  G  N  W  L
gtaccactaataattggtgcccctgatatagccttcccgcgaatgaataatatgagcttc
 V  P  L  I  I  G  A  P  D  I  A  F  P  R  M  N  N  M  S  F
tgacttctaccaccctcattcctcctcctcctcgcatcttctggagtagaagcagggggct
 W  L  L  P  P  S  F  L  L  L  L  A  S  S  G  V  E  A  G  A
ggtacaggttgaaccgtctacccccctctcgcaagcaatttagcccacgctggaccctcc
 G  T  G  W  T  V  Y  P  P  L  A  S  N  L  A  H  A  G  P  S
gtagatctaactattttttcacttcacctggcaggtatttcctcatcctagggggcaatc
 V  D  L  T  I  F  S  L  H  L  A  G  I  S  S  I  L  G  A  I
aactttattaccaccatcattaatataaaaccccctgcagcatctcagtaccaaacaccc
 N  F  I  T  T  I  I  N  I  K  P  P  A  A  S  Q  Y  Q  T  P
ctatttgtctgagctgtaatgattacagctgtactcctacttctctctcttcctgtcctc
 L  F  V  W  A  V  M  I  T  A  V  L  L  L  L  S  L  P  V  L
gccgctggtatcaccatgcttctaacagatcgaaatctaaacaccactttctttg
 A  A  G  I  T  M  L  L  T  D  R  N  L  N  T  T  F  F
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Figure 1A. Nucleotide sequence and deduced amino acid of (A) *Poecilia sphenops* (JF752336).

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ggcatagtgggaacagctctgagtctttttaatccgagccgaactcagtcaaccaggatcc
 G  I  V  G  T  A  L  S  L  L  I  R  A  E  L  S  Q  P  G  S
ctcctaggtgatgatcaaatttataatgtaatcgtcacagctcatgcctttgtaataatc
 L  L  G  D  D  Q  I  Y  N  V  I  V  T  A  H  A  F  V  I  I
ttttttatagtcatgccaattataattggcggcttcggtaattgactagtaccactaata
 F  F  I  V  M  P  I  I  I  G  G  F  G  N  W  L  V  P  L  I
attggtgcccctgatatagccttcccgcgaatgaataatatgagcttctgacttctacca
 I  G  A  P  D  I  A  F  P  R  M  N  N  M  S  F  W  L  L  P
ccctcattcctcctcctcctcgcatcttctggagtagaagcagggggctggtacaggttga
 P  S  F  L  L  L  L  A  S  S  G  V  E  A  G  A  G  T  G  W
accgtctacccccctctcgcaagcaatttagcccatgctggaccctccgtagatctaact
 T  V  Y  P  P  L  A  S  N  L  A  H  A  G  P  S  V  D  L  T
attttctcacttcacctagcaggtatttcctcatcctaggagcaatcaactttattacc
 I  F  S  L  H  L  A  G  I  S  S  I  L  G  A  I  N  F  I  T
accatcattaatataaagcccctgcagcatctcagtaccaaacaccctgtttgtctga
 T  I  I  N  I  K  P  P  A  A  S  Q  Y  Q  T  P  L  F  V  W
gctgtaatgattacagctgtactcctacttctctctcttcctgtcctcgccgctgggatc
 A  V  M  I  T  A  V  L  L  L  L  S  L  P  V  L  A  A  G  I
accatgcttctaacagatcgaaatctaaacaccactttcttcgaccctgcaggaggggga
 T  M  L  L  T  D  R  N  L  N  T  T  F  F  D  P  A  G  G  G
gacccaattctttaccaacacttattctgattctttggccaccca
 D  P  I  L  Y  Q  H  L  F  W  F  F  G  H  P
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Figure 1B. *Poecilia latipinna* (JF752337).

Table 1. Result of BLASTn analysis of COI nucleotide sequence of *Poecilia sphenops* (JF752336).

Closest species	Accession number	E-Value	%Query	% Identity
Poecilia sphenops	JN028268	0	100	100
Poecilia velifera	JQ667585	0	100	100
Poecilia latipinna	JN028262	0	99	98
Poecilia petenensis	EU751941	0	99	96
Poecilia mexicana	EU751927	0	99	96
Poecilia latipinna	HQ557464	0	90	98

Table 2. Result of BLASTn analysis of COI nucleotide sequence of *Poecilia latipinna* (JF752337).

Closest species	Accession number	E-Value	% Query	% Identity
Poecilia latipinna	JN028262	0	97	100
Poecilia sphenops	JN028268	0	97	98
Poecilia velifera	JQ667585	0	96	98
Poecilia petenensis	EU751941	0	96	96
Poecilia mexicana	EU751927	0	96	95

Figure 2A. Multiple alignment of (A) nucleotide sequence.

BLAST results obtained for the nucleotide sequences of *P. latipinna* (JF752337) confirmed the identity of the experimental organism to be *P. latipinna.* The COI sequence of *P. latipinna* also showed identity to COI sequence of other Poeciliid fishes viz., *P. sphenops* and *P. velifera* (98%), *P. petenensis* (96%), *P. mexicana* and *P. orri* (95%), *L. vittata* and *P. hollandi* (89%), *C. decemmaculatus* (87%), *H. jonesii, X. variatus* and *P.*

reticulata (86 %) and *B. belizanus* and *G. falcatus* (85%) (Table 2).

Multiple alignments performed for the nucleotide sequence of the COI gene of *P. sphenops* (JF752336) and *P. latipinna* (JF752337) showed variations in the nucleotide sequences at 11 positions *viz.,* 12th, 129th, 156th, 336th, 366th, 378th, 402nd, 438th, 471st, 537th and 582nd positions (Figure 2A). Translation of the nucleotide

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                    *         20        *         40        *
p.lat : GIVGTALSLLIRAELSQPGSLLGDDQIYNVIVTAHAFVIIFFIVMPIIIGGFGNWLV : 57
p.sph : GIVGTALSLLIRAELSQPGSLLGDDQIYNVIVTAHAFVIIFFMVMPIIIGGFGNWLV : 57
        GIVGTALSLLIRAELSQPGSLLGDDQIYNVIVTAHAFVIIFF6VMPIIIGGFGNWLV

           60        *         80        *        100        *
p.lat : PLIIGAPDIAFPRMNNMSFWLLPPSFLLLLASSGVEAGAGTGWTVYPPLASNLAHAG : 114
p.sph : PLIIGAPDIAFPRMNNMSFWLLPPSFLLLLASSGVEAGAGTGWTVYPPLASNLAHAG : 114
        PLIIGAPDIAFPRMNNMSFWLLPPSFLLLLASSGVEAGAGTGWTVYPPLASNLAHAG

          120        *        140        *        160        *
p.lat : PSVDLTIFSLHLAGISSILGAINFITTIINIKPPAASQYQTPLFVWAVMITAVLLLL : 171
p.sph : PSVDLTIFSLHLAGISSILGAINFITTIINIKPPAASQYQTPLFVWAVMITAVLLLL : 171
        PSVDLTIFSLHLAGISSILGAINFITTIINIKPPAASQYQTPLFVWAVMITAVLLLL

          180        *
p.lat : SLPVLAAGITMLLTDRNLNTTFF : 194
p.sph : SLPVLAAGITMLLTDRNLNTTFF : 194
        SLPVLAAGITMLLTDRNLNTTFF
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Figure 2B. Deduced amino acid sequence of *Poecilia sphenops* (JF752336) and *Poecilia latipinna* (JF752337) obtained using GenDoc programme Version 2.7.0. Black and grey indicates conserved sequences.

sequences were performed using ExPASy tools. BLASTp analysis of the deduced amino acid sequences of COI gene was carried out, which confirmed the presence of COI super family domain, possessing heme binding sites in the translated sequences (Frame-1) of *P. sphenops* (JF752336) and *P. latipinna* (JF752337). Multiple alignments performed for the amino acid sequence of the COI gene of *P. sphenops* (JF752336) and *P. latipinna* (JF752337) also shared great similarity. Though 11 variations could be noticed in the nucleotide sequences of *P. sphenops* and *P. latipinna*; only one variation could be noticed at the amino acid level that is, at 43rd position methionine in *P. sphenops* being replaced by isoleucine in *P. latipinna* (Figure 2B). Multiple alignments performed for the nucleotide sequence and amino acid sequence of *P. sphenops* (JF752336) and *P. latipinna* (JF752337) with other Poeciliid fishes showed that the sequences shared high degree of similarity among Poeciliid fishes (Figure 3).

The phylogenetic relationships of *P. sphenops* and *P. latipinna* were also established, based on nucleotide sequence comparison of the COI genes. The molecular phylogenetic trees were compared with morphology based classification. As shown in Figure 4A, the phylogenetic relationships among the *Poecilia* species in the maximum likelihood tree were virtually identical to those in the neighbor joining tree (Figure 4B). The neighbor joining tree could be broadly divided into two clusters. Cluster 1 included fishes *viz., P. latipinna*, *P. sphenops*, *P. petenensis*, *P. mexicana*, *P. hollandi*, *L. vittata*, *P. reticulate, B. belizanus*, *H. jonesii*, *Xiphophorus* sp. and *C. decemmaculatus*. *P. sphenops* (JF752336) and *P. latipinna* (JF752337) were found to be more

closely related to each other. Cluster 1 could again be divided into two subclusters. Subcluster 1 included *Poecilia, Limia* and *Pamphorichthys* sp.; Subcluster 2 included *Cnesterodon, Belonesox, Heterandria* and *Xiphophorus* sp. Cluster 2 was found to include only two genera of Poeciliid fishes *viz., Girardinus* sp. and *Gambusia* sp. (Figure 4A).

However, in case of maximum likelihood, the tree could be broadly divided into three clusters. Cluster 1 included fishes *viz., Poecilia, Limia* and *Pamphorichthys* sp. and Cluster 2 included *Cnesterodon, Girardinus* and *Gambusia* whereas, Cluster 3 included *Belonesox, Heterandria* and *Xiphophorus* sp. (Figure 4B).

DISCUSSION

Several species of Poeciliid fishes, in particular mollies, are used as model systems for studying the effects of sexual and natural selection on the evolution of natural populations because of their extensive morphological and behavioural variation within and between species; short generation time and ease of culturing this genus in the lab (Endler, 1983; Houde, 1997; Schluter et al., 1998; Hamilton, 2001). Genus *Poecilia* includes at least 20 named species that are commonly referred as mollies and have been divided into two species complexes: *P. latipinna* (Sailfin) and *P. sphenops* (Shortfin) (Hubbs, 1933; Miller, 1983). There are only a few morphological characters that distinguish Sailfins readily from Shortfin species. Sailfin species are characterized by a sexual dimorphism in which males have a greatly enlarged dorsal fin that is erected and presented to the female in a courtship display. Whereas, Shortfin species show

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                    *        20        *        40        *        60        *        80
JF752336-P : GGTGCTTGAGCCGGCATAGTGGGGACAGCTCTGAGTCTTTTAATCCGAGCCGAACTCAGTCAACCAGGATCCCTCCTAGGT :  81
JF752337-P : ------------GGCATAGTGGGAACAGCTCTGAGTCTTTTAATCCGAGCCGAACTCAGTCAACCAGGATCCCTCCTAGGT :  69
JX111725-C : GGTGCTTGAGCCGGTATAGTCGGGACAGCTCTTAGCCTTCTAATCCGAGCTGAACTGAGTCAACCTGGCACCCTCCTAGGT :  81
JQ840428-B : GGTGCTTGAGCCGGCATAGTCGGAACTGCCCTAAGGCTGTTAATCCGAGCGGAACTAAGCCAGCCTGGCTCCCTCCTTAGGA :  81
JQ935963-X : GGCGCTTGGGCCGGTATCGTGGGAACAGCCTTAAGCCTCTTAATTCGAGCCGAACTAAGTCAACCTGGCCACCCTCCTAGGT :  81
JQ935962-X : GGCGCTTCGGCCGGGATAGTGGGGACAGCCTTAAGCCTCTTAATTCGAGCCGAACTAAGTCAACCTGGCGCCCTCTTCGGT :  81
JQ935960-X : GGCGCTTCGGCCGGTATAGTGGGGACACGCCTTAAGCCTCTTAATTCGAGCCGAACTAAGTCAACCAGGCACCCTCCTGGGT :  81
JQ935955-X : GGCGCTTCGGCCGGTATCGTGGGGACAGCCTTAAGTTCGAGCCGAACTAAGTCAACCGGGCACCCTCCTGGGT :  81
JQ935948-X : GGCGCTTCGGCCGGTATCGTGGGGACCGCCTTAAGGCTTTTAATTCGAGCCGAACTAAGTCAACCGGCGCACTCTCCTGGGT :  81
JQ935929-P : GGTGCTTGAGCCGGCATAGTGGGAACAGCTCTGAGTCTTTTAATCCGAGCCGAACTCAGTCAACCAGGATCCCTCCTAGGC :  81
JQ935928-P : GGTGCTTGAGCCGGCATAGTGGGAACAGCTGTGAGTCTTTAATCCGAGCCGAACTCAGTCAACCAGGATCCCTCCTAGGC :  81
JQ935925-H : GGCGCTTCGGCCGGCATAGTTGGAACAGCTTTAAGCCTTTTAATCCGAGCGGAACTTAGTCAACCGGCTCTCTCTTAGGT :  81
JQ935865-G : GGTGCTTGAGCTGGTATAGTTGGAACAGCTCTGAGCCTACTGATCCGGGCGAACTCAGTCAGCCAGGCACACTCCTGGGT :  81
JQ665833-P : ---------------AATAGTAGGAACAGCCTTCGATCCGAGCCGAACTCAGCCAACCAGGGCCCTCCTGGGA :  67
JQ665831-P : ---------------AATAGTAGGAACAGCTTTAAGCCTTCTGATCCGAGCCGAACTCAGCCAACCGGGGCCCTCCTAGGA :  67
JQ667593-X : GGCGCTTCGGCCGGTATAGTGGGAACAGCCTTAAGCCTCTTAATTCGGGCGAACTAAGTCAACCGGGCACCCTCCTGGGT :  81
JQ432022-P : GGTGCTTGAGCCGGAATAGTAGGAACAGCCTTAAGCCTTCTCATCCGAGCCGAACTCAGCCAACCTGGGGCCCTCCTGCGG :  81
JN028268-P : GGTGCTTGAGCCGGCATAGTGGGAACAGCTCTGAGTCTTTAATCCGAGCCGAACTCAGTCAACCCAGGATCCCTCCTAGGT :  81
JN028266-P : GGTGCTTGAGCCGGAATAGTAGGAACAGCTCTTAAGCCTTCTCGATCCGAGCCGAACTCAGTCAACCTGGGGCCCTCCTGGGG :  81
JN028261-P : GGTGCTTGAGCCGGCATAGTGGGAACAGCTCTGAGTCTTTTAATCCGAGCCGAACTCAGTCAACCAGGATCCCTCCTAGGT :  81
HM906030-P : GGTGCTTGAGCCGGCATAGTAGGAACAGCTCTAAGCTCTTCTAATCCGAGCCGAACTTAGTCAACCAGGATCCCTCCTAGGT :  81
HM405172-P : ------------GCATCGTAGGAACAGCTCTAAGCTCTTCTAATCCGAGCCGAACTTAGTCAACCAGGATCCCTCCTAGGT :  68
HM404991-P : GGTGCTTGAGCCGGAATAGTAGGAACAGCTTTAAGCCTTCTGATCCGAGCTGAACTCAGCCAACCTGGGGCCCTCCTGGGG :  81
HM404990-P : GGTGCTTGAGCCGGAATAGTAGGAACAGCTCTAAGCCTTTAAGCCTCTCGATCCGAGCTGAACTCAGCCAACCTGGGGCCCTCCTGGGG :  81
FN545684-G : GGTGGCTCGAGCCGGCATCGTGGGCACGCGCTGTCAGCCTCTTAATCCGAGCTGAGCTCAGTCAACCGGCCGCACTCCTGGGT :  81
FN545665-L : GGTGGCTCGAGCCGGCATAGTAGGAACAGCCCTGAGCTCTTTGATTCGAGCGGAACTTAGTCAACCAGGATCCCTCCTGGGT :  81
FN545659-L : GGTGGCTCGAGCCGGCATAGTAGGAACAGCCCTGAGCTCTTTGATTCGAGCGGAACTTAGTCAACCAGGATCCCTCCTGGGT :  81
FN545615-G : GGTGGCTCGAGCCGGCATCGTGGGACGGCGCTCTCAGCCTCTTAATCCGAGCGGAGCTCAGTCAACCGGCCGCACTCCTGGGT :  81
FN545601-G : GGTGGCTCGAGCCGGCATCGTCGGAACGGCGCTGTCAGCCTCTTAATCCGAGCGTGAGCTCAGTCAACCGGCGCACTCCTGGGT :  81
GU702171-P : ---------------TAGTAGGAACAGCTTTAAGCCTTCTGATCCGAGCCGAACTCAGCCAACCTGGGGCCCTCCTGGGG :  65
EU751941-P : GGTGCTTGAGCCGGCATAGTGGGAACAGCTCTGAGTCTTTTAATCCGAGCCGAACTCAGTCAACCAGGATCCCTCCTAGGT :  81
EU751921-P : GGTGCTTGAGCCGGCATAGTGGGAACAGCTCTGAGTCTTTTAATCCGAGCCGAACTCAGTCAACCAGGATCCCTCCTAGGC :  81
EU751911-P : GGTGCTTGAGCCGGCATAGTGGGAACAGCTCTGAGTCTTTTAATCCGAGCCGAACTCAGTCAACCAGGATCCCTCCTAGGC :  81
EU751664-B : GGTGCTTGGGCCGGCGAGCTGCCTAAGCCTAAGCCTGTTAATCCGGGCGGGACGAACTAAGCCAGCCTGGCCACCCTCCTTAGGA :  81
             qq  qc  tq  qccqg  aTaGT GG AC GC   T AG CT   T AT CGaGC GAaCT AG CAaCC  GG   CcCTCcT GG
                    *        100       *        120       *        140       *        160
JF752336-P : GATGATCAAATTTATAATGTAATCGTCACAGCTCATGCCTTTGTAATAATCTTTTTTATGGTCATCCCAATTATAATTGGC : 162
JF752337-P : GATGATCAAATTTATAATGTAATCGTCACAGCTCATGCCTTTGTAATAATCTTTTTTATAGTCATGCCAATTATAATTGGC : 150
JX111725-C : GACGATCAAATTTATAATGTAATCGTTACAGCTCACGCCTTTGTAATAATCTTTTTGATAGTGTATACCAATTCATAATTGGT : 162
JQ840428-B : GATGATCAGATCTACAATGTGATCGTTACAGCTCATGCCTTTGTAATAATCTTTTTTATAGTGTATACCCATTATAATCGGG : 162
JQ935963-X : GATGACCAAATCTACAATGTGATCGTCACAGCTCATGCCTTTGTAATAATTTTTTTTATAGTGTATACCAATTATGATTGGC : 162
JQ935962-X : GATGACCAAATCTACAATGTGATCGTCACAGCTCATGCCTTTGTAATAATTTTTTTTATAGTCATACCAATCATCATTGGC : 162
JQ935960-X : GATGACCAAATCTACAATGTGATCGTCACAGCTCATGCCTTTGTAATAATTTTTTTTATAGTCATGCCAATCATCATTGGC : 162
JQ935955-X : GATGACCAAATCTACAATGTGATCGTCACAGCTCATGCCTTTGTAATAATTTTTTTTATAGTCATACCAATCATCATTGGC : 162
JQ935948-X : GATGACCAAATCTACAATGTGATCGTCACAGCTCATGCCTTTGTAATAATTTTTTTTATAGTCATGCCAATCATCATTGGC : 162
JQ935929-P : GATGACCAAATTTATAATGTAATCGTCACAGCTCATGCCTTTGTAATAATCTTTTTTATAGTTATGCCAATTATAATTGGC : 162
JQ935928-P : GATGACCAAATTTATAATGTAATCGTCACAGCTCATGCCTTTGTAATAATCTTTTTTATAGTTATGCCAATTATAATTGGC : 162
JQ935925-H : GATGATCAAATCTATAATGTCATCGTCACAGCTCATGCCTTTGTAATAATTTTTTTTATAGTTATACCGTAATCATTGGC : 162
JQ935865-G : GACGATCAAATCTACAATGTAATCGTCACAGCTCATGCTTTTGTAATAATCTTTTTTATAGTTATGCCAATTATAATTGGA : 162
JQ665833-P : GATGATCAAATTTATAATGTAATTGTTACAGCTCATGCCTTTGTAATAATCTTTTTTATAGTTATGCCAATCATCATTGGA : 148
JQ665831-P : GATGATCAAATTTATAATGTAATTGTTACAGCTCATGCCTTTGTAATAATCTTTTTTATAGTTATGCCAATCATCATTGGA : 148
JQ667593-X : GACGACCAAATCTACAATGTGATCGTCACAGCTCATGCCTTTGTAATAATTTTTTTTATAGTTATACCCATCATAATTGGC : 162
JQ432022-P : GATGATCAAATTTATAATGTAATCGTTACAGCTCATGCCTTTGTAATAATCTTTTTTATAGTTATGCCAATCATAATCGGA : 162
JN028268-P : GATGATCAAATTTATAATGTAATCGTCACAGCTCATGCCTTTGTAATAATCTTTTTTATGGTCATGCCAATTATAATCGGA : 162
JN028266-P : GATGATCAAATTTATAATGTAATCGTCACAGCTCATGCCTTTGTAATAATCTTTTTTATAGTCATCCCAATTATAATCGGA : 162
JN028261-P : GATGATCAAATTTATAATGTAATCGTCACAGCTCATGCCTTTGTAATAATCTTTTTTATAGTCATGCCAATTATAATTGGC : 162
HM906030-P : GACGATCAAATCTACAATGTAATCGTCACAGCTCATGCCTTTGTAATAATCTTTTTTATAGTCATACCAATTATAATCGGC : 162
HM405172-P : GACGATCAAATCTACAATGTAATCGTTACGGCTCATGCCTTTGTAATAATCTTTTTTATAGTCATACCAATTATAATTGGT : 149
HM404991-P : GATGATCAAATTTATAATGTAATCGTTACAGCTCATGCCTTTGTAATAATCTTTTTTATAGTTATGCCAATCATAATAGGA : 162
HM404990-P : GATGATCAAATTTATAATGTAATCGTTACAGCTCATGCCTTTGTAATAATCTTTTTTATAGTTATGCCAATCATAATAGCA : 162
FN545684-G : GACGACCAAATTTATAATGTGATCGTCACAGCTCATGCCTTTGTAATAATCTTTTTTATAGTAATACCAATTATGATCGGC : 162
FN545665-L : GACGACCAAATCTACAATGTGATCGTCACAGCTCATGCCTTTGTAATAATCTTTTTTATAGTCATACCAATTATAATTGGC : 162
FN545659-L : GACGACCAAATCTACAATGTGATCGTCACAGCTCATGCCTTTGTAATAATCTTTTTTATAGTCATGCCAATTATAATTGGC : 162
FN545615-G : GACGACCAAATCTATAATGTAATCGTCACAGCTCACGCCTTTGTAATAATCTTTTTTATAGTAATGCCCATTATGATCGGC : 162
FN545601-G : GACGACCAAATTTATAATGTGATCGTCACAGCTCATGCCTTTGTAATAATCTTTTTTATAGTAATACCAATTATAATCGGC : 162
GU702171-P : GATGATCAAATTTATAATGTAATCGTTACAGCTCATGCCTTTGTAATAATCTTTTTTATAGTCATACCAATCATAATCGGA : 146
EU751941-P : GATGATCAAATTTATAATGTAATCGTCACAGCTCATGCCTTTGTAATAATCTTTTTTATAGTCATGCCAATTATAATTGGC : 162
EU751921-P : GATGATCAAATTTATAATGTAATCGTCACAGCTCATGCCTTTGTAATAATCTTTTTTATAGTTATGCCAATTATAATTGGC : 162
EU751911-P : GATGATCAAATTTATAATGTAATCGTCACAGCTCATGCCTTTGTAATAATCTTTTTTATAGTTATGCCAATTATAATTGGC : 162
EU751664-B : GACGATCAAATTTATAATGTAATCGTCACAGCTCATGCCTTTGTAATAATCTTTTTTATAGTTATACCAATTATAATTGGC : 162
             GA GA CAaAT TA AATGT ATcGT ACaGCTCAtGCCTTTGTAATAATcTTTTTtATaGT AT CCaaT AT AT GG
```

neither sexual dimorphism in fin morphology nor perform courtship display behaviours. These variations imply an important role for sexual selection in promoting premating reproductive isolation. The *P. latipinna* complex, or Sailfin mollies, includes three species, *P. latipinna, P. petenensis,* and *P. velifera*; and males of all three species are sexually dimorphic. Possession of the Sailfin distinguishes these species from the remaining Shortfin molly species in the *P. sphenops* complex. The *P.*

sphenops complex includes *P. sphenops* and *P. Mexicana.*

In the present study molecular phylogeny for 13 species of Poeciliid fishes has been done, based on nucleotide sequence data from the mitochondrial genome of the COI gene.

The neighbor-joining and maximum likelihood trees has been presented (Figure 4A) from 1000 and 100 bootstrap iterations respectively. Neighbor-joining and maximum

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                        *         260       *         280       *         300       *         320
JF752336-P : CTTCTACCACCCTCATTCCTCGCTCCTCCTCGCATCGTTCTGGAGTAGAAGCAGCGGCTGGTACAGGTTCAACCGTCTACCGC : 324
JF752337-P : CTTCTACCACCCTCATTCCTCGCTCCTCCTCGCATCGTTCTGGAGTAGAAGCAGCGGCTGGTACAGGTTGAACCGTCTACCGC : 312
JX111725-C : CTACTCCCGCCCTCATTCCTCGTTCTTCTTCCTAGCATCGTCCGGAGTAGAAGCAGCGGCTGGAACAGGCTGAACTGTTTACCGC : 324
JQ840428-B : CTTGTTCCCCCCTCATTCCTCGCTCCTCCTTGCATCGTTCTGCTGTTGAAGCAGCTGCTGGAACAGGGTCAAGAGTGTATCGC : 324
JQ935963-X : CTCCTTCCGCCCTCATTCTTCTTCTTCCTAGCCATCCTCTGGGGTTGAAGCGGCGGCTGGAACAGGGTGAAGTGTCTATCGC : 324
JQ935962-X : CTCCTTCCCCCCTCATTCTTCTTCTTCCTAGCCATCCTCGGGGTTGAAGCAGCAGCCGGCTGGAAGTGGATGAACTGTCTTTACCGC : 324
JQ935960-X : CTCCTTCCCCCCTCATTCCTTCTTCTTCCTGCATCCTCGGGGGTTGAAGCGGCGGCTGGAACTGGATGAACTGTCTACCGC : 324
JQ935955-X : CTCCTTCCCCCCTCATTCCTCTTTCTCCTAGCCATCCTCGGGAGTTGAAGCAGCAGAGCGGGAGCTGGAACTGGATGAACTGTCTTACCGC : 324
JQ935948-X : CTCCTTCCCCCCTCATTCCTTCCTTCTTCCTAGCCATCCTCAGCGGTTGAAGCAGCAGCTGCGAAGTGGATCGCACTGTTTATCGC : 324
JQ935929-P : CTTCTACCGCCCTCATTCCTCGCTCCTCCTGTTAGCATCGTTCTGGGGTAGAAGCAGCGGCGTGGTACAGGTTGAACCGTCTACCGA : 324
JQ935928-P : CTTCTACCGCCCTCATTCCTCGCTCCTCCTCTTAGCATCGTTCTGGGGTAGAAGCAGCGGCTGGTACAGGTTGAACCGTCTACCCA : 324
JQ935925-H : CTCCTTCCCCCTTCATTCCTCGCTTCTCTTAGCCATCGTTCTGGAGTAGAAGCAGGAGAGCAGCGGCTGGAACAGGATCAACTGTTTACCGA : 324
JQ935865-G : TTACTTCCCCCTCATTTCCTCGCTCCTCCTCGCATCGTTCTGGGGGTTGAGCAGCGGCGCAGGACAGGCTGAACTGTGTTTATCGC : 324
JQ665833-P : CTTTTACCACCCTCATTCCTCGCTCCTCCTATCATCCTCTGGAGTCGAAGCAGCAGCCGGTACAGGATCAACTGTTTATCGT : 310
JQ665831-P : CTTTTACCACCCTCATTCCTCGTTCCTCCTATCATCCTCTGGGGTTGAAGCAGCAGAGCCCGGTACAGGGTCAACTGTTTATCGT : 310
JQ667593-X : CTCCTTCCCCCCTCATTCCTTCTTCTTCTCCTGCCATCCTCGGGGGTTGAAGCAGCGGCTGGAACAGGGTCAACTGTTTATCGC : 324
JQ432022-P : CTTTTACCACCCTCATTTCCTCGCTCCTCCTATCATCCTCGGGGGTCGAAGCAGCGGCGTACAGGATCAACTGTTTATCGC : 324
JN028268-P : CTTGTACCACCCTCATTCCTCGCTCCTCCTCGCATCGTTCTGGAGTAGAAGCAGCGGCTGGTACAGGTTCAACCGTCTACCGC : 324
JN028266-P : CTTTTACCACCCTCATTCCTCGCTCCTCCTATCATCCTCTGGAGTCGAAGCAGCGGCGTACAGGGTCAACTGTTTATCGC : 324
JN028261-P : CTTGTACCACCCTCATTCCTCGCTCCTCCTCGCATCGTCTGGAGTAGAAGCAGCGGCTGGTACAGGTTGAACCGTCTACCGC : 324
HM906030-P : CTTCTACCTCCATCGTTCCTTCTCGCTTTTAGCATCGTTCCGGAGTAGAAGCAGCGGCGCTGGAACAGGCTCAACTGTTTACCGC : 324
HM405172-P : CTTCTACCTCCATCGTTCCTTCTCCTTTTAGCCATCGTTCTGGAGTTGAACAGCGGCTGGAACAGGCTGAACTGTTTACCGC : 311
HM404991-P : CTTTTACCACCCTCATTCCTTCTTCCTCCTATCATCCTCCGGGGGTCGAAGCAGCAGCCGGTACAGGATCAACTGTTTATCGC : 324
HM404990-P : CTTTTACCACCCTCATTCCTTCTTCTTCCTAGCCATCCTCGGGGGTCGAAGCAGCAGAGCCCGGTACAGGATCAACTGTTTATCGC : 324
FN545684-G : CTCCTCCCGCCCTCATTCCTCGTTCTTCTTCTCGCCATCCTCGGGGGTTGAAGCAGCGGCTGGGCCCGAACTGGTTCAACCGTCTACCGC : 324
FN545665-L : CTCCTACCCCCTCATTCCTCGCTCCTCCTTCTGGCATCGTTCCGGAGTAGAGGCGGCGGTACAGGGTCAACTGTTTATCGT : 324
FN545659-L : CTCCTACCCCCTCATTCCTCGCTCCTCCTAGCCATCGTTCCGGGGTAGAGGCGGCGGTACAGGGTTCAGTGTTTATCGT : 324
FN545615-G : CTCCTCCCCCCTCATTCCTTCGCTTCTTCTCTCGGGGGGTTGAAGCAGCGGCGGCCGGAATGGGTCAACCGTCTACCGC : 324
FN545601-G : CTCCTCCCCCCCTCATTCCTTCCTTCTCTCTCGCCATCCTCGGGAGTAGAGGCTGGCGCCGGAACTGGGTCAACCGTCTACCGC : 324
GU702171-P : CTTTTACCACCCTCATTCCTCGCTCCTCCTATCATCCTCCGGGGGTGGAAGCAGCAGCCGGTACAGGGTCAACTGTTTATCGC : 308
EU751941-P : CTTCTACCGACCTTCATTCCTCGCTCCTCCTCGCATCGTCCGGAGTAGAAGCGGCGGCTGGTACAGGTTGAACCGTCTACCCT : 324
EU751921-P : CTTCTACCGCCCTCATTCCTCGCTCCTCCTCGCATCGTTCTGGTGTAGAAGCAGCGGCTGGTACAGGTTCAACCGTCTACCGA : 324
EU751911-P : CTTCTACCGCCCTCATTCCTCGCTCCTCCTAGCATCGTTCTGGGGGTAGAAGCAGCGGCGCTGGTACAGGTTGAACCGTCTACCGA : 324
EU751664-B : CTTGTTCCCCCCCTCATTCCTCGCTCCTCCTGGCATCGTTCTGCTGTTGAAGCAGCTGCTGGAACAGGGTCAAGAGTATATCGC : 324
             cT  cT  CC  CCcTCaTT CT  CT  CTccT  qCATC  TC  GG  GT  GAaGCaGG  GC  GG  ACaGG  TGaAC  GT  TA  CC
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                     *        180       *         200       *         220       *         240
JF752336-P : GGCTTTGGTAATTGACTAGTACCACTAATAATTGGTGCCCCTGATATAGCCTTCCCGCGAATGAATAATATGCAGCTTCTGA : 243
JF752337-P : GGCTTCGGTAATTGACTAGTACCACTAATAATTGCTGCCCCTGATATAGCCTTCCCGCGAATCAATAATATCAGCTTCTGA : 231
JX111725-C : GGTTCGGTAATTGACTCATCCCACTAATAATTGCCGCCCCGACATAGCTTTCCCCCGCGATAAATAAATAATGCAGCTTCTGA : 243
JQ840428-B : GGATTTGGTAATTGACTTGTTCCACTTATAATTGCGCCCCTGACATGGCCTTCCCGCGCGATGAATAACATAAGCTTCTGA : 243
JQ935963-X : GGCTTTGGTAACTGACTAATCCCACTAATAATTGCCGCCCCTGACATAGCCTTCCCCCGCATGAATAACATAAGCTTTTGA : 243
JQ935962-X : GGCTTTGGTAACTGATTGATCCCACTAATAATTGCGCCCCTGACATAGCCTTCCCCCGCGAATCAATAACATAAGTTTTTGA : 243
JQ935960-X : GGCTTTGGTAACTGTAGTACCCACTAATAATTGCCGCCCCTGACATAGCCTTCCCCCGAATGAATAACATAAGCTTTGA : 243
JQ935955-X : GGCTTTGGCAACTGATTGGTCCCACTAATAATTGCCGCCCCTGACATAGCCTTTCCCCGAATAAATAACATAAGTTTTTGA : 243
JQ935948-X : GGCTTTGGTAACTGATAGATCCCACTAATAATTGCGCCCCTGACATAGCCTTTCCCCGAATCAATAATATCAGTTTTTGA : 243
JQ935929-P : GGCTTCGGTAATTGATTAGTACCACTAATAATTGCTGCCCCTGATATGGCCTTTCCACGAATCAATAATATGCAGCTTCTGA : 243
JQ935928-P : GGCTTCGGTAATTGATTAGTACCACTAATAATTGCTGCCCCTGATATGGCCTTTCCACGAATCAATAATATGCAGCTTCTGA : 243
JQ935925-H : GGCTTTGGTAATTAAATCCCACTAATAATTGCTGCTCCTGACATGGCCTTTCCCCGAATAAATAACATCAGTTTCTGA : 243
JQ935865-G : GGGTTTGGTAACTGATTAGTACCCACTAATAATTGCTGCCCCAGACATAGCCTTTCCACGAATAAACAATATAAGCTTCTGA : 243
JQ665833-P : GGCTTCGGTAATTGATTAGTCCCATTAATAATCGGCGCTCCTGACATAGCTTTTCCCCGAATAAATATATAAGCTTCTGA : 229
JQ665831-P : GGCTTCGGTAATTGATTAATTCCATTAATAATCGGCGCTCCTGACATGGCTTTTCCCCGAATAAACAACATAAGCTTTTGA : 229
JQ667593-X : GGCTTTGGTAACTGACTAATCCCACTAATAATTGCGCCCCTGACATAGCCTTTCCCCGAATAAACAACATAAGCTTTTGA : 243
JQ432022-P : GGCTTCGGTAATTGATTAGTCCCATTAATAATTGCTGCCCCTGACATGGCCTTTCCCCGAATAAATAATATAAGCTTCTGA : 243
JN028268-P : GGCTTTGGTAATTGACTAGTACCACTAATAATTGCTGCCCCTGATATAGCCTTTCCCGCGAATGAATAATATGCAGCTTCTGA : 243
JN028266-P : GGCTTCGGTAATTGATTAGTCCCATTAATAATTGCTGCTCCTGACATAGCCTTTCCCCGAATAAATAATATAAGCTTCTGA : 243
JN028261-P : GGCTTCGGTAATTGACTAGTACCACTAATAATTGCTGCCCCTGATATAGCCTTTCCCGCGAATGAATAATATGCAGCTTCTGA : 243
HM906030-P : GGCTTCGGTAATTGACTAGTCCCATTAATAATTGCTGCCCCTGACATAGCCTTCCCGCGAATAAATAATATAAGCTTCTGA : 243
HM405172-P : GGCTTCGGTAATTGACTAGTCCCATTAATAATTGCTGCCCCTGACATGGCCCTCGCAATAAATAATATAAGCTTCTGA : 230
HM404991-P : GGCTTCGGTAATTGATTAGTCCCATTAATAATTGCTGCCTCCTGACATGGCCTTTCCCCGAATAAATAATAAGCTTCTGA : 243
HM404990-P : GGCTTCGGTAATTGATTAGTCCCATTAATAATTGCTGCCTCCTGACATGGCCTTTCCCCGAATAAATAATATAAGCTTCTGA : 243
FN545684-G : GGCTTCGGTAATTGACTAGTCCCATTAATAATTGCGCCCCGATATGGCCTTCCCTCGCATGAATAATATAAGCTTCTCG : 243
FN545665-L : GGCTTCGGTAATTGATTGGTGCCATTAATAATTGCTGCCCCCGATATGGCCTTCCCACGAATCAATAATATAAGCTTCTGA : 243
FN545659-L : GGCTTCGGTAATTGATTGGTGCCCATTAATAATTGCTGCCCCGATATGGCCTTCCCACGAATCAATAATATAAGCTTCTGA : 243
FN545615-G : GGCTTTGGTAATTGACTAGTTCCACTAATAATTGCGCCTCCGACATAGCCTTCCCCCGAATAAATAATATAAGCTTTGA : 243
FN545601-G : GGCTTCGGTAATTGACTAGTCCCATTAATAATTGCGCCCCCGATATGGCCTTCCCTCGGATAAATAATATAAGCTTCTCG : 243
GU702171-P : GGCTTCGGTAATTGATTAGTCCCATTAATAATTGCTGCCCCTGACATAGCCTTTCCCCGAATAAATAATATAAGCTTCTGA : 227
EU751941-P : GGTTTCGGTAATTGATTAGTACCACTAATAATTGCTGCCCCTGATATAGCCTTCCCGCGAATGAATAATATGCAGCTTCTGA : 243
EU751921-P : GGCTTCGGTAATTGATTAGTACCACTAATAATTGCTGCCCCTGACATAGCCTTCCACGAATCAATAATATCAGCTTCTGA : 243
EU751911-P : GGCTTCGGTAATTGATTAGTACCACTAATAATTGCTGCCCCTGACATAGCCTTTCCCCGAATAAATAATAATAAGCTTCTGA : 243
EU751664-B : GGGTTTGGTAATTGACTCGTTCCACTTATAATTGCGCCCCGACATGGCCTTTCCGCGGATAAATAACATAAGCTTCTGA : 243
             GGcTT  GGtAAtTGa  T  gT  CCa  TaATAATtGG  GC  CC  GA  ATaGC  TT  CC  CGaaT  AAtAA  AT  AGcTTcTGa
```

likelihood analyses recovered similar topologies (Figure 4A and B).

In the present study, approximately 595 and 645 bp COI fragments were obtained from *P. sphenops* (JF752336) and *P. latipinna* (JF752337) respectively (Figure 1A and B). Deduced amino acid sequences showed the presence of conserved COI domain with heme binding sites, during BLASTp analysis of the deduced amino acid sequences. The COI nucleotide sequence obtained for *P. sphenops* and *P. latipinna* along with 11 other COI sequences obtained from GenBank were included for analysis in the present study.

Multiple alignments (Figure 3) were performed with COI nucleotide sequences from the GenBank database of other Poeciliid fishes that was obtained during the BLAST analysis and the molecular phylogenetic trees were

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                     *        340        *        360        *        380        *        400
JF752336-P : CCTCTCGCAAGCAATTTAGCCCACGCTGGACCCTCCGTAGATCTAACTATTTTTCACTTCACCTGCCAGGTATTTTCCTGC : 405
JF752337-P : CCTCTCTGCAAGCAATTTAGCCCATGCTGGACCCTCCGTAGATCTAACTATTTTCTCACTTCACCTAGCAGGTATTTCCTGC : 393
JX111725-C : CCTCTTGCAGGGAATCTAGCCCATGCCGGACCCTCCGTAGATTTAACCTATTCTCACTTCACCTAGCAGGTATCTCCTGT : 405
JQ840428-B : CCTCTCGCAGGTAATTTAGCACACGGCTGGACCCTCCGTAGATTTAACTATTTTTTCACTTCCACCTAGCAGGAATCTCCTGC : 405
JQ935963-X : CCTCTTGCAGGTAATTTAGCGCATGCTGGACCCTCCGTGGGACTTGACTATTTTTCACTTCCTCTCTGGTGTGGTATTTCCTGC : 405
JQ935962-X : CCTCTTGCAGGTAACTTAGCGCATGCGGACCCTCCGTGGGACTTAACTATTTTTTCCCTCCATCTCGCTGGTGGTATTTCCTGC : 405
JQ935960-X : CCTCTTGCAGGTAACTTTAGCGCATGCTGGGCCTCTCGGTGGACTTAACTATTTTTTTCCTCCATCTCGCTGGTATTTCCTGC : 405
JQ935955-X : CCTCTTGCAGGTAACTTAGCGCATGCGGACCCTCCGTGGGACTTAACTATTTTTTCCTCTCCATCTCGGTGGTATTTCCTGC : 405
JQ935948-X : CCTCTTGCAGGCAACTTAGCACATGCTGGACCCTCCGTGGGACTTAACTATTTTTTTCTCTCCATCTCGGTGGTATTTCCTGC : 405
JQ935929-P : CCTCTTGCAGGCAATTTAGCCCACGCTGGTCCCTCTGTAGATCTAACTATTTTTTCACTCCACCTGCAGGTATTTCCTGC : 405
JQ935928-P : CCTCTTGCAGGCAATTTAGCCCACGCTGGTCCCTCTGTAGATCTAACTATTTTTTCACTCCACCTGCCAGGTATTTCCTGC : 405
JQ935925-H : CCCCTTGCAGGTAATTTAGCACACGGCCGGGCCCTCTGTAGATTTAACTATTTTCTCACTTCATTTCTGCTGGTATTTCTTGC : 405
JQ935865-G : CCTCTAGCAGGGCAACCTAGCACACGCGGACCCTCTGTAGACTTGACCCTCTTCTCCCTTCATTTGCGGGGCATTTCCTGC : 405
JQ665833-P : CCCCTTGCAAGCAATTTAGCCCACGCTGGACCATCTGTAGATCTTAACTATTTTTTCACTTCACTTGCCGGTATTTCTTCC : 391
JQ665831-P : CCCCTTGCAGGCAATTTAGCCCACGCTGGACCCTCCGTGGACTTGACTATTTTTTCACTTCCATCTTGCGGGTATTTCTTCC : 391
JQ667593-X : CCTCTTGCAGGCAATTTAGCCCACGCTGGACCCTCCGTGGACTTGACTATTTTTTCACTTCCATCTGCGGGTATTTCCTCC : 405
JQ432022-P : CCTCTTGCAGGCAATTTAGCCCACGCTGGCCATCCGTGGGATTTAACTATTTTTTCCTCTCCACCTGCAGGTATTTCTTCC : 405
JN028268-P : CCTCTCGCAGGCAATTTAGCCCACGCTGGACCCTCCGTAGATCTAACTATTTTTTCACTTCCACCTGCCAGGTATTTCCTGC : 405
JN028266-P : CCTCTTGCAAGCAATTTAGCCCACGCTGGACCATCCGTGGATTTAACTATTTTTTCACTTCCACCTGCGGGTATTTCTTGC : 405
JN028261-P : CCTCTCGCAAGCAATTTAGCCCACGCTGGACCCTCCGTAGATCTAACTATTTTTTCACTTCCACCTAGCAGGTATTTCCTGC : 405
HM906030-P : CCCCTCGCAGGTAACCTAGCACACGCGGACCCTCCGTGGATCTAACTATTTTTTCACTTCACCTAGCAGGTATTTCTTGT : 405
HM405172-P : CCCCTCGCAGGGCAATTTAGCACACGGCTGGTCCTTCCGTGGGATTCTAACTATCTTTTTCACTTCACCTAGCAGGTATTTCTTGT : 392
HM404991-P : CCTCTTGCAGGCAATTTAGCCCACGCTGGGCCATCCGTGGGATTTAACTATTTTTTCACTTCACCTGCCAGGTATTTCCTGC : 405
HM404990-P : CCTCTTGCAGGCAATTTAGCACACGCTGGACCATCCGTGGGATTTAACTATTTTTTTCACTTCCACCTGCCAGGTATTTCTTCC : 405
FN545684-G : CCTCTTGCAGGCAATCTGGCCCATGCGGACCATCTGTGTGGACTTAACTATCTTCTCCCTTCACTAGCCGGTATCTCTTCA : 405
FN545665-L : CCCCTAGCAGGCAACTTAGCACACGGCGGGACCATCCGTGGGATTTAACTATTTTCTCTCCTCCACCTAGCAGGTATCTCCTGA : 405
FN545659-L : CCCCTAGCAGGCAACTTTAGCACACGGCGGGACCATCCGTGGGATTTAACTATTTTCTCTCCTCCACCTAGCGGTATCTCCTGA : 405
FN545615-G : CCCCTTGCAGGTAACCTGGCTCACGCGGGGCCCATCCGTGGACTTGGTATCTATCTTCTCCTCCCTCCATCTGCGGGTATCTTTGA : 405
FN545601-G : CCTCTTGCAGGCAATCTGGCCCATGCGGACCCATCCGTGGACTTAACTATCTTCTCCCTTCACCTAGCCGGTATCTCTTGA : 405
GU702171-P : CCTCTTGCAGGCAATTTAGCCCACGCTGGACCATCCGTGGGATTTAACTATTTTTTCACTTCACCTGCGGGTATTTCTTCC : 389
EU751941-P : CCTCTCGCAGGCAATTTAGCCCACGCTGGACCCTCCGTGGACTTAACTATTTTTTCACTTCCACCTGCCAGGTATTTCCTGC : 405
EU751921-P : CCTCTTGCAGGCAATTTAGCCCACGCTGGTCCTGTAGATCTAACTATTTTTTCACTCCACCTGCAGGTATTTCCTGC : 405
EU751911-P : CCTCTTGCAGGCAATTTAGCCCACGCTGGACCCTCTGTAGATCTAACTATTTTTTTCACTCCACCTGCCAGGTATTTCCTGC : 405
EU751664-B : CCTCTCGCAGGCAATTTAGCACACGCTGGACCCTCGTGTGATTTAACTATTTTTTCACTCCACCTAGCAGGGATCTCCTGC : 405
             CC  CT  GCA  G AA  tTaGC  CA  GC  GGaCC  TC  GT  GA    TaaCtATtTT  TC  CT  CA  cT  GC  GGtAT  TC  TC
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                     *        420        *        440        *        460        *        480
JF752336-P : ATCCTAGGCGCAATCAACTTTATTACCACCATCATTAATATAAAACCCCCTGCAGCCATCTCAGTACCAAACACCCCTATTT : 486
JF752337-P : ATCCTAGGAGCAATCAACTTTATTACCACCATCATTAATATAAAACCCCCCTGCAGCCATCTCGTACCAAACACCCCTGTTT : 474
JX111725-C : ATCTTAGGAGCAATTAACTTTATTACCACAATTATTAATATGAAACCCCCCGCAGCCATCTCAATACCAAACACCTTCTTT : 486
JQ840428-B : ATTCTTGGAGCCATCAACTTTATTACTACCATTATTAACATAAAACCCCCCGCAGCCATCTCAATACCAAACACCTCTTTT : 486
JQ935963-X : ATTCTGGGCGCCATCAACTTTATCACCACTATAATCAACATAAAACCCCCCGCAGCCATCTCAATACAGACACCCCGTTTT : 486
JQ935962-X : ATTCTGGGCGCAATCAACTTTATCACCACTATAATTAACATAAAACCCCGCAGCCATCTCAATACCAGAGCCCCGTTTT : 486
JQ935960-X : ATTCTAGGCGCAATCAACTTTATCACCACCATATAATTAACATAAAACCCCGCAGCCATCTCAATACCAAACGCCCCTGTTT : 486
JQ935955-X : ATTCTAGGGGCAATCAACTTTATCACCACTATAATTAACATAAAACCCCCCGCAGCCATCTCAATACCAAAGCCCCCGTTTT : 486
JQ935948-X : ATTCTAGGCGCAATCAACTTTATCACCACTATAATTAACATAAAACCCCCCGCAGCCATCTCAATACCAAAGCCCCCGTGTTT : 486
JQ935929-P : ATTCTAGGCGCAATCAACTTTATTACCACTATTATTAATATAAAACCCCCTGCAGCCATCTCAATACCAAACACCCCTATTT : 486
JQ935928-P : ATTCTAGGCGCAATCAACTTTATTACCACTATTATTAATATAAAACCCCCTGCAGCCATCTCAATACCAAACACCCCTATTT : 486
JQ935925-H : ATTTTAGGCGCAATCAACTTTATTACCACTATTATTAATATAAAACCCCCTGCAGCCATCTCAATACCAAACACCCTTGTTT : 486
JQ935865-G : ATTGTTGGGGCTATTAATTTTATTACCACCATCATTAATATAAAACCTCCCGCAGCCGTCCAGTACCAGACACCCTTGTTT : 486
JQ665833-P : ATTCTAGGAGCAATTAACTTCATTACGACAATTATTAATATAAAACCCCCTGCCCCATCTCAATATCAAACACCCTTTATTT : 472
JQ665831-P : ATTCTAGGAGCAATCAACTTCATTACCACTATTATTAATATAAAACCACCTGCAGCCATCACAATATCAAACACCTTTATTT : 472
JQ667593-X : ATTCTGGGCGCAATCAACTTTATCACCACTATAATTAACATAAAACCCCGCAGCCATCTCAATACCAGACACCCCGTTTT : 486
JQ432022-P : ATTTTAGGCGCAATTAACTTCATTACCACTATCATTAATATAAAACCCCCTGCAGCCATCTCAATATCAAACACCTTTATTT : 486
JN028268-P : ATCCTAGGCGCAATCAACTTTATTACCACTATTATTAATATAAAACCCCCTGCAGCCATCTCAGTACCAAACACCCCTATTT : 486
JN028266-P : ATTTTAGGAGCAATTAACTTCATCACCACTATTATTAACATAAAACCCCCTGCAGCCATCTCAATATCAAACACCCTTTATTT : 486
JN028261-P : ATCCTAGGCGCAATCAACTTTATTACCACTATCATTAATATAAAGCCCCCTGCAGCCATCTCAGTACCAAACACCCCTGTTT : 486
HM906030-P : ATCTTAGGAGCAATTAATTTTATTACCACTATTATCAATATAAAACCCCCCGCAGCCATCTCAATACCAGACACCACTGTTT : 486
HM405172-P : ATCTTAGGAGCAATCAACTTTATTACCACTATTATCAATATAAAACCCCCGCAGCCATCTCAATACCAACACCACGTGTTT : 473
HM404991-P : ATTTTAGGAGCAATTAACTTCATTACCACTATTATTAATATAAAACCCCCTGCAGCCATCTCAATATCAAACACCTTTATTT : 486
HM404990-P : ATTTTAGGAGCAATTAACTTCATTACCACTATTATTAATATAAAACCCCCTGCAGCCATCTCAATATCAAACACCTTTATTT : 486
FN545684-G : ATTCTAGGAGCAATTAATTTCATCACCACTATTATTAACATAAAACCCCCCGCAGCCATCCTATACCAGACGCCTTTGTTT : 486
FN545665-L : ATCCTCGGAGCAATCAACTTTATTACTACTATTATTAAATATAAAACCCCCTGCAGCCATCTCAATACCAGACACCCTTGTTT : 486
FN545659-L : ATCCTCGGAGCAATCAACTTTATTACTACTATTATTAAATATAAAACCCCCTGCAGCCATCTCAATACCAGACACCCTTGTTT : 486
FN545615-G : ATTTTAGGCGCAATTAACTTCATCAGTACCACTATTAGGATAAAACCCCCCGCAGCCGTCCAGTACCAAACACCCCTATTT : 486
FN545601-G : ATTCTAGGAGCAATTAATTTCATCACCACTATTATTAACATAAAACCCCCCGCAGCCATCTCCTTATACCAGACGCCTTTGTTT : 486
GU702171-P : ATTTTAGGAGCAATTAACTTCATCACCACTATTATTAATATAAAACCCCCTGCAGCCATCTCCTTATACCAGACACCCTTTATTT : 470
EU751941-P : ATCCTGGGAGCAATCAACTTTATTACCACAATTATTAATATAAAACCCCCTGCAGCCATCTCAATACCAAACACCCCTATTT : 486
EU751921-P : ATCCTAGGGGCAATCAACTTTATTACCACTATTATTAATATAAAACCCCCTGCAGCCATCTCAATACCAAACACCCCTGTTT : 486
EU751911-P : ATCCTAGGCGCAATCAACTTTATTACCACTATTATTAATATAAAACCCCCTGCAGCCATCTCAATACCAAACACCCCTATTT : 486
EU751664-B : ATTCTTGGAGCCATCAACTTTATTACCACCATTATTAACATAAAACCTCCCGCAGCCCTCTCAATACCAAACACCTCGTTTT : 486
             AT    T  GG  GCaAT  AAcTT  AT  ACcAC  AT  ATtAA  ATaAAaCCcCC  GCagCaTCtCaaTAcCA  ACaCC    T  TTT
```

constructed using MEGA 5.0 software (Figure 4A and B). Multiple alignments performed for the nucleotide sequence of the COI gene of *P. sphenops* (JF752336) and *P. latipinna* (JF752337) showed variations at 11 positions. Whereas, multiple alignments, performed for the amino acid sequences of the COI genes showed variation at only one position, which shows that the COI genes of *P. latipinna* and *P. sphenops* are highly conserved (Figure 2B). Multiple alignments were performed for the nucleotide sequence and amino acid sequence of *P. sphenops* and *P. latipinna* to other Poeciliid fishes also showed that the sequences shared high degree of similarity among Poeciliid fishes. However, higher degree of similarity could be noticed within the genus *Poecilia*.

Phylogenetic analysis was performed for the COI sequences of 13 Poeciliid fishes, obtained during BLAST analysis. For a robust and reliable phylogenetic analysis,
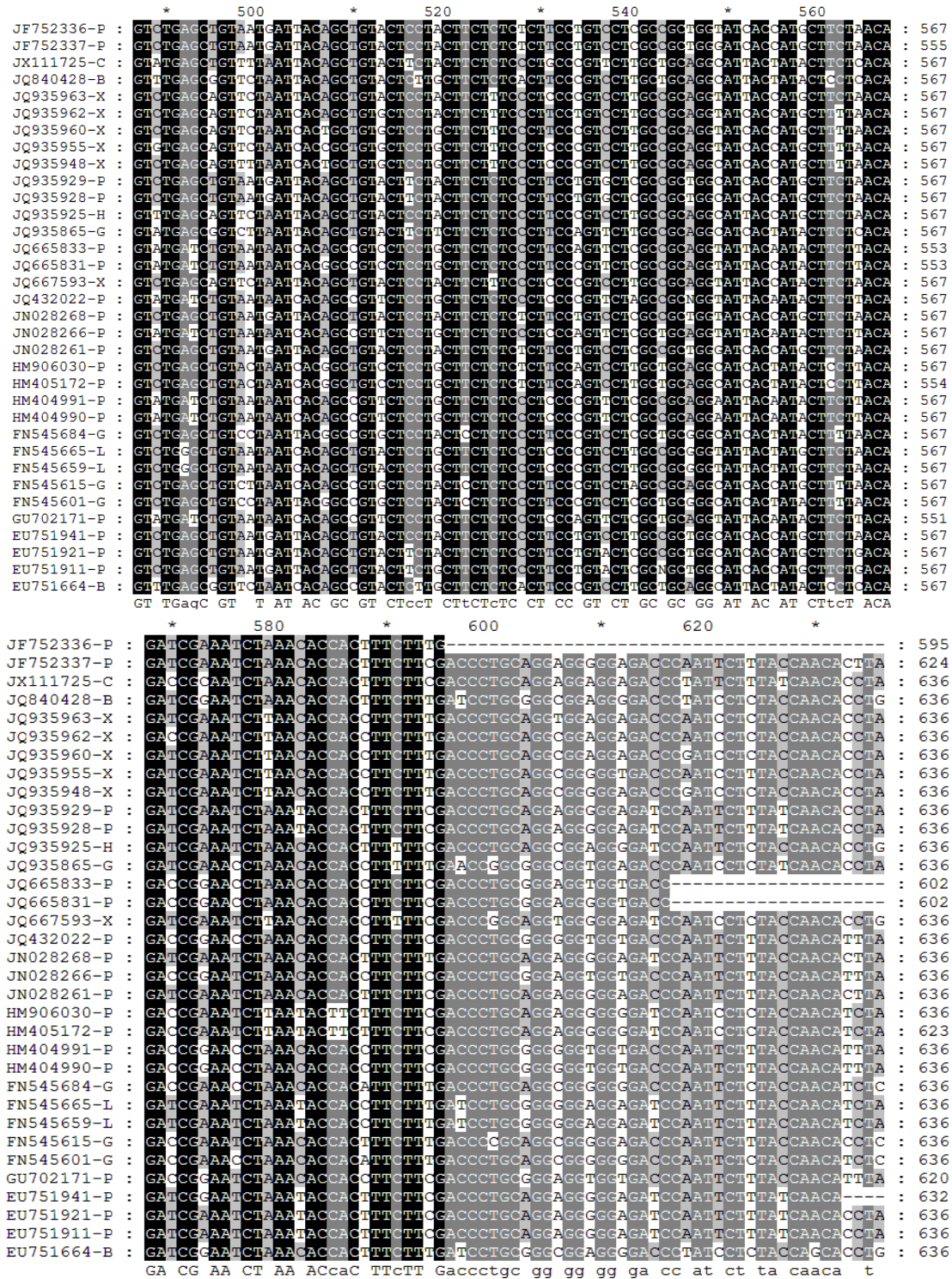
Figure 3. Multiple alignment of nucleotide sequence of *Poecilia sphenops* (JF752336) and *Poecilia latipinna* (JF752337) with other similar sequences obtained using GenDoc programme Version 2.7.0. Black and grey indicates conserved sequences.

two methods were used, *viz.,* neighbor joining and maximum likelihood approaches. Neighbor joining and maximum likelihood trees was constructed using

bootstrap test by 1000 and 100 replicates respectively (Figure 4A and B).

The results of phylogenetic analysis based on the COI
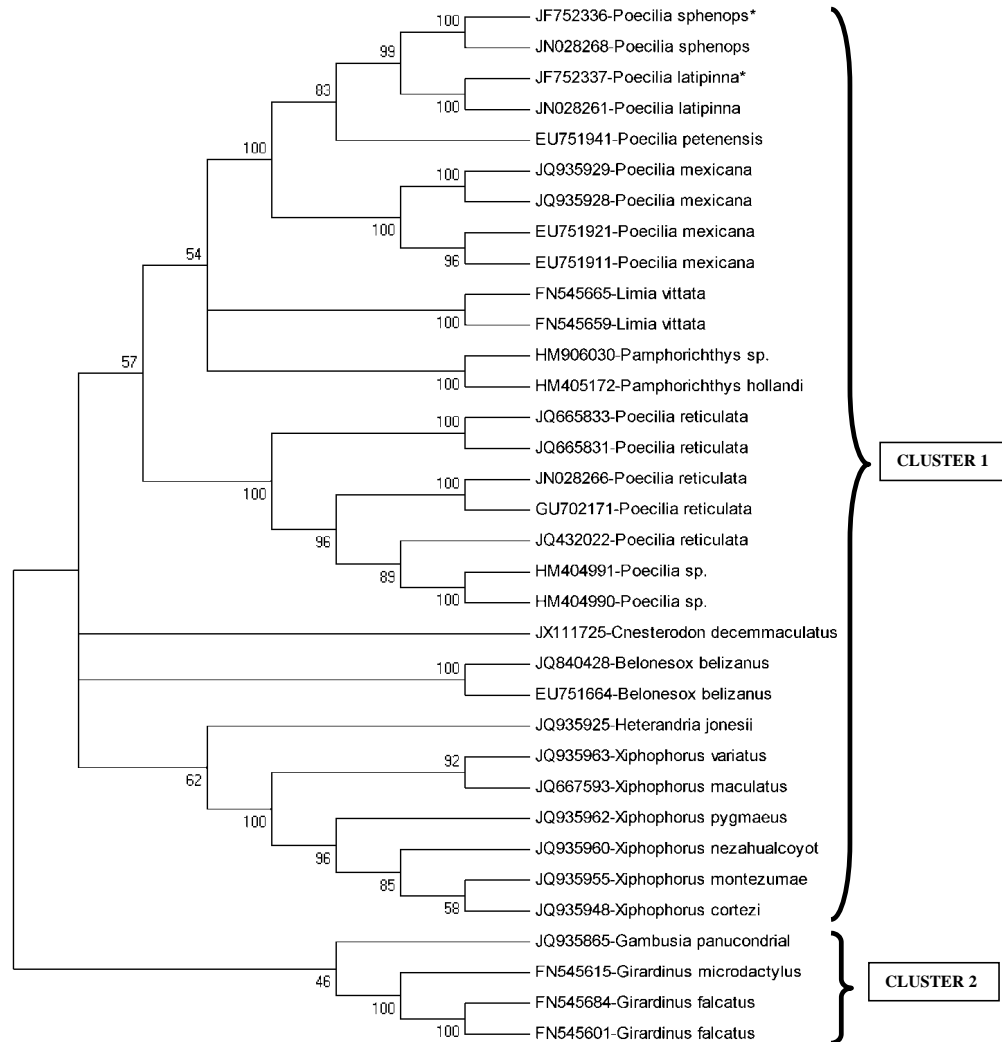
Figure 4A. A bootstrapped neighbour-joining tree obtained using MEGA version 5.0 illustrating relationships between the nucleotide sequences of *Poecilia sphenops* (JF752336) and *Poecilia latipinna* (JF752337) with other Poeciliid fishes. Values at the node indicate the percentage of times that the particular node occurred in 1000 trees generated by bootstrapping the COI nucleotide sequences.

sequences confirmed the basal placement of mollies in the fish phylogeny. Analysis of the results from neighbor joining and maximum likelihood analyses for 13 species of *Poeciliids* support the categorisation by Rosen and Bailey (1963). The present study shows that *P. sphenops* and *P. latipinna* are more closely related to each other than to other Poeciliid fishes. The phylogenetic relationships among the *Poeciliid* fishes in the maximum likelihood tree were virtually identical to those in the neighbor joining tree.

The neighbor joining tree could be broadly divided into two clusters. Cluster 1 included fishes *viz., P. latipinna*, *P. sphenops*, *P. petenensis*, *P. mexicana*, *Pamphorichthys hollandi*, *L. vittata*, *P. reticulate, Belonesox belizanus*, *Heterandria jonesii*, *Xiphophorus* sp. and *C.*

decemmaculatus. P. sphenops (JF752336) and *P. latipinna* (JF752337) were found to be more closely related to each other. Cluster 1 could again be divided into two subclusters. Subcluster 1 included *Poecilia, Limia* and *Pamphorichthys* species. Subcluster 2 included *Cnesterodon, Belonesox*, *Heterandria* and *Xiphophorus* species. Cluster 2 included *Girardinus falcatus* and *Gambusia panucondrial*. However, in case of maximum likelihood, the tree could be broadly divided into three clusters. Cluster 1 included fishes *viz., Poecilia, Limia* and *Pamphorichthys* species. Cluster 2 included fishes *viz., Cnesterodon, Girardinus* and *Gambusia*, whereas, Cluster 3 included *Belonesox, Heterandria* and *Xiphophorus* species.

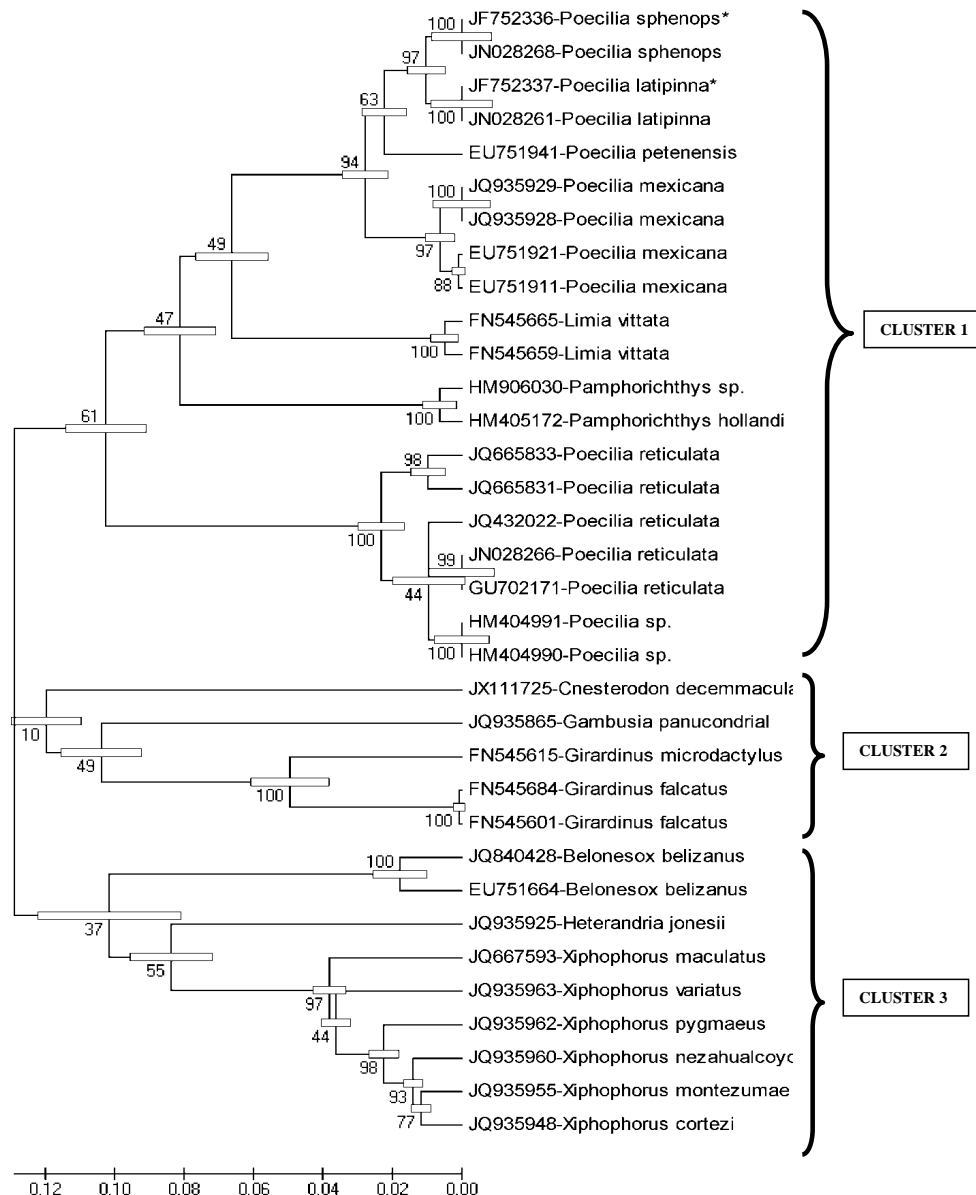Several groups within the genus *Poecilia viz.*, four

Figure 4B. A bootstrapped maximum-likelihood tree obtained using MEGA version 5.0 illustrating relationships between the nucleotide sequences of *Poecilia sphenops* (JF752336) and *Poecilia latipinna* (JF752337) with other Poeciliid fishes. Values at the node indicate the percentage of times that the particular node occurred in 100 trees generated by bootstrapping the COI nucleotide sequences.

species of *Poecilia*, *Limia* and *Pamphorichthys* were found to be supported at the 99 to 100% level in both neighbour joining and maximum likelihood analyses. Within this *Mollienesia* group, the sailfin mollies, *P. latipinna, P. petenensis* and *P. mexicana,* form a strongly supported group (94 to 100%). Also, according to both neighbor joining tree and maximum likelihood analysis, *P. sphenops, P. latipinna, P. petensis* and *P. mexicana* were found to be closely related to each other than to *P. reticulata.* Interestingly, *P. sphenops* and *P. latipinna*

were found to be more closely related to *Limia* sp. and *Pamphorichthys* sp. than to *P. reticulata* belonging to the same genus. These results are in agreement with the observation by Breden et al. (1999) and with the definition of subgenera of *Poecilia* by Rosen and Bailey (1963), which state that they are morphologically quite different from each other, and also that there are monophyletic assemblages within *Poecilia* (*e.g.*, *Limia, Pamphorichthys*). Analysis of the phylogenetic tree also showed that the inferred position of *Cnesterodon,*

Girardinus, Gambusia, Belonesox, *Heterandria* and *Xiphophorus* sp. also changes depending on the approach taken. Only the maximum likelihood criterion places this species in a group when compared to that of neighbour joining analysis and this relationship is upheld by very low bootstrap support (10 to 40%).

Molecular phylogenetic arrangements as inferred from the present study suggest the presence of single origin of Sailfin species from a Shortfin ancestor. Several lines of evidence implicate an important role for sexual selection in the divergence between Shortfin and Sailfin species. Hence, the enlarged dorsal fin and associated courtship behaviour found in males of all three Sailfin species evolved from a common ancestor (1999).

Conclusion

The present study reports results of phylogenetic analyses based on nucleotide sequence data from the mitochondrial cytochrome oxidase gene of two Poeciliid fishes *viz.*, *P. latipinna* (Sailfin species) and *P. sphenops* (Shortfin species). The phylogenetic relationships among the *Poeciliid* fishes in the maximum likelihood tree were virtually identical to those in the neighbor joining tree. The results of phylogenetic analysis based on the COI sequences confirmed the basal placement of mollies in the fish phylogeny. The molecular data was found to support a close relationship between Sailfin species and Shortfin species. Molecular phylogenetic arrangements as inferred from the present study suggest the presence of single origin of Sailfin species from a Shortfin ancestor. The study also shows that the faster evolving genes viz., COI, is useful in differentiating closely-related species and may thus be more used as a universal marker for fish identification.

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REFERENCES

Breden F, Ptacek MB, Rashed M, Taphorn D, Figueiredo CA (1999). Molecular phylogeny of the live-bearing fish genus *Poecilia* (Cyprinodontiformes: Poeciliidae). Mol. Phylogenet. Evol., 12: 95-104.

Endler JA (1983). Natural and sexual selection on color patterns in poeciliid fishes. Environ. Biol. Fishes, 9: 173-190.

Hamilton A (2001). Phylogeny of *Limia* (Teleostei: Poeciliidae) based on NADH dehydrogenase subunit 2 sequences. Mol. Phylogenet. Evol., 19: 277–289.

Houde AE (1997). Sex, Color, and Mate Choice in Guppies. Princeton University Press, Princeton, New Jersey.

Hubbs CL (1933). Species and hybrids of *Mollienisia.* Aquarium, 1: 263–268.

Lucinda PHF, Reis RE (2005). Systematics of the subfamily Poeciliinae Bonaparte (Cyprinodontiformes: Poeciliidae), with an emphasis on the tribe Cnesterodontini. Neotrop. Ichthyol., 3: 1-60.

Lucinda PHF (2003). Family Poeciliidae. In: Reis, R.E., Kullander SO, Ferraris CJ (Eds.) Check List of the Freshwater Fishes of South and Central America. Edipucrs, Porto Alegre, Brazil. pp. 555–581.

Meffe GK, Snelson FF (1989). Ecology & Evolution of Livebearing Fishes (Poeciliidae). Prentice Hall. Upper Saddle River, NJ. p. 453.

Meyer A, Lydeard C (1993). The evolution of copulatory organs, internal fertilization, placentae and viviparity in killifishes (Cyprinodontiformes) inferred from a DNA phylogeny of the tyrosine kinase gene X-src. Proc. R. Soc. London B. 254: 153–162.

Miller RR (1983). Checklist and key to the mollies of Mexico (Pisces: Poeciliidae: *Poecilia,* subgenus *Mollienesia*). Copeia, pp. 817-822.

Parenti LR (1981). A phylogenetic and biogeographic analysis of cyprinodontiform fishes. Bull. Amer. Mus. Nat. Hist., 168: 335–557.

Rauchenberger M (1989). Annotated species list of the subfamily Poeciliinae. *In* "Ecology and Evolution of Livebearing Fishes (Poeciliidae)" (Meffe GK and Snelson FF (Eds.)), pp. 359–367, Prentice Hall International, Englewood Cliffs, NJ.

Rosen DE, Bailey RM (1963). The poeciliid fishes (Cyprindontiformes), their structure, zoogeography, and systematics. Bull. Amer. Mus. Nat. Hist. 126:1–176.

Schluter A, Parzefall J, Schlupp I (1998). Female preference for symmetrical vertical bars in male sailfin mollies. Anim. Behav., 56: 147-153.