



The complete mitochondrial genome of the small yellow croaker and partitioned Bayesian analysis of Sciaenidae fish phylogeny

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Abstract

To understand the phylogenetic position of *Larimichthys polyactis* within the family Sciaenidae and the phylogeny of this family, the organization of the mitochondrial genome of small yellow croaker was determined herein. The complete, 16,470 bp long, mitochondrial genome contains 37 mitochondrial genes (13 protein-coding, 2 ribosomal RNA and 22 transfer RNA genes), as well as a control region (CR), as in other bony fishes. Comparative analysis of initiation/termination codon usage in mitochondrial protein-coding genes of Percoidae species, indicated that COI in Sciaenidae entails an ATG/AGA codon usage different from other Percoidae fishes, where absence of a typical conserved domain or motif in the control regions is common. Partitioned Bayesian analysis of 618 bp of COI sequences data were used to infer the phylogenetic relationships within the family Sciaenidae. An improvement in harmonic mean $-\ln L$ was observed when specific models and parameter estimates were assumed for partitions of the total data. The phylogenetic analyses did not support the monophyly of *Otolithes*, *Argyrosomus*, and *Argyrosominae*. *L. polyactis* was found to be most closely related to *Collichthys niveatus*, whereby, according to molecular systematics studies, the relationships within the subfamily Pseudosciaenidae should be reconsidered.

Key words: *Larimichthys polyactis*, mitochondrial genome, control region, partitioned Bayesian analysis, Sciaenidae.

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Introduction

Animal mitochondrial DNA, a 16-19 kb long circular molecule, comprises 37 genes encoding 22 transfer RNAs (tRNAs), 13 proteins, 2 ribosomal RNAs, and a putative control region (CR) (Brown, 1985; Boore, 1999). Due to its compactness, maternal inheritance, fast evolutionary rate compared to nuclear DNA, and the resulting short coalescence time, mitochondrial DNA sequence data have been widely used in phylogeny (Miya *et al.*, 2003), taxonomic resolution (Poulin *et al.*, 2004), and population genetic studies (Habib *et al.*, 2010).

The small yellow croaker, *Larimichthys polyactis*, a very popular fish among consumers, is one of the most important commercial benthopelagic fishes in China and Korea. The species is extensively distributed in the Bohai, Yellow and East China Seas, global landing having reached 320 thousand metric tons in 2000 (Seikai National Fisheries Research Institute, 2001). Nevertheless, intense fishing has deflated catches in the Yellow and East China Seas, to such an extent that resources are now considered over-exploited (Lin *et al.*, 2008). In the past, the focus has been on catch statistics, size composition, early life history and

feeding habits (Xue *et al.*, 2004; Yan *et al.*, 2006; Wan and Sun, 2006). However, over recent years, the genetic characteristics of the small yellow croaker have also come under investigation by means of molecular markers (Meng *et al.*, 2003; Lin *et al.*, 2009; Xiao *et al.*, 2009), although, information is still limited, and the complete mitochondrial genome sequence, critical in studies of genome evolution and species phylogeny, continues unavailable. Thus, much additional work is required to furnish important genetic and evolutionary data, essential for species conservation, management and phylogenetic analysis.

Sciaenidae is a diverse and commercially important family, comprising 68 genera and about 311 species (Nelson, 2006). Despite specific studies of morphological and molecular phylogeny, instabilities of the phylogenetic relationships within the group have not, as yet, been resolved, the methods employed in previous research on phylogenetic reconstruction based on molecular data having been, to some extent, empirical and simple. The non-implementation of advanced methods, such as ML or BI, best-fit evolutionary models for specific data, and the statistical testing of the different topologies derived from the same data matrix, has probably contributed to mismodeling and systematic error in analysis. Mismodeling itself commonly occurs when a single model, inappropriate for multiple-gene regions involving several models of evolution, as stem and

loop in RNA, or codon positions in protein genes, is employed for those multiple data partitions best explained by separate models of DNA evolution. Another form of mis-modeling is the case, when multiple data partitions, defined by the same general model, differ drastically in the specific model parameter estimates that maximize the likelihood score (Reeder, 2003; Brandley *et al.*, 2005). An overall solution would be to apply appropriate models and their specified parameter estimates to each data partition, and subsequently incorporate this into a single ML tree-search (Yang, 1996). Methods for reconstructing phylogeny, based on partitioned data using Bayesian/Markov chain Monte Carlo (MCMC) methods, are now available (Huel- senbeck and Ronquist, 2001; Nylander *et al.*, 2004). Since it more accurately models the data, the use of partition- specific modeling - in other words, partitioned Bayesian analysis - should reduce systematic error, thereby resulting in better likelihood scores and more accurate posterior probability estimates (Brandley *et al.*, 2005).

In the present study, pre-defined complete mitochondrial genome sequences of the small yellow croaker were compared with those reported for Percoidei species, where- upon partitioned Bayesian analysis was applied to infer the phylogeny of Sciaenidae fishes.

Materials and Methods

Fish sample and DNA extraction

L. polyactis individuals were collected by trawling in the Zhoushan fishing grounds, East China Sea (Zhejiang Province, China). They were identified by morphology. Muscle tissue was removed and immediately preserved at -80 °C. Total DNA was extracted as per standard phenol-chloroform method (Sambrook and Russell, 2001) and visualized on 1.0% agarose gels.

PCR amplification and sequencing

As shown in Table S1, 11 sets of primers that amplify contiguous, overlapping segments of the complete mitochondrial genome of *L. polyactis*, were used. The primers were designed from reported complete mitochondrial genome sequences for the large yellow croaker (Cui *et al.*, 2009). Worthy of note, these primers are also very useful for amplifying the mitochondrial genomes of two other Sciaenidae species, *Miichthys miiuy* and *Collichthys lucidus*. Of the 11 pairs of primers, six (1F/R, 4F/R, 6F/R, 7F/R, 8F/R, and 10F/R) are capable of perfectly amplifying the mitochondrial genomes of both species. The total length of amplified products is approximately 12,300 bp. PCR assays were carried out in a final volume of 50 µL, each containing 5.0 µL of a 10x *Taq* Plus polymerase buffer, 0.2 mM of dNTP, 0.2 µM of the forward and reverse primers, 2 units of *Taq* Plus DNA polymerase with proof-reading characteristic (TIANGEN, Beijing, China), and 1 µL of DNA template. Cycling conditions were 94 °C for 4 min,

followed by 35 cycles of 94 °C for 50 s, 60 °C for 60 s and 72 °C for 2-3 min, followed by 1 cycle of 72 °C for 10 min, PCR was performed on a PTC-200 thermocycler (MJ Research, USA). The resultant PCR products, first electro- phoresed on a 1% agarose gel to check integrity, were then visualized by the Molecular Imager Gel Doc XR system (BioRad), followed by purification using a QIAEX II Gel Extraction Kit (Qiagen). The purified fragments were li- gated into PMD18-T vectors (Takara, Japan) used to trans- form to TOP10 *E. coli* cells, according to standard protocol. Positive clones were screened via PCR with M13+/- primers. Amplicons were sequenced using an ABI 3730 auto- mated sequencer (Applied Biosystems) with M13+/- primers.

Sequence analysis

The sequence fragments so obtained were edited in the Seqmen program (DNASTar, Madison, WI, USA) for contig assembly to obtain a complete mitochondrial ge- nome sequence. Annotation of protein-coding and ribo- somal RNA genes, and definition of their respective gene boundaries were carried out with DOGMA software (Wy- man *et al.*, 2004) through reference sequences of Percoidei, available in GenBank. tRNA genes and their secondary structures, were identified by means of tRNAscan-SE 1.21 software (Lowe and Eddy, 1997). Base composition, ge- netic distances, and codon usage were calculated in MEGA 4.0 software (Tamura *et al.*, 2007). Putative O_L and CR, and conserved motifs were identified via sequence homology and proposed secondary structure. The complete mitochon- drial genome sequence, deposited in the GenBank data- base, can be accessed through Accession Number GU586227.

Phylogenetic analysis

Multiple alignments of the COI sequences from 30 Sciaenidae species were performed using the MEGA 4.1 version. The data matrix of COI sequences was partitioned by codon position (Table 1). The appropriate model of se- quence evolution for each partition (Table 2) was deter- mined using the jModeltest program (Guindon and Gas- cueil, 2003; Posada, 2008), under the Akaike Information Criterion (AIC). Bayesian analysis for each data partition- ing strategy consisted of two separate runs with four Markov chains in the MrBayes3.1 program (Huelsenbeck and Ronquist, 2001). Each run, comprising 10 million gen-

Table 1 - Partition strategies used in this study.

Partition strategy	Partition identity
P ₁	All codon positions together
P ₂	First and second codon positions; third codon position
P ₃	First codon position; second codon position; third codon position

Table 2 - Data partitions, their estimated models of sequence evolution, and total number of characters of each partition used in phylogenetic analysis.

Partition	Evolutionary model	Number of characters in partition
COI	HKY+I+G	618
COI 1 st codon	GTR+G	206
COI 2 nd codon	GTR+I	206
COI 3 rd codon	HKY+I+G	206
COI 1 st +2 nd codon	GTR+I+G	412

erations, was systematically sampled at every 100. The first 25% of the trees were discarded as part of a burn-in procedure, and the remainder employed in constructing a 50% majority rule consensus tree. The results for each partitioning strategy were then compared using the Bayes factor as an aid in accepting the best phylogeny hypothesis for the sequence data matrix.

Results and Discussion

Gene content, arrangement and base composition

The complete mitochondrial genome of *L. polyactis* was 16,470 bp long (Table 3), which is similar to those of not only teleost species but also terrestrial vertebrates. Its gene content conforms to the vertebrate consensus, by containing the highly conserved set of 37 genes encoding 2 ribosomal RNAs (rRNAs), 22 transfer RNAs (tRNAs) and 13 proteins, that are essential in mitochondrion respiration and adenosine triphosphate (ATP) production. Although, as in other vertebrates, most of the genes are encoded on the H-strand, besides ND6 and eight tRNAs (Glu, Ala, Asn, Cys, Tyr, Ser-UCN, Gln, and Pro) being also encoded on the L-strand, the genes are all similar in length to those of bony fishes (Oh *et al.*, 2007, 2008). As reported in other vertebrates, there are four notable ‘overlaps’ between genes, as reported in other vertebrates, with the lengths of these overlaps are generally being fixed. Whereas ATPase 8 and ATPase 6 overlap by 10 bp, overlapping was also observed between ND4L and ND4 (seven bp), ND5 and ND6 (four bp), and ATPase 6 and COIII (one bp). The remainders are located between tRNA genes themselves, and between tRNA and protein-coding genes. The sizes of non-coding spacers range from 1 to 37 bp (Table 3). The largest of these, located between tRNA-Asn and tRNA-Cys, was recognized as the putative replicate origin of the L-strand[s]. These non-coding spacers are interesting in the study of mtDNA evolutionary mechanisms. The base composition of *L. polyactis* was analyzed separately for rRNA, tRNA, and protein-coding genes (Table S2). In the latter, pronounced anti-G bias was observed at the third codon positions (8.5%), which are free from selective constraints on nucleotide substitution. Besides the A+T composition of the second codon position being relatively

higher than in most Percoidei fishes, pyrimidines were over-represented in this position (61.5%). Already observed in other vertebrate mitochondrial genomes, this has been attributed to the hydrophobic character of the proteins (Naylor *et al.*, 1995). *L. polyactis* tRNA genes are A+T rich (54.5%), as in other vertebrates, whereas rRNAs are A+C rich (59.3%), as in other bony fishes (Zardoya and Meyer, 1997; Cheng *et al.*, 2010).

Protein-coding genes

As expected, 13 large open-reading frames were detected in the mitochondrial genome of *L. polyactis*. The T:C:A:G base composition of the mitochondrial 13 protein-coding-gene sequence, 26.9: 32.4: 24.8: 15.9, is summarized in Table S2. Bias of nucleotide frequencies is strand specific (Broughton and Reneau, 2006). Moreover, in contrast to H-stranded genes, in L-stranded ND6 genes, the most prominent anti-C bias is at the third position (7.5%). The lengths of 13 protein-coding genes of *L. polyactis* mitochondrial DNA were compared with the corresponding sequences of other Percoidei species, whereat it was found that these are conserved. Moreover, there is almost no variation among species. By comparing predicted initiation and termination codons of the 13 protein-coding genes among 23 percoidei species (Table S3), it was apparent that most use ATG as the initiation codon (92.6%), with GTG in second place. In a few species, initiation codons are even absent (shown by “?”). The situation in termination codons is also similar. TAA, TAG and incomplete TA- or T-- are commonly used, but AGA and AGG rarely so. This condition is apparently common among vertebrate mitochondrial genome, and it also appears that TAA stop codons are created via posttranscriptional polyadenylation (Ojala *et al.*, 1981). Furthermore, each protein has its preferable initiation and termination codons, although there are exceptions. For example, in most species, COII, COIII, Cytb, ND4L and ND4 choose ATG/T--, ATG/TA-, ATG/T--, ATG/TAA and ATG/T--, respectively. But in COI there are two types of initiation/termination codon usage. COI proteins in Sciaenidae fish mitochondrial DNA bear ATG/AGA as initiation and termination codons, whereas in other Percoidei fishes, this is not the case (they possess GTG/TAA, GTG/AGG or GTG/T--). Studies in insects have shown positive correlations between the incidence of canonical initiation and termination codons and the relative rate of gene evolution (Szafranski, 2009). Whether this relationship also applies to fishes requires confirmation.

Codons in the 13 protein-coding genes identified in *L. polyactis* are shown in Table S4. As regards amino acids with the fourfold degenerate third position, codons ending in C are mostly seen, followed by codons ending in A and T for alanine, proline, glycine, valine and threonine. However, for arginine and serine, A is more frequent than C. Among codons with twofold degenerate positions, C appears to be

Table 3 - Characteristics of the mitochondrial genome of *L. polyactis*.

Gene	Position		Size (bp)		Codon		Intergenic nucleotide ^{II}	Strand
	From	To	Nucleotide	Amino acid	Initiation	Stop ^I		
tRNA-Phe	1	69	69					H
12S rRNA	70	1019	950					H
tRNA-Val	1020	1090	71				2	H
16S rRNA	1093	2789	1697				1	H
tRNA-Leu(UUR)	2791	2864	74					H
ND1	2865	3839	975	324	ATG	TAG	4	H
tRNA-Ile	3844	3913	70				-1	H
tRNA-Gln	3913	3984	72				-1	L
tRNA-Met	3984	4053	70					H
ND2	4054	5099	1046	348	ATG	TA-		H
tRNA-Trp	5100	5170	71				1	H
tRNA-Ala	5172	5240	69				2	L
tRNA-Asn	5243	5315	73				37	L
tRNA-Cys	5353	5419	67				-1	L
tRNA-Tyr	5419	5488	70				1	L
CO1	5490	7046	1557	518	ATG	AGA	-5	H
tRNA-Ser(UCN)	7042	7113	72				3	L
tRNA-Asp	7117	7185	69				8	H
CO2	7194	7884	691	230	ATG	T--		H
tRNA-Lys	7885	7959	75				1	H
ATPase8	7961	8128	168	55	ATG	TAA	-10	H
ATPase6	8119	8802	684	227	ATG	TAA	-1	H
CO3	8802	9586	785	261	ATG	TA-		H
tRNA-Gly	9587	9657	71					H
ND3	9658	10006	349	116	ATG	T--		H
tRNA-Arg	10007	10075	69					H
ND4L	10076	10372	297	98	ATG	TAA	-7	H
ND4	10366	11746	1381	460	ATG	T--		H
tRNA-His	11747	11815	69					H
tRNA-Ser(AGY)	11816	11882	67				5	H
tRNA-Leu(CUN)	11888	11960	73					H
ND5	11961	13799	1839	612	ATG	TAA	-4	H
ND6	13796	14314	519	172	ATG	TAA		L
tRNA-Glu	14315	14383	69				4	L
Cytb	14388	15528	1141	380	ATG	T--		H
tRNA-Thr	15529	15600	72				2	H
tRNA-Pro	15603	15672	70					L
Control region	15673	16470	798					H

I: TA- and T-- represent incomplete stop codons.

II: Numbers correspond to the nucleotides separating adjacent genes. Negative numbers indicate overlapping nucleotides.

more used than T in the pyrimidine codon family, whereas the purine codon family ends mostly with A. Except for arginine, G is the least common third position nucleotide in all the codon families. All these features are very similar to those observed in vertebrates (Hu *et al.*, 2010; Yang *et al.*, 2010).

Ribosomal RNA gene and transfer RNA gene

As with other mitochondrial genomes, the genome contains a small (12S) and large (16S) subunits of rRNA,

which are 950 bp and 1697 bp long, respectively (Table S2). As in the other vertebrates, they are located between tRNA-Phe and tRNA-Leu(UUR), and are separated by tRNA-Val (Figure S1, Table 3). When compared with other genes reported for Sciaenidae, and similar to other vertebrates, both rRNA genes are conserved either in A+T content or gene length and location. As with *Gonostoma gracile* (Miya and Nishida, 1999), preliminary assessment of the secondary structure of *L. polyactis* indicated that the present sequence could be reasonably superimposed on the

proposed secondary structures of carp 12S and cow 16S rRNA (Gutell *et al.*, 1993). Twenty-two tRNA genes, with lengths varying from 67 bp (tRNA-Cys and tRNA-Ser(AGY) to 75 bp(tRNA-Lys), were interspersed throughout the entire genome. As reported in some other vertebrates (Miya *et al.*, 2003; Kim and Lee, 2004; Oh *et al.*, 2007), with the known exception of the tRNA-Ser (AGY) gene, all tRNA gene transcripts can be folded into typical cloverleaf secondary structures (Figure S2). Besides harboring anticodons identical to those used in other vertebrate mitogenomes, they conserve the aminoacyl, DHU (dihydrouridine), anticodon and T Ψ C (thymidine-pseudouridine-cytidine) stems. As shown in the rock bream (Oh *et al.*, 2007) and *Pseudolabrus* fishes (Oh *et al.*, 2008), the tRNA-Ser (AGY) found in the *L. polyactis* mitochondrial genome bore no complete DHU arm. Similar to usual tRNAs (Ohtsuki *et al.*, 2002), aberrant tRNAs can also fit into the ribosome by adjusting their structural conformation and function.

Main non-coding regions of Percoidei species

The putative O_L was confirmed in *L. polyactis*. When compared with other Percoidei fishes, they were almost identical and are located in a cluster of five tRNA genes (the WANCY region) between the tRNA-Asn and tRNA-Cys gene. The putative O_L, besides serving as the initiation site of Light-strand replication, is capable of folding into a stable stem-loop secondary structure with 13 bp in the stem and 11 bp in the loop. Furthermore, there is a C-rich sequence in the loop, whereby RNA primer synthesis can be initiated. This C-rich sequence has also been found in the O_L loop of other fishes, such as *Gadus morhua* (Johansen *et al.*, 1990) and *Oncorhynchus mykiss* (Zardoya *et al.*, 1995). This feature supports the hypothesis that in vertebrates, primer synthesis is most probably initiated by a polypyrimidine tract (Taanman, 1999), and not by a stretch of thymines, as previously suggested (Wang and Clayton, 1985). The conserved sequence motif, 5'-GCCGG-3', was found at the base of the stem within tRNA-Cys. This motif seems to be involved in the transition from RNA to DNA synthesis (Hixson and Brown, 1986).

The mitochondrial control region is located between tRNA-Pro and tRNA-Phe in mitochondrial DNA. Besides being the most variable region, it also contains certain conserved motifs that are associated with the initiation of DNA replication and transcription (Zhao *et al.*, 2006). The control region of *L. polyactis* was identified and compared with those of other Percoidei fishes. They are also located between the two tRNAs (Pro and Phe), and range in size from 533 bp (*Parargyrops edita*, EF107158) to 1354 bp (*Pagrus major*, AP002949), all having an overall base composition rich in A and T (A+T= 60%). The variation in length is largely due to the number of conserved domains inserted in these species. Long tandem repeats were recognized in *Monodactylus argenteus* and *Pagellus bogaraveo*, with

lengths of 56 bp and 183 bp, respectively. Slippage and mispairing during mitogenome replication may explain tandem repeats in the control region (Brought and Dowling, 1997). Although this region is a unique and highly variable area in mitochondrial DNA, conserved domains and motifs are recognizable by multiple homologous sequence alignment and recognition site comparison. Control regions are also divided into a typical tripartite structure with an extent termination association sequence (ETAS), central conserved-block domains (CSB-F, CSB-D, and CSB-E), and conserved sequence block domains (CSB-1, CSB-2, and CSB-3) (Sbisa *et al.*, 1997). The conserved ETAS motif in most fishes is TACAT, with one palindromic sequence, ATGTA. In *Coreoperca kawamebari*, there is some variation in ETAS having the conserved TGCAT motif. The consensus sequences of ETAS in Percoidei fishes was identified as TACAT-TATGTAT---CACCAT----ATATTAACAT, where “-” indicating nucleotide variations such as transitions, transversions, or deletions, similar to reported in siniperine fishes (Zhao *et al.*, 2006). CSB domains that are associated with the initiation of mitochondrial DNA replication, and other important functions of control regions, were detected. Consensus sequences have been summarized in Table S5. While all these conserved blocks can be easily identified in most of the Percoidei species, the incomplete structure of control regions with the absence of conserved domains, was also detected (Table 4). Such obvious insertions and deletions implied the rapid evolution of the control region in Percoidei fishes, a possible source of information for dissecting the structure-function-evolution relationships of control regions.

Effect of partitioning on harmonic mean -lnL, topology, posterior probabilities and bayes factors

Harmonic mean -lnL was used when measuring the aptitude of data partitioning in defining the entire data set. Partitioning the COI data set by codon position greatly improved harmonic mean -lnL (Table 5). The same results were reported (Brandley *et al.*, 2005; Brown and Lemmon, 2007), when partitioning by codon position and RNA gene specific stems and loops. The inference is that, as the different data partitions may evolve quite variably, partitioning can be considered as a useful method for accommodating heterogeneity in the processes of molecular evolution. Consensus tree topologies inferred from the three analyses differed, yet all of these difference involved alternative placements of weakly supported nodes (Bayesian posterior probabilities < 95%). These distinct differences were dependent on whether the COI sequences were partitioned. The most dramatic differences could be noted, not only in the deep nodes in the tree, but also in the relationships among the three main groups (Groups1, 2 and 3 in Figures 1-3). No obvious differences were observed in posterior probabilities among analyses depending on different partition-strategies All Bayes-factor estimates were much

Table 4 - Characteristics and recognized conserved domains of control regions in Percoidae species.

Species	Accession number	Length	A+T content	Recognized conserved domains
<i>Carangoides armatus</i>	AP004444	865	64	ETAS, CSB-F, CSB-E, CSB-D, CSB-1, CSB-2, CSB-3
<i>Caranx melampygus</i>	AP004445	893	65	CSB-E, CSB-D, CSB-1, CSB-2, CSB-3
<i>Centropyge loricula</i>	AP006006	651	60	ETAS, CSB-E, CSB-D
<i>Chaetodon auripes</i>	AP006004	860	65	ETAS, CSB-F, CSB-E, CSB-D, CSB-2, CSB-3
<i>Chaetodontoplus septentrionalis</i>	AP006007	1007	64	CSB-E, CSB-D, CSB-2, CSB-3
<i>Collichthys lucida</i>	NC_014350	771	63	ETAS, CSB-1, CSB-2, CSB-3
<i>Collichthys niveatus</i>	HM219223	799	63	ETAS, CSB-1, CSB-2, CSB-3
<i>Coreoperca kawamebari</i>	AP005990	849	62	ETAS, CSB-F, CSB-E, CSB-D, CSB-1, CSB-2, CSB-3
<i>Diagramma pictum</i>	AP009167	840	61	ETAS, CSB-E, CSB-D, CSB-1, CSB-2, CSB-3
<i>Doederleinia berycooides</i>	AP009181	835	63	ETAS, CSB-F, CSB-E, CSB-D, CSB-2, CSB-3
<i>Emmelichthys struhsakeri</i>	AP004446	832	62	ETAS, CSB-F, CSB-E, CSB-D, CSB-1, CSB-2, CSB-3
<i>Etheostoma radiosum</i>	AY341348	938	62	ETAS, CSB-F, CSB-E, CSB-D, CSB-1, CSB-2, CSB-3
<i>Heniochus diphreutes</i>	AP006005	1000	65	CSB-E, CSB-D, CSB-1, CSB-2, CSB-3
<i>Larimichthys crocea</i>	EU339149	795	61	ETAS, CSB-1, CSB-2, CSB-3
<i>Larimichthys polyactis</i>	GU586227	798	62	ETAS, CSB-1, CSB-2, CSB-3
<i>Lates calcarifer</i>	DQ010541	768	64	CSB-E, CSB-D, CSB-1, CSB-2
<i>Lethrinus obsoletus</i>	AP009165	967	60	ETAS, CSB-F, CSB-E, CSB-D, CSB-1, CSB-2, CSB-3
<i>Lutjanus rivulatus</i>	AP006000	842	62	ETAS, CSB-F, CSB-E, CSB-D, CSB-1, CSB-2, CSB-3
<i>Miichthys miui</i>	HM447240	845	63	ETAS, CSB-F, CSB-E, CSB-D, CSB-1, CSB-2, CSB-3
<i>Monodactylus argenteus</i>	AP009169	873	65	ETAS, ETAS, CSB-F, CSB-E, CSB-D, CSB-1, CSB-2, CSB-3
<i>Pagellus bogaraveo</i>	AB305023	1195	63	ETAS, CSB-F, CSB-F, CSB-E, CSB-D, CSB-1, CSB-2, CSB-3
<i>Pagrus auriga</i>	AB124801	948	61	CSB-F, CSB-E, CSB-D, CSB-1, CSB-2, CSB-3
<i>Pagrus major</i>	AP002949	1354	61	CSB-F, CSB-E, CSB-D, CSB-1, CSB-2, CSB-3
<i>Parapristipoma trilineatum</i>	AP009168	845	63	ETAS, CSB-E, CSB-D, CSB-1, CSB-2, CSB-3
<i>Parargyrops edita</i>	EF107158	533	62	CSB-D
<i>Percina macrolepida</i>	DQ536430	941	64	ETAS, CSB-F, CSB-E, CSB-D, CSB-1, CSB-2, CSB-3
<i>Plectropomus leopardus</i>	DQ101270	1065	66	CSB-F, CSB-E, CSB-D, CSB-2
<i>Pterocaesio tile</i>	AP004447	822	61	ETAS, CSB-F, CSB-E, CSB-D, CSB-1, CSB-2, CSB-3
<i>Spicara maena</i>	AP009164	961	64	ETAS, CSB-F, CSB-E, CSB-D, CSB-1, CSB-2, CSB-3
<i>Trachurus japonicus</i>	AP003091	862	63	CSB-F, CSB-E, CSB-D, CSB-1, CSB-2, CSB-3
<i>Trachurus trachurus</i>	AB108498	862	62	CSB-F, CSB-E, CSB-D, CSB-1, CSB-2, CSB-3

higher than the criterion for strong evidence against a hypothesis. According to the Bayes factors, analysis employing the P_3 partition-strategy provided a decisively better explanation of the data than the remainder (Table 5 and Table 6). Thus, as this is the preferred hypothesis of the

Table 5 - Harmonic mean $-\ln L$ of each partition strategy (top matrix), and $2\ln$ Bayes factor results from comparisons between all the partitioning strategies (the left bottom matrix). The Bayes factor was calculated from estimated harmonic means of likelihood by the *sump* command in MrBayes3.1

	Partition strategy		
	P_1	P_2	P_3
Harmonic mean $-\ln L$	5427.68	5124.09	5058.92
P_1	-		
P_2	607.18	-	
P_3	737.52	130.34	-

phylogeny of Sciaenidae fishes based on the present data, subsequent discussion will be limited to this tree (Figure 3).

The phylogeny of the Sciaenidae family

Based on the characters of the gas bladder, sagitta, and mental pores, Zhu *et al.* (1963) divided the family into seven subfamilies, viz., Johniinae, Megalonibinae, Bahabiinae, Sciaeninae, Otolithinae, Argyrosominae and Pseudos-

Table 6 - Interpretation of the $2\ln$ Bayes factor. Modified from Kass and Raftery (1995).

$2\ln$ Bayes factor	Evidence for H_1
< 0	Negative (supports H_0)
0 to 2	Barely worth mentioning
2 to 6	Positive
6 to 10	Strong
> 10	Very strong

ciaeniae. In this study, phylogenetic analysis revealed three distinct monophyletic groups (Groups 1, 2 and 3), thus very different from the results of Zhu *et al.* (1963). Monophyly of the genera *Otolithes* and *Argyrosomus* is not supported. The proposition (Zhu *et al.*, 1963) of grouping *Argyrosomus* and *Nibeia* into the subfamily Argyrosominae is also without support, for, based on the phylogenetic tree presented herein, *Argyrosomus* and *Nibeia* have been placed into two distinct groups (Figure 3). Even though monophyly of Pseudosciaenidae is supported, herein, Bayesian posterior probability is relatively weak. Notably, within the subfamily Pseudosciaeninae, *L. polyactis* was found to be most closely related to *Collichthys niveatus*, and then to its congeneric species, *L. crocea*. Although previous molecular phylogenetic analyses had the same opinion on the phylogenetic positions of *Otolithes*, *Argyrosomus*, and Argyrosominae, the phylogenetic relationships within the subfamily Pseudosciaeninae are still far from clear (Meng *et al.*, 2004; Xu *et al.*, 2010). Based on different data and methods, once again our results suggested that *Collichthys*

and *Larimichthys* may be merged into a single genus. These results are consistent with Chen (Chen QM, 2007, Disserta-

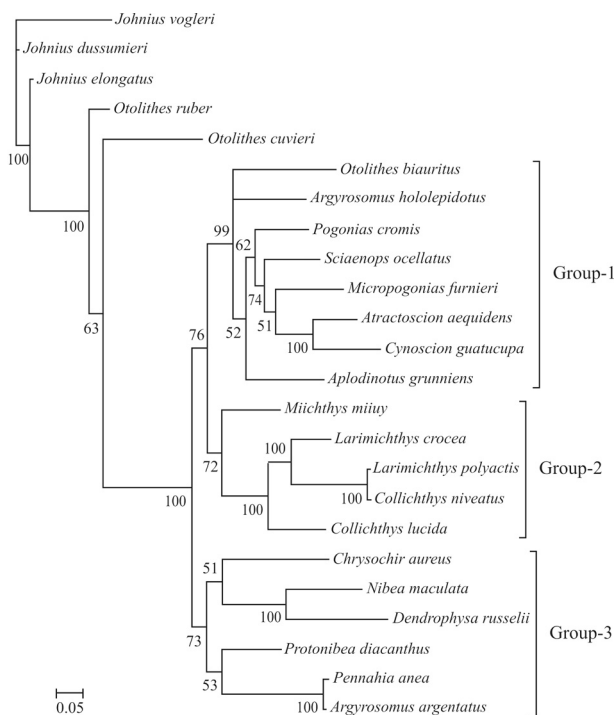


Figure 1 - Consensus trees of Sciaenidae constructed using Bayesian analysis based on P₁ partition-strategies. The accession numbers of species used in this study are flowing: *Aplodinotus grunniens*, EU523922; *Atractoscion aequidens*, DQ107824; *Argyrosomus argentatus*, HQ890946; *Argyrosomus hololepidotus*, DQ107810; *Chrysochir aureus*, EF607347; *Collichthys lucidus*, HM447239; *Collichthys niveatus*, NC_014263; *Cynoscion guatucupa*, EU074398; *Dendrophysa russelii*, EF607358; *Johnius dussumieri*, EF534120; *Johnius elongates*, EF534124; *Johnius vogleri*, EF534112; *Larimichthys crocea*, EU871688; *Larimichthys polyactis*, EU266386; *Micropogonias furnieri*, EU074482; *Nibeia maculate*, EU014249; *Miichthys miuy*, EU266377; *Otolithes cuvieri*, EF534116; *Otolithes ruber*, EF607450; *Otolithoides biauritus*, EF536890; *Pogonias cromis*, EU074550; *Protonibeia diacanthus*, EF528232; *Pennahia anea*, EF607487; *Sciaenops ocellatus*, EU180148.

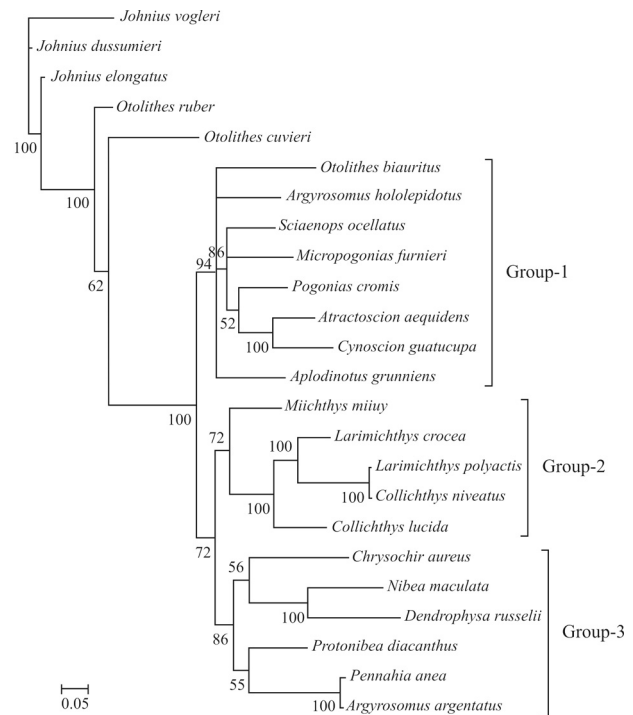


Figure 2 - Consensus trees of Sciaenidae constructed using Bayesian analysis based on P₂ partition-strategies. The accession numbers of species are those listed in Figure 1.

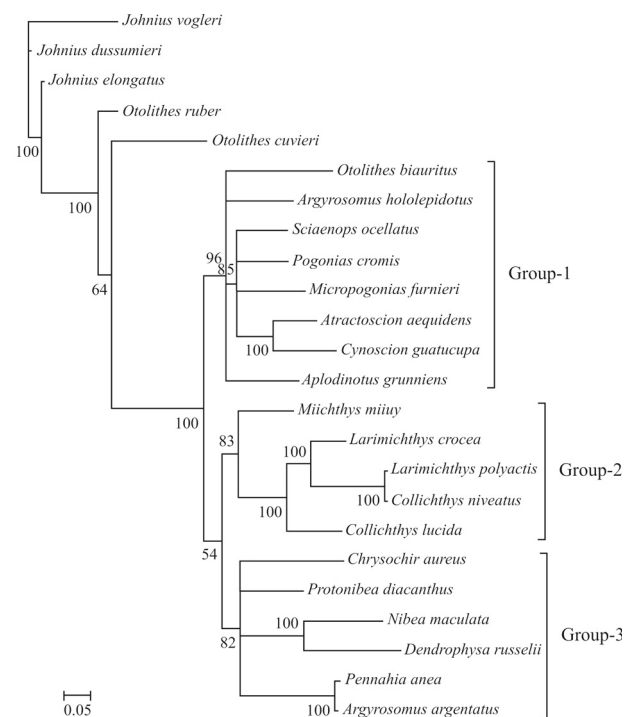


Figure 3 - Consensus trees of Sciaenidae constructed using Bayesian analysis based on P₃ partition-strategies. The accession numbers of species are those listed in Figure 1.

tion, Jinan University, China) and Tong *et al.* (2007), where, respectively, non-monophyletic *Larimichthys* and *Collichthys* were recovered. Nevertheless, Cheng *et al.* (2011) recently recovered monophyletic *Collichthys* and *Larimichthys*, thus, in common with the morphological results of Zhu *et al.* (1963). Sampling errors, scarce data and mismodeling may have contributed to these disputes. Thus, the inclusion of further data from the mitochondrial and nuclear genomes, more accurate evolutionary models, and extensive taxonomic sampling, with careful identification integrated with information on morphological characters, is required for reconstructing the phylogeny of Sciaenidae.

Acknowledgments

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Supplementary Material

The following online material is available for this article:

Figure S1 - Gene map of the *L. polyactis* mitochondrial genome

Figure S2 - Sequences of *L. polyactis* mitochondrial tRNA genes, represented in the clover-leaf form.

Table S1 - PCR primers in the analysis of the *L. polyactis* mitochondrial genome

Table S2 - Base composition of the *L. polyactis* mitochondrial genome

Table S3 - Comparison of predicted initiation and termination condons of the 13 mitochondrial protein-coding genes among 23 species of Percoidei.

Table S4 - Codon usage in *L. polyactis* mitochondrial protein-coding genes

Table S5 - Consensus sequences of conserved domains in control regions of Percoidei species.

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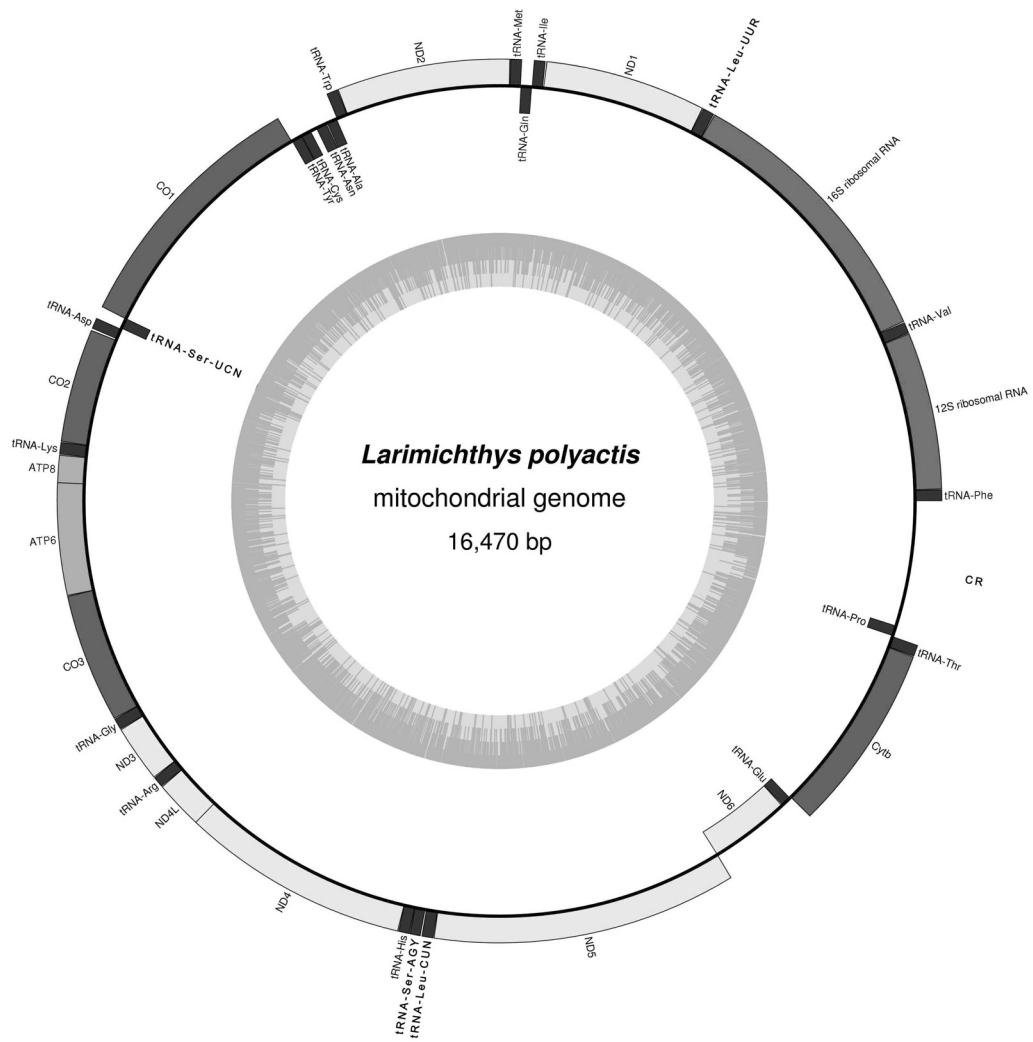


Figure S1 - Gene map of the *L. polyactis* mitochondrial genome.

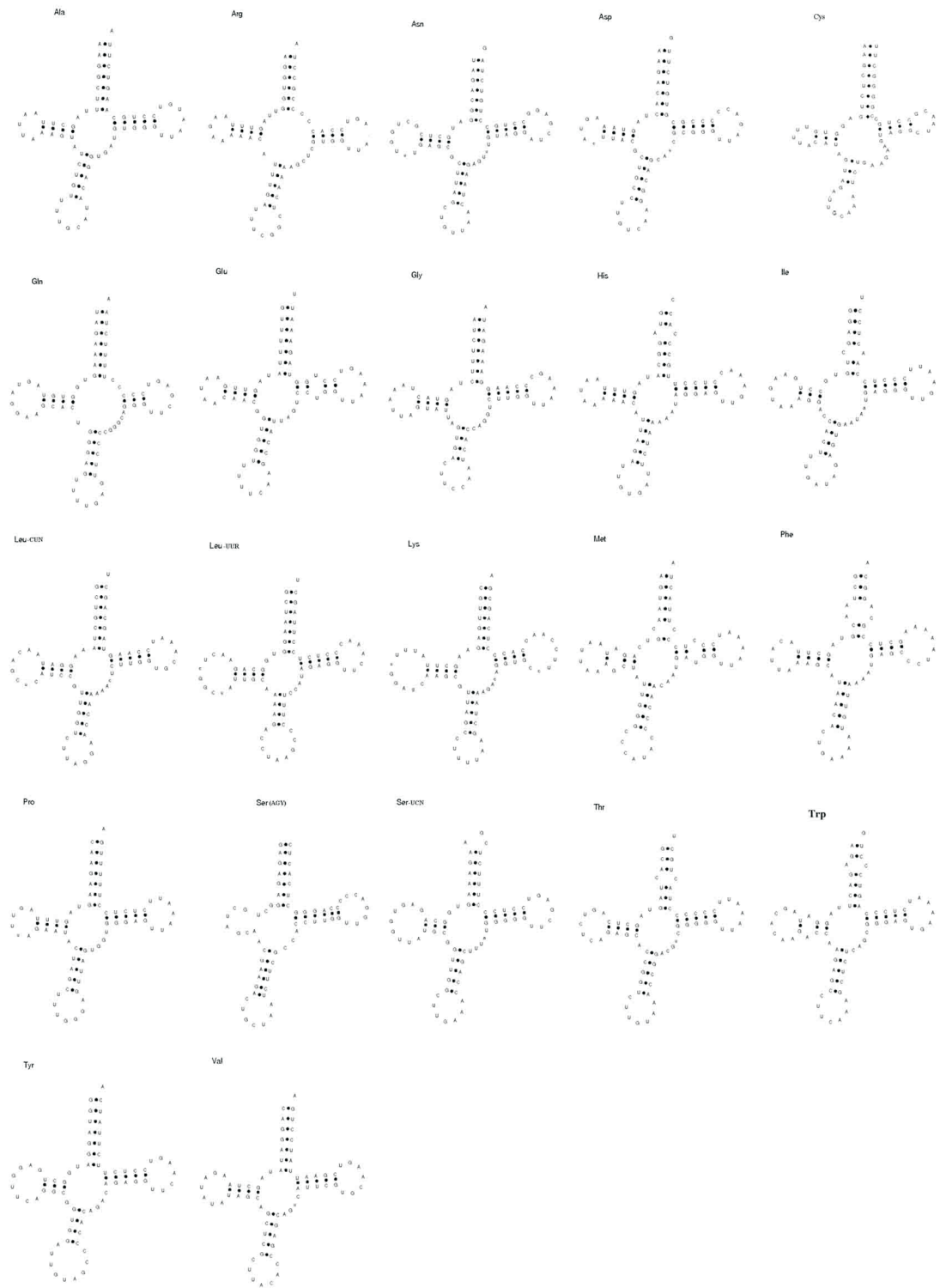


Figure S2 - Sequences of *L. polyactis* mitochondrial tRNA genes, represented in the clover-leaf form.

Table S1 - PCR primers in the analysis of the *L. polyactis* mitochondrial genome

Primer	Sequence (5'-3')
1F	TAGAGGCATTTAGGCACAAGGT
1R	TTCTGTTTATTAGAGCGGAGGC
2F	AACCAACCATAGCCCACGACA
2R	ATTCATTTCCCAGGCAACCAG
3F	ATAAAGACCCGTATGAATGGC
3R	ATGAGCGGTAAGATAGCAAGG
4F	CCCTCCAACCTCCTTAGAAAAG
4R	GGTGACCGAAGAATCAGAATA
5F	GACATTGGCACCCCTCTATCTAA
5R	AGGCAAGGTCTTCGTAATCAGT
6F	ACTTCTTCACTTTCACGACCA
6R	AGAGTCAAACGACATCTACGA
7F	TCATCATCGGCTCTACATTCCTG
7R	GGCGAGACTGGCAATAAATCATC
8F	TATGGCGTTCACCTCTGACTTC
8R	TTGATTGAGCCCGATGGTTACT
9F	CAACTGGCAAATCAGCACAA
9R	TTACAAGGAGAAAGAGCACGAC
10F	GACTTGAAAAACCACCGTTG
10R	CTCCGATCTCCGGATTACAAGAC
11F	TGATACTTCCTATTTCGCCTAC
11R	TCAGATGACAAAGCCACAG

Table S2 - Base compositions of the *L. polyactis* mitochondrial genome

	A	C	G	T	A+T
Protein					
1st	25.9	29.0	25.3	20.0	45.9
2nd	18.2	27.7	13.8	40.0	58.2
3rd	30.2	40.5	8.5	21.0	51.2
Total	24.8	32.4	15.9	26.9	51.7
tRNAs	27.4	21.5	24.0	27.1	54.5
rRNAs	32.6	26.7	20.4	20.3	52.9
Control region	32.2	23.2	15.0	29.6	61.8
Complete genome	27.6	31.2	16.2	25.0	52.6

Table S3 - Comparison of predicted initiation and termination condons of the 13 mitochondrial protein-coding genes among 23 species of Percoidaei.

Gene	Predicted initiation codon and termination codon												
	ATPase6	ATPase8	COI	COII	COIII	Cytb	ND1	ND2	ND3	ND4	ND4L	ND5	ND6
1	ATG/TA-	ATG/TAA	ATG/AGA	ATG/T--	ATG/TA-	ATG/TAA	ATG/TAG	ATG/TA-	ATG/T--	ATG/T--	ATG/TAA	ATG/TAA	ATG/TAA
2	ATG/TAA	ATG/TAA	ATG/AGA	ATG/T--	ATG/TA-	ATG/T--	ATG/TAG	ATG/TA-	ATG/T--	ATG/T--	ATG/TAA	ATG/TAA	ATG/TAA
3	ATG/TAA	ATG/TAA	ATG/AGA	ATG/T--	ATG/TA-	ATG/T--	ATG/TAG	ATG/TA-	ATG/T--	ATG/T--	ATG/TAA	ATG/TAA	ATG/TAG
4	ATG/TAA	ATG/TAA	ATG/AGA	ATG/T--	ATG/TA-	ATG/T--	ATG/TAG	ATG/TA-	ATG/T--	ATG/T--	ATG/TAA	ATG/TAA	ATG/TAA
5	ATG/TAA	ATG/TAA	ATG/AGA	ATG/T--	ATG/TA-	ATG/T--	ATG/TAG	ATG/TA-	ATG/T--	ATG/T--	ATG/TAA	ATG/TAA	ATG/TAA
6	ATG/TA-	ATG/TAA	GTG/AGG	ATG/T--	ATG/TA-	ATG/T--	ATG/TAA	ATG/TA-	ATG/T--	ATG/T--	ATG/TAA	ATG/TAG	ATG/TAA
7	ATG/TAA	ATG/TAG	GTG/TAA	ATG/T--	ATG/T--	ATG/T--	ATG/TAA	ATG/T--	ATG/T--	ATG/T--	ATG/TAA	ATG/TAA	ATG/TAA
8	ATG/TAA	ATG/TAG	GTG/AGG	ATG/T--	ATG/TA-	ATG/T--	ATG/TAG	ATG/TA-	ATG/T--	ATG/T--	ATG/TAA	ATG/TAA	ATG/TAG
9	ATG/TAA	ATG/TAA	GTG/TAA	ATG/T--	ATG/TA-	ATG/T--	ATG/TAA	ATG/TAA	ATG/TAG	ATG/T--	ATG/TAA	ATG/TAA	ATG/TAA
10	ATG/TA-	ATG/TAA	GTG/TAA	ATG/T--	ATG/TA-	ATG/T--	ATG/TAA	ATG/TAA	ATG/TAG	ATG/T--	ATG/TAA	ATG/TAG	ATG/TAA
11	ATG/TAA	ATG/TAA	GTG/TAA	ATG/T--	ATG/TA-	ATG/T--	ATG/TAG	ATG/TA-	ATG/TAG	ATG/T--	ATG/TAA	ATG/TAA	ATG/TAA
12	?/TA-	ATG/TAA	GTG/TAA	ATG/T--	ATG/TA-	ATG/T--	ATG/TAA	ATG/TA-	ATG/T--	GTG/T--	ATG/TAA	ATG/TAA	ATG/TAG
13	ATG/TAA	ATG/TAA	GTG/TAA	ATG/TA-	ATG/TA-	ATG/T--	ATG/TAA	ATG/TAA	ATG/T--	ATG/T--	GTG/TAA	ATG/TAA	ATG/TAG
14	ATG/TA-	ATG/TAA	GTG/TAA	ATG/T--	ATG/TA-	ATG/T--	ATG/TAA	ATG/TA-	ATG/T--	ATG/T--	ATG/TAA	ATG/TAG	ATG/TAA
15	ATG/TA-	ATG/TAA	GTG/TAA	ATG/T--	ATG/TA-	ATG/T--	ATG/TAA	ATG/TA-	ATG/T--	ATG/T--	ATG/TAA	ATG/TAG	ATG/TAA
16	ATG/TA-	ATG/TAA	GTG/TAA	ATG/TAG	ATG/TA-	ATG/T--	ATG/TAG	ATG/TA-	ATG/T--	ATG/T--	ATG/TAA	ATG/TAG	ATG/TAA
17	ATG/TA-	ATG/TAA	GTG/TAA	ATG/T--	ATG/TA-	ATG/T--	ATG/TAA	ATG/TA-	ATG/T--	ATG/T--	ATG/TAA	ATG/TAA	ATG/TAG
18	ATG/TAA	ATG/TAA	GTG/TAA	ATG/T--	ATG/TAG	ATG/T--	ATG/TAG	ATG/TA-	ATG/TAG	ATG/T--	ATG/TAA	ATG/TAA	ATG/TAG
19	ATG/TA-	ATG/TAA	GTG/TAA	ATG/T--	ATG/TA-	ATG/T--	ATG/TAA	ATG/TA-	ATG/T--	ATG/T--	ATG/TAA	ATG/TAA	ATG/TAG
20	?/TA-	ATG/TAA	GTG/T--	ATG/T--	ATG/TA-	ATG/T--	ATG/TAA	ATG/TA-	ATG/T--	ATG/T--	ATG/TAA	ATG/TAA	ATG/TAG
21	ATG/TAA	ATG/T--	GTG/TAA	ATG/T--	ATG/TAA	ATG/T--	ATG/TAA	ATG/TA-	ATG/T--	ATG/T--	ATG/TAA	ATG/TAA	ATG/AGA
22	ATG/TA-	ATG/TAA	GTG/TAA	ATG/T--	ATG/TA-	ATG/T--	ATG/TAA	ATG/TA-	ATG/T--	ATG/T--	ATG/TAA	ATG/TAA	ATG/TAG
23	ATG/TAA	ATG/TAA	GTG/AGG	ATG/T--	ATG/TAA	ATG/T--	ATG/TAA	ATG/TAA	ATG/TAG	ATG/T--	ATG/TAA	ATG/TAA	ATG/TAA

Notes: 1: *Larimichthys crocea* 2: *Larimichthys polyactis* 3: *Mitichthys miyu* 4: *Collichthys lucida* 5: *Collichthys niveatus* 6: *Doederleinia berycoidea* 7: *Trachurus trachurus* 8: *Spicara maena* 9: *Micropterus salmoides* 10: *Hemiochus diphreutus* 11: *Emmelichthys struhsakeri* 12: *Parapristipoma trilineatum* 13: *Lates calcarifer* 14: *Lethrinus obsoletus* 15: *Luijanus rivulatus* 16: *Pterocaesio tile* 17: *Monodactylus argenteus* 18: *Etheostoma radiosum* 19: *Chaetodontoplus septentrionalis* 20: *Labracinus cyclophthalmus* 21: *Plectropomus leopardeus* 22: *Coreoperca kawamebari* 23: *Pagrus auriga*. The interrogation shows for non-determined initiation condons.

Table S4 - Codon usage in *L. polyactis* mitochondrial protein-coding genes

Amino acid	Codon	Number	Amino acid	Codon	Number
Phe	TTT	105	Tyr	TAT	46
	TTC	132		TAC	62
Leu	TTA	58	Cys	TGT	9
	TTG	18		TGC	22
	CTT	127	His	CAT	28
	CTC	224		CAC	79
	CTA	183		CAA	89
Ile	CTG	65	Asn	CAG	9
	ATT	124		AAT	32
	ATC	145	AAC	87	
Met	ATA	96	Lys	AAA	61
	ATG	61		AAG	10
Val	GTT	58	Asp	GAT	22
	GTC	58		GAC	55
	GTA	52	Glu	GAA	74
	GTG	30		GAG	28
Ser	TCT	32	Trp	TGA	101
	TCC	65		TGG	20
	TCA	66	Ser	AGT	10
	TCG	9		AGC	42
Pro	CCT	48	Gly	GGT	29
	CCC	127		GGC	109
	CCA	43		GGA	70
	CCG	5		GGG	35
Thr	ACT	53	Arg	CGT	8
	ACC	141		CGC	18
	ACA	112		CGA	42
	ACG	13		CGG	10
Ala	GCT	58	Stop ^I	TAA	5
	GCC	180		TAG	1
	GCA	94		AGA	1
	GCG	12			

Table S5 - Consensus sequences of conserved domains in control region of Percoidei species.

Conserved domains	Consensus sequences
CSB-F	ATGYAGTAAGARCCCKACCAWCM
CSB-E	AGGGRCAA----WB-GTGGGG
CSB-D	TATTVCTGRCAWYTGGYTCCT
CSB-1	ATT-TCA-G--CATA--
CSB-2	A-CCCCCCTACCCCC
CSB-3	AAACCCCC----AAACAG