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Complete Mitochondrial Genome of Three Fish Species (Perciformes: Amblyopinae): Genome Description and Phylogenetic Relationships

Zisha Liu[1], Na Song[1], Takashi Yanagimoto[2], Zhiqiang Han[3], Bonian Shui[3] and Tianxiang Gao[3]*

[1]*Institute of Evolution and Marine Biodiversity, Ocean University of China, Qingdao 266003, China*

[2]*National Research Institute of Fisheries Science, Fisheries Research Agency, Yokohama 2368648, Japan*

[3]*Fishery College, Zhejiang Ocean University, Zhoushan 316022, China*

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ZL and NS performed the experiment, analyzed data and wrote the manuscript. TG designed the experiments. TY, ZH, TG and BS collected samples and proofread the manuscript.

Key words
Mitochondrial genome, Phylogenetic relationship, *O. lacepedii, O. rebecca, O.* sp.

ABSTRACT

The complete mitogenomes of three species (*O. lacepedii*, *O. rebecca* and *O.* sp.) were sequenced, their genomic structure examined, and their genome organization, arrangement and codon usage analyzed. Phylogenetic Bayesian and ML analyses were conducted, using a concatenated set of 12 protein-coding genes, and adding 16 other species of gobies (Gobiidae). The mitogenome sequences of *O. lacepedii*, *O. rebecca* and *O.* sp. were all circular double-strand molecules, 17245 bp, 17009 bp and 17004 bp long, respectively. Compared with other bony fishes, the three species shared the similar features in gene arrangements, base composition and tRNA structure. The control region spanned 1571 bp, 1336 bp and 1332 bp in *O. lacepedii*, *O. rebecca* and *O.* sp., respectively, and was A+T-rich. Three species were only detected termination-associated sequence domain (TAS) and conserved sequence blocks domain (CSB-1, CSB-2 and CSB-3). The Bayesian and ML tree topologies of 19 Gobiidae represented two groups: one large group consisted of Amblyopinae, Gobionellinae, Oxudercinae and Sicydiinae, and the other was the monophyletic Gobiinae. Phylogenetic analysis also demonstrated clade Amblyopinae included all three species and that *O. lacepedii* has been proved to be a much closer affinity to *O.* sp. than *O. rebecca*. Our study theoretically provided a supplementary proof and significant information for future taxon studies.

INTRODUCTION

The vertebrate mitochondrial DNA is a double-stranded circular molecule of 16-18kb in length consisting of genes for 22 transfer RNA genes (tRNA), 2 ribosomal RNA genes (rRNA) and 13 protein-coding genes as well as two non-coding regions: origin of light strand replication (O_L) and control region (Bibb *et al*., 1981). The usefulness of mitogonomes was becoming increasingly important in solving the long-term controversial phylogenetic relationship and confusing taxon (Cheng *et al*., 2012). In recent years, longer mitochondrial DNA sequences were used to reconstruct higher level phylogenetic relationships (Boore *et al*., 2005) and those studies were necessary to resolve these controversial problems.

The suborder Gobioidei consisted of six divided families (Thacker, 2009), and Gobiidae was the largest family including five subfamilies (Gobiidae, Gobionellinae, Sicydiinae, Oxudercinae and Amblyopinae) (Hoese, 1984). Moreover, studies on Gobiidae taxonomy have been controversial and attract extensive attention for a long time. The subfamily Amblyopinae are usually inshore, mud-dwelling fishes which are generally given the vernacular name of "eel gobies" or "worm gobies". The genus *Odontamblyopus* within Amblyopinae was firstly proposed by Bleeker (1874). Since then, Norman (1966) was the only recent author who clarified *Odontamblyopus* into more than two species. Murdy and Shibukawa (2001) demonstrated that *Odontamblyopus* comprised 4 species. In 2003, *Odontamblyopus rebecca* was found in Vietnam by Murdy and Shibukawa. Up to now, the genus *Odontamblyopus* consists of 5 species including *Odontamblyopus rubicundus*, *Odontamblyopus roseus*, *Odontamblyopus lacepedii*, *Odontamblyopus tenuis* and *Odontamblyopus rebecca*. Due to the similar morphological characters, some taxon problems still exist.

In this paper, we sequenced the complete mitogenome sequences and examined the genomic structure of three species (*O. lacepedii*, *O. rebecca* and *O.* sp.), and we analyzed the main features in terms of the

* Corresponding author: gaotianxiang0611@163.com
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genome organization, gene arrangement and codon usage. Furthermore, combined with 12 protein-coding genes of other Gobiidae fishes, the phylogenetic relationships were conducted using Bayesian and ML analyses. This study aims to solve confusing taxon problems using longer sequences and comparison between gobies.

MATERIALS AND METHODS

Sample collection

The three species (*O. lacepedii*, *O. rebecca* and *O.* sp.) were collected from Ariake Bay in Japan and from Zhujiang and Zhoushan in China, respectively. All samples were morphologically discriminated to the species level based on Wu and Zhong (2008), Murdy and Shibukawa (2001) and (2003). Muscles excised were used for DNA extraction and preserved in 95% ethanol until use.

DNA extraction, PCR amplification and sequencing

Whole genomic DNA was extracted from muscle tissue by proteinase-K digestion followed by the standard phenol/chloroform method and used as a template for subsequent PCR reactions to determine the complete mitogenome sequences of *O. lacepedii*, *O. rebecca* and *O.* sp. Six sets of primers were designed for long-PCR amplification based on the aligned mitogenome sequences of *Trypauchen vagi*na (NC_016693) as previously determined. New pairs of primers were designed for the subsequent amplification by primer walking method. Thirty-two normal PCR primer sets were used to accomplish the entire mitogenome. Essentially every contiguous segment overlapped by at least 50 bp to explicit the accuracy of the sequences.

All PCRs were performed in an Eppendorf thermal cycler. TaKaRa Ex-Taq and LA-Taq Kits (Takara Biomedical) were used for normal and long-PCR reactions, respectively. Long-PCR reactions were carried out in 25 µl reaction mixture containing 15.25 µl of sterile distilled H_2O, 2.5 µl of 10×Buffer, 4 µl of dNTP, 1µl of each primer (5µM), 0.25 µl of LA Taq polymerase (1 unit/µl, Takara), and 1 µl of DNA template. The long-PCR reactions consisted of an initial denaturing step at 94°C for 2 min, followed by 30 cycles of denaturing at 94°C for 30 s, annealing at 58°C for 3 min and a final extension at 72°C for 10 min. The normal PCR was performed following the standard procedure (Liu *et al*., 2007). Negative controls were included in all PCR amplifications to conform the absence of contaminants. PCR product was purified with a Gel Extraction Mini Kit (Watson BioTechnologies Inc., China). The purified product was then sequenced on ABI Prism 3730 (Applied Biosystems) from both strands with the same primers as those used for PCRs.

Table I.- Species downloaded from GenBank for phylogenetic analysis

Family	Subfamily	Species	GenBank accession number
Gobiidae	Gobiinae	*Glossogobius olivaceus* Temminck and Schlegel, 1845	JQ001860
		Acentrogobius pflaumii Bleeker, 1853	JX029961
		Amoya chusanensis Herre, 1940	NC_020347
	Oxudercinae	*Boleophthalmus pectinirostris* Linnaeus, 1758	JN631352
		Oxuderces dentatus Eydoux and Souleyet, 1850	JN831381
		Scartelaos histophorus Valenciennes, 1837	JQ654459
		Periophthalmus minutus Eggert, 1935	LK391944
	Amblyopinae	*Trypauchen vagina* Bloch and Schneider, 1801	NC_016693
	Gobionellinae	*Tridentiger bifasciatus* Steindachner, 1881	JN244650
		Lophiogobius ocellicauda Günther, 1873	KC480264
		*Mugilogobius abe*i Jordan and Snyder, 1901	KF128984
		*Rhinogobius giurin*us Rutter, 1897	KF371534
		Acanthogobius hasta Temminck and Schlegel, 1845	KJ958906
		Chaenogobius gulosus Sauvage, 1882	KP696748
	Sicydiinae	*Stiphodon alcedo* Maeda, Mukai and Tachihara, 2012	AB613000
		Sicyopterus japonicus Tanaka, 1909	JX628620
Odontobutidae		*Micropercops swinhonis* Dabry de Thiersant, 1872	NC_021763
		Perccottus glenii Dybowski, 1877	KM657956

Sequence assembling and sequence analysis

Sequences of overlapping fragments were assembled manually and aligned initially against the complete mitochondrial genome sequences of *T. vagina* using SEQMAN software and DNASTAR software with default parameters and further adjusted manually. The boundaries for rRNAs and protein-coding genes were determined by DOGMA comparing with published mitochondrial sequences. The codon usage of the 13 protein-coding genes and the base composition of 37 genes for three species were analyzed using the program MEGA 4.0. Translation initiation and translation termination codons were identified using genetic codon table for mitochondrion in MEGA 4.0. The putative O_L and control region were determined by sequence homology and proposed secondary structures. The secondary structure of the putative O_L was analyzed with the software RNAstructure 5.4. The complete mitochondrial sequences of three species (*O. lacepedii*, *O. rebecca* and *O.* sp.) have been deposited in GenBank with accession number KR815520, KT633953 and KT633954.

Phylogenetic analysis

Phylogenetic relationship using homologous sequences downloaded from GenBank (Table I) were performed. Only 12 protein-coding regions were used for the subsequent phylogenetic analyses with the exception of ND6 (Fig. 1) because of its heterogeneous base composition and consistently poor performance (Miya *et al*., 2003). Multiple alignments of sequences were performed using Clustal X version 1.8. Alignment of all protein coding genes was triplet and no internal stop codons were found in any fragments. To explore the degree of saturation present in the datasets, we plotted sequence divergence (GTR-based distance) vs. number of transition and transversion substitutions for all pairwise comparisons among taxa for each codon position in DAMBE. If the codon position sites were saturated, we would expect to see a plateau in such a plot, where little or no additional substitution is detectable with increased p distance.

Phylogenetic analyses of the concatenated genes were conducted under Bayesian Inferences (BI) and maximum likelihood (ML) with the programs MrBayes and PAUP 4.0, respectively. Appropriate evolution substitution models were screened by the ‘‘decision-theoretic performance-based’’ approach (DT; Minin *et al*., 2003) selection strategy in jModeltest 2.1.1 with partition strategies of all nucleotide sites and each codon. The best-fit model of all nucleotide sites was used for reconstructed phylogenetic trees in BI and ML analyses. BI was also conducted for the combined dataset using the optimal models with each codon position. *Micropercops swinhonis* and *Perccottus glenii* were chosen as outgroups. A total of 100 ML bootstrap replicates (MLBS) were performed using PAUP. Bayesian inferences using Markov chain Monte Carlo (MCMC) sampling were performed with four chains which ran simultaneously for 2,000,000 generations with tree sampled every 100 generations. The average standard deviation of split support (ASDOSS) and effective sample size (ESS) were used to assess convergence of MCMC. When value of ASDOSS was less than 0.01, the runs were stopped; ESSs detected by Tracer v.1.5 were more than 200, achieving convergence. The burn-in trees sampled prior to convergence (25% of trees from each run; 5000 sampled trees) were discarded, and the subsequent trees sampled independently from the posterior probability distribution were combined to produce phylogram and 50% majority-rule consensus trees.

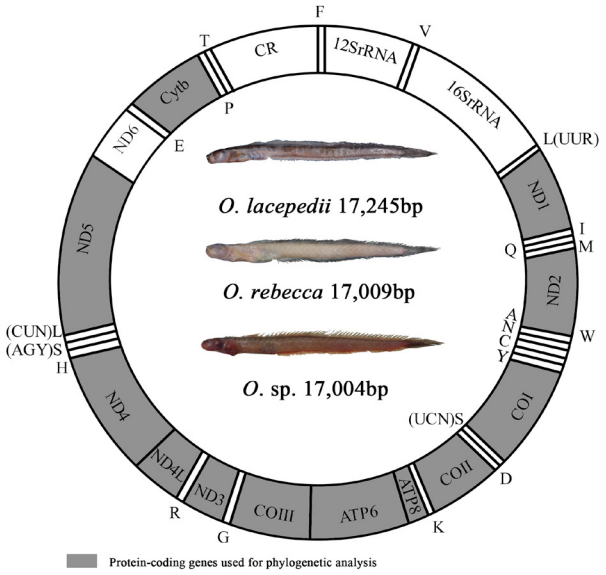

Fig. 1. Gene organization of the mitochondrial genome of *O. lacepedii*, *O. rebecca* and *O.* sp. All protein-coding genes are encoded on the H-strand, with the exception of ND6 which is encoded on the L-strand. The two ribosomal RNA genes are encoded on the H-strand. Transfer RNA genes are designated by single-letter amino acid codes. Genes encoded on the H-strand and L-strand are shown outside and inside the circular gene map, respectively.

RESULTS AND DISCUSSION

Genome content

The complete mitogenome sequences were 17245bp, 17009bp and 17004bp in *O. lacepedii*, *O.rebecca* and *O.* sp., respectively (Supplementary Table S1, Fig. 1). The length and gene order of protein-coding genes were highly

in agreement with previous reports (Cui *et al*., 2009; Kim *et al*., 2004; Wang *et al*., 2008). The length variation of mitochondrial DNA was mainly due to the different length of control region for most vertebrates (Randi *et al*., 1998; Ketmaier and Bernardini, 2005).

The overall base compositions of three species were listed in Supplementary Table S2. The G content was 15.3% (*O. lacepedii*), 15.3% (*O. rebecca*) and 15.4% (*O.* sp.) showing an obvious bias against G, supporting by previous studies (Miya *et al*., 2003; Mabuchi *et al*., 2007; Wang *et al*., 2008). The A+T content exhibited higher values than G+C content indicating that the codon usage with A and T nucleotides preferred to C and G nucleotides at the third codon position (Supplementary Table S2).

Protein-coding genes and codon usage

The total length of 13 protein-coding genes was 11415 bp, 11412 bp and 11415 bp in *O. lacepedii*, *O. rebecca* and *O.* sp., respectively. As with the common features of other bony fishes (Miya *et al*., 2003; Cheng *et al*., 2012), three reading-frames overlap were noted on the same strand: ATPase8 and ATPase6 overlapped by 4 nucleotides, ATPase6 and COIII overlapped by 1 nucleotide, and ND4L and ND4 overlapped by 7 nucleotides (Supplementary Table S1). The pair of genes ND5-ND6 encoding on the different strands overlapped by 4 nucleotides.

All the initiation and stop codons were examined according to the corresponding genes and proteins of other goby fishes (Cheng *et al*., 2012). Most protein-coding genes

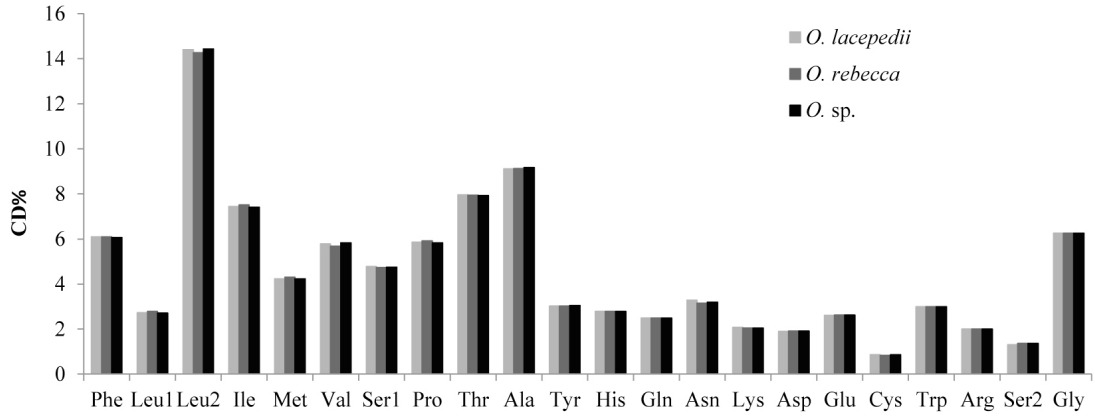

Fig. 2. Codon distribution in 13 protein-coding genes of *O. lacepedii, O. rebecca* and *O.* sp. with 3805 codons, 3804 codons and 3805 codons, respectively, for each mitochondrial sequence analysis, excluding all stop codons. CDs%, the percentage of codons.

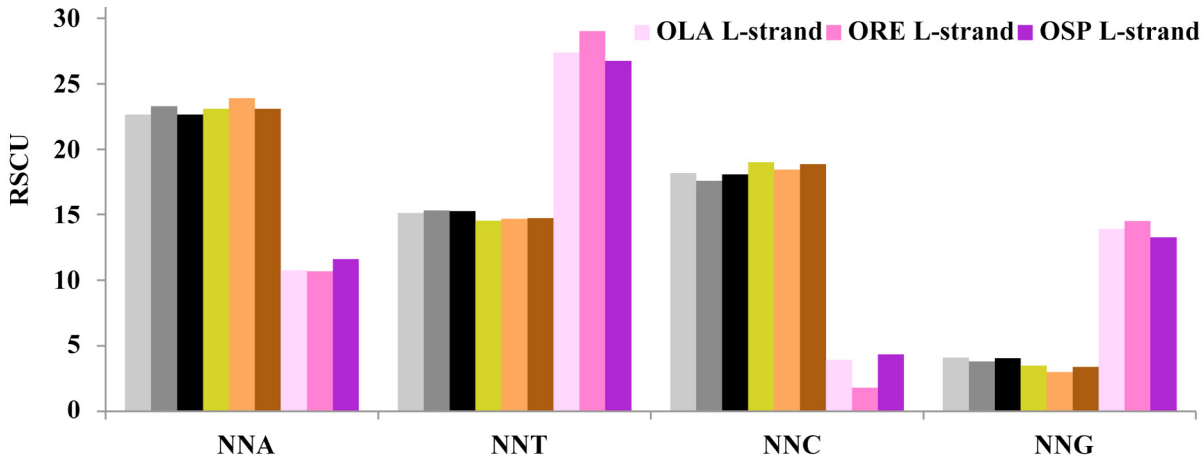

Fig. 3. Frequencies of codons ended with the same nucleotide on total and each strand (H, L) of *O. lacepedi*, *O. rebecca* and *O.* sp. mitogenomes. Values on the y-axis indicate the sum of RSCU values of codons ended with the same nucleotide across all codon families (x-axis). RSCU, relative synonymous codon usage. OLA, *O. lacepedi*. ORE, *O. rebecca*. OSP, *O.* sp.

began with ATG codon (Supplementary Table S1) except COI gene and ATP6 gene used the initiation codon GTG and ATA. Differing from initiation codons (Catanese *et al*., 2010; Oh *et al*., 2007), complete stop codons and incomplete codons were identified. ATG seemed to be the most common initiation codon, though there were exceptions (Miya *et al*., 2003). The open reading frames for ND6 genes ended with TAG in *O. lacepedii* and *O. rebecca*, however, *O.* sp. ended with TAA. All other reading frames of three species ended with TAA (ND1, ND2, ND4L and ND5), AGG (COI) and TAG (ATPase8). Six protein-coding genes ended with incomplete stop codons TA (ATPase6), as well as in complete stop codons T (COII, COIII, ND3, ND4 and Cyt*b*). Based on Ojala *et al*. (1981), the presence of such noncanonical stop codons can be converted into a fully functional TAA stop codon via post-transcriptional polyadenylation.

Comparative base composition analysis at each codon position (Supplementary Table S2) reflected that the proportion of G at third codon position showed a relatively low value (Miya *et al*., 2003; Mabuchi *et al*., 2007; Oh *et al*., 2007) and a clearly excess of pyrimidines over purines probably as a result of the hydrophobic character of the proteins (Naylor *et al*., 1995). The patterns of codon usage were exhibited in Figures 2, 3 and 4. Codons for Leucine accounted for the highest percent value, however codon usage for Cysteine were the least (Fig. 2). The overall codons ending with A or C were all used more frequently than those ending with T and G (Fig. 3). Moreover, evidence also can be observed codon usage of genes oriented in opposite directions. For amino acids with fourfold degenerate codons, third position ending with A were the most frequently employed, followed by codon families ending with C and U. Otherwise, among twofold degenerate codons, C seemed to be more often used than T in pyrimidine codon families; purine codons encoded mostly with A (Fig. 4). The codon usage pattern

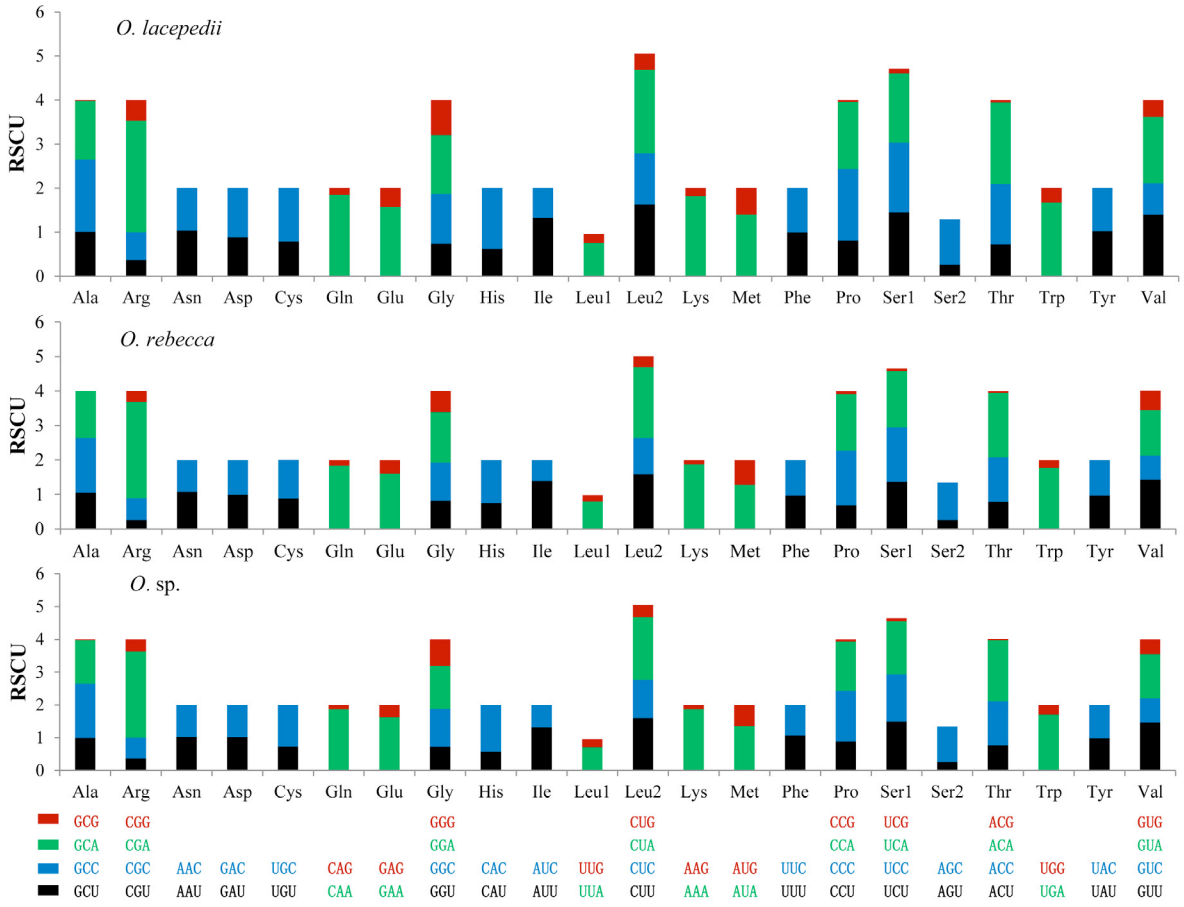

Fig. 4. RSCU in 13 protein-coding genes of *O. lacepedii*, *O. rebecca and O.* sp. Codon families are provided on the x-axis.

of 13 protein-coding genes of the three species was in common with other fishes (Cheng *et al*., 2010).

Transfer and ribosomal RNA genes

Mitogenomes of three species examined included 22 typital tRNA genes dispersed between rRNA and protein-coding genes (Table I). The tRNA genes varied from 64 bp to 76 bp and gene inversion of tRNA genes has been observed in some fishes (Lin *et al*., 2006; Zhu and Yue, 2008). Among these tRNA genes, two forms of tRNA-Leu (UUR and CUN) and tRNA-Ser (UCN and AGY) were determined (Table I). The three tRNA clusters-IQM (isoleucine, glutamine and methionine), WANCY (tryptophan, alanine, asparagines, cysteine and tyrosine) and HSL (histidine, serine and leucine) were well conserved. The A:T:C:G base composition of 22 tRNA genes was exhibited in Supplementary Table S2, among which A+T content was the highest in *O. rebecca* (56.6%).

Similar to other Gobiidae fishes (Jin *et al*., 2012, 2015), small encoding subunit (12S rRNA) and large subunit (16S rRNA) located between the tRNA-Phe and tRNA-Leu (UUR) genes on the H-strand and separated by the tRNA-Val genes (Supplementary Table S1, Fig. 1) were identified among three species. The 12S rRNA genes were 950 bp in *O. rebecca* and 949bp in *O. lacepedii* and *O.* sp. However, the 16S rRNA genes were in different length (1693 bp in *O. lacepedii*, 1695 bp in *O. rebecca* and 1692 bp in *O.* sp., respectively). The rRNA genes showed a slightly lower A+T contents comparing with the protein-coding genes and tRNA genes (Supplementary Table S2), however, slightly richer than other bony fishes (Kim *et al*., 2004).

Non-coding regions

The O_L region located between tRNA-Asn and tRNA-Cys comprised 35 bp in length within the WANCY region (Supplementary Table S1) and predictably had the capacity to fold into a stable stem-loop hairpin structure consisting of 13 bp in the stem and 11 bp in the loop. While, both *O. lacepedii* and *O.* sp. had single base difference with *O. rebecca* in the loop (Supplementary Fig. S1). In addition, the O_L region had a strong asymmetry in the codon usage in the stem with an obvious over-representation of pyrimidines in the 5' side in the sequence (Supplementary Fig. S1). The conserved motif (5'-GCCGG-3') at the base of the stem within tRNA-Cys among three species seemed to be in connection with the transition from RNA synthesis to DNA synthesis (Hixson *et al*., 1986). T-rich or C-rich loop was highly in agreement with previous reports (Zardoya *et al*., 1995; Cheng *et al*., 2010). It was suggested that tRNA genes can be functional as origins of replication as they can form O_L-like structure without loss of other main functions (Desjardins and Morais, 1990).

Control region was characterized by discrete and conserved sequence blocks and exhibited the typical tripartite structure with termination-associated sequence domain (TAS), central conserved sequence blocks domain (CSB) and conserved sequence block domain (Kim *et al*., 2005; Lin *et al*., 2006). Three species were detected termination-associated sequence domain (TAS) (Fig. 5), which may be predicted to be treated as an identification for the termination of H-strand. In addition, conserved sequence blocks domain (CSB-1, CSB-2 and CSB-3) (Fig. 5) were identified and thought to be related to positioning RNA polymerse for both transcription and priming replication

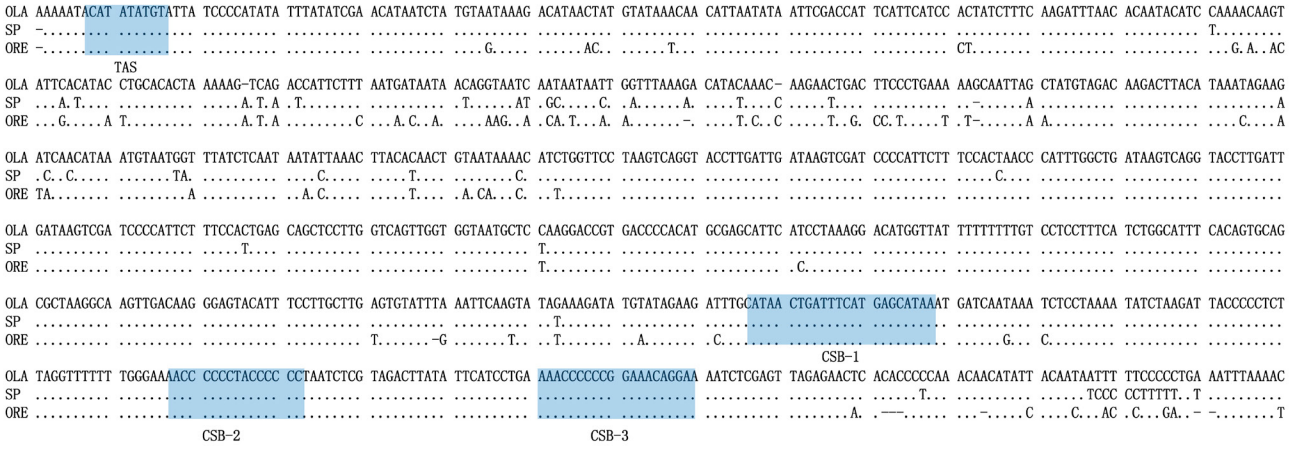

Fig. 5. Comparison of the characteristics of mitochondrial control region of three gobies. TAS: termination-associated sequence; CSB-1, 2, 3: conserved sequence blocks 1, 2 and 3.

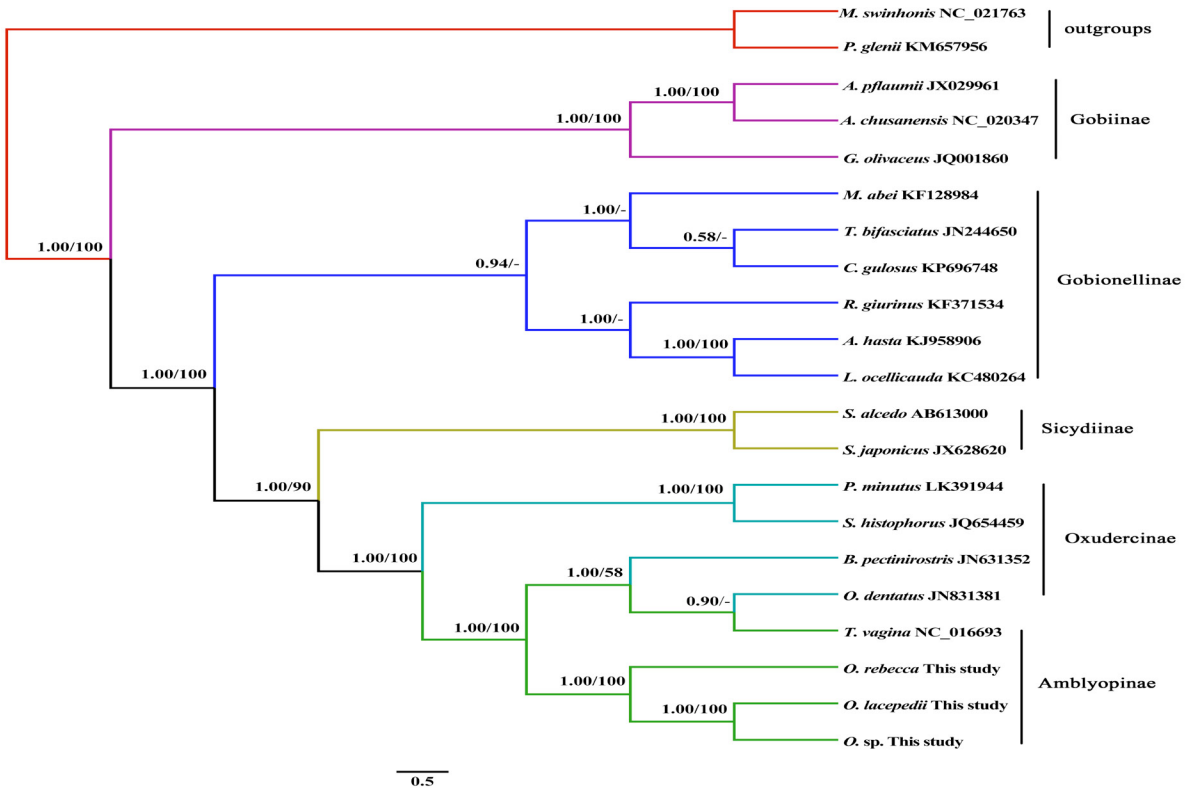

Fig. 6. Phylogenetic tree of Gobiidae fishes based on 12 protein-coding genes excluding ND6 gene. The tree was built based on the TIM3+I+G model (-ln L= 119,585.39, A:C:G:T= 0.3172, 0.3187, 0.1016, 0.2625, I= 0.2830, G= 0.4140). The topology generated by Bayesian inference is shown, and is identical to the result of maximum likelihood analyses. Bayesian posterior probabilities and bootstrap support values above 50% replicates are shown adjacent to each internode. *M. swinhonis* and *P. glenii* were used as outgroups.

(Shadel and Clayton, 1997). Normally, transcripts originating from the promoters were responsible for priming replication (Chang and Clayton, 1986). While the most striking central conserved sequences (CSB-D, CSB-E and CSB-F) and conserved motifs commonly emerging in fish control region cannot be observed in the three species.

Phylogenetic analysis

Substitution saturation decreased phylogenetic information contained in sequences and had plagued the phylogenetic analysis (Xia and Lemey, 2009). When sequences had experienced full substitution saturation, the similarity between the sequences will depend entirely on the similarity in nucleotide frequencies (Xia and Xie, 2001), which often didn't reflect phylogenetic relationships. To identify the mutation saturation in phylogeny, an analysis was conducted using GTR-based models by ploting transitions and transversions (Supplementary Fig. S2). The results implied that the 1st positions, 2nd positions and 3rd positions were in unsaturation. Thus, the whole 12 protein-coding genes will be used for phylogeny.

Phylogenetic relationships were constructed based on the concatenated 12 protein-coding genes adding 16 Gobiidae species. As a result, Bayesian analysis was practically identical to ML analysis. Bayesian analysis with each codon indicated insignificant difference (data not shown). The Bayesian analyses was supported by high Bayesian posterior probabilities, however, the bootstrap values for ML analyses were low in some nodes (Fig. 6). The Gobiidae comprised five subfamilies (Amblyopinae, Gobionellinae, Gobiinae, Oxudercinae and Sicydiinae). According to previous studies, the relationship among Gobiidae has been poorly resolved. We provided a more comprehensive relationship using longer mitochondrial DNA especially among the three species. Consequently, the tree topologies represented two groups (Group A and Group B): the large group A consisted of Amblyopinae, Gobionellinae, Oxudercinae and Sicydiinae, however

group B was only the Gobiinae (Jin *et al*., 2015). In group A, four clades were found in which clade Amblyopinae and Oxudercinae were paraphyletic with high supported value and the result was endorsed by Thacker (2003), Thacker and Roje (2011) and Tornabene *et al*. (2013). However, the topological relationship in Oxudercinae was diverged from You *et al*. (2014) and Murdy (1989). In this study, *Boleophthalmus* was sister to *Oxuderces*, while You *et al*. (2014) suggested *Boleophthalmus* and *Scartelaos* were in close relationship and Murdy (1989) indicated *Boleophthalmus* was clustered with *Periophthalmus*. Also, an unexpected relationship that Amblyopinae contained *B. pectinirostris* and *O. dentatus*, was not supported by traditional taxon (Wu and Zhong, 2008). Moreover, the result seemed to suggest that the relationship between Amblyopinae and Oxudercinae was the closest in Gobiidae. In group B, the monophyletic Gobiinae was sister to the clade Gobionellinae (Thacker, 2009).

In this paper, we discussed the phylogenetic relationship based on the 12 protein-coding genes and the analysis demonstrated that clade Amblyopinae included three species (*O. lacepedii*, *O. rebecca* and *O.* sp.). In addition, *O. lacepedii* has been proved to be a much closer affinity to *O.* sp. than *O. rebecca*. The result was in agreement with previous records that Tang *et al*. (2010) found the cryptic species *O.* sp. which was similar to *O. lacepedii* based on collected molecular and morphological data; The two species were sister species and cannot easily be differentiated with naked eye. Agorreta *et al*. (2013) analyzed sequences data of five molecular makers (two mitochondrial DNA and three nuclear DNA) averaging 222 species of gobioids, while the phylogenetic relationship included only *O.* sp. within *Odontamblyopus* and the relationship among three species hasn't been described with such numberous species studied. Briefly, the phylogenetic relationship among the Godiidae species still remains poorly understood, and our study is confirmed to be a validly supplementary proof and expected to provide significant information for future taxon studies.

CONCLUSION

The mtDNA genome of three species (*O. lacepedii*, *O. rebecca* and *O.* sp.) were all similar with other bony fishes including genome organization, gene arrangement and codon usage. An unexpected structure in the control region among the three species was discovered that only termination-associated sequence domain (TAS) and conserved sequence blocks domain (CSB-1, CSB-2 and CSB-3) were found. Phylogenetic relationships based on the concatenated 12 protein-coding genes indicated the tree topologies represented two groups, and also demonstrated clade Amblyopinae included all three goby species and that *O. lacepedii* has been proved to be a much closer affinity to *O.* sp. than *O. rebecca*. Three species are distinguished authoritatively by analysis of the complete mitochondrial DNA.

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Conflict of Interest Statement

The authors declare that there is no conflict of interests regarding the publication of the manuscript.

Supplementary Material

There is supplementary material associated with this article. Access the material online at: http://dx.doi.org/10.17582/journal.pjz/2017.1.111.120

REFERENCES

Agorreta, A., San Mauro, D., Schliewen, U., Van Tassell, J.L., Kovačić, M., Zardoya, R. and Rüber, L., 2013. Molecular phylogenetics of Gobioidei and phylogenetic placement of European gobies. *Mol. Phylogen. Evol.,* **69**:619-633. http://dx.doi.org/10.1016/j.ympev.2013.07.017

Bibb, M.J., Van Etten, R.A., Wright, C.T., Walberg, M.W. and Clayton, D.A., 1981. Sequence and gene organization of mouse mitochondrial DNA. *Cell*, **26**:167-180. http://dx.doi.org/10.1016/0092-8674(81)90300-7

Bleeker, P., 1874. Esquisse d'un système naturel des Gobioïdes. *Archiv. Nerland. Sci. Exact. Natur*., **9**:289-331.

Boore, J.L., Macey, J.R. and Medina, M., 2005. Sequencing and comparing whole mitochondrial genomes of animals. *Method. Enzymol.,* **395**:311-348. http://dx.doi.org/10.1016/S0076-6879(05)95019-2

Catanese, G., Manchado, M. and Infante, C., 2010. Evolutionary relatedness of mackerels of the genus *Scomber* based on complete mitochondrial genomes: strong support to the recognition of Atlantic *Scomber colias* and Pacific *Scomber japonicus* as distinct species. *Gene*, **452**:35-43.

http://dx.doi.org/10.1016/j.gene.2009.12.004

Chang, D.D. and Clayton, D.A., 1986. Precise assignment of the light-strand promoter of mouse mitochondrial DNA: A functional promoter consists of multiple upstream domains. *Mol. Cell. Biol.,* **6**:3253-3261. http://dx.doi.org/10.1128/MCB.6.9.3253

Cheng, Y.Z., Xu, T.J., Shi, G. and Wang, R.X., 2010. Complete mitochondrial genome of the miiuy croaker *Miichthys miiuy* (Perciformes, Sciaenidae) with phylogenetic consideration. *Mar. Genom.,* **3**:201-209. http://dx.doi.org/10.1016/j.margen.2010.10.003

Cheng, J., Ma, G.Q., Miao, Z.Q., Shui, B.N. and Gao, T.X., 2012. Complete mitochondrial genome sequence of the spinyhead croaker *Collichthys lucidus* (Perciformes, Sciaenidae) with phylogenetic considerations. *Mol. Biol. Rep.,* **39**:4249-4259. http://dx.doi.org/10.1007/s11033-011-1211-6

Cui, Z., Liu, Y., Li, C.P., You, F. and Chu, K.H., 2009. The complete mitochondrial genome of the large yellow croaker, *Larimichthys crocea* (Perciformes, Sciaenidae): Unusual features of its control region and the phylogenetic position of the Sciaenidae. *Gene*, **432**:33-43. http://dx.doi.org/10.1016/j.gene.2008.11.024

Desjardins, P. and Morais, R., 1990. Sequence and gene organization of the chicken mitochondrial genome. A novel gene order in higher vertebrates. *J. mol. Biol.,* **212**:599-634. http://dx.doi.org/10.1016/0022-2836(90)90225-B

Hixson, J.E., Wong, T.W. and Clayton, D.A., 1986. Both the conserved stem-loop and divergent 5′-flanking sequences are required for initiation at the human mitochondrial origin of light-strand DNA replication. *J. biol. Chem.,* **261**:2384-2390.

Hoese, D.F., 1984. *Gobioidei: relationships*. *Ontogeny and systematics of fishes*. American Society Ichthyologists and Herpetologists, pp. 588-591.

Jin, X.X., Wang, R.X., Xu, T.J. and Shi, G., 2012. Complete mitochondrial genome of *Oxuderces dentatus* (Perciformes, Gobioidei). *Mitochond. DNA*, **23**:142-144. http://dx.doi.org/10.3109/19401736.2012.660930

Jin, X., Wang, R., Wei, T., Tang, D. and Xu, T., 2015. Complete mitochondrial genome sequence of *Tridentiger bifasciatus* and *Tridentiger barbatus* (Perciformes, Gobiidae): A mitogenomic perspective on the phylogenetic relationships of Gobiidae. *Mol. Biol. Rep.,* **42**:253-265. http://dx.doi.org/10.1007/s11033-014-3768-3

Ketmaier, V. and Bernardini, C., 2005. Structure of the mitochondrial control region of the Eurasian Otter (*Lutra lutra*; Carnivora, Mustelidae): Patterns of genetic heterogeneity and implications for conservation of the species in Italy. *J. Hered.,* **96**:318-328. http://dx.doi.org/10.1093/jhered/esi037

Kim, I.C., Kweon, H.S., Kim, Y.J., Kim, C.B., Gye, M.C., Lee, W.O., Lee, Y.S. and Lee, J.S., 2004. The complete mitochondrial genome of the javeline goby *Acanthogobius hasta* (Perciformes, Gobiidae) and phylogenetic considerations. *Gene*, **336**:147-153. http://dx.doi.org/10.1016/j.gene.2004.04.009

Kim, I.S., Choi, Y., Lee, C.L., Lee, Y.J., Kim, B.J. and Kim, J.H., 2005. *Illustrated book of Korean fishes*. Kyo-Hak Publishing Co. Seoul, pp. 615.

Lin, G., Lo, L.C., Zhu, Z.Y., Feng, F., Chou, R. and Yue, G.H., 2006. The complete mitochondrial genome sequence and characterization of single-nucleotide polymorphisms in the control region of the Asian seabass (*Lates calcarifer*). *Mar. Biotechnol.,* **8**:71-79. http://dx.doi.org/10.1007/s10126-005-5051-z

Liu, J.X., Gao, T.X., Wu, S.F. and Zhang, Y.P., 2007. Pleistocene isolation in the Northwestern Pacific marginal seas and limited dispersal in a marine fish, *Chelon haematocheilus* (Temminck and Schlegel, 1845). *Mol. Ecol.,* **16**:275-288. http://dx.doi.org/10.1111/j.1365-294X.2006.03140.x

Mabuchi, K., Miya, M., Azuma, Y. and Nishida, M., 2007. Independent evolution of the specialized pharyngeal jaw apparatus in cichlid and labrid fishes. *BMC Evol. Biol.,* **7**:1-12. http://dx.doi.org/10.1186/1471-2148-7-10

Minin, V., Abdo, Z., Joyce, P. and Sullivan, J., 2003. Performance-based selection of likelihood models for phylogeny estimation. *System. Biol.,* **52**:674-683. http://dx.doi.org/10.1080/10635150390235494

Miya, M., Takeshima, H., Endo, H., Ishiguro, N.B., Inoue, J.G., Mukai, T., Satoh, T.P., Yamaguchi, M., Kawaguchi, A., Mabuchi, K., Shirai, S.M. and Nishida, M., 2003. Major patterns of higher teleostean phylogenies: a new perspective based on 100 complete mitochondrial DNA sequences. *Mol. Phylogenet. Evol.,* **26**:121-138. http://dx.doi.org/10.1016/S1055-7903(02)00332-9

Murdy, E.O., 1989. A taxonomic revision and cladistic analysis of the oxudercinae gobies (Gobiidae: Oxudercinae). Rec. Austral. Mus. Suppl. **11**:1-93.

Murdy, E.O. and Shibukawa, K., 2001. A revision of the gobiid fish genus *Odontamblyopus* (Gobiidae: Amblyopinae). *Ichthyol. Res.,* **48**:31-43. http://dx.doi.org/10.1007/s10228-001-8114-9

Murdy, E.O. and Shibukawa, K., 2003. *Odontamblyopus*

rebecca, a new species of amblyopine goby from Vietnam with a key to known species of the genus (Gobiidae: Amblyopinae). *Zootaxa*, **138**:1-6.

Naylor, G.J., Collins, T.M. and Brown, W.M., 1995. Hydrophobicity and phylogeny. *Nature*, **373**:565-566. http://dx.doi.org/10.1038/373565b0

Norman, J.R., 1966. *A draft synopsis of the orders, families and genera of recent fishes and fishlike vertebrates*. Bristish Museum (Natural History), London.

Oh, D.J., Kim, J.Y., Lee, J.A., Yoon, W.J., Park, S.Y. and Jung, Y.H., 2007. Complete mitochondrial genome of the rock bream *Oplegnathus fasciatus* (Perciformes, Oplegnathidae) with phylogenetic considerations. *Gene*, **392**:174-180. http://dx.doi.org/10.1016/j.gene.2006.12.007

Ojala, D., Montoya, J. and Attardi, G., 1981. tRNA punctuation model of RNA processing in human mitochondria. *Nature*, **290**:470-474. http://dx.doi.org/10.1038/290470a0

Randi, E., Mucci, N., Pierpaoli, M. and Douzery, E., 1998. Organization and evolution of the mitochondrial DNA control region in the avian genus *Alectoris*. *J. mol. Evol.,* **47**:149-162.

Shadel, G.S. and Clayton, D.A., 1997. Mitochondrial DNA maintenance in vertebrates. *Annu. Rev. Biochem.,* **66**:409-435. http://dx.doi.org/10.1146/annurev.biochem.66.1.409

Tang, W., Fu, C., Yin, W., Li, G., Chen, H., Wu, Q. and Li, B., 2010. Cryptic species and historical biogeography of eel gobies (Gobioidei: *Odontamblyopus*) along the northwestern Pacific coast. *Zool. Sci.,* **27**:8-13. http://dx.doi.org/10.2108/zsj.27.8

Thacker, C.E., 2003. Molecular phylogeny of the gobioid fishes (Teleostei: Perciformes: Gobioidei). *Mol. Phylogenet. Evol.,* **26**:354-368. http://dx.doi.org/10.1016/S1055-7903(02)00361-5

Thacker, C.E., 2009. Phylogeny of Gobioidei and placement within Acanthomorpha, with a new classification and investigation of diversification and character evolution. *Copeia*, **2009**:93-104. http://dx.doi.org/10.1643/CI-08-004

Thacker, C.E. and Roje, D.M., 2011. Phylogeny of Gobiidae and identification of gobiid lineages. *System. Biodiv.,* **9**:329-347. http://dx.doi.org/10.1080/14772000.2011.629011

Tornabene, L., Chen, Y. and Pezold, F., 2013. Gobies are deeply divided: phylogenetic evidence from nuclear DNA (Teleostei: Gobioidei: Gobiidae). *System. Biodiv.,* **11**:345-361. http://dx.doi.org/10.1080/14772000.2013.818589

Wang, C., Chen, Q., Lu, G., Xu, J., Yang, Q. and Li, S., 2008. Complete mitochondrial genome of the grass carp (*Ctenopharyngodon idella*, Teleostei): insight into its phylogenic position within Cyprinidae. *Gene*, **424**:96-101. http://dx.doi.org/10.1016/j.gene.2008.07.011

Wu, H.L. and Zhong, J.S., 2008. *Fauna Sinica-Osteichthyes: Perciformes Gobioidei*. Science Press, Beijing.

Xia, X. and Xie, X., 2001. DAMBE: software package for data analysis in molecular biology and evolution. *J. Hered.,* **0**:1-3. http://dx.doi.org/10.1093/jhered/92.4.371

Xia, X. and Lemey, P., 2009. *Assessing substitution saturation with DAMBE*. Cambridge University Press, pp. 611-626. http://dx.doi.org/10.1017/cbo9780511819049.022

You, X.X., Bian, C., Zan, Q.J., Xu, X., Chen, J.M., Wang, J.T., Ying, Q., Li, W.J., Zhang, X.H., Sun, Y., Chen, S.X., Hong, W.S., Li, Y.X., Cheng, S.F., Fan, G.Y., Shi, C.C., Liang, J., Tang, Y.T., Yang, C.Y., Ruan, Z.Q., Bai, J., Peng, C., Mu, Q., Lu, J., Fan, M.J., Yang, S., Huang, Z.Y., Jiang, X.T., Fang, X.D., Zhang, G.J., Zhang, Y., Polgar, G., Yu, H., Li, J., Liu, Z.J., Zhang, G.Q., Rav, V., Coon, S.L., Wang, J., Yang, H.M., Venkatesh, B., Wang, J. and Shi, Q., 2014. Mudskipper genomes provide insights into the terrestrial adaptation of amphibious fishes. *Nature Commun.,* 5. http://dx.doi.org/10.1038/ncomms6594

Zardoya, R., Garrido-Pertierra, A. and Bautista, J.M., 1995. The complete nucleotide sequence of the mitochondrial DNA genome of the rainbow trout, *Oncorhynchus mykiss*. *J. mol. Evol.,* **41**:942-951.

Zhu, Z.Y. and Yue, G.H., 2008. The complete mitochondrial genome of red grouper *Plectropomus leopardus* and its applications in identification of grouper species. *Aquaculture*, **276**:44-49. http://dx.doi.org/10.1016/j.aquaculture.2008.02.008