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Comparative Analysis of Complete Mitogenomes of Two *Oxyurichthys* Gobies and Their Phylogenetic Implication

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Oxyurichthys is a genus of goby that is widespread in the tropical Indo-West Pacific region. *Oxyurichthys* species are usually found in estuarine and coastal marine habitats. In Southeast Asia, they are commercial fishes and often collected by trawling to serve the market's demand. The mitogenome serves as a good marker for investigating the systematics and evolution of fishes, but the mitogenome of *Oxyurichthys* species remains unknown. In this study, mitogenomes of two *Oxyurichthys* gobies, *O. ophthalmonema* and *O. microlepis*, were characterized and compared. The sizes of the mitogenomes were 16,504 bp and 16,506 bp for *O. ophthalmonema* and *O. microlepis*, respectively. Mitogenomes of these two species were similar in gene content and structure. Both included 37 genes and a control region. The two *Oxyurichthys* mitogenomes shared similar gene features and base composition with other recorded gobies. Typical conserved blocks (CSB-1, CSB-2, CSB-3 and CSB-D) were found in the control region of both species. Phylogenetic analyses based on concatenation of 13 protein-coding genes and 2 rRNAs revealed that the two *Oxyurichthys* species clustered together and were sister to species of the genera *Sicydium*, *Sicyopterus* and *Stiphodon*. The findings of the present study support previous evolutionary studies of gobies using other molecular markers.

Key words: Gobies, Mitogenome, Gene structure, Control region, Phylogeny.

BACKGROUND

Gobies are relatively small fishes with a typical length of less than 10 cm that are ubiquitous in most seas and estuaries of the world (Patzner et al. 2011). The majority of gobies belong to two families, Gobiidae and Oxudercidae, comprising roughly 2,000 species in about 200 genera (Thacker 2015; Betancur et al. 2017). Gobies are not only important for aquatic ecosystems, but also for humans. Gobies play a key role in the ecosystem as prey for larger, more commercially important fishes (Yokoo et al. 2012). Some gobies are also important as direct sources of food for humans (*e.g.*, Bell 1999; Zarev et al. 2013). Gobies are also popular in the aquarium fish trade, particularly species of the genus *Brachygobius* (Larson et al. 2008). Despite their ubiquity and economic and ecological significance, phylogenetic studies of goby evolution have been hampered by the simplification and

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loss of many morphological characteristics (Thacker 2003; Jin et al. 2015). Therefore, analyses of molecular data have been considered attractive tools for resolving goby relationships (Akihito et al. 2000; Jin et al. 2015; Adrian-Kalchhauser et al. 2017).

Oxyurichthys species are large gobies found in the tropical Indo-west Pacific region (Pezold and Larson 2015). Species of this genus are usually distributed in shallow estuarine and coastal marine habitats. *Oxyurichthys* species are commercially important for fishermen in Southeast Asia (Pezold and Larson 2015). Two species, *O. ophthalmonema* and *O. microlepis* are common in mangroves, estuaries and lagoons. Both species are important sources of livelihood for the people living around the Tam Giang–Cau Hai lagoon in Vietnam.

Considerable progress has been made in diagnosing *Oxyurichthys* over the last half century (Gilbert and Randall 1979; Hoese 1986; Pezold 1991 1998), but the genus had never been adequately revised until the comprehensive review of Pezold and Larson (2015). *Oxyurichthys* is distinguished from other gobioid genera in having a transversely broad third neural spine, an A'BCDFH' pattern of oculoscapular canal pores and no preopercular canal. All but one species have a single row of teeth in the upper jaw. The genus is a member of the *Stenogobius* lineage in the family Oxudercidae (Agorreta et al. 2013; Thacker 2015) and twenty species are currently recognized (Pezold and Larson 2015). While more morphological and biological data of *Oxyurichthys* has been accumulated, little is known about the genetic characteristics of species in the genus.

The mitogenome has been used as an effective marker for identification, phylogeny and population genetics in vertebrates. The vertebrate mitogenome is circular and compact, generally spanning 16–17 kb in size (Satoh et al. 2016). Typically, it contains 37 genes, including 13 protein-coding genes (PCGs), two rRNA genes, and 22 tRNA genes. In addition, two non-coding regions, origin of L-strand replication (OL) and control region (CR), are found in the vertebrate mitogenome. CR contains regulatory elements that control the transcription and replication of the mitogenome (Yu et al. 2021). With the development of sequencing technology, the number of mitogenomes of fishes, including gobies, has rapidly increased. Based on available sequences, some studies have been performed to investigate the interrelationships among species and genera of gobies (Jin et al. 2015; Adrian-Kalchhauser et al. 2017). Nevertheless, many genera, including *Oxyurichthys*, have never been sampled for these analyses. This lack of data has limited the investigation of goby phylogeny based on mitogenomes.

This study was performed to sequence and analyze the mitogenomes of *O. ophthalmonema* and *O. microlepis* from Tam Giang–Cau Hai lagoon, Vietnam. Content and structure of the mitogenomes were investigated and compared with available mitogenomes of other gobies. In addition, phylogenetic trees were reconstructed to determine the position of *Oxyurichthys* in relation to other gobies.

MATERIALS AND METHODS

Fish sampling and genomic DNA extraction

The specimens of *Oxyurichthys* species were collected from Tam Giang–Cau Hai lagoon, Thua Thien Hue province, Vietnam (16°36'38.49"N–107°31'40.95"E) in April 2021. Taxonomic identification of the collected specimens was performed based on morphological characteristics guided by Pezold and Larson (2015) together with *cox1* sequences (data not shown). Whole fin clips of fishes were removed and preserved in 95% ethanol. Total genomic DNA was extracted from fin clips using DNeasy Blood & Tissue Kit following manufacturer's instructions (Qiagen, Germany).

PCR amplification and sequencing

PCR amplification was conducted using different sets of primers shown in table S1. Universal primers for the control region (Cheng et al. 2012) and fragments of *cox1* (Ward et al. 2005) were adopted from previous studies. Additional primers for overlapping sequences were designed with Primer3 ver. 4.1.0 program based on available goby mitogenomes (Untergasser et al. 2012). The mixture of 20 µl reaction volume contained 10 µL of 2X TOPsimple™ DyeMIX-Tenuto (Enzynomics, South Korea), 1 µL of each primer (10 pmoles/µL), 100 ng of extracted DNA, and distilled water. PCR reaction was performed under the following conditions: initial denaturation at 95°C for 5 min, followed by 35 cycles at 95°C for 45 s, annealing at 50°C for 1 min, and extension at 72°C for 1 min, with a final extension at 72°C for 5 min. PCR products were visualized on 1% agarose gel under a UV transilluminator. Sequencing of the products was conducted with an ABI 3730 DNA Analyzer (Applied Biosystems, USA).

Sequence alignment and mitogenome annotation

The overlapping fragments of mitogenome sequences were aligned and analyzed using Geneious Prime ver. 2022.1 (Kearse et al. 2012). Mitogenome annotation was conducted using MitoFish ver. 3.72

(Iwasaki et al. 2013) and MITOS web server (Bernt et al. 2013). The secondary structure of tRNAs was predicted with tRNAscan-SE ver. 2.0 (Chan and Lowe 2019). The complete mitogenome map of the two species was generated using Geneious Prime ver. 2022.1 (Kearse et al. 2012). The nucleotide compositions and genetic code were estimated in MEGA X ver. 10.2.4 (Kumar et al. 2018). Circular maps of complete mitogenomes were generated and annotated using Geneious Prime ver. 2022.1 (Kearse et al. 2012). The secondary structure of the putative origin of L-strand replication was predicted using Mfold web server (Zuker 2003). Skewness was assessed as follows: AT skew = [A − T] / [A + T]; GC skew = [G − C] / [G + C] (Perna and Kocher 1995). RSCU values were calculated using MEGA X ver. 10.2.4 to evaluate the level of nucleotide bias in each codon (Kumar et al. 2018).

Phylogenetic analyses

The mitogenomes of *Oxyurichthys* species from the present study and mitogenomes from different genera of Gobiidae obtained from GenBank were used for phylogenetic investigation (Table S2). *Gobiomorus maculatus*, *Eleotris fusca* and *Odontobutis platycephala* were used as outgroups. Phylogenetic trees were reconstructed for different concatenated datasets: 13 PCGs and 13 PCGs + 2 rRNAs. First, each sequence was extracted and aligned using MAFFT ver. 7 (Katoh and Standley 2013) in Geneious Prime ver. 2022.1 (Kearse et al. 2012). Subsequently, GBlocks 0.91b was applied to exclude poorly aligned regions (Castresana 2000). PartitionFinder 2 was used to identify the best partition scheme and the best fit model (Lanfear et al. 2017).

The phylogenetic trees were constructed using Maximum Likelihood (ML) and Bayesian Inference (BI) approaches. Maximum Likelihood (ML) trees were constructed using IQ-tree ver. 2.1.2 with 1,000 bootstrap replicates (Minh et al. 2020). Meanwhile, Bayesian Inference (BI) trees were constructed using MrBayes ver. 3.2.7 with four chains, 10,000,000 generations and sampling every 100 generations (Ronquist et al. 2012). The first 25,000 trees before stationarity were excluded as burn-in, and the remaining trees were used to generate consensus trees. Effective sample size (ESS) values for the convergence of MCMC runs were assessed in Tracer ver. 1.7 (Rambaut et al. 2018). The convergence was accepted with ESS values above 200. The generated tree was visualized using FigTree ver. 1.4.4 (Rambaut 2018).

RESULTS

General features of *Oxyurichthys* mitogenome

The mitogenomes of *Oxyurichthys ophthalmonema* and *O. microlepis* (GenBank accession numbers: ON755178 and ON755179) were 16,504 bp and 16,506 bp in length, respectively. Gene order and direction of the two mitogenomes are shown in figures 1–2. Both mitogenomes contained 13 PCGs (*nd1–6*, *nd4l*, *cox1–3*, *cytb*, *atp6* and *atp8*), two rRNA genes (12S rRNA and 16S rRNA) and 22 tRNA genes (Tables 1–2). In addition, two non-coding regions (OL and CR) that are important for replication and transcription were also found in the two mitogenomes. While *nd6* and eight tRNA genes (tRNA-Gln, tRNA-Ala, tRNA-Asn, tRNA-Cys, tRNA-Tyr, tRNA-Ser, tRNA-Glu and tRNA-Pro) were encoded on the L-strand, remaining genes of *O. ophthalmonema* and *O. microlepis* mitogenomes were encoded on the H-strand (Tables 1–2). The base composition of *O. ophthalmonema* was 28.7% A, 26.1% C, 16.7% G and 28.5% T, while the base composition of *O. microlepis* was 28% A, 25.9% C, 17.2% G and 28.9% T. Overlapping regions in *O. ophthalmonema* and *O. microlepis* mitogenomes ranged from 1 to 7 bp, with the longest overlapping region (7 bp) located between *atp8* and *atp6* as well as between *nd4l* and *nd4* (Tables 1–2). Meanwhile, intergenic regions ranged from 1 to 34 bp, and the largest spacer was located between tRNA-Asn and tRNA-Cys (Tables 1–2).

The analysis of nucleotide frequencies revealed a slight bias for A and T. A-T content accounted for 57.2% in *O. ophthalmonema* and 56.9% in *O. microlepis*. The control region was the main A+T-rich region with 63.8% in *O. ophthalmonema* and 63.1% in *O. microlepis*. A-T skew was 0.003 and -0.016 in *O. ophthalmonema* and *O. microlepis*, respectively. This indicates that *O. ophthalmonema* had A > T, while *O. microlepis* had A < T. In contrast, both species had negative G-C skew, -0.220 in *O. ophthalmonema* and -0.202 in *O. microlepis*, indicating that G < C.

There were 3,799 amino acids of PCGs encoded in the mitogenomes of *O. ophthalmonema* and *O. microlepis*. The amino acids Leu (16.46%), Ala (8.75%), and Thr (7.96%) were most abundant in the two mitogenomes. Among these, the frequencies of Leu (CUC, CUA), Ala (GCC), and Thr (ACC) were highest. The frequencies of Cys (UGU), and Arg (CGG) in PCGs were lowest. Analysis of relative synonymous codon usage (RSCU) showed that the codons UCC for Ser, CUC and CUA for Leu and GCC for Ala occurred most frequently, while UUG for Leu, AGU for Ser and GCG for Ala were rare in the mitogenome of *Oxyurichthys* (Figs. 3–4).

Protein-coding genes (PCGs)

The total length of 13 protein-coding genes was 11,426 bp for both *O. ophthalmonema* and *O. microlepis*, ranging from *atp8* 165 (bp) to *nd5* (1,839 bp). Most of 13 PCGs were encoded on the H-strand, except for *nd6*, which was encoded on the L-strand. Twelve PCGs initiated with a typical ATG codon, except for *atp6* gene, which was GTG. The two species had almost similar stop codons, except for the *nd5* gene. For this gene, *O. ophthalmonema* had TAG for termination while *O. microlepis* had TAA. For the remaining genes, TAA was the common termination codon, while incomplete T-- was the termination for *cox2*, *cox3*, *cytb*, *nd3* and *nd4*.

Ribosomal RNA and transfer RNA genes

In total, the tRNA genes were 1,556 bp in length, varying from 66 bp (tRNA-Cys) to 76 bp (tRNA-Leu) in size. Of 22 tRNA genes, 8 genes were encoded on the L-strand and 14 genes were encoded on the H-strand. The prediction for the secondary structure of tRNA genes is presented in figures S1 and S2. The prediction result revealed that all tRNAs could be folded into a typical cloverleaf secondary structure except for tRNA-Ser (GCT). There was no recognizable dihydrouridine (DHU) stem found for tRNA-Ser. In vertebrate mitogenomes, the lack of a DHU stem in tRNA-Ser is commonly observed (Lee and Kocher 1995). The three tRNA clusters (IQM, WANCY and HSL) showed

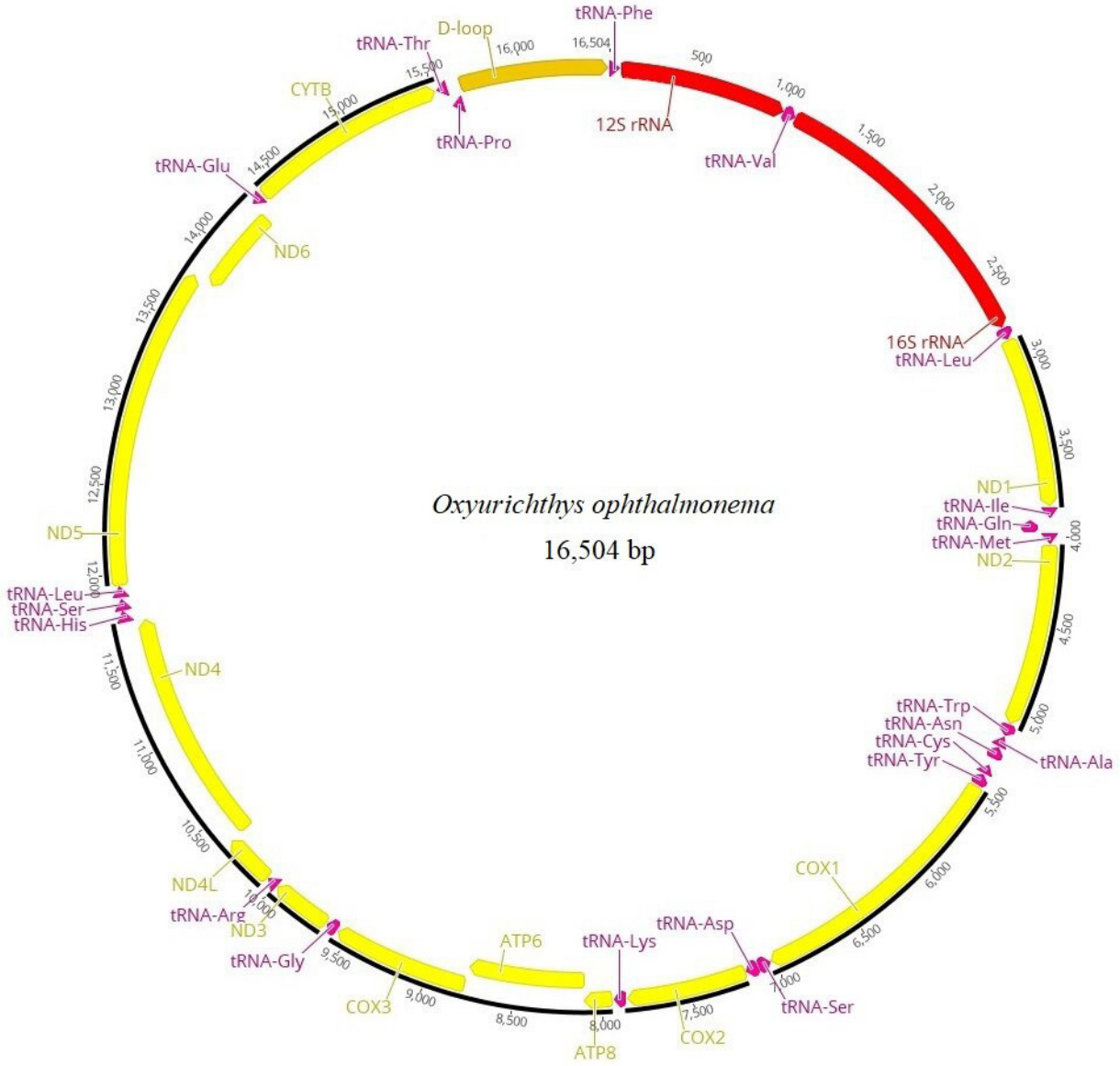

Fig. 1. Gene map of *Oxyurichthys ophthalmonema* mitogenome.

conservative features in the two *Oxyurichthys* species typical of vertebrate mitogenomes.

12S rRNA was located between tRNA-Phe and tRNA-Val, with a length of 952 bp. Meanwhile, 16S rRNA was located between tRNA-Val and tRNA-Leu, with a length of 1,675 bp. The G-C content of rRNA genes was 44.8% and 45.4% for *O. ophthalmonema* and *O. microlepis*, respectively.

Non-coding region

The putative origin of L-strand replication (OL) was determined in the mitogenomes of the two *Oxyurichthys* species (Fig. 5). OL was found between tRNA-Asn and tRNA-Cys in the WANCY region that includes a block of five tRNA genes (Trp, Ala, Asp, Cys, and Tyr). OL could fold into the secondary structure as a stable stem-loop, with 13 bp in the stem and 11 bp in the loop.

The control region of both species was found between tRNA-Pro and tRNA-Phe. Control region sizes of *O. ophthalmonema* and *O. microlepis* were 847 bp and 848 bp, respectively. The region showed high A-T content with A-T = 63.8% for *O. ophthalmonema* and 63.1% for *O. microlepis*, which was higher than the A-T content of the whole mitogenome (57.2% and 56.9%). Conservative elements, including Termination-associated sequence (TAS), the conserved sequence block domain (CSB-1, CSB-2 and CSB-3), the central conserved sequences (CSB-D) and A GTGGG box were

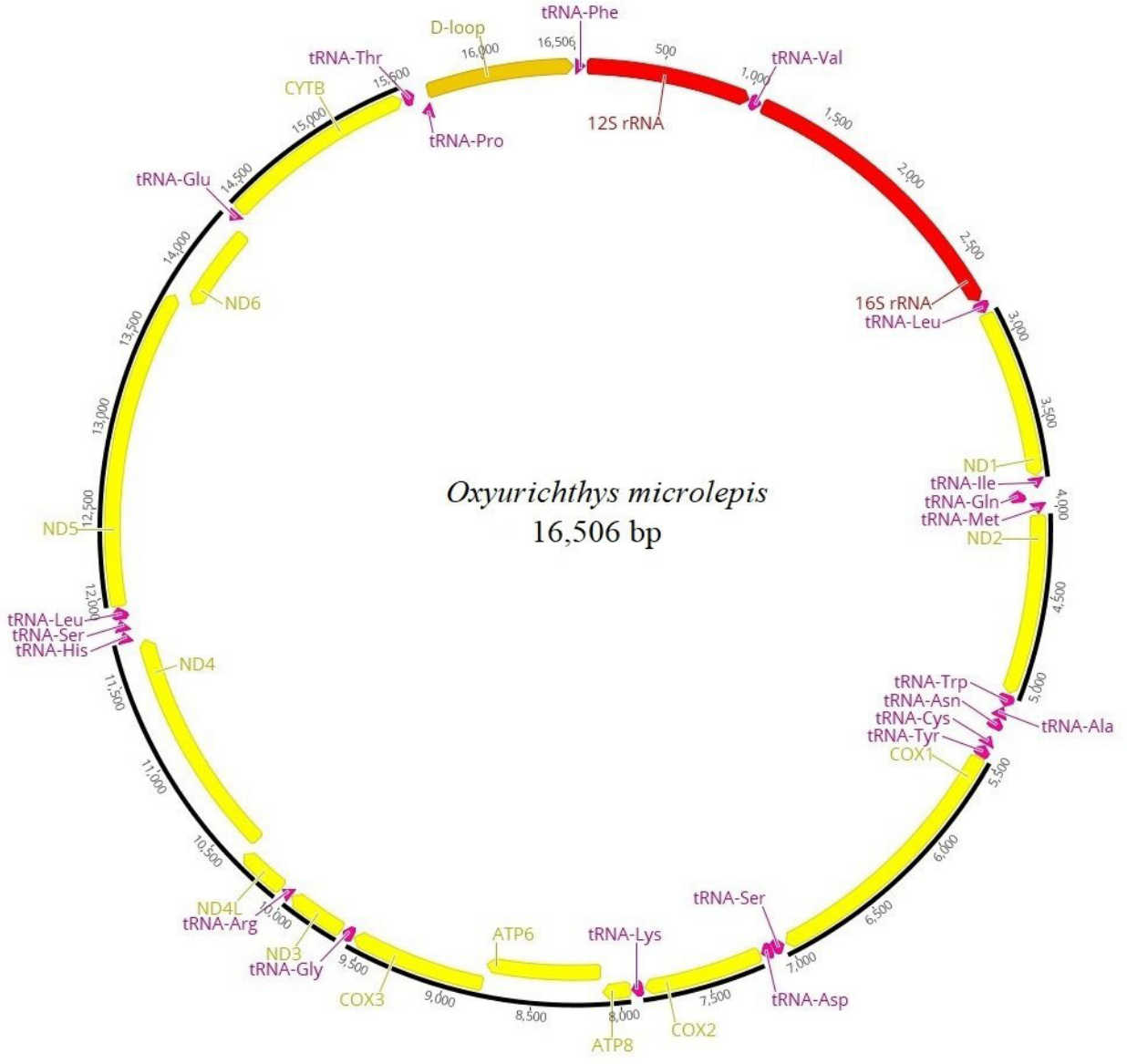

Fig. 2. Gene map of *Oxyurichthys microlepis* mitogenome.

also observed in the mitogenomes of *O. ophthalmonema* and *O. microlepis* (Fig. 6).

Phylogenetic analyses

For investigation of the phylogenetic position of *Oxyurichthys*, the concatenated sets of nucleotide sequences of available mitogenomes were used for phylogenetic analyses. Bayesian Inference and Maximum Likelihood approaches were performed for tree reconstruction. The phylogenetic position of *Oxyurichthys* presented in figures 7–8 and figures S3–4 was relative to goby species based on mitogenome sequences. The ML and BI analyses indicated a slightly different topology, but all lineages as recognized by Agorreta et al. (2013) and Thacker (2015) were recovered as monophyletic. The two species of *Oxyurichthys* from our samples were clustered together with high values of posterior probability (PP = 1) and ultrafast bootstrap (UFBoot = 100) and were sister to a clade containing the genera *Sicydium*, *Sicyopterus* and *Stiphodon* (PP = 1, UFBoot = 100). Monophyly for the clade of sicydiines was well-supported with high strong posterior probability and ultrafast bootstrap support

Table 1. Gene organization of *Oxyurichthys ophthalmonema* mitogenome

Gene	Position		Size	Codon		Intergenic nucleotide	Strand
	From	To		Start	Stop		
tRNA-Phe	1	68	68			0	H
12S rRNA	69	1,021	953			0	H
tRNA-Val	1,022	1,093	72			0	H
16S rRNA	1,094	2,780	1,687			0	H
tRNA-Leu	2,781	2,856	76			0	H
nd1	2,857	3,831	975	ATG	TAG	0	H
tRNA-Ile	3,835	3,904	70			3	L
tRNA-Gln	3,904	3,974	71			-1	H
tRNA-Met	3,974	4,042	69			-1	H
nd2	4,043	5,089	1,047	ATG	TAA	0	H
tRNA-Trp	5,091	5,161	71			1	H
tRNA-Ala	5,164	5,232	69			2	L
tRNA-Asn	5,234	5,306	73			1	L
tRNA-Cys	5,341	5,406	66			34	L
tRNA-Tyr	5,407	5,477	71			0	L
cox1	5,479	7,032	1,554	GTG	TAA	1	H
tRNA-Ser	7,033	7,103	71			0	L
tRNA-Asp	7,107	7,178	72			3	H
cox2	7,181	7,871	691	ATG	T--	2	H
tRNA-Lys	7,872	7,946	75			0	H
atp8	7,948	8,112	165	ATG	TAA	1	H
atp6	8,106	8,789	684	ATG	TAA	-7	H
cox3	8,789	9,572	784	ATG	T--	-1	H
tRNA-Gly	9,573	9,644	72			0	H
nd3	9,645	9,993	349	ATG	T--	0	H
tRNA-Arg	9,994	10,062	69			0	H
nd4l	10,063	10,359	297	ATG	TAA	0	H
nd4	10,353	11,733	1,381	ATG	T--	-7	H
tRNA-His	11,734	11,802	69			0	H
tRNA-Ser	11,803	11,870	68			0	H
tRNA-Leu	11,875	11,947	73			4	H
nd5	11,948	13,786	1,839	ATG	TAG	0	H
nd6	13,783	14,301	519	ATG	TAA	-4	L
tRNA-Glu	14,302	14,370	69			0	L
cytb	14,376	15,516	1,141	ATG	T--	5	H
tRNA-Thr	15,517	15,588	72			0	H
tRNA-Pro	15,588	15,657	70			-1	L
D-loop	15,658	16,504	847			0	H

values (Figs. 7–8). *Oxyurichthys formosanus* however was revealed to be sister to a clade of *Gobiopsis* lineage species of the family Gobiidae.

DISCUSSION

The mitogenomes of the two *Oxyurichthys* species sequenced here contain 37 genes typical of the metazoan mitogenome. The total length between the two sequences is comparable, differing only by 2 bp. The length of goby mitogenomes range from 16,396 bp (*Pomatoschistus minutus*) to 18,999 bp (*Neogobius melanostomus*) (Adrian-Kalchhauser et al. 2017). The mitogenome lengths for *O. ophthalmonema* (16,504 bp) and *O. microlepis* (16,506 bp) fall within this range. Gene arrangement of the two *Oxyurichthys* species examined in this study is similar to the majority of recorded goby mitogenomes. In general, gene arrangement in goby mitogenomes is pretty conservative with few exceptions. For example, in the case of the gene arrangement of *O. ophthalmonema*, *O. microlepis* and most goby species, the order is Ile/Gln/Met, while it is Gln/Ile/Met in *Neogobius melanostomus*

Table 2. Gene organization of *Oxyurichthys microlepis* mitogenome

Gene	Position		Size	Codon		Intergenic nucleotide	Strand
	From	To		Start	Stop		
tRNA-Phe	1	68	68			0	H
12S rRNA	69	1,021	953			0	H
tRNA-Val	1,022	1,093	72			0	H
16S rRNA	1,094	2,780	1,687			0	H
tRNA-Leu	2,781	2,856	76			0	H
nd1	2,857	3,831	975	ATG	TAG	0	H
tRNA-Ile	3,835	3,904	70			3	L
tRNA-Gln	3,904	3,974	71			-1	H
tRNA-Met	3,974	4,042	69			-1	H
nd2	4,043	5,089	1,047	ATG	TAA	0	H
tRNA-Trp	5,091	5,161	71			1	H
tRNA-Ala	5,164	5,232	69			2	L
tRNA-Asn	5,234	5,306	73			1	L
tRNA-Cys	5,341	5,406	66			34	L
tRNA-Tyr	5,407	5,477	71			0	L
cox1	5,479	7,032	1,554	GTG	TAA	1	H
tRNA-Ser	7,033	7,103	71			0	L
tRNA-Asp	7,107	7,178	72			3	H
cox2	7,182	7,872	691	ATG	T--	3	H
tRNA-Lys	7,873	7,947	75			0	H
atp8	7,949	8,113	165	ATG	TAA	1	H
atp6	8,107	8,790	684	ATG	TAA	-7	H
cox3	8,790	9,573	784	ATG	T--	-1	H
tRNA-Gly	9,574	9,645	72			0	H
nd3	9,646	9,994	349	ATG	T--	0	H
tRNA-Arg	9,995	10,063	69			0	H
nd4l	10,064	10,360	297	ATG	TAA	0	H
nd4	10,354	11,734	1,381	ATG	T--	-7	H
tRNA-His	11,735	11,803	69			0	H
tRNA-Ser	11,804	11,871	68			0	H
tRNA-Leu	11,876	11,948	73			4	H
nd5	11,949	13,787	1,839	ATG	TAA	0	H
nd6	13,784	14,302	519	ATG	TAA	-4	L
tRNA-Glu	14,303	14,371	69			0	L
cytb	14,377	15,517	1,141	ATG	T--	5	H
tRNA-Thr	15,518	15,589	72			0	H
tRNA-Pro	15,589	15,658	70			-1	L
D-loop	15,659	16,506	848			0	H

and *Ponticola kessleri* (Adrian-Kalchhauser et al. 2017).

The putative origin of L-strand replication and control region of *O. ophthalmonema* and *O. microlepis* identified here were similar to those found for other goby mitogenomes. The control region showed rich A-T content with 63.8% for *O. ophthalmonema* and 63.1% for *O. microlepis*. These values are comparable to previous records such as 64.2 % for *Tridentiger bifasciatus* and 64.5% for *T. barbatus* (Jin et al. 2015). Conservative elements in the control region are expected as they play an important role in duplication and transcription of mitogenome. A number of conservative elements were identified in the two *Oxyurichthys* mitogenomes, consistent with previously recorded goby mitogenomes. The termination-associated sequence (TAS) is reported to be a recognition site for termination of heavy strand synthesis (Jin et al. 2015). Meanwhile, the conserved sequence block domains (CSB-1, CSB-2 and CSB-3) may function in positioning RNA polymerase for transcription as well as for priming replication (Clayton 1991; Shadel and Clayton 1997). In addition, the central conserved sequence (CSB-D) was recorded in *O. ophthalmonema* and *O. microlepis* just as it had been in mitogenomes of other gobies (Jin et al. 2015; Adrian-Kalchhauser et al. 2017). CSB-D is well-known as a universally conserved block in the mitogenomes of teleost fishes (Lee et al. 1995).

For phylogenetic analyses, 87 mitogenome sequences belonging to the Gobiidae and Oxudercidae and relatives were included (Table S1). The BI and ML

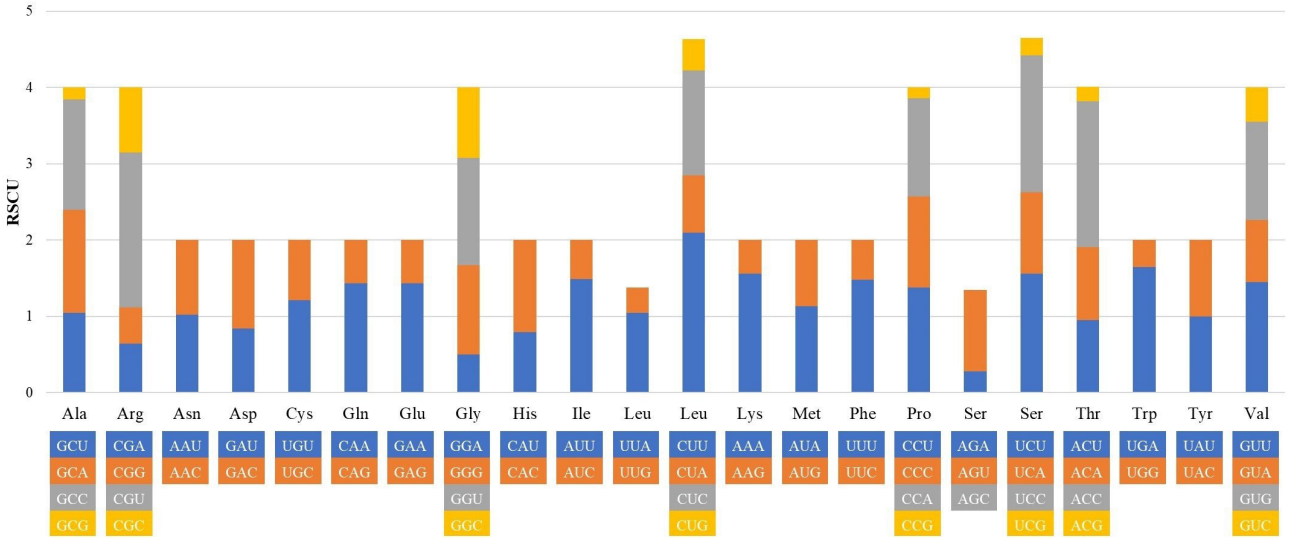

Fig. 3. Relative synonymous codon usage of Protein-coding genes in *Oxyurichthys ophthalmonema*.

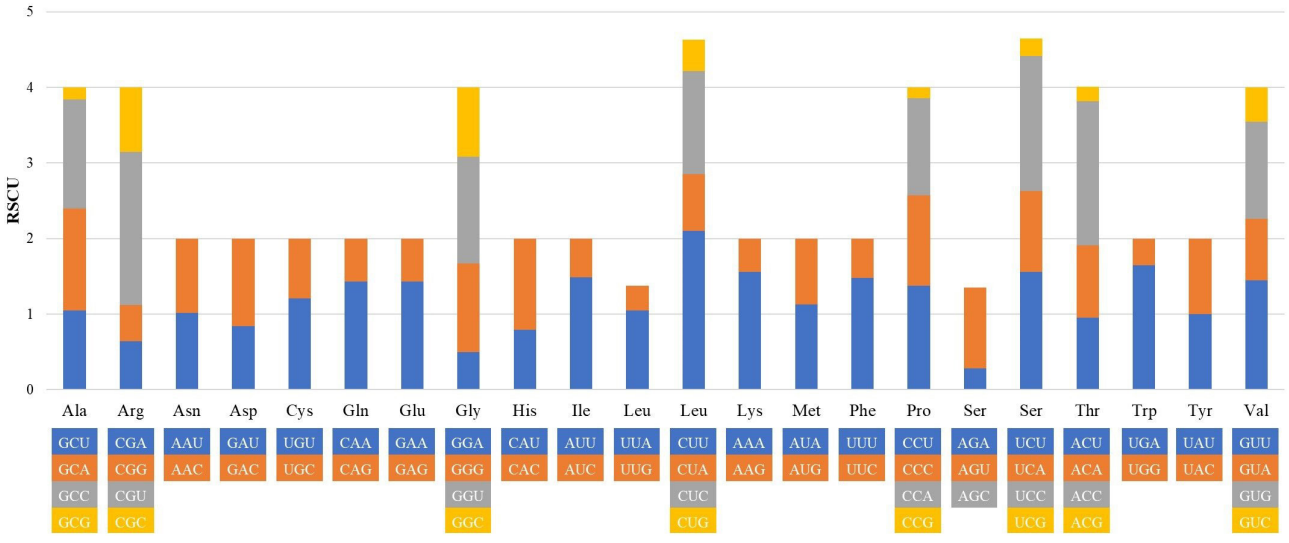

Fig. 4. Relative synonymous codon usage of Protein-coding genes in *Oxyurichthys microlepis*.

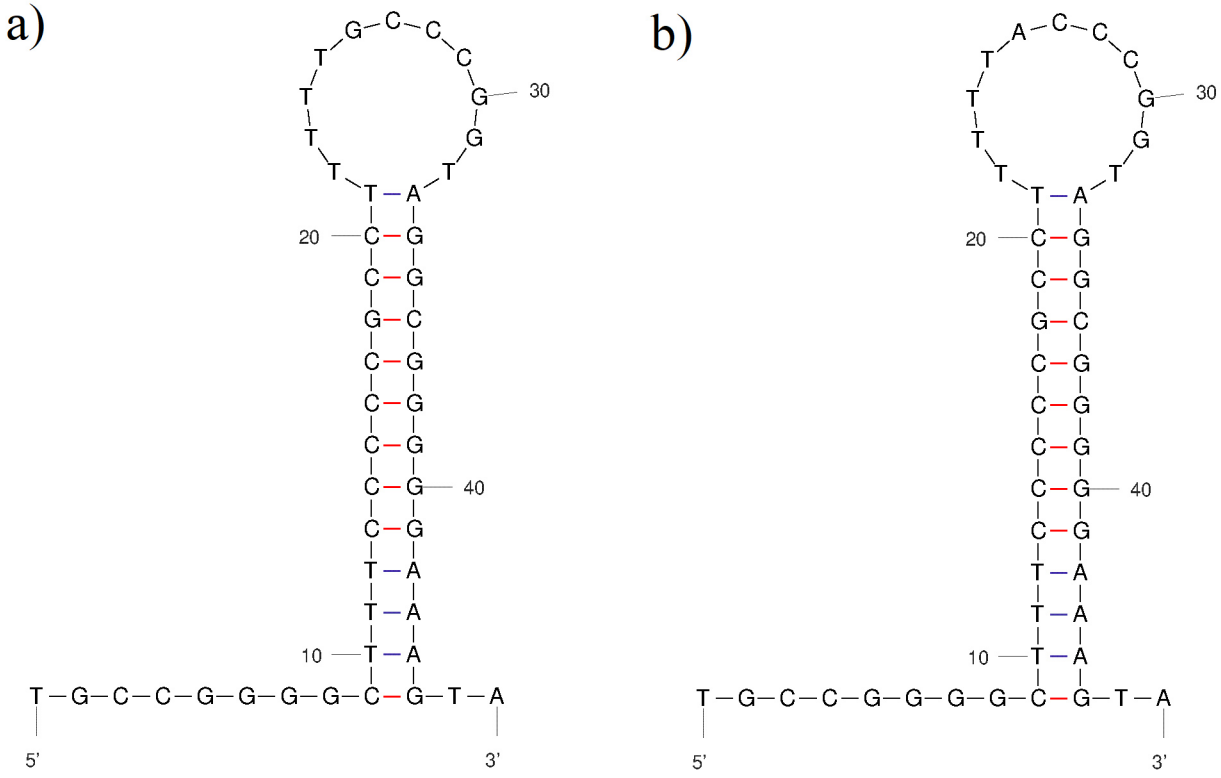

Fig. 5. The putative origin of L-strand replication (OL) of *Oxyurichthys ophthalmonema* (a) and *Oxyurichthys microlepis* (b).

Fig. 6. Termination-associated sequences (TAS), conserved sequence blocks (CSB-1, CSB-2, and CSB-3) and central conserved sequences (CSB-D) and GTGGG box in control region of two *Oxyurichthys* species mitogenomes.

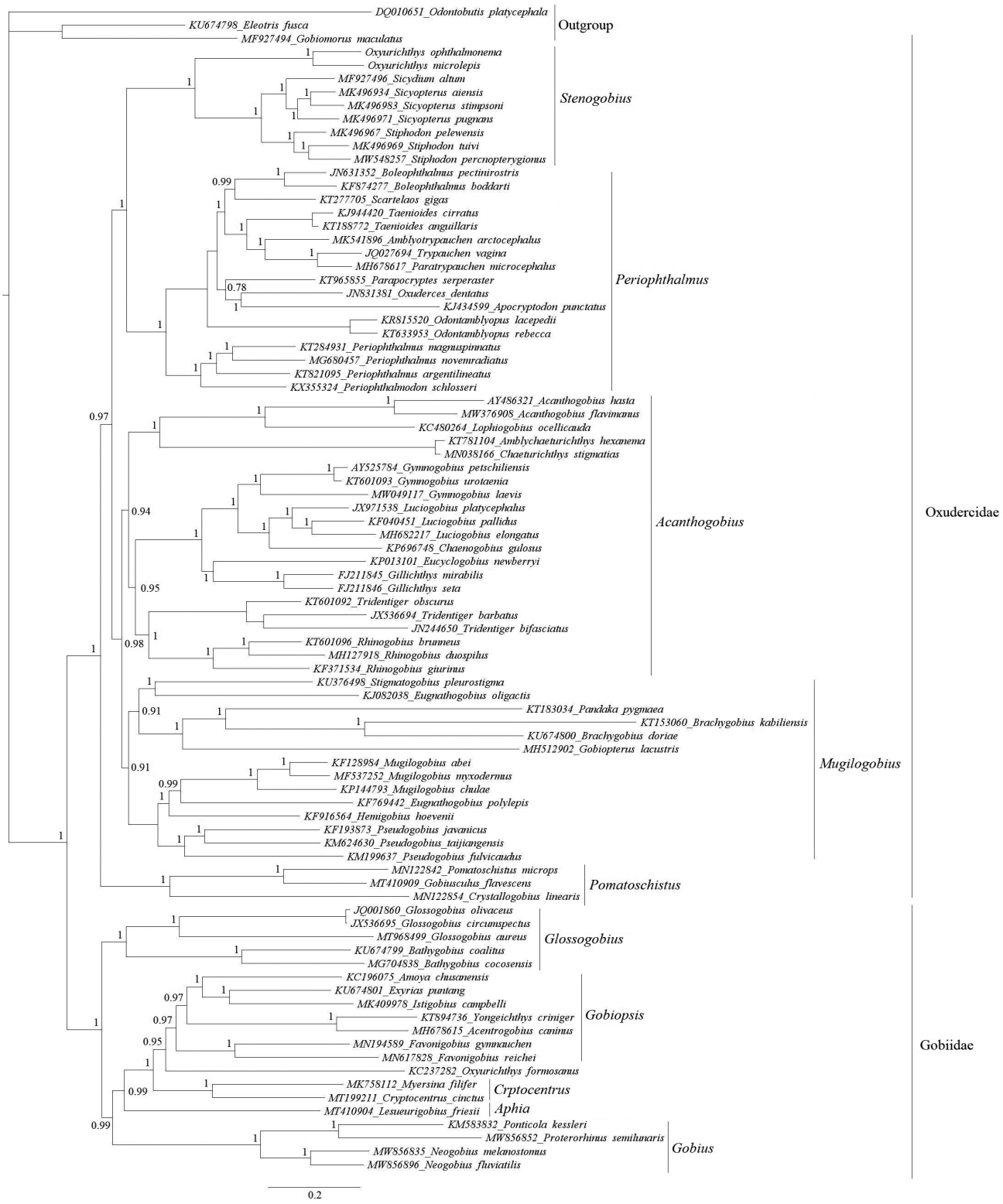

Fig. 7. Phylogenetic trees of goby derived from Bayesian Inference (BI) method based on 13 PCGs + 2 rRNAs. The numbers at nodes are posterior probability values. GenBank accession numbers are placed in front of species names.

phylogenetic tree with various representatives showed almost similar topologies. Gobiidae and Oxudercidae species formed separate monophyletic clades as observed in previous studies (Thacker 2009; Tornabene et al. 2013; Agorreta et al. 2013). The phylogenetic analyses also recovered monophyletic lineages consistent with previous studies by Thacker and Roje (2011), Agorreta et al. (2013) and Tornabene et al. (2013).

An unusual case observed in the present study

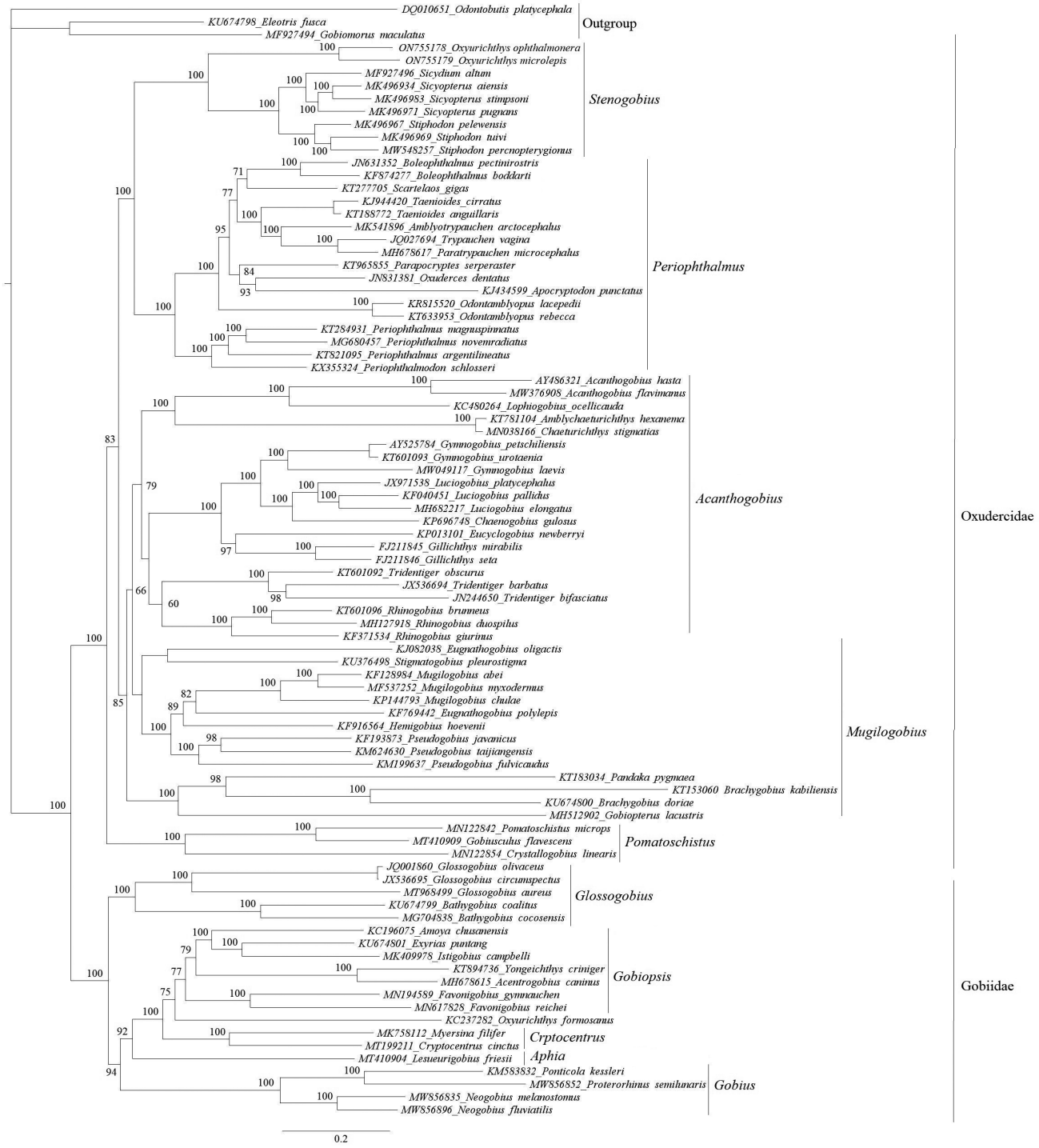

Fig. 8. Phylogenetic trees of goby derived from Maximum Likelihood (ML) method based on 13 PCGs + 2 rRNAs. The numbers at nodes are ultrafast bootstrap values. GenBank accession numbers are placed in front of species names.

was the position of *Oxyurichthys formosanus* (GenBank accession number: KC237282). While *O. formosanus* was expected to cluster with the other species of the genus represented here, it was found among the Gobiidae sister to representatives of the *Gobiopsis* lineage. This clade was in turn sister to two members of the *Cryptocentrus* lineage, *Cryptocentrus cinctus* and *Myersina filifer*. A similar result for the phylogenetic placement of *O. formosanus* was recorded in a previous mitogenome study (Adrian-Kalchhauser et al. 2017). It is worth noting that in the original publication, authors of this mitogenome labeled it as *Cryptocentrus yatsui* (Sun et al. 2013). Additionally, *Oxyurichthys formosanus* is an *Oligolepis* species (Pezold and Larson 2015). The genus *Oligolepis* has been demonstrated both in morphological and molecular analyses to be a member of the *Stenogobius* lineage (Pezold 2004; Tornabene et al. 2013). With two mitogenomes of *Oxyurichthys* sequenced from the present study, we can confirm that the mitogenome of *O. formosanus* available in GenBank does not belong to the genus *Oxyurichthys*.

CONCLUSIONS

The present study sequenced and characterized the mitogenomes of two species of *Oxyurichthys*, *O. ophthalmonema* and *O. microlepis*. Comparative analyses of *O. ophthalmonema* and *O. microlepis* mitogenomes showed high similarities in total length, gene arrangement and control region. Our study revealed conservative characteristics observed in mitogenomes of other gobies in previous studies. Phylogenetic analyses indicated that *O. ophthalmonema* and *O. microlepis* formed a cluster that was sister to other members of the *Stenogobius* lineage. In addition, our phylogenetic trees supported lineages of goby species recovered as monophyletic in recent studies using other molecular markers. Our study provides insight into the relationships between *Oxyurichthys* and related species. It also demonstrates that the mitogenome is a promising marker for investigation of goby systematics. In future studies, comprehensive sampling for mitogenomes is necessary to clarify the relationships among goby genera.

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Competing interests: The authors declare that they have no conflicts of interest.

Availability of data and materials: Mitogenome sequences of *Oxyurichthys ophthalmonema* and *Oxyurichthys microlepis* are available in GenBank with accession numbers: ON755178 and ON755179.

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Ethics approval consent to participate: Not applicable.

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Supplementary materials

Fig. S1. Secondary structures of tRNAs of *Oxyurichthys ophthalmonema* mitogenome. (download)

Fig. S2. Secondary structures of tRNAs of *Oxyurichthys microlepis* mitogenome. (download)

Fig. S3. Phylogenetic trees of goby derived from Bayesian Inference (BI) method based on 13 PCGs. The numbers at nodes are posterior probability values. GenBank accession numbers are placed in front of species names. (download)

Fig. S4. Phylogenetic trees of goby derived from Maximum Likelihood (ML) method based on 13 PCGs. The numbers at nodes are ultrafast bootstrap values. GenBank accession numbers are placed in front of species names. (download)

Table S1. Primers used for amplification of *Oxyurichthys ophthalmonema* and *Oxyurichthys microlepis* mitogenomes. (download)

Table S2. Mitogenome sequences obtained from GenBank for phylogenetic analyses. (download)