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MITOGENOME REPORT

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Complete mitochondrial genome of larged-eye pygmy goby *Trimma macrophthalmus* (Teleostei, Gobiidae) and its phylogenetic implications

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ABSTRACT

Trimma macrophthalmus is a colorful pygmy goby that is widely associated with coral and distributed in the Indo-Pacific Ocean. Here, the complete mitochondrial genome of *T. macrophthalmus* was first sequenced and annotated. The entire mitogenome is a typical circular molecule (16,943 bp in total length) containing 13 protein-coding genes, 2 ribosomal RNA genes, 22 transfer RNA genes, and 1 control region (CR). The overall base composition is 29.6% for A, 15.6% for G, 27.9% for C and 26.9% for T. Phylogenetic tree was reconstructed using concatenated 13 protein coding genes sequences of the 38 related taxa. The phylogenetic analysis indicated that *T. macrophthalmus* was most closely related to *T. okinawae*. These findings will contribute for further genetic studies such as evolution, DNA barcoding and molecular phylogeny of genus *Trimma* and related gobiid fishes.

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Mitogenome; Gobiinae; phylogeny; molecular DNA; *Trimma*

1. Introduction

The gobiid fishes of the genus *Trimma* Jordan and Seale 1906, the so-called pygmy gobies, are small-sized and colorful fishes that are found widely associated with corals and rocky reefs at shallow and deep water in the Indo-Pacific Ocean (Winterbottom and Hoese 2015; Winterbottom 2019, Winterbottom and Pyle 2022a, 2022b). With 110 taxonomically valid species worldwide and this number is expected to grow, *Trimma* is currently the second most speciose marine genus in the family Gobiidae (Winterbottom and Hoese 2015; Winterbottom 2019; Winterbottom and Pyle 2022a, 2022b). To date, complete mitochondrial genomes are only available for *Trimma caesiura* Jordan and Seale 1906 and *T. okinawae* (Aoyagi 1949) collected from Japan (Hanahara et al. 2019). *Trimma macrophthalmus* (Tomiyama 1936) has wide range of geographical distribution, ranging from the Cocos Island in the Indian Ocean, across to Indonesia, Taiwan, Japan, New Guinea and Fiji in Pacific Ocean (Shao and Chen 1993; Winterbottom et al. 2014; 2020; Winterbottom and Hoese 2015). In the present study, we sequenced for the first time the complete genome of *T. macrophthalmus* to give better understanding for future molecular studies of this gobiid fish group.

Figure 1. Freshly collected specimen of *T. macrophthalmus*, NTOUP-2022-01-040. Photo by tonisman Harefa.

2. Materials and methods

2.1. Sample collection and DNA extraction

Fish specimens of *Trimma macrophthalmus* (Figure 1) were collected alive from Hongchaikeng, Hengchun Township, Pingtung County, Taiwan (location: 21.97027° N, 120.71480° E), in January 2022 using hand net while SCUBA diving. Samples for DNA analysis were preserved in 95% ethanol and fish specimens deposited at National Taiwan Ocean University (http://imb.ntou.edu.tw/, I-Shiung Chen, iscfish@gmail.com) with voucher number NTOUP-2022-01-040. Total genomic DNA was obtained from fins tissue using Tissue and Cell Genomic DNA Purification Kit (GenMark, Taichung, Taiwan).

2.2. PCR amplification and sequencing

The complete mitogenome of *T. macrophthalmus* was amplified and sequenced using 12 pairs of primer designed based

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Table 1. Nucleotide composition comparison of three complete mitogenomes of *Trimma*, including *T. macrophthalmus* (TM), *T. caesiura* (TC) and *T. okinawae* (to).

	Length (bp)			A%			T%			C%			G%			A + T%		
	TM	TC	TO	TM	TC	TO	TM	TC	TO	TM	TC	TO	TM	TC	TO	TM	TC	TO
Genome	16943	17137	17969	29.6	27.4	28.6	26.9	27.3	26.7	27.9	28.4	28.4	15.6	17.0	16.2	57.5	54.7	55.3
PCGs	11496	11456	11481	26.6	24.6	26.1	28.9	29.6	28.9	29.0	29.1	28.9	15.5	16.8	16.0	55.5	54.2	55.0
rRNA	2648	2787	2642	34.1	32.6	33.5	21.0	22.1	21.2	25.7	25.9	26.4	19.3	19.5	18.8	55.1	54.7	54.7
tRNA	1551	1549	1629	31.2	30.1	30.5	24.6	23.8	25.2	25.1	25.4	24.2	19.0	20.6	20.0	55.8	53.9	55.7
Dloop	1103	1008	1201	36.9	32.1	31.1	34.9	30.1	33.5	15.0	21.9	19.1	13.2	15.9	16.3	71.8	62.2	64.6

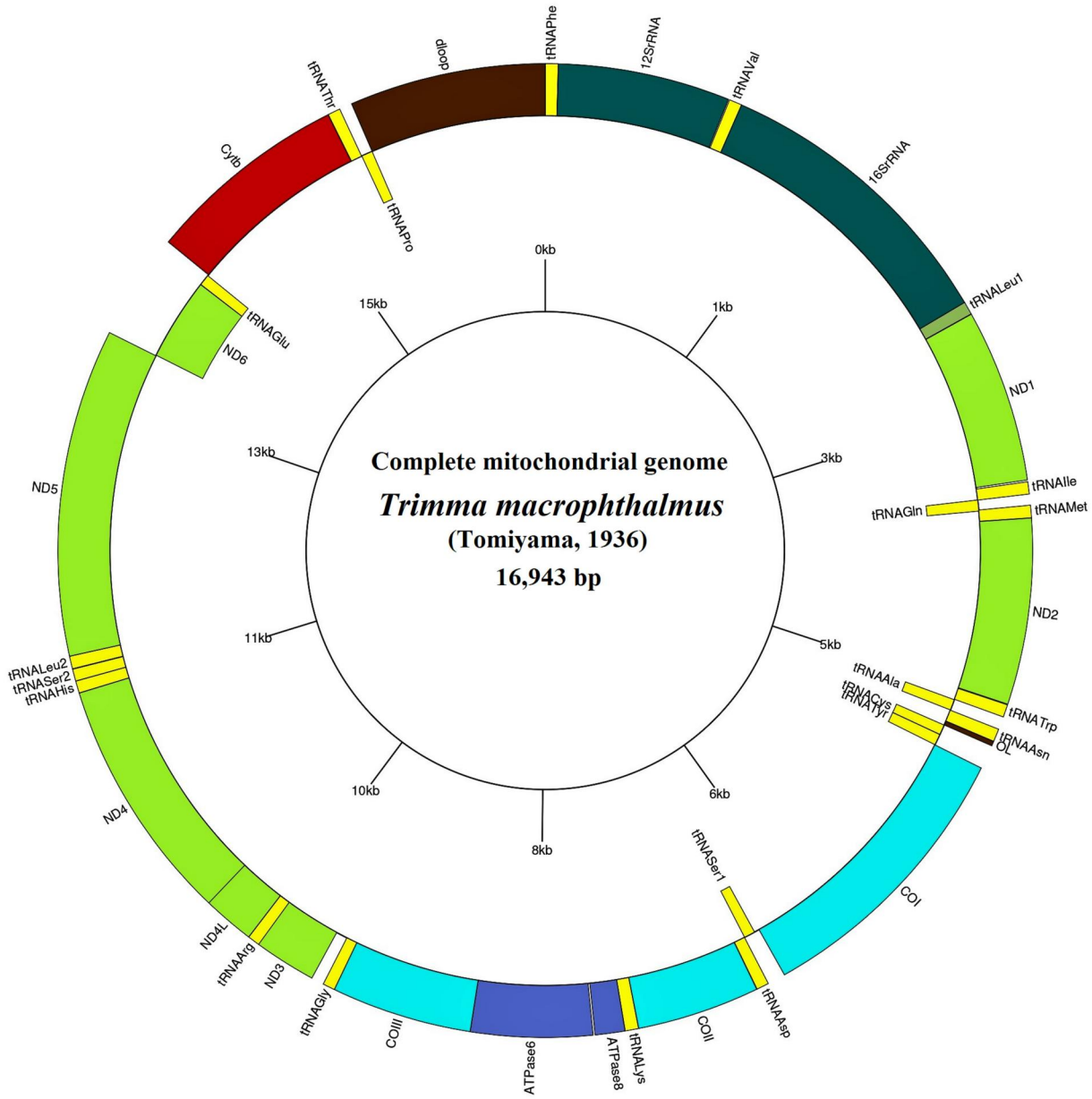

Figure 2. Circular map of the *T. macropthalmus* complete mitogenome.

on conserved nucleotide sequences of two available *Trimma* mitogenomes (*i.e. T. caesiura* and *T. okinawae*) downloaded from GenBank. PCR product were purified using High Pure PCR Product Purification Kit (Roche, Germany). Purified PCR products were sequenced using the same as PCR primer and some new designed by authors on an ABI3730XL DNA Analyzer at Academia Sinica (Nangang, Taiwan).

2.3. Annotation and analysis

All available DNA fragments of mitogenomic sequences were assembled using BIOEDIT (Hall 1999). Identification of protein-coding genes (PCGs) and rRNA genes were refined by comparing to corresponding complete mitogenome *Trimma* and other closed related species using CLUSTAL W (Thompson et al. 1994). All tRNA genes were verified by their

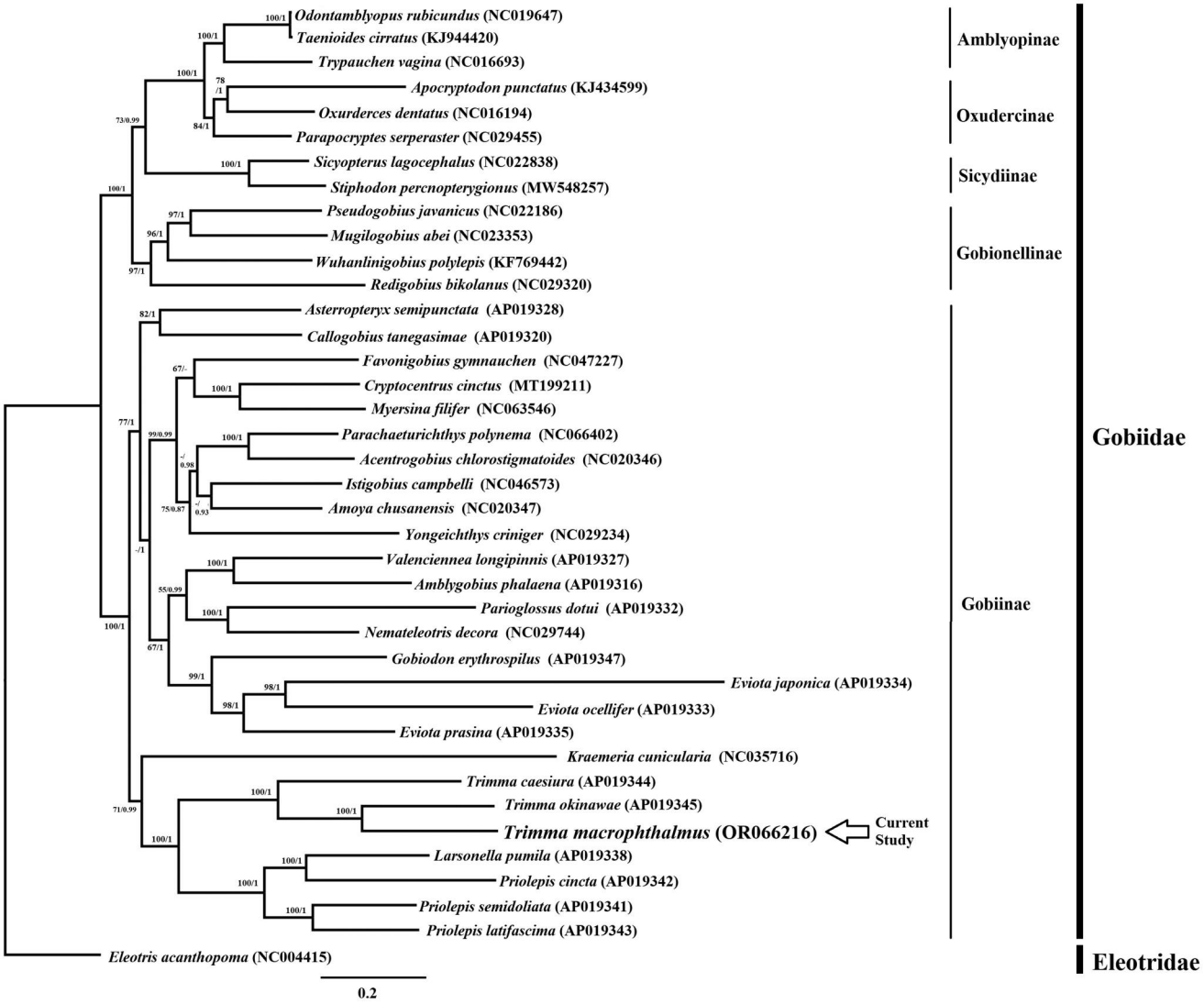

Figure 3. Phylogenetic tree of *T. macrophthalmus* with other members of Gobiidae family based on 13 PCGs using GTR + G + I model of ML and BI analysis. Bootstrap values >50 and posterior probabilities are shown on at the nodes. Each species is followed by its corresponding accession number. *Eleotris acanthopoma* as outgroup.

proposed cloverleaf secondary structures and anticodons using tRNA-scan SE 2.0 (Lowe and Chan 2016). Base composition was estimated using MEGA X (Kumar et al. 2018). Phylogenetic relationship was analyzed based on concatenated 13 PCGs sequences of *T. macrophthalmus* and other 38 members of Gobiidae fish downloaded from GenBank (Table S2). Additionally, one eleotrid fish *Eleotris acanthopoma* Bleeker 1853 was chosen as outgroup Maximum likelihood (ML) was performed using RAXML (Stamatakis 2016) with 1000 bootstrap. Bayesian inference was performed using MrBayes (Ronquist et al. 2012) with Marcov Chain Monte Carlo (MCMC) set 1,000,000 generations and were sampled every 100 generations and the first 500 of samples were discarded as burn-in. MRMODELTEST (Nylander 2004) was used to find best model parameter according to the Akaike Information Criterion (AIC) which was performed using PAUP (Swofford 2003). Phylogenetic tree was viewed and edited in FIGTREE v 1.3.1 (Rambaut 2009).

3. Results and discussions

3.1. Genome characteristic and organization

The complete mitochondrial of *T. macropthalmus* (GenBank accession number: OR066216) has 16,943 bp in total length and is relatively small compared to other two *Trimma* mitogenomes (Table 1). This mitogenome contains coding regions with 37 genes (13 PCGs 22 tRNAs, 2rRNA) and non-coding region (d-loop) (Figure 2, Table S1). Gene arrangement is identical to other gobiid fish (Chiang et al. 2013, 2015; Huang et al. 2015a, 2015b; Chen and Wen 2016; Wen and Chen 2016; Huang et al. 2016a, 2016b, 2016c; Harefa and Chen 2022). Overall base composition of T, C, A and G was 26.9%, 27.9%, 29.6% and 15.6%, respectively. In this genome, total A + T content is relatively high compared to other two *Trimma* mitogenomes. The 13 PCGs, overall length 11,496 bp, were comprised of one subunit of cytochrome b (*CYTB),* two subunits of ATP synthases (*ATP6, ATP8*), three subunits of

cytochrome c oxidase (*COI-III*), and seven subunits of NADH (*ND1-6, ND4L*). Among the available *Trimma* mitogenomes, *T. macrophthalmus* has the largest total PCGs. Twelve PCGs sequences were located on H-strand whereas *ND6* is located on L-strand. Except for *COI* which started with GTG, all other PCGs contained the same start codon ATG, however, codon terminations were found to vary, either with TAA (*ND2, ND4L, ND5, COI, ATP8*), TAG (*ND1, ND3, ND6*), TA- (*ATP6, COIII*), or T– (*COII, ND4, CYTB*). 22 sets of tRNA genes, lengths ranging from 64 to 76 bp, were distributed through the mitogenome of *T. macrophthalmus*. Eight tRNAs were located on the L-strand (tRNA-*Gln,* tRNA-*Ala,* tRNA-*Asn,* tRNA-*Cys,* tRNA-*Tyr,* tRNA-*Ser1*, tRNA-*Glu* and tRNA-*Pro*) and the remaining were located on the H-strand. Two rRNA genes, 12S rRNA (969 bp in length, located between tRNA-*Phe* and tRNA-*Val*) and 16S rRNA (1679 bp in length, located between tRNA-*Val* and tRNA-*Leu1*). In this mitogenome, *ATP 8* and 16S rRNA genes are relatively small, while COIII gene is relatively large compared to other two *Trimma* mitogenomes (Table S3). The dloop was 1103 bp in length and located between tRNA-*Pro* and tRNA-*Phe*.

3.2. Phylogenetic consideration among other gobiid fish

The phylogenetic tree resulting from ML and BI analysis based on concatenated sequences of 13 PCGs showed the same topology and revealed that 38 taxa were found within Gobiidae lineage which divided into five clades (Amblyopinae, Oxudercinae, Sicydiinae, Gobionellinae, and Gobiinae subfamily). This result is consistent with previous classification of Gobiidae based on morphological characters (Pezold 1993; Gill and Mooi 2012) (Figure 3). The mitogenomic phylogenetic perspective confirms that *Trimma* is a considerably monophyletic group based on the current data of three available species. Furthermore, phylogenetic analysis revealed that *T. macrophthalmus* were most closely related to *T. okinawae*. The genetic distance among *T. macrophthalmus* and two other congeners were analyzed based on Kimura 2 parameter model (K2P) (Kimura 1980), ranging from 24.5-30.7%. In addition, the tree also revealed that *Larsonella pumilus* (Larson and Hoese 1980) nested within the typical *Priolepis* clade which is similar with previous molecular phylogenetic study based on whole mitogenome (Hanahara et al. 2019). As monotypic genus, *Larsonella* distinguishing merely by fewer extension of squamation on its body (Hoese and Larson 2010) which feature is not always set for gobiid generic criteria. Therefore, the molecular phylogenetic result strongly suggests that *Larsonella* might be reconsidered a subgenus within *Priolepis*. Extensive materials or detailed morphological survey would help us to completely resolve the relationship within the gobiid members of *Priolepis*, *Larsonella*, even *Lubricogobius* and other related taxa (Figure 3).

4. Conclusions

In this study, we successfully sequenced and annotated the complete mitochondrial genome of *Trimma macrophthalmus*. Total length sequence was 16,943 bp (GenBank: OR066216), consist of 37 genes and 2 non-coding regions. A phylogenetic tree was reconstructed using concatenated 13 PCGs sequences showed that *Trimma* formed a stable monophyletic group. The tree also revealed that *T. macrophthalmus* was most closely related to *T. okinawae*. The information of the present study will provide an essential and important data for further genetic study among all members of *Trimma*.

Author contributions

TH: Sample collection, PCR experiments, data analysis, drafting of manuscript, revising manuscript, final approval of the version to be published. **ISC**: Conception and design, data analysis, revising manuscript, final approval of the version to be published. All authors agree to be accountable for all aspects of the work.

Ethic Approval

Approval of the field experiment and sample collection was provided by Kenting National Park under research permit (1101019460).

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

Mitochondrial genome sequence can be accessed *via* accession number OR066216 in GenBank of NCBI (https://www.ncbi.nlm.nih.gov/nuccore/OR066216). The associated BioProject, SRA, and Bio-Sample numbers are PRJNA976490, SRR24743753, and SAMN35370829, respectively.

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