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Mitogenome of *Gymnothorax minor* and Phylogenetic Relationship with Its Congeners and Related Genera (Anguilliformes: Muraenidae)

(Mitogenom *Gymnothorax minor* dan Hubungan Filogenetik dengan Genus yang Berhubung Kait (Anguilliformes: Muraenidae))

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ABSTRACT

Gymnothorax minor *is a moray eel of the family Muraenidae found in the Western Pacific Ocean. We report here its complete mitogenome as determined by Illumina next-generation sequencing and the phylogenetic relationship with its congeners and other taxa of the family Muraenidae. The whole mitogenome of* G. minor *had a total length of 16,574 bp, comprising 37 genes - 13 protein-coding genes (PCGs), two ribosomal ribonucleic acid (rRNA) and 22 transfer ribonucleic acid (tRNA) genes - and a control region. Excepting* cox1 *with GTG, the other 12 PCGs had ATG start codon. Seven of its PCGs had incomplete stop codon - five (*nad2*;* cox1*;* cox2*;* nad3 *and* nad4*) with T and two (*atp6 *and* cox3*) with TA. Molecular phylogeny based on 13 PCGs was concordant with 15 mitochondrial genes (13 PCGs and 2 rRNA genes). The subfamily Muraeninae as well as the subfamily Uropterygiinae were monophyletic. However, the genus* Gymnothorax *was paraphyletic, with* G. minor *forming a sister group with* Rhinomuraena quaesita *in the lineage containing also* G. kidako *and* G. formosus *forming a sister group with* Enchelynassa canina. *The phylogenetic relationship of the genus* Gymnothorax *and related taxa of the family Muraenidae, based on the mitochondrial* cob *gene, was in general similar to that based on 15 mt-genes. The mitogenome is useful for future studies on phylogenetics and systematics of eels of the family Muraenidae and other taxa of the order Anguilliformes.*

Keywords: Gymnothorax*; mitogenome; moray eels; Muraenidae; next-generation sequencing (NGS)*

ABSTRAK

Gymnothorax minor *adalah belut laut dalam famili Muraenidae yang boleh dijumpai di Lautan Pasifik Barat. Kami melaporkan di sini mitogenom lengkap* G. minor *yang ditentukan dengan menggunakan penjujukan generasi akan datang Illumina dan hubungan filogenetik* G. minor *dengan genus yang berhubung kait dan takson yang lain dalam famili Muraenidae. Kepanjangan keseluruhan mitogenom* G. minor *adalah 16,574 bp yang terdiri daripada 37 gen - 13 gen pengkodan protein (PCGs), dua asid ribonukleik ribosoma (rRNA) dan 22 gen pemindahan ribonukleik ribosoma (tRNA) - dan satu kawasan kawalan. Dua belas PCGs mempunyai kodon pemula ATG kecuali* cox1 *dengan kodon pemula GTG. Tujuh PCGs mempunyai kodon penamat yang tidak lengkap - lima gen (*nad2*;* cox1*;* cox2*;* nad3 *dan* nad4*) dengan T dan dua gen (*atp6 *dan* cox3*) dengan TA. Filogeni molekul berdasarkan 13 PCGs adalah konsisten dengan 15 gen mitokondria (13 gen PCGs dan 2 rRNA). Subfamilli Muraeninae dan subfamili Uropterygiinae adalah monofiletik. Walau bagaimanapun, genus* Gymnothorax *adalah parafiletik dengan* G. minor *membentuk kumpulan beradik dengan* Rhinomuraena quaesita *dalam garis keturunan yang mengandungi* G. kidako *manakala* G. formosus *membentuk kumpulan beradik dengan* Enchelynassa canina*. Berdasarkan gen mitokondria* cob*, hubungan filogenetik genus* Gymnothorax *dan takson lain dalam famili Muraenidae adalah sama dengan hubungan filogenetik yang berdasarkan 15 gen mitokondria. Mitogenom adalah penting untuk kajian filogenetik dan sistematik belut dalam famili Muraenidae dan takson yang lain dalam order Anguilliformes.*

Kata kunci: Belut laut; Gymnothorax*; mitogenom; Muraenidae; penjujukan generasi akan datang (NGS)*

INTRODUCTION

Moray eels of the genus *Gymnothorax* Bloch 1795 are members of the family Muraenidae (order Anguilliformes). The genus is represented by 126 recognised species, distributed in the Atlantic, Indian and Pacific oceans (Froese & Pauly 2017). An earlier checklist documented 123 species by Smith (2012).

The genus *Gymnothorax* is distinguished from other genera of the family by the possession of a line of pigment along the dorsal midline before the dorsal fin origin (Smith & Böhlke 1997). Members of the genus range in total length from 6.5 cm (*G. parini*) to 300 cm (*G. favagineus* and *G. javanicus*) (www.fishbase.org). Most of the species (13/16) studied to date possess a diploid number of 2n =

42 chromosomes, excepting 2n = 40 for *G. javanicus* and 2n = 36 for *G. flavimarginatus* and *G. kidako* (Coluccia et al. 2015).

Two species of *Gymnothorax* eels (*G. hepaticus* and *G. undulatus*) from the Pacific, both sympatric with two species of sea snakes (*Aipysurus laevis* – a dietary generalist, and *Laticauda colubrina* – an eel specialist) were more resistant to the venom of the diet specialist snake than to that of the generalist (Heatwole & Poran 1995). On the other hand, another eel *G. moringa* from the Caribbean, where no sea snakes occur, was sensitive to sea krait venom (Heatwole & Powell 1998). The resistance of the Pacific *Gymnothorax* to sea krait venom has been attributed to coevolution of predator and prey (Heatwole & Powell 1998). There is no report of sea krait venom resistance on *Rhinomuraena*.

Eels of the genus *Gymnothorax* are principally marine inhabitants. An exception is *G. polyuranodon*, the juveniles and adults inhabit primarily fresh and mildly brackish habitats (Ebner et al. 2011), or are able to reside in fresh water for extended periods of time (Tsukamoto et al. 2014). Another species, *G. tile* lives in marine conditions but travels to fresh water for breeding and spawning (Froese & Pauly 2017).

Although the genus *Gymnothorax* is represented by 126 species, its complete mitochondrial genome (mitogenome) has not received much study. There are only two entries in the GenBank – *G. formosus* (KP874184) and *G. kidako* (NC_004417). We report here the complete mitogenome of *G. minor* and phylogenetic relationship with its congeners and related genera.

Materials and Methods

Specimen and mitochondrial DNA extraction

Tissues of *G. minor* were preserved in 95% ethanol and stored at -20°C until use. The mitochondria were isolated by standard differential centrifugation method (White & Desmore 1992) and the mtDNA was extracted using Mitochondrial DNA Isolation Kit (Abnova, Taiwan) following the manufacturer's instructions.

Genome sequencing and analysis

A library was prepared using Nextera DNA Sample Preparation Kit and the mitochondrial genome was sequenced using the Illumina MiSeq Desktop Sequencer (2 × 250 bp paired-end reads) (Illumina, USA). Raw sequences were extracted from the Illumina MiSeq system in FASTQ format and the quality of sequences was evaluated using the FastQC software (Andrews 2010). All the ambiguous nucleotides and reads with an average quality value lower than Q20 were excluded from further analysis. De novo assembly was performed using the CLC Genomic Workbench v.8.0.1 (https://www.qiagenbioinformatics.com/). Contigs greater than 16 kbp were subjected to BLAST (Altschul et al. 1990) alignment against the nucleotide database at the National Center for Biotechnology Information (NCBI). Contigs with hits to mitochondrial genes or genomes were identified and extracted from the CLC Genomic Workbench.

Mitogenome identification, annotation and visualization

The assembled contig identified as the mitogenome was manually examined for overlap at the beginning and end of the sequence to assess circularity. The mitogenome was then annotated by manual validation of the coding regions using the NCBI ORF Finder (https://www.ncbi.nlm.nih.gov/orffinder). Transfer RNA (tRNA) genes were identified by tRNAscan-SE software v.1.21 (Schattner et al. 2005). The sequin file generated from MITOS was edited and submitted to NCBI according to ORF Finder result. The circular mitogenome of *G. minor* was visualized with Blast Ring Image Generator (BRIG) (Alikhan et al. 2011). The mitogenome sequence has been deposited in GenBank – accession number MF448350.

Mitogenomes and cytochrome b nucleotide sequences from GenBank

The mitogenomes of the *Gymnothorax* genus and other Muraenidae taxa available in GenBank were *Gymnothorax formosus* (KP874184), *Gymnothorax kidako* (NC_004417), *Gymnomuraena zebra* (NC_027240), *Enchelynassa canina* (NC_027234), *Rhinomuraena quaesita* (NC_013610), *Anarchias* sp. Ansp (NC_013613) and *Scuticaria tigrina* (KP874183). *Pythonichthys microphthalmus* (NC_013601) (Heterenchelyidae), *Anguilla bengalensis* (NC_006543) (Anguillidae) and *Anguilla luzonensis* (NC_011575) were used as outgroup taxa. In addition to complete mitogenomes, nucleotide sequences of cytochrome *b* (*cob*) gene of over 1100 bp for various *Gymnothorax* taxa in the GenBank (Figure 3) were used to reconstruct molecular phylogeny.

Phylogenetic analysis

Alignment of nucleotide sequences and reconstruction of phylograms based on 13 PCGs, two rRNA genes, 15 mt-genes and cytochrome *b* nucleotide sequences followed Yong et al. (2015a). The total nucleotide sequences of 15 mt-genes was 14,133 bp with AIC model = GTR+Gamma and BIC model = SYM+Gamma while the total nucleotide sequences of the *cob* gene was 1140 bp with AIC model = J3+Gamma and BIC model = HKY85+Gamma.

Results

Mitogenome analysis and features

The complete mitogenome of *G. minor* had a total length of 16,574 bp, comprising 37 genes (13 protein-coding genes – PCGs, two rRNA genes and 22 tRNA genes) and a non-coding region (A+T-rich control region) (Table

1; Figure 1). The total GC content was 44.5%, with base composition of 29.3% A, 26.2% T, 17.8% G and 26.7% C. Spacing sequences ranged from 1 to 23 bp in 10 regions, the largest was between *trnN* and *trnC* genes. The overlaps in five regions ranged from 1 to 10 bp, the largest being between *atp8* and *atp6* genes (Table 1).

Excepting *nad6* located on the N-strand, the other 12 PCGs were located on the major J-strand (Table 1). Both the rRNA genes, 14 of the 22 tRNA genes and the control region were located on the J-strand. The control region (924 bp) was flanked by *trnP* and *trnF* genes. All the tRNAs of *G. minor*, *G. kidako* (NC_004417) and *R. quaesita* (NC_013610) had typical cloverleaf structure (Supplementary Figures S1, S3, S4). However, the *trnS1* of *G. formosus* (KP874184) lacked a DHU-stem (Supplementary Figure S2). Twelve PCGs had ATG start codon, except *cox1* with GTG (Table 1). Seven of the 13 PCGs had incomplete stop codon – five (*nad2*, *cox1*, *cox2*, *nad3* and *nad4*) with T and two (*atp6* and *cox3*) with TA (Table 1).

The supplementary Table S1 summarizes the base composition of the mitochondrial whole genome, protein-coding genes, rRNA genes and control region. The A+T content was higher than G+C content in all the genes including the control region except in *rrnS*. The A+T content for PCGs ranged from 52.5% (*nad4l*) to 57.5% (*nad5*). The GC skewness values for the whole genome, PCGs, rRNA genes and control region were negative (–0.514 to –0.017) indicating bias toward the use of Cs over Gs. The AT skewness value was variable for individual genes.

PHYLOGENETIC RELATIONSHIP

Figure 2 depicts the molecular phylogeny of *G. minor* in relation to other taxa of Muraenidae based on 15 mt-genes (13 PCGs + 2 rRNA genes). The phylogram based on 13 PCGs was congruent with that based on 15 mt-genes. Most of the nodes were well-supported. The subfamily Muraeninae as well as the subfamily Uropterygiinae were monophyletic. The genus *Gymnothorax* was paraphyletic, with *G. minor*

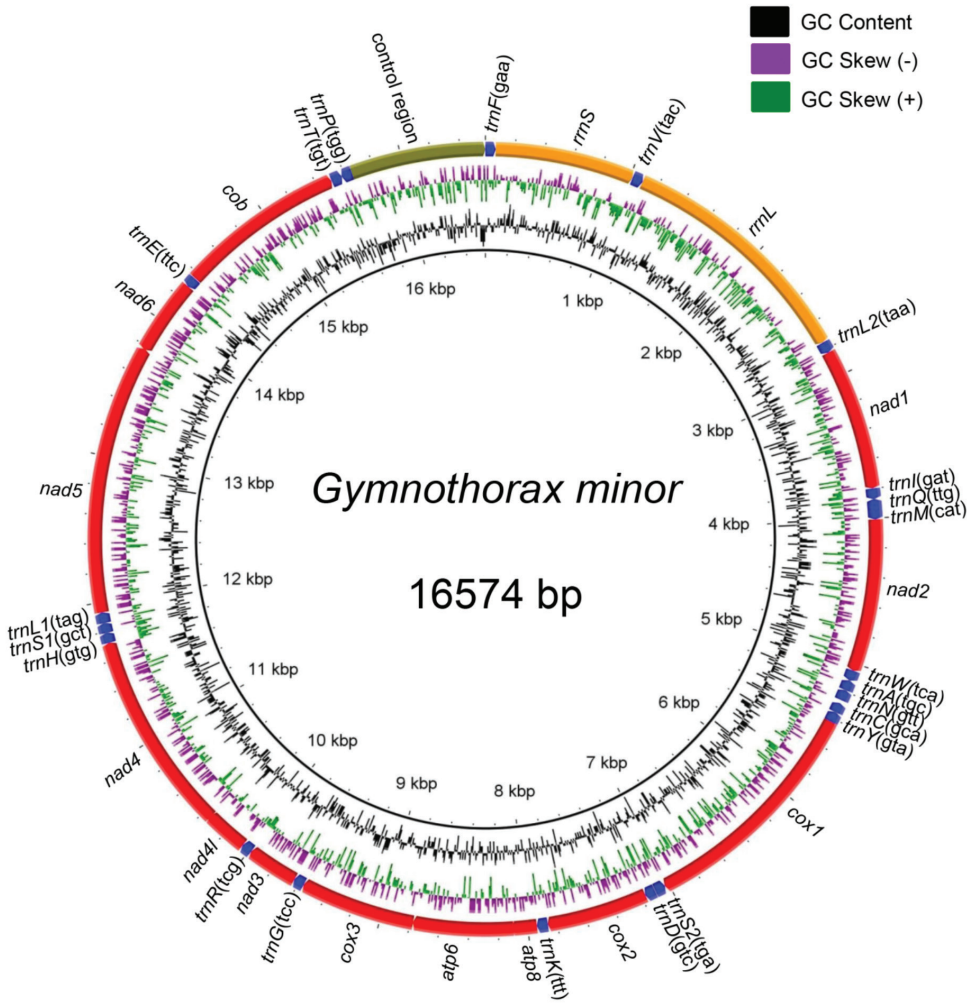

FIGURE 1. Complete mitogenome of *Gymnothorax minor* with BRIG visualization showing the protein coding genes, rRNAs and tRNAs. GC skew is shown on the outer surface of the ring whereas GC content is shown on the inner surface

TABLE 1. Gene order of mitochondrial genome of *Gymnothorax minor*

Gene	Location	Strand	Size (bp)	Intergenic Sequence	Start/stop codon
trnF(gaa)	1 – 69	J	69		
rrnS	70 – 1020	J	951		
trnV(tac)	1021 – 1091	J	71		
rrnL	1092 – 2751	J	1660		
trnL2(taa)	2752 – 2827	J	76		
nad1	2828 – 3799	J	972	1	ATG/TAA
trnI(gat)	3801 – 3871	J	71	-1	
trnQ(ttg)	3871 – 3941	N	71	-1	
trnM(cat)	3941 – 4009	J	69		
nad2	4010 – 5051	J	1042		ATG/T
trnW(tca)	5052 – 5120	J	69	1	
trnA(tgc)	5122 – 5190	N	69	1	
trnN(gtt)	5192 – 5264	N	73	23	
trnC(gca)	5288 – 5354	N	67		
trnY(gta)	5355 – 5425	N	71	1	
cox1	5427 – 7029	J	1603	0	GTG/T
trnS2(tga)	7030 – 7100	N	71	4	
trnD(gtc)	7105 – 7174	J	70		
cox2	7175 – 7865	J	691		ATG/T
trnK(ttt)	7866 – 7938	J	73	1	
atp8	7940 – 8107	J	168	-10	ATG/TAA
atp6	8098 – 8780	J	683		ATG/TA
cox3	8781 – 9565	J	785		ATG/TA
trnG(tcc)	9566 – 9636	J	71		
nad3	9637 – 9985	J	349		ATG/T
trnR(tcg)	9986 – 10055	J	70		
nad4l	10056 – 10352	J	297	-7	ATG/TAA
nad4	10346 – 11723	J	1378		ATG/T
trnH(gtg)	11724 – 11792	J	69		
trnS1(gct)	11793 – 11860	J	68		
trnL1(tag)	11861 – 11933	J	73		
nad5	11934 – 13775	J	1842	-4	ATG/TAA
nad6	13772 – 14290	N	519		ATG/TAG
trnE(ttc)	14291 – 14359	N	69	5	
cob	14365 – 15504	J	1140	3	ATG/TAA
trnT(tgt)	15508 – 15579	J	72	1	
trnP(tgg)	15581 – 15650	N	70		
Control region	15651 – 16574	J	924		

TABLE 2. Start/stop codons of protein-coding genes of various taxa of family Muraenidae

Gene	Gm	Gf	Gk	Gz	Ec	Rq	As	St
nad1	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAG	ATG/TAG	ATG/TAG
nad2	ATG/T	ATG/T	ATG/T	ATG/T	ATG/T	ATG/T	ATG/T	ATG/T
cox1	GTG/T	GTG/T	GTG/T	GTG/T	GTG/T	GTG/T	GTG/T	GTG/TAA
cox2	ATG/T	ATG/T	ATG/T	ATG/T	ATG/T	ATG/T	ATG/T	ATG/T
atp8	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAA
atp6	ATG/TA	ATG/TAA	ATG/TA	ATG/TAA	ATG/TAA	ATG/TA	ATG/TA	ATG/TAA
cox3	ATG/TA	ATG/TA	ATG/TA	ATG/TA	ATG/TA	ATG/TA	ATG/TA	ATG/TA
nad3	ATG/T	ATG/T	ATG/T	ATG/T	ATG/T	ATG/T	ATG/T	ATG/T
nad4l	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAA
nad4	ATG/T	ATG/T	ATG/T	ATG/T	ATG/T	ATG/T	ATG/T	ATG/T
nad5	ATG/TAA	ATG/TAG	ATG/TAG	ATG/TAA	ATG/TA	ATG/TAA	ATG/TAA	ATG/TAA
nad6	ATG/TAG	ATG/TAG	ATG/TAG	ATG/TAA	ATG/TAG	ATG/TAG	ATG/TAG	ATG/TAG
cob	ATG/TAA	ATG/TAA	ATG/TAG	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAA	ATG/TAA

Gm, *Gymnothorax minor*; Gf, *Gymnothorax formosus*; Gk, *Gymnothorax kidako*; Gz, *Gymnomuraena zebra*; Ec, *Enchelynassa canina*; Rq, *Rhinomuraena quaesita;* As, *Anarchias* sp. Ansp; St, *Scuticaria tigrina*

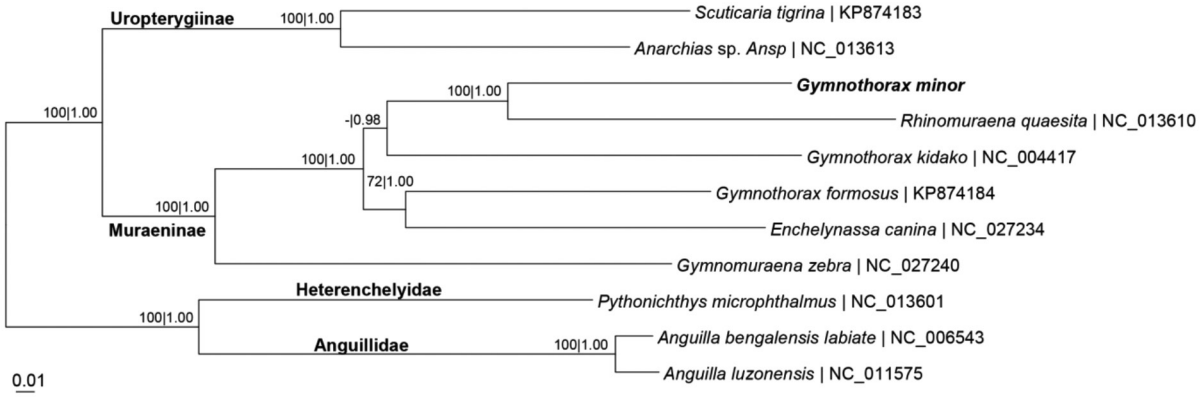

FIGURE 2. Bayesian inference and maximum likelihood tree based on 15 mt-genes (13 PCGs and 2 rRNA genes) of the whole mitogenomes of *Gymnothorax* and other taxa of Muraenidae with *Pythonichthys microphthalmus* and *Anguilla* species as outgroup. Numeric values at the nodes are Bayesian posterior probabilities/ML bootstrap

forming a sister group with *R. quaesita* in the lineage containing also *G. kidako* and *G. formosus* forming a sister group with *E. canina*.

Phylogenetic analysis based on *cob* sequences indicated that the subfamily Muraeninae as well as the subfamily Uropterygiinae were monophyletic and the genus *Gymnothorax* was paraphyletic (Figure 3). The subfamily Muraeninae contained four lineages. *G. minor* formed a sister group with *R. quaesita*, while *Gymnomuraena zebra* formed a lineage with two species of *Gymnothorax* (*G. unicolor* and *G. maderensis*).

DISCUSSION

G. minor occurs in Northwestern and Southwestern Pacific (Smith 2012), from southern Honshu, Japan to southern China and Taiwan and in Australia from Western Australia to New South Wales (Tawa & Mochioka 2009). It has recently been recorded in Vietnam (Hibino et al. 2016). The record of *G. minor* from the Gulf of Thailand (Yoshida 2013) has been attributed to *G. annulatus* (Hibino et al. 2016).

To the best of our knowledge, there appears to be no report on the complete mitogenome of *G. minor*. The complete mitogenome of *G. minor* (16,574 bp) is longer than that of *G. formosus* (16,558 bp) but shorter than *G. kidako* (16,579 bp). The seven incomplete stop codons (T and TA) in the PCGs of *G. minor* (Tables 1 & 2) can be converted to TAA by post-translational polyadenylation (Ojala et al. 1981; Yong et al. 2015a, 2015b).

Various mitochondrial and nuclear markers have been used for phylogenetic studies involving *G. minor* – cytochrome *c* oxidase subunit I (*cox1*), cytochrome *b* (*cob*), recombination activating protein 1 (*rag1*) and

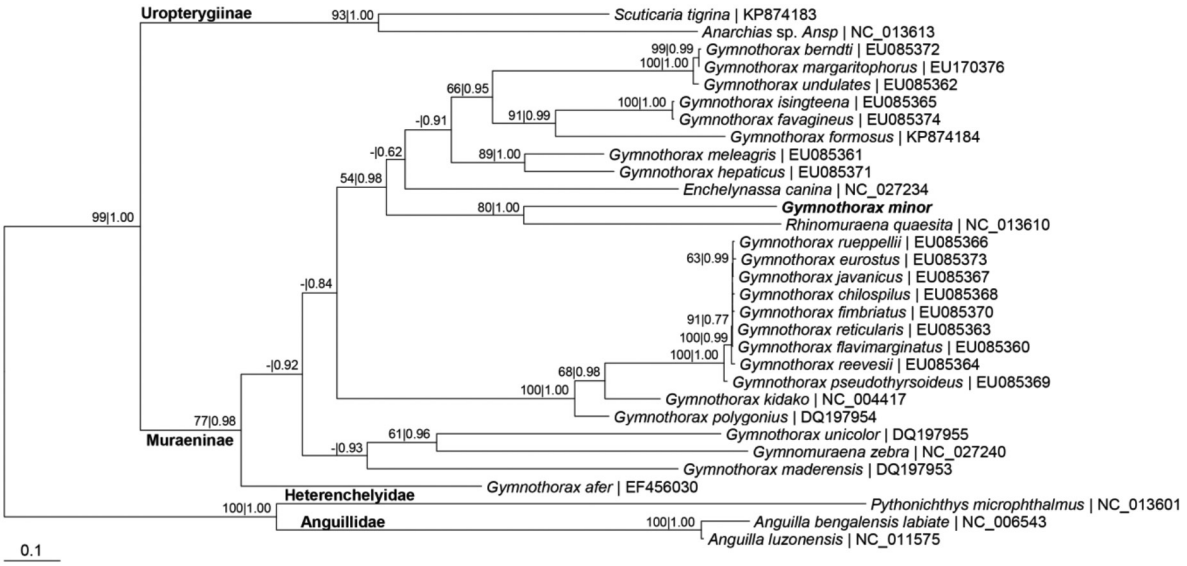

FIGURE 3. Bayesian inference and maximum likelihood tree based on mitochondrial *cob* gene of *Gymnothorax* and other taxa of Muraenidae with *Pythonichthys microphthalmus* and *Anguilla* species as outgroup. Numeric values at the nodes are Bayesian posterior probabilities/ML bootstrap

recombination activating protein 2 (*rag2*) genes (Reece et al. 2010); early growth response 2B (*egr2b*), 12 ribosomal RNA (*rrnS*), 16S ribosomal RNA (*rrnL*) and *cox1* genes (Chen et al. 2014). These studies included very small number of the *Gymnothorax* taxa, one involving three species - *G. minor*, *G. albimarginatus* and *G. reevesii* (Reece et al. 2010) and the other involving two species - *G. minor* and *G. tile* (Chen et al. 2014). Three other studies on *Gymnothorax* - 24 species based on *rrnS* and *rrnL* genes (Tang & Fielitz 2013), 20 species based on the complete *cob* gene (Du et al. 2016) and 7 species based on the *cox1* gene (Peninal et al. 2017) - did not include *G. minor*.

Based on concatenated datasets of partial *cox1*, *cob*, *rag1* and *rag2* genes, the subfamily Muraeninae as well as the subfamily Uropterygiinae of Muraenidae are monophyletic (Reece et al. 2010). The monophyly of the subfamilies of Muraenidae is reflected in the phylogenetic analysis based on *rrnS* and *rrnL* genes (Tang & Fielitz 2013) as well as complete mitogenomes (Inoue et al. 2010; Loh et al. 2016a,b,c). However, the genus *Gymnothorax* is paraphyletic and some species of the genus *Echidna* and the genera *Enchelycore*, *Enchelynassa* and *Rhinomuraena* have been suggested to be placed in the genus *Gymnothorax* to reflect a monophyletic grouping (Reece et al. 2010).

The present study based on 15 mt-genes and the *cob* gene supports the monophyletic status of the subfamily Muraeninae as well as the subfamily Uropterygiinae and the paraphyletic status of the genus *Gymnothorax* (Figures 2 & 3). Unlike the finding of an earlier study by Reece et al. (2010), our study suggested that *E. canina* and *R. quaesita* belong to different sister lineages (Figures 2 & 3). Similarly, the present finding based on broader taxa sampling indicates closer relationship of *G. kidako* to *R. quaesita* than to *E. canina* (Figures 2 & 3) instead of closer relationship to *E. canina* than to *R. quaesita* (Loh et al. 2016a). In addition, *G. kidako* forms a sister lineage with *R. quaesita* (Figures 2 & 3) instead of with *G. formosus* (Loh et al. 2016c).

In comparison to *G. minor,* the distribution of *Rhinomuraena* is in East Africa to the Tuamoto Islands, north to southern Japan, south to New Caledonia and French Polynesia, including Marianas and Marshalls (www.fishbase.org). Separation between *Rhinomuraena* and *Gymnothorax* is based on special features of keratinous fan-like outgrowths of nostrils, very delicate jaws and other distinguishing marks present only in the genus *Rhinomuraena* (Fishelson 1990).

In the present analysis based on the *cob* gene with broader taxa sampling, *Gymnomuraena zebra* formed a lineage with two *Gymnothorax* species (*G. unicolor* and *G. maderensis*) and this differs from the earlier finding of *Gymnomuraena zebra* having diverged from a common ancestor of all other Muraeninae (Reece et al. 2010; Tang & Fielitz 2013). In addition, two other species of *Gymnothorax* (*G. undulatus* and *G. pseudothyrsoideus*) belong to different lineages, in contrast to the close affinity of these species based on the *cox1* gene of small number of taxa (Peninal et al. 2017). It is clear that a broader taxa sampling will result in a better understanding of the phylogenetic relationship of the genus *Gymnothorax* and related taxa of the subfamily Muraeninae.

In summary, we have successfully sequenced the complete mitogenome of *G. minor* and confirmed the monophyletic status of the subfamily Muraeninae as well as the subfamily Uropterygiinae (family Muraenidae) and the paraphyletic status of the genus *Gymnothorax*. The mitogenome will prove useful for future studies on phylogenetics and systematics of eels of the family Muraenidae and other taxa of order Anguilliformes.

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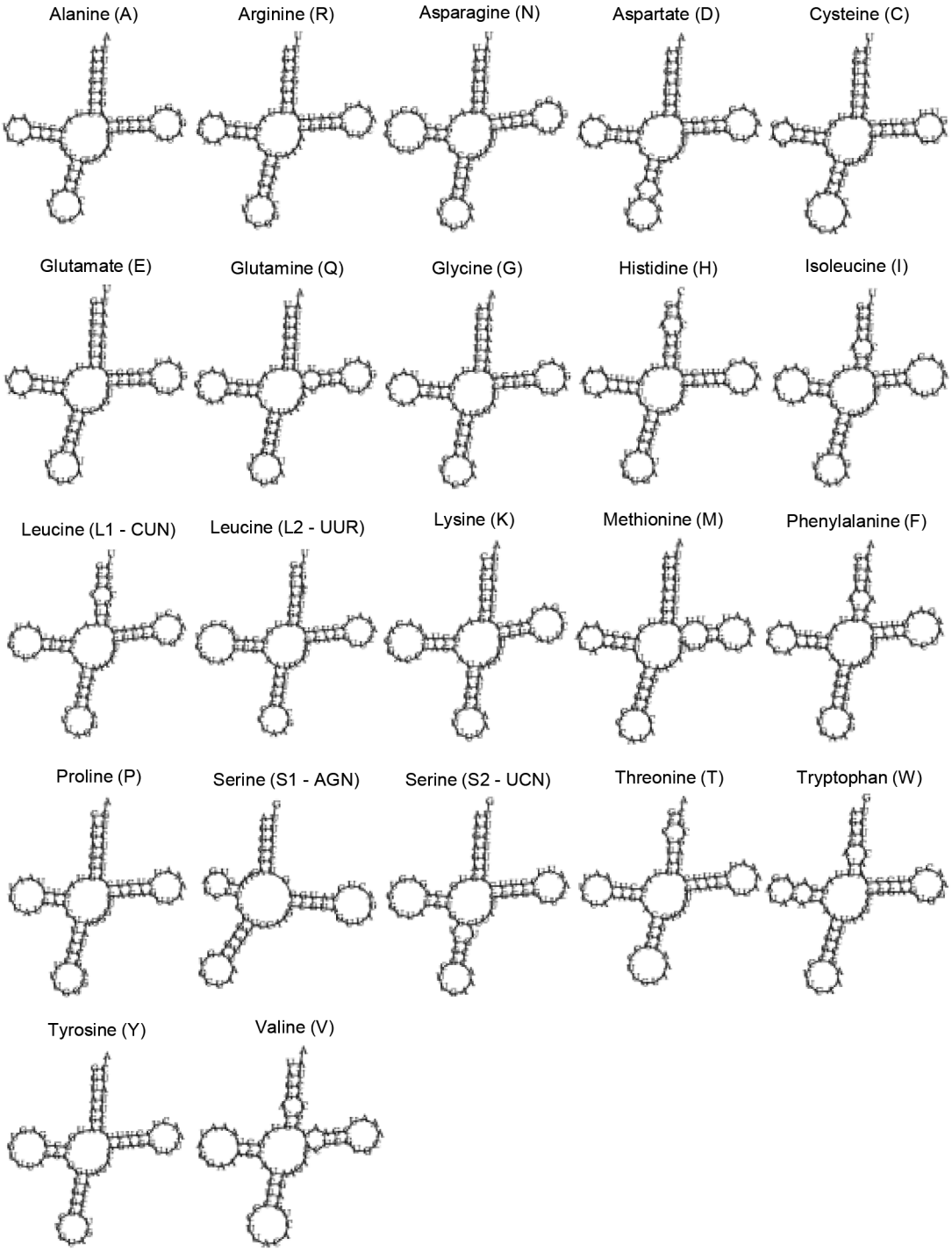
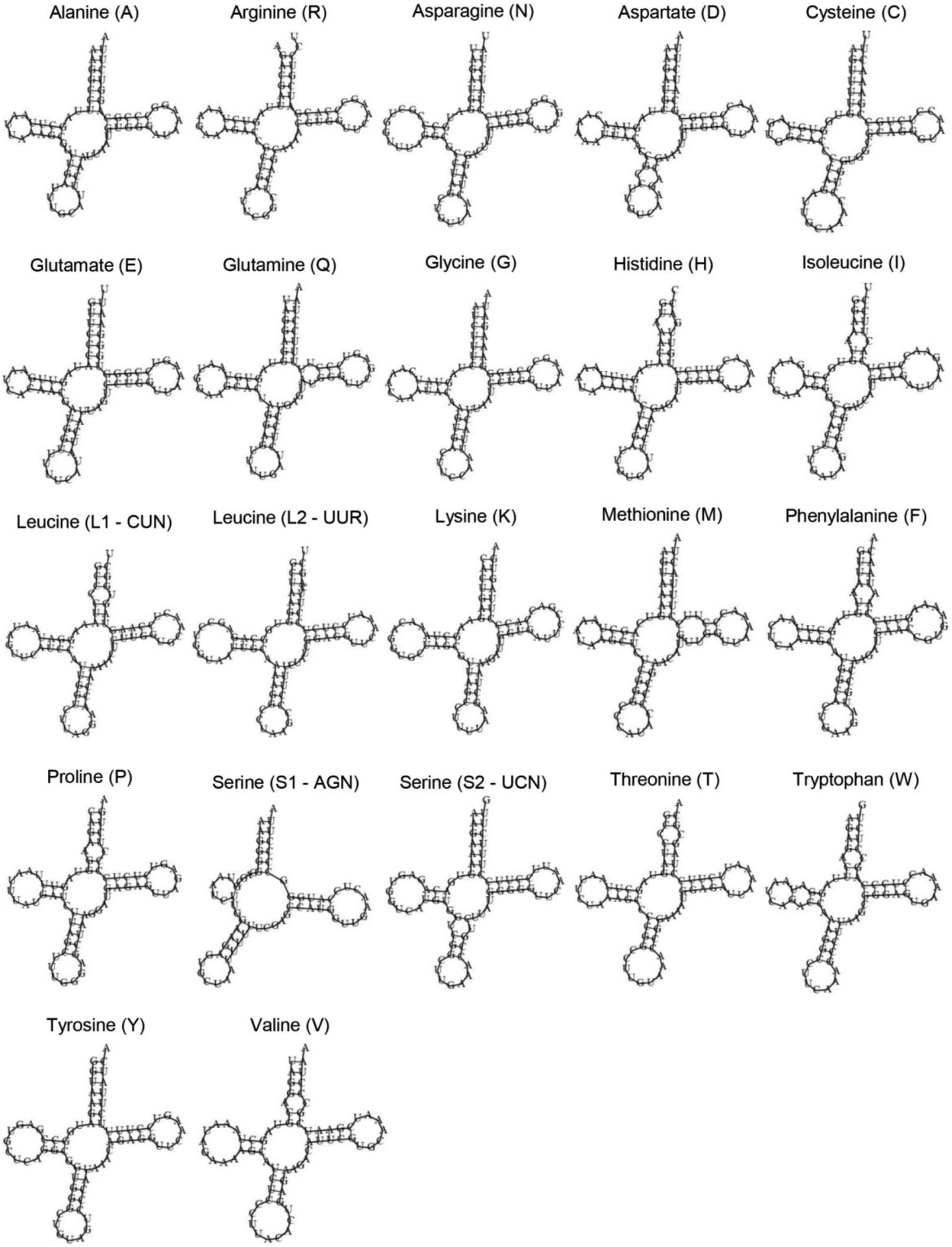
SUPPLEMENTARY TABLE S1. Base composition of mitochondrial whole genome, protein-coding genes, rRNA genes and control region of *Gymnothorax minor*

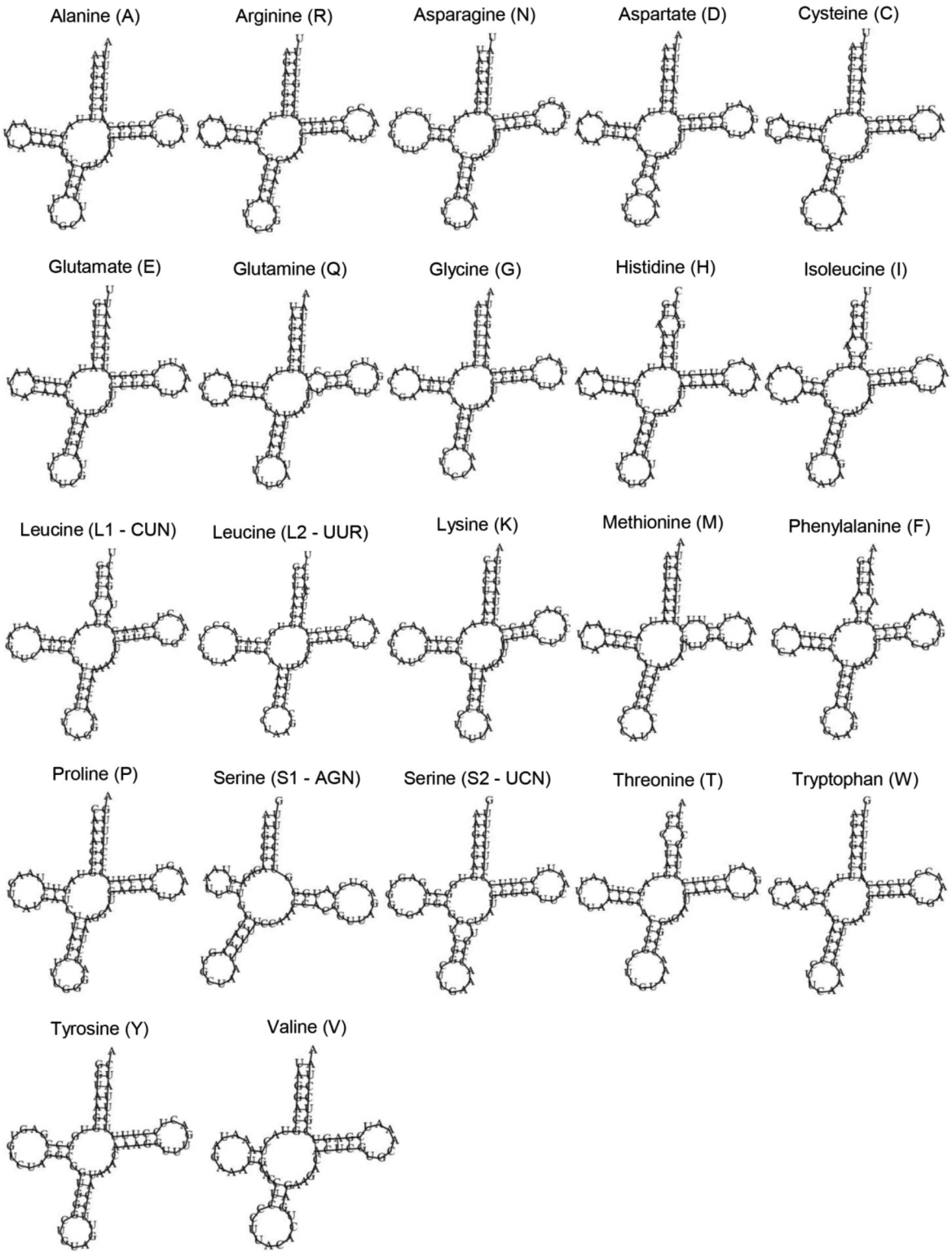
Region	A/%	C/%	G/%	T/%	A+T/%	G+C/%	AT skew	GC skew
Whole mitogenome	29.3	26.7	17.8	26.2	55.5	44.5	0.056	-0.200
nad2	29.6	29.7	15.8	24.9	54.5	45.5	0.086	-0.305
cox1	25.7	25.3	18.3	30.7	56.4	43.6	-0.089	-0.161
cox2	30.1	27.2	16.4	26.3	56.4	43.6	0.067	-0.248
atp8	31.0	33.3	10.7	25.0	56.0	44.0	0.107	-0.514
atp6	26.5	28.4	15.1	30.0	56.5	43.5	-0.062	-0.306
cox3	26.2	26.9	19.0	27.9	54.1	45.9	-0.031	-0.172
nad3	26.6	29.3	14.0	30.1	56.7	43.3	-0.062	-0.353
nad5	30.3	27.4	15.1	27.2	57.5	42.5	0.054	-0.289
nad4	27.9	27.9	16.6	27.6	55.5	44.5	0.005	-0.254
nad4l	23.9	30.7	16.8	28.6	52.5	47.5	-0.090	-0.293
nad6	36.0	32.0	14.1	17.9	53.9	46.1	0.336	-0.388
cob	26.0	28.9	15.0	30.1	56.1	43.9	-0.073	-0.317
nad1	25.1	27.1	18.1	29.7	54.8	45.2	-0.084	-0.199
rrnS	31.5	26.8	23.3	18.4	49.9	50.1	0.263	-0.070
rrnL	34.6	23.5	22.7	19.2	53.8	46.2	0.286	-0.017
Control region	30.8	21.9	17.6	29.7	60.5	39.5	0.018	-0.109



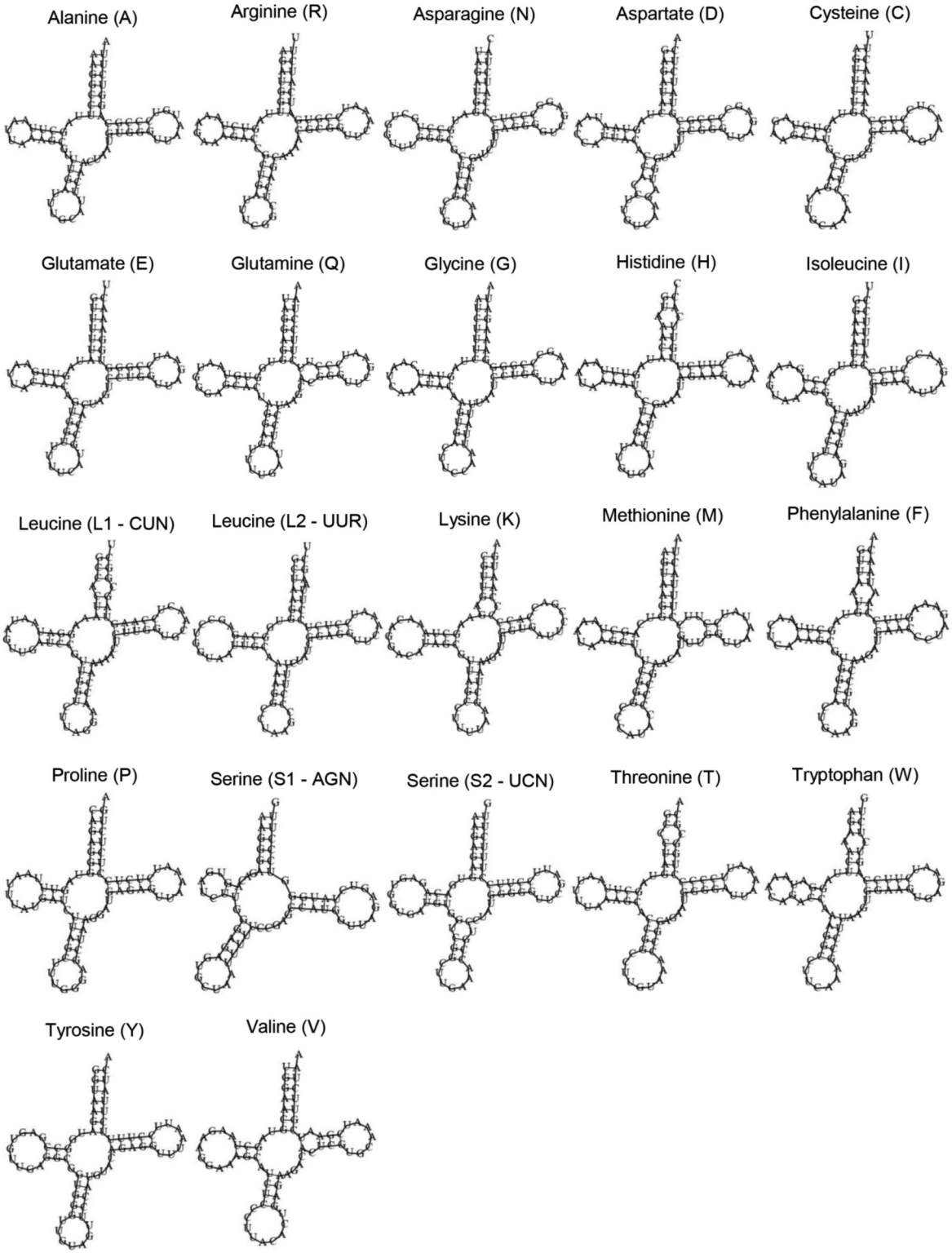
SUPPLEMENTARY FIGURE S1. Cloverleaf structure of the 22 inferred tRNAs in the mitogenome of *Gymnothorax minor*



SUPPLEMENTARY FIGURE S2. Cloverleaf structure of the 22 inferred tRNAs in the mitogenome of *Gymnothorax formosus* (KP874184). The cloverleaf structure for *trnS1* lacked a Dihydrouridine (DHU)-stem



SUPPLEMENTARY FIGURE S3. Cloverleaf structure of the 22 inferred tRNAs in the mitogenome of *Gymnothorax kidako* (NC_004417)



SUPPLEMENTARY FIGURE S4. Cloverleaf structure of the 22 inferred tRNAs in the mitogenome of *Rhinomuraena quaesita* (NC_013610)