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First report of complete mitogenome for an Itarinae species (Orthoptera, Grylloidea) with phylogenetic analysis

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ABSTRACT
The subfamily Itarinae Shiraki, 1930 belongs to the cricket family Gryllidae Laicharting, 1781 based on morphology. However, there is no phylogenetic research on Itarinae and the relationships of Itarinae within Gryllidae remain to be solved. Mitochondrial genomes (mitogenomes) have been extensively used as markers to infer the phylogenetic relationships between some families in Orthoptera. Here, we reported the first complete mitogenome of subfamily Itarinae and the mitogenome features of *Itara minor* Chopard, 1925. The mitogenome of this species is 17342 bp which contains 37 genes (13 protein-coding genes, 2 ribosomal RNA and 22 transfer RNA) and a control region, and is conserved in structure as most of species in Gryllidae. The composition of nucleotide has high content of A + T, presenting a significant bias towards A and T. Except *trnS1* with missing DHU arm structure, the remaining of tRNAs presents typical clover-leaf secondary structure. Moreover, we performed the phylogenetic trees based on 37 genes of the 35 mitogenomes along with *I. minor* in this study. The results of phylogenetic analysis strongly supported that Itarinae is the sister group of Gryllinae with the following topology ((((((Gryllinae + Itarinae) + Eneopterinae) + (Oecanthinae + Podoscirtinae)) + Cacoplistinae) + (Nemobiinae + Trigonidiinae)) + Mogoplistinae) in superfamily Grylloidea Laicharting, 1781.

KEY WORDS
Itarinae,
Gryllidae,
Grylloidea,
mitochondrial genome,
phylogeny.

RÉSUMÉ
Premier relevé sur le mitogénome complet d'une espèce d'Itarinae (Orthoptera, Grylloidea) avec analyse phylogénétique.
La sous-famille Itarinae Shiraki, 1930 appartient à la famille de grillons Gryllidae Laicharting, 1781 sur la base de la morphologie. Cependant, il n'existe pas de recherche phylogénétique sur les Itarinae et les relations des Itarinae au sein des Gryllidae n'ont pas encore été élucidées. Les génomes mitochondriaux (mitogénomes) ont été largement utilisés comme marqueurs pour déduire les relations phylogénétiques entre certaines familles d'Orthoptères. Ici, nous présentons le premier mitogénome complet de la sous-famille des Itarinae et les caractéristiques du mitogénome d'*Itara minor* Chopard, 1925. Le mitogénome de cette espèce est de 17342 pb et contient 37 gènes (13 gènes codant pour des protéines, 2 ARN ribosomiques et 22 ARN de transfert) et une région de contrôle, et sa structure est conservée comme celle de la plupart des espèces de Gryllidae. La composition des nucléotides a un contenu élevé en A + T, présentant un biais significatif vers A et T. À l'exception de trnS1 avec une structure de bras DHU manquante, le reste des ARNt présente une structure secondaire typique en feuille de trèfle. En outre, nous avons réalisé les arbres phylogénétiques sur la base de 37 gènes des 35 mitogénomes avec *I. minor*. Les résultats de l'analyse phylogénétique soutiennent fortement que les Itarinae sont groupe frère des Gryllinae avec la topologie suivante ((((((Gryllinae + Itarinae) + Eneopterinae)) + (Oecanthinae + Podoscirtinae))) + Cacoplistinae) + (Nemobiinae + Trigonidiinae)) + Mogoplistinae) dans la superfamille des Grylloidea Laicharting, 1781.

MOTS CLÉS
Itarinae,
Gryllidae,
Grylloidea,
génome mitochondrial,
phylogénie.

INTRODUCTION

Shiraki (1930) established subfamily Itarinae including *Itara* Walker, 1869, and Gorochov (1997) transferred genus *Parapentacentrus* Shiraki, 1930 under this subfamily. Itarinae is a subfamily under family Gryllidae Laicharting, 1781 including 63 species currently (Cigliano *et al.* 2022). Species of this subfamily are distributed in tropical region in Asia (Ma & Zhang 2015). Gorochov (2001a, b, 2004, 2007, 2008, 2009, 2012, 2013) systematically studied Itarinae with the additions to new species and some classifications based on male genitalia between *Itara*. Other studies reported new species and the distribution of subfamily Itarinae (Ma & Zhang 2015; He & Ma 2021).

Itarinae, Gryllomiminae, Gryllomorphinae and Gryllinae were included in 'Gryllinae subfamily group' which were based on the morphological characters by Gorochov (1995a, b, 1997, 2001c, 2010a, b, 2015). Their characters present the existence of the hypopharyngeal proboscis and the similarity of copulatory apparatus (Gorochov 1997, 2015). Subsequently, the molecular phylogeny of superfamily Grylloidea Laicharting, 1781 and Gryllotalpoidea Leach, 1815 was reported based on four nuclear and three mitochondrial markers (Chintauan-Marquier *et al.* 2016). Their results with 88% subfamilies of the crickets *sensu lato* suggested that crickets should be divided into four families (Gryllidae, Phalangopsidae, Trigonidiidae and Mogoplistidae) and one subfamily *incertae sedis* (Pteroplistinae) (Chintauan-Marquier *et al.* 2016). Campos *et al.* (2022) proposed the fifth family in Grylloidea, Oecanthidae, as sister-group of the Gryllidae (= clades F and G of Chintauan-Marquier *et al.* 2016) based on molecular data and morphological characters. Although Chintauan-Marquier *et al.* (2016) used the fragments of nuclear and mitochondrial genes to classify most of crickets *sensu lato*, some groups were not taken into account. More specifically, the relationship between Itarinae and other subfamilies of Gryllidae were not examined. The goal of the present paper is to test the phylogenetic position of Itarinae using the mitochondrial genome (mitogenome).

The mitogenome is considered as the informative marker which has been extensively used in phylogenetic analysis of insects (Cameron 2014). Since Flook *et al.* (1995) sequenced the first mitogenome of Orthoptera, *Locusta migratoria* (Linnaeus, 1758), the researches focusing on the organization and phylogeny of Orthoptera using mitogenomes have increased. Zhou *et al.* (2017) described the arrangements in 59 ensiferan mitogenomes and their phylogenetic tree recovered the sister relationship between Grylloidea and Gryllotalpoidea based on four Grylloidea species (including Oecanthinae and Gryllinae) and three Gryllotalpoidea species (including Myrmecophilinae and Gryllotalpinae). Dong *et al.* (2017) presented complete mitogenome of *Cardiodactylus muiri* Otte, 2007 and constructed phylogenetic trees of Eneopterinae and other species in the family Gryllidae based on the sequences of *cytb* and *rrnS* respectively. Chang *et al.* (2020) reported the phylogenetic relationship of 171 species in Orthoptera by using mitogenomes and represented their divergence time and evolution rate along with wing types. Sanno *et al.* (2021) investigated the phylogenetic analysis of Myrmecophilidae with complete mitogenomes in infraorder Gryllidea.

In this study, we sequenced and assembled the complete mitogenome of *Itara minor* Chopard, 1925 and analyzed the mitogenome features. In addition, the phylogenetic analysis of current mitogenomes of superfamily Grylloidea was performed to test the relationship between Itarinae and other subfamilies of Grylloidea combining public data of complete mitogenomes.

MATERIAL AND METHODS

Sampling and mitogenome sequencing

The sample of *Itara minor* Chopard, 1925 was collected from Jinping, Honghe, Yunnan Province, China (22°40'12.0"N, 103°05'24.0"E) and preserved in 100% ethanol at – 40°C. The specimen sampled is deposited in the Museum of Biology, East China Normal University (ECNU) under the inventory number ECNU-3864. The total genomic DNA was extracted from muscle tissue of the right hind leg with FastPure Cell/Tissue DNA Isolation Mini Kit (Vazyme Inc., Nanjing, China). The genome was sequenced using Illumina NovaSeq platform (Yao'en Bio Technology Inc., Shanghai, China) with 150 bp paired-end and constructed sequencing library with Illumina TruSeq DNA Sample Prep Kit (Illumina Inc., San Diego, CA, USA).

Mitogenome assembly, annotation and analysis

The mitogenome assembly was made with NOVOPlasty v 4.3.1 in k-mer size of 39. The annotation of mitogenome was performed by MITOS2 Web Server (Donath *et al.* 2019) with invertebrate mitochondrial genetic code. All PCGs and rRNA were aligned with extant mitochondrial data of Grylloidea (Table 1) by MEGA 7.0 (Kumar *et al.* 2016) and PCGs were also translated into amino acids for comparing. The positions and secondary structures of rRNA were recognized through MITOS2 Web Server (Donath *et al.* 2019). The composition of each base and usage of codons were measured by MEGA 7.0 (Kumar *et al.* 2016). The nucleotide biases were performed by the formulae of AT-skew = (A – T) / (A + T) and GC-skew = (G – C) / (G + C) (Perna & Kocher 1995). The circular mitogenome map were generated by CGView Server (http://cgview.ca/). The substitution of nucleotide was measured for testing the substitution saturation (Iss) by DAMBE v7.3.0 (Xia 2018).

Phylogenetic analysis

Itara minor in this study along with 34 species in Grylloidea and Gryllotalpoidea, obtained from National Center for Biotechnology Information (NCBI), were selected as ingroups. One species in infraorder Tettigoniidea Kevan, 1982, *Cyphoderris monstrosa* Uhler, 1864 (Hagloidea, Prophalangopsidae) (Song *et al.* 2015), was treated as the outgroup. Each gene of mitogenomes was extracted by PhyloSuite (Zhang *et al.* 2020). The 13 PCGs were aligned by MAFFT (Katoh & Standley 2013) and MASCE (Ranwez *et al.* 2011). All genes were removed ambiguously aligned fragments by G-blocks (Castresana 2000) with minimum number of sequences for a conserved/flank position (19/19), maximum number of contiguous non-conserved positions (8), minimum length of a block (10), allowed gap positions (with half). The trimmed data would be concatenated with PhyloSuite (Zhang *et al.* 2020). The dataset was generated for the phylogenetic analysis with PCG + rRNA + tRNA matrix containing 13 PCGs, two rRNA and 22 rRNA. The best partitioning strategies and substitution model for partition were determined by using PartitionFinder2 (Lanfear *et al.* 2017) with greedy algorithm and Bayesian Information Criterion (BIC). Bayesian inference (BI) and the maximum likelihood (ML) approaches were used for phylogenetic analysis. The BI analysis was performed by MrBayes 3.2.6 (Ronquist *et al.* 2012) with four simultaneous Markov chain Monte Carlo (MCMC), running for 2 million generations, in which the first 25% of trees were discarded as burn-in. The ML method was conducted by IQ-TREE (Nguyen *et al.* 2015) with 10 000 ultrafast (Minh *et al.* 2013) bootstraps along with the Shimodaira-Hasegawa-like approximate likelihood-ratio test (Guindon *et al.* 2010). The Bayesian posterior probabilities (BPP) and ML bootstrap support values (MLB) were considered as the values of confidence for BI and ML tree respectively. FigTree 1.4.4. was used to generate the figures of phylogenetic trees.

RESULTS

Mitogenome features

The complete mitochondrial genome of *Itara minor* is 17342 bp in size (Fig. 1) with 37 typical mitochondrial genes, including 13 protein-coding genes (PCGs), two ribosomal RNA (rRNA) and 22 transfer RNA (tRNA), and a control region (Table 2). Among the mitochondrial genes, 20 genes (including 9 PCGs and 11 tRNAs) are coded on the majority strand (J strand), while other 17 genes (including four PCGs, 11 tRNAs and two rRNAs) are coded on the minority strand (N strand). The seven basis pairs (BP) overlaps are detected between *atp8-atp6* (ATGATAA) and *nad4-nad4L* (ATGTTAA).

The compositions of nucleotide are A (37.09%), T (32.23%), C (9.32%), G (21.36%), which present a significant bias towards A and T. Besides, the mitogenome shows positive AT-skew (0.0701) and negative GC-skew (– 0.3933).

Protein coding genes and codon usage

The total length of 13 PCGs is 11167 bp, which encodes 3710 amino acids. The composition of A + T is 68.47%, suggesting a strong bias towards A and T.

The 12 PCGs start with typical ATN codons, except for *cox1* which starts with TTG. The ATG start codon is found in *cox2*, *atp6*, *cox3*, *nad4*, *nad4L* and *cytb*, five PCGs (*nad2*, *atp8*, *nad5*, *nad6* and *nad1*) start with ATT, and *nad3* uses ATA as the initiation codon. Nine PCGs (*nad2*, *cox2*, *atp8*, *atp6*, *cox3*, *nad3*, *nad5*, *nad4L* and *nad6*) use TAA as stop codons and three PCGs (*cox1*, *cytb* and *nad1*) terminate with TAG. Only *nad4* uses T as the partial stop codon.

The analysis of codon usage shows that the most frequently used codons are UUA-Leu (7.90%), AUU-Ile (7.47%) and UUU-Phe (5.63%), UUA-Leu shows the highest relative synonymous codon usage (RSCU) in the mitogenome of *I. minor* (Fig. 2). The test of sequence saturation shows that the *Iss* is significantly lower than *Iss.c*. In this way, the codon position is not saturated in the PCGs of the mitogenome which indicates that the dataset of mitogenome is suitable for the phylogenetic analysis.

TABLE 1. — GenBank accession number and taxonomic information for phylogenetic analysis (taxonomy according to Cigliano *et al.* 2022). **In bold:** the species sequenced in this study.

Superfamily, Family, Subfamily, Species	Accession	References
GRYLLOIDEA		
Gryllidae		
Eneopterinae		
Cardiodactylus muiri Otte, 2007	NC_037914	Dong *et al.* 2017
Xenogryllus marmoratus (Haan, 1844)	NC_041236	Ma *et al.* 2019a
Pseudolebinthus lunipterus Salazar, Murphy, Guillaume, Nattier & Robillard, 2020	MN414243	Salazar *et al.* 2020
Gryllinae		
Acheta domesticus (Linnaeus, 1758)	MZ440654	Sanno *et al.* 2021
Gryllodes sigillatus (Walker, 1869)	NC_057195	Yang *et al.* 2021
Gryllus bimaculatus De Geer, 1773	NC_053546	Park *et al.* 2021
Gryllus veletis (Alexander & Bigelow, 1960)	NC_057053	Torson *et al.* 2021
Gryllus lineaticeps Stål, 1861	NC_057052	Torson *et al.* 2021
Loxoblemmus doenitzi Stein, 1881	NC_033985	Zhou *et al.* 2017
Loxoblemmus equestris Saussure, 1877	KU562919	Yang *et al.* 2016
Tarbinskiellus portentosus (Lichtenstein, 1796)	MZ427921	Sanno *et al.* 2021
Teleogryllus emma (Ohmachi & Matsuura, 1951)	KU562917	Yang *et al.* 2016
Teleogryllus occipitalis (Serville, 1838)	MZ440652	Sanno *et al.* 2021
Teleogryllus infernalis (Saussure, 1877)	MK903574	Chang *et al.* 2020
Turanogryllus eous Bey-Bienko, 1956	MK656322	Ma *et al.* 2019b
Velarifictorus hemelytrus (Saussure, 1877)	NC_030762	Yang *et al.* 2016
Itarinae		
***Itara minor* Chopard, 1925**	**OM935774**	**This study**
Oercanthidae		
Oecanthinae		
Oecanthus rufescens Serville, 1838	KX057720	Zhou *et al.* 2017
Oecanthus sinensis Walker, 1869	NC_034799	Li *et al.* 2019
Podoscirtinae		
Truljalia hibinonis (Matsumura, 1917)	NC_034797	Li *et al.* 2019
Phalangopsidae		
Cachoplistinae		
Cacoplistes rogenhoferi Saussure, 1877	NC_039664	Ma & Li 2018
Meloimorpha japonica (Haan, 1844)	NC_039665	Ma & Li 2018
Trigonidiidae		
Nemobiinae		
Dianemobius fascipes (Walker, 1869)	NC_045846	Ma *et al.* 2019c
Dianemobius furumagiensis (Ohmachi & Furukawa, 1929)	NC_045847	Ma *et al.* 2019c
Polionemobius taprobanensis (Walker, 1869)	NC_045848	Ma *et al.* 2019c
Trigonidiinae		
Homoeoxipha nigripes Xia & Liu, 1993	NC_045841	Ma *et al.* 2019c
Natula pravdini (Gorochov, 1985)	MG701239	Ma *et al.* 2019c
Svistella anhuiensis He, Li & Liu, 2009	MG701238	Ma *et al.* 2019c
Trigonidium sjostedti (Chopard, 1925)	NC_032077	Song *et al.* 2016
Mogoplistidae		
Mogoplistinae		
Ornebius fuscicerci (Shiraki, 1930)	NC_039739	Ma & Li 2018
Ornebius bimaculatus (Shiraki, 1930)	NC_039666	Ma & Li 2018
GRYLLOTALPOIDEA		
Myrmecophilidae		
Myrmecophilinae		
Myrmecophilus manni Schimmer, 1911	NC_011301	Fenn *et al.* 2008
Myrmecophilus kubotai Maruyama, 2004	MZ440658	Sanno *et al.* 2021
Gryllotalpidae		
Gryllotalpinae		
Gryllotalpa orientalis Burmeister, 1838	NC_006678	Kim *et al.* 2005
Gryllotalpa pluvialis (Mjöberg, 1913)	NC_011302	Fenn *et al.* 2008
HAGLOIDEA		
Prophalangopsidae		
Cyphoderrinae		
Cyphoderris monstrosa Uhler, 1864	NC_028059	Song *et al.* 2015

TRANSFER RNA, RIBOSOMAL RNA GENES AND CONTROL REGION

The 22 tRNA genes detected in the mitogenome were found in *I. minor*. The length of tRNA is 1489 bp with each of the tRNA ranging from 64 bp (*trnT*) to 72 bp (*trnC*). The mitogenome shows A + T bias with positive AT-skew (0.0137) and GC-skew (0.1633). The secondary structure of tRNA exhibit typical clover-leaf structure except *trnS1* with the DHU arm missing (Fig. 3). The lengths of large rRNA subunit gene (*rrnL*) and rRNA subunit gene (*rrnS*) are 1359 bp and 780 bp

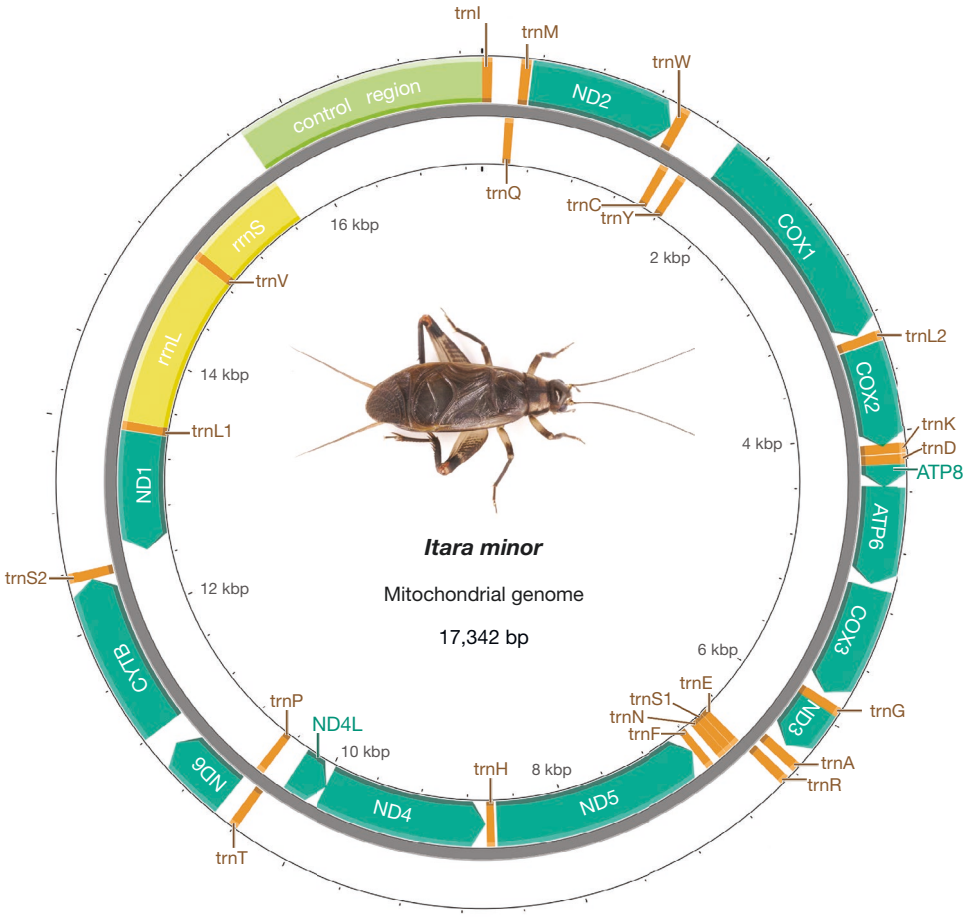

Fig. 1. — The circular mitochondrial genome map of *Itara minor* Chopard, 1925.

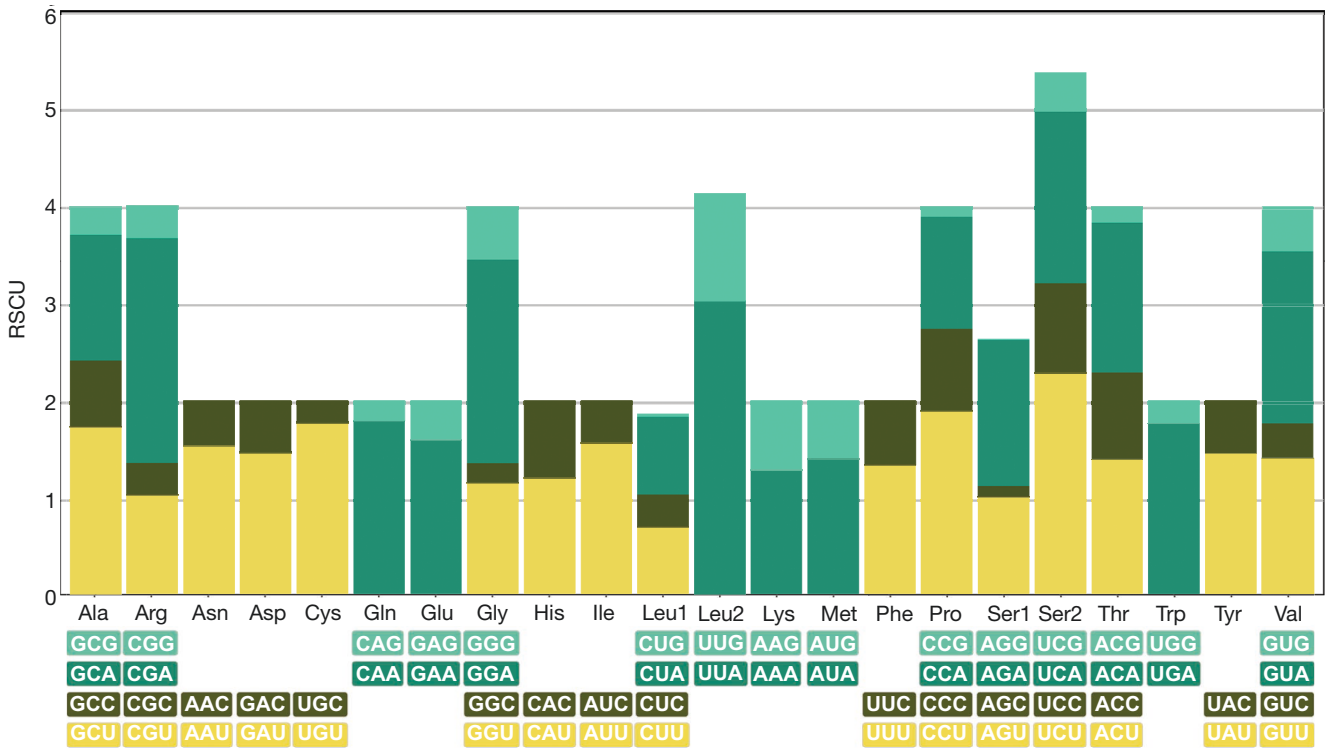

Fig. 2. — The relative synonymous codon usage (RSCU) of *Itara minor* Chopard, 1925.

TABLE 2. —Mitogenomic organization and annotation of *Itara minor* Chopard, 1925.

Gene	Strand	Region	Length (bp)	Start codon	Stop codon	Anticodon	Intergenic nucleotides (bp)
trnI	J	1-69	69	–	–	GAT	104
trnQ	N	174-242	69	–	–	TTG	15
trnM	J	258-326	69	–	–	CAT	9
nad2	J	336-1349	1014	ATT	TAA	–	-2
trnW	J	1348-1418	71	–	–	TCA	-8
trnC	N	1411-1482	72	–	–	GCA	85
trnY	N	1568-1638	71	–	–	GTA	144
cox1	J	1783-3318	1536	TTG	TAG	–	9
trnL2	J	3328-3392	65	–	–	TAA	6
cox2	J	3399-4097	699	ATG	TAA	–	-20
trnK	J	4078-4147	70	–	–	CTT	4
trnD	J	4152-4217	66	–	–	GTC	0
atp8	J	4218-4376	159	ATT	TAA	–	-7
atp6	J	4370-5047	678	ATG	TAA	–	22
cox3	J	5070-5858	789	ATG	TAA	–	30
trnG	J	5889-5953	65	–	–	TCC	-3
nad3	J	5951-6304	354	ATA	TAA	–	30
trnA	J	6335-6403	69	–	–	TGC	45
trnR	J	6449-6515	67	–	–	TCG	-6
trnE	N	6510-6574	65	–	–	TTC	1
trnS1	N	6576-6642	67	–	–	GCT	0
trnN	N	6643-6709	67	–	–	GTT	58
trnF	N	6768-6832	65	–	–	GAA	3
nad5	N	6836-8554	1719	ATT	TAA	–	18
trnH	N	8573-8636	64	–	–	GTG	6
nad4	N	8643-9978	1336	ATG	T	–	-7
nad4L	N	9972-10268	297	ATG	TAA	–	108
trnT	J	10377-10440	64	–	–	TGT	14
trnP	N	10455-10521	67	–	–	TGG	31
nad6	J	10553-11062	510	ATT	TAA	–	139
cytb	J	11202-12335	1134	ATG	TAG	–	-2
trnS2	J	12334-12400	67	–	–	TGA	56
nad1	N	12457-13398	942	ATT	TAG	–	3
trnL1	N	13402-13470	69	–	–	TAG	0
rrnL	N	13471-14829	1359	–	–	–	0
trnV	N	14830-14898	69	–	–	TAC	-2
rrnS	N	14897-15676	780	–	–	–	0
control region	-	15677-17342	1666	–	–	–	–

respectively. The two rRNA gene exhibit negative AT-skew (– 0.1393) and positive GC-skew (0.4615). The length of control region is 1666 bp. The A + T content of control region is 65.85% with the position afterwards by the end of *rrnS* and shows the strong bias towards A and T in control region.

PHYLOGENETIC ANALYSIS

The BI and ML methods result in two phylogenetic trees with concordant topologies for the concatenated dataset (Fig. 4). The phylogenetic trees distinctively sort out five families within Grylloidea, i.e., Mogoplistidae, Trigonidiidae, Phalangopsidae, Oecanthidae and Gryllidae, with high nodal support values for both BI and ML analyses (1.00 BPP and 100% MLB). The results of the phylogenetic analysis show that Itarinae is the sister group to Gryllinae with high support values (1.00 BPP and 99% MLB). Within the superfamily Grylloidea, the same topology was observed in BI and ML trees, with the following topology of the relationship of subfamilies: ((((((Gryllinae + Itarinae) + Eneopterinae) + (Oecanthinae + Podoscirtinae)) + Cacoplistinae) + (Nemobiinae + Trigonidiinae)) + Mogoplistinae).

DISCUSSION

THE MITOGENOME FEATURE OF *ITARA MINOR*

Most of species of Insecta share the same mitogenome arrangement of ancestral pancrustacean (Cameron 2014). However, many specific rearrangements of mitogenome are reported in some orders as well as in Orthoptera. For instance, the order of *trnK-trnD* is the character in infraorder Ensifera and most of the Insecta, while the rearrangement of *trnD-trnK* has been found in most of the infraorder Acrididea except in *Erianthus versicolor* (Leavitt *et al.* 2013; Cameron 2014). The inversion of *trnE-trnS-trnN* is common in superfamily Grylloidea which is also found in *I. minor* in this study. Ma *et al.* (2019c) reported that the ancestral arrangement of *trnN-trnS-trnE* is found in family Mogoplistidae which could mean that Mogoplistidae is sister to other three families in Grylloidea.

The gene *cox1* represents the varied start codons found in Grylloidea. The codons of *cox1* in some species of Grylloidea start with ATT or ATC. The Oecanthidae *Oecanthus sinensis* (Oecanthinae), *Truljalia hibinonis* (Podoscirtinae),

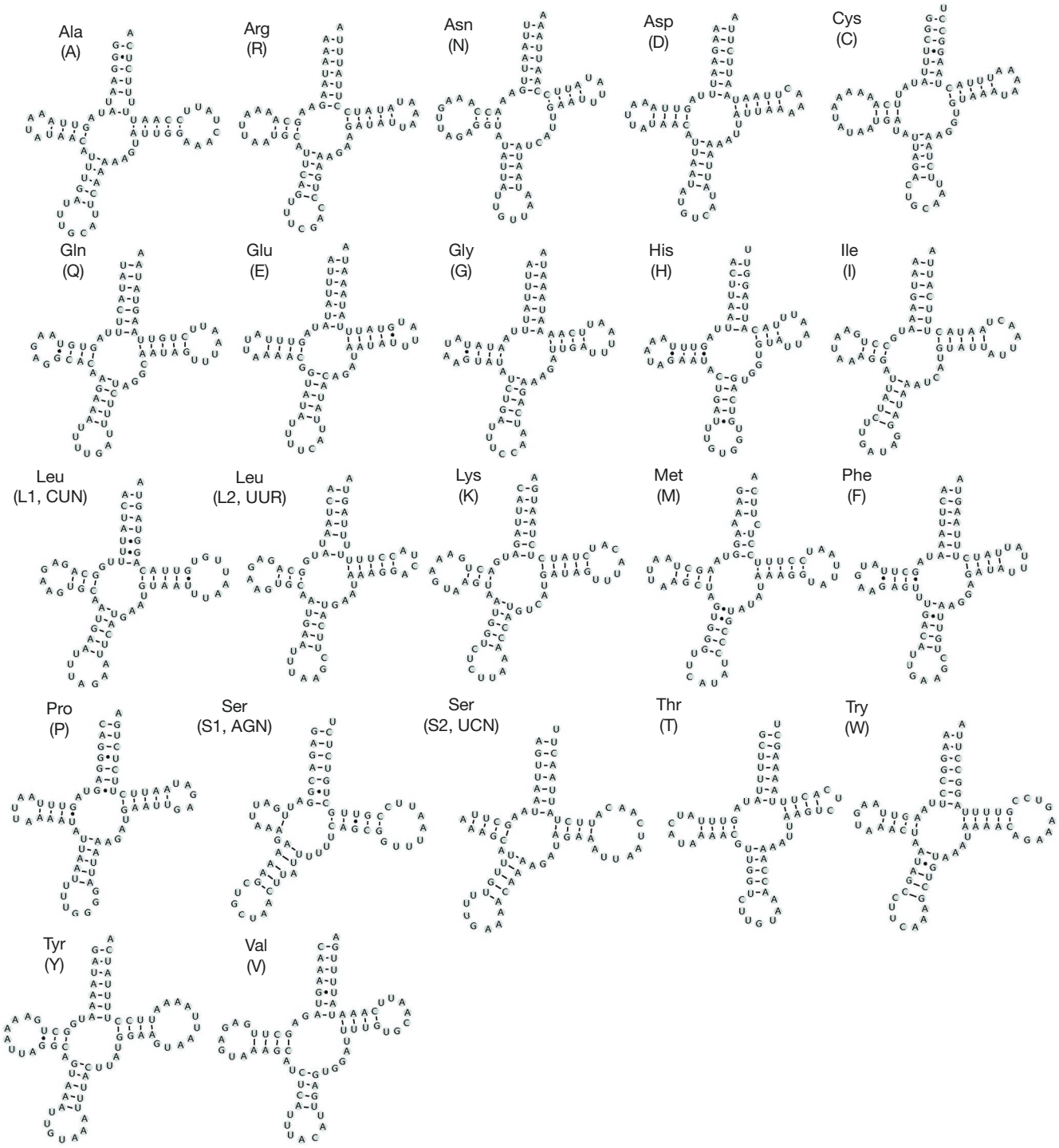

FIG. 3. — Predicted secondary structures of 22 tRNAs in *Itara minor* Chopard, 1925. Dash (–) represents Watson-Crick bonds and dot (•) represents trans Watson-Crick GU bonds.

and the Trigonidiidae *Trigonidium sjostedti* (Trigonidiinae) use ATT as the start codon of *cox1*; ATC is the start codon of *cox1* in the two Gryllidae Eneopterinae *Cardiodactylus muiri* and *Pseudolebinthus lunipterus* (Song *et al.* 2016; Dong *et al.* 2017; Li *et al.* 2019; Salazar *et al.* 2020). Most species in Gryllidae Gryllinae have TCG as the start codon of *cox1* (Yang *et al.* 2016; Zhou *et al.* 2017; Ma *et al.* 2019b; Chang *et al.* 2020; Sanno *et al.* 2021; Torson *et al.* 2021; Yang *et al.* 2021), which is also found in the Gryllidae Eneopterinae *Xenogryllus marmoratus* and Oecanthidae Oecanthinae *Oecanthus rufescens*, and in the Phalangopsidae *Cacoplistes rogenhoferi* and *Meloimorpha japonica* (Cacoplistinae) (Ma *et al.* 2019a; Zhou *et al.* 2017; Ma & Li 2018). There are also some unusual start codons of *cox1*, e.g. CAA in the Gryl-

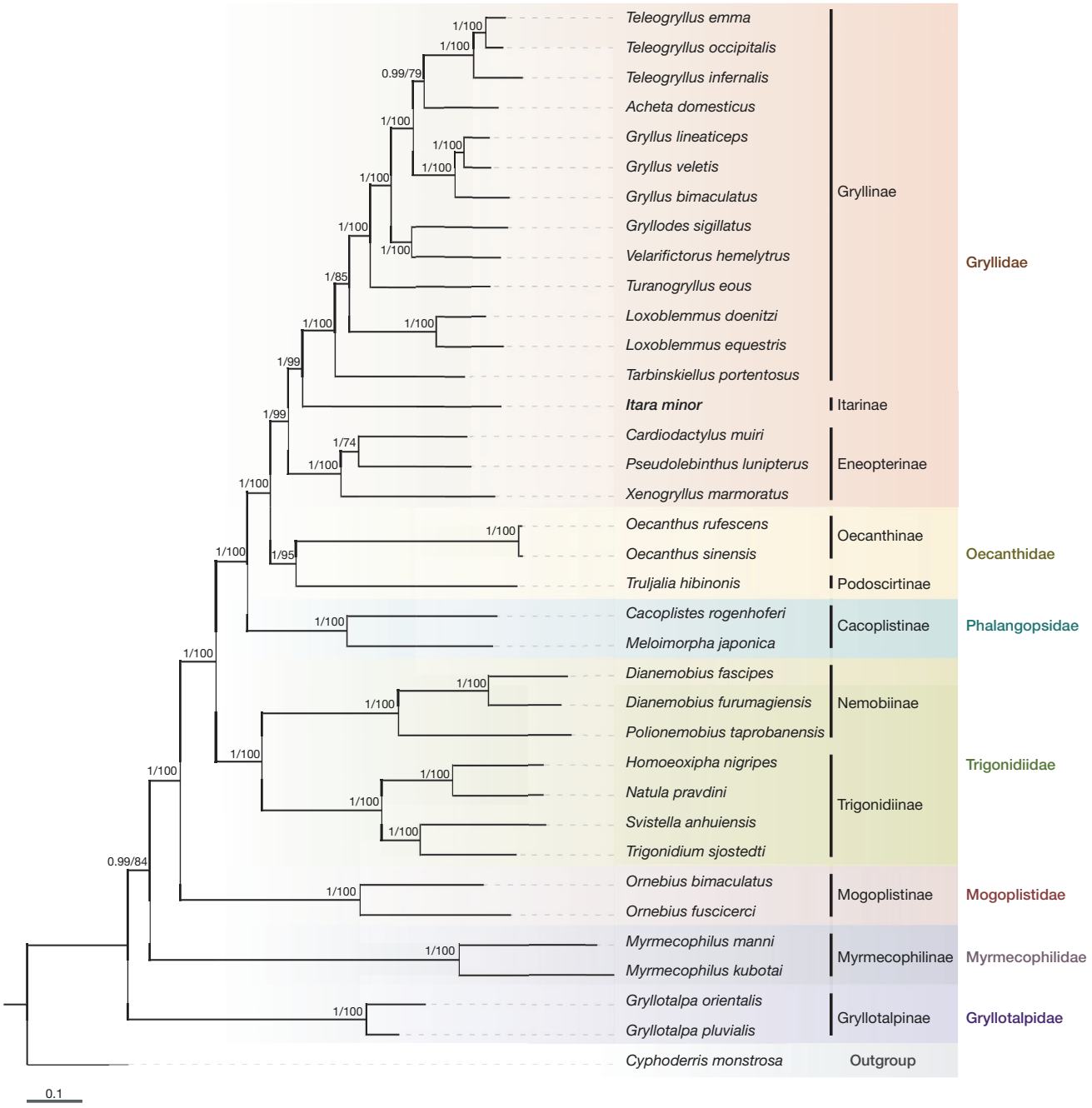

FIG. 4. — Phylogeny of the superfamily Grylloidea with Bayesian inference (**BI**) and maximum likelihood (**ML**) based on PCGs + rRNA + tRNA matrix. The numbers on each node are Bayesian posterior probabilities (**BPP**, left) and ML bootstrap support values (**MLB**, right) respectively.

lidae Gryllinae *Acheta domesticus* and *Teleogryllus occipitalis*, and in the Trigonidiidae Nemobiinae *Dianemobius fascipes* and *D. furumagiensis*; TTA in the Gryllidae Gryllinae *Gryllus bimaculatus* and *Velarifictorus hemelytrus*; CCG in the Trigonidiidae Trigonidiinae *Homoeoxipha nigripes*, *Natula pravdini* and *Svistella anhuiensis*, and in the Mogoplistidae Mogoplistinae *Ornebius bimaculatus* and *O. fuscicercis* (Yang *et al.* 2016; Ma & Li 2018; Ma *et al.* 2019c; Park *et al.* 2021; Sanno *et al.* 2021). The *cox1* of *Itara minor* (Itarinae) in our study starts with TTG codon which was not reported in other species of Grylloidea up to now. Meanwhile, 7 bp overlap found in *atp8-atp6* (ATGATAA) and *nad4-nad4L* (ATGTTAA) had also been detected in almost all species in Grylloidea, but some exceptions exist for the *atp8-atp6* (ATGATAG and ATTATAA) overlap of *Pseudolebinthus lunipterus* (Gryllidae, Eneopterinae) and *Truljalia hibinonis* (Oecanthidae, Podoscirtinae) respectively (Salazar *et al.* 2020; Li *et al.* 2019). The *nad4-nad4L* overlap is GTGTTAA in the mitogenome of *Ornebius fuscicercis* (Mogoplistidae, Mogoplistinae), while no overlap has been detected between *nad4*

and *nad4L* in the mitogenome of *Dianemobius fascipes* and *D. furumagiensis* (Trigonidiidae, Nemobiinae) (Zhou *et al.* 2017; Ma *et al.* 2019c).

Phylogenetic relationships between Itarinae and other subfamilies in Gryllidae

Our results document for the first time the phylogenetic position of subfamily Itarinae with well support by using mitochondrial data. Although with a much smaller ingroup, the phylogenetic analysis based on mitogenome data comforts the results of Chintauan-Marquier *et al.* (2016) for the general structures of cricket phylogeny, and further documents the Gryllidae node with the addition of the Itarinae in the topology. Our study also supports the results of Campos *et al.* (2022) that Gryllidae is sister to Oecanthidae.

The phylogenetic trees by Sanno *et al.* (2021) were based on concatenated sequences of 13 PCGs analysed either as nonpartitioned or partitioned data: the genera *Loxoblemmus* and *Tarbinskiellus* (Gryllidae, Gryllinae) were then found sister groups in these results. We use 37 genes including 22 tRNA and 2 rRNA to reconstruct a phylogenetic tree and the genus *Tarbinskiellus* was found at the base among the representatives of the subfamily Gryllinae. In the same way, we found Myrmecophilidae grouped together with Grylloidea (including Gryllidae, Oecanthidae, Phalangopsidae, Trigonidiidae and Mogoplistidae), which is consistent with the results based on partitioned data from Sanno *et al.* (2021). However, the cluster of Myrmecophilidae and Gryllotalpidae was monophyletic based on transcriptome and PCGs data from mitogenomes (Song *et al.* 2020) and nonpartitioned data from Sanno *et al.* (2021). The previous studies used fewer sample size of infraorder Gryllidea and different scales of data compared to our study. Our results are based on larger mitogenomic data (PCG + rRNA + tRNA matrix) and the sample size was designed to resolve the phylogenetic relation of Itarinae in Grylloidea, which was obtained with high support value, although further research using adequate data and sample size is still needed.

Gorochov (2015) proposed a systematic classification of Grylloidea based on morphology and Itarinae was in the so-called 'Gryllinae subfamily group' including Gryllomiminae, Gryllomorphinae and Gryllinae. Later, Chintauan-Marquier *et al.* (2016) presented the phylogeny of Gryllidea based on multilocus analysis: they showed that Gryllomorphinae is a polyphyletic clade, with some genera belonging to a monophyletic Gryllinae clade including *Sclerogryllus*, while the genera *Gryllomorpha* and *Petaloptila* should be excluded from the Gryllidae. Landrevinae was moreover obtained polyphyletic by Chintauan-Marquier *et al.* (2016). The relationship among subfamilies in Gryllidae is clearly still in need of revision for further discussion. Our result is the first molecular study testing the sister-group relationship between Itarinae and Gryllinae proposed by Gorochov (2015), and providing the placement of Itarinae in the phylogeny of the whole cricket clade. Further research should focus on the phylogeny of Gryllidae and untangle the nested relationship among Gryllinae and its related groups.

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