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ORIGINAL ARTICLE

The complete mitochondrial genomes of five blind scolopendromorph centipedes and phylogenetic within Scolopendromorpha (Chilopoda)

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Abstract Mitochondrial genomes of five Tykhepoda scolopendromorphs (Cryptopidae, Scolopocryptopidae and Plutoniumidae) are sequenced and analyzed using methods of comparative genomics to provide more information on the phylogeny of Scolopendromorpha. The locations of 22 tRNAs, 13 PCGs, and 2 rRNAs are annotated. The heavy chain of mitochondrial genomes are 14,841–15,619 bp in length. A+T % vary from 64.79% to 77.40%, and mitochondrial genomes are CG and AT skewed. The phylogenetic reconstructions showed the blind clade, recently named Tykhepoda, is monophyletic with high support values (BS = 100% and PP = 1).

Key words Mt DNA, phylogenomic, Cryptopidae, Scolopocryptopidae, Plutoniumidae.

1 Introduction

The order Scolopendromorpha (Chilopoda) consists of five families: Cryptopidae, Scolopocryptopidae, Mimopidae, Plutoniumidae and Scolopendridae (Schileyko *et al.*, 2020). The blind clade, including Cryptopidae, Scolopocryptopidae and Plutoniumidae, was strongly supported by phylogenetic analyses (Vahtera *et al*, 2012; Fernández *et al.*, 2016) and recently named as Tykhepoda (Benavides *et al.*, 2023). The presence of four eyeless species (in the genera *Tonkinodentus* and *Cormocephalus*) within the ocellate family Scolopendridae suggests the multiple origins of blindness in Scolopendromorpha (Edgecombe *et al.*, 2019; Schileyko & Solovyeva, 2019). A comparative genomic analyses for mitochondrial genomes of the Tykhepoda species can provide a basis for the taxonomic and phylogenetic study of scolopendromorphs, and establish a taxonomic system more consistent with centipede evolution.

Mitochondria have characteristics of independent replication and stable inheritance. COI, 16S rRNA and complete mitochondrial genomes are frequently used in the identification of uncertain species and phylogenetic analyses (Negrisolo *et al.*, 2004; Spelda *et al.*, 2011; Wesener *et al.*, 2016; Siriwut *et al.*, 2018; Qiao *et al.*, 2019; Ganske *et al.*, 2020; Ayivi *et al.*, 2021; Xu *et al.*, 2021; Zhang *et al.*, 2021). Mitochondrial genomes in Scolopendromorpha are in length varying from 14,014 bp to 15,030 bp (Gai *et al.*, 2014; Sun *et al.*, 2018; Ding *et al.*, 2022; Pan *et al.*, 2023), and also various in the relative arrangements, so do the subclass Chilopoda. For instance, the arrangement in *Scolopendra mutilans* (Scolopendromorpha: Scolopendridae) is cox1–cox2–atp8–atp6–cox3–nad3–nad1–l-rRNA–s-rRNA–nad5–nad4–nad4l–nad6–cob–nad2 in, while cox1–cox2–atp8–atp6–cox3–nad3–nad5–nad4–nad4l–nad6–cob–nad1–l-rRNA–s-rRNA–nad2 in *Scolopocryptops* sp. (Scolopendromorpha: Scolopocryptopidae) (Gai *et al.*, 2014; Hu *et al.*, 2020), and cox1–cox2–atp8–atp6–cox3–nad5–nad4l–nad6–nad1–nad3–nad4–cob–l-rRNA–s-rRNA–nad2 in *Thereuonema tuberculata* (Scutigeromorpha: Scutigeridae) (Yang *et al.*, 2022). Therefore, studying the arrangement pattern of genes on the mitochondrial genome can provide more

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support for species classification.

In this study, five species, *Theatops chuanensis* Di, Cao, Wu, Yin, Edgecombe & Li, 2010 (Plutoniumidae), *Scolopocryptops rubiginosus* L. Koch, 1878 (Scolopocryptopidae), *Scolopocryptops nigrimaculatus* Song, Song & Zhu, 2004 (Scolopocryptopidae), *Cryptops doriae* Pocock, 1891 (Cryptopidae) and *Cryptops songi* Song, Zhu & Liang, 2010 (Cryptopidae), representing three Tykhepoda families, are sequenced. Their mitochondrial genome arrangement patterns are compared with those of the genus *Scolopendra* for further discussion on the phylogenetic relationship between the Tykhepoda and other centipedes. In addition, phylogenetic trees (BI and ML trees) are established using 13 PCGs and 2 rRNAs, further verifying the phylogenetic relationships of Tykhepoda at the level of mitochondrial genomics.

2 Materials and methods

2.1 **Sample information** (Fig. 1)

Figure 1. Tykhepoda spp. A. *Scolopocryptops nigrimaculatus*; B. *Theatops chuanensis*; C. *Cryptops songi*.

T. chuanensis: China, Gansu, Longnan, CMMI 20190606004, 06 June 2019, leg. Quanyu Ji.

S. nigrimaculatus: China, Hubei, Wuhan, Maanshan, CMMI 20210408143, 30.5146°N, 114.4394°E, elev. 106 m, 08 April 2021, leg. Tianyun Chen and Zhidong Wang.

S. rubiginosus: China, Liaoning, Dalian, Lüshun, CMMI 20190906031, 31 June 2019, leg. Junduo Zhang.

C. doriae: China, Yunnan, Dehong, CMMI 20200224002, 24 February 2020.

C. songi: China, Beijing, Fengtai, CMMI 20191021007, 21 October 2019, leg. Chao Jiang.

2.2 DNA extraction, mitogenome sequencing and assembly

Legs from each sample were dried and ground to powder before being put in a 2.0 ml centrifuge tube. DNA extraction was carried out using the Promega Wizard® SV Genomic DNA Purification kit (Promega, USA).

Genomic DNA libraries were built using Invitrogen Collibri NGS (Thermofisher, USA) and sequenced based on the double-terminal 150 bp protocol (PE 150 bp) using Illumina HiSeq 2500. Data from the mitogenome of each species are 5–6G. To obtain the mitochondrial genome sequence, clean sequence reads were assembled using the software GetOrganelle 1.7.1 (Jin *et al.*, 2020).

2.3 Mitogenome annotation and comparative analysis

MITOS2 was used (Donath *et al.*, 2019) (http://mitos2.bioinf.uni-leipzig.de/index.py) to predict loci of 12S and 16S ribosomal RNAs (rRNAs), 13 PCGs and 22 transfer RNAs (tRNAs). Unannotated tRNAs and PCGs were predicted using comparisons of closely related species. The secondary structure of tRNA was predicted by rtools (http://rtools.cbrc.jp/cgi-bin/index.cgi) (Hamada *et al.*, 2016). The coding regions of 13 PCGs were adjusted using the NCBI Conserved Domains (Marchler-Bauer & Bryant, 2004; Marchler-Bauer *et al.*, 2010, 2015, 2017; Lu *et al.*, 2020) (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi).

Strand asymmetry of all available mitogenomes was evaluated using AT Skew and GC Skew. Composition skew was calculated according to the following formulae: AT skew=(A−T)/(A+T) and GC skew=(G−C)/(G+C). Relative synonymous codon usage (RSCU) was calculated using codonW 1.3 (Bennetzen & Hall, 1982; Wright, 1990; Sharp & Cowe, 1991; Sharp & Lloyd, 1993).

2.4 Phylogenetic analyses

Toatlly 13 PCG and 2 rRNA sequences were selected to construct a phylogenetic tree. The sequence data used originates both from this work as well as from GenBank and other scholars (Table 1). 15 partitioned genes from mitogenomes were aligned in Bioedit 7.1.3.0 (11/4/2011) (Hall, 1999) using Clustal W (Thompson *et al.*, 1994). Bayesian inference (BI) and maximum likelihood (ML) methods were used to construct phylogenetic trees. Modelfinder (Kalyaanamoorthy, *et al.*, 2017) chose GTR+F+I+G4 as the preferred substitution model. ML analysis was performed using IQ-Tree 1.6.8 (Guindon *et al.*, 2010; Minh *et al.*, 2013; Nguyen *et al.*, 2015) in Phylosuite 1.2.2 (Zhang *et al.*, 2020) with 500,000 ultrafast bootstraps. Modelfinder was also used to evaluate the best-fit substitution models of BI, and GTR+F+I+G4 was chosen as the optimal substitution model. Mrbayes 3.2.6 (Ronquist *et al.*, 2012) was used to run Bayesian analyses with 10,000,000 generations, sampling every 1000 generations, and discarding 25% of trees as burn-in. A split frequency of less than 0.01 was used to determine stationarity, and a consensus tree was constructed from the remaining trees. To evaluate branch support, standard statistical tests were used (bootstrap support and posterior probability).

3 Result

3.1 Composition of mitochondrial genome

The length of mitochondrial genomes of representative species in the Tykhepoda ranges from 14,444 bp to 15,741 bp. Genes for 12S and 16S rRNAs, 22 tRNAs, 13 PCGs and the control region were identified (Fig. 2). The distribution of PCGs in the concentrated on the positive strand of the mitochondrial genome, and only 4 PCGs (nad5, nad4, nad4, nad1) were located on the negative strand. In *C. doriae*, only 3 PCGs (nad4, nad4, nad1) were distributed on the negative strand.

In *C. doriae*, 16 tRNAs located on the positive strand and 6 tRNAs on the negative strand. The trnF and trnY of *C. doriae* were located on positive strand that different from other Tykhepoda species. The trnI of *C. songi* located on negative

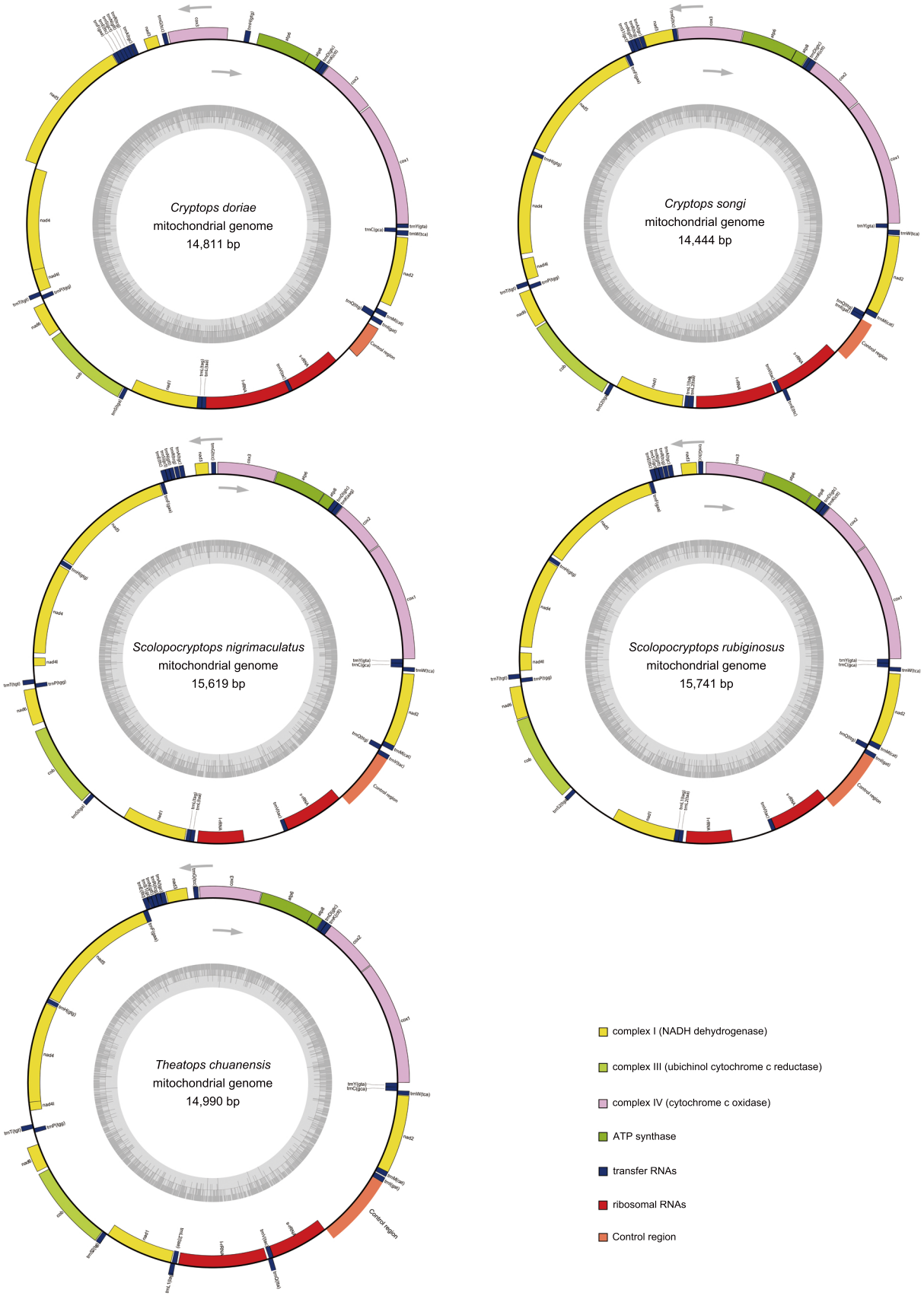

Figure 2. Mitochondrial genomes of *Cryptops doriae*, *C. songi*, *Scolopocryptops nigrimaculatus*, *S. rubiginosus* and *Theatops chuanensis*.

strand and the trnE located on positive strand were different from other Tykhepoda species. The trnQ of Scolopocryptopidae and *C. songi* were located on negative strand that different from *T. chuanensis* and *C. doriae*. In *T. chuanensis*, trnL1 was located on positive strand that different from other Tykhepoda species.

The composition proportion of four bases is imbalanced (Table 2), and A+T% are higher than G+C% in all species. A+T% are 64.79–77.40%, the mean being 71.63%. The (A+T) content is 77.40% in *T. chuanensis* which is the highest among the studied species, while it is lowest in *C. doriae* (64.79%). The GC contents were 23–35%, with the mean 27.9% (Table 2). Strand-specific bias is represented by AT skewness ((A–T)/(A+T)) and GC skewness((G–C)/(G+C)). AT skewness is positive and GC skewness is negative in all species, indicating that all studied motocondrial genomes are CG and AT skewness. Among them, AT skewness of Scolopocryptopidae is lower than in other species (0.045 in *S. nigrimaculatus* and 0.053 in *S. rubiginosus vs.* 0.082–0.094 in other species). The absolute value of GC skewness in Plutoniumidae is the lowest 0.29, followed by Scolopocryptopidae (0.32–0.33), and Cryptopidae is the highest (0.34).

Table 1. Information of mitochondrial genomes used in phylogenetic analysis.

Organism	Length (bp)	GenBank No.	Reference
Theatops chuanensis	14,990	ON513426	This study
Scolopocryptops nigrimaculatus	15,619	ON513424	This study
Scolopocryptops rubiginosus	15,741	OP680784	This study
Scolopocryptops sp.	15,119	KC200076	Gai *et al.*, 2014
Cryptops doriae	14,841	ON513425	This study
Cryptops songi	14,444	OP680783	This study
Scolopendra dehaani	14,538	KY947341	Zhang *et al.*, 2021
Lithobius forficatus	15,437	AJ270997	Hwang *et al.*, 2001
Lithobius forficatus	15,038	MT862427	Yang *et al.*, 2022
Lithobius forficatus	15,695	AF309492	Lavrov *et al.*, 2000
Bothropolys sp.	15,139	AY691655	-
Mecistocephalus marmoratus	15,279	KX774322	Wang *et al.*, 2022
Scutigera coleoptrata	14,922	AJ507061	Negrisolo *et al.*, 2004
Scolopendra hainanum	15,057	MZ569035	Ding *et al.*, 2022
Scolopendra mutilans	15,011	MT175377	Hu *et al.*, 2020
Scolopendra morsitans	14,019	MW810062	Ding *et al.*, 2022

Table 2. Base composition of mitochondrial genome.

Species	A	C	G	T	G+C%	A+T%	AT skewed	GC skewed
Cryptops doriae	5259	3495	1731	4356	35%	65%	0.094	-0.338
Cryptops songi	5465	2929	1433	4617	30%	70%	0.084	-0.343
Scolopocryptops nigrimaculatus	5990	2736	1424	5469	27%	73%	0.046	-0.315
Scolopocryptops rubiginosus	6088	2777	1404	5472	27%	73%	0.053	-0.328
Theatops chuanensis	6283	2189	1198	5320	23%	77%	0.083	-0.293

3.2 Mitochondrial gene rearrangement and RSCU analysis

According to the results of MITOS2, the arrangements of 13 functional PCGs and 2 ribosomal DNA in the Tykhepoda and in the genus *Scolopendra* are conserved. Most species conform to the arrangement pattern: cox1–cox2–ATP8–ATP6–cox3–nad3–nad5–nad4–nad4l–nad6–cob–nad1–l-rRNA–s-rRNA–nad2. Only NAD5 of *C. doriae* aligned in the opposite direction compared to other species, and nad5–nad4–nad4l–nad6–cob and nad1–l-rRNA–s-rRNA of *S. mutilans* are swapped (Fig. 3).

Rearrangements of mitochondrial genomes among Tykhepoda families occurs mainly on tRNAs. Compared to the other studied families. The trnH of *C. doriae* was in front of gene NAD4 but in the rear of gene ATP6 in the other. The trnE of *C. songi* was located on negative strand and overlap with s-rRNA. The trnQ of *T. chuanensis* was located on positive strand and between l-rRNA and s-rRNA while the other was located on negative strand and between trnM and trnI.

Relative synonymous codon usage (RSCU) of mitochondrial genes were calculated (Fig. 4). UUA is the most commonly used ending codons inside the Tykhepoda. The most abundantly used codons are filtered by RSCU≥1. There are

28–32 codons identified as abundantly used codons in these species, among them 21 codons being the overlap codons (AAA AAU ACA ACU AGA AUA CAA CAU CCA CCU GAA GAU GCA GCU GUA UAA UAU UCA UCU UUA UUU). The results of RSCU also showed that codons ending in A/U were used more frequently than those ending in G/C, consistent with the high AT specificity of the mitochondrial genome.

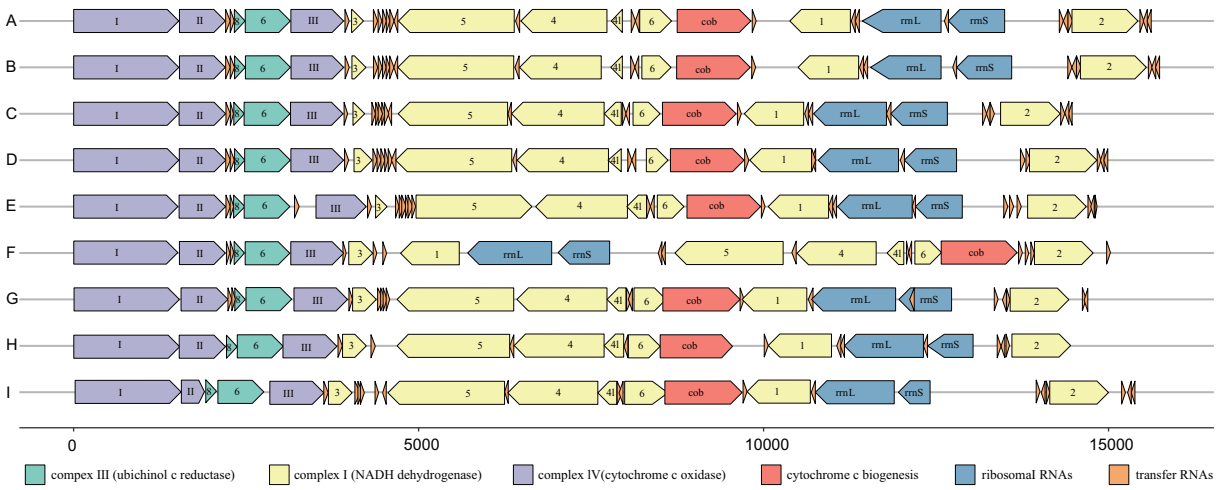

Figure 3. Gene arrangement of Tykhepoda and Scolopendridae. A. *Scolopocryptops nigrimaculatus*; B. *Scolopocryptops rubiginosus*; C. *Cryptops songi*; D. *Theatops chuanensis*; E. *Cryptops doriae*; F. *Scolopendra multilans*; G. *Scolopendra dehaani*; H. *Scolopendra morsitans*; I. *Scolopendra hainanum*.

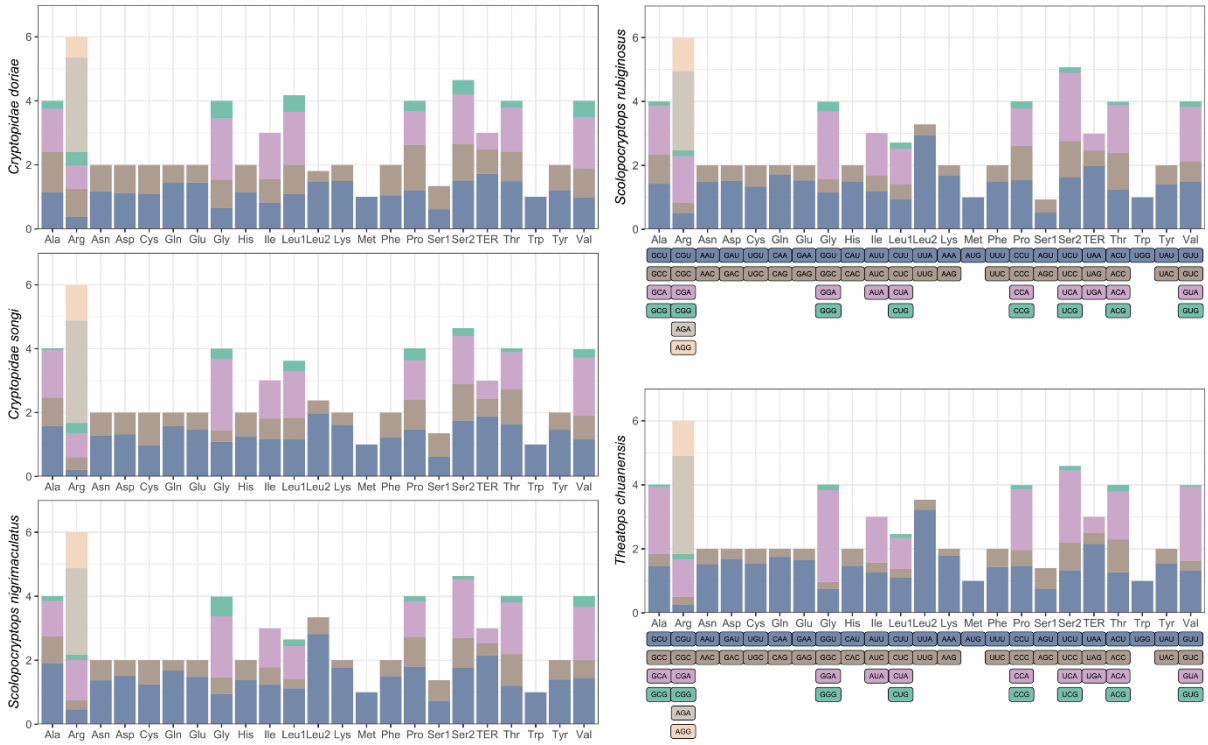

Figure 4. RSCU of mitochondrial genomes of five species from Tykhepoda.

3.3 Phylogenetic analyses

Eighteen mitochondrial genomes of four orders of Chilopoda (Scolopendromorpha, Geophilomorpha, Lithobiomorpha and Scutigeromorpha) are used in this study. Phylogenetic trees are constructed for the combined dataset (all thirteen PCGs and two ribosomal genes) using ML and BI analyses (Fig. 5). The phylogenetic analyse shows high support values for the

monophyletic of the Tykhepoda (BS = 93% and PP = 1) and is sister to Scolopendridae. Within the Tykhepoda, all three families are monophyletic. Cryptopidae is resolved basal as sister to the two other families (Scolopocryptopidae + Plutoniumidae), but with strong support for the latter grouping (BS = 100%, PP = 1). According to the phylogenetic tree, Lithobiomorpha is the sister group of Scolopendromorpha and Geophilomorpha. The poor support of the clade Epimorpha, which unites Geophilomorpha and Scolopendromorpha (BS = 62, PP = 0.97), may result from the small number of Geophilomorpha samples.

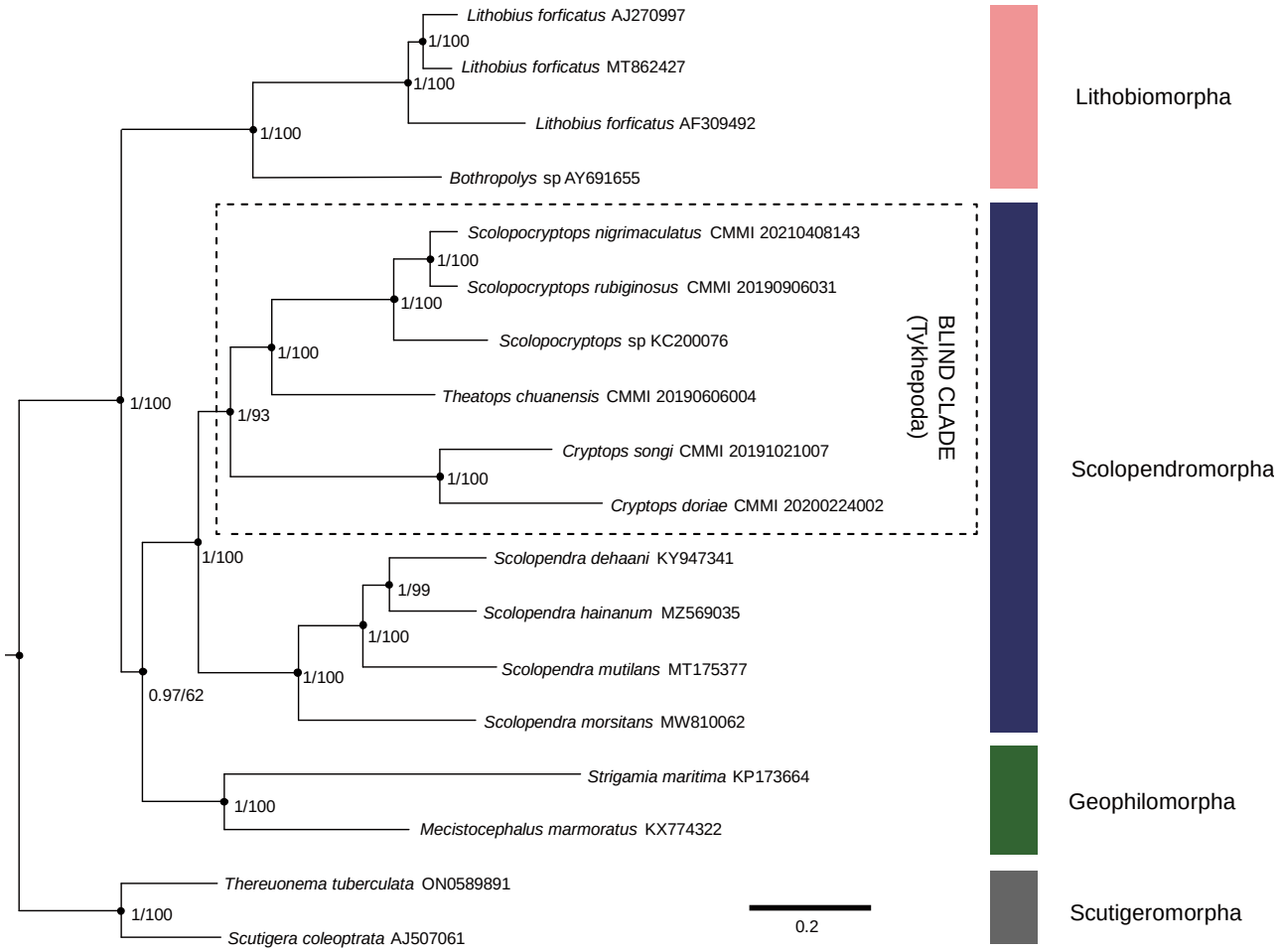

Figure 5. Phylogenetic relationship of Chilopoda inferred from BI analysis based on 13 mitochondrial PCGs and 2 rRNAs. Support values (PP/BS) are shown on the nodes.

4 Dicussion

The mitochondrial genomes of the Tykhepoda are 14,841–15,619 bp in length. A+T% vary from 64.79% to 77.40%, and show obvious CG and AT skewness. The distribution of PCGs in the Scolopendromorpha mainly concentrated on the positive strand of the mitochondrial genome, and only PCGs (nad5, nad4, nad4, nad1) were located on the negative strand (Sun *et al.*, 2018; Pan *et al.*, 2023). In this study, most species in the Tykhepoda also conform to this rule, but in *C. doriae* only 3 PCGs (nad4, nad4, nad1) were distributed on the negative strand. In *Scolopendra*, *S. mutilans* also showed a different arrangement pattern from most of the other species (Vahtera *et al.*, 2012). More species of Scolopendromorpha need to be sequenced to find out whether these new mitochondrial gene arrangement patterns are unique and whether there are other mitochondrial gene arrangement patterns.

According to BI and ML phylogenetic trees, Tykhepoda was monophyletic with strong support (BS = 93% and PP = 1). (Hu *et al.*, 2020) recovered the certainly spurious result of *Scolopocryptops* nesting within *Scolopendra* through mitogenomic phylogenies, but with the improved sampling in this study a morphologically and taxonomically sensible result

of *Scolopocryptops* nesting in the blind clade was recover. (Lavrov *et al.*, 2000; Edgecombe & Koch, 2009; Fernández *et al.*, 2016; Yang *et al.*, 2022). In addition to being blind, this clade shares a distinct morphology of the digestive tract, with a "sieve-type" gizzard at the boundary between the foregut and midgut (Koch *et al.*, 2009). There was no obvious relationship between the PCGs arrangement and phylogeny. but *Theatops* showed different arrangement pattern from the other two species in the distribution of tRNA.

The genetic information revealed by gene rearrangements and phylogenetic relationships reconstructed by mitochondrial genomes may help to solve controversial phylogenetic problems. Prior to our investigation, studies on the mitochondrial genome of the order Scolopendromorpha focused only on the familes Scolopendridae and Scolopocryptopidae (Gai *et al.*, 2014; Ding *et al.*, 2022), and data on other families (Cryptopidae and Plutoniumidae) were lacking. Although mitochondrial genome sequenced now cover almost all families of the Scolopendromorpha, the data are still very limited to allow more comparative studies between the scolopendromorph families.

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