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Paraphyly of the subgenus *Micronecta* (*Micronecta*) Kirkaldy, 1897 (Hemiptera: Heteroptera: Micronectidae) based on mitochondrial genomes and nuclear rDNAs

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Abstract

The genus *Micronecta* Kirkaldy, 1897 is the most species-rich genus in the family Micronectidae, containing more than 160 species. *Micronecta* is currently divided into 11 subgenera, five of which are monotypic. Moreover, the subgenus *Micronecta* is an empirical mixture group. The definitions of some subgenera were based on only a few aberrant morphological features, which are specializations with few phylogenetic significances. The relationship between these subgenera remains unclear. In this study, we newly sequenced mitochondrial genomes (mitogenomes) and nuclear rDNAs (nrDNAs) for 13 *Micronecta* species, representing seven subgenera, and those for ten other water bugs. Our phylogenetic analyses showed that the subgenus *Lundbladella* represents the sister group to all other studied subgenera of *Micronecta*. The subgenus *Unguinecta* was the sister group to the clade that contains *Dichaetonecta* and *Sigmonecta*. More importantly, the subgenus *Micronecta* represents a paraphyletic group, which further forms a monophyletic group together with the subgenera *Basileonecta* and *Ctenonecta*. This is for the first time that the phylogeny of the genus *Micronecta* was investigated based on molecular data and the paraphyly of the subgenus *Micronecta* was revealed. Such evidence suggested the necessity of the revision of the taxonomic system of the genus in the future, and may also serve as a reference for the delimitation of subgeneric characters.

Key words

Aquatic insects; Corixoidea; Nepomorpha; water boatmen; phylogeny; subgenus

1. Introduction

Micronectidae, commonly known as pygmy water boatmen due to their minute size (0.8−5 mm), is a family of aquatic bugs (Nepomorpha) and with representatives in all temperate, subtropical and tropical biogeographical regions (Chen et al. 2005). Micronectids undergo five nymphal instars as do the majority of aquatic and semi-aquatic true bugs. Most species inhabit nearly stagnant or shallow stagnant water, preferring an open sandy or clayey bottom with little or no plant debris (Chen et al. 2015). Usually, we can find a large quantity of individuals in paddy fields. While the diet and feeding habits of micronectid species are unclear, and they probably feed

on fish eggs, algae, detritus, or mosquito larvae (Hädicke et al. 2017). Their complex feeding habits are likely to correlate with the modified spoon- or scoop-like "pala". Males of micronectid species always have stridulatory structures on the right paramere and can produce sound, which are likely to play important role in mating (King 1999) and is a character distinguishing from other corixoids (Jansson 1989; Nieser 2002). In addition, *Micronecta* is the only nepomorphan genus besides *Aphelocheirus* (Aphelocheiridae) known to produce spermatophores (Andersen and Weir 2004).

Two subfamilies are currently recognized: Synaptogobiinae with two species of *Synaptogobia* Nieser and Chen 2006 from the Neotropical Region and Micronectinae with six genera and approximately 210 species predominantly from the Old World (Wróblewski 1972; Nieser and Chen 2006; Tinerella 2008, 2013). *Synaptonecta* Lundblad, 1933 is represented by three species in the Oriental Region, *Papuanecta* Tinerella, 2008 is represented by four species from New Guinea, and *Austronecta* Tinerella, 2013 is represented by four species in Australia. The largest and most widespread genus, *Micronecta* Kirkaldy, 1897 comprises 11 subgenera and more than 160 species is occurring throughout the temperate, subtropical and tropical parts of the Old World, but the fauna of Africa is poorly known and the number of species is doubtful (Nieser and Chen 2006; Ha and Tran 2021). Many species of *Micronecta* have excellent dispersal abilities, and some have enormous distribution ranges, fx. *M.* (*Micronecta*) *ludibunda* Breddin 1905, which is recorded from India, throughout South-East Asia, and eastwards to the Solomon Islands, while others seem to have a much more limited distribution, fx. *M.* (*Micronecta*) *jennferae* Tinerella 2008, which is recorded only from Fiji. No member of Micronectidae have been reported from further East in Oceania, and no records exist from either New Zealand or New Caledonia, both of which are inhabited by numerous species water boatmen (Larivière and Larochelle 2004; Damgaard and Zettel 2014). In the New World, Micronectinae is represented by *Monogobia* Nieser and Chen, 2006, including a single species from Brazil, and *Tenagobia* Bergroth 1899 with seven subgenera and almost 30 species distributed in South- and Central America and with a single species reaching northern Mexico (Nieser 1977; Nieser and Chen 2008). Interestingly, Micronectidae is absent from the Nearctic Region, except for two introduced Old World species from Florida (Polhemus and Rutter 1997; Polhemus and Golia 2006; Epler and Denson 2017).

Currently, the genus *Micronecta* is divided into 11 subgenera: *Basileonecta* Hutchinson, 1940, *Ctenonecta* Wróblewski, 1962, *Dichaetonecta* Hutchinson, 1940, *Indonectella* Hutchinson, 1940, *Lundbladella* Wróblewski, 1967, *Mesonecta* Poisson, 1938, *Micronecta* Kirkaldy, 1897, *Micronectella* Lundblad, 1933, *Pardanecta* Horváth, 1904, *Sigmonecta* Wróblewski, 1962, and *Unguinecta* Nieser, Chen et Yang, 2005 (Hutchinson 1940; Wróblewski 1962, 1967; Nieser et al. 2005; Ha and Tran 2021). It is worth noting that nearly half of all described species had not been formally assigned to any subgenera. Moreover, there are species that were placed tentatively into the subgenus *Micronecta* (Jansson 1995) or left as members of informal species group (Tinerella 2008, 2013). Eight out of the 11 subgenera have been recorded in China, except *Mesonecta*, *Micronectella*, and *Pardanecta*. Up to date, no study has investigated the phylogeny of the genus *Micronecta* and the relationships among those subgenera based on molecular data. While a robust phylogeny is vital to support both the taxonomy and biogeography.

Mitochondrial genomes (mitogenomes) have been widely used in molecular systematics and molecular evolutionary studies (Cameron 2014). The typical insect mitogenome is a circular double-strand molecule about 14-20 kb and encodes 37 genes, including 13 protein coding genes (PCGs), 22 transfer RNAs (tRNAs), and 2 ribosomal RNA genes (rRNAs) (Wolstenholme 1992; Cameron 2014). Comparing with nuclear genomes, the features of mitogenomes, e.g. fast evolutionary rates, small size, low recombination rates and conserved gene arrangements (Curole and Kocher 1999), make it frequently-used in phylogenetic studies in insects (Li et al. 2014; Wang et al. 2016; Li et al. 2017; Chang et al. 2020; Dong et al. 2022; Ye et al. 2022). Besides, nuclear ribosomal DNA (nrDNA) also plays an important role in phylogenetic studies in insects (Kjer 2004; Wang et al. 2016; Ye et al. 2022). As both mitogenomes and nrDNAs have defects in phylogenetic studies, i.e., the former is sensitive to taxon sampling while the latter is too conservative in family and lower levels, we combined the two data types to overcome these disadvantages in this study. Up to now, only one mitogenome of the species *Micronecta* (*Dichaetonecta*) *sahlbergii* (Zhang et al. 2018) and a few nrDNA sequences of various species of the Micronectidae have been released in public databases as of November 30, 2022.

In this study, we sequenced the mitogenomes of 13 *Micronecta* species covering all 37 genes, and comprehensively analyzed the characteristic of these mitogenomes. Meanwhile, ten complete mitogenomes were also sequenced for other water boatmen representing Corixidae (Corixoidea) and Diaprepocoridae (Corixoidea). We also newly provided the corresponding nrDNAs of those 23 species. The nrDNAs for *Lethocerus* sp. (Belostomatidae), *Laccotrephes* sp. (Nepidae), *Enithares* sp. and *Notonecta* sp. (both Notonectidae) were provided for the first time as well. The phylogeny of the genus *Micronecta* was reconstructed based on the whole mitogenomes and nrDNAs.

2. Methods

2.1. Sampling and DNA extraction

Our taxon sampling included 31 species, of which 13 species of *Micronecta* were in-groups and 10 species of other water boatmen and 8 species of the remaining true water bugs were out-groups (Table 1). The 13 species covered seven out of the 8 subgenera distributed in China. They were all preserved in 100% ethanol under –20°C until

Table 1. Taxon sampling used in this study.

Family	Genus	Subgenus	Species	GenBank Accession		
				Mitogenome	18S nrDNA	28S nrDNA
Micronectidae	*Micronecta*	*Basileonecta*	*Micronecta orientalis* *	OQ606211	OQ598531	OQ598681
		Ctenonecta	*Micronecta jaczewskii* *	OQ606210	OQ598532	OQ598676
		Dichaetonecta	*Micronecta sahlbergii* *	OQ606212	OQ598530	OQ598687
		Lundbladella	*Micronecta guttatostriata* *	OQ606215	OQ598533	OQ598675
		Micronecta	*Micronecta wui wui* *	OQ581713	OQ598526	OQ598678
		Micronecta	*Micronecta anatolica* *	OQ606213	OQ598529	OQ598679
		Micronecta	*Micronecta vietnamica**	OQ606216	OQ598534	OQ598684
		Micronecta	*Micronecta drepani* *	OQ606217	OQ598535	OQ598685
		Micronecta	*Micronecta erythra* *	OQ606218	OQ598536	OQ598683
		Micronecta	*Micronecta tuberculata* *	OQ606219	OQ598537	OQ598682
		Micronecta	*Micronecta ornitheia* *	OQ606220	OQ598538	OQ598680
		Micronecta	*Micronecta griseola*	OP850016(COI) OP850221(16S)	/	OP849810
		Micronecta	*Micronecta minutissima*	OP849995(COI) OP850197(16S)	/	OP849786
		Micronecta	*Micronecta poweri*	OP849996(COI) OP850198(16S)	/	OP849787
		Sigmonecta	*Micronecta quadristrigata* *	OQ587936	OQ598527	OQ598686
		Unguinacta	*Micronecta melanochroa* *	OQ606214	OQ598528	OQ598677
		Unguinacta	*Micronecta khasiensis*	OP849907(COI) OP850107(16S)	/	OP849696
	Tenagobia	*Incertagobia*	*Tenagobia incerta* *	OR545228	OR544013	OR552402
Corixidae	*Sigara*		*Sigara striata* *	OQ606224	OQ598548	OQ598671
	Paracorixa		*Paracorixa concinna* *	OQ606223	OQ598547	OQ598672
	Cymatia		*Cymatia coleopterata* *	OQ606225	OQ598542	OQ598668
	Callicorixa		*Callicorixa praeusta* *	OQ606221	OQ598543	OQ598673
	Corixa		*Corixa punctata* *	OQ606226	OQ598544	OQ598670
	Glaenocorisa		*Glaenocorisa propinqua* *	OQ606222	OQ598545	OQ598674
	Hesperocorixa		*Hesperocorixa linnaei* *	OQ606227	OQ598546	OQ598669
Diaprepocoridae	*Diaprepocoris*		*Diaprepocoris barycephalus* *	OQ612738	OQ598549	OQ598666
			Diaprepocoris zealandiae *	OQ612739	OQ598550	OQ598667
Belostomatidae	*Diplonychus*		*Diplonychus rusticus*	FJ456940	KJ461265	KJ461227
	Lethocerus		*Lethocerus indicus*	KM588201	OQ598541*	OQ598663*
Nepidae	*Laccotrephes*		*Laccotrephes* sp.	FJ456948	OQ598540*	OQ598662*
Gelastocoridae	*Nerthra*		*Nerthra indica*	NC012838	KJ461313	KJ461276
Ochteridae	*Ochterus*		*Ochterus marginatus*	FJ456950	KJ461251	KJ461315
Notonectidae	*Enithares*		*Enithares* sp.	FJ456949	OQ598539*	OQ598664*
	Notonecta		*Notonecta* sp.	KX034086	FJ372662	OQ598665*
Aphelocheiridae	*Aphelocheirus*		*Aphelocheirus ellipsoideus*	FJ456939	KJ461184	KJ461297

* Species with newly sequenced mitogenomes and nrDNAs, or newly sequenced nrDNAs in the present study.

used for DNA extraction. These species of *Micronecta* were identified using morphological characteristic provided by Nieser et al. (2005) and Ha and Tran (2021). Whole genomic DNA was extracted from the heads and thoraces using the CTAB method (Reineke et al. 1998).

2.2. Low-coverage genomic sequencing and assembly, annotation, and analysis

An independent DNA library was constructed for each species with an insert size of 250 base pairs (bp), and then sequenced with a 150 bp paired end (PE) using the Illumina HiSeq 4000 Platform at Biomarker Technologies (Qingdao, China). The purified reads were filtered from raw data by removing adaptor contamination and low-quality sequences. To better distinguish the repeat fragment brought by the assemble process, two approaches were employed to assemble the complete mitogenome for each species. For the first method, SOAPDENOVO2 (Luo et al. 2012) were applied to conduct de novo assembly under different settings respectively (-*k* = 61&71). Then the mitogenome and nrDNA assemblies were identified using the program BLAST+ (Camacho et al. 2009) against local databases. All the reference sequences of mitogenome and nrDNA used for constructing local databases were downloaded from the GenBank database. For the second method, MITOBIM (Hahn et al. 2013) was employed to bait and assemble mitogenomes directly referring to the

mitogenomes of closely related species. As for the nrDNAs, only the first method under different *k* values was employed.

The online webserver of MITOS (Bernt et al. 2013) was used to annotate each mitogenome, as well as predict and determine tRNA structures with invertebrate mitochondrial genetic codes. The boundary of protein-coding genes (PCGs) were re-confirmed through Open Reading Frame Finder (ORF Finder) (https://www.ncbi.nlm.nih.gov/orffinder) and verified manually by an alignment with homologous genes from published heteropteran mitogenomes. The boundaries of 12S and 16S rRNAs were delimitated by the boundaries of *tRNA*-Leu (L1) and *tRNA-Val* (V) and compared with homologous regions of known nepomorphan mitogenomes. Boundary definitions of 18S and 28S nrDNAs were also realized by alignment with homologous genes.

Base composition and relative synonymous codon usage (RSCU) were calculated using MEGA 11 (Tamura et al. 2021). Base compositional skews were measured using the formulae AT-skew = (A−T)/(A+T) and GC-skew = (G−C)/(G+C) (Perna and Kocher 1995). DNASP v5 (Librado and Rozas 2009) was used to calculate the rate of non-synonymous substitutions (Ka) and synonymous substitutions (Ks), and the ratio of Ka/Ks for each PCG, in order to evaluate the evolutionary rate of micronectid mitochondrial PCGs. ALIGROOVE (Kück et al. 2014) was used to analyze the compositional heterogeneity across sequences.

2.3. Phylogenetic analyses

Phylogenetic relationships of *Micronecta* were reconstructed based on 37 genes from mitochondrion and 18S and 28S nrDNAs. Individual genes were aligned using MUSCLE integrated in MEGA. The ambiguously aligned sites from both protein and nucleotide alignments were removed using GBlocks (Talavera and Castresana 2007). Then all individual matrixes were concatenated into three datasets for phylogenetic analyses: (1) the PCGNTRNA matrix, including nucleotide sequences of 13PCGs, 22 tRNAs, and two nrDNAs (File S1: PCGNTRNA); (2) the PCGNT12RNA matrix, including the first two codons of nucleotide sequences of 13PCGs, 22 tRNAs, and two nrDNAs (File S2: PCGNT12RNA); (3) the PCGAARNA matrix, comprising amino-acid sequences of 13PCGs and nucleotide sequences of 22 tRNAs and two nrDNAs (File S3: PCGAARNA).

Phylogenetic analyses were conducted using MRBAYES 3.2.6 (Ronquist et al. 2012) for Bayesian inference (BI) and RAXML 8.2.12 in PThreads version (Stamatakis 2014) for Maximum likelihood (ML). We used IQ-TREE (Nguyen et al. 2015) to obtain the best matched substitution model and partitioning schemes. For the BI inference with PCGAARNA matrix, a "mixed" substitution model for amino-acids and a GTR model for nucleotides were employed with a discrete gamma model (G) allowing for a proportion of invariable sites (I). While for the ML analysis with PCGAARNA matrix, the substitution model GTR+G+I for nrDNAs, rRNAs, and tRNAs; amino acid substitution models mtArt+G+I for COI and mtZOA+G+I for the remaining PCGs turned out to be the most appropriate ones. For phylogenetic analyses with matrixes PCGNTRNA and PCGNT12RNA, the substitution model GTR+G+I was employed. In BI analyses, we conducted 2,000,000 generations with sampling every 100 generations. The generations with values of the standard deviation greater than 0.01 were discarded. The numbers of burned generations were also checked with the help of Tracer (available at http://beast.bio.ed.ac.uk/Tracer). In ML analyses, the best ML tree and bootstrap trees were assessed by 1,000 rapid bootstrap replicates (*-f a* option).

3. Results

3.1. Genome organization and nucleotide composition

In this study, lengths of the 13 newly obtained mitogenomes of *Micronecta* species range from 14,825 bp to 15,405 bp (Table 2). The mitogenomes of *M.* (*Micronecta*) *wui wui*, *M.* (*Unguinacta*) *melanochroa*, *M.* (*Micronecta*) *anatolica*, *M.* (*Micronecta*) *vietnamica*, and *M.* (*Micronecta*) *ornitheia* were complete, and the rest mitogenomes were nearly complete with a partial control region (CR). All mitogenomes included 37 genes (13 PCGs, 22 tRNAs, and 2 rRNAs) and a control region, sharing the same strand distribution pattern of coding genes: 23 genes located on the majority strand; the remaining 14 genes located on the minority strand (Fig. 1, Fig. S1). Comparison of the mitogenomes of *Micronecta* species indicated that the PCGs, tRNAs, and rRNAs are relatively conservative in length (14,367–14,482 bp). Detailed statistics for the mitogenomes of the remaining water boatmen were showed in the supplementary Table S1.

The nucleotide composition of *Micronecta* mitogenomes biased toward A/T, with A+T contents ranging from 69.65% to 74.0% (Fig. S2). The mitogenomes of *M.* (*Dichaetonecta*) *sahlbergii* and *M. vietnamica* exhibit the lowest and highest A+T contents, respectively. The AT skew and GC skew present similar patterns in all *Micronecta* mitogenomes, with positive AT skews (from 0.14 to 0.23) and negative GC skews (from –0.27 to –0.11) (Table 2).

The total length of all 13 PCGs ranges from 10,981 bp in *M. anatolica* to 11,187 bp in *M. melanochroa* (Table 2). The A+T content of the 13 PCGs ranges from 68.54% (*M. vietnamica*) to 73.63% (*M. sahlbergii*). The majority of the PCGs in the thirteen *Micronecta* mitogenomes initiate with conventional star codons (ATN), except for *ND2*, *ND4L*, *ND5*, which use TTG as the star codon in several species. The most frequently used stop codon is TAA, followed by T and TAG. Meanwhile, the most prevalent codons are UUA(L), AUU(I), UUU(F),

Table 2. Length of *Micronecta* mitochondrial genomes, AT-skew and GC-skew were measured for the 37 genes except the control regions.

Species	PCGs (bp)	tRNAs (bp)	12S rRNA (bp)	16S rRNA (bp)	CR (bp)	Total (bp)	AT-skew	GC-skew
Micronecta (*Basileonecta*) *orientalis*	11029	1425	730	1127	681	15163	0.16	–0.14
Micronecta (*Ctenonecta*) *jaczewskii*	11044	1425	761	1226	590	14998	0.16	–0.13
Micronecta (*Dichaetonecta*) *sahlbergii*	11017	1425	759	1241	664	15074	0.22	–0.22
Micronecta (*Lundbladella*) *guttatostriata*	10996	1430	768	1242	944	15311	0.22	–0.27
Micronecta (*Micronecta*) *wui wui*	11033	1425	761	1224	589	14992	0.15	–0.12
Micronecta (*Micronecta*) *anatolica*	10981	1423	761	1226	589	14990	0.15	–0.11
Micronecta (*Micronecta*) *vietnamica*	11024	1425	762	1223	666	15072	0.16	–0.14
Micronecta (*Micronecta*) *drepani*	11018	1428	759	1271	939	15405	0.21	–0.14
Micronecta (*Micronecta*) *erythra*	11023	1427	761	1224	777	15195	0.23	–0.16
Micronecta (*Micronecta*) *tuberculata*	11023	1425	761	1225	414	14825	0.14	–0.12
Micronecta (*Micronecta*) *ornitheia*	11028	1424	760	1262	592	15032	0.16	–0.14
Micronecta (*Sigmonecta*) *quadristrigata*	11014	1431	761	1248	578	15000	0.16	–0.25
Micronecta (*Unguinacta*) *melanochroa*	11187	1428	763	1244	519	14948	0.19	–0.23

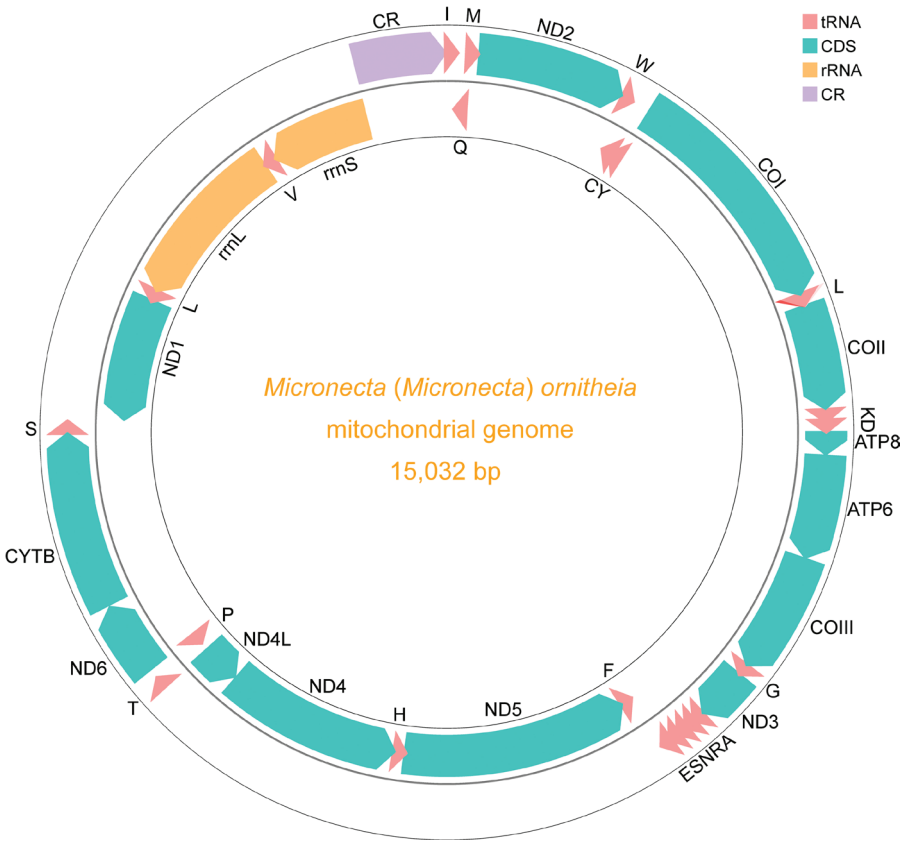

Figure 1. Circular diagram of the *Micronecta* (*Micronecta*) *ornitheia* mitogenome. The transcriptional direction is denoted by arrows.

AUA(M), UAU(Y) and AAU(N), whereas AGG(S), CGC(R) and CGG(R) are rarely used (Fig. S3).

The Ka/Ks ratio is used to evaluate the evolutionary rate of 13 PCGs of the *Micronecta* species (Fig. S4). The results showed that the average Ka/Ks ratios are lower than 1, indicating that these PCGs evolved likely under the purifying selection (Hurst 2002; Ye et al. 2021). Among which the *COI* had the lowest evolutionary rate (0.020), while *ATP8* had the highest evolutionary rate (0.471). The average Ka/Ks ratios of *COI*, *COII*, *COIII*, and *CYTB* (Ka/Ks<0.1) are lower than that of the remaining genes, indicating these four genes are usually under stronger selection and constraints.

There are 22 tRNA genes in the *Micronecta* mitogenomes, as observed in other heteropteran mitogenomes. All tRNAs display the classic clover-leaf secondary structure except *tRNA*-Ser (GCU), with the dihydrouridine (DHU) stem simply forms a loop (Fig. S5). The A+T content of tRNAs ranges from 72.54% (*M.* (*Unguinacta*) *quadristrigata*) to 76.0% (*M. vietnamica*).

The 12S and 16S rRNA genes in *Micronecta* species are encoded on the J-strand and located at conserved

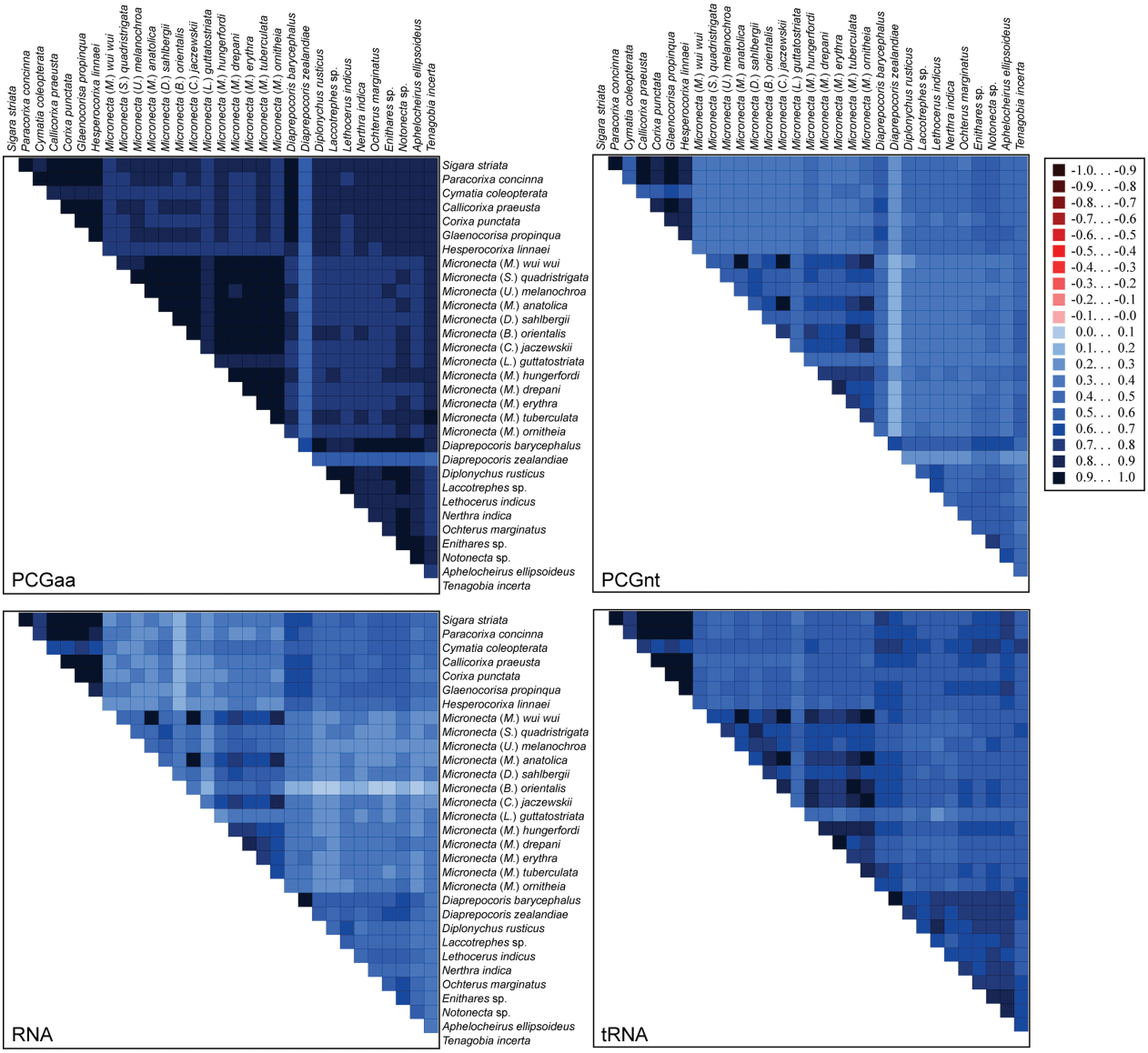

Figure 2. The compositional heterogeneity of mitochondrial sequences used in phylogenetic analyses. The mean similarity score between sequences is represented by a colored square, based on the AliGROOVE scores from -1, indicating great differences in rates from the remainder of the datasets (red), to +1, indicating rates match all other comparisons (blue).

positions between *trnL1* and *trnV* and between *trnV* and control region, respectively. The length of 12S rRNA varies from 730 bp in *M.* (*Basileonecta*) *orientalis* to 768 bp in *M.* (*Lundbladella*) *guttatostriata*, with A+T content from 71.22% in *M. guttatostriata* to 76.61% in *M.* (*Micronecta*) *tuberculata*. The length of 16S rRNA ranges from 1,127 bp in *M. orientalis* to 1,271 bp in *M.* (*Micronecta*) *drepani*, with A + T content from 73.67% in *M. guttatostriata* to 78.09% in *M. vietnamica*. Hence, there is no substantial size variation in 12S and 16S rRNA among the mitogenomes of the thirteen *Micronecta* species (Table 2).

Heterogeneous composition of amino-acid or nucleotide sequences may bias results of likelihood based tree reconstructions. The AliGROOVE analyses showed a low heterogeneity in both nucleotide sequences and amino-acid sequences of PCGs (Fig. 2). Thus, our phylogenetic results were hardly influenced by sequence heterogeneity.

3.2. Phylogenetic analyses

Phylogenetic analyses using both BI and ML approaches based on different datasets produced a congruent and well-resolved tree (Fig. 3, Figs S6–S11). All families of Corixoidea, i.e., Micronectidae, Corixidae and Diaprepocoridae, were consistently recovered as monophyletic groups.

Within Micronectidae, the genus *Micronecta* was strongly supported as a monophyletic group and split into three well-supported clades. Subgenus *Lundbladella* was recovered as the sister group to all other *Micronecta* by all analyses. Subgenus *Unguinaccta* were supported as the sister group to subgenera *Dichaetonecta* and *Sigmonecta*. Subgenus *Micronecta* together with subgenera *Ctenonecta* and *Basileonecta* formed a monophyletic clade. Subgenus *Micronecta* were recovered as paraphyly based on both BI and ML analyses. For this clade, the relationship among the three groups, i.e., (*M.*

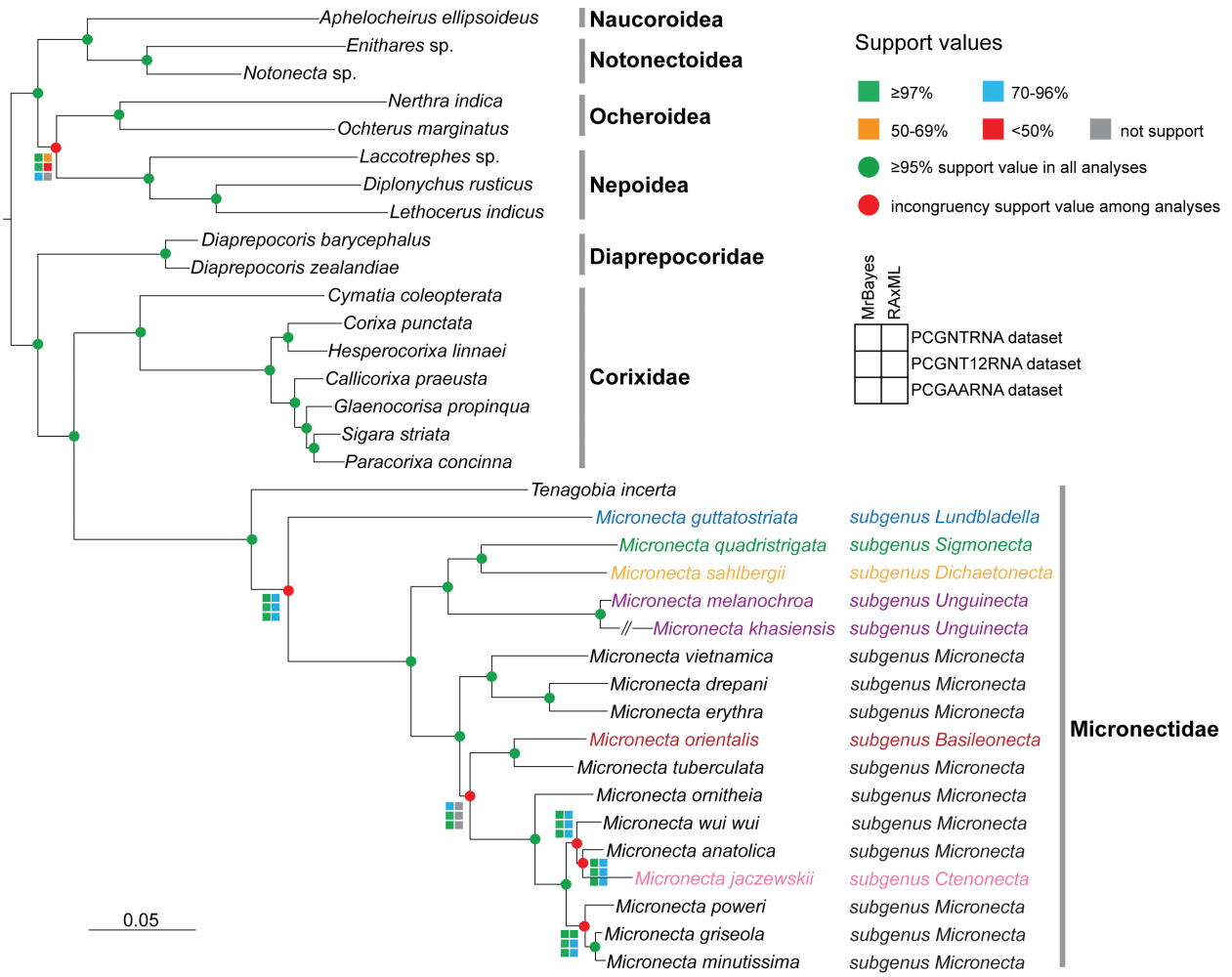

Figure 3. Phylogenomic relationships of *Micronecta*. The tree was constructed using the PCGNT12RNA dataset with Bayesian analysis. The bootstrap values of maximum-likelihood analyses and posterior probabilities of Bayesian analyses are summarized and labelled around each node. Higher taxa are indicated as taxon labels on the right of the tree.

vietnamica + *M. drepani* + *M.* (*Micronecta*) *erythra*), (*M. orientalis* + *M. tuberculata*), and (*M. ornitheia*, *M. wui wui*, *M. anatolica*, *M.* (*Ctenonecta*) *jaczewskii*, *M. poweri*, *M. griseola*, *M. minutissima*) are controversial among different analyses (Fig. S6–S11). The results of all BI analyses support the sister relationship between (*M. orientalis* + *M. tuberculata*) and (*M. ornitheia*, *M. wui wui*, *M. anatolica*, *M. jaczewskii*, *M. poweri*, *M. griseola*, *M. minutissima*), while the ML analyses exhibit different topologies.

4. Discussion

Our study presents 13 newly sequenced mitogenomes of the genus *Micronecta*, 1 that of the genus *Tenagobia* (Micronectidae) and ten those of the remaining water boatmen (Corixidae, Diaprepocoridae). All mitogenomes exhibited the similar putative pattern as in other heteropteran insects (Cameron et al. 2014; Ye et al. 2021).

Phylogenetic trees based on mitogenomes and nrDNAs are largely congruent among different analyses, which laid a foundation for further phylogenetic analyses and taxonomic studies. Before this study, only two works involved the phylogenetic relationships between micronectid genera of continental Australia based on morphological characters (i.e., Tinerella, 2008, 2013), in which the subgenera *Dichaetonecta* and *Sigmonecta* were also recovered as sister groups. They share the same shape of the left paramere shaft, which is long, straight and narrow.

Among the 11 nominated subgenera, male individuals of three subgenera, i.e., *Lundbladella*, *Indonectella*, *Micronectella*, lack the strigil structure on abdominal tergite VI (Wróblewski 1967; Ha and Tran 2021). While within Micronectidae, both the genera *Monogobia* and *Tenagobia* lack this structure as well. Presence and absence of strigil is likely a secondary character, which cannot serve as the evidence for the close relationship among the three subgenera mentioned above. Both the subgenera *Lundbladella* and *Indonectella* are monotypic subgenus, while *Micronectella* include two species. Unfortunately, it was not possible to analyze representatives of subgenera *Indonectella* and *Micronectella*. The status of the subgenus *Lundbladella* as sister group to all other *Micronecta* in this study needs to be verified with more

taxa sampling from other subgenera, especially *Indonectella* and *Micronectella*.

According to the identification key provided by Hutchinson (1940) and Ha and Tran (2021), diagnostic features of current subgenera of *Micronecta* were only applicable to male specimens, i.e., the shape of the palar claw, the setae of seventh abdominal sternite, the free lobe of eighth abdominal tergite and the morphology of the left paramere, some of which are potentially homoplasious characters. For example, the free lobe of subgenus *Dichaetonecta* and *Micronecta*, is nearly rectangular. The left paramere of subgenera *Basileonecta* and *Ctenonecta* is styliform. As the phylogenetic results shown, both the subgenera *Basileonecta* and *Ctenonecta* imbedded within the subgenus *Micronecta* and therefore their subgeneric status is questionable. It probably need more stable characteristics to identify or redefine current subgenera.

The genus *Micronecta* is the most diverse and speciose group of Micronectidae, which is the same condition with the subgenus *Micronecta*. Although 11 subgenera have been proposed to accompany the extant species of the genus *Micronecta*, there are still some species which do not fit any known subgenus. As a result, they were just placed tentatively into the subgenus *Micronecta* (see Jansson 1995). There are also some species which do not fit any known subgenus were left as incertae sedis or species groups (Ha and Tran 2021). In future, a more comprehensive taxon sampling including all subgenera even those species that were not assigned to any subgenera is still expected via a broad range of international collaborations.

As a result, the current taxonomy of *Micronecta* does not yet satisfactorily reflect natural relationships among subgeneric taxa. As more and more species are being describ, it is necessary to redefine the subgenus *Micronecta* or split it into more subgenera. The taxon sampling might not be that complete, although the paraphyly of the subgenus *Micronecta* can be revealed convincingly.

5. Conclusion

In this study, we investigated the phylogenetic relationships concerning the genus *Micronecta* based on the species sampled in China. This is the first time that the subgeneric relationships among *Micronecta* were investigated based on molecular evidence. Our main findings are the paraphyly of the subgenus *Micronecta*, the status of the subgenus *Lundbladella* as sister group to all other studied *Micronecta*, and the sister relationship between the subgenera *Dichaetonecta* and *Sigmonecta*. This study provided a chance to redefine the subgenera level classification of the genus *Micronecta* and laid a foundation for further molecular studies with complete taxon sampling to fully resolve the phylogeny of *Micronecta* via a broad range of international collaborations.

6. Authors' contributions

Conceptualization, Y.W., Q.X.; funding acquisition, Y.W.; formal analysis, B.X., Y.W. and J.X.; writing—original draft preparation, B.X., Y.W., Q.X.; writing—review and editing, J.D., P.C. and Q.X. All authors have read and agreed to the published version of the manuscript.

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Supplementary Material 1

Figures S1–S11

Authors: Xie B-J, Chen P-P, Damgaard J, Xie J-Y, Xie Q, Wang Y-H (2024)
Data type: .docx
Explanation notes: Figure S1. Circular diagram of the mitochondrial genomes of *Micronecta* spp. and *Tenagobia incerta*. — **Figure S2.** The A+T content of *Micronecta* spp. mitochondrial genomes. — **Figure S3.** Relative synonymous codon usage (RSCU) of mitochondrial genomes of *Micronecta* spp. — **Figure S4.** Average evolutionary rate of *Micronecta* mitochondrial PCGs. — **Figure S5.** Universal models of *Micronecta* mitochondrial tRNAs. — **Figure S6.** Phylogenetic tree inferred from PCGNTRNA matrix using ML analysis. Numbers at the nodes are bootstrap values. — **Figure S7.** Phylogenetic tree inferred from PCGNTRNA matrix using BI analysis. Numbers at the nodes are Bayesian posterior probabilities. — **Figure S8.** Phylogenetic tree inferred from PCGNT12RNA matrix using ML analysis. Numbers at the nodes are bootstrap values. — **Figure S9.** Phylogenetic tree inferred from PCGNT12RNA matrix using BI analysis. Numbers at the nodes are Bayesian posterior probabilities. — **Figure S10.** Phylogenetic tree inferred from PCGAARNA matrix using ML analysis. Numbers at the nodes are bootstrap values. — **Figure S11.** Phylogenetic tree inferred from PCGAARNA matrix using BI analysis. Numbers at the nodes are Bayesian posterior probabilities.

Link: https://doi.org/asp.82.e108906.suppl1

Supplementary Material 2

Tables S1, S2

Authors: Xie B-J, Chen P-P, Damgaard J, Xie J-Y, Xie Q, Wang Y-H (2024)
Data type: .docx
Explanation notes: Table S1. Mitochondrial genome statistics for the other water boatmen. AT-skew and GC-skew were measured for the 37 genes except the control regions. Only partial mitogenome of *Diaprepocoris zealandiae* was available, so it was not included in this table. — **Table S2.** Locality data for each *Micronecta* species used in this study.

Link: https://doi.org/asp.82.e108906.suppl2

Supplementary Material 3

Files S1–S3

Authors: Xie B-J, Chen P-P, Damgaard J, Xie J-Y, Xie Q, Wang Y-H (2024)
Data type: .zip
Explanation notes: File S1. PCGNTRNA matrix. — **File S2.** PCGNT12RNA matrix. — **File S3.** PCGAARNA matrix.

Link: https://doi.org/asp.82.e108906.suppl3