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A new species of the genus *Hilethera* (Orthoptera: Acrididae: Oedipodinae) from China and its complete mitochondrial genome

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Abstract

The new species, *Hilethera xinjiangensis* **sp. nov.** (Orthoptera: Acrididae: Oedipodinae) is described based on specimens collected from Xinjiang, northern China. The new species is similar to *H. brevipennis* Zheng & Lu, 2002 and *H. turanica* Uvarov, 1925, but differs from: (1) dark brown in general coloration, (2) darker coloration in forewings, (3) forewings longer than *H. brevipennis* but shorter than *H. turanica*, (4) cubital area of forewings boarder than *H. brevipennis* and *H. turanica*, (5) hind tibiae dark brown with two light yellow pre-basal rings, while dark with one fade pre-basal ring in *H. brevipennis* and light yellow with three dark rings in *H. turanica.*

In addition, the complete mitogenome of holotype was sequenced using next-generation sequencing technology. The total length of the assembled mitogenome is 16,145 bp, representing 13 protein-coding genes, 22 transfer RNA genes, two ribosomal RNA genes and one noncoding region (D-loop region). Besides, the new mitogenome sequence is compared with published Oedipodinae mitogenomes and the phylogenetic relationships within the subfamily are reconstructed. The results infer that the gene cox1 could be a useful marker for higher phylogenetic level, while the genes *nd5* and *rrnL* could be a potentially useful markers between closely related species.

Key words: new species, mitogenome, phylogenetic marker, China, Asia

Introduction

The subfamily Oedipodinae is one of the few nearly globally distributed subfamilies within the short-horned grasshoppers (Caelifera), with major radiations in the Palaearctic as well as in the Nearctic, occupying every conceivable terrestrial habitats outside of the polar regions and playing important roles in the ecosystem (Uvarov, 1966; Vickery, 1977; Vickery, 1997; Vickery & Kevan, 1983; Cigliano *et al.*, 2018). Its diversity in numerous lineages, form and function has attracted attention in many biological fields, including systematics, evolutionary ecology and biogeography (e.g., Chapco & Contreras, 2011; Husemann *et al.* 2012; Song *et al.*, 2015, 2018).

According to the early study, the subfamily Oedipodinae had an ancient origin, before the separation of Laurasia from Africa (Vickery, 1989) and Asiamerica was the center of initial oedipodine radiation about 94 Mya (million years ago) (Fires *et al.*, 2007). Based on recent available worldwide biogeographical framework of Orthopteran insects (Song *et al.*, 2015, 2018), oedipodine grasshoppers, known as strong fliers and colonizers, may diverge from Africa ancestor in the late Paleocene, and undergo an explosive adaptive radiation, powered by the evolution of a new niche space (grasslands) and frequent founder events after the colonization of new habitats.

Within Oedipodinae, the genus *Hilethera* Uvarov, 1923 is characterized by long band wings and stubby legs. It is distributed widely, from Africa to Asia and containing habitat from Asia-temperate to tropical Africa (Cigliano *et al.*, 2018). This wide distribution and high diversity in habitat suggest that *Hilethera* may constitute a valuable model to address questions about patterns of endemism, species diversification, evolution and biogeography.

To better understand the origin and evolution of the genus *Hilethera*, basic data are needed for as many species as possible, and this in turn necessitates taxonomic and biological studies, e.g., molecular markers for species identification (Hajibabaei *et al.*, 2007; Crampton-Platt *et al.*, 2016). Since the genus was established by B. P. Uvarov in 1923, 11 species were recorded on the Orthoptera Species File (OSF, Cigliano *et al.*, 2018). However, there is not yet molecular work related to *Hilethera* species.

In this study, we report a new species and present its complete mitogenome. Furthermore, comparative and phylogenetic analyses within Oedipodinae

mitogenomes are given to help understand the evolution characterization of mitogenome structure of Oedipodinae and provide a preliminary phylogeny of Oedipodinae for future research.

Materials and Methods

Materials. The new material was collected in the expedition of Xinjiang, China, and deposited in the museum of Shaanxi Normal University.

Molecular techniques. Total genomic DNA was extracted from the hind femur muscles using the traditional cetyltrimethylammonium bromide (CTAB) method. Total genomic DNA was sequenced using the Illumine HiSeq^{TM}2500 platform (Biomarker technologies Co., Ltd., Beijing, China). In all, 7.48 million raw reads of 250 bp each were retrieved. They were quality-trimmed with Trimmomatic v0.35 (Bolger *et al.,* 2014), and then were used to assemble the mitochondrial genome using a MIRA4/Mitobim combined pipeline (Hahn *et al.,* 2013) with a complete mitochondrial genome of *Aiolopus thalassinus* (GenBank accession Number: NC_034674) as the initial reference. The molecular service including total genomic DNA extraction and sequencing were completed at the Biomarker technologies company in China. The mitogenome assembly was carried out on the computer workstation of Shaanxi University, China.

Sequence analysis. Annotation of the mitogenome was performed using the MITO webserver with invertebrate genetic code (Bernt *et al*., 2013) and modified after comparisons with other mitogenomes from Oedipodine species. The validation of tRNA sequences was performed in tRNAscan-SE using the invertebrate mitogenome genetic codon (http://lowelab.ucsc.edu/tRNAscan-SE/; Lowe & Chan, 2016). The nucleotide base composition of the complete mitogenome was calculated in GENEIOUS R9 (Biomatters Ltd., Auckland, New Zealand). The nucleotide compositional skew was calculated following the formula (Konstantionv *et al.*, 2016): AT-skew=(A-T)/(A+T) and GC-skew=(G-C)/(G+C), where A, C, G and T are the frequencies of the four bases.

Besides the mitochondrial genomes of *Hilethera xinjiangensis* sp. nov., the nucleotide base composition comparison analysis for additional 15 Oedipodinae grasshopper mitogenomes from GenBank (see in Table 3) were also calculated, at

the level of the whole mitogenomes, for the two ribosomal genes (the large and small ribosomal subunit, *rrnL* and *rrnS*) and at the level of the A+T-rich region.

Pairwise genetic distances within 16 Oedipodine species based on single protein coding genes (PCGs) and two ribosomal genes (*rrnL* and *rrnS*) were calculated in GENEIOUS R9 (Biomatters Ltd., Auckland, New Zealand). Besides, the comparison analysis of synonymous and non-synonymous substitutions (Ka/Ks) for all PCGs in Oedipodinae were calculated using MEGA 7.0 (Kumar *et al.*, 2016).

Phylogenetic analyses. In addition to the newly generated mitogenome of *Hilethera xinjiangensis* sp. nov., we used all available mitogenomes of Oedipodinae in GenBank to reconstruct the phylogeny of the subfamily. According to the previous studies (Chapco & Contreras, 2011; Song *et al.*, 2018), the subfamily Oedipodinae are not monophyletic, and was nested in the subfamilies Acridinae and Gomphocerinae. Therefore, we also add all available mitogenomes of the two latter families recorded in GenBank. The mitogenome of *Ommexecha virens* (accession number: NC_020778) and *Tristira magellanica* (accession number: NC_020773) were selected as outgroup. The dataset contained all three codon positions of 13 PCGs and two rRNAs (*rrnL* and *rrnS*). The final alignment implemented by GENEIOUS R9 (Biomatters Ltd., Auckland, New Zealand), and the concatenated dataset resulted in 13,451 bp sites. The concatenated dataset was then analyzed using Bayesian inference (BI) and maximum likelihood (ML). For both BI and ML we used PartitionFinder V2.1.1 (Lanfear *et al.*, 2017) to determine best-fit partitioning schemes and the associated substitution models. Bayesian inference analyses were performed with MrBayes 3.2.6 (Ronquist *et al.*, 2012) whereas ML analyses were performed with IQ-TREE 1.6.2 (Nguyen *et al.*, 2015). All corresponding analyses were performed using the CIPRES Science Gateway 3.3 (Miller *et al.*, 2015).

Abbreviations

Measurements --- PronL: pronotum length; PronW: pronotum width; FWL: forewing length; FWW: forewing width; FIIIL: length of hind femora; FIIIW: width of hind femora; Is: inner spines on TIII dorsal side; Os: outer spines on TIII dorsal side; TIIIL: length of hind tibiae.

Taxonomic part

Subfamily Oedipodinae Walker, 1871

Tribe Epacromiini Brunner von Wattenwyl, 1893

Genus *Hilethera* Uvarov, 1923

Lerina Bolivar, 1902: 602 (nom. Praeocc.).

Lerinnia Uvarov, 1940: 176 (Syn. nov.);

Hilethera Uvarov, 1923: 82; 1925: 33; B.-Bienko & Mistshenko, 1951: 570; Dirsh, 1958: 56; Dirsh, 1965: 398; Zheng, 1993: 442; Yin *et al.*, 1996: 332; Zheng & Xia, 1998: 616; Zheng & Lu, 2002: 16; Bughio et al., 2012.

Type species: *Hilethera hierichonica* Uvarov, 1923

Diagnosis. Size medium to small among the subfamily Oedipodinae with compressed black spots. Head shorter than pronotum. Vertex wide and short. Face slightly sloping backward. Fastigium of vertex protruded and flat, lacking median keel. Eyes large and oval shaped. Foveolae distinctly triangular. Antennae slender, filiform. Median keel of pronotum depressed and thin, cut only by posterior transverse sulcus located above the middle of pronotum, while lateral keel weak or nearly absent in the metazona; posterior margin of pronotum right-angled or obtusely angulate. Interspace of mesosternal lobes wide. Tegmina and hind wings well developed, surpassing apex of hind femora. Median intercalary vein of forewings approaching to median vein or between median vein and cubitus, without cross veins before median intercalary vein; cubital area wider than medial area, with or without intercalary vein. Median vein and precubitus of hing wings close, without dark transverse stripe. Hind femora short and thick, mostly dark in the inner side. Hind tibiae distinctly shorter than hid formora. Aroliums between claws of tarsus small. Subgenital plate of male short conical.

Distribution. Central and western Asia, Africa.

***Hilethera xinjiangensis* sp. nov.**

(Figs 1-2)

Material examined. Holotype (male): **China**, Xinjiang, Burqin Kanas, 48.2244° N, 87.0053° E, 1172 m, 23 June 2017, coll. Zhenning Chen & Haisheng Chen. Paratypes. **China**, same locality as holotype, Xinjiang, Burqin Kanas, 48.2244° N, 87.0053° E, 1172 m, 23 June 2017, 2 males, coll. Zhenning Chen & Haisheng Chen.

Etymology. The species is named after the type locality.

Distribution. North of Xinjiang, China.

Diagnosis. Species of small size for the genus, close to *H. turanica* from Xinjiang, China and *H. brevipennis* from Shanxi, China, from which it differs by general coloration darker, length of tegmina shorter, coloration-banded in forewings and hind tibiae, and cubital area of forewings boarder. The major differences are listed in Table1.

Description. Male (holotype). Body small, stubby and short. Head slightly higher than pronotum in lateral view but shorter in dorsal view. Apex of vertex slightly protruding before eyes, anterior margin straight, weakly depressed in middle area; median keel of vertex slender, lateral margin prominent and distinctly higher than upper margin of eyes; vertex and frons forming roundness in profile. Foveolae elongate-triangular, with sharp, raised margins, the lower margin slightly incurved; foveola and vertex forming obtuse angle in dorsal view. Antennae filiform but incrassate, not extending to posterior margin of pronotum. Eyes large and prominent, oval, their longitudinal diameter about 1.4 times their horizontal diameter and about 1.5 times length of subocular furrow; median ocellus located between lower margins of eyes. Frontal ridge flat with a faint constriction near the ocellum; lateral margins of frontal ridge protruding and nearly parallel, the width of frontal ridge between the antennae slightly smaller than below median ocellus. In dorsal view, anterior margin of pronotum slightly round, posterior margin curved, forming nearly a right angle; median keel obvious, lateral keel absent in prozona while clearly present but irregularly curved in metazona; the length of prozona smaller than that of metazoan; anterior and media transverse sulci indistinct whereas posterior transverse sulcus distinct and cut by median keel. Lateral lobe of pronotum saddle-shaped with a distinct incision on posterior transverse sulcus, anterior and posterior margins of lateral lobe nearly parallel, lower margin S-shaped, anterior lower angle obtuse but

posterior lower angle right-angle, lateral lobe of pronotum with two longitudinal and one short transverse sulci in prozona, and two long transverse sulci in metazona. Tegmina large, exceeding apex of femora but not reaching median of hind tibiae; length of tegmina about 6 times wider than wide. Cubital area of forewings about 1.7 times wider than medial area. Medial area with one distinct intercalary vein. Hind femur robust, about 3.7 times longer than wide, tip of lower knee lobe rounded. Posterior tibia with 11 spines on inner side and 11-12 spines on outer side, outer apical spine present. Arolium shorter than claw. Tympanal organ developed, tympanic cavity oval. Epiproct triangular, with broad median longitudinal sulcus, apex acute. Cercus conical, surpassing apex of epiproct. Subgenital plate long and conical, apex acute, surpassing apex of epiproct in dorsal view. Epiphallus with arch bridge, ancorae curved inward, apex pointed, lophi curved.

Coloration. Body dark brown. Eyes brown. Antennae yellowish brown with dark brown base. Pronotum dark brown with yellowish V-shape. Forewings dark brown with melanin spots on basal and anterior margin, and two fading stripes on median part; hind wings dark brown on anterior part and transparent on remaining part. Hind femora black dark with a preapical yellowish ring; knee lobes light yellow. Hind tibia dark brown with yellowish base and two light yellow pre-basal rings.

Female unknown.

Measurements. See Table 2.

TABLE 1. Comparison of *Hilethera xinjiangensis* sp. nov. with *H. brevipennis* and *H. turanica*.

Characters	*Hilethera xinjiangensis* sp. nov.	*H. brevipennis*	*H. turanica*
General coloration	dark brown	brown	brown
Apex of forewing	exceeding apex of femur but not reaching the middle of hind tibiae	reaching to the apex of hind femur	reaching to the middle of hind tibiae
Coloration of forewing	dark brown with two fading stripes on median part	two indistinct black stripes on median part	two black stripes on median part
Cubital area of forewing	1.7 times wider than medial area	1.5 times wider than medial area	1.3 times wider than medial area
Hind tibiae	dark brown with two light yellow pre-basal rings	dark with one fade pre-basal ring	light yellow with three dark rings

TABLE 2. Measure of *Hilethera xinjiangensis* sp. nov. (in mm, mean values in brackets).

	PronL	PronW	FWL	FWW	FIIIL	FIIIW	TIIIL	TIIIs	
								Is	Os
Holotype	3.6	2.8	17.0	2.8	4.8	2.3	10.0	11	11
Male paratype (n=2)	(3.6)	(2.7)	(17.1)	(2.8)	4.7	2.2	10.1	11	12
Male (n=3)	(3.6)	(2.8)	(17.0)	(2.8)	(4.8)	(2.3)	(10.5)	(11)	(11)

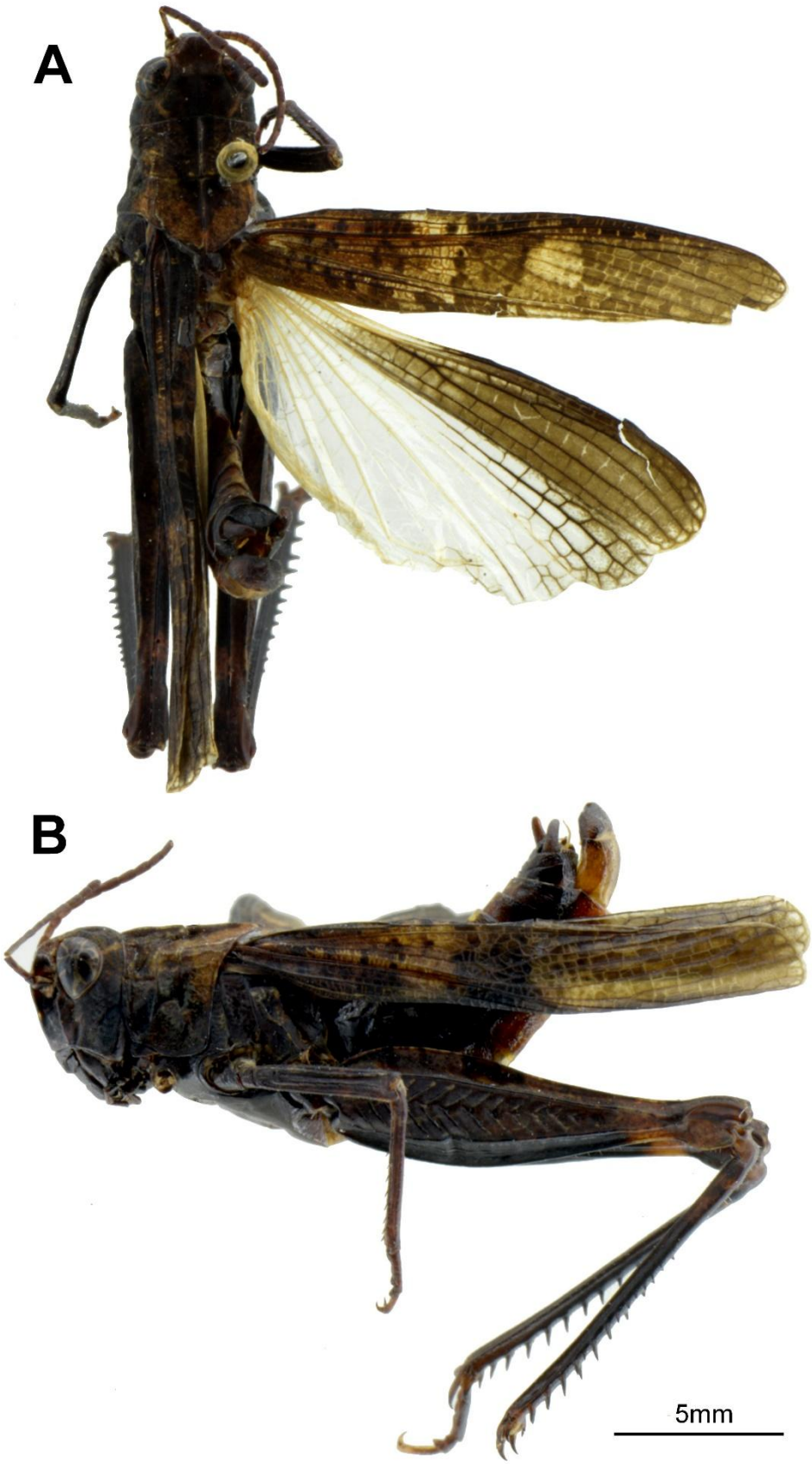

FIGURE 1. *Hilethera xinjiangensis* **sp. nov.** male habitus: dorsal (A) and lateral (B) views. Scale bar: 5 mm.

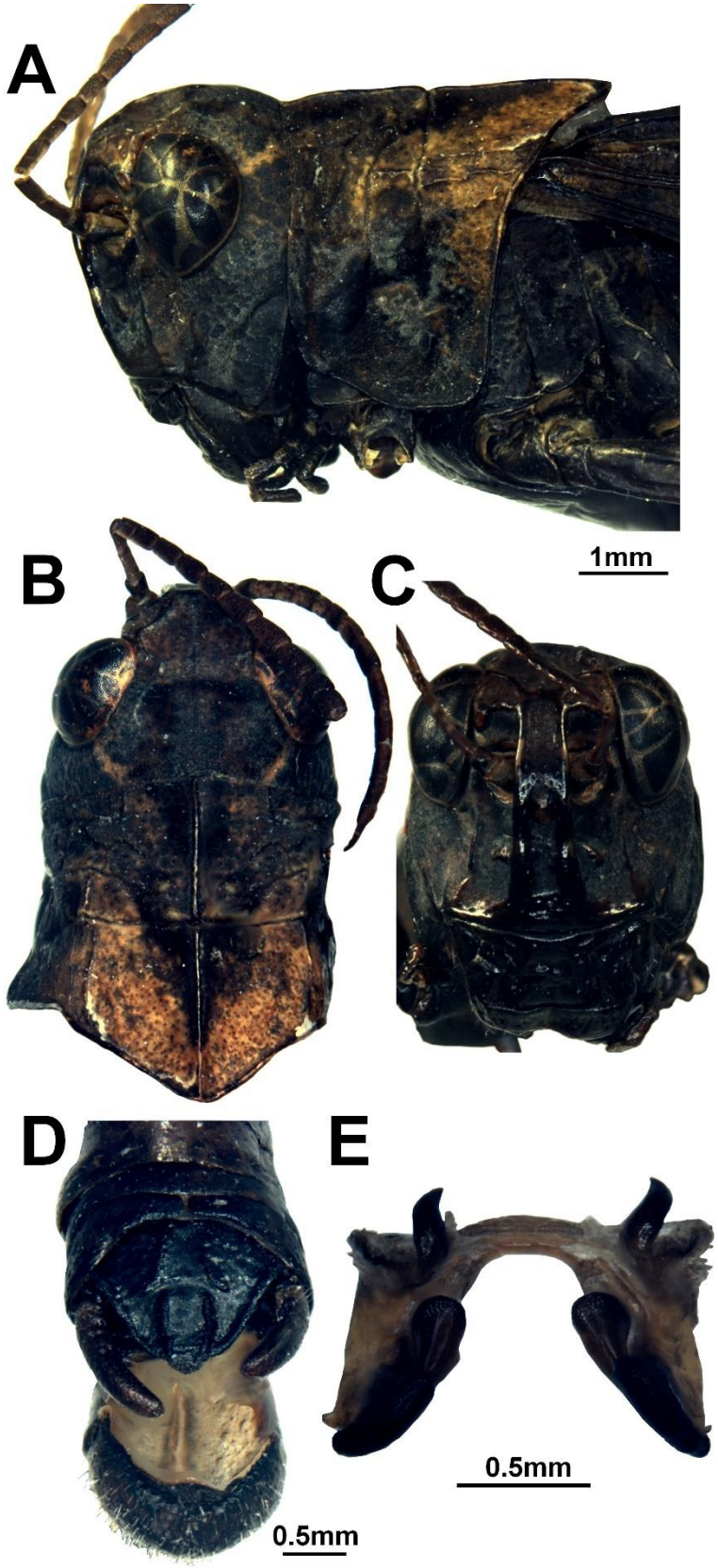

FIGURE 2. *Hilethera xinjiangensis* **sp. nov.** male: head and pronotum in lateral (A) and dorsal (B) views; head in facial view (C); abdomen in dorsal view (D); epiphallus (E). Scale bar: 1mm (A, B and C), 0.5mm (D and E).

Molecular Results

Genome organization, structure, and composition. The mitogenome of *Hilethera xinjiangensis* **sp. nov.** is 16,145 bp long (Fig. 3), organized in the typical set of 37 genes (13 protein-coding genes, 22 transfer RNA genes and two ribosomal RNA genes) and one A+T-rich control region (Table 3). There are 137 bp intergenic nucleotides in total, in 17 locations, and the length of the intergenic spacers ranges in size from 1 to 30 bp. The longest intergenic spacer (30 bp) was located between the tRNA-Leu$^{(UAG)}$ and tRNA-Val genes. Nine pairs of genes overlapped each other, with a length ranging from 1 to 8 bp, and with the longest overlap (8 bp) located between *nd2* and tRNA-Cys, and tRNA-Cys and *cox1*, respectively. Twelve pairs of genes are directly adjacent.

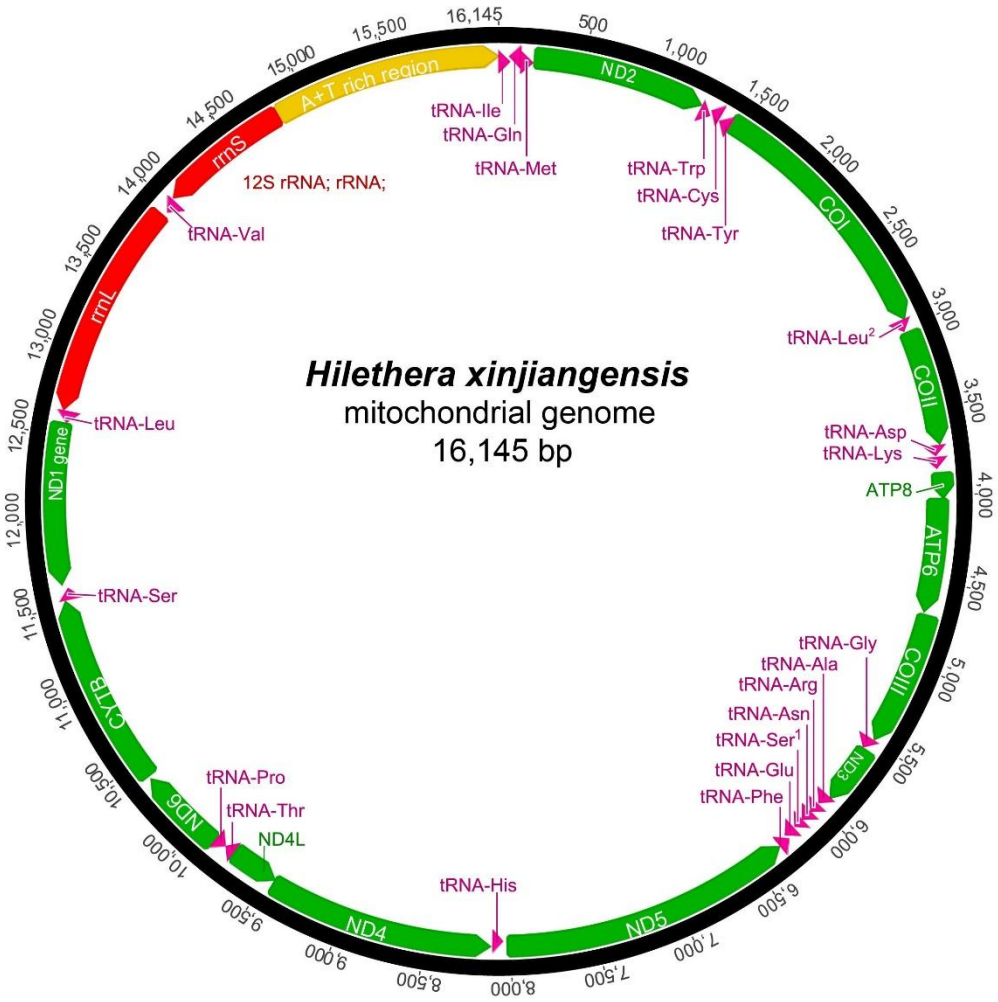

FIGURE 3. Map of the mitochondrial genome of *Hilethera* sp. nov.

TABLE 3. Annotation of the mitochondrial genome of *Hilethera xinjiangensis* sp. nov.

Gene	Strand	Anticodon	Start codon	Stop codon	Position	Length
tRNA-Ile	+	AUC			1-67 (-3)	67
tRNA-Gln	-	UUG			65-133 (-1)	69
tRNA-Met	+	AUG			133-203 (0)	71
nd2	+		ATG	T	204-1,224 (0)	1,021
tRNA-Trp	+	UCA			1,225-1,290 (-8)	66
tRNA-Cys	-	GCA			1,283-1,348 (8)	66
tRNA-Tyr	-	GUA			1,357-1,423 (-8)	67
cox1	+		ATC	TAA	1,416-2,955 (0)	1,540
tRNA-Leu^{2}	+	UUA			2,956-3,021 (7)	66
cox2	+		ATG	T	3,029-3,710 (0)	682
tRNA-Asp	+	GAC			3,711-3,775 (3)	65
tRNA-Lys	+	AAG			3,779-3,848 (17)	70
atp8	+		ATC	TAA	3,866-4,024 (-7)	159
atp6	+		ATG	TAA	4,018-4,695 (4)	678
cox3	+		ATG	TAA	4,700-5,491 (3)	792
tRNA-Gly	+	GGA			5,495-5,560 (0)	66
nd3	+		ATT	TAG	5,561-5,914 (-2)	354
tRNA-Ala	+	GCA			5,913-5,977 (5)	65
tRNA-Arg	+	CGA			5,983-6,047 (0)	65
tRNA-Asn	+	UUC			6,048-6,113 (0)	66
tRNA-Ser^{1}	+	UGC			6,114-6,180 (3)	67
tRNA-Glu	+	GUU			6,184-6,249 (-2)	66
tRNA-Phe	-	GAA			6,248-6,311 (0)	64
nd5	-		ATT	T	6,312-8,031 (15)	1,720
tRNA-His	-	GUG			8,047-8,112 (2)	66
nd4	-		ATG	TAA	8,115-9,449 (-7)	1,335
nd4l	-		ATG	TAA	9,443-9,736 (2)	294
tRNA-Thr	+	ACA			9,739-9,801 (0)	63
tRNA-Pro	-	UGG			9,802-9,864 (2)	63
nd6	+		ATG	TAA	9,867-10,388 (8)	522
cytb	+		ATG	TAA	10,397-11,536 (-1)	1,140
tRNA-Ser^{2}	+	UCA			11,536-11,605 (20)	70
nd1	-		ATA	TAG	11,626-12,570 (3)	945
tRNA-Leu^{1}	-	UAG			12,574-12,638 (0)	65
rrnL	-				12,639-13,925 (30)	1,287
tRNA-Val	-	UAC			13,956-14,026 (5)	71
rrnS	-				14,032-14,813 (0)	782
A+T-rich region	+	N/A			14,814-16,145 (0)	1,332

Note: The number of intergenic nucleotides is shown in parentheses. The positive numbers indicate intergenic spacers between genes, the negative numbers indicate overlaps between genes, and the zero value means two genes are adjacent.

The nucleotide composition of *H. xinjiangensis* sp. nov. is typically insect A+T biased, (75.1%). Either strong A skew (AT-skew=0.16) or C skew (GC-skew=-0.17) are not shown in the whole mitogenome. However, the slightly A skew (*rrnL*: 0.14; *rrnS*: 0.17) and strongly C skew (*rrnL*: -0.26; *rrnS*: -0.28) are detected in *rrnL* and *rrnS* genes. In addition, there are strong A skew (AT-skew=0.11) and slight C skew (GC-skew=-0.05) in the A+T-rich region. The comparison of nucleotide compositional

skew of the whole mitogenomes, *rrnL* genes, *rrnS* genes and the A+T-rich regions of Oedipodinae grasshoppers are represented in Table 4.

TABLE 4. General nucleotide composition of complete mitogenome and nucleotide compositional skew of the Oedipodinae mitogenomes, *rrnL* genes, *rrnS* genes and the A+T-rich regions in Oedipodinae grasshoppers. A (%), C (%), G (%) and T (%) mean the percentage of adenine, thymine, guanine and cytosine. The number with asterisk (*) means the average nucleotide compositional skew in the subfamily Oedipodinae.

			whole mitogenome					
Species	Accession No.	Length (bp)	A (%)	T (%)	G (%)	C (%)	AT-skew	GC-skew
Aiolopus thalassinus	NC_034674	15,753	44.4	30.9	10.4	14.3	0.18	-0.16
Angaracris barabensis	NC_025558	15,930	43.6	31.7	10.1	14.4	0.16	-0.18
Angaracris rhodopa	NC_025946	15,930	43.8	31.6	10.2	14.4	0.16	-0.17
Bryodema luctuosum luctuosum	HQ833839	15,946	43.6	31.3	10.3	14.8	0.16	-0.18
Bryodema miramae miramae	KP889242	15,919	43.8	31.7	10.1	14.4	0.16	-0.18
Compsorhipis davidiana	NC_029408	16,085	43.9	31.5	10.0	14.6	0.16	-0.19
Gastrimargus marmoratus	NC_011114	15,924	45.6	29.6	9.6	15.2	0.21	-0.23
Hilethera xinjiangensis **sp. nov.**	in this study	16,145	43.5	31.6	10.3	14.6	0.16	-0.17
Locusta migratoria	NC_001712	15,722	44.5	30.8	10.1	14.6	0.18	-0.18
Locusta migratoria manilensis	NC_014891	15,895	44.7	30.7	10.0	14.6	0.19	-0.19
Locusta migratoria migratoria	NC_011119	16,053	44.8	30.7	9.9	14.6	0.19	-0.19
Locusta migratoria tibetensis	NC_015624	15,568	44.3	31.0	10.2	14.5	0.18	-0.17
Oedaleus infernalis	NC_029327	16,259	45.0	30.4	10.0	14.6	0.19	-0.19
Oedaleus decorus asiaticus	NC_011115	15,898	45.5	30.6	9.8	14.2	0.20	-0.18
Pternoscirta caliginosa	NC_035227	15,598	44.6	30.7	10.1	14.6	0.18	-0.18
Trilophidia annulata	NC_027179	15,775	43.2	31.7	10.6	14.5	0.15	-0.16

continued.

	rrnL genes		*rrnS* genes		A+T-rich region	
Species	AT-skew	GC-skew	AT-skew	GC-skew	AT-skew	GC-skew
Aiolopus thalassinus	0.18	-0.24	0.21	-0.27	0.13	-0.13
Angaracris barabensis	0.16	-0.27	0.19	-0.21	0.04	-0.08
Angaracris rhodopa	0.16	-0.27	0.18	-0.20	0.05	-0.09
Bryodema luctuosum luctuosum	0.15	-0.24	0.19	-0.23	0.05	-0.12
Bryodema miramae miramae	0.16	-0.27	0.19	-0.21	0.05	-0.11
Compsorhipis davidiana	0.16	-0.28	0.18	-0.20	0.08	-0.21
Gastrimargus marmoratus	0.17	-0.28	0.21	-0.28	0.23	-0.32
Hilethera xinjiangensis **sp. nov.**	0.14	-0.26	0.17	-0.28	0.11	-0.05
Locusta migratoria	0.14	-0.24	0.20	-0.24	0.17	-0.27
Locusta migratoria manilensis	0.16	-0.25	0.19	-0.22	0.21	-0.36
Locusta migratoria migratoria	0.16	-0.25	0.20	-0.24	0.23	-0.46
Locusta migratoria tibetensis	0.16	-0.25	0.21	-0.22	0.06	-0.05
Oedaleus infernalis	0.18	-0.29	0.22	-0.22	0.16	-0.14
Oedaleus decorus asiaticus	0.19	-0.31	0.20	-0.20	0.19	-0.23
Pternoscirta caliginosa	0.17	-0.27	0.21	-0.26	0.10	-0.24
Trilophidia annulata	0.15	-0.26	0.19	-0.25	0.10	-0.13

The conventional start/stop codons and incomplete stop codons are represented in the PCGs (see details in Table 3). All the protein-coding genes (PCGs) in the mitogenome start with a typical ATN codon but end with different stop codons. The complete stop codon TAA are found in genes *atp8*, *atp6*, *cox1*, *cox3*, *cytb*, *nd4*, *nd4l*, *nd6*, and TAG are in genes *nd1* and *nd3*, respectively. Whereas, the incomplete stop codons (T) are found in *cox2*, *nd2* and *nd5*. The total number of codons in PCGs is 3,717, and the most common amino acids in mitochondrial proteins are leucine (Leu, 504), isoleucine (Ile, 391), and phenylalanine (Phe, 351) (Table 5).

TABLE 5. Codon number and RSCU in *Hilethera xinjiangensis* sp. nov. mitochondrial PCGs. A total of 3,717 codons were analyzed, excluding the initiation and termination codons. Amino acids encoded by these codons are labelled according to the IUPAC-IUB single-letter amino acid codes.

Codon	Count	RSCU	Codon	Count	RSCU	Codon	Count	RSCU	Codon	Count	RSCU
UUU(F)	286	1.63	UCU(S)	108	2.39	UAU(Y)	149	1.75	UGU(C)	43	1.83
UUC(F)	65	0.37	UCC(S)	19	0.42	UAC(Y)	21	0.25	UGC(C)	4	0.17
UUA(L2)	312	3.71	UCA(S)	115	2.55	UAA(*)	0	0	UGA(W)	91	1.82
UUG(L2)	46	0.55	UCG(S)	3	0.07	UAG(*)	0	0	UGG(W)	9	0.18
CUU(L1)	59	0.7	CCU(P)	59	1.74	CAU(H)	54	1.5	CGU(R)	19	1.41
CUC(L1)	2	0.02	CCC(P)	6	0.18	CAC(H)	18	0.5	CGC(R)	1	0.07
CUA(L1)	76	0.9	CCA(P)	66	1.94	CAA(Q)	58	1.87	CGA(R)	32	2.37
CUG(L1)	9	0.11	CCG(P)	5	0.15	CAG(Q)	4	0.13	CGG(R)	2	0.15
AUU(I)	333	1.7	ACU(T)	49	0.97	AAU(N)	151	1.68	AGU(S)	26	0.58
AUC(I)	58	0.3	ACC(T)	19	0.37	AAC(N)	29	0.32	AGC(S)	3	0.07
AUA(M)	249	1.7	ACA(T)	131	2.58	AAA(K)	73	1.51	AGA(S)	81	1.8
AUG(M)	44	0.3	ACG(T)	4	0.08	AAG(K)	24	0.49	AGG(S)	6	0.13
GUU(V)	94	2.09	GCU(A)	67	1.77	GAU(D)	62	1.68	GGU(G)	91	1.7
GUC(V)	9	0.2	GCC(A)	8	0.21	GAC(D)	12	0.32	GGC(G)	5	0.09
GUA(V)	73	1.62	GCA(A)	75	1.99	GAA(E)	64	1.66	GGA(G)	101	1.89
GUG(V)	4	0.09	GCG(A)	1	0.03	GAG(E)	13	0.34	GGG(G)	17	0.32

The lengths of the 22 rRNA genes range from 63 to 71 bp. Two rRNA genes (*rrnL* and *rrnS*), separated by tRNA-Val, are located between tRNA-Leu[1] and A+T-rich region. The lengths of *rrnL* and *rrnS* are 1,287 and 782 bp, respectively. Their A+T contents are 76.9% and 74.5%, respectively. The A+T-rich region of 1,332 bp, with an A+T content of 83.2%, is located between *rrnS* and tRNA-Ile.

Mitogenomic comparisons within Oedipodinae. The nucleotide base composition comparison for 16 Oedipodinae species were represented in Table 4. The highest and lowest A+T content of the whole mitogenome are *Oedaleus decorus asiaticus* (76.1%) and *Bryodema luctuosum luctuosum* (74.9%), respectively; and *H. xinjiangensis* ranked in the third place. The nucleotide compositional skew of the whole mitogenomes, *rrnL* genes, *rrnS* genes and the A+T-rich regions of Oedipodinae species are also represented (Table 4, Fig. 4).

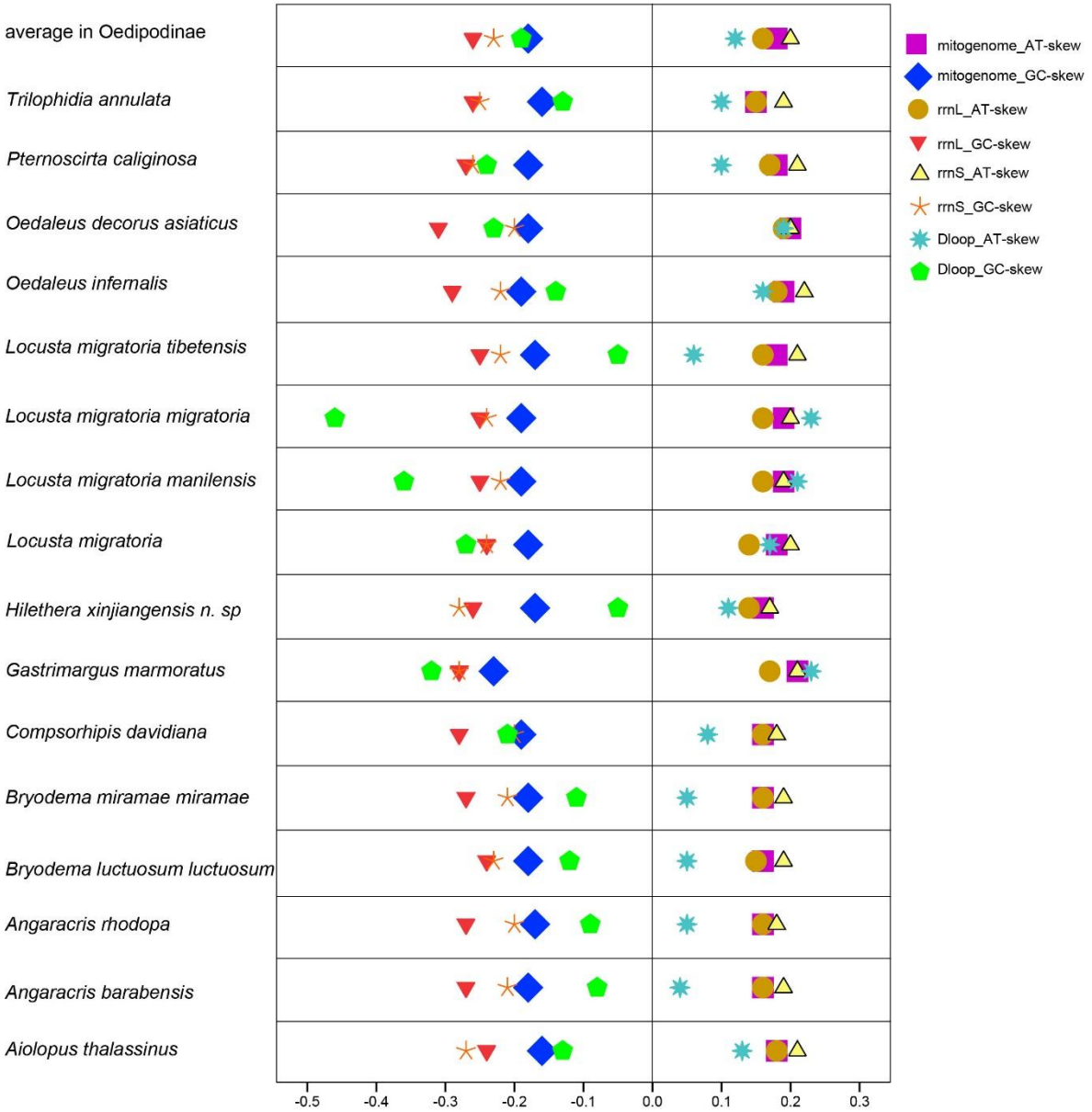

FIGURE 4. Comparison of AT-skew and GC-skews of Oedipodinae species.

The pairwise genetic distance within 16 species based on single PCG, *rrnL* and *rrnS* are shown in Fig. 5. The gene *rrnL* is the least conserved gene (median 0.850, 25-75 percentile range 0.840-0.910) and *cox1* is the most conserved gene (median 0.87, 25-75 percentile range 0.855-0.885).

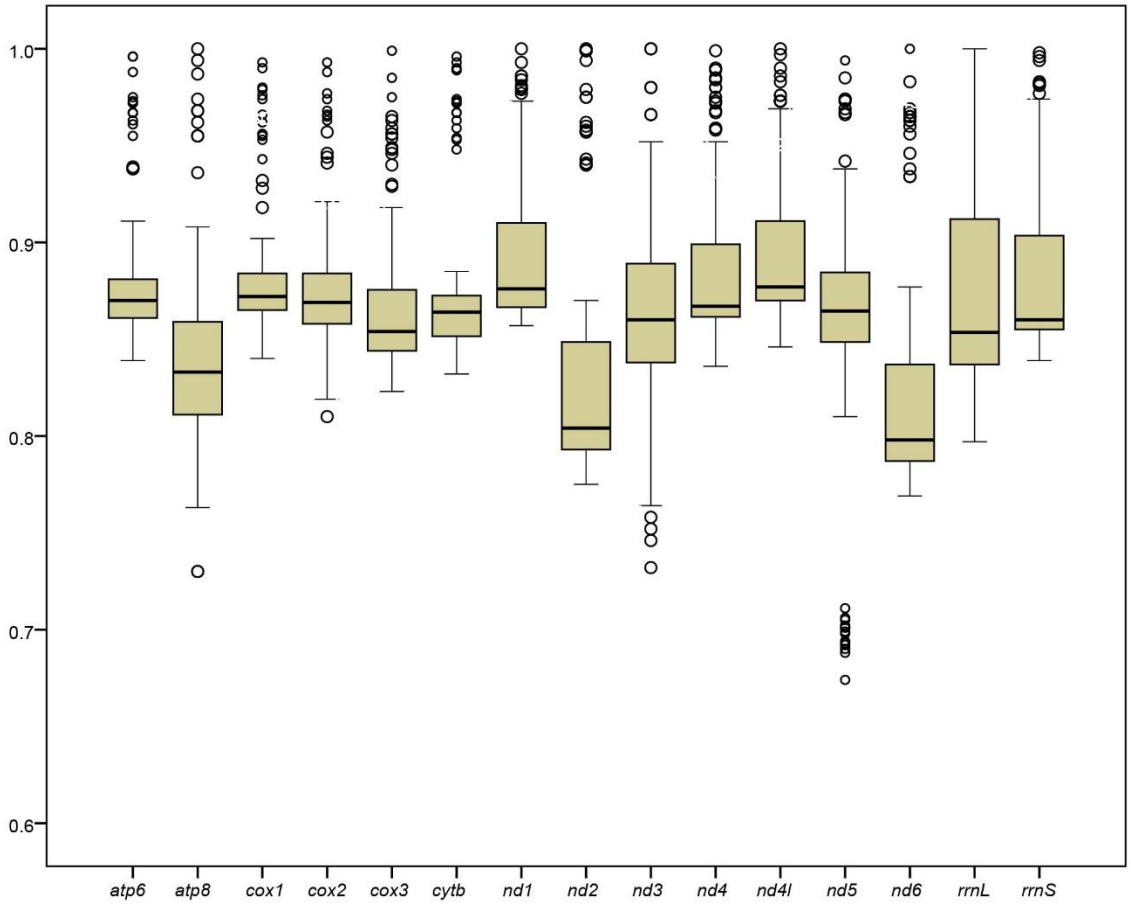

FIGURE 5. Genetic distance of individual genes within Oedipodinae. Each boxplot represents P distance for 13 PCGs, *rrnL* and *rrnS* in sixteen Oedipodinae species. Lower horizontal bar, non-outlier smallest observation; lower edge of rectangle, 25 percentile; central bar within rectangle, median; upper edge of rectangle, 75 percentile; upper horizontal non-outlier largest observation; open circle, outlier.

The evolution rate of Ka/Ks for all PCGs within Oedipodinae were shown in Fig. 6. The overall Ka/Ks values are less than 1.0 within Oedipodinae, indicating the existence of purifying selection in these species. The evolutionary rate of cytochrome c oxidase complex genes (*cox1*, *cox2*, *cox3*) is overall lower than that of NADH dehydrogenase complex genes (*nd1-nd6*). Thereinto, the highest rate is *nd5* and the lowest is *cox1*. In addition, the evolutionary rate of gene *cytb* is located in the second lowest place, whereas, the gene *atp8* was in the second highest place following *nd5*.

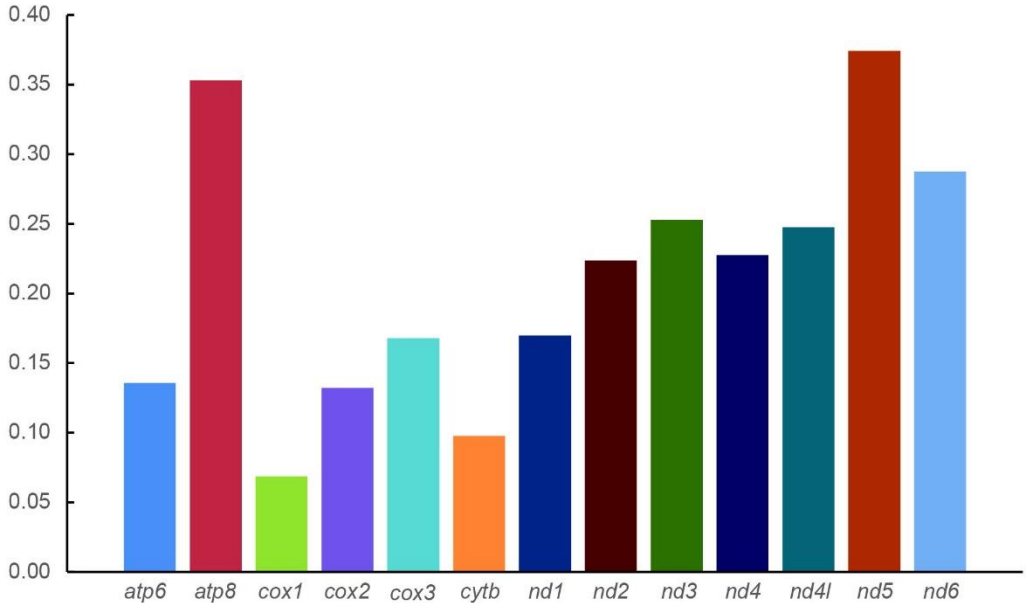

FIGURE 6. Evolution rates of each protein-coding (PCG) in the oedipodine mitogenomes.

Phylogenetic analyses. The best-fit partition schemes and substitution models used in BI and ML analyses of the combine dataset are shown in Table 6. Both BI and ML phylogenetic analyses yielded robust and largely congruent topologies (Fig. 7; see also supporting information Fig. S1 for original outputs of both BI and ML analyses). Both BI and ML analyses inferred that subfamilies Acridinae, Gomphocerinae and Oedipodinae are not monophyletic, in accordance with the previous studies (Chapco & Contreras, 2011; Song *et al.*, 2018). In BI analyses, except for *Orinhipps tibetanus*, the remaining species in the subfamily Gomphocerinae form one separated clade with well supported (PP=1.0), whereas the ML result inferred that they were divided into two different clades (Fig 7; see ML original output in Figure S1). Except for one species of Gomphocerinae (*Orinhipps tibetanus*), the subfamily Oedipodinae formed one clade and is sister to Acridinae.

Within Oedipodinae subfamily, all species belonging to the same genus form clades, except for *Bryodema* genus (Fig. 7). The subfamily Oedipodinae includes three main clades with high support (1.0 for PP and 100% for BV). The genera *Trilophidia* and *Aiolopus* form a clade independently. Remaining genera and species are formed the third clade with two main clades (hereby referred as clade A and B in Fig. 7). Within the clade A, two *Bryodema* species are nested in the clade of (*Compsorhipis* + *Angaracris*), then forming a sister group to the genus *Hilethera*. Within the clade B, the genus *Pternoscirta* is the first lineage to branch off, sister to

the remaining three genera. The phylogenetic relationship among these three genera is not congruent in BI and ML analyses. In BI analyses, the genus *Gastrimargus* grouped together with the genus *Locusta* (0.81 for PP), sister to the genus *Oedaleus* (1.0 for PP). Whereas the position of two genera *Locusta* and *Oedaleus* was swapped in ML analyses with high support (84% for BV).

TABLE 6. Best-fit models of sequence evolution and partitioning schemes selected with PartitionFinder for phylogenetic reconstructions using Bayesian Inference (MrBayes) and Maximun Likelihood (IQ-TREE). Codon position is denoted by pos1, pos2 and pos3. Subsets are denoted by p1, p2 and p3.

Analyses	Partitions	Models
Bayesian inference (BI) analyses	p1: *atp6*_pos1, *atp6*_pos2, *atp6*_pos3, *atp8*_pos1, *atp8*_pos2, *atp8*_pos3, *cox1*_pos1, *cox1*_pos2, *cox1*_pos3, *cox2*_pos1, *cox2*_pos2, *cox2*_pos3, *cox3*_pos1, *cox3*_pos2, *cox3*_pos3, *cytb*_pos1, *cytb*_pos2, *cytb*_pos3, *nd1*_pos1, *nd1*_pos2, *nd2*_pos1, *nd2*_pos2, *nd2*_pos3, *nd3*_pos1, *nd3*_pos2, *nd3*_pos3, *nd4*_pos1, *nd4*_pos2, *nd4l*_pos1, *nd4l*_pos2, *nd6*_pos1, *nd6*_pos2, *nd6*_pos3, *rrnL*	GTR+I+G
	p2: *nd5*_pos3, *nd1*_pos3, *nd4*_pos3, *nd4l*_pos3	HKY+G
	p3: *nd5*_pos2, *nd5*_pos1, *rrnS*	GTR+G
Maximum likelihood (ML) analyses	p1: *atp6*_pos1, *atp6*_pos2, *atp8*_pos1, *atp8*_pos2, *cox1*_pos1, *cox1*_pos2, *cox2*_pos1, *cox2*_pos2, *cox3*_pos1, *cox3*_pos2, *cytb*_pos1, *cytb*_pos2, *nd1*_pos1, *nd1*_pos2, *nd2*_pos1, *nd2*_pos2, *nd2*_pos3, *nd3*_pos1, *nd3*_pos2, *nd4*_pos1, *nd4*_pos2, *nd4l*_pos1, *nd4l*_pos2, *nd6*_pos1, *nd6*_pos3, *rrnL*	GTR+I+G
	p2: *atp6*_pos3, *atp8*_pos3, *cox1*_pos3, *cox2*_pos3, *cox3*_pos3, *cytb*_pos3, *nd1*_pos3, *nd3*_pos3, *nd4*_pos3, *nd4l*_pos3, *nd5*_pos1, *nd5*_pos2, *nd5*_pos3, *nd6*_pos2, *rrnS*	GTR+G

Discussion

The new species *H. xinjiangensis* sp. nov. was described, which supplements the basic data for the genus and expands its distribution to the northern part of China. The new species resembles the species *H. turanica* from Xinjiang and *H. brevipennis* from Shanxi province, from which the new species differs by general coloration darker, shorter tegmina, two faint coloration-banded strip in forewings and hind tibiae, and broader cubital area in forewings.

The first mitogenome of the genus *Hilethera* grasshopper was sequenced, which allows comparison with existing mitogenomes from the diverse subfamily Oedipodinae. The gene order and orientation within the mitogenome of *H. xinjiangensis* sp. nov. are identical with those described for other oedipodine species. The length of the whole mitogenome and the length range for tRNAs in *H. xinjiangensis* sp. nov. are also very close to Oedipodinae. The genes *rrnL* of these

16 Oedipodinae species are weakly AT-skewed (0.14 to 0.19) and strongly GC-skewed (-0.24 to -0.29). However, the nucleotide compositional skew between the whole mitogenome, *rrnS* and A+T-rich region differ amongst species. It is logical that the nucleotide compositional skew in different genes slightly different, but more mitogenomes of other Oedipodine species are necessary to test whether this tendency is consistent between species.

The pairwise genetic distance shows that *cox1* is the most conserved gene in the mitogenomes of oedpodines, confirming it as a useful marker to infer phylogenetic relationships at higher taxonomic levels. Meanwhile, the gene *rrnL* could be a useful marker for barcoding approaches or for inferring phylogenetic relationships between closely related species since it is the most variable gene among the species of oedipodines. According to the Ka/Ks ratio, the evolutionary rate of the Cytochrome Oxidase complex (*cox1*, *cox2* and *cox3*) and cytb genes are lower than that of in NADH dehydrogenase complex. In addition, the disparity of the divergence evolutionary rates in the latter is not significant, expect for the gene *nd5*. However, there is an obvious evolutionary rate discrepancy in ATP synthase. The evolutionary rate of the gene *atp8* is far higher than that of *apt6*, ranking at the secondary place after *nd5*. In view of lowest conserved gene *rrnL* and the highest evolutionary rate of the gene *nd5*, the present study revealed these two genes as potential markers for future phylogenetic study between the closely related species within Oedipodinae. Our results confirm that the mitogenomes in grasshoppers have a relatively stable structural and functional evolution, as suggested by studies in Orthoptera (Song *et al.*, 2015; Dong *et. al.*, 2017) and in insects in general (e.g. Simon *et al.*, 2006; Cameron, 2014; Konstantinov *et al.*, 2016).

The phylogenetic relationship among the subfamilies Acridinae, Gomphocerinae and Oedipodinae using mitogenomes inferred in this study, are mostly congruent with the previous studies dealing with the phylogeny of Acrididae (Chapco & Contreras, 2011; Song *et al.*, 2018). The previous studies inferred that the three subfamilies were not monophyletic. However,our BI analyses showed that except for one species (*Orinhippus tibetanus*), all gomphocerine species form one clade. Similarly, within Oedipodinae, the positions of the genera *Bryodema* and *Gastrimargus* are incongruent in BI and ML analyses, respectively. More complete mitogenome or genomic data are needed to reconstruct phylogenetic relationship within Oedipodinae, and for the further study within Acrididae.

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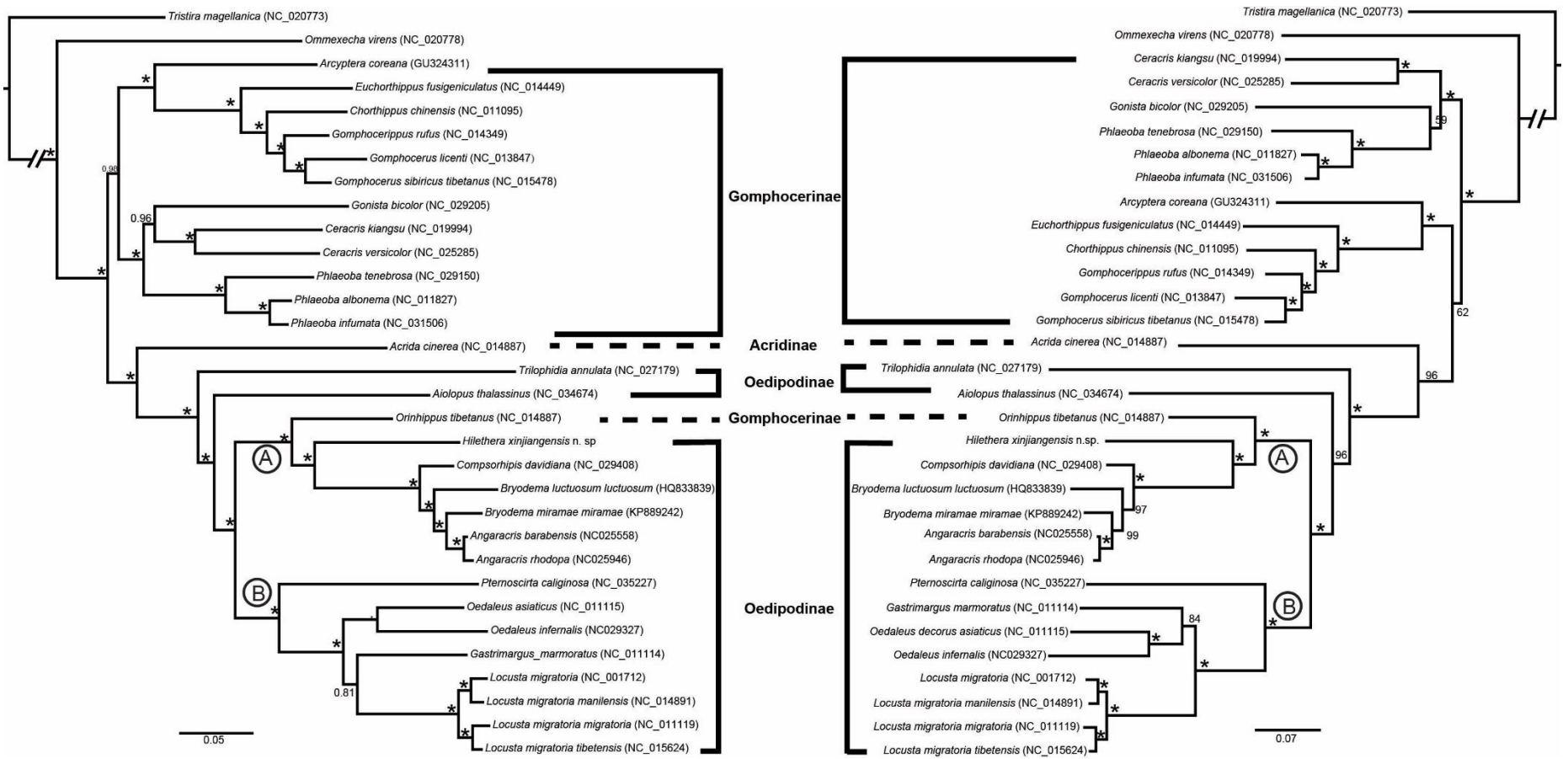
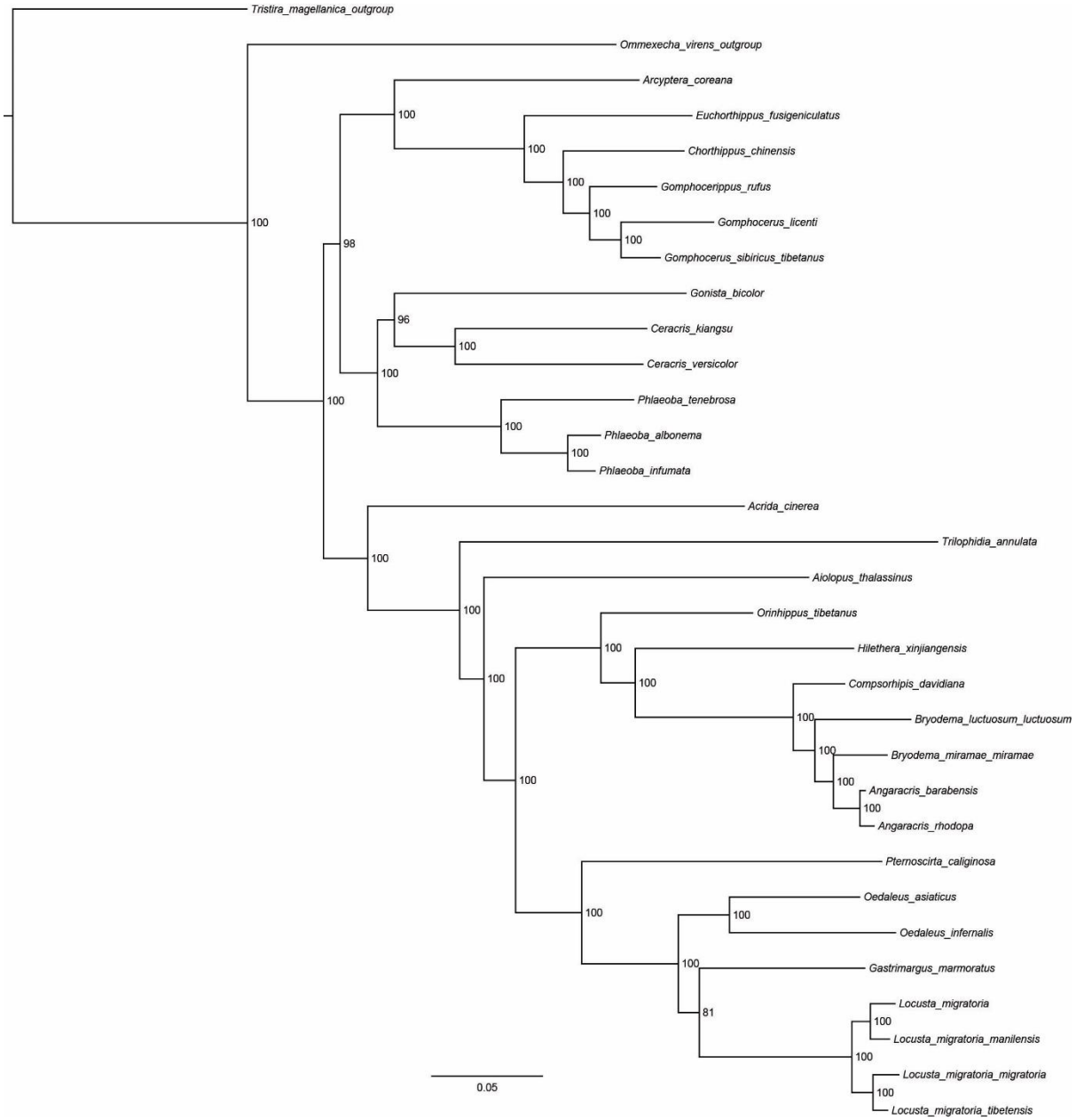


FIGURE 7. Phylogenetic reconstruction of Oedipodinae using mitochondrial PCGs and rRNAs concatenated dataset inferred from Bayesian inference (BI, on the left of figure) and Maximum likelihood (ML, on the right of figure). Values on nodes indicate branch support, BI posterior probabilities (PP) / Maximum likelihood Bootstrap support values (BV). Asterisks are used to indicate maximum support (1.0 for PP and 100% for BV). Accession number of each species recorded in GenBank was indicated in the parentheses.

Supporting Information

Figure S1. The original output results from BI (A) and ML (B) analysis.

(A) BI analysis result



(B) ML analysis result