RESEARCH ARTICLE

Comparative mitochondrial genome analysis of *Dendrolimus houi* (Lepidoptera: Lasiocampidae) and phylogenetic relationship among Lasiocampidae species

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Abstract

Dendrolimus houi is one of the most common caterpillars infesting Gymnosperm trees, and widely distributed in several countries in Southeast Asia, and exists soley or coexists with several congeners and some Lasiocampidae species in various forest habitats. However, natural hybrids occasionally occur among some closely related species in the same habitat, and host preference, extreme climate stress, and geographic isolation probably lead to their uncertain taxonomic consensus. The mitochondrial DNA (mtDNA) of *D. houi* was extracted and sequenced by using high-throughput technology, and the mitogenome composition and characteristics were compared and analyzed of these species, then the phylogenetic relationship was constructed using the maximum likelihood method (ML) and the Bayesian method (BI) based on their 13 protein-coding genes (PCGs) dataset, which were combined and made available to download which were combined and made available to download among global Lasiocampidae species data. Mitogenome of *D. houi* was 15,373 bp in length, with 37 genes, including 13 PCGs, 22 tRNA genes (tRNAs) and 2 rRNA genes (rRNAs). The positions and sequences of genes were consistent with those of most known Lasiocampidae species. The nucleotide composition was highly A+T biased, accounting for ~80% of the whole mitogenome. All start codons of PCGs belonged to typical start codons ATN except for *COI* which used CGA, and most stop codons ended with standard TAA or TAG, while *COI*, *COII*, *ND4* ended with incomplete T. Only *tRNA*[Ser (AGN)] lacked DHU arm, while the remainder formed a typical "clover-shaped" secondary structure. For Lasiocampidae species, their complete mitochondrial genomes ranged from 15,281 to 15,570 bp in length, and all first genes started from *trnM* in the same direction. And base composition was biased toward A and T. Finally, both two methods (ML and BI) separately revealed that the same phylogenetic relationship of *D*. spp. as ((((*D. punctatus* + *D. tabulaeformis*) + *D. spectabilis*) + *D. superans*) + (*D. kikuchii* of Hunan population + *D. houi*) as in previous research, but results were different in that *D. kikuchii* from a Yunnan population was included, indicating that different geographical populations of insects have differentiated. And the phylogenetic

relationship among Lasiocampidae species was ((((*Dendrolimus*) + *Kunugia*) + *Euthrix*) +
Trabala). This provides a better theoretical basis for Lasiocampidae evolution and classification for future research directions.

Introduction

Dendrolimus houi Lajonquiere (Lepidoptera: Lasiocampidae), being one of the most abundant
phytophagous caterpillar in southern China and some countries in Southeast Asia, voraciously
feeds on about 12 species of coniferous trees, including *Cryptomeria fortunei*, *Pinus yunnanensis*, *Platycladus orientalis*, *P. kesiya* var.*langbianensis* and *Cupressus funebris*, causing thousands
hectares of dead or dying forests, and it tends to be continuously spreading rapidly [1]. Biologically, different geographical populations of *D. houi* have different life cycles, host preference
and adaptation to local extreme climatic factors [2,3], which might lead to population differentiation or taxonomic controversy based on previous reasearches on *D. kikuchii* [4] and *D.
punctatus* [5].

Moreover, insects in the family Lasiocampidae are some of the most serious phytophagous
pests worldwide, causing the host withering and rapid death, having serious impact on the ecological environment during their outbreaks [6–15]. Examples include such *D. pini* infesting
Scots pines in Europe [16], *D. houi* in Yunnan, Sichuan, Fujian and Zhejiang province [2,9–
10], and *D. punctatus* at fifteen provinces in the south of China [11]. Other speices like *D. tabulaeformis*, *D. kikuchii*, *D. spectabilis*, *Euthrix laeta* and *Trabala vishnou guttata* often occur in
China as well [8,11], and thus face the stress of multiple and complex host and environmental
factors. Consequently, they could potentially evolve in two directions: firstly, some *Dendrolimus* species may inevitably share the same host species and tend to inhabit the same forest,
which might lead to taxonomically mis-discrimination [9] and hybridization [8]. (For example, *D. tabulaeformis* and *D. spectabilis* are the subspecies of *D. punctatus* probably due to
hybridization of these three species [11,12]). Or different populations of same species might
differentiated and evolved into separate species as a result of long-term adaptation to different
hosts and other climatic factors [2]. (For example, *D. kikuchii* and *D. houi* is thought to have
evolved from ta commmon ancestor, and evolved separately into different species as revealed
by phylogenetic analysis [9]). Transcriptome analysis of antenna still showed that they still
have high similarity and close phylogenetic relationship, however, they have different sex pheromone components [13], indicating somehow interspecific reproductive isolation. However,
how do we identify and evaluate their ecological function and phylogenetic relationship? Fortunately, many previous studies have focused on the taxonomic relationship of Lasiocampidae,
especially the taxonomic relationship within genera [5–15], but there are still some species
whose taxonomic status is controversial and without a complete consensus [5–15]. Currently,
some taxonomic relationships focus on the comparison with Lepidoptera [16–19], however
very few reports have focused on the phylogenetic relationship among Lasiocampidae genera,
so the taxonomy remains unclear.

Technically, mitochondrial genes and genomes have been generally adopted as an informative molecular marker for diverse evolutionary research of animals [5–21], and the complete
and nearly complete mitogenome from hundreds of insect species has currently been determined. Generally, the length of most animal mitogenomes is 15.4–18.3 Kb [16]. It is usually a
highly compact and covalently bonded closed-ring molecule composed of 37 genes, including
13 PCGs, 22 tRNAs, 2 rRNAs and a non-coding control region (D-loop region) [15–20]. In

addition, animal mtDNA displays maternally inheritance, and mitogenomes have a very compact gene arrangement, high coding efficiency and overlap among some genes. Furthermore, there are no gene recombinations, translocations or inversion mutations in the genetic process. Therefore, mitogenomes have been widely used in the classification, identification, interspecific molecular evolution, population genetic and phylogenetic evolution relationships [16–23].

In this study, we obtained the complete mitogenome of *D. houi* and downloaded the available mtDNA data of nine species (four genera in Lasiocampidae) from the public database, containing some other congenetic species of *D.* spp., *Kunugia undans*, *E. laeta* and *T. vishnou guttata* to compare mitogenome composition and structure. Then, we reconstructed phylogenetic tree and analyzed phylogenetic relationship between *D. houi* and other Lasiocampidae species.

Materials and methods

Ethics statement

There is no endangered or protected species involved in this study, no specific permissions were required for this serious and widespread forest pest, feeding on leaves of *Cryptomeria fortunei* and causing thousands hectares of dying and dead forests. Additionally, this study is sponsored and permitted by NSFC (National Natural Science Foundation of China). We confirm that the locations are not privately owned or otherwise protected.

Samples collection mitochondria DNA extraction

Samples of *D. houi* were collected in Yongtai, Fuzhou, Fujian Province, China. Mitochondrial DNA of *D. houi* was extracted from muscle tissue using the GENMED Mitochondrial DNA Extraction kit (Genmed Scientifics Inc., Arlington, MA, USA). Muscle tissue of *D. houi* was crushed under ice bath condition following instruction of Extraction kit. The quality of DNA was assessed using NanoDrop2000, qubit3.0, and 1% agarose gel electrophoresis.

Sequencing and assembling of the mitochondrial genome

After DNA isolation, 1 μg of purified DNA was fragmented and used to construct short-insert libraries (430 bp) according to the manufacturer's instructions (Illumina, Hercules, CA, USA), and then sequenced on the Illumina Hiseq 4000 platform [24]. In order to obtain high quality clean reads, the raw reads were filtered to remove adaptors, the reads containing unknown nucleotide "N" over 10% and the duplicated sequences. Then, the clean reads were assembled into contigs using SOAPdenovo2.04 [25].

Gene annotation and analysis

13 PCGs of *D. houi* mitogenome were annotated by utilizing the online program ORF, while 2 rRNAs and 22 tRNAs were annotated using the online software MITOS2 Web Server (http://mitos2.bioinf.uni-leipzig.de/index.py). The unpredicted tRNA genes used tRNAscan-SE [26] to predict secondary structure. We determined the location of each gene, and corrected the annotation based on data from the reported related species mitogenomes. Then, the genome was aligned with the Nr (non-redundant protein sequence), Swiss-Prot (a manually annotated, non-redundant protein sequence), COG (clusters of orthologous groups of proteins), GO (gene ontology) and KEGG (kyoto encyclopedia of genes and genomes) databases by BLAST v2.2.31 with a cut-off e-valve of 10^{-5} [2].

Table 1. Comparative analysis of mitogenomes and phylogenetic relationship of Lasiocampidae species information.

Family	Genus	Species	Size(bp)	GenBank accession no.	Sample sources
Lasiocampidae	*Dendrolimus*	*Dendrolimus punctatus* (DP)	15,411	NC_027156.1	-
		Dendrolimus tabulaeformis (DT)	15,411	NC_027157.1	-
		Dendrolimus spectabilis (DSP)	15,410	KJ913816.1	Taian, Shandong
		Dendrolimus superans (DSU)	15,417	NC_039841.1	-
		Dendrolimus kikuchii (DK1)	15,422	NC_036347.1	Puer, Yunnnan
		Dendrolimus kikuchii (DK2)	15,385	MF100138.1	Zhuzhou, Hunan
		Dendrolimus houi (DH)	**15,373**	**This study**	**Fuzhou, Fujian**
	Kunugia	*Kunugia undans* (KU)	15,570	KX822016.1	-
	Euthrix	*Euthrix laeta* (EL)	15,368	NC_031507.1	Jiujiang, Jiangxi
	Trabala	*Trabala vishnou guttata* (TVG)	15,281	KU884483.1	Jiujiang, Jiangxi
Bombycidae	*Bombyx*	*Bombyx mori* (BM)	15,635	AB083339.1	-

Comparative mitogenome analyses of lasiocampidae

We downloaded available mtDNA data of nine species (four genera in Lasiocampidae) from the public database, containing *D. punctatus* (DP) NC_027156.1, *D. tabulaeformis* (DT) NC_027157.1, *D. spectabilis* (DSP) KJ913816.1, *D. superans* (DSU) NC_039841.1, *D. kikuchii* (DK1) NC_036347.1, *D. kikuchii* (DK2) MF100138.1, *K. undans* (KU) KX822016.1, *E. laeta* (EL) NC_031507.1 and *T. vishnou guttata* (TVG) KU884483.1) (Table 1). To obtain the information of gene loss, duplication, rearrangement, and horizontal transfer in Lasiocampidae, multiple genome alignments were conducted using Mauve software [27]. The base composition, codon usage and relative synonymous codon usage (RSCU) frequency within these 10 species of mitogenomes were analyzed by MEGA 7.0 software. The formula for calculating the composition skew in mitogenomes was as follows, AT-skew = (A-T) / (A+T), GC-skew = (G-C) / (G+C).

Phylogenetic analysis

The dataset of 13 PCGs, from these ten species plus *Bombyx mori* as the reference outgroup, were used to reconstructed the phylogenetic tree. Sequences blast was conducted by MAFFT of Translator X online server, the empty spaces and fuzzy sites were removed by GBlocks, and the single genes were combined to obtain the mitochondrial gene datasets, then an optimal evolution model was calculated by Modeltest 3.7 for the subsequent phylogenetic analysis. We also used RAXML 7.2.6 [28] to build a ML tree (bootstrap value is 1000), and BI analysis was carried out by using Mr Bayes 3.2.2 [29] (Markov chains were run for 1×10^5 generations, sampling every 100 generations) to construct the phylogenetic tree of Lasiocampidae.

Results

Genome structure and nucleotides composition of *D. houi*

The complete mitogenome of *D. houi* was 15,373 bp in length (Fig 1), which contained 37 genes (13 PCGs, 22 tRNAs, 2 rRNAs) and a non-coding control region (A+T-rich region) related to replication and transcription. The gene sequences were similar to that of some known related species. Among them, 23 genes were located in J-strand, including 9 PCGs (*COI-COIII, ATP6, ATP8, ND2, ND3, ND6, CYTB*) and 14 tRNAs, while the remaining 14 genes were located in the N-strand.

The genes in the mitogenome were closely arranged with overlapping and interval phenomena. Typically, there were 7 overlapping regions with total length of 25 bp, and the longest
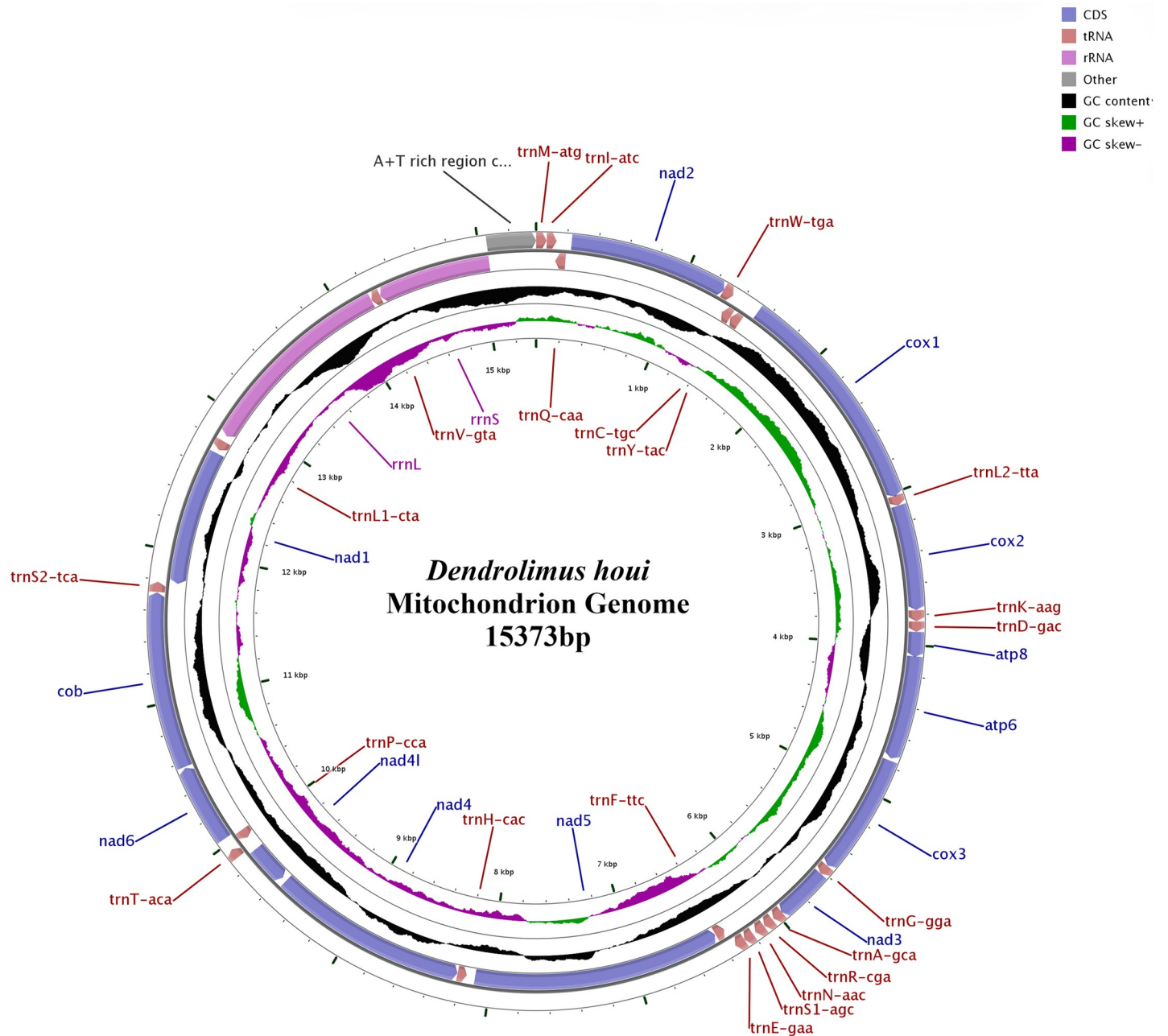
Fig 1. Mitochondrial genomic structure of _D. houi_.

overlapping region was 8 bp, while there were 23 intergenic spacers with a total length of 372 bp, with the longest one 60 bp between _COI_ and _tRNA_^{Tyr}, followed by another one with 57 bp between _tRNA_^{His} and _ND5_. Six regions did not have overlaps or intervals (S1 Table), and the mitogenome structure of _D. houi_ was basically similar to those of most species within Lasiocampidae.

The number of A, T, G and C in the mitogenome within _D. houi_ were 6,321 (41.12%), 5,954 (38.73%), 1,175 (7.64%) and 1,923 (12.51%) respectively, and the content of A was the highest while G was lowest; A+T accounted for 79.85%, while G+C was 20.15%; AT skewness was

0.0299 and GC skewness was -0.2417 (S2 Table). Furthermore, the A+T content at codon site 2 of PCGs (79.70%) was slightly higher than that at the first site (79.43%), while the A+T content at site 3 was the highest (80.43%). The non-coding region was especially A+T -rich, with the total content was as high as 91.85%. AT skewness was negative, which indicated that the content of T was higher than A.

The PCGs sequence was 11,091 bp, accounting for 72.15% of the complete mitogenome. A, T, G, C bases were 3,749 (33.8%), 4,924 (44.4%), 1,239 (11.17%), 1,179 (10.63%), respectively. The A+T content was 78.20%, which was 1.65% lower than that of the whole mitogenome. The complementary G+C content accounted for 21.80%, which was different from the whole mitogenome, with the highest T content and the lowest C content. AT skewness was -0.1356, GC skewness was 0.0248. A total of 4,617 codons (excluding stop codon) were encoded by 13 PCGs within the mitogenome. The most frequently encoded amino acids were Leu (13.26%), Ile (10.83%), Asn (10.66%), Phe (9.75%), and Lys (9.18%) respectively. The frequency of relative synonymous codons showed that the mitogenome of *D. houi* had obvious bias towards A and T, for example when it encoded the same amino acid, it was preferred to use UUU(Phe), UUA(Leu), AUU(Ile), AUA(Met), GUA(Val), UCU(Ser), CCU(Pro), ACU(Thr), GCA(Ala), UAU(Tyr), CAU(His), CAA(Gln), AAU(Asn), AAA(Lys), GAU(Asp), GAA(Glu), UGU(Cys), UGG(Trp), AGA(Arg), GCA(Gly). Most bases within each codon were composed of A and U, and the RSCU values of each codon varied significantly, which indicated that the codon frequency was biased in the mitogenome of *D. houi* (S1 Fig).

Mitogenome of *D. houi* contained 22 tRNA genes with total length 1,475 bp, and the sequences lengths were between 64–71 bp. Twenty-two secondary structures of tRNA genes were basically similar to those of other species of Lepidoptera, and twenty-one of them had typical clover structures consisting of amino acid receiving arms, DHU arm, anticodon arm, variable ring and TΨC arm. Only *tRNA*$^{Ser (AGN)}$ could not form a complete structure due to absence of dihydrouridine (DHU) arm. Based on the predicted 22 tRNA secondary structures, there were also nonstandard T-C, G-T and T-T in additional to mismatches except for the standard A-T and G-C matches (S2 Fig). Two rRNAs (*rRNAL* and *rRNAS*) of mitogenome between *tRNA*$^{Leu (CUN)}$ and control region (CR) were encoded by L-strand and separated by *tRNA*^{Val} gene. The length of *rRNAL* was 1,365 bp, A+T content was 83.51%, AT skewness value was -0.0470. The length of *rRNAS* was 776 bp, A+T content was 85.70%, AT skewness value was 0.0345.

The A+T -rich region was the main non-coding region of mitogenomes, located between *rRNAS* and *tRNA*^{Met} genes, with a total length of 319 bp. The A+T content was 91.85%, which was significantly higher than other genes of the mitogenome (S2 Table). There were also typical structural features of Lepidopteran mitogenomes in the CR. The results also showed that there was a 14 bp poly-T stretch with motif ATAGA that was 15,075–15,093 bp downstream of *rRNAS* gen, and 4 microsatellite-like repeat sequences containing AT, AAT and AAT in this region. Additionally, there were also multiple poly-T stretch in this region (S3 Fig) except for the poly-T stretch at the beginning of replication.

Comparative analysis of mitogenomes of lasiocampidae species

Complete genome alignment using Mauve software was done for 10 species of Lasiocampidae (*Dendrolimus* spp., 1 *Kunugia*, 1 *Euthrix* and 1 *Trabala*) (Table 1, Fig 2). Generally, most of the genes within these ten species maintain a consistent position and direction, and no rearrangement or inversion events were found in the locally-collinear blocks (LCBs). Interestingly, there were two specific *tRNA*^{Arg} (Fig 3A) within the mitogemone of *K. undans*, which was absent among Lasiocampidae (Fig 3B).

Fig 2. Mauve (multiple alignment of sequence with rearrangements) alignment of mitogenomes of ten species. The *D. houi* mitogenome was shown at the top as the reference genome. Within each of the alignments, local collinear blocks are represented by blocks of the same color connected by lines.

The complete mitochondrial genomes of Lasiocampidae ranged from 15,281 to 15,570 bp in length, TVG was 15,281 bp and KU was 15,570 bp (Table 2). The first genes of these species all started from *trnM*, and had same direction. Moreover, there was base composition bias toward A and T in these mitogenomes. The A+T content of these mitogenomes ranged from 78.64% to 80.87%, KU was 78.64% and TVG was 80.87% (Table 3).

Start and stop codon usage is an important characteristic in the annotation of PCGs. We compared the start and stop codons across the 10 species of Lasiocampidae (Table 4). The start
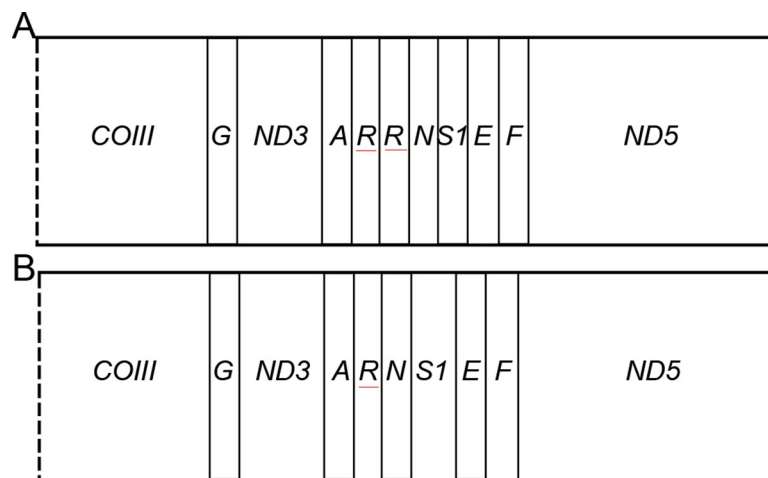

Fig 3. Arrangement of mtDNA genes in Lasiocampidae species. A: Arrangement of *Kunugia undans* mtDNA, B: Arrangement of Lasiocampidae mtDNA.

Table 2. Genome organization of ten species.

Gene	D	DP	DT	DSP	DSU	DK1	DK2	DH	KU	EL	TVG
tRNAMet	F	1–67 (-)	1–67 (-)	1–67 (-)	1–68 (-)	1–67 (-)	1–67 (-)	1–67 (-)	1–68 (-)	1–68 (-)	1–64 (-)
tRNAIle	F	71–134 (3)	71–134 (3)	71–134 (3)	72–135 (3)	71–134 (3)	71–134 (3)	69–132 (1)	72–135 (3)	72–135 (3)	70–133 (5)
tRNAGln	R	132–200 (-3)	132–200 (-3)	132–200 (-3)	133–201 (-3)	132–200 (-3)	132–200 (-3)	130–198 (-3)	136–205 (0)	133–201 (-3)	131–199 (-3)
ND2	F	259–1266 (58)	259–1266 (58)	259–1266 (58)	256–1266 (54)	259–1266 (58)	250–1263 (49)	230–1264 (31)	263–1276 (57)	272–1276 (70)	254–1261 (54)
tRNATrp	F	1265–1333 (-2)	1265–1333 (-2)	1265–1333 (-2)	1265–1336 (-2)	1265–1333 (-2)	1263–1333 (-1)	1263–1332 (-2)	1275–1344 (-2)	1275–1342 (-2)	1260–1327 (-2)
tRNACys	R	1326–1391 (-8)	1326–1391 (-8)	1326–1391 (-8)	1329–1394 (-8)	1326–1391 (-8)	1334–1391 (0)	1325–1391 (-8)	1337–1402 (-8)	1335–1400 (-8)	1320–1385 (-8)
tRNATyr	R	1392–1459 (0)	1392–1459 (0)	1392–1459 (0)	1395–1460 (0)	1392–1459 (0)	1392–1459 (0)	1393–1463 (1)	1412–1479 (9)	1418–1483 (17)	1386–1448 (0)
COI	F	1487–3017 (27)	1494–3024 (34)	1485–3015 (25)	1493–3023 (32)	1494–3024 (34)	1500–3030 (-10)	1524–3026 (60)	1500–3057 (20)	1490–3020 (6)	1464–2994 (15)
tRNALeu (UUN)	F	3018–3084 (0)	3025–3091 (0)	3016–3082 (0)	3024–3090 (0)	3025–3091 (0)	3031–3097 (0)	3022–3088 (-5)	3058–3125 (0)	3021–3087 (0)	2995–3061 (0)
COII	F	3085–3766 (0)	3092–3773 (0)	3083–3764 (0)	3091–3772 (0)	3092–3773 (0)	3098–3779 (0)	3089–3772 (0)	3125–3806 (-1)	3088–3769 (0)	3062–3743 (0)
tRNALys	F	3767–3837 (0)	3774–3844 (0)	3765–3835 (0)	3773–3843 (0)	3774–3844 (0)	3780–3850 (0)	3774–3844 (1)	3807–3877 (0)	3770–3840 (0)	3744–3814 (0)
tRNAAsp	F	3841–3908 (3)	3848–3915 (3)	3839–3906 (3)	3847–3914 (3)	3848–3915 (3)	3851–3918 (0)	3846–3912 (1)	3879–3947 (1)	3846–3912 (5)	3815–3879 (0)
ATP8	F	3909–4070 (0)	3916–4074 (0)	3907–4065 (0)	3915–4076 (0)	3916–4074 (0)	3919–4080 (0)	3913–4074 (0)	3948–4109 (0)	3913–4077 (0)	3880–4044 (0)
ATP6	F	4064–4741 (-7)	4068–4745 (-7)	4059–4736 (-7)	4070–4747 (-7)	4068–4745 (-7)	4074–4751 (-7)	4071–4745 (-4)	4103–4780 (-7)	4071–4748 (-7)	4038–4715 (-7)
COIII	F	4757–5545 (15)	4761–5549 (15)	4748–5536 (11)	4760–5548 (12)	4761–5549 (15)	4758–5546 (6)	4762–5550 (16)	4787–5575 (6)	4753–5541 (4)	4735–5523 (19)
tRNAGly	F	5548–5613 (2)	5552–5617 (2)	5539–5604 (2)	5551–5616 (2)	5552–5617 (2)	5549–5615 (2)	5553–5618 (2)	5578–5644 (2)	5544–5609 (2)	5526–5591 (2)
ND3	F	5614–5967 (0)	5618–5971 (0)	5605–5958 (0)	5617–5970 (0)	5618–5971 (0)	5616–5969 (0)	5619–5972 (0)	5645–5998 (0)	5610–5963 (0)	5592–5945 (0)
tRNAAla	F	5967–6033 (-1)	5971–6037 (-1)	5958–6024 (-1)	5975–6041 (4)	5971–6037 (-1)	5972–6037 (2)	5971–6038 (-2)	6003–6070 (4)	5974–6039 (10)	5944–6010 (-2)
TRNAArg	F	6049–6112 (15)	6053–6116 (15)	6045–6108 (20)	6055–6118 (13)	6058–6121 (20)	6064–6128 (6)	6048–6112 (9)	6084-6147/6175-6241(13/27)	6052–6120 (12)	6024–6088 (13)
tRNAsn	F	6117–6182 (4)	6121-6186 (4)	6113–6178 (4)	6120–6185 (1)	6126–6191 (4)	6150–6216 (21)	6117–6183 (4)	6242–6308 (0)	6122–6186 (1)	6094–6159 (5)
TRNASer (AGN)	F	6194–6261 (11)	6198–6265 (11)	6197–6264 (18)	6202–6269 (16)	6211–6276 (19)	6232–6297 (15)	6204–6269 (20)	6308–6375 (-1)	6192–6257 (5)	6160–6220 (0)
tRNAGlu	F	6261–6326 (-1)	6265–6329 (-1)	6264–6328 (-1)	6269–6333 (-1)	6277–6342 (0)	6298–6361 (0)	6270–6338 (0)	6376–6440 (0)	6258–6323 (0)	6221–6286 (0)
tRNAPhe	R	6331–6396 (4)	6334–6399 (4)	6337–6402 (8)	6346–6412 (12)	6351–6416 (8)	6375–6441 (13)	6347–6413 (8)	6471–6537 (30)	6328–6395 (4)	6285–6349 (-2)
ND5	R	6399–8141 (2)	6402–8144 (2)	6406–8148 (3)	6416–8158 (3)	6420–8162 (3)	6446–8185 (4)	6418–8103 (4)	6538–8275 (0)	6394–8133 (-2)	6349–8088 (-1)
tRNAHis	R	8142–8209 (0)	8145–8212 (0)	8149–8216 (0)	8159–8226 (0)	8163–8230 (0)	8186–8250 (0)	8161–8224 (57)	8276–8343 (0)	8134–8197 (0)	8089–8154 (0)
ND4	R	8210–9548 (0)	8213–9551 (0)	8217–9555 (0)	8227–9565 (0)	8231–9569 (0)	8251–9592 (0)	8226–9563 (1)	8348–9682 (4)	8197–9537 (-1)	8155–9493 (0)
ND4L	R	9573–9866 (24)	9576–9869 (24)	9579–9872 (23)	9604–9897 (38)	9593–9880 (23)	9625–9918 (2)	9595–9861 (31)	9688–9981 (5)	9562–9855 (24)	9503–9796 (9)

(Continued)

Table 2. (Continued)

Gene	D	DP	DT	DSP	DSU	DK1	DK2	DH	KU	EL	TVG
tRNAThr	F	9874–9937 (7)	9877–9940 (7)	9880–9943 (7)	9905–9970 (7)	9894–9957 (13)	9923–9987 (4)	9893–9957 (31)	9986–10050 (4)	9860–9923 (4)	9801–9864 (4)
tRNAPro	R	9938–10002 (0)	9941–10005 (0)	9944–10008 (0)	9971–10035 (0)	9958–10022 (0)	9988–10052 (0)	9958–10022 (0)	10051–10115 (0)	9924–9988 (0)	9865–9929 (0)
ND6	F	10011–10541 (8)	10014–10544 (8)	10017–10547 (8)	10044–10574 (8)	10043–10561 (20)	10061–10591 (8)	10040–10561 (17)	10124–10654 (8)	9997–10524 (8)	9941–10465 (11)
CytB	F	10546–11694 (4)	10549–11697 (4)	10552–11700 (4)	10579–11727 (4)	10569–11714 (7)	10595–11743 (3)	10570–11715 (8)	10662–11807 (7)	10537–11685 (12)	10478–11626 (12)
TRNASer (UCN)	F	11698–11764 (3)	11701–11767 (3)	11704–11769 (3)	11731–11797 (3)	11718–11783 (3)	11742–11807 (-2)	11719–11784 (3)	11809–11875 (1)	11688–11754 (2)	11625–11692 (-2)
ND1	R	11764–12717 (-1)	11767–12720 (-1)	11769–12722 (-1)	11797–12750 (-1)	11783–12736 (-1)	11807–12760 (-1)	11784–12713 (-1)	11869–12825 (-7)	11753–12709 (-2)	11691–12647 (-2)
tRNALeu (CUN)	R	12719–12786 (1)	12722–12789 (1)	12724–12791 (1)	12752–12820 (1)	12738–12805 (1)	12762–12832 (1)	12739–12806 (25)	12827–12892 (1)	12711-12780 (1)	12648–12718 (0)
rRNAL	R	12787–14247 (0)	12790–14248 (0)	12792–14245 (0)	12821–14253 (0)	12907–14263 (101)	12831–14214 (-2)	12847–14211 (40)	12893–14406 (0)	12766–14155 (-15)	12704–14057 (-15)
tRNAVal	R	14248–14312 (0)	14249–14313 (0)	14246–14311 (0)	14254–14319 (0)	14260–14325 (-4)	14216–14280 (1)	14211–14278 (1)	14407–14471 (0)	14155–14220 (-1)	14056–14120 (-2)
rRNAS	R	14313–15091 (0)	14314–15091 (0)	14312–15090 (0)	14320–15101 (0)	14326–15102 (0)	14282–15062 (1)	14279–15054 (0)	14472–15253 (0)	14221–14996 (0)	14121–14898 (0)
Control region	F	15092–15411	15092–15411	15091–15410	15102–15417	15103–15422	15063–15382	15055–15373	15254–15570	14997–15368	14936–15281

The value in parentheses: the positive number indicates interval base pairs between genes, while the negative indicates the overlapping base pairs between genes. "D" means that "Direction".

codon of these ten species was the typical ATN codons except for *COI* (CGA), and there were some different start codons on genes of *ND2*, *ATP8*, *ND3*, *ND5*, *ND4*, *ND4L* and *ND1*. The PCGs were used the same stop codon except for *ND3*, *ND5*, *ND4* and *ND4L*. Furthermore, we compared codon usage and RSCU of ten available Lasiocampidae mitogenomes (S3 Table). Examination of these ten individual Lasiocampidae mitogenomes showed that Leu2 (UUA), Ser2 (UCU), Ala (GCU), Ser1 (AGA) were the four most frequent relatively synonymous codons.

Table 3. A+T content of Lasiocampidae.

Species	Size (bp)	A+T content (%)			
		Whole genome	1st codon positions	2nd codon positions	3rd codon positions
DP	15411	79.5	80.5	75.4	81.7
DT	15411	79.5	77.7	79.8	82.3
DSP	15410	79.38	80.42	78.45	79.26
DSU	15417	80.13	82.72	79.47	78.21
DK1	15422	79.20	76.62	82.01	78.97
DK2	15383	78.70	78.35	83.01	74.74
DH	15373	79.85	79.43	79.70	80.43
KU	15570	78.64	79.23	81.43	75.26
EL	15368	80.19	83.12	77.57	79.89
TVG	15281	80.87	81.94	82.69	77.97

Table 4. **Start codon and stop codon of 13 PCGs in Lasiocampidae species.**

Species	ND2	COI	COII	ATP8	ATP6	COIII	ND3	ND5	ND4	ND4L	ND6	CytB	ND1
DP	ATT/TAA	CGA/T	ATA/T	ATT/TAA	ATG/TAA	ATG/TAA	ATG/TAA	ATT/TAA	ATG/T	ATG/TAA	ATA/TAA	ATG/TAA	ATG/TAA
DT	ATT/TAA	CGA/T	ATA/T	ATT/TAA	ATG/TAA	ATG/TAA	ATG/TAA	ATT/TAA	ATG/T	ATG/TAA	ATA/TAA	ATG/TAA	ATG/TAA
DSP	ATT/TAA	CGA/T	ATA/T	ATC/TAA	ATG/TAA	ATG/TAA	ATC/TAA	ATT/TAA	ATG/T	ATG/TAA	ATA/TAA	ATG/TAA	ATG/TAA
DSU	ATC/TAA	CGA/T	ATA/T	ATT/TAA	ATG/TAA	ATG/TAA	ATC/TAA	ATT/TAA	ATG/T	ATG/TAA	ATA/TAA	ATG/TAA	ATG/TAA
DK1	ATT/TAA	CGA/T	ATA/T	ATC/TAA	ATG/TAA	ATG/TAA	ATT/TAA	ATT/TAA	ATG/T	ATT/TAA	ATA/TAA	ATA/TAA	ATG/TAA
DK2	ATT/TAA	CGA/T	ATA/T	ATA/TAA	ATG/TAA	ATG/TAA	ATT/TAA	ATA/TAA	ATG/T	ATG/TAA	ATA/TAA	ATG/TAA	ATG/TAA
DH	ATA/TAA	CGA/T	ATA/T	ATT/TAA	ATG/TAA	ATG/TAA	ATT/TAG	ATA/TAA	ATG/T	ATG/TAA	ATA/TAA	ATG/TAA	ATG/TAA
KU	ATT/TAA	CGA/T	ATA/T	ATC/TAA	ATG/TAA	ATG/TAA	ATC/TAA	ATT/T	ATG/TAG	ATG/TAG	ATA/TAA	ATG/TAA	ATG/TAA
EL	ATA/TAA	CGA/T	ATA/T	ATC/TAA	ATG/TAA	ATG/TAA	ATT/TAA	ATT/TAA	ATG/TAA	ATA/TAA	ATA/TAA	ATG/TAA	ATG/TAA
TVG	ATT/TAA	CGA/T	ATA/T	ATC/TAA	ATG/TAA	ATG/TAA	ATT/TAA	ATT/TAA	ATG/T	ATG/TAA	ATA/TAA	ATG/TAA	ATT/TAA

Phylogenetic relationship

The result showed that the best model of ML and BI trees was GTR+I+G, where 1nL value was -163,261.5358, AIC value was 326,791.0716, and ΔAIC value was 0. Two types of phylogenetic trees were constructed by using the 13 PCGs dataset having the same structure as Fig 4, which indicate that both methods (ML and BI) and results were consistent and reliable. The phylogenetic relationship among four genera was ((((*Dendrolimus*) + *Kunugia*) + *Euthrix*) + *Trabala*). Interestingly, we found two different phylogenetic trees can be constructed by using two groups data of *D. kikuchii*. When we use the data of DK1 to construct tree, the relationship was ((((*D. punctatus* + *D. tabulaeformis*) + (*D. spectabilis* + DK1)) + *D. superans*) + *D. houi*) (Fig 4A). However, the relationship have changed as ((((*D. punctatus* + *D. tabulaeformis*) + *D. spectabilis*) + *D. superans*)+ (DK2 + *D. houi*) (Fig 4B) by using the data of DK2. If two groups of *D. kikuchii* data were used simultaneously to construct phylogenetic tree, their phylogenetic relationship demonstrated that those two groups of DK data have different genetic relationship with *D. houi* (Fig 4C).

Discussion

In this study, the complete mitogenome of *D. houi* was obtained, with a total length of 15,373 bp. The mitogenome of *D. houi* had similar structural composition and gene arrangement, which indicated that the mitogenome was stable and suitable for the study of phylogenetic relationships. Technically, we obtained the mitogenome by using high-throughput sequencing which was different from previous research [30–33]. The traditional mitogenome sequencing is mainly Sanger sequencing method based on PCR amplification products [34]. However, this method requires primer information of each segment of the genome, and the experimental process is complex, time-consuming and laborious. With the development of next generation sequencing technology (NGS) [33–35], the use of high-throughput sequencing technology has provided great convenience for the rapid acquisition of mitogenome [35–37], and the sequencing cost has also been significantly reduced in recent years, which provides an alternative choice for the sequencing of small genomes such as animal mitochondria [37].

Mitogenomes are more comprehensive and accurate in species identification and phylogeny, avoiding artificial biases caused by blindness of a single gene [38–43], and even the differences among species can be determined based on genes structure and arrangement, which has higher reliability [31,43]. Methodologically, using the 13 PCGs in the sequencing genome was superior in obtaining reliable results compared with clustering analysis by using individual genes [44–50], while both two clustering trees by using both methods were consistent, and the
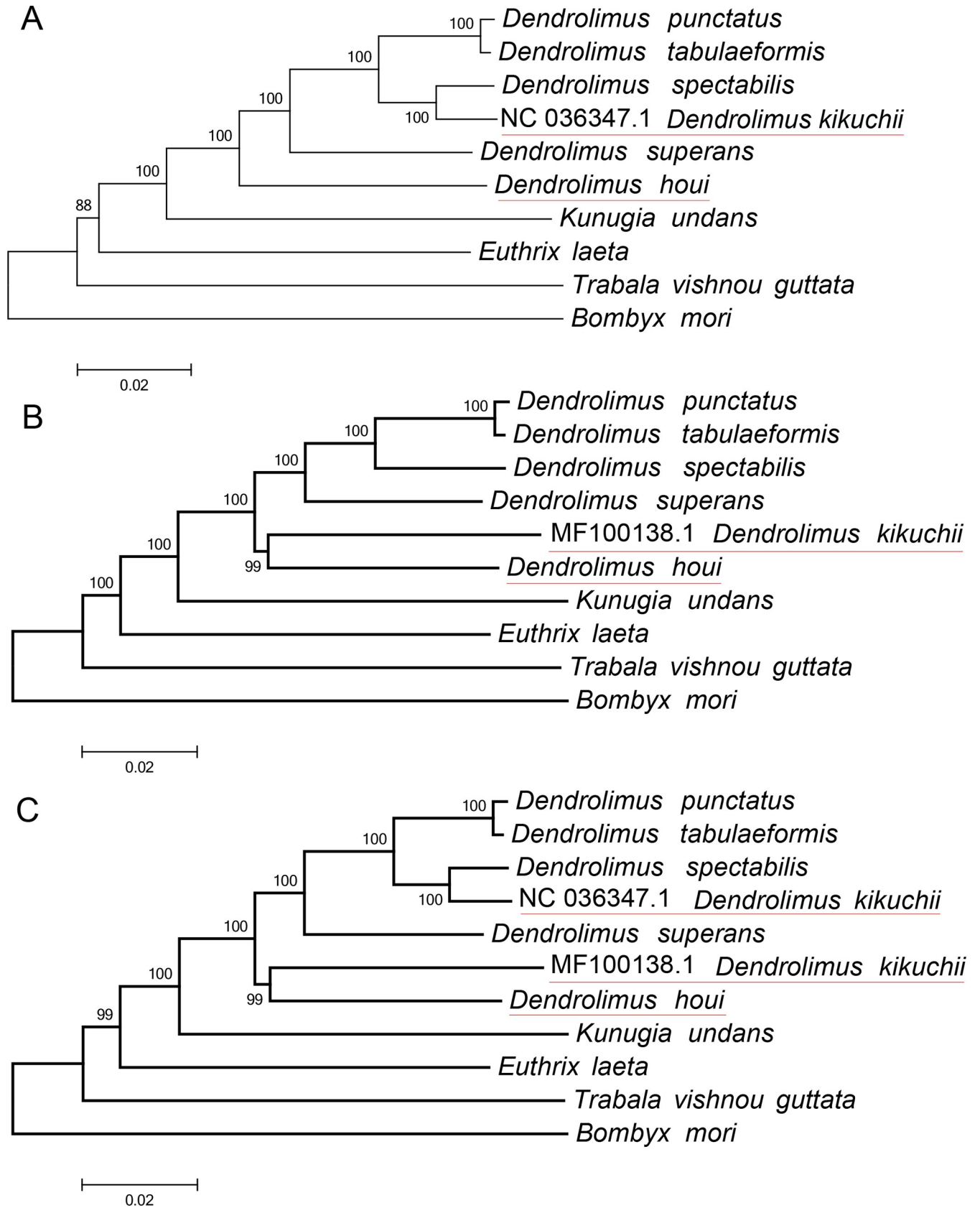
Fig 4. Lasiocampidae phylogenetic tree. A: using the data of DK1, B: using the data of DK2, C: using the data of DK1 and DK2.

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phylogenic relationship among 4 genera in Lasiocampidae was consistent with some previous studies [15,50–52]. In this study, the mitogenome total lengths of *D. houi* from two geographical populations (Yongtai and Jingdong [10]) were various, which may be caused by individual differences of samples. Furthermore, two original data of *D. kikuchii* were used to compare mitogenome structures and construct phylogenetic trees. Interestingly, we obtained the different phylogenetic trees concerning the relationship between *D. houi* and *D. kikuchii*. The phylogenetic relationship between *D. kikuchii* and *D. spectabilis* was closer than that between *D. kikuchii* and *D. houi* by using DK1 data (Fig 4A), which was different from previous research [7–8,10]. However, we obtained the same result as previous reports [10] by utilizing DK2 data (Fig 4B), and show *D. houi* and *D. kikuchii* have a close relationship (Fig 4B) and evolved earlier than the other four pine caterpillar species. Obviously, some populations of *D. kikuchii* from different locations can not be clustered as one group (Fig 4C) whereas DK1 and DK2 data were clustered simultaneously, indicating differentiation occurred due to geographical isolation [4].

These differences were thought to be mostly caused by individual differences or geographic population variation, because the samples of DK1 and DK2 were originally collected originally from Yunnan and Hunan Province respectively [6,14], and some previous studies have shown that insects have generated genetic variation after long-term living in different geographical habitats or from feeding on different hosts [4,5,53]. The degree of genetic differentiation and gene exchange within and between populations have an impact on the geographical populations' genetic diversity of insects. However, even now, little is known about the different geographical populations genetic diversity of *D. houi*. Effective molecular markers should be used to prove whether there is differentiation among different populations, or to better determine the genetic relationship and variation at the genus level.

In this study, all species looked at have the start codon CGA with *COI* gene, which were reported in previous research [11–19,54,55]. Theoretically, it is considered a typical trait due to it being high conservation in Lepidoptera specicies. Similarly, the stop codon ended with TAA in the majority of PCGs, which was also consistent with the results of most Lepidoptera species [6–10]. Currently, there was only one *trnR* within the mitochondrial structure of *D. houi*, which is similar to most species of Lasiocampidae speices, and different from that of *K. undans* with two *trnR*s. Therefore, we presume that the structure of two *trnR*s is unique in *Kunugia* spp., which requires further clarification because more species' genomes are not available at this time.

Currently, the sequencing of animal mitogenomes is rapidly increasing, but the data of insect mitogenomes are obviously still insufficient. Actually, several species of pine caterpillars periodically occur and outbreak [1–3,9,10], endangering local coniferous forests in China [56] under the reasonable climate. Their phylogenetic relationship might be complicated because of homogenization or hybrid of congeneric species. Therefore, the acquisition of the complete mitogenome of *D. houi* and comparison to Lasiocampidae species may provide more genetic varieties for future research.

Supporting information

S1 Fig. The relative synonymous codon usage frequency of the mitogenome of *Dendrolimus houi* (RSCU).
(TIF)

S2 Fig. Secondary structure of mitogenome tRNA of *Dendrolimus houi*.
(TIF)

S3 Fig. Structure of A +T-rich region of *Dendrolimus houi*.
(TIF)

S1 Table. Composition and characteristics of mitogenome of *Dendrolimus houi*.
(XLS)

S2 Table. Mitogenomic nucleotides composition of *Dendrolimus houi*.
(XLS)

S3 Table. The relative synonymous codon usage frequency of the mitogenome of ten Lasiocampidae species (RSCU).
(XLS)

S1 File. Mitochondrial genome of *Dendrolimus houi*.
(FA)

Acknowledgments

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