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[Short communication]

Complete mitochondrial genome of the Japanese oak silkmoth, *Antheraea yamamai* (Lepidoptera: Saturniidae), from Jeju Island, Korea

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Abstract

The wild silkmoth *Antheraea yamamai* Guérin-Méneville, 1861 (Lepidoptera: Saturniidae) is an important producer of silk that is superior to the silk produced by traditional domesticated silkworm. In this study, we sequenced the complete mitochondrial genome (mitogenome) of *An. yamamai* collected from Jeju Island, which is the southernmost island approximately 100 km offshore southward from the Korean Peninsula. Determining this sequence will be necessary for tracing the biogeographic history of the species and developing molecular markers for identifying the origin of commercial products. Comparison of the sequence divergence among two available and the current mitogenomes revealed a low but substantial number of substitutions, totaling 23 nucleotides in the whole genome. *CytB* and *ND5* showed the highest variability with five and four variations, respectively, suggesting that these regions will be prior regions to target for subsequent biogeographic and diagnosis study. Phylogenetic reconstruction based on all available sequences of Saturniidae showed that *An. yamamai* is a sister to the congeneric species *An. pernyi*, corroborating that *Antheraea* is a highly supported monophyletic group. The tribe Saturniini was clearly non-monophyletic and interrupted by Attacini and Bunaeini.

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Introduction

The wild silkmoth *Antheraea yamamai* Guérin-Méneville, 1861 is a large silkmoth endemic to east Asia including Korea (Park *et al*., 1999). Adults eclose once a year, from the middle of July to the middle of September, in Korea. The larvae feed on the leaves of the sawtooth oak (*Quercus acutissima*), chestnut (*Castanea crenata*), jolcham oak (*Quercus serrata*), etc. Compared to the silk yarn of the domestic silkworm *Bombyx mori*, that of *An. yamamai* shows superior gloss, durability, and wrinkle and, thus, was imported to Europe (Park *et al*., 1999; Yoon *et al*., 2006).

Because of its commercial value, the genetic diversity and population genetic structure of *An. yamamai* was determined in a previous study (Kim *et al*., 2017). Evaluation of two partial mitochondrial genes (COI and ND4) provided a maximum

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sequence divergence of only 0.46% (3 bp) and 0.32% (2 bp), respectively, from 79 individuals collected from 12 locations in Korea (Kim *et al*., 2017). Thus, analysis of the full mitochondrial genomes (mitogenomes) from geographic samples to detect further variable regions is required, in that such variable regions selected from full mitogenome sequences provide further scrutinized inference on population history (Jeong *et al*., 2021; Lee *et al*., 2021; Kim *et al*., 2022). Currently, two mitogenome sequences each from northern regions of South Korea and China are available (Kim *et al*., 2009; Sun *et al*., 2021). In this study, we sequenced the complete mitogenome of a sample collected from a remote island, which is ~430 km distant from previous sampling site in South Korea. Our and previous data collectively will be useful for tracing the biogeographic history of the species after more sequence information is accumulated. Furthermore, such variance information can be used to develop molecular markers to trace the origin of commercial products made from *An. yamamai*.

Materials and Methods

Sampling and sequencing

In 2021, *An. yamamai* eggs were collected from mulberry leaves on Jeju Island, which is a remote subtropical island approximately 100 km offshore and southward from the Korean Peninsula (33°25′58.0″ N, 126°38′25.6″ E). DNA was extracted from one egg using a commercial kit (Qiagen, Hilden, Germany), from which three long overlapping fragments (*COI-ND4*, *ND5-lrRNA*, and *lrRNA-COI*) were amplified (Kim *et al*., 2012). The PCR products were purified with a PCR purification Kit (Bioneer, Daejeon, Korea). These long fragments were sequenced using barcode-tagged sequencing technology (Celemics, Inc., Seoul, Korea) and the Illumina MiSeq platform (Illumina, San Diego, CA, USA). The remaining DNA was deposited as a voucher specimen at the Chonnam National University, Gwangju, Korea, under accession no. CNU7299.

Genome construction and phylogenetic analysis

The genome was constructed using MITObim ver. 1.9 (Hahn *et al*.,) through de novo assembly with one conspecies (Kim *et al*., 2009). Phylogenetic analysis was performed using 13 protein-coding genes (PCGs) and 2 rRNA genes (13,305 bp, including gaps) from all available mitogenome sequences in the family Saturniidae. The Bayesian inference method, which is implemented in CIPRES Portal v. 3.1 (Miller *et al*., 2010) was used for phylogenetic analysis. For the analysis, two independent runs of four incrementally heated Markov and Monte Carlo chains (one cold chain and three hot chains) were simultaneously run for one million generations, with tree sampling conducted every 1000 generations. The first 25% of the sampled trees was discarded as burn-in with 48 hrs of maximum running time. The average of the split frequencies under 0.01 was the criterion to decide the reach of convergence from two simultaneous runs. Confidence values were obtained from the Bayesian posterior probabilities (BPPs). Substitution model selection, which was conducted by the comparison of AIC scores (Akaike, 1974) using Modeltest ver. 3.7 (Posada and Crandall, 1998) provided the GTR substitution model with Gamma-distributed rate heterogeneity (G) and invariable sites (I) (GTR + G + I) and was applied to BI analyses.

Results and Discussion

Genome summary

The complete 15,340-base pair (bp) mitogenome of *An. yamamai* is composed of typical gene sets (two rRNAs, 22 tRNAs, and 13 PCGs) and a major non-coding A+T-rich region (Table 1; GenBank acc. no. OM214002). The gene arrangement of the *An. yamamai* mitogenome is identical to that of most lepidopteran genomes including geographic samples of *A. yamamai* mitogenomes (Kim *et al*. 2009; Sun *et al*. 2021). The size of the genome is highly similar to two other geographic samples of *An. yamamai* (15,341 bp from China by Sun *et al*., 2021; and 15,338 bp from Korean peninsula by Kim *et al*., 2009) and is well within the range found in Saturniidae, which ranges from 15,236 (*Actias selene*; Liu *et al*., 2012) to 15,573 (*An. pernyi* Qinghuang1; Li *et al*., 2021; Table 2). Twelve of 13 PCGs had the typical ATN start codon, whereas COI had an atypical CGA codon frequently found in the start region of the lepidopteran COI (Kim *et al*., 2012). The stop codons were incomplete in COII, COIII, ND5, and ND4, being present only as a single thymine, whereas the remaining ones were complete as TAA (Table 1). The length of A+T-rich region of *A. yamamai* is 334 bp and is identical to two other *A. yamamai* mitogenomes. Within the region no detectable repeat unit was found. The A/T content of the whole mitogenome was 80.31%; however, it

Table 1. Summary of *Antheraea yamamai* mitochondrial genome.

Gene	Direction	Nucleotidenumber	Size	Anticodon	Start codon	Stop codon
trnM	F	1-67	67	CAT 32-34		
trnI	F	77-140	64	GAT 106-108		
trnQ	R	138-206	69	TTG 174-176		
ND2	F	260-1273	1014		ATT	TAA
trnW	F	1282-1349	68	TCA1313-1315		
trnC	R	1342-1404	63	GCA 1373-1375		
trnY	R	1415-1481	67	GTA 1446-1448		
COI	F	1488-3018	1531		CGA	T-tRNA
trnL$_2$	F	3019-3085	67	TAA 3049-3051		
COII	F	3086-3767	682		ATG	T-tRNA
trnK	F	3768-3838	71	CTT 3798-3800		
trnD	F	3855-3923	69	GTC 3886-3888		
ATP8	F	3924-4091	168		ATT	TAA
ATP6	F	4085-4762	678		ATG	TAA
COIII	F	4762-5550	789		ATG	TAA
trnG	F	5553-5619	67	TCC 5583-5585		
ND3	F	5617-5971	355		ATA	T-tRNA
trnA	F	5973-6036	64	TGC 6002-6004		
trnR	F	6037-6101	65	TCG 6063-6065		
trnN	F	6102-6166	65	GTT 6132-6134		
trnS1	F	6169-6234	66	GCT 6194-6196		
trnE	F	6236-6301	66	TTC 6265-6267		
trnF	R	6308-6376	69	GAA 6340-6342		
ND5	R	6377-8117	1741		ATT	T-tRNA
trnH	R	8118-8183	66	GTG 8151-8153		
ND4	R	8196-9536	1341		ATG	TAG
ND4L	R	9541-9831	291		ATG	TAA
trnT	F	9837-9901	65	TGT 9867-9869		
trnP	R	9902-9966	65	TGG 9934-9936		
ND6	F	9969-10505	537		ATA	TAA
CytB	F	10505-11659	1155		ATA	TAA
trnS$_2$	F	11685-11751	67	TGA 11713-11715		
ND1	R	11772-12710	939		ATG	TAA
trnL1	R	12712-12782	71	TAG 12750-12752		
lrRNA	R	12783-14162	1380			
trnV	R	14163-14230	68	TAC 14198-14200		
srRNA	R	14231-15006	776			
A+T–rich region		15007-15340	334			

Note: The abbreviations for the genes are as follows: *COI*, *COII* and *COIII* refer to the cytochrome oxidase subunits, *CytB* refers to cytochrome b, and *ND1-6* refers to NADH dehydrogenase components. *srRNA* and *lrRNA* denote small and large subunit ribosomal RNA (rRNA) genes. tRNAs are denoted as one-letter symbols in accordance with the IUPAC-IUB single-letter amino acid codes except those encoding leucine and serine, which are labeled L_1 for CTN codon family, L_2 for TTR codon family, S_1 for AGN codon family, and S_2 for TCN codon family.

Table 2. Characteristics of Saturniidae mitochondrial genomes.

Taxon/Strain	Size (bp)	A/T content (%)	PCG		srRNA		lrRNA		tRNA		A+T-rich region		GenBank accession no.
			No. codons[a]	AT (%)	Size (bp)	AT (%)	Size (bp)	AT (%)	Size (bp)	AT (%)	Size (bp)	AT (%)	
Saturniidae													
Saturniini													
Actias selene	15,236	78.91	3,728	77.30	762	83.99	1,364	83.58	1,459	80.60	339	87.91	JX186589
Actias artemis aliena	15,243	78.62	3,729	76.85	777	84.30	1,363	83.13	1,462	80.78	328	91.77	KF927042
Actias dubernardi	15,270	78.24	3,726	76.36	779	84.08	1,371	83.30	1,472	80.71	330	90.00	MW133617
Actias luna	15,259	78.73	3,729	76.99	758	83.91	1,360	83.75	1,463	80.59	345	89.57	MN832537
Antheraea yamamai	15,338	80.29	3,729	78.89	776	84.41	1,380	83.99	1,473	81.33	334	89.52	EU726630
Antheraea yamamai	15,341	80.30	3,727	78.87	786	84.35	1,382	84.08	1,473	81.33	334	89.52	MW009051
Antheraea yamamai	15,340	80.31	3,730	78.90	776	84.41	1,380	84.13	1,469	81.28	334	89.52	This study
Antheraea frithi	15,338	80.19	3,725	78.65	778	84.58	1,380	83.99	1,476	81.78	333	89.19	KJ740437
Antheraea pernyi	15,566	80.16	3,727	78.46	775	84.13	1,369	83.86	1,472	81.39	552	90.40	AY242996
Antheraea pernyi	15,537	80.10	3,727	78.41	775	83.87	1,370	83.87	1,487	81.64	516	90.89	HQ264055
*Antheraea pernyi*_Yu6	15,569	80.28	3,727	78.58	775	84.26	1,369	84.00	1,472	81.45	552	90.94	KP762788
*Antheraea pernyi*_Yu7	15,572	80.11	3,727	78.41	775	84.00	1,369	83.78	1,472	81.52	554	89.89	KP999979
*Antheraea pernyi*_731	15,570	80.19	3,727	78.53	775	83.87	1,369	83.86	1,472	81.45	553	90.24	KP881616
*Antheraea pernyi*_Luhong	15,563	80.21	3,727	78..54	775	84.13	1,368	83.70	1,468	81.34	552	90.58	MW364566
*Antheraea pernyi*_Qing6	15,572	80.22	3,727	78.53	775	84.13	1,368	83.70	1,475	81.49	552	90.76	MT890592
*Antheraea pernyi*_Qinghuang1	15,573	80.22	3,727	78.54	775	84.13	1,369	83.64	1,471	81.44	552	90.58	MN064713
*Antheraea pernyi*_Dingzhou1	15,573	80.21	3,727	78.52	775	84.13	1,368	83.70	1,475	81.49	553	90.60	MW940851
Antheraea assama	15,312	80.18	3,726	78.74	779	84.72	1,371	84.17	1,466	80.56	332	90.66	KU301792
Antheraea assama	15,272	80.19	3,725	78.76	779	84.72	1,344	84.00	1,465	80.75	328	91.16	KU379695
Eriogyna pyretorum	15,327	80.82	3,731	79.35	778	84.45	1,338	84.60	1,477	81.86	358	92.18	FJ685653
Neoris haraldi	15,383	79.51	3,725	77.95	776	79.38	1,363	85.25	1,473	80.86	417	90.89	MF664471
Cricula trifenestrata	15.425	77.68	3,728	75.54	777	83.53	1,384	83.60	1,453	80.45	447	89.71	KY644697
Rhodinia fugax	15,334	80.57	3,731	79.14	772	83.94	1,371	84.46	1,480	81.55	370	91.62	MT548575
Saturnia jonasii	15,261	79.48	3,727	77.87	764	84.29	1,366	83.89	1,468	81.20	339	90.27	MF346379
Saturnia boisduvalii	15,257	79.32	3,726	77.57	772	84.33	1,363	84.15	1,470	81.36	332	90.66	MF034742
Saturnia japonica	15,376	80.67	3,727	79.12	772	84.33	1,407	84.93	1,478	81.80	332	91.87	MT614593
Saturnia japonica	15,368	80.71	3,727	79.16	772	84.33	1,400	84.86	1,477	81.86	331	92.45	MW405443
Saturnia japonica	15,360	80.62	3,733	79.11	774	84.11	1,391	84.76	1,478	81.87	330	91.52	EF622227
Attacini													
Samia cynthia ricini	15,384	79.78	3,732	78.26	779	83.83	1,358	84.02	1,463	80.59	361	90.86	JN215366
Samia cynthia cynthia	15,345	79.86	3,726	78.33	778	84.19	1,359	84.18	1,465	80.75	359	91.09	KC812618
Samia canningi	15,384	79.88	3,731	78.38	779	83.95	1,358	84.02	1,465	80.48	361	91.14	KJ159909
Attacus atlas	15,282	79.30	3,727	77.68	777	83.14	1,368	84.80	1,436	81.20	357	90.48	KF006326
Bunaeini													
Gonimbrasia belina	15,295	80.57	3,728	78.89	773	85.25	1,371	84.83	1,472	82.81	357	91.04	MN832539
Gonimbrasia belina	15,308	80.60	3,728	79.03	774	84.88	1,366	84.41	1,471	82.80	355	88.73	MN832538
Gynanisa maja	15,275	80.73	3,726	79.24	759	84.85	1,365	84.76	1,458	82.72	350	87.71	MN832540
Gynanisa maja	15,287	80.92	3,725	79.27	761	85.55	1,359	85.36	1,458	82.51	358	91.90	MN832541

[a]Termination codons were excluded in total codon count.

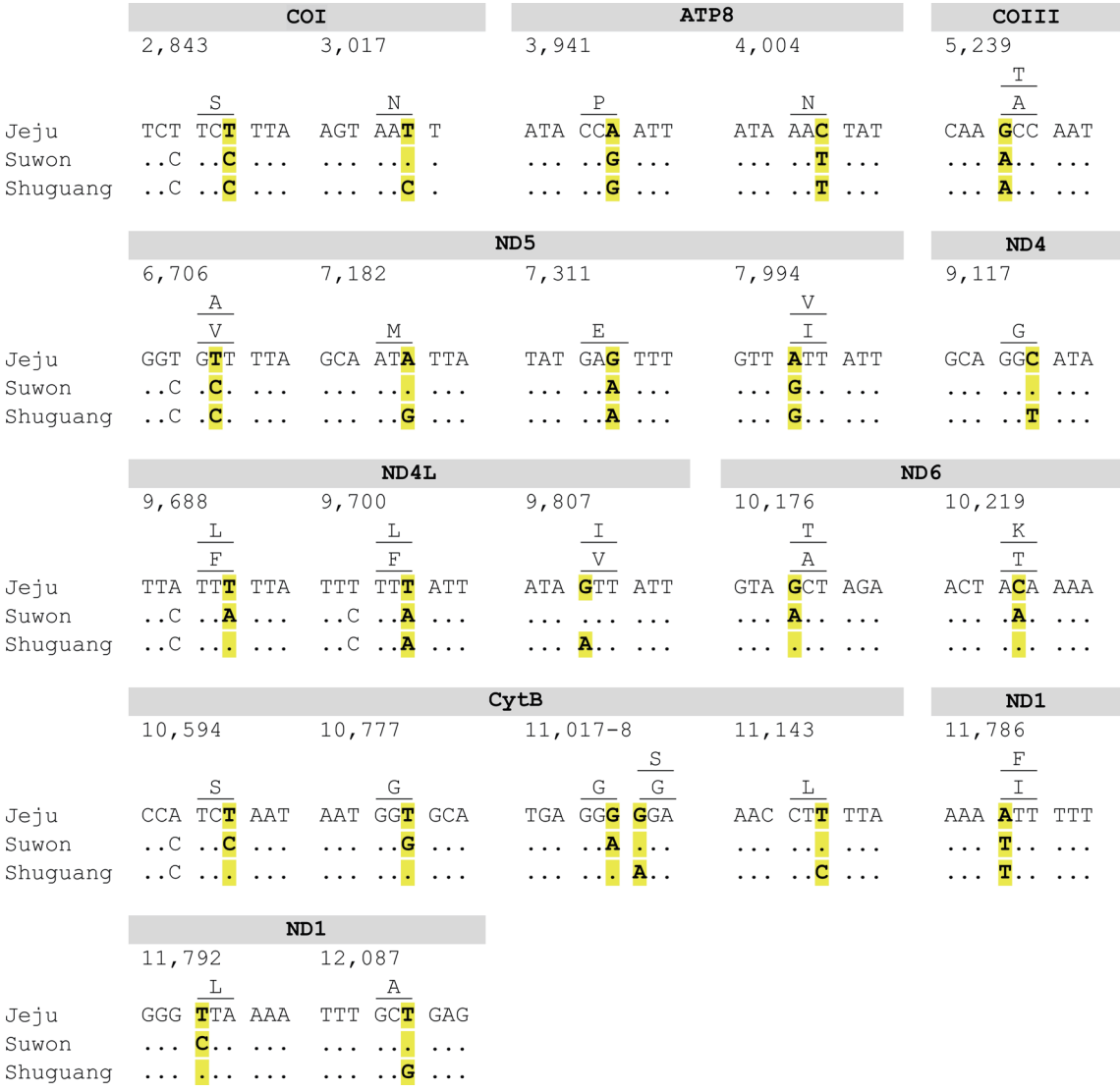

Fig. 1. Sequencing alignment of three mitochondrial genome sequences of *Antheraea yamamai* collected in Jeju, Suwon, and Shuguang, China, respectively. Only nucleotide positions that differ to each other are indicated. The letters on the sequences indicate amino acid. The numbers correspond to the positions, where sequence variation is detected, with respect to the mitochondrial genome of Jeju individual.

varied among the genes as follows: A+T-rich region, 89.52%; lrRNA, 84.18%; srRNA, 81.41%; tRNAs, 81.28%; PCGs, 78.90% (Table 2).

Variability among *An. yamamai* sequences

Comparison of DNA barcoding region among *A. yamamai* individuals showed a maximum of only 0.13% (two bp) of sequence divergence, suggest that the geographic samples of *A. yamamai* are very closer genetically. However, comparison of whole genome sequences provided a substantial number of substitutions, with 23 nucleotides, mainly in PCGs (Fig. 1). CytB and ND5 showed the highest variability, with each containing five and four variations, respectively, whereas other PCGs showed less and other genic region provided no variation. Thus, these two PCGs should be further evaluated in biogeographic and diagnosis studies.

Phylogenetic analysis

Phylogenetic analysis indicated a very close group among geographic samples of *An. yamamai*, a sister relationship between *An. pernyi* and *An. yamamai*, and *Antheraea* as a monophyletic group with the highest nodal supports (Bayesian

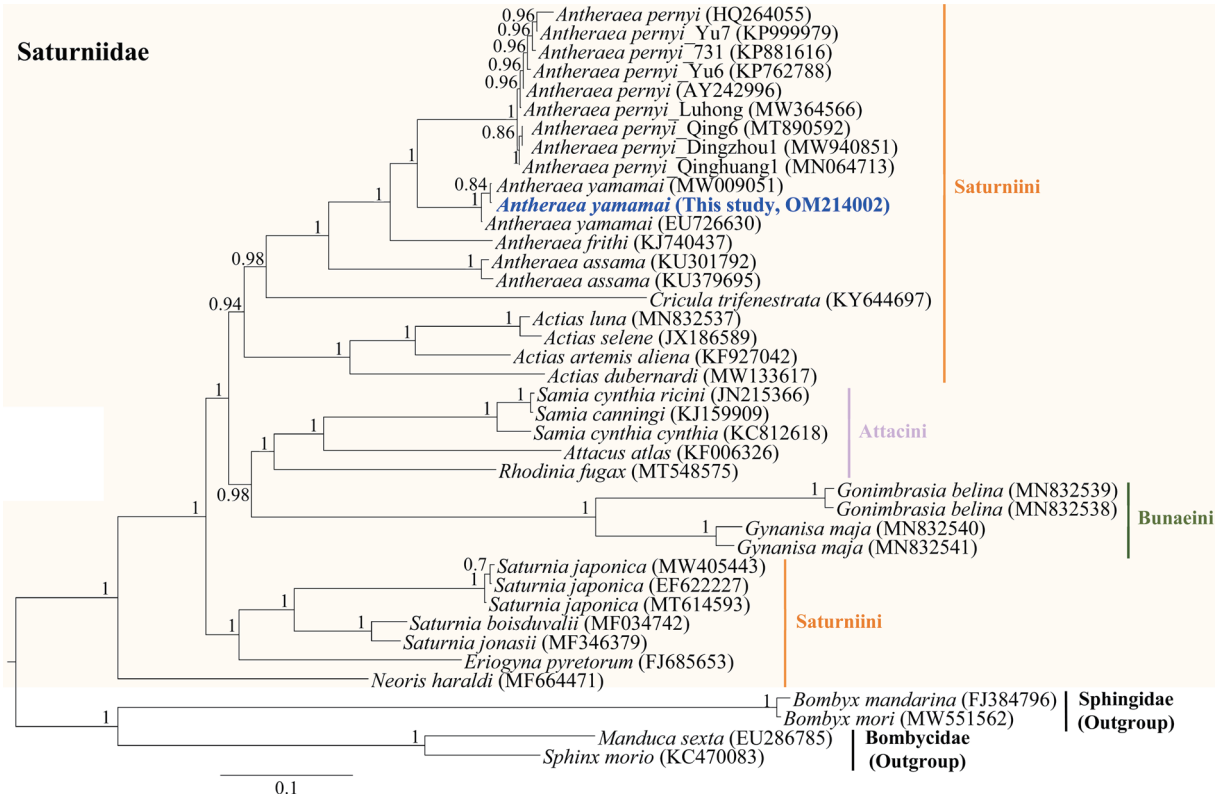

Fig. 2. Phylogenetic tree obtained using the Bayesian inference method. The numbers at each node specify Bayesian posterior probabilities. The species Bombycidae and Sphingidae were used as outgroups. GenBank accession number of each species is shown in brackets after the scientific names.

posterior probabilities = 1; Fig. 1). Consistent to previous studies *Neoris haraldi* was placed as the most basal lineage of Saturniidae (He *et al*., 2017; Chen *et al*., 2021). The tribe Saturniini was clearly found to be a non-monophyletic group, interrupted by Attacini and Bunaeini, as detected previously using mitogenome sequences (Langley *et al*., 2020; Chen *et al*., 2021). However, our result did not place Bunaeini, which includes the African Saturniidae, as the most basal lineage, which is not consistent with the results of previous studies (Langley *et al*., 2020; Chen *et al*., 2021). As more mitogenome sequences are available further scrutinized analysis, which will provide further robust phylogenetic inference might be feasible.

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Data availability statement

The genome sequence data supporting the findings of this study are openly available in GenBank of NCBI at https://www.ncbi.nlm.nih.gov under accession no. OM214002. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA799902, SRP356406, and SAMN25211519, respectively.

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