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The first mitochondrial genome for Sterictiphorinae (Hymenoptera: Argidae) and insights into argid phylogeny

Bia Park[1] & Ui Wook Hwang[1,2,3,4,5]✉

The family Argidae is a significant group of agricultural and forest pests, yet its mitochondrial genome (mitogenome) diversity remains only partially understood. To expand current knowledge, we sequenced and characterized, for the first time, the mitogenome of *Sterictiphora koreana* Lee and Wei 2017 involving in subfamily Sterictiphorinae, an endemic species in Korea. Comparative analyses with four argid species (one Sterictiphorinae and three Arginae) reveal a conserved gene arrangement generally matching the basal hymenopteran form, except for one or four tRNA gene translocations. Whole mitogenomes, protein-coding genes (PCGs), tRNAs, and rRNAs of Arginae, show higher AT content than those of Sterictiphorinae. GC skew values range from − 0.376 to − 0.110 for all four argid species, though a subset of PCGs, tRNAs, and rRNAs in *Arge aurora* exhibit positive. Phylogenetic analyses based on nucleotide sequences of the 13 PCGs strongly support the monophyly of Argidae within superfamily Tenthredinoidea. Divergence time estimation suggests Argidae originated around 166.38 Ma in the Middle Jurassic, with subsequent diversification occurring from the Early Cretaceous to Eocene. These findings enhance our understanding of mitogenome evolution in Argidae and provide a foundation for broader investigations in symphytan phylogenetics and taxonomy.

Keywords AT content, Phylogeny, Divergence time, Symphyta, Tenthredinoidea

Order Hymenoptera is among the most diverse and ecologically important insect groups, having undergone substantial radiation during the Mesozoic Era[1]. Many extant sawfly families likely originated in the mid- to late Cretaceous[2,3]. The family Argidae is the second-largest within the superfamily Tenthredinoidea, encompassing seven subfamilies, 60 genera, and 913 species worldwide[4]. In Korea, Argidae comprises two subfamilies (Arginae and Sterictiphorinae), five genera, and 40 species[5]. Adults are distinguished by a one-segmented flagellum—often forked in males—that sets them apart from other sawfly families.

Argidae is regarded as a monophyletic group closely related to Pergidae and is generally placed as a basal lineage within Tenthredinoidea in most phylogenetic analyses of the paraphyletic suborder Symphyta[6–15].

Mitogenomes are widely utilized in molecular systematics of insects due to their conserved gene content and structure, maternal inheritance, and high mutation rates[16–21]. Despite the taxonomic and ecological importance of Argidae, mitogenomic data exist for only three species—*Arge aurora*, *A. bella*, and *A. similis*—all belonging to the subfamily Arginae[22–24]. To date, no mitogenomic data have been available for Sterictiphorinae or any Korean representatives.

In this study, we present the first complete mitogenome of *Sterictiphora koreana* Lee and Wei 2017, a Korean endemic species in Sterictiphorinae. We compare its mitogenomic features with those of three previously published Arginae species, focusing on gene arrangement, nucleotide composition, codon usage, and strand asymmetry. Phylogenetic analyses based on nucleotide sequences of the 13 PCGs were conducted to evaluate the monophyly of Argidae and clarify its evolutionary relationships within Tenthredinoidea. Furthermore, we estimated divergence times to elucidate the evolutionary history of Argidae, emphasizing its diversification from

[1]Department of Biology Education, Teachers College and Institute for Phylogenomics and Evolution, Kyungpook National University, Daegu 41566, Republic of Korea. [2]Department of Advanced Bioconvergence, Kyungpook National University, Daegu 41566, Republic of Korea. [3]Department of Biomedical Convergence Science and Technology, Kyungpook National University, Daegu 41566, Republic of Korea. [4]Institute for Korean Herb-Bio Convergence Promotion, Kyungpook National University, Daegu 41566, Republic of Korea. [5]Phylomics Inc, Daegu 41910, Republic of Korea. ✉email: uwhwang1@gmail.com

the Jurassic to the Paleogene. Our findings fill a critical gap in the mitogenomic data for Sterictiphorinae and provide fresh insights into symphytan evolution.

Results

General mitochondrial genome features of *Sterictiphora koreana*

The complete mitogenome of *S. koreana* (GenBank accession number PV464956) is 17,872 bp long, comprising 11,150 bp for PCGs, 1,458 bp for tRNAs, and 2,199 bp for rRNAs (Fig. 1a; Tables 1 and 2). This is substantially longer than the average (15,652 bp) of the three *Arge* species—*A. aurora* (15,675 bp; MN913350), *A. bella* (15,576 bp; MF287761), and *A. similis* (15,707 bp; MG923484)—primarily due to variation in the A + T-rich region. *S. koreana* possesses the standard 37 mitochondrial genes (13 PCGs, 22 tRNAs, and 2 rRNAs) plus an A + T-rich region (Fig. 1a; Table 1). Most genes reside on the heavy strand, whereas four PCGs, eight tRNAs, and both rRNA genes are on the light strand, paralleling the arrangement found in other argid species.

All 22 tRNAs (including two each for Leucine and Serine) were identified. They range from 64 to 72 bp in length, leading to slight structural variations in D- and variable-loop regions (Table 1). Each tRNA forms a typical cloverleaf secondary structure, except for *trnS1*, which lacks a stable DHU arm (Fig. 1b). A total of 28 mismatched base pairs were found in the tRNA stems, including 18 wobble G–U pairs, seven U–U pairs, two A–C pairs, and one A–A pair, a common phenomenon in hymenopteran mitogenomes[25–29]. The large (*rrnL*) and small (*rrnS*) rRNA genes measure 1,409 bp and 814 bp, respectively, positioned in a conserved order (*trnL1–rrnL–trnV–rrnS*–A + T-rich region) across Argidae (Table 1).

Nucleotide composition and codon usage patterns of *Sterictiphora koreana*

The overall mitogenome of *S. koreana* exhibits a high AT bias of 79.06%, slightly lower than that of *Arge* species (80.71–83.09%; Fig. 2a; Table 2). AT richness compared to GC is a typical feature shown in hymenopteran insects[30]. AT biases are also reflected in PCGs (76.45%), tRNAs (80.11%), rRNAs (82.45%), and the A + T-rich region (85.26%). Additionally, the species shows slightly positive AT skew (0.038–0.078) and strongly negative GC skew (–0.326 to – 0.110), indicating a relative excess of adenine (A) and cytosine (C).

When compared across Argidae, most species also exhibit positive AT skew and negative GC skew (0.018–0.078 and – 0.376 to – 0.110, respectively), except *A. aurora*, which has negative AT skew in PCGs, rRNAs, and the A + T-rich region but positive GC skew in PCGs, tRNAs, and rRNAs (Fig. 2a; Table 2). This indicates that overall strand asymmetry generally exhibits an excess of A and C relative to thymine (T) and guanine (G) in the Argidae family, except for *A. aurora*. Unlike the whole mitogenome, PCGs, tRNAs, and rRNAs, the A + T-rich region in *S. koreana* shows negative AT (–0.044) and GC (–0.256) skews, akin to *A. aurora* (Table 2).

All PCGs start with typical ATN codons (ATT, ATG, ATA, or ATC) and terminate with either complete (TAA) or truncated (T–) stop codon, a common pattern in insect mitogenomes (Table 1). Relative synonymous

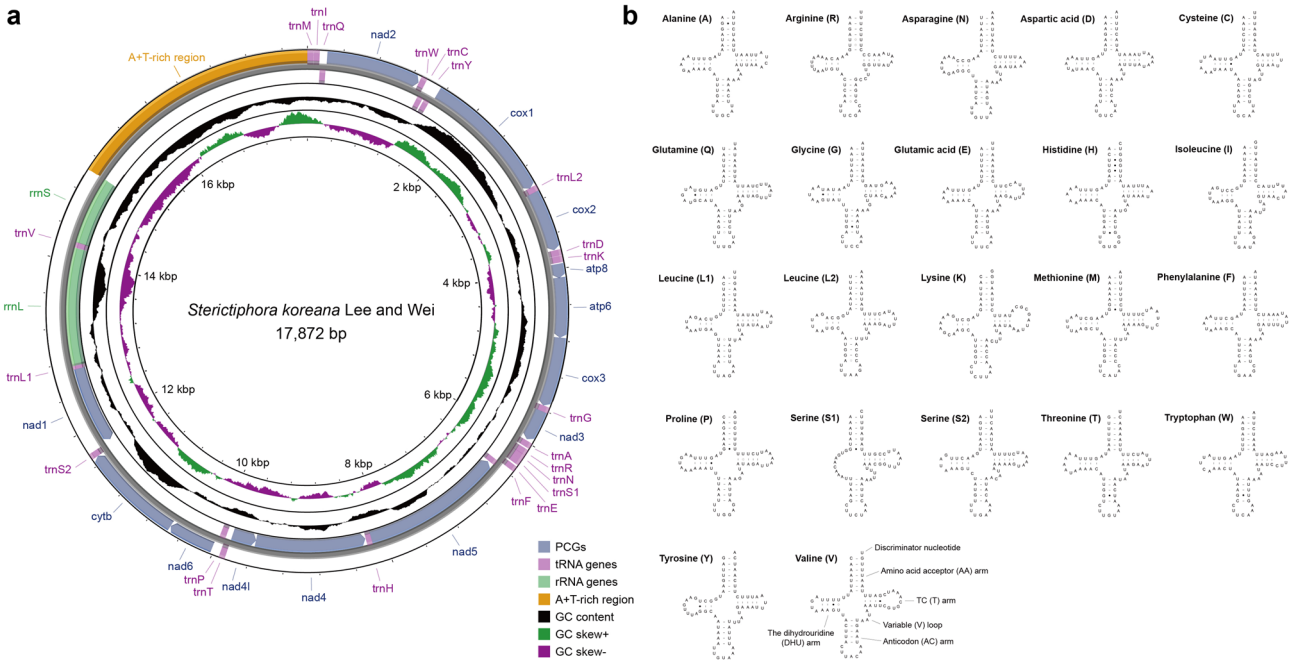

Fig. 1. Complete mitogenome map and potential tRNA secondary structures of *Sterictiphora koreana*: **a** A newly sequenced circular mitogenome is shown, comprising 37 genes: 13 PCGs, 22 tRNA genes, and 2 rRNA genes. Of these, 23 genes reside on the heavy strand, and 14 on the light strand. Standard abbreviations are used for PCGs and rRNA genes, while tRNA genes are indicated by single-letter amino acid codes; **b** Secondary structures of 22 tRNAs. Dashes represent canonical base pairs (A–U, G–C), filled circles denote G–U wobble pairs, and empty circles indicate non-canonical base pairs (A–A, A–C, U–U).

Gene	Strand	Position		Length (bp)	Codon		Anticodon	IGN
		from	to		start	stop		
trnM	H	1	70	70			CAT	
trnI	H	73	137	65			GAT	−2
trnQ	L	146	214	69			TTG	−8
nad2	H	223	1,287	1,065	ATG	TAA		−8
trnW	H	1,293	1,358	66			TCA	−5
trnC	L	1,351	1,414	64			GCA	8
trnY	L	1,435	1,500	66			GTA	−20
cox1	H	1,535	3,037	1,503	ATT	TAA		−34
trnL2	H	3,033	3,098	66			TAA	5
cox2	H	3,099	3,779	681	ATT	TAA		0
trnD	H	3,789	3,856	68			GTC	−9
trnK	H	3,860	3,931	72			CTT	−3
atp8	H	3,944	4,102	159	ATC	TAA		−12
atp6	H	4,096	4,770	675	ATG	TAA		7
cox3	H	4,770	5,549	780	ATG	TAA		1
trnG	H	5,549	5,614	66			TCC	1
nad3	H	5,615	5,968	354	ATT	TAA		0
trnA	H	5,984	6,049	66			TGC	−15
trnR	H	6,067	6,133	67			TCG	−17
trnN	H	6,133	6,199	67			GTT	1
trnS1	H	6,201	6,265	65			TCT	−1
trnE	H	6,286	6,350	65			TTC	−20
trnF	L	6,364	6,429	66			GAA	−13
nad5	L	6,442	8,155	1,714	ATT	T*		−12
trnH	L	8,156	8,220	65			GTG	0
nad4	L	8,227	9,562	1,336	ATG	T*		−6
nad4l	L	9,562	9,852	291	ATT	TAA		1
trnT	H	9,855	9,921	67			TGT	−2
trnP	L	9,922	9,992	71			TGG	0
nad6	H	10,025	10,546	522	ATA	TAA		−32
cytb	H	10,550	11,686	1,137	ATG	TAA		−3
trnS2	H	11,686	11,752	67			TGA	1
nad1	L	11,768	12,706	939	ATT	TAA		−15
trnL1	L	12,707	12,773	67			TAG	0
rrnL	L	12,751	14,159	1,409				23
trnV	L	14,162	14,230	69			TAC	−2
rrnS	L	14,230	15,043	814				1
A + T-rich region	-	15,044	17,872	2,829				0

Table 1. Genome organization of *Sterictiphora koreana* (Argidae, Sterictiphorinae). *IGN* stands for intergenic nucleotide. Incomplete termination codons extended by post-transcriptional adenylation are marked with an asterisk.

codon usage (RSCU) analysis reveals that Asparagine (Asn), Isoleucine (Ile), Leucine2 (Leu2), Methionine (Met), and Phenylalanine (Phe) are the most frequently encoded amino acids, with Leu2 exhibiting the highest RSCU values, consistent with other argid mitogenomes (Fig. 2b, Supplementary Table S3).

Gene arrangement and substitution rate estimation in the family Argidae

Insect mitochondrial gene order is generally conserved[31–34], with tRNA genes displaying the highest rearrangement frequency. Within Argidae, tRNA rearrangements are often subfamily-specific (Fig. 3). In *Arge* species, *trnW* is translocated between the A + T-rich region and *trnI*, whereas *Sterictiphora koreana* shows rearrangements of *trnK–trnD* and *trnI–trnM* clusters, resulting in *cox2–trnD–trnK–atp8* and A + T-rich region–*trnM–trnI–trnQ*.

Ka and Ks values, and ratio of Ka/Ks were calculated for PCGs of each argid mitogenomes (Supplementary Table S4). Ka/Ks ratios for concatenated PCGs among argid species ranged from 0.230 to 0.335, with a mean of 0.283 (Fig. 4, Supplementary Table S4), indicating predominantly purifying selection. Individual genes also

Region	Species	Length (bp)	Nucleotide composition (%)						AT-skew	GC-skew
			A	G	C	T	A + T	G + C		
WM	Sk	17,872	41.56	8.21	12.71	37.50	79.06	20.92	0.051	−0.215
	Aa	15,675	42.97	7.36	10.44	39.23	82.20	17.80	0.045	−0.173
	Ab	15,576	43.20	7.05	12.23	37.51	80.71	19.28	0.070	−0.269
	As	15,707	44.12	6.86	10.05	38.97	83.09	16.91	0.062	−0.189
PCGs	Sk	11,150	41.20	9.38	14.15	35.25	76.45	23.53	0.078	−0.203
	Aa	11,188	35.66	9.80	9.55	44.99	80.65	19.35	−0.116	0.013
	Ab	11,246	42.74	7.66	12.98	36.62	79.36	20.64	0.077	−0.258
	As	11,195	43.73	7.55	10.51	38.20	81.93	18.06	0.067	−0.164
tRNAs	Sk	1,458	41.56	8.85	11.04	38.55	80.11	19.89	0.038	−0.110
	Aa	1,496	42.11	10.03	7.22	40.64	82.75	17.25	0.018	0.163
	Ab	1,499	43.30	7.20	10.27	39.23	82.53	17.47	0.049	−0.176
	As	1,489	43.59	7.32	9.13	39.96	83.55	16.45	0.043	−0.110
rRNAs	Sk	2,199	43.84	5.91	11.64	38.61	82.45	17.55	0.063	−0.326
	Aa	2,266	40.56	9.84	5.16	44.44	85.00	15.00	−0.046	0.312
	Ab	2,229	44.64	5.11	11.26	38.99	83.63	16.37	0.068	−0.376
	As	2,282	45.00	5.00	9.90	40.10	85.10	14.90	0.058	−0.329
AT	Sk	2,829	40.76	5.44	9.19	44.50	85.26	14.63	−0.044	−0.256
	Aa	254	43.70	2.36	4.72	49.21	92.91	7.08	−0.059	−0.333
	Ab*	> 412	45.28	3.39	7.26	43.83	89.11	10.65	0.016	−0.363
	As*	> 445	46.29	1.35	7.64	44.72	91.01	8.99	0.017	−0.700

Table 2. Genome composition and skewness of four argid species. *WM* Whole mitochondrial genomes, *PCGs* protein-coding genes, *tRNAs* transfer RNA genes, *rRNAs* ribosomal RNA genes, *AT* A + T-rich region, *Sk Sterictiphora koreana*, *Aa Arge aurora*, *Ab Arge bella*, *As Arge similis*. Nearly complete mitochondrial genome is marked with an asterisk.

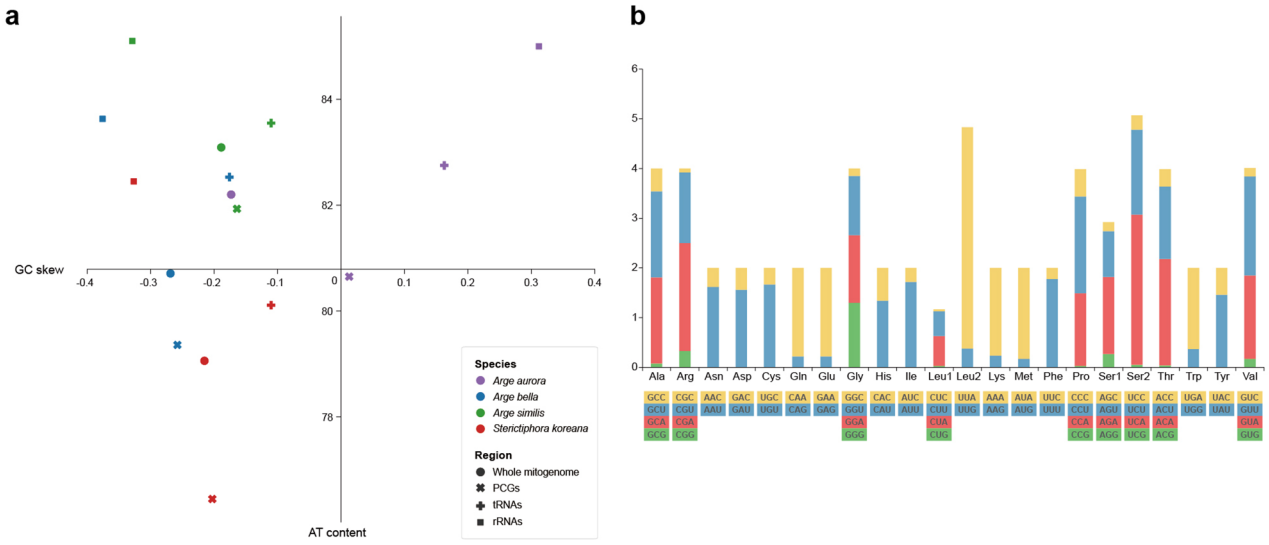

Fig. 2. AT content, GC skew, and relative synonymous codon usage (RSCU) in *Sterictiphora koreana*: **a** Comparisons of AT content and GC skew in the whole mitogenome, PCGs, tRNA genes, and rRNA genes across four argid species. Arginae species display higher AT content than *S. koreana* (Sterictiphorinae). All GC skew values are negative except for those of PCGs, tRNA genes, and rRNA genes in *Arge aurora*; **b** RSCU of the 13 PCGs, showing that UUA (Leu), AUU (Ile), UUU (Phe), and AUA (Met) are the most frequently used codons in argid mitogenomes.

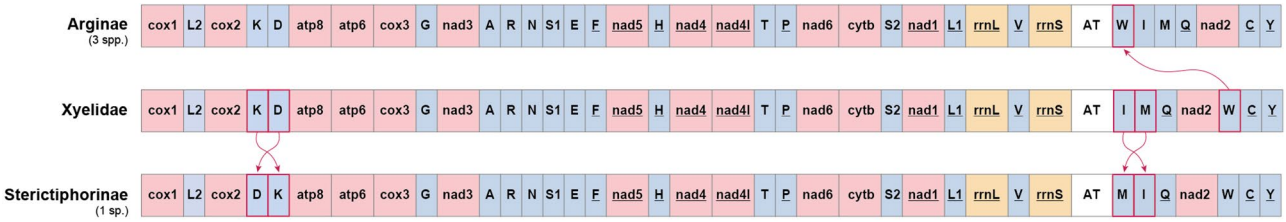

Fig. 3. Comparison of mitochondrial gene arrangements in four argid species and a basal hymenopteran form. Arginae species (*Arge aurora*, *A. bella*, and *A. similis*) have a translocation of *trnW* from its typical basal hymenopteran position, whereas *Sterictiphora koreana* (Sterictiphorinae) exhibits rearrangements of *trnK*, *trnD*, *trnI*, and *trnM*. Underlined labels indicate genes encoded on the light strand, and arrows highlight gene translocations. PCGs are shown in red, tRNA genes in blue, rRNA genes in yellow, and the A + T-rich region (AT) in white.

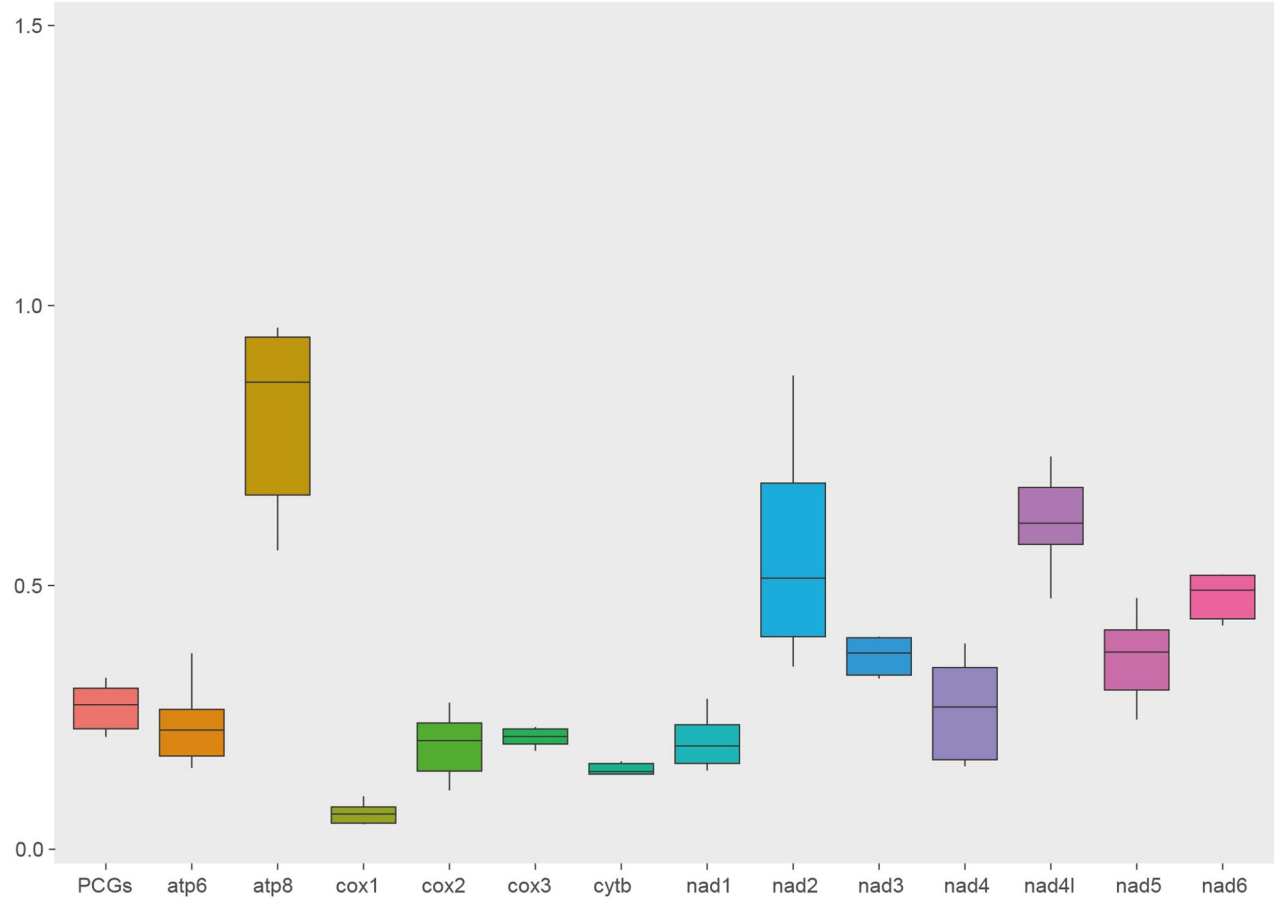

Fig. 4. Box plot of pairwise divergence (Ka/Ks ratios) for the 13 PCGs of argid mitogenomes. Ka/Ks ratios (mean ± SD, and range) for three *Arge* species and *Sterictiphora koreana* were generated in DnaSP v6.12.03[67].

showed Ka/Ks ratios below 1 (mean value 0.094–0.888), with *cox1* having the lowest ratio, suggesting its utility as a barcoding marker for species differentiation.

Phylogenetic relationships at the family level within the superfamily Tenthredinoidea

Phylogenetic trees for 70 sawfly mitogenomes were inferred from the nucleotide sequence alignment set of 13 PCGs using both maximum likelihood (ML) and Bayesian inference (BI) methods (Fig. 5, Supplementary Figs. S1–S2, Supplementary Table S1). The dataset was 11,877 nucleotide sites, which contained the three codon positions of mitochondrial PCGs. Four xyelid species—*Macroxyela ferruginea* (NC_045902), *Megaxyela euchroma* (OL794667), *Pleroneura* sp. (OL794666), and *Xyela* sp. (MG923517)—were chosen as outgroups. Mitogenome data proved effective in resolving relationships among higher taxa of Symphyta[13,14,20,22,35]. All analyses produced nearly identical topologies, strongly supporting the monophyly of each tenthredinoid family

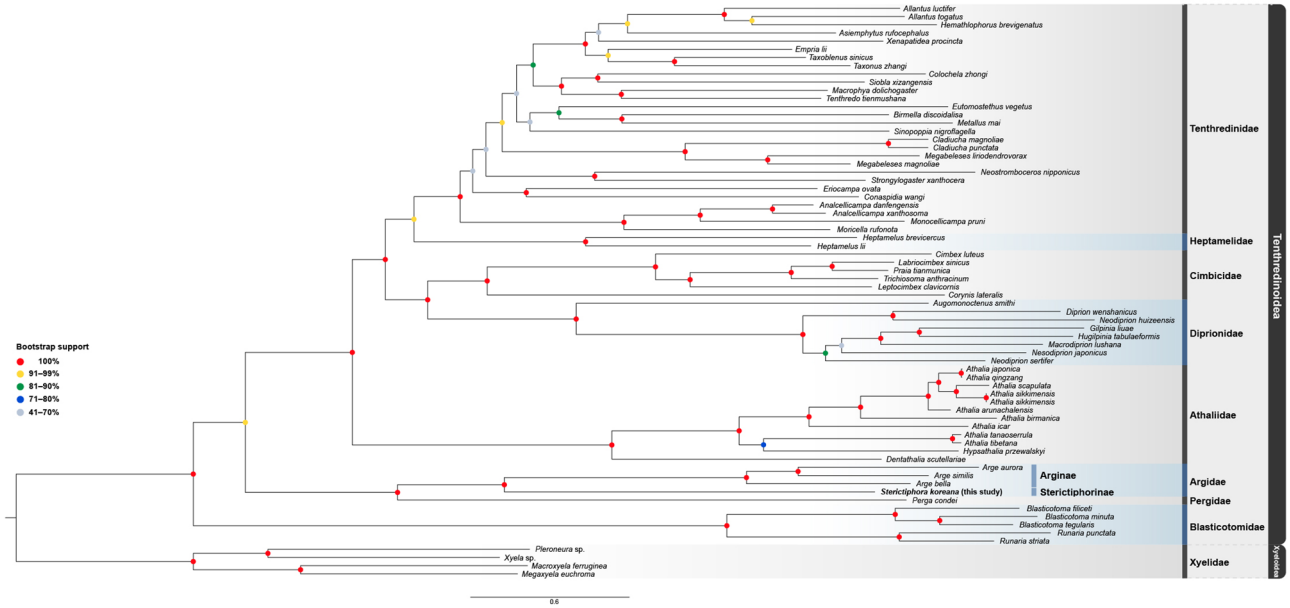

Fig. 5. Phylogenetic placement of Argidae within Tenthredinoidea. ML and BI trees, inferred from the nucleotide sequences of 13 PCGs, illustrate familial relationships in Tenthredinoidea (Supplementary Figs. S1–S2). The presented phylogeny is an ML tree based on the partitioned dataset using IQ-TREE v.2.4.0[70]. Node confidence values are displayed in different colors for each node depending on its range. Four xyelid species, *Macroxyela ferruginea*, *Megaxyela euchroma*, *Pleroneura* sp., and *Xyela* sp., serve as outgroups.

with high bootstrap (BP) and posterior probability (BPP) values (100 BP, 1.00 BPP; Fig. 5, Supplementary Figs. S1–S2).

Family Blasticotomidae consistently occupied the most basal position in Tenthredinoidea, followed by Argidae + Pergidae as a sister group to the remaining non-blasticotomid tenthredinoids. These findings align with previous studies[7–15,36–38]. Athaliidae was originally a subfamily of Tenthredinidae, but is recently treated as a family. This family emerged as a well-supported lineage, separate from Tenthredinidae. The phylogenetic placement of Athaliidae was consistently confirmed in all analyses as sister to the remaining four tenthredinoids, Cimbicidae, Diprionidae, Heptamelidae, and Tenthredinidae, with high support values (100 BP, 1.00 BPP). Within the latter clade, Cimbicidae + Diprionidae formed a monophyletic group sister to Heptamelidae + Tenthredinidae. Argidae, the focal taxon, was strongly monophyletic with distinct subfamily-level clusters (Arginae and Sterictiphorinae) and was consistently placed immediately after Blasticotomidae (100 BP, 1.00 BPP).

Divergence time estimations for tenthredinoid lineages

Molecular dating of Tenthredinoidea based on the nucleotide sequence alignment is shown in Fig. 6 and Supplementary Fig. S3. The estimated stem group age for Tenthredinoidea was 243.49 Ma (231.94–263.72) in the Middle Triassic, followed by diversification from the Late Triassic into the Early Quaternary. The earliest split separated Blasticotomidae from other tenthredinoids at 223.74 Ma (205.94–239.56). Argidae + Pergidae diverged from the remaining tenthredinoids at 206.28 Ma (190.10–211.35) in the Late Triassic. Athaliidae subsequently diverged from the rest at 165.92 Ma (160.51–188.85), whereas Cimbicidae + Diprionidae separated from Heptamelidae + Tenthredinidae at 149.93 Ma (142.82–169.66). Within Tenthredinidae, the largest and most species-rich lineage, the earliest divergence event occurred at 127.43 Ma (118.85–135.71) in the Early Cretaceous.

The Argidae + Pergidae lineage originated around 166.38 Ma (159.82–170.51) in the Middle Jurassic, coinciding with the split between Argidae and Pergidae. Within Argidae, the subfamilies Arginae and Sterictiphorinae diverged at approximately 126.02 Ma (113.90–128.22), corresponding to the Early Cretaceous.

Discussion

Many researchers have used mitogenomes as powerful markers for studying insect phylogeny, population genetics, and phylogeography[13,14,20,34,35,39–47]. Over the past two decades, phylogenetic analyses based on the mitogenomes of sawflies and woodwasps have been widely conducted, and recent advances in whole-genome sequencing have further accelerated this research[13,14,20,22,35]. Within Hymenoptera, Symphyta is considered paraphyletic to Apocrita, yet most higher symphytan taxa are strongly supported as monophyletic[6–15,20,22,35,38,48–50]. Nevertheless, the phylogenetic placement of some families, subfamilies, and genera within Tenthredinoidea remains uncertain.

To date, more than 60 tenthredinoid mitogenomes have been reported (Supplementary Table S1), including only three from Argidae (*Arge aurora*, *A. bella*, and *A. similis*)[22–24]. These known argid mitogenomes belong to Arginae, and no studies have addressed the internal phylogenetic relationships of Argidae across subfamilies.

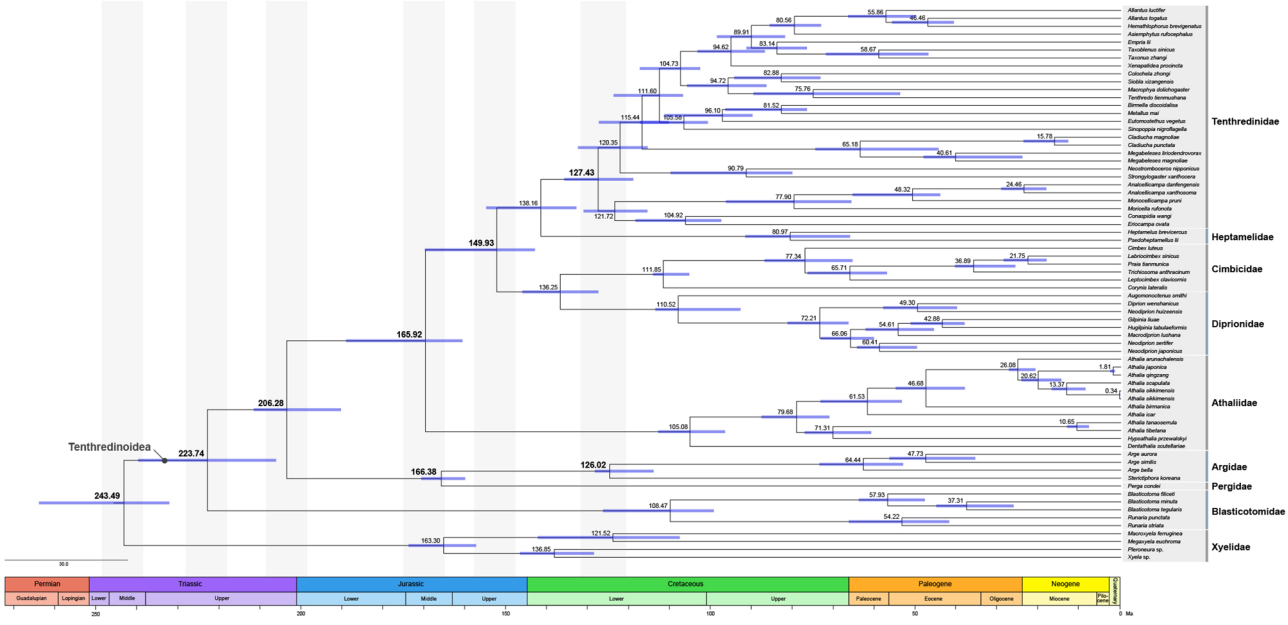

Fig. 6. Dated phylogeny of Tenthredinoidea inferred from the partitioned nucleotide sequence alignment in BEAST v2.7.7[73]. The x-axis indicates millions of years, and the colored time scale corresponds to geological epochs. Blue bars on nodes represent 95% of the highest posterior density intervals of divergence times inferred via MCMC. Mean node ages (in black) are indicated at each node. The gray shading highlights major divergence events at the family level within Tenthredinoidea.

Here, we present the first fully sequenced mitogenome of the Korean endemic species *Sterictiphora koreana* Lee and Wei 2017 (Sterictiphorinae). We also conducted comparative analyses of four argid mitogenomes—*S. koreana* and three Arginae species—constituting the first subfamily-level comparison in Argidae.

The mitogenome of *S. koreana* (17,872 bp) is notably longer than those of other argid species, primarily due to an expanded A + T-rich region (Fig. 1a; Table 1). Consistent with the high AT bias common in hymenopteran mitogenomes[30,51], all four argid species exhibit 79.06–83.09% AT content in the whole mitogenome (Fig. 2a; Table 2). This indicates that A and T are much more favored than G and C in codon usage. In the subfamily level, Sterictiphorinae contains relatively low AT content than that of Arginae, showing the lowest AT content in PCGs (76.45%). Although tRNA gene rearrangements frequently occur in hymenopteran mitogenomes, rearrangement patterns are relatively stable in high-level symphytan groups[13,14,20,42,43,52–56]. Similarly, Argidae shows subfamily-specific rearrangement patterns. In Arginae (3 spp.), *trnW* is relocated between the A + T-rich region and *trnI*, whereas in Sterictiphorinae (1 sp.; *S. koreana*), rearrangements occur among *trnK*–*trnD* and *trnI*–*trnM* gene clusters (Fig. 3). These rearrangements may represent synapomorphic events at the subfamily level, although further studies are necessary to clarify their evolutionary significance.

The Ka/Ks ratio is used as an indicator of natural selection acting on mitochondrial PCGs. Ka/Ks ratios for argid PCGs range from 0.094 to 0.888 (Fig. 4, Supplementary Table S4), indicating purifying selection. This finding is consistent with other symphytan mitogenomes (Ka/Ks < 1; Supplementary Table S5), suggesting generally slower evolutionary rates in Symphyta compared to Apocrita[42,57]. Because mitochondrial PCGs encode enzymes essential for oxidative phosphorylation, their mutations are shaped by both ecological and physiological factors. Symphytan larvae are predominantly herbivorous, whereas many apocritans display parasitic or predatory lifestyles, potentially subjecting them to distinct selective pressures on mitochondrial function. Moreover, variations in habitat stability—such as temperature, oxygen availability, or host plant interactions—can lead to differences in the rate and direction of mitochondrial evolution.

Tenthredinoidea is the most species-rich superfamily of Symphyta[4]. Numerous morphological and molecular studies have supported the monophyly of most tenthredinoid families[6–15,20,35,38,49]. Among these, Argidae has consistently emerged as monophyletic and closely allied to Pergidae[6–15,35,38,48,49], diverging at approximately 166.38 Ma (159.82–170.51) during the Jurassic.

Argidae is distinguished by its one-segmented flagellum, absence of 2r vein in the fore wing, and a prominently long-constricted anal cell in the fore wing[58,59]. Of the seven extant argid subfamilies, Arginae and Sterictiphorinae together comprise roughly 85.13% of species: 13 genera and 443 species in Arginae, and 27 genera and 330 species in Sterictiphorinae[4]. In this study, Arginae and Sterictiphorinae are clearly separable by morphological traits (e.g., male flagellum morphology, R1 cell condition in the hind wing) and by molecular features (e.g., specific tRNA rearrangements, contrasting AT content). Arginae shows a simple male flagellum, a closed R1 cell in the apex of the hind wing, relocation of *trnW*, and a higher AT content (79.36–85.10%), whereas Sterictiphorinae exhibits a forked male flagellum, an open R1 cell, multiple tRNA rearrangements (*trnK*, *trnD*, *trnI*, *trnM*), and comparatively lower AT content (76.45–82.45%). However, the scarcity of mitogenomic data available for Argidae still limits our ability to fully interpret the structural and compositional evolution of their

mitogenomes. Because the present conclusions are based on only four currently reported argid mitogenomes, additional sampling from diverse argid lineages will be essential for validating these patterns and may reveal new lineage-specific genomic features.

In addition, the positions of Heptamelidae and Athaliidae within Tenthredinoidea remain variable depending on the dataset and analytical methodology[11,14,15]. Likewise, relationships among Cimbicidae, Diprionidae, and Tenthredinidae are not fully resolved[6–12,14,15,48]. Additional taxonomic challenges include the potential elevation of some subgroups within Tenthredinidae to subfamily or family status, ambiguous phylogenetic placements of certain taxa, and questions about the monophyly of specific subfamilies. Broader sampling in future studies will likely refine our understanding of this diverse superfamily's evolutionary history.

Materials and methods

Sample collection and DNA extraction

An adult female *Sterictiphora koreana* was collected using an insect net on 20 April 2020 in Dae-dong, Gyeongsan-si, Gyeongsangbuk-do, Republic of Korea. The specimen was immediately preserved in 99% ethanol and identified using external morphological characteristics. Total genomic DNA was extracted from whole-body muscle tissue with a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions.

Mitogenome sequencing, assembly, and annotation

The complete mitogenome was sequenced on an Illumina, HiSeq 4000 or HiSeq X system (GnCBIO, Daejeon, Republic of Korea) in 150 bp paired-end 5G mode. NOVOPlasty[60]—a seed-based *de novo* assembler specifically designed for organelle genomes—was employed to assemble the mitogenome. The assembly was conducted using paired-end Illumina sequencing data comprising two FASTQ files of approximately 1.5 Gb and 1.7 Gb, respectively. A total of 39,768,010 raw reads were provided as input to NOVOPlasty[60]. Of these, 238,634 reads were aligned to the seed sequence and contributed to the assembly process. The final assembly yielded a single circular contig of 17,872 bp and 175,094 reads. The average insert size was estimated at 309 bp, and the assembled mitogenome showed an average sequencing depth of 1,966 reads per base. Overall, mitochondrial reads accounted for approximately 0.6% of the total dataset.

All 13 PCGs, 22 tRNA genes, and two rRNA genes were identified by comparing them with homologous sequences of other argid species (MF287761, MG923484, and MN913350). PCGs were further confirmed by translating nucleic acid sequences into amino acid sequences using the invertebrate mitochondrial genetic code with the EMBOSS transeq server[61]. ARWEN v1.2[62] and tRNAscan-SE 2.0[63] were used to locate the 22 tRNAs, and their secondary structures were manually plotted in Adobe Illustrator 2021, following ARWEN's predictions. The two rRNA genes (*rrnL* and *rrnS*) and the A + T-rich region were verified based on their proximity to tRNA genes (*trnL1* and *trnV*) and alignment with the reference mitogenome. Circular mitogenomic maps were generated in GenomeVx[64]. The complete mitogenome sequence was submitted to GenBank under accession number PV464956.

Mitogenome sequence analyses

Using BioEdit v7.0.5.3[65], we calculated the length, base composition, AT and GC content, and skewness (AT skew = [A − T]/[A + T], GC skew = [G − C]/[G + C]) for the whole mitogenome, PCGs, tRNA genes, and rRNA genes[66]. Nonsynonymous (Ka) and synonymous (Ks) substitution rates of the PCGs were estimated in DnaSP v6.12.03[67].

Phylogenetic analyses

A total of 66 available tenthredinoid mitogenomes were retrieved from the NCBI GenBank to investigate phylogenetic relationships, with four xyelid species designated as outgroups (Supplementary Table S1). Nucleotide sequences of the 13 PCGs were aligned using ClustalX v2.1[68], and the individual gene alignments were subsequently concatenated to generate a combined dataset for ML and BI analyses. An optimal partitioning strategy and substitution models were identified using PartitionFinder v2.1.1[69], applying the greedy algorithm under the Bayesian information criterion (BIC) with branch lengths estimated as unlinked. The best-fitting scheme partitioned the dataset by codon position, with the following models assigned to each partition: GTR + I+G for the 1st codon positions of 13 PCGs and the 2nd codon positions of *atp8*; TVM + I+G for the 2nd codon positions of the remaining 12 PCGs; and TIM + I+G for the 3rd codon positions of 13 PCGs.

ML tree was reconstructed with IQ-TREE v2.4.0[70] running locally, applying the optimal partitioning scheme and substitution models with partition merging. Nodal support was assessed with 1,000 ultrafast bootstrap replicates. BI analysis was performed in MrBayes 3.2.7a[71], with two independent runs of 10 million generations, each consisting of four Markov chains, and sampling every 1,000 generations. The first 25% of sampled trees were discarded as burn-in. Partition-specific substitution models were applied in the BI analysis. Consensus phylogenetic trees were visualized using FigTree v1.4.4[72].

Divergence time estimations

Divergence times among major lineages of the superfamily Tenthredinoidea were estimated using the partitioned dataset in BEAST v2.7.7[73] under the uncorrelated relaxed lognormal clock algorithm and a BirthDeath speciation tree prior. Ten fossil-based node calibration points were applied based on the recent previous study[15], representing the oldest available and reliable calibrations for each tenthredinoid family and for subfamilies within Argidae.

The applied calibrations were as follows: (1) *Xyelotoma nigricornis* for the split of the clade Argidae + Pergidae from the remaining five tenthredinid members except for Blasticotomidae (mean age of 160 Ma and ranging

from 155.7 to 164.7 Ma, lognormal prior distribution); (2) *Sterictiphora konowi* for the stem calibration of Argidae (36 Ma, 33.9–37.2 Ma, uniform); (3) *Cenocimbex menatensis* for the stem calibration of Cimbicidae (60 Ma, 58.7–61.7 Ma, uniform); (4) *Eodiprion groehni* for the stem calibration of Diprionidae (36 Ma, 33.9–37.2 Ma, uniform); (5) *Athalia vetuecclesiae* for the stem calibration of Athaliidae (31 Ma, 28.4–33.9 Ma, uniform); (6) *Paremphytus ostentus* for the stem calibration of Blasticotomidae (36 Ma, 33.9–37.2 Ma, uniform); (7) *Palaeathalia laiangensis* for the stem calibration of the clade Tenthredinoidea except for Blasticotomidae, Argidae, and Pergidae (119 Ma, 112.6–125.5 Ma, lognormal); (8) *Pseudoxyelocerus bascharagensis* for the crown calibration of Tenthredinoidea (183 Ma, 182.0–183.0 Ma, lognormal); (9) *Dauhogoa bella* for the stem calibration of Xyelidae (160 Ma, 155.7–164.7 Ma, lognormal); (10) *Triassoxyela foveolata* for the originated age of Hymenoptera (228 Ma, 221.5–235.0 Ma, lognormal).

All calibrated nodes were constrained as monophyletic in the BEAUti v10.5.0 (part of the BEAST v2.7.7[73] package), and detailed calibration settings are provided in Supplementary Table S2. Although optimal partition-specific substitution models were identified using PartitionFinder v2.1.1[69], the GTR + I+G model was applied uniformly in the divergence-time analyses because several selected models are not directly implemented in BEAST. The Markov chain was totally run for 400 million generations, sampling every 1,000 generations, with the initial 10% of samples discarded as burn-in. Run convergence was evaluated with Tracer v1.7.2[74] by confirming that effective sample sizes (ESS) exceeded 200 for all estimated parameters, including the posterior, tree likelihood, ucldStdev, BDBirthRate, and BDDeathRate. A maximum clade credibility (MCC) tree was combined and produced using LogCombiner v2.7.7 and TreeAnnotator v2.7.7 (part of the BEAST v2.7.7[73] package) after removing 10% of post burn-in trees. The final time-calibrated phylogeny was visualized in FigTree v1.4.4[72].

Data availability

The data underlying this article are available in NCBI GenBank at [https://www.ncbi.nlm.nih.gov/], and can be accessed with the accession number PV464956. The associated BioProject, SRA, and BioSample numbers are PRJNA1247704, SRR33031097, and SAMN47827871, respectively.

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Author contributions

B.P. and U.W.H designed the research, wrote and reviewed the main manuscript. B.P. performed experiments, data analysis, visualization, and research funding acquisition. U.W.H. provided funding and supervision.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to U.W.H.