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Description and mitochondrial genome sequencing of a new species of inquiline gall wasp, *Synergus nanlingensis* (Hymenoptera, Cynipidae, Synergini), from China

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Abstract

A new species of inquiline gall wasp, *Synergus nanlingensis* Wang & Zeng, **sp. nov.**, which was reared from galls on *Castanopsis eyrei* Tutch (Fagaceae) collected in Guangdong Province, China, is described and illustrated herein along with its mitochondrial genome. The mitogenome of *S. nanlingensis* is 16,604 base pairs in length and comprises 37 genes, which is typical of mitogenomes. One large control region was detected in the *S. nanlingensis* mitogenome, which differed from that reported for other Cynipidae species. Similar to other Cynipidae species, *S. nanlingensis* has the same four common gene rearrangement events; however, it shows some differences, as follows: *trnS1* is downstream of *Cytb*; *trnS2* is upstream of *nad1*; and *trnC* is downstream of *rrnS*. Phylogenetic analysis using *COI*, *CytB*, and *28S-D2* sequences confirmed that *S. nanlingensis* is a distinct species belonging to the genus *Synergus* Hartig.

Keywords

Castanopsis, gall wasp, mitogenome, morphology, phylogenetic analysis

* These authors contributed equally.

Introduction

Cynipids or gall wasps (Hymenoptera: Cynipoidea, Cynipidae) are the second largest radiation of gall-inducing insects, with about 1400 described species (Ronquist et al. 2015; Lobato-Vila et al. 2022a). They are widely distributed worldwide, mainly throughout the Holarctic region (Nearctic and Palearctic), and most species are gall inducers on different host plants (Melika and Abrahamson 2002; Ronquist et al. 2015; Lobato-Vila et al. 2022b). Gall induction starts after the oviposition where this interaction between the female wasp and the plant tissue triggers gall formation, and larval activity (chewing, feeding) promotes gall growth and subsequent transformations of the gall structure (Csóka et al. 2005). This can affect the growth of the host plant, even causing host death (Duffet 1968). The gall protects the larvae from not only predatory insects, but also insecticides, posing difficulties for their chemical control (Moriya et al. 2003; Chiara et al. 2018). Some cynipoids can significantly impact the forestry industry. For example, *Dryocosmus kuriphilus* Yasumatsu is a worldwide invasive pest that causes serious damage to chestnut trees (Zhu et al. 2007; Yang et al. 2021), while damage by *Diplolepis abei* Pujade-Villar & Wang causes significant economic losses to the rose horticulture industry in Northwest China (Guo et al. 2013; Lobato-Vila et al. 2020).

By contrast, nearly 240 species of cynipids (Lobato-Vila et al. 2022b), termed inquilines, are unable to trigger gall growth; instead, they develop inside galls induced by other gall wasps, forming an advantageous relationship that benefits only the inquilines and that can even cause the death of the gall inducer (Duffet 1968; Pénzes et al. 2009; Bozsó et al. 2015). Inquilines are distributed into four tribes: Synergini *sensu stricto*, Ceroptresini, Diastrophini, and Rhoophilini (Ronquist et al. 2015; Lobato-Vila et al. 2022a). *Synergus* Hartig is the most speciose genus in Synergini, with about 130 species known worldwide (Schwéger et al. 2015; Pujade-Villar et al. 2017; Lobato-Vila et al. 2020). Most *Synergus* species are associated with galls induced by gall wasps of the tribe Cynipini on Fagaceae (principally *Quercus* spp.). In Europe, the 22 species of Fagaceae (Schwarz 1993; Tutin 1993; Tutin and Akeroyd 1993) are known to host at least 30 species of *Synergus* (Melika 2006; Pénzes et al. 2012). By comparison, there are 294 species of Fagaceae in seven genera in mainland China, including 163 endemic and at least three introduced (Huang et al. 1999). However, only 17 *Synergus* species are known from mainland China (Lobato-Vila et al. 2022a), thus, it is thought that the species diversity of *Synergus* in mainland China is likely to be higher than current estimates (Abe 2007; Liu et al. 2012).

The mitochondrial genome of most insects is a double-stranded circular structure DNA molecule comprising 13 protein-coding genes, 22 transfer RNAs (tRNAs), two ribosomal RNA (rRNA) genes, and a major noncoding sequence called 'Control Region' (CR) (Cameron 2014). Given the maternal mode of inheritance and conserved gene components, the insect mitochondrial genome is a molecular marker widely used in phylogenetic construction (Cameron 2014). However, gene rearrangements have been found frequently in Hymenoptera (Wei et al. 2014; Chen et al. 2018), not only for tRNA genes but also for protein-coding genes (PCGs) (Simon et al. 2006; Tang et

al. 2019). Gene rearrangements, including transpositions, inversions, and inverse transpositions in the mitogenome, are common in certain insect groups, can be an informative feature for phylogenetic reconstruction (Cameron 2014; Feng et al. 2020). For example, Tang et al. (2019) analyzed 83 full or partial mitochondrial genomes to resolve relationships among all major clades of Hymenoptera with high support, confirming the phylogenetic position of Cynipoidea in Proctotrupomorpha as previously hypothesized by Heraty et al. (2011). Despite these advances, complete or nearly complete mitogenome sequences remain scarce for Cynipidae, with just seven species documented, including only two from Synergini (Tang et al. 2019; Xue et al. 2020; Pang et al. 2022; Shu et al. 2022; Zhong and Zhu 2022; Mozhaitseva et al. 2023; Su et al. 2023).

In this study, we describe a new species of the genus *Synergus* from China. The completed mitogenome of this new species was sequenced and annotated, and mitogenome structure and gene rearrangements in this lineage were analyzed. Additionally, phylogenetic analyses were conducted using *COI*, *Cytb*, and *28S-D2* sequences to delineate the evolutionary relationships between this new species and existing species from the Palearctic region within *Synergus*.

Materials and methods

Specimen collection

A total of 142 galls were collected in September 2023, from branches of *Castanopsis eyrei* Tutch on the summit of Xiaohuang Mountain, Guangdong Province, China. The galls were kept in insect mesh bags with moistened cotton and placed in meshed rearing cages. These cages were placed in the laboratory environment under room temperature conditions. To maintain humidity, the cages were misted with water every 1.5 days, and the humidifying cotton was frequently replaced until the emergence of insects. Adult wasps were directly preserved in 100% ethanol within two days after emergence and frozen at –80 °C for morphological and molecular studies.

Morphological observations

Specimens for conventional morphological examination were air-dried at room temperature and mounted to pinned triangular card paper. They were then photographed with a Leica M205C microscope system equipped with Leica DMC6200 digital camera (Leica Inc.,Wetzlar, Germany) attached to a computer. The illustration was made using the Procreate application on an iPad Air 3, utilizing an Apple Pencil and based on a magnified photograph of the tarsal claw.

The terminology used to describe the morphology of specimens follows that used in other studies on gall wasps (Harris 1979; Ronquist and Nordlander 1989; Ronquist 1995; Melika 2006) as follows: abbreviations: F1–F13 = 1st and subsequent flagellomeres; post-ocellar distance (POL) = distance between the posterior ocelli; ocellar-ocular

distance (OOL) = distance from the outer margin of a posterior ocellus to the inner margin of the compound eye; lateral-frontal ocelli distance (LOL) = distance between the lateral and frontal ocellus. The width of the radial cell of the forewing was measured from the margin of the wing to the Rs vein.

Type specimens are housed in the Insect Collection of the Central South University of Forestry and Technology (CSUFT), Changsha city, Hunan province, China.

DNA extraction and sequencing

Before DNA extraction, specimens were washed in sterile water to avoid surface contamination. Total DNA was then extracted using SDS/proteinase K digestion and phenol-chloroform extraction. The extracted DNA pellets were air-dried, resuspended in 20 μL sterile water, and stored at 4 °C for PCR and sequencing. Insect universal primers designed by Folmer et al. (1994), Simon et al. (1994), Schwéger et al. (2015), and Tavakoli et al. (2019) (Suppl. material 1) were used to amplify partial fragments of the mitochondrial *rrnL*, *COI*, *Cytb*, and *28s-D2* genes. The PCR products were purified and sequenced using the Sanger method by Wuhan Icongene Co, Ltd (Wuhan, China). GDcox1F, GDcox1R, GDrrnLF, GDrrnLR, and GDcytbR were designed to amplify the remaining genome by long PCR (Suppl. material 2). The reaction mixture comprised: 0.4 μL APEX (AG, Dalian, China), 10 μL buffer mixture, 0.4 μL of each primer, and 0.5 μL of DNA; water was added to each reaction to a final volume of 20 μL. Amplification was conducted using a C1000 Touch Thermal Cycler (Bio-Rad, Hercules, CA, USA). The cycling conditions were as follows: 98 °C for 1 min, 34 cycles of 98 °C for 10 s, 55 °C for 30 s, and 68 °C for 10 min. Two amplification strategies were used to obtain the complete mitogenome sequence. First, PCR amplification was performed using four specific long PCR primer combinations: GDrrnLF/GDcox1F, GDrrnLR/GDcox1R, GDrrnLF/GDcox1R, and GDrrnLR/GDcox1F. However, only GDrnnLF/GDcox1F resulted in a desired outcome. A clear single band was obtained using the primer combination GDcytbR/GDcox1R. These PCR products were then purified and sequenced.

The primer walking method was used to determine the sequence for each long PCR product using an ABI 3730XL DNA sequencer (Applied Biosystems, Foster City, CA, USA) by Wuhan Icongene Co, Ltd. Long PCR fragments were sequenced directly with the PCR primers and internal primers (Suppl. material 3). Sequences were assembled using SeqMan Pro 7.1.0 (Burland, 2000), then checked and corrected manually. The same site with different nucleotides was used to check the original sequencing peak map or to resequence the products to determine the nucleotides of the site.

Genome annotation and analyses

The initial mitogenome annotations were conducted using MITOS on Galaxy (https://usegalaxy.org/root?tool_id=toolshed.g2.bx.psu.edu%2Frepos%2Fiuc%2Fmitos2%2Fmitos2%2F2.1.3%20galaxy0). PCGs were identified by ORFFinder in NCBI (www.ncbi.nlm.nih.gov). rRNA genes were confirmed by sequence comparison with

published mitochondrial rRNA sequences from *Synergus* sp. (Tang et al. 2019), and *Dryocosmus liui* Pang, Su & Zhu (Hymenoptera: Cynipidae) (Su et al. 2023). Control regions (CRs) were confirmed by the boundaries of *trnS2* and *trnC*. Codon usage and relative synonymous codon usage (RSCU) of 13 PCGs in the specimens were calculated using PhyloSuite v1.2.2. The RSCU figure was drawn using the ggplot2 package (Hadley 2009); a plugin of Rscript 3.4.4 (Zhong and Zhu 2022). The nucleotide composition and AT/GC skew were calculated using PhyloSuite.

Analyses of phylogenetic relationship and pairwise genetic distance

To assess the taxonomic position of *Synergus nanlingensis* within the genus *Synergus*, we incorporated *S. nanlingensis* into the clade of *Synergus* species from the Palearctic region as recovered by Lobato-Vila et al. (2022a). This clade was strongly supported as monophyletic. New species specificity and whether the morphological similarities reflected the phylogenetic relationship based on molecular data were determined using the method of Lobato-Vila et al. (2021). Specifically, the *COI*, *Cytb*, and *28S-D2* sequences of 31 Palearctic *Synergus* species and two additional species of other cynipid genera were used as outgroups (Suppl. material 4). Sequences were aligned using MAFFT (Katoh et al. 2002) and those from each gene (660 bp of *COI*, 450 bp of *Cytb*, and 574 bp of *28S-D2*) were concatenated in a single matrix (1684 bp) using PhyloSuite.

This concatenated matrix of molecular data sets was analyzed based on the model-based phylogenetic approaches Bayesian Inference (BI) and Maximum Likelihood (ML). To determine the best partitions and models, the data sets were also analyzed using ModelFinder (Kalyaanamoorthy et al. 2017). For BI analysis, four simultaneous Markov chains were run for 10 million generations, with tree sampling occurring every 1,000 generations, and a burn-in of 25% of the trees in MrBayes 3.2.7 (Huelsenbeck and Ronquist 2001). For ML analyses, a total of 10,000 bootstrap replicates were obtained with the auto model applied to all partitions in IQ-tree2.2.2.7 (Nguyen et al. 2015). The final tree was rooted using the outgroup.

Results

Morphology-based taxonomy

***Synergus nanlingensis* Wang & Zeng, 2023, sp. nov.**
https://zoobank.org/982D4466-0B8A-4F8B-BD6B-938871C8417B
Figs 1, 2

Holotype. Female, China, Guangdong Province, Shaoguan City, 24-09-2022, reared from galls collected in 1-9-2022, leg. Y. Zeng, L. Liu and Y. Duan. Paratypes: three females and 13 males, same as holotype, housed in CSUFT (the holotype and two male paratypes were dried and mounted, while the other paratypes were deposited in 99% ethanol in a freezer at –80 °C).

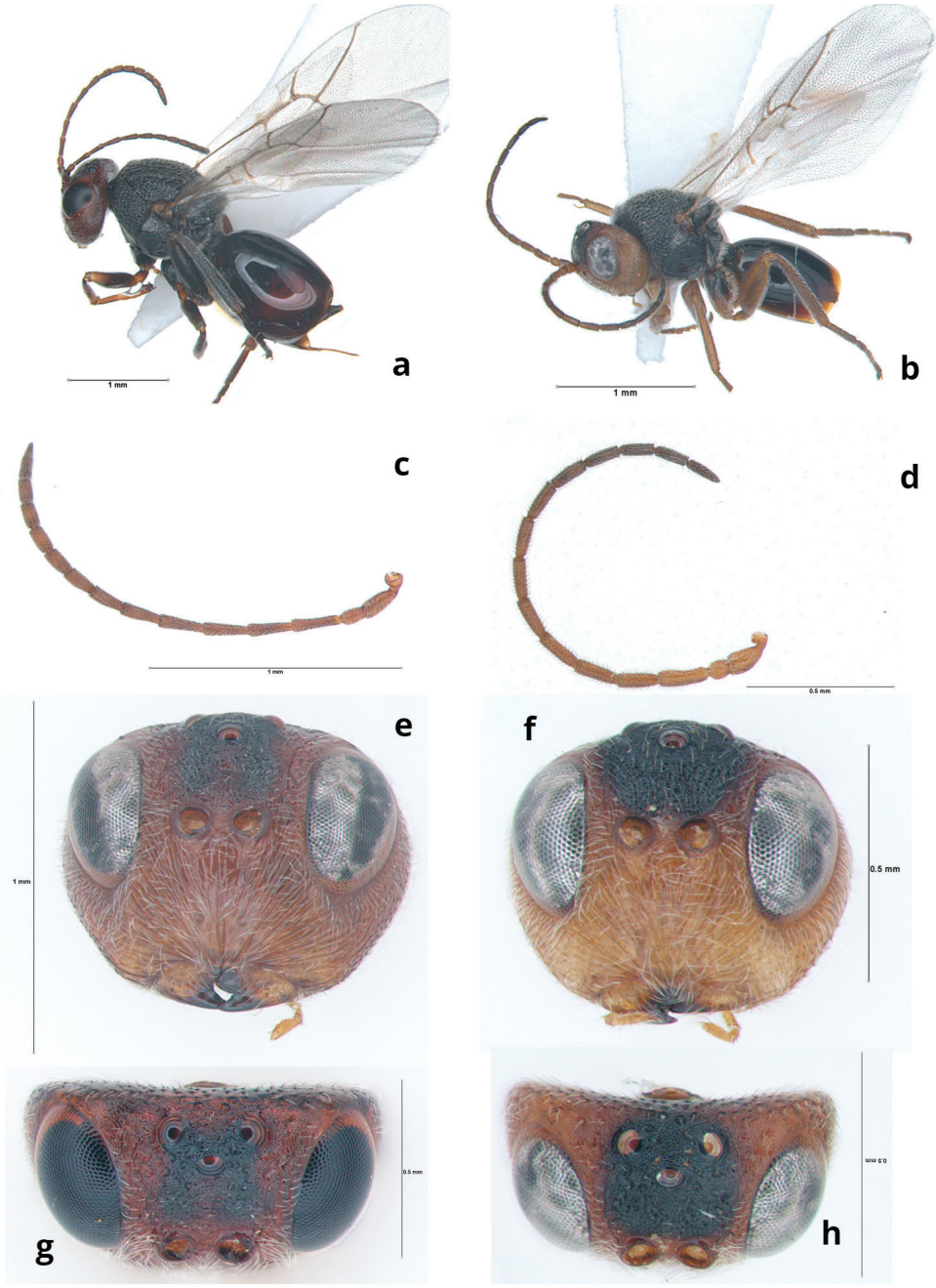

Figure 1. *Synergus nanlingensis* Wang & Zeng, 2023, sp. nov. **a** general habitus (♀) **b** general habitus (♂) **c** antenna (♀) **d** antenna (♂) **e** head in anterior view (♀) **f** head in anterior view (♂) **g** head in dorsal view (♀) **h** head in dorsal view (♂).

Diagnosis. *Synergus nanlingensis* Wang & Zeng, sp. nov., most closely resembles *Synergus hupingshanensis* (Liu, Yang & Zhu) is part of a group characterized by a completely opened radial cell, tarsal claws with a basal lobe and lateral pronotal carina present. However, it can be differentiated from *S. hupingshanensis* by the following morphological features: (1) The first flagellomere (F1) of *S. nanlingensis* is nearly equal in length to the second flagellomere (F2), whereas in *S. hupingshanensis*, F1 1.3× as long as F2; (2) the head of *S. nanlingensis* reddish brown with the frons and the center of the occiput being black, whereas head of *S. hupingshanensis* entirely orange without such black markings; and (3) scutellar foveae in *S. nanlingensis* are smooth and shiny at the bottom, whereas in *S. hupingshanensis* are roughly sculptured.

Description. Female; body length: 2.6–3.2 mm (N = 10).

Color (Figs 1a, 2c): head reddish brown, except frons, mandible teeth, and center of occiput black; antennae reddish brown. Mesosoma, legs, and metasoma black, with tarsus and distal part of body reddish brown. Wings hyaline with distinct brown veins.

Head (Figs 1e, g, 2a, c): transverse ellipse in front view (the widest of head near middle), 1.2× as wide as high, slightly broader than mesosoma in the anterior view, 1.2× wider than long as seen from above; frons slightly elevated from lateral view; lateral frontal carinae inconspicuous or absent, with rugose sculpture between the compound eye and frons; frons surface densely punctate with deep punctures and sparse setae (Fig. 1g). Eyes 1.6× as high as wide; height of eye 1.5× as high as length of malar space (Fig. 1e). Lower face densely setose, radiating from the clypeus toward basal margin of compound eye and antennal toruli. Gena broadened behind eyes, with punctures and white sparse setae. Middle of clypeus slightly impressed; anterior tentorial pit large and distinct; epistomal sulcus and clypeopleurostomal line indistinct; malar sulcus absent. Transfacial distance longer than the height of the compound eye; diameter of torulus shorter than the diameter of toruli and about half the distance between the inner margin of the eye and torulus (Fig. 1e). POL: OOL: LOL=2.2:1.8:1; LOL approximately as long as the diameter of the lateral ocellus. Ocelli ovate, all three similar in size (Fig. 1e). Occiput smooth; postgena with setae.

Antenna (Fig. 1c): 12 flagellomeres, pedicel 1.8× as long as broad, F1 longer than F2. F1–F12:14:13:13:13:11:10:9:8:8:7:7:10. Placoid sensillae distinct on F5–F12.

Mesosoma (Fig. 2d–f): 1.3× as long as high on the lateral view (Fig. 2d), with dense pubescence. Length of the middle part of pronotum is one-third that of the outer lateral margin; pronotum punctate, laterally areolate-rugulose, lateral carina distinct. Mesoscutum 1.4× as wide as long (measuring along the anterior edge of tegulae), surface areolate-rugose, center with a transverse rugae, covered with densely yellow setae. Notauli percurrent and distinct, somewhat convergent posteriorly; anterior parallel line, parapsidal line, and median mesoscutal line indistinct, barely traceable (Fig. 2e). Scutellar foveae elongate ovate, bottom smooth and shiny, deeply impressed, with short sparse white setae, separated by distinct central carina. Mesopleuron hairless, finely striated ventrally and carinate-rugose dorsally. Metapleural sulcus reaches posterior margin of mesopectus in the most upper 1/4 of its height (Fig. 2d). Propodeum smooth coriaceous, with short sparse white setae. Lateral propodeal carinae slightly impressed basally and slightly convergent distally (Fig. 2f).

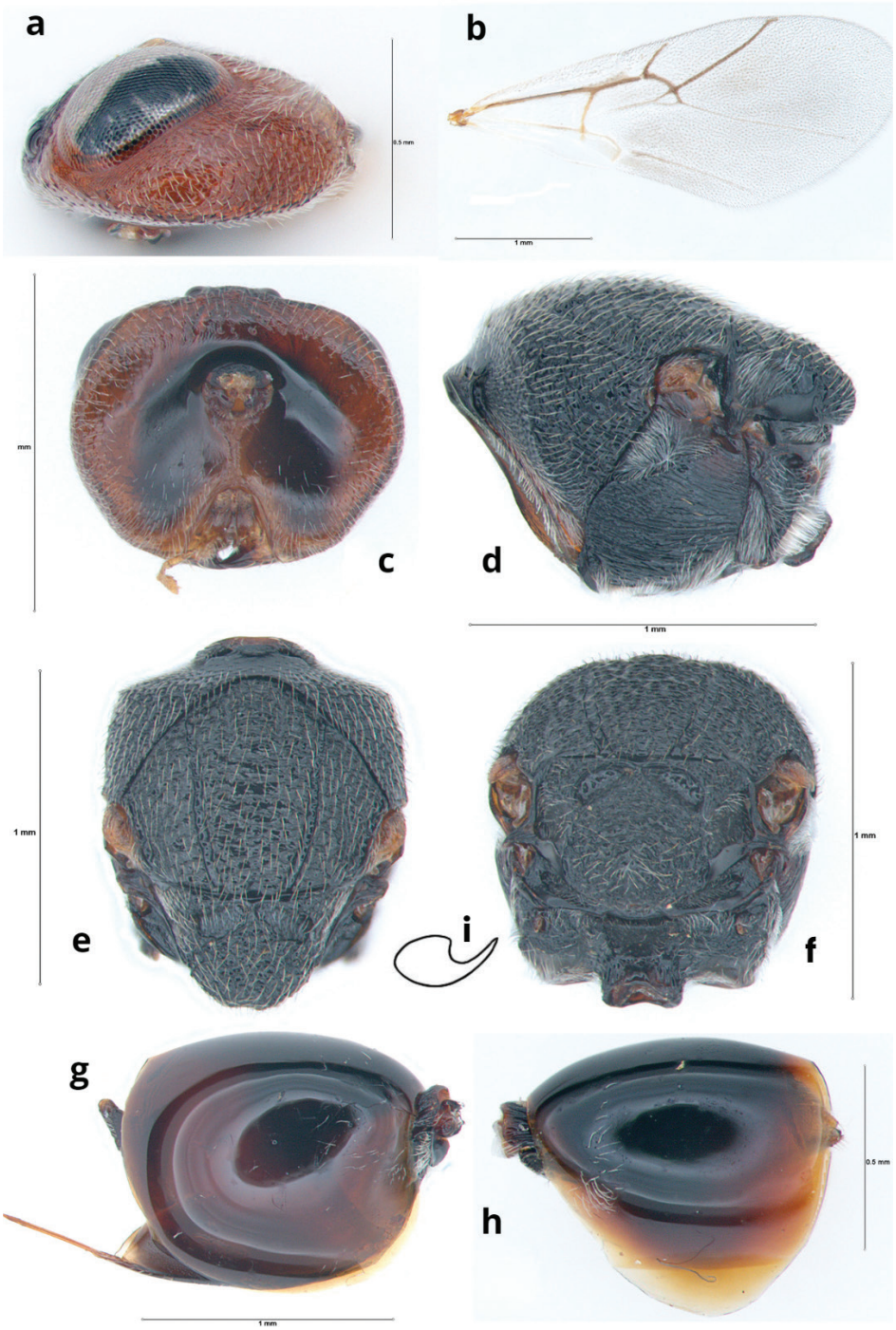

Figure 2. *Synergus nanlingensis* Wang & Zeng, 2023, sp. nov. **a** head in lateral view (♀) **b** fore wing (♀) **c** head in posterior view (♀) **d** mesosoma, lateral view (♀) **e** mesosoma, dorsal view (♀) **f** propodeum, dorsal view (♀) **g** metasoma, lateral view (♀) **h** metasoma, lateral view (♂) **i** tarsal claw.

Legs: Tarsal claws with a small basal lobe (Fig. 2i).

Forewing (Fig. 2b): hyaline and densely setose, approximately as long as body length. All veins well pigmented. Radial cell open, about 2.9× as long as broad; R1 does not reach wing margin; Rs curved toward to posterior distally.

Metasoma (Fig. 2g): slightly shorter than the head and mesosoma combined, and 1.2× as long as high; petiole sulcate; syntergite almost completely covering remaining tergites, surface smooth and mainly glabrous, with few white setae anterolaterally, and a posterodorsal area without setae and micropunctures. Subsequent tergites and hypopygium micropunctate; prominent part of ventral spine of hypopygium small, with few lateral setae.

Male (Figs 1b, d, f, g, 2h): similar to the female, but body length 1.9–2.2 mm ($N = 6$); head, legs, and distal part of abdomen yellowish brown; frons, mandible teeth, mesosoma, basal part of abdomen, and hind coax black.

***Antenna*:** 13 flagellomeres, pedicel 1.4 times as long as broad. F1–F13: 16:13:14 :14:14:13:13:13:12:11:10: 11. Metasoma elongated, shorter than the head and mesosoma combined.

Biology. Specimens of *S. nanlingensis* were collected from galls found on branches of *Castanopsis eyrei* on the summit of Xiaohuang Mountain 1,600 m above sea level. Galls are nearly spherical in shape, range in diameter from 15 to 35 mm, and are hard and strongly lignified (Fig. 3). Galls appear in July and inquilines emerged from late September to October. The gall inducer of the gall which yielded *S. nanlingensis* is unconfirmed.

Distribution. Shaoguan City, Guangdong Province, China.

Etymology. The specific epithet refers to the type locality.

Genome organization and base composition

The total length of the complete mitogenome of *S. nanlingensis* is 16,604 bp (GenBank accession OR978581). The mitochondrial genome contains the typical gene repertoire of 13 PCGs, two rRNA genes, and 22 tRNA genes (Fig. 4). There are eight overlapping regions, ranging in size from 1 to 7 bp. The mitogenome contains 20 intergenic spacers, with lengths ranging from 1 to 336 bp. The longest gene spacer is between *Cox2* and *trnD* (Table 1). The nucleotide content of the *S. nanlingensis* mitogenome is as follows: 44.2% A, 5.6% G, 42% T, and 8.2% C; the total A + T percentage is 86.2%,.AT skew is 0.026 and GC skew is –0.191, which is consistent with that in other Hymenoptera (Wei et al. 2010; Chen et al. 2018).

Protein-coding genes and codon usage

The total length of the 13 PCGs of *S. nanlingensis* is 11,037 bp. Five PCGs (*nad1*, *nad2*, *nad4L*, *nad4*, and *nad5*) are encoded by the minority strand (N-strand), and the other eight genes are encoded by the majority strand (J-strand) (Table 1). The overall A + T content in PCGs is 84.7%, ranging from 77.4% (*cox1*) to 91.8% (*nad6*). The AT skew of the PCGs is –0.102, and the GC skew is 0.03. A very high A + T content (94.9%) is found at the third codon of PCGs.

Figure 3. Gall of *Synergus nanlingensis* Wang & Zeng, 2023, sp. nov. on *Castanopsis eyrei* Tutch.

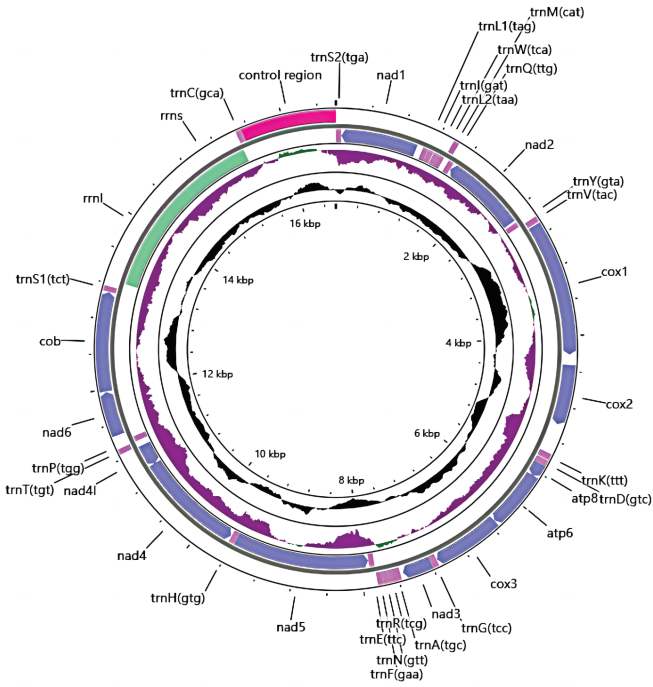

Figure 4. Mitochondrial genome of *Synergus nanlingensis* Wang & Zeng, 2023, sp. nov. sequenced in this study. Genes outside the circle are encoded by the majority strand, and genes inside are encoded by the minority strand. The tRNA genes are indicated by their one-letter corresponding amino acids. The GC content is plotted using a black sliding window. Abbreviations: atp6 and atp8, ATP-synthase subunits 6 and 8; cob, cytochrome b; cox1–3, cytochrome oxidase subunits 1–3; nad1–6 and nad4L, NADH dehydrogenase subunits 1–6 and 4 L; rrnL and rrnS, large and small rRNA subunits.

Table 1. Annotation of the *Synergus nanlingensis* Wang & Zeng, 2023, sp. nov. mitochondrial genome.

Gene	Positions	Size	Strand	Nucleotides Intergenic	Anti or Start codon	Stop codon	A+T(%)
trnS2	1–68	68	–	-2	TGA		89.7
nad1	67–1005	939	–	73	ATT	TAG	85
trnL1	1079–1144	66	–	1	TAG		92.4
trnI	1146–1215	70	–	5	GAT		85.7
trnL2	1221–1291	71	–	-1	TAA		90.1
trnW	1291–1358	68	–	3	TCA		91.2
trnM	1362–1427	66	+	-5	CAT		89.4
trnQ	1423–1491	69	–	2	TTG		87
nad2	1494–2501	1008	–	24	ATT	TAA	91.7
trnY	2526–2592	67	–	4	GTA		86.6
trnV	2597–2664	68	+	12	TAC		94.1
cox1	2677–4212	1536	+	117	ATT	TAA	77.4
cox2	4330–5016	687	+	336	ATA	TAA	83.9
trnK	5353–5424	72	+	6	TTT		87.5
trnD	5431–5503	72	+	0	GTC		94.5
atp8	5504–5665	161	+	-6	ATT	TAA	87.7
atp6	5659–6333	675	+	1	ATG	TAA	83.7
cox3	6335–7122	788	+	3	ATG	TA	80.5
trnG	7126–7198	73	+	0	TCC		94.5
nad3	7199–7534	336	+	31	ATT	TAA	87.8
trnA	7566–7636	71	+	-3	TGC		87.3
trnR	7634–7703	70	+	0	TCG		88.6
trnN	7704–7771	68	+	1	GTT		83.8
trnF	7773–7836	64	+	-2	GAA		92.2
trnE	7835–7901	67	–	0	TTC		97
nad5	7902–9572	1671	–	0	ATT	TAA	87.4
trnH	9573–9646	74	–	7	GTG		89.2
nad4	9654–10967	1314	–	-7	ATG	TAA	85.5
nad4L	10961–11236	276	–	12	ATT	TAA	90.6
trnT	11249–11312	64	+	-1	TGT		92.2
trnP	11312–11378	67	–	81	TGG		88.1
nad6	11460–11969	510	+	3	ATT	TAA	91.8
cytb	11973–13109	1137	+	0	ATG	TAA	80.9
trnS1	13110–13171	62	+	76	TCT		87.1
rrnL	13248–14628	1381	–	0			88.8
rrnS	14629–15461	833	–	0			90.1
trnC	15462–15530	69	+	0	GCA		91.3
CR	15531–16604	1066					84.6

Notes: + indicates the gene is coded on majority strand while – indicates the gene is coded on minority strand.

In *S. nanlingensis*, eight genes (*cox1*, *nad1*, *nad2*, *nad3*, *nad4L*, *nad5*, *nad6*, *atp8*) are initiated with ATT, four genes (*atp6*, *nad4*, *cob*, and *cox3*) with ATG, and *Cox2* initiated with ATA. All PCGs use ATN as the starting codon, similar to that reported for other Hymenoptera (Tang et al. 2019). Most PCGs from *S. nanlingensis* terminate with stop codons TAA, whereas *Cox3* ends with TA and *nad1* with TAG.

The relative synonymous codon usage in all 13 PCGs is shown in Fig. 5. As reported for previously studied Cynipidae species, the most common amino acids are leucine (Leu2) and serine (Ser2). The least common codons are CUG-Leu1 and CUC-Leu1.

tRNA and rRNA

In total, 22 tRNA genes were identified in the mitogenome of *S. nanlingensis*, ranging in size from 62 bp to 74 bp and accounting for 1,507 bp in total concatenated length (Table 1). Of the tRNA genes, 12 are located on the H-strand whereas ten tRNA genes are located on the L-strand. Of these tRNA genes, 21 can be folded into a conventional cloverleaf secondary structure, whereas *trnS1* lack the dihydrouridine arm (D-arm). This feature has also been reported for *Andricus mairei* (Kieffer) (Zhong and Zhu 2022). The lack of the D-arm in trnS1 is a common feature of most metazoans (Kahnt et al. 2015; Du et al. 2017). In the mitochondrial tRNA secondary structures of *S. nanlingensis*, seven mismatched base pairs were detected: five G-U pairs, one G-A pair, and one A-A pair. As reported for other Cynipoidea species (Mao et al. 2015; Tang et al. 2019; Xue et al. 2020; Su et al. 2023), *rrnL* and *rrnS* are next to each other and both are located in the L-strand in *S. nanlingensis*, with lengths of 1,381 bp and 833 bp, respectively.

Noncoding sequences (CR)

A large CR was detected in *S. nanlingensis* mitogenome, located between *trnC* and *trnS2*. The CR is 1073 bp in length and its AT content was 84.6%. It has three 166-bp non-tandem repeat units, one 36-bp A + T-rich region (AT% = 94.3%) and one 32-bp A + T-rich region (AT% = 90.6%) (Fig. 6).

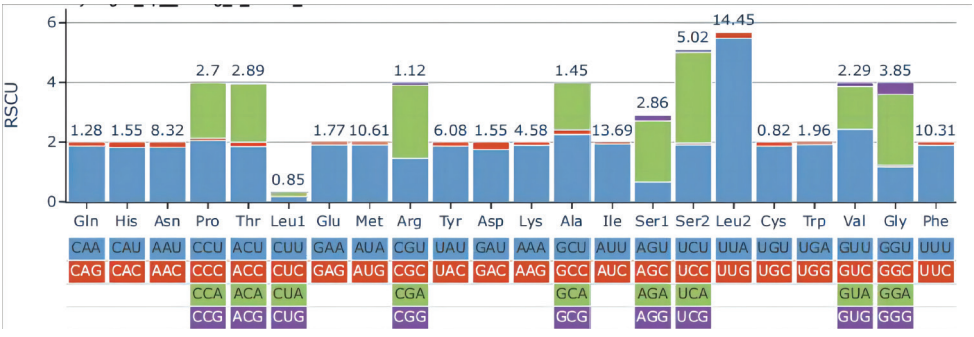

Figure 5. Relative synonymous codon usage (RSCU) of *Synergus nanlingensis* Wang & Zeng, 2023, sp. nov. mitochondrial genome. Codon families are labeled on the x-axis. Values on the top of the bars indicate the percentage of each amino acid used for the construction of 13 protein-coding genes (PCGs).

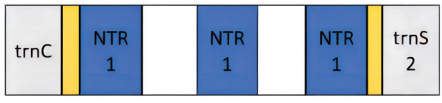

Figure 6. Structures of control regions in the mitogenome of *Synergus nanlingensis* Wang & Zeng, 2023, sp. nov. Abbreviation: NTR, nontandem repeat. Yellow shows A + T-rich regions.

Gene arrangements

Compared with the ancestral mitogenome arrangement, rearrangements of *S. nanlingensis* mitogenome involve tRNA genes, rRNA genes, and PCGs. Su et al. (2023) compared the reported mitochondrial gene rearrangements of gall wasps and found four rearrangement events: *trnE* and *trnF* had inverted and swapped positions; *rrnL* and *rrnS* moved into the *cob–nad1* junction; a novel tRNA gene cluster *trnL1–trnI–trnL2–trnW–trnM–trnQ* was formed between *nad1* and *nad2*; and *trnV* was inverted and moved to the *nad2–cox1* junction. These four rearrangements are also found in *S. nanlingensis*. However, unlike gall wasps with two CRs (Xue et al. 2020; Pang et al. 2022; Zhong and Zhu 2022; Su et al. 2023), mitochondrial genes of *S. nanlingensis* have the following differences: *trnS1* is downstream of *Cytb*; *trnS2* is upstream of *nad1*; and *trnC* is downstream of *rrnS* (Fig. 7).

DNA taxonomy and phylogenetic relationship

The genetic distance between *Synergus nanlingensis* and other *Synergus* species is long (Suppl. material 5). The topology of our phylogenetic tree mostly coincides with that recovered by Lobato-Vila et al. (2022a) (Fig. 8) and supports *S. nanlingensis* as a distinct species, clustering it with other Palearctic *Synergus.* Different analytical approaches (BI and ML) did not affect the topology but did affect the level of node support (Suppl. material 7). *Synergus nanlingensis* is recovered as a sister species of *Synergus itoensis* Abe, Ide & Wachi, although this relationship is not highly supported.

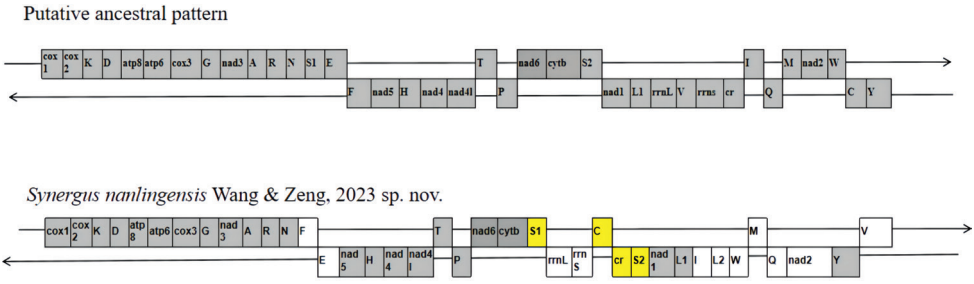

Figure 7. Mitochondrial genome organization and gene rearrangement in *Synergus nanlingensis* Wang & Zeng, 2023, sp. nov. compared with the ancestral type of the insect mitochondrial genome. All abbreviations are the same as in Table 1 in the main text. Arrow pointing to the right represents the J-strand and arrow pointing to the left represents the N-strand. Genes are drawn in their original order; intergenic distances are not included, and sizes of genes are not to scale. Yellow boxes indicate genes with different positions from two control regions reported in Cynipidae. and white boxes indicate genes that are different in terms of both position and strand associations from the putative ancestral pattern. Gray boxes show conserved gene blocks.

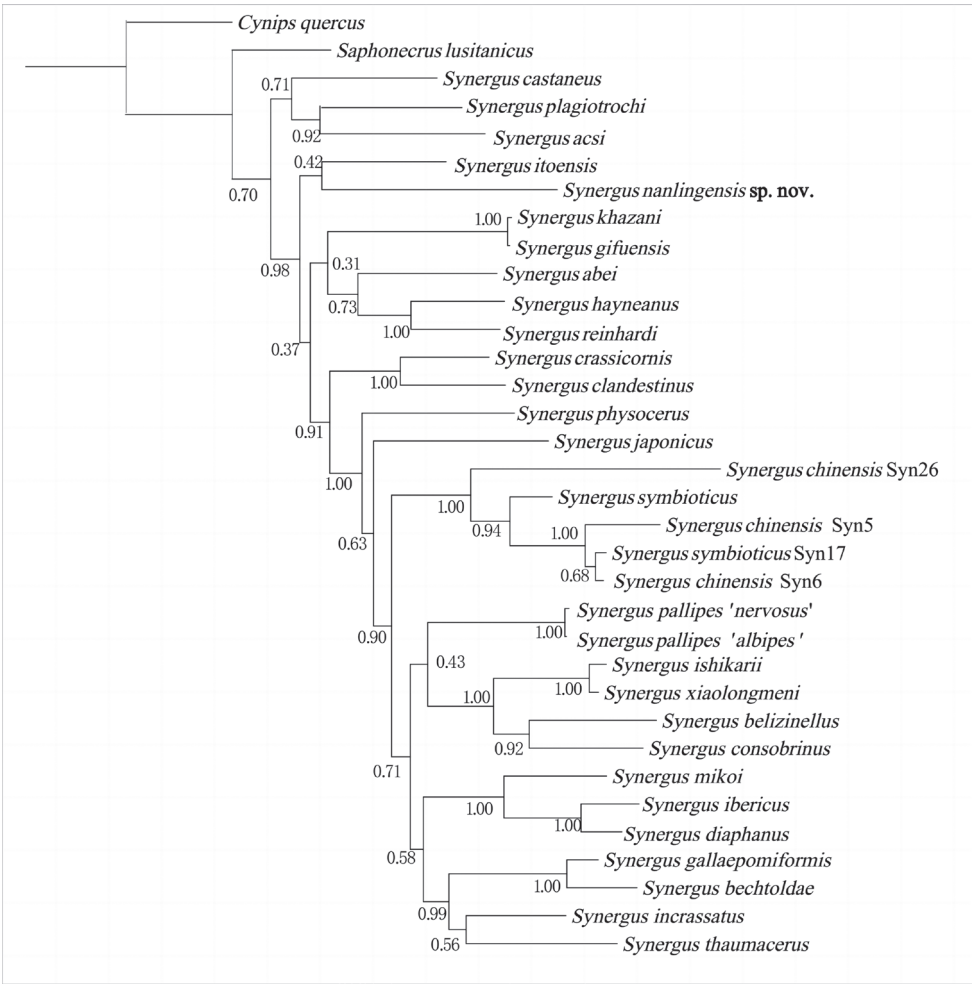

Figure 8. Bayesian analysis of the MAFFT alignment data set inferred from the *COI* + *Cytb* + *28S-D2* data sets. Posterior probabilities are shown at each node.

Discussion

The discovery of *Synergus nanlingensis*, a new species found in China, marks a significant contribution to the biodiversity of the family Cynipidae, especially among inquilines. Currently, little is known about the gall wasp species associated with *Castanopsis*, with only two *Synergus* species, *S. hupingshanensis* and *S. kawakamii* (Tang & Melika), reported so far (Schwéger et al. 2015; Lobato-vila et al. 2022a). Given the rich diversity of species and endemics within *Castanopsis* and Fagaceae in China (Xu et al. 2022), further research in this region is expected to uncover more new species associated with these hosts.

Phylogenetic tree analysis robustly confirms the status of *S. nanlingensis* as a member of the genus *Synergus*. Although an open radial cell in the forewing is not a typical characteristic of *Synergus*, the species was confirmed as a member based on the presence of the female antenna with 12 flagellomeres, the complete notaulus, and presence of an incomplete lateral frontal carina (Schweger et al. 2015); placing it within a group characterized by a fully open radial cell, basally lobed claws, and the presence of a lateral thoracic carina (Lobato-Vila et al. 2020). Interestingly, no Cynipini were reared from the same galls as *S. nanlingensis*, despite three years of collections made in different months. This phenomenon was also observed in the breeding records of *S. hupingshanensis*, where no expected gall-inducers were reared from two years of field collections (Liu et al. 2012). The reasons for the lack of expected gall inducers for these two species are still unknown and warrant further experimental investigation to determine whether or not they are inducers or inquilines as its currently known sister species *S. itoensis* is one of the few known rare cases of Synergus that have secondarily reverted back to gall induction (Abe and Wachi 2011). Gobbo et al. (2020) have compared the genome of *S. itoensis* with that of three other related *Synergus* inquilines, and found that there were distinct genetic differences between gall inducers and inquilines. Therefore, further study on the mitogenome or genome comparison between Chinese *Synergus* and known gall inducers and inquilines will provide molecular evidence for speculating whether they have gall inducing ability.

This study presents the first complete mitochondrial genome reported for a species of *Synergus.* In the mitochondrial genome of S. *nanlingensis*, general characteristics and typical rearrangement events of Cynipidae species were observed (Zhong and Zhu 2022; Su et al. 2023), but some differences were also noted. For instance, a long intergenic spacer of 366 bp between Cox2 and trnD was observed in *S. nanlingensis*. Such long intergenic spacers have also been found in the mitochondrial genomes of other Hymenoptera insects, possibly as a result of gene rearrangement (Chen et al. 2018; Zhong and Zhu 2022). While TAA is commonly used as a stop codon in arthropod mitogenomic PCGs, variations such as TA, a single T, and more uniquely, TAG, have been observed (Yamauchi et al. 2002). In *S. nanlingensis*, Cox3 ends with TA and nad1 with TAG, aligning with stop codon usage in two previously known Synergini mitogenomes (Tang et al. 2019; Shu et al. 2022). Prior research identified two control regions (CR1 and CR2) in Cynipoidea, with CR2 being a partially inverted repeat of CR1 (Mao et al. 2015; Zhong and Zhu 2022; Su et al. 2023). This led to speculation that inverted, duplicated CRs might be characteristic of the Cynipoidea mitochondrial genome. However, only one control region was found in the *S. nanlingensis* mitogenome, consistent with the two known mitogenomes of Synergini. Remarkably, this study provides a complete sequencing of the control region, which were never described before. Whether these features and gene rearrangement serve as distinguishing characteristics within Synergini requires further data support.

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References

Abe Y (2007) Parallelism in secondary loss of sex from a heterogonic life cycle on different host plants in the *Andricus mukaigawae* complex (Hymenoptera: Cynipidae), with taxonomic notes. Journal of Natural History 41(8): 473–480. https://doi.org/10.1080/00222930701192122

Abe Y, Ide T, Wachi N (2011) Discovery of a new gall-inducing species in the inquiline tribe Synergini (Hymenoptera: Cynipidae): inconsistent implications from biology and morphology. Annals of the Entomological Society of America 104: 115–120. https://doi.org/10.1603/AN10149

Bozsó M, Tang CT, Pénzes Z, Yang, MM, Bihari P, Pujade-Villar J, Schwéger S, Melika G (2015) A new genus of cynipid inquiline, *Lithosaphonecrus* Tang, Melika & Bozsó (Hymenoptera: Cynipidae: Synergini), with description of four new species from Taiwan and China. Insect Systematics & Evolution 46(1): 79–114. https://doi.org/10.11646/zootaxa.5060.1.6

Burland TG (2000) DNASTAR's Lasergene sequence analysis software. Methods in Molecular Biology 132: 71–91. https://doi.org/10.1385/1-59259-192-2:71

Cameron SL (2014) Insect mitochondrial genomics: Implications for evolution and phylogeny. Annual Review of Entomology 59: 95–117. https://doi.org/10.1146/annurev-ento-011613-162007

Chen L, Chen PY, Xue PY, Hua HQ, Li YX, Zhang F, Wei SJ (2018) Extensive gene rearrangements in the mitochondrial genomes of two egg parasitoids, *Trichogramma japonicum* and *Trichogramma ostriniae* (Hymenoptera:Chalcidoidea:Trichogrammatidae). Scientific Reports 8(1): 7034. https://doi.org/10.1038/s41598-018-25338-3

Chiara F, Ester F, Marianna P, Matteo AS, Alberto A (2018) Effectiveness of *Torymus sinensis*: a successful long-term control of the Asian chestnut gall wasp in Italy. Journal of Pest Science 92: 353–359. https://doi.org/10.1007/s10340-018-0989-6

Csóka G, Stone GN, Melika G (2005) Biology, ecology and evolution of gall inducing Cynipidae. In: Raman A, Schaefer CW, Withers TM (Eds) Biology, ecology and evolution of gall-inducing arthropods. Science Publishers, Inc. Enfield, New Hampshire, 569–636.

Duffet GH (1968) Some new interrelationships of Hymenoptera over-wintering within the galls of *Andricus kollari* (Hartig). The Entomologist's Monthly Magazine 105: 1259–1261.

Du YM, Dai W, Dietrich CH (2017) Mitochondrial genomic variation and phylogenetic relationships of three groups in the genus *Scaphoideus* (Hemiptera: Cicadellidae: Deltocephalinae). Scientific Reports 7: 16908. https://doi.org/10.1038/s41598-017-17145-z

Feng Z, Wu Y, Yang C, Gu X, Wilson JJ, Li H, Cai W, Yang H, Song F (2020) Evolution of tRNA gene rearrangement in the mitochondrial genome of ichneumonoid wasps (Hymenoptera, Ichneumonoidea). International Journal of Biological Macromolecules 164: 540–547. https://doi.org/10.1016/j.ijbiomac.2020.07.149

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochromec oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3(5): 294–299.

Guo R, Wu BM, Zhang WL, Wang YQ, Wang YP (2013) First discovery of an invasive gall-former insect pest *Diplolepis rosae*, in China. Chinese Journal of Applied Entomology 50(2): 500–504. [in Chinese] https://doi.org/10.7679/j.issn.2095-1353.2013.069

Gobbo E, Lartillot N, Hearn J, Stone GN, Abe Y, Wheat CW, Ide T, Ronquist F (2020) From inquilines to gall inducers: Genomic signature of a life-style transition in synergus gall wasps. Genome Biology and Evolution 12(11): 2060–2073. https://doi.org/10.1093/gbe/evaa204

Hadley W (2009) ggplot2, elegant graphics for data analysis. Springer, New York, America. https://doi.org/10.1111/j.1467-985X.2010.00676_9.x

Harris R (1979) A glossary of surface sculpturing. State of California, Department of Food and Agriculture. Occasional Papers in Entomology 28: 1–31.

Heraty J, Ronquist F, Carpenter JM, Hawks D, Schulmeister S, Dowling AP, Murray D, Munro J, Wheeler WC, Schiff N, Sharkey M (2011) Evolution of the hymenopteran megaradiation. Molecular Phylogenetics and Evolution 60:73–88. https://doi.org/10.1016/j.ympev.2011.04.003

Huang C, Chang Y, Hsu Y, Jen H (1999) Fagaceae. In: Chun WY, Huang CC (Eds) Fl. Reipubl. Popularis Sin, 332 pp.

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17(8): 764–755. https://doi.org/10.1093/bioinformatics/17.8.754

Kahnt B, Gerth M, Paxton RJ, Bleidorn C, Husemann M (2015) The complete mitochondrial genome of the endemic and highly specialized South African bee Species *Rediviva intermixta* (Hymenoptera: Melittidae), with a comparison with other bee mitogenomes. Biological Journal of The Linnean Society 116(4): 940–953. https://doi.org/10.1111/BIJ.12627

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods 14: 587–589. https://doi.org/10.1038/nmeth.4285

Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30(14): 3059–3066. https://doi.org/10.1093/nar/gkf436

Liu Z, Yang XH, Zhu DH, He YY (2012) A new species of *Saphonecrus* (Hymenoptera, Cynipoidea) associated with plant galls on *Castanopsis* (Fagaceae) in China. Annals of the Entomological Society of America 105(4): 555–561. https://doi.org/10.1603/AN12021

Lobato-Vila I, Caicedo G, Rodríguez PA, Pujade-Villar J (2020) The inquiline oak gall wasp (Hymenoptera: Cynipidae) fauna from Colombia: new data and species. The Canadian Entomologist 152(2): 131–144. https://doi.org/10.4039/tce.2019.77

Lobato-Vila I, Wang YP, Melika G, Guo R, Ju XX, Pujade-Villar J (2021) A review of the species in the genus *Synergus* Hartig (Hymenoptera: Cynipidae: Synergini) from mainland

China, with an updated key to the Eastern Palaearctic and Oriental species. Journal of Asia-Paific Entomology 24(1): 341–362. https://doi.org/10.1016/j.aspen.2020.11.014

Lobato-Vila I, Bae J, Roca-Cusachs M, Kang M, Jung S, Melika G, Pénzes Z, Pujade-Villar J (2022a) Global phylogeny of the inquilinous gall wasp tribe Synergini (Hymenoptera: Cynipoidea: Cynipidae): first insights and establishment of a new cynipid tribe. Zoological Journal of the Linnean Society 195(4): 1338–1354. https://doi.org/10.1093/zoolinnean/zlab085

Lobato-Vila I, Sala-Nishikawa A, Melika G, Stone GN, Tang CT, Yang MM, Fang ZQ, Zhu Y, Wang YP, Jung S, Nicholls JA, Pujade-Villar J (2022b) A catalogue, revision, and regional perspective of Eastern Palaearctic and Oriental oak gall wasps and their inquilines (Hymenoptera: Cynipidae: Cynipini, Synergini, Ceroptresini). Zootaxa 5161: 1–71. https://doi.org/10.11646/zootaxa.5161.1.1

Mao M, Gibson T, Dowton M (2015) Higher-level phylogeny of the Hymenoptera inferred from mitochondrial genomes. Molecular Phylogenetics and Evolution 84: 34–43. https://doi.org/10.1016/j.ympev.2014.12.009

Melika G (2006) Gall wasps of Ukraine, Cynipidae. Vestnik zoologii (Supplement) 21(1): 300.

Melika G, Abrahamson WG (2002) Review of the world genera of oak cynipid wasps (Hymenoptera: Cynipidae: Cynipini). In: Melika G, Thuróczy C (Eds) Parasitic Wasps: Evolution, Systematics, Biodiversity and Biological Control. Agroinform, Budapest, 150–190.

Moriya S, Shiga M, Adachi I (2003) Classical biological control of the chestnut gall wasp in Japan. In: Van Driesche RG (Eds) Proceedings of the 1st international symposium on biological control of arthropods, Hawaii, 14–18 January 2002. USDA Forest Service, Washington, DC, USA, 407–415.

Mozhaitseva K, Tourrain Z, Branca A (2023) Population genomics of the mostly thelytokous *Diplolepis rosae* (Linnaeus, 1758) (Hymenoptera: Cynipidae) reveals population-specific selection for sex. Genome Biology and Evolution 15(10): evad185. https://doi.org/10.1093/gbe/evad185

Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32(1): 268–274. https://doi.org/10.1093/molbev/msu300

Pang Y, Su CY, Zhu JQ, Yang XH, Zhong JL, Zhu DH, Liu ZW (2022) A new species of *Andricus* Hartig, 1840 (Hymenoptera, Cynipidae) from China, with references to DNA taxonomy and Wolbachia infection. ZooKeys 1134: 52–73. https://doi.org/10.3897/zookeys.1134.89267

Pénzes Z, Tang P, Bihari P, Bozsó M, Schwéger S, Melika G (2012) Oak associated inquilines (Hymenoptera, Cynipidae, Synergini). TISCIA Mono 11: 1–76.

Pénzes Z, Melika G, Bozsóki Z, Bihari P, Mikó I, Tavakoli M, Pujade-Villar J, Fehér B, Fülöp D, Szabó K, Bozsó M, Sipos B, Somogyi K, Stone GN (2009) Systematic re-appraisal of the gall-usurping wasp genus *Synophrus* Hartig, 1843 (Hymenoptera: Cynipidae: Synergini). Systematic Entomology 34(4): 688–711. https://doi.org/10.1111/j.1365-3113.2009.00482.x

Pujade-Villar J, Wang YP, Chen T, Shen J, Ferrer-Suay M (2017) Description of a new *Synergus* species from China and comments on other inquiline species (Hymenoptera: Cynipidae: Synergini). Zootaxa 4341(1): 56–66. https://doi.org/10.11646/zootaxa.4341.1.4

Ronquist F (1995) Phylogeny and early evolution of the Cynipoidea (Hymenoptera). Systematic Entomology 20: 309–335. https://doi.org/10.1111/j.1365-3113.1995.tb00099.x

Ronquist F, Nieves-Aldrey JL, Buffington ML, Liu Z, Liljeblad J, Nylander JA (2015) Phylogeny, evolution and classification of gall wasps: the plot thickens. PLOS ONE 10(5): 1–40. https://doi.org/10.1371/journal.pone.0123301

Ronquist F, Nordlander G (1989) Skeletal morphology of an archaic cynipoid, *Ibalia rufipes* (Hymenoptera: Ibaliidae). Entomologica Scandinavica 19(4): 1–60. https://doi.org/10.1002/arch.940100408

Schwarz O (1993) *Quercus* L. In: Tutin TG, Heywood VH, Burges NA, Valentine DH, Walters SM, Webb DA (Eds) Flora Europaea, Vol. 1: Lycopodiaceae to Platanaceae. Cambridge University Press, Cambridge, 72–76.

Schwéger S, Melika G, Tang CT, Bihari P, Bozsó M, Stone GS, Nicholls JA, Pénzes Z (2015) New species of cynipid inquilines of the genus *Synergus* (Hymenoptera: Cynipidae: Synergini) from the Eastern Palaearctic. Zootaxa 3999(4): 451–497. https://doi.org/10.11646/zootaxa.3999.4.1

Shu X, Li Z, Yuan R, Tang P, Chen X (2022) Share novel gene rearrangements in the mitochondrial genomes of cynipoid wasps (Hymenoptera: Cynipoidea). Genes (Basel) 13(5): 914. https://doi.org/10.3390/genes13050914

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America 87: 651–701. https://doi.org/10.1093/aesa/87.6.651

Simon C, Buckley TR, Frati F, Stewart JB, Beckenbach AT (2006) Incorporating molecular evolution into phylogenetic analysis, and a new compilation of conserved polymerase chain reaction primers for animal mitochondrial DNA. Annual Review of Ecology, Evolution, and Systematics 37(1): 545–579. https://doi.org/10.1146/annurev.ecolsys.37.091305.110018

Su CY, Zhu DH, Abe Y, Ide T, Liu ZW (2023) The complete mitochondrial genome and gene rearrangements in a gall wasp species, *Dryocosmus liui* (Hymenoptera: Cynipoidea: Cynipidae). Peer J 11: e15865. https://doi.org/10.7717/peerj.15865

Tang P, Zhu JC, Zheng BY, Wei SJ, Michael S, Chen X.X, Alfried P (2019) Mitochondrial phylogenomics of the Hymenoptera. Molecular Phylogenetics and Evolution 131(1): 8–18. https://doi.org/10.1016/j.ympev.2018.10.040

Tavakoli M, Khaghaninia S, Melika G, Stone GN, Hosseini-Chegeni (2019) Molecular identification of *Andricus* species (Hymenoptera: Cynipidae) inducing various oak galls in Central Zagros of Iran., Mitochondrial DNA Part A 30(5): 713–720. https://doi.org/10.1080/24701394.2019.1622693

Tutin TG (1993) *Castanea* Miller. In: Tutin TG, Heywood VH, Burges NA, Valentine DH, Walters SM, Webb DA (Eds) Flora Europaea, vol. 1: Lycopodiaceae to Platanaceae. - Cambridge University Press, Cambridge, 72 pp.

Tutin TG, Akeroyd JR (1993) *Fagus* L. In: Tutin TG, Heywood VH, Burges NA, Valentine DH, Walters SM, Webb DA (Eds) Flora Europaea, Vol. 1: Lycopodiaceae to Platanaceae. - Cambridge University Press, Cambridge, 72 pp.

Wei SJ, Li Q, van Achterberg K, Chen XX (2014) Two mitochondrial genomes from the families Bethylidae and Mutillidae: Independent rearrangement of protein-coding genes and

higher-level phylogeny of the Hymenoptera. Molecular Phylogenetics and Evolution 77: 1–10. https://doi.org/10.1016/j.ympev.2014.03.023

Wei SJ, Shi M, Sharkey MJ, van Achterberg C, Chen XX (2010) Comparative mitogenomics of Braconidae (Insecta, Hymenoptera) and the phylogenetic utility of mitochondrial genomes with special reference to holometabolous insects. BMC Genomics 11: 371. https://doi.org/10.1186/1471-2164-11-371

Xu W, Yang T, Li ZH, Zhou P (2022) Distribution pattern of plant community species diversity along altitudinal gradients in Nanling mountains, Guangdong. Forestry and Environmental Science 38(1): 9–17. [in Chinese]

Xue S, Zhang Y, Gao S, Lu S, Wang J, Zhang K (2020) Mitochondrial genome of *Trichagalma acutissimae* (Hymenoptera: Cynipoidea: Cynipidae) and phylogenetic analysis. Mitochondrial DNA B 5(1): 1073–1074. https://doi.org/10.1080/23802359.2020.1721366

Yamauchi MM, Miya MU, Nishida M (2002) Complete mitochondrial DNA sequence of the Japanese spiny lobster *Panulirus japonicus* (Crustacea, Decapoda). Gene 295(1): 89–96. https://doi.org/10.1016/s0378-1119(02)00824-7

Yang XH, Li XM, Zhu DH, Zeng Y, Zhao LQ (2021) The diversity and dynamics of fungi in *Dryocosmus kuriphilus* community. Insects 12(5): 426. https://doi.org/10.3390/insects12050426

Zhong JL, Zhu DH (2022) Detection of two different mitochondrial genomes in a gall wasps species, *Andricus mairei* (Hymenoptera: Cynipoidea: Cynipidae). Journal of Asia-Paific Entomology 25(4): 101987. https://doi.org/10.1016/j.aspen.2022.101987

Zhu DH, He YY, Fan YS, Ma MY, Peng DL (2007) Negative evidence of parthenogenesis induction by *Wolbachia* in a gallwasp species, *Dryocosmus kuriphilus*. Entomologia Experimentalis et Applicata 124(3): 279–284. https://doi.org/10.1111/j.1570-7458.2007.00578.x

Supplementary material 1

List of universal insect mitochondrial short fragments of the *cox1*, *cob*, *rrnL* and *D2* genes primers used for long PCR primer developments

Authors: Yu-Bo Duan, Yan-Jie Wang, Dao-Hong Zhu, Yang Zeng, Xiu-Dan Wang

Data type: docx

Link: https://doi.org/10.3897/jhr.97.119433.suppl1

Supplementary material 2

List of PCR primers used in this study

Authors: Yu-Bo Duan, Yan-Jie Wang, Dao-Hong Zhu, Yang Zeng, Xiu-Dan Wang

Data type: docx

Link: https://doi.org/10.3897/jhr.97.119433.suppl2

Supplementary material 3

List of PCR primers and sequencing primers used in this study

Authors: Yu-Bo Duan, Yan-Jie Wang, Dao-Hong Zhu, Yang Zeng, Xiu-Dan Wang

Data type: docx

Link: https://doi.org/10.3897/jhr.97.119433.suppl3

Supplementary material 4

Summary of taxonomic groups used in Fig. 8

Authors: Yu-Bo Duan, Yan-Jie Wang, Dao-Hong Zhu, Yang Zeng, Xiu-Dan Wang

Data type: docx

Link: https://doi.org/10.3897/jhr.97.119433.suppl4

Supplementary material 5

Pair-wise COI sequence distances in *Synergus*
Authors: Yu-Bo Duan, Yan-Jie Wang, Dao-Hong Zhu, Yang Zeng, Xiu-Dan Wang
Data type: docx

Link: https://doi.org/10.3897/jhr.97.119433.suppl5

Supplementary material 6

Predicted folding pattern for tRNAs of Synergus nanlingensis mitochondrial genome
Authors: Yu-Bo Duan, Yan-Jie Wang, Dao-Hong Zhu, Yang Zeng, Xiu-Dan Wang
Data type: docx

Link: https://doi.org/10.3897/jhr.97.119433.suppl6

Supplementary material 7

Maximum Likelihood tree were inferred from the datasets *COI* + *Cytb* + *28S-D2* using IQ-tree
Authors: Yu-Bo Duan, Yan-Jie Wang, Dao-Hong Zhu, Yang Zeng, Xiu-Dan Wang
Data type: doc

Link: https://doi.org/10.3897/jhr.97.119433.suppl7