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Nearly complete mitochondrial genome of *Trachelus stipa* Budak, Blank & Basibuyuk, 2017 (Hymenoptera: Cephidae)

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Abstract

The family Cephidae is a relatively modest phytophagous lineage within the suborder Symphyta of the order Hymenoptera. Members of this exclusively endophytophagous lineage feed on graminoids, berry canes, shrubs, and arboreal species. In this study, the nearly complete mitogenome of *Trachelus stipa* (Hymenoptera: Cephidae) was assembled and annotated as a third-party annotation and compared with the mitogenome of *T. iudaicus* and *T. tabidus*. The obtained length of the partial mitogenome of *T. stipa* was 16,085 bp with an A+T content of 79.02%. The mitogenome was containing 13 protein-coding, two rRNA, 22 tRNA genes and all of the protein coding genes (PCGs) are initiated with ATN as start codon, and all are ended with TAA or T− stop codon. All tRNA genes folded into the usual clover-leaf structure except for *tRNA-Ser1*. Compared with the inferred insect ancestral mitogenome, the gene orders are mostly conserved, except for the translocation of *tRNA-Met* and swapped positions of *tRNA-Ile* and *tRNA-Gln*. Phylogenetic analysis confirmed the position of *T. stipa* within genus *Trachelus* and recovered a relationship of *Stenocephus* + (*Janus* + ((*Phylloecus* + *Pachycephus*) + (*Syrista* + (*Calameuta* + (*Trachelus* + *Cephus*)))) in Cephidae.

Keywords: Cephidae phylogeny, mitogenome, *Trachelus*, Symphyta

1. Introduction

The family Cephidae constitutes a relatively modest phytophagous lineage within the Hymenoptera, comprising 165 described species across three subfamilies, of which Cephinae is the most diverse, encompassing 159 species distributed among 20 genera (Taeger et al., 2018). Members of this obligately endophytophagous lineage display varied host-plant associations and are conventionally referred as stem sawflies, reflecting the lifestyle of the larvae from a range of hosts, including graminoids, berry canes, shrubs, and arboreal species (Shanower & Hoelmer, 2004; Budak et al., 2011; Korkmaz et al., 2018). From an economic perspective, a number of species are knowns as important pests, such as larval stem borers inside grass stems or twigs of woody plants (Middlekauff, 1969; Shanower and Hoelmer, 2004).

The genus *Trachelus* Jurine, 1807, resides within the subfamily Cephinae and tribus Cephini, comprising nine described species primarily distributed across the Palearctic region, with one species (*T. tabidus*) introduced to North America since the 1880s (Taeger et al., 2018). The phylogenetic analyses confirm the monophyly of *Trachelus*, positioning it as closely allied to genera such as *Calameuta* and *Cephus*, within

a broader clade where traditional tribal divisions (Cephini, Hartigiini, Pachycephini) are not fully monophyletic (Budak et al., 2011; Korkmaz et al., 2018). The species of this genus exhibit specialized morphologies, including elongate antennae and genitalic structures adapted for oviposition in grass stems, with host associations predominantly involving Poaceae. Notably, *Trachelus stipa* Budak, Blank & Basibuyuk, 2017 is a recently described endemic species from Central Anatolia and displays morphological affinities to *T. flavicornis* and *T. troglodyta* in antennal segmentation and penile valve configuration, while specializing on *Stipa holosericea* (feather grass) as its larval host (Budak et al., 2017). Speciation dynamics in *Trachelus*, are most probably driven by host-plant transitions, as verified by phylogenetic reconstructions that highlight clade-specific adaptations to the host plants, with localized endemics such as *T. stipa* representing ecological speciation through niche adaptation within grassland biomes (Budak et al., 2011, 2017; Korkmaz et al., 2018).

Here, the nearly complete mitogenome of the stem sawfly *T. stipa* was assembled and annotated for the first time as a third-party annotation. So far, only two mitogenomes from *T. tabidus* and *T. iudaicus*, have been reported for this genus (Korkmaz et al., 2017). The mitogenome of *T. stipa* was compared with these previously reported mitogenomes of *Trachelus* to investigate the mitogenomic architecture and features within the genus.

2. Materials and Methods

Mitogenome Assembly and Annotation

The raw next generation sequencing (NGS) data of *T. stipa* was downloaded from the NCBI Sequence Read Archive (SRA) database with the accession number SRR27337724 using SRA Toolkit (https://trace.ncbi.nlm.nih.gov/Traces/sra/sra.cgi?view=software). The quality of the raw reads was evaluated using FastQC v0.12.1 (Andrews, 2010). Adapter sequences, low quality reads and possible reads including too much "N"s were eliminated from raw reads using fastp v0.20.0 (Chen et al., 2018). Clean reads were then assembled into mitogenomic contigs using MitoZ v3.6 (Meng et al., 2019) by using MEGAHIT (Li et al., 2015) as an assembler.

The annotation of the obtained mitogenome was performed using MitoZ v3.6 and MITOS2 (Al Arab et al., 2017; Donath et al., 2019) tool on Galaxy web server (Abueg et al., 2024). The decision on the locations and boundaries of the rRNA genes and PCGs was made manually by comparing with the *T. iudaicus* (NC032071) and *T. tabidus* (NC032072) mitochondrial genomes. The overlapping and intergenic regions between all genes were predicted manually. Finally, the nearly complete mitogenome of *T. stipa* was deposited in GenBank. The mitogenome is visualized using OrganellarGenomeDRAW (OGDRAW) (Greiner et al., 2019).

The nucleotide compositions were computed using MEGA v12.1 (Stecher et al., 2025). The strand asymmetries were calculated using the formulas: AT-skew = [A - T]/[A + T] and GC-skew = [G - C]/[G + C] (Perna and Kocher, 1995). To estimate the nucleotide and amino acid divergences between *Trachelus* species, the nucleotide and amino acid identities were calculated MEGA v12.1.

Phylogenetic analyses

Phylogenetic analyses were conducted on the mitogenome dataset of cephid species, including one newly annotated mitogenome of *T. stipa* (Table 1) to verify the phylogenetic position of *T. stipa*. *Allantus luctifer* (Tenthredinidae) was included as an outgroup (Table 1). The mitogenome sequences of the species that used in the phylogenetic analyses were retrieved from GenBank. Mitochondrial PCGs were aligned individually using "translation align" option implemented in Geneious R9 (Kearse et al., 2012) with MAFFT (Katoh and Standley, 2013) algorithm. Each rRNA gene was aligned individually using MAFFT implementation in Geneious R9. The individual alignments of each gene were then concatenated using PhyloSuite (Zhang et al., 2020). The optimal partition strategy and nucleotide substitution model of each partition were selected using ModelFinder (Kalyaanamoorthy et al., 2017) in PhyloSuite under the Bayesian Information Criterion (BIC). Best partitioning scheme and evolutionary model were used in subsequent phylogenetic analyses. The phylogenetic relationships were inferred with Maximum Likelihood (ML) approach. The ML analysis was carried out in IQ-TREE 2 (Minh et al., 2020) with 1000 bootstrap replicates using the proposed evolutionary model by PartitionFinder2. The produced phylogenetic trees were visualised by FigTree (Rambaut, 2014).

3. Results and Discussion

Mitochondrial genome architecture and nucleotide composition

The nearly complete mitogenome of *T. stipa* was assembled, annotated and compared with the mitogenome sequences of *T. iudaicus* and *T. tabidus* (Korkmaz et al., 2017). Sequence information for the part comprising the AT-rich region could not be obtained (Figure 1, Table 2). The length of the obtained mitogenome was 16,085 bp and including the 13 PCGs, 22 tRNAs and two rRNA genes (Figure 1, Table 2). The mitogenome organisation of the *T. stipa* was exactly the same with those of other reported *Trachelus* mitogenomes (Korkmaz et al., 2017). The mitogenome was mostly arranged in the same direction and order of the insect ancestral mitogenome, with only exception of the location of several tRNA genes, similar to previously reported cephid mitogenomes (Korkmaz et al., 2015; Korkmaz et al., 2016; Korkmaz et al., 2018). In total, twenty-three genes were encoded on the majority (J) strand, while the remaining fourteen genes were found on the minority (N) strand (Table 2).

The nucleotide composition of the mitogenome of *T. stipa* was remarkably biased towards A and T nucleotides, with a 79.02% A+T content, and similar to other reported *Trachelus* mitogenomes, varying between 79.03% A+T in *T. iudaicus* and 80.70% A+T in *T. tabidus* (Korkmaz et al., 2017) (Table 3). A similar bias towards A and T nucleotides was also detected in PCGs of the T. stipa mitogenome with 77.00% A+T content. The percentage of A+T of the 3rd codon position (86.18%) was substantially higher than those of the 1st (73.68%) and also the 2nd codon positions (71.14%) (Table 3). Similar to other reported *Trachelus* mitogenomes (Table 3), the AT- and GC- skews were found slightly positive (0.0750) and moderately negative (-0.2786) in the whole mitogenome of *T. stipa*, respectively.

Protein coding genes

The total length of the 13 PCGs was 11,240 bp which accounts for about 69.88% of the mitogenome (Table 2). Among the PCGs the *ND5* was the longest (1686 bp) and the *ATP8* was the shortest (177 bp). The lengths of PCGs were mostly similar to those of other *Trachelus* mitogenomes, except for *ND2*, *ATP8*, *COX3*, *ND4* and *ND6* (Table 2). The gene exhibiting the most length variation was the *COX3* with 9 codon differences between *T. stipa* (789 bp) and *T. iudaicus* (816 bp). As widely reported for the metazoan mitogenomes (Crozier and Crozier 1993), the PCGs was using the ATN-Ile/Met inititation codon. As frequently seen in invertebrate mitogenomes, TAA canonical termination codon was found in eleven PCGs (Table 2), while *ND2* and *COX1* have truncated termination codon (T−). The products of these two genes were possibly completed with the posttranscriptional polyadenylation process (Ojala et al. 1980, 1981). Among *Trachelus* species, based on both amino acid and nucleotide identities the least conserved PCG was *ATP8* (53.85% amino acid identity, 58.46% nucleotide identity), whereas the most conserved was *COX1* based on amino acid identity (94.52%), and was *ND5* based on nucleotide identity (82.29%) (Table 4).

tRNA and rRNA genes

All of the tRNA genes folded into usual clover-leaf secondary structure, except for *tRNA-Ser1*. Their locations and orientations are identical between *Trachelus* species, and the lengths were ranging from 65 bp (*tRNA-Arg* and *tRNA-Asn*) to 75 bp (*tRNA-His*). The nucleotide composition of the tRNA genes was biased towards A + T similar to mitogenome, with an average 84.14% A+ T.

The locations of rRNA genes were identical with those of other reported cephid mitogenomes (Figure 1) (Korkmaz et al., 2015; Korkmaz et al., 2016; Korkmaz et al., 2018; Liu et al., 2022). The *16S rRNA* was located between *tRNA-Leu1* and *tRNA-Val* and was 1396 bp in length (Table 2). The *12S rRNA* was found between *tRNA-Val* and *tRNA-Met* and its length was 1007 bp. The lengths of the rRNA genes were comparable with the reported *Trachelus* mitogenomes (*16S rRNA:* ranging between 1370 bp and 1406 bp, *12S rRNA:* ranging between 1021 bp and 1036 bp) (Table 2). The A+T nucleotide content of the rRNA genes was 83.98% on average in *T. stipa* mitogenome (Table 3).

Overlapping and noncoding regions

The total length of the overlapping regions between genes in the *T. stipa* mitogenome was 27 bp in six locations ranging between 1 and 8 bp (Table 2). The common reported "ATNNTAA/termination codon-N-initiation codon" overlap motif in insect mitogenomes was found in *ATP8-ATP6* and *ND4-ND4L* overlaps. The total length of intergenic regions was 261 bp and distributed in 18 different locations with a size ranging from 1 to 58 bp (Table 2). The longest intergenic region was located between *tRNA-Ile* and *ND2* genes (Table 2). There was also a non-coding region with a 585 bp length downstream of *tRNA-Gln*, which is possibly a part of AT-rich region, however the entire sequence of AT-rich region could not be obtained.

Phylogeny of Cephidae

The phylogenetic analysis has recovered the tree topologies with high bootstrap support values (Figure 2). The recovered tree confirmed the taxonomic position of *T. stipa* within genus *Trachelus* with high support (BS: 100%). The tree supported the monophyly of all included genera (Figure 2), and the topology was mostly congruent with the previously reported Cephidae and Hymenoptera phylogenies (Korkmaz et al., 2017; Korkmaz et al., 2018; Liston and Prous, 2021; Liu et al., 2022). The tree revealed sister group relationship of the genus *Cephus* and *Trachelus* (Figure 2). The genus *Calameuta* was found as sister to *Cephus* + *Trachelus* clade. In the Cephidae, a relationship of *Stenocephus* + (*Janus* + ((*Phylloecus* + *Pachycephus*) + (*Syrista* + (*Calameuta* + (*Trachelus* + *Cephus*)))) was recovered with high support values (Figure 2).

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Table 1. List of mitogenomes used in phylogenetic analysis

	Species	Family	Accession Number	References
	Trachelus stipa	Cephidae	Pending	This study
	Trachelus iudaicus	Cephidae	NC032071	Korkmaz et al., 2017
	Trachelus tabidus	Cephidae	NC032072	Korkmaz et al., 2017
	Calameuta filiformis	Cephidae	NC028445	Korkmaz et al., 2016
	Calameuta idolon	Cephidae	NC028446	Korkmaz et al., 2016
	Cephus cinctus	Cephidae	NC012688	Dowton et al., 2009
	Cephus fumipennis	Cephidae	PX255344	An, 2025
	Cephus nigrinus	Cephidae	BK071385	Hastaoglu Orgen,2025
	Cephus pygmeus	Cephidae	KM377623	Korkmaz et al., 2015
Ingroup	*Cephus sareptanus*	Cephidae	KM377624	Korkmaz et al., 2015
	Janus compressus	Cephidae	KX907844	Korkmaz et al., 2018
	Janus sp.	Cephidae	OL757402	Niu and Wei, 2025
	Janus sp. 1 GYN-2022e	Cephidae	OL757400	Niu et al., 2025
	Pachycephus cruentatus	Cephidae	KX907845	Korkmaz et al., 2018
	Pachycephus smyrnensis	Cephidae	KX907846	Korkmaz et al., 2018
	Phylloecus linearis	Cephidae	KX907843	Korkmaz et al., 2018
	Stenocephus fraxini	Cephidae	NC069641	Niu and Wei, 2025
	Syrista parreyssii	Cephidae	OK104785	Liu et al., 2022
	Syrista sp. 1 GYN-2021c	Cephidae	OL889929	Niu, 2025
Outgroup	*Allantus luctifer*	Outgroup	NC024664	Wei et al., 2014

Table 2. Mitogenome summary of *Trachelus* species

		Trachelus stipa						***Trachelus iudaicus***						***Trachelus tabidus***					
Gene	Strand	Start	End	Size (bp)	IGN	Start codon	Stop codon	Start	End	Size	IGN	Start codon	Stop codon	Start	End	Size	IGN	Start codon	Stop codon
tRNA-Gln	N	586	654	69	32			1	69	69	59			1	69	69	33		
tRNA-Ile	J	687	754	68	58			129	196	68	55			103	170	68	87		
ND2	J	813	1836	1024	0	ATT	T--	252	1290	1039	0	AAA	T--	258	1299	1042	0	ATT	T--
tRNA-Trp	J	1837	1907	71	-8			1291	1363	73	-8			1300	1368	69	-8		
tRNA-Cys	N	1900	1973	74	16			1356	1424	69	70			1361	1431	71	1		
tRNA-Tyr	N	1990	2057	68	28			1495	1563	69	70			1433	1499	67	15		
COX1	J	2086	3619	1534	0	ATG	T--	1634	3167	1534	0	ATG	T--	1515	3048	1534	0	ATG	T--
tRNA-Leu2	J	3620	3688	69	0			3168	3236	69	0			3049	3117	69	0		
COX2	J	3689	4372	684	6	ATG	TAA	3237	3920	684	11	ATG	TAA	3118	3801	684	15	ATG	TAA
tRNA-Lys	J	4379	4450	72	6			3932	4003	72	5			3817	3888	72	10		
tRNA-Asp	J	4457	4526	70	0			4009	4082	74	0			3899	3968	70	0		
ATP8	J	4527	4703	177	-7	ATT	TAA	4083	4265	183	-7	ATT	TAA	3969	4136	168	-7	ATT	TAA
ATP6	J	4697	5371	675	13	ATG	TAA	4259	4933	675	3	ATG	TAA	4130	4804	675	6	ATG	TAA
COX3	J	5385	6173	789	21	ATG	TAA	4937	5752	816	75	ATA	TAA	4811	5608	798	8	ATA	TAA
tRNA-Gly	J	6195	6263	69	0			5828	5897	70	0			5617	5685	69	0		
ND3	J	6264	6617	354	2	ATT	TAA	5898	6249	352	0	ATC	T--	5686	6038	353	0	ATT	TA-
tRNA-Ala	J	6620	6685	66	14			6250	6316	67	1			6039	6104	66	15		

tRNA-Arg	J	6700	6764	65	-3			6318	6374	57	8			6120	6188	69	-5		
tRNA-Asn	J	6762	6826	65	0			6383	6451	69	0			6184	6252	69	0		
tRNA-Ser1	J	6827	6897	71	-1			6452	6522	71	0			6253	6323	71	-1		
tRNA-Glu	J	6897	6963	67	1			6523	6592	70	11			6323	6383	61	15		
tRNA-Phe	N	6965	7031	67	8			6604	6669	66	4			6399	6466	68	0		
ND5	N	7040	8725	1686	0	ATG	TAA	6674	8359	1686	0	ATG	TAA	6467	8152	1686	0	ATG	TAA
tRNA-His	N	8726	8800	75	-1			8360	8436	77	9			8153	8230	78	1		
ND4	N	8800	10152	1353	-7	ATG	TAA	8446	9801	1356	-7	ATG	TAA	8232	9584	1353	-7	ATG	TAA
ND4L	N	10146	10439	294	5	ATG	TAA	9795	10088	294	9	ATG	TAA	9578	9871	294	4	ATG	TAA
tRNA-Thr	J	10445	10518	74	3			10098	10165	68	0			9876	9948	73	0		
tRNA-Pro	N	10522	10588	67	6			10166	10234	69	3			9949	10016	68	3		
ND6	J	10595	11179	585	0	ATG	TAA	10238	10828	591	7	ATG	TAA	10020	10607	588	4	ATG	TAA
CYTB	J	11180	12319	1140	22	ATG	TAA	10836	11975	1140	10	ATG	TAA	10612	11751	1140	12	ATG	TAA
tRNA-Ser2	J	12342	12410	69	17			11986	12053	68	16			11764	11831	68	19		
ND1	N	12428	13372	945	3	ATA	TAA	12070	13014	945	5	ATA	TAG	11851	12795	945	0	ATG	TAA
tRNA-Leu1	N	13376	13443	68	0			13020	13088	69	0			12796	12865	70	0		

16S rRNA	N	1344	1483	1396	0		1308	1445	1370	0		1286	1427	1406	0
tRNA-Val	N	1484	1490	67	0		1445	1452	70	0		1427	1434	69	0
12S rRNA	N	1490	1591	1007	0		1452	1547	1021	0		1434	1537	1036	0
tRNA-Met	J	1591	1598	69	0		1555	1617	69	0		1537	1445	69	0

Table 3. Nucleotide composition of the mitogenomes of *Trachelus* species

	T	C	A	G	AT%	GC%	AT-skew	GC-skew
Whole genome								
T. stipa	36.55	13.41	42.47	7.57	79.02	20.98	0.0750	-0.2786
T. tabidus	38.21	12.18	42.50	7.12	80.70	19.30	0.0532	-0.2621
T. iudaicus	36.55	13.58	42.48	7.39	79.03	20.97	0.0750	-0.2952
Protein coding genes								
T. stipa	42.44	11.63	34.56	11.37	77.00	23.00	-0.1022	-0.0113
T. tabidus	42.73	11.47	34.71	11.09	77.44	22.56	-0.1036	-0.0166
T. iudaicus	41.43	12.74	34.00	11.84	75.42	24.58	-0.0985	-0.0369
Protein coding genes-1st								
T. stipa	35.48	10.60	38.21	15.72	73.68	26.32	0.0371	0.1943
T. tabidus	35.81	10.40	38.08	15.71	73.89	26.11	0.0307	0.2037
T. iudaicus	34.99	10.95	38.86	15.19	73.85	26.15	0.0523	0.1621
Protein coding genes-2nd								
T. stipa	49.32	16.63	21.82	12.24	71.14	28.86	-0.3865	-0.1521
T. tabidus	49.52	16.44	21.62	12.43	71.14	28.86	-0.3922	-0.1389
T. iudaicus	49.16	16.72	21.43	12.69	70.59	29.41	-0.3927	-0.1369
Protein coding genes-3rd								
T. stipa	42.52	7.66	43.67	6.16	86.18	13.82	0.0134	-0.1085
T. tabidus	42.88	7.56	44.43	5.13	87.30	12.70	0.0178	-0.1916
T. iudaicus	40.12	10.56	41.70	7.62	81.82	18.18	0.0192	-0.1613
rRNAs								
T. stipa	46.61	5.04	37.37	10.99	83.98	16.02	-0.1100	0.3714
T. tabidus	45.41	5.28	38.33	10.97	83.74	16.26	-0.0846	0.3501
T. iudaicus	46.08	5.62	36.01	12.30	82.08	17.92	-0.1227	0.3724
tRNAs								
T. stipa	41.91	6.64	42.24	9.21	84.14	15.86	0.0039	0.1618
T. tabidus	41.83	7.03	41.56	9.59	83.39	16.61	-0.0031	0.1542
T. iudaicus	40.28	7.38	42.52	9.82	82.79	17.21	0.0271	0.1418

Table 4. Nucleotide and amino acid identities of mitochondrial protein coding genes among *Trachelus* species

	Nueclotide identity (%)	**Amino acid identity (%)**
ATP6	79,91	88,39
ATP8	58,46	53,85
COX1	81,41	94,52
COX2	77,68	85,02
COX3	78,35	87,82
CYTB	78,1	89,45
ND1	78,77	84,08
ND2	72,57	67,14
ND3	75,21	80,34
ND4	74,87	80,04
ND4L	74,57	69,07
ND5	82,29	83,96
ND6	66,16	61,73

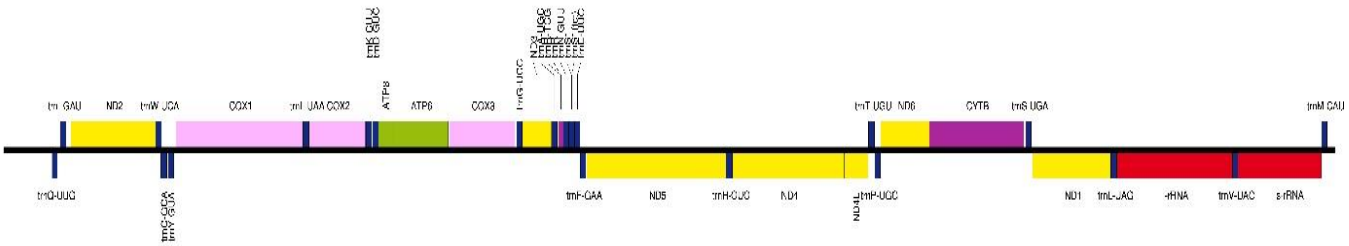

Figure 1. Mitogenome organization of *Trachelus stipa*. Genes transcribed from the J and N strands are shown above and below the line, respectively. PCGs coding complex I, complex III, complex IV and complex V components are marked with yellow, purple, pink and green, respectively. tRNA genes are painted with dark blue and labelled by the single letter amino acid code and rRNA genes are painted with red.

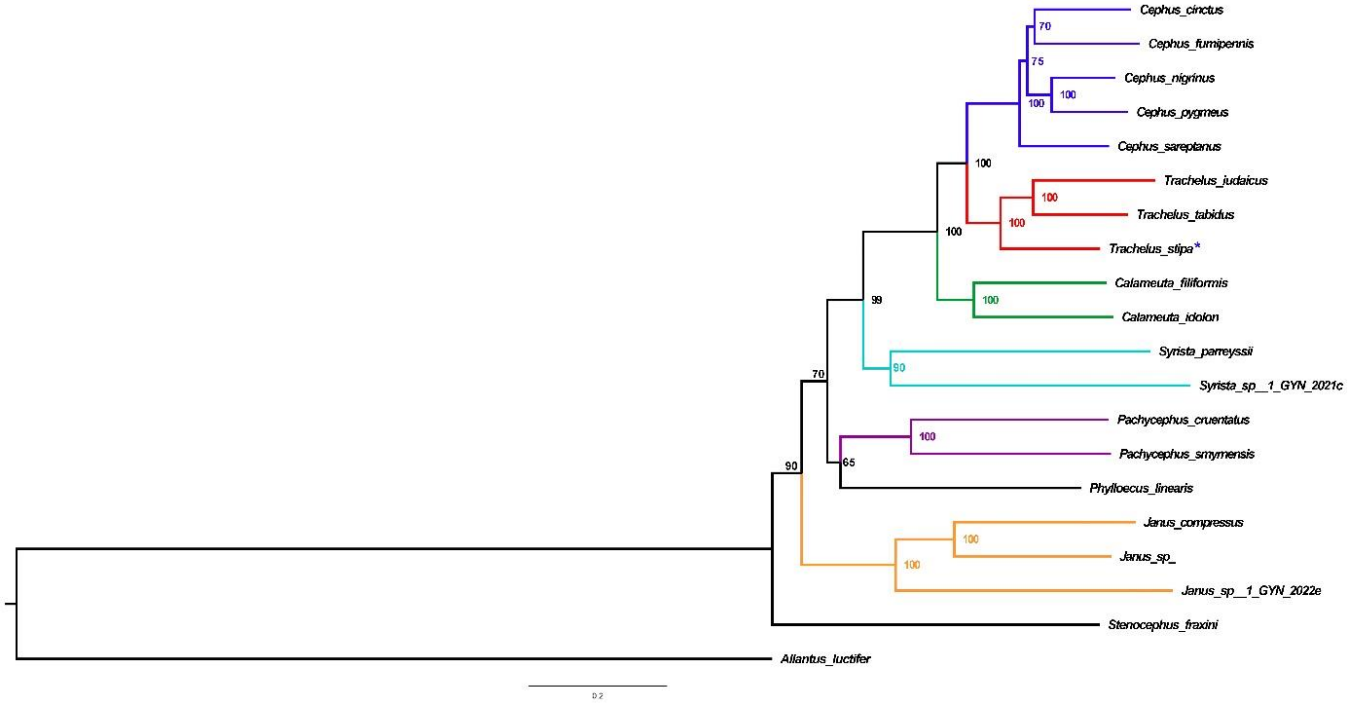

Figure 2. The phylogenetic tree constructed under ML using the mitochondrial gene dataset. *Allantus luctifer* (Tenthredinidae) was used as outgroup. Bootstrap support values were shown on the nodes.