Characterization of the mitochondrial genome of *Arge bella* Wei & Du sp. nov. (Hymenoptera: Argidae)

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We describe *Arge bella* Wei & Du sp. nov., a large and beautiful species of Argidae from south China, and report its mitochondrial genome based on high-throughput sequencing data. We present the gene order, nucleotide composition of protein-coding genes (PCGs), and the secondary structures of RNA genes. The nearly complete mitochondrial genome of *A. bella* has a length of 15,576 bp and a typical set of 37 genes (22 tRNAs, 13 PCGs, and 2 rRNAs). Three tRNAs are rearranged in the *A. bella* mitochondrial genome as compared to the ancestral type in insects: *trnM* and *trnQ* are shuffled, while *trnW* is translocated from the *trnW-trnC-trnY* cluster to a location downstream of *trnl*. All PCGs are initiated by ATN codons, and terminated with TAA, TA or T as stop codons. All tRNAs have a typical cloverleaf secondary structure, except for *trnS1*. H821 of *rrnS* and H976 of *rrnL* are redundant. A phylogenetic analysis based on mitochondrial genome sequences of *A. bella*, 21 other symphytan species, two apocritan representatives, and four outgroup taxa supports the placement of Argidae as sister to the Pergidae within the symphytan superfamily Tenthredinoidea.
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ABSTRACT

We describe *Arge bella* Wei & Du sp. nov., a large and beautiful species of Argidae from south China, and report its mitochondrial genome based on high-throughput sequencing data. We present the gene order, nucleotide composition of protein-coding genes (PCGs), and the secondary structures of RNA genes. The nearly complete mitochondrial genome of *A. bella* has a length of 15,576 bp and a typical set of 37 genes (22 tRNAs, 13 PCGs, and 2 rRNAs). Three tRNAs are rearranged in the *A. bella* mitochondrial genome as compared to the ancestral type in insects: *trnM* and *trnQ* are shuffled, while *trnW* is translocated from the *trnW-trnC-trnY* cluster to a location downstream of *trnl*. All PCGs are initiated by ATN codons, and terminated with TAA, TA or T as stop codons. All tRNAs have a typical cloverleaf secondary structure, except for *trnS1*. H821 of *rrnS* and H976 of *rrnL* are redundant. A phylogenetic analysis based on mitochondrial genome sequences of *A. bella*, 21 other symphytan species, two apocritan representatives, and four outgroup taxa supports the placement of Argidae as sister to the Pergidae within the symphytan superfamily Tentredinoidea.

INTRODUCTION

With about 950 valid species in the world, Argidae is the second-largest family of the paraphyletic suborder Symphyta of the order Hymenoptera (Choi et al., 2016). Eastern Asia is one of the three main centers of diversity of the family (Wei and Nie, 1997). Within China, about 170 species and 16 genera have been recorded (Choi et al., 2016). However, there are probably many more species to be described or newly recorded from this vast country.

Symphyta is the predominantly herbivorous and relatively less diverse suborder of the Hymenoptera, and contains more than 8500 described species (Taeger et al., 2010). The systematic arrangement of Symphyta, including the numbers of families and superfamilies are quite uncertain. Most symphytan researchers have divided the extant Symphyta into 14 families (Benson, 1938; Königsmann, 1977; Abe and Smith, 1991; Taeger et al., 2010) under four (Benson, 1938), six (Königsmann, 1977; Abe and Smith, 1991) or seven superfamilies (Taeger et al., 2010), and the six superfamilies in the system of Königsmann and of Abe and Smith are also different. Rasnitsyn (1988) divided extant taxa of Symphyta into two suborders, five infraorders, and 13 families. Wei and Nie (1997, 1998) divided the original Symphyta into five suborders, eleven superfamilies, and 20 families. It seems that a consensus on the systematics of Symphyta among sawfly researchers is difficult to get just based on morphological analysis, so using molecular-genetic data would be critical to test the different proposed systems and to approach a natural system of Symphyta.
The monophyly of Tenthredinoidea is supported by both morphological (Wei and Nie, 1997) and molecular data (Malm and Nyman, 2015) as well as combined analyses (Ronquist et al., 2012a; Sharkey et al., 2012; Klopfstein et al., 2013), but relationships among core tenthredinoids are less clear. Argidae was inferred as the sister to the remaining tenthredinoids by Wei and Nie (1997), but the discord of this result with several recent studies may be arisen from the limited dataset. Malm and Nyman (2015) analyzed nine protein-coding genes of 164 taxa to reconstruct the phylogenetic backbone of the Hymenoptera. In their analysis, 13 taxa were included to represent five out of seven subfamilies within Argidae; in the tree, Argidae and Pergidae form a monophylum as sister to the other non-blasticotomid tenthredinoids, which supports more comprehensive morphological (Vilhelmsen, 1997; Schmidt et al., 2006) and combined studies (Ronquist et al., 2012a; Sharkey et al., 2012; Klopfstein et al., 2013). A recent analysis of whole-body transcriptomes also inferred the monophylum of Argidae and Pergidae (Peters et al., 2017).

The mitochondrial genomes of 21 symphytan species have been reported (Table 1; data were collected at NCBI, available at https://www.ncbi.nlm.nih.gov/; accessed 3 Nov. 2017). Five phylogenetic analyses have been conducted based on nucleotide sequences of symphytan mitochondrial genomes (Castro and Dowton, 2005; Dowton et al., 2009b; Song et al., 2015b, 2016; Doğan and Korkmaz, 2017), but none of them have provided clear insights into symphytan relationships because of the taxonomically restricted representation of sawfly families in the datasets: the mitochondrial genomes of Argidae, Xyelidae, Diprionidae, Heptameliidae, Blastocotomidae, Megalodontesidae, Pamphilidae, Xiphydriidae, Siricidae, and Anaxyelidae have not been previously reported.

The small number of available sawfly mitochondrial genomes also limits our understanding of their genomic architecture. Compared with the ancestral gene arrangement of insects, only translocated and swapped are exhibited in A. bella. The conservation of rRNA secondary structures exceeds that of its nucleotides and, therefore, it is recommended that secondary structures guide decisions about the alignment of homologous positions for phylogenetic studies (Kjer, 1995). The secondary structures of \textit{rrnS} of \textit{A. bella} and \textit{Cephus} species are conservative in H821, but previous researchers supported that Argidae and Pergidae form a monophylum as sister to the remaining tenthredinoids (Malm and Nyman, 2015), instead of Argidae formed a sister group with \textit{Cephus} species. However, inferred secondary structures can only be considered as working hypotheses, and would be almost impossible to estimate without using a comparative approach (Misof and Fleck, 2003). Our understanding of the secondary structures of symphytan rRNAs has been developed only from seven Cepheid species (Dowton et al., 2009a; Korkmaz et al., 2015, 2016, 2017). More representatives and comparative analyses are therefore required within the Symphyta.

Here, we describe a large and beautiful new species of \textit{Arge} Schrank (1802) and report its near-complete mitochondrial genome sequence, as the first representative of the family Argidae. We characterize the nucleotide composition, codon usage and secondary structure of tRNAs of this mitochondrial genome. We compare the gene rearrangement of \textit{A. bella} with the ancestral gene arrangement of insects. We also analyze two rRNAs secondary structures across the sequenced symphytan mitochondrial genomes. The structural differences of rRNAs between \textit{A. bella} and \textit{Cephus} species are described to establish structural features as potentially useful characters for symphytan systematics. Finally, we report the results of phylogenetic analyses that we used to verify the phylogenetic placement of \textit{A. bella} based on sequences of 13 protein-coding genes and two rRNA genes of 22 species of Symphyta, two representatives of Apocrita, and four outgroup taxa. Our results support the placement of Argidae as sister to the Pergidae within the symphytan superfamily Tenthredinoidea.

**MATERIALS AND METHODS**

**Description of new species**

Specimens were examined with a Leica S8APO dissection microscope. Adult images were taken with a Nikon D700 digital camera, and sequentially focused images were montaged using Helicon Focus (HeliconSoft), while detailed images were taken with Leica Z16 APO/DFC550. All images were further processed with Adobe Photoshop CS 6.0.

The terminology of sawfly genitalia follows Ross (1945), and that of general morphology follows Viitasaari (2002). Abbreviations used are: OOL = distance between the eye and outer edge of lateral ocellus; POL = distance between the mesal edges of the lateral ocelli; OCL = distance between a lateral ocellus and the occipital carina or hind margin of the head.
The holotype and all paratypes of the new species are deposited in the Insect Collection of Central South University of Forestry and Technology, Changsha, Hunan, China (CSCS).

All nomenclatural acts, authors and literature are registered in ZooBank as per the recent proposed amendment to the International Code of Zoological nomenclature for a universal register for animal names (Polashek et al., 2005a,b; Pyle et al., 2008; ICZN, 2008). Rules for spelling Chinese personal and place names follow GB/T 16159-1996 and ISO 7098: 1991: “Chinese people’s names are to be written separately with the surname first, followed by the personal name written as one word, with the initial letters of both capitalized.”. “Chinese place names should be alphabetized according to the “Spelling Rules for Chinese Geographical Place Names,” document no. 17 (1984) of the State Committee on Chinese Geographical Place Names. Separate the geographical proper name from the geographical feature name and capitalize the first letter of both” (Niu et al., 2012).

The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The LSID for this publication is: urn:lsid:zoobank.org:pub:A94BD62A-D4BE-40F9-8718-84F425875C7C. The online version of this work is archived and available from the following digital repositories: PeerJ, PubMed Central and CLOCKSS.

Library construction and sequencing
Total genomic DNA of a single specimen was used for library preparation with insert size of 250 bp following the manufacturer’s instructions, and then 150 bp PE sequenced on an Illumina HiSeq 4000 platform for around 2.5 Gb of data at BGI-Shenzhen, China. The sequencing reads have been deposited in NCBI SRA database under accession number: PRJNA493965.

Mitochondrial genome assembly
Pre-analysis data filtering included: (i) Clean data was generated following published protocols (Zhou et al., 2013; Tang et al., 2014, 2015), by removing reads with adaptor contamination, >10% low-quality bases, or >5 bp Ns; (ii) clean data was then compared with reference mitochondrial genomes downloaded from GenBank (716 RefSeq genomes, including 699 arthropods, seven starfish and 10 cyprinid fish; accessed on 10 March 2014) to screen out candidate mitochondrial reads using relaxed criteria: BLAST identity >30% and E-value <0.00001; (iii) a 51-mer set was then generated from these candidate mito-reads and used as reference for a second round of data filtering for the discarded reads from step 2; (iv) De novo assembly was performed using SOAPdenovo-Trans (Xie et al., 2014) (-K 71, -L 100, -t 1), SOAPdenovo 2.0 (Luo et al., 2012; Li et al., 2010) (-K 61, -k 45), IDBA-UD (Peng et al., 2012) (kMaxShortSequence = 256, –num threads 12), and mitochondrial protein-encoding assemblies and mitochondrial genome-sequence candidates were annotated by a custom Perl script (Zhou et al., 2013) using RefSeq mitochondrial genomes of target animal taxa (604 arthropod species, two asteriid starfish and the zebrafish; downloaded from GenBank on 13 June 2013) downloaded from NCBI as reference. The mitochondrial genome was constructed, corrected and manually checked as previously described (Tang et al., 2015).

Mitochondrial genome annotation and secondary structure prediction
All of the tRNAs were identified using MITOS (http://mitos.bioinf.uni-leipzig.de/index.py) (Bernt et al., 2013) using the default settings. The initiation and termination codons of PCGs were determined in Geneious v8.0.5 (Kearse et al., 2012) (available from http://www.geneious.com) using reference sequences from other symphytan species, and then checked manually. Secondary structures of rRNAs were inferred using alignment to the models predicted for Trichiosoma anthracinum and Labriocimbex sinicus (Yan et al., in press). The predicted secondary structures of tRNAs and rRNAs were drawn using VARNA v3-93 (Darty et al., 2009) and RNAviz 2.0.3 (De Rijk et al., 2003). Helix numbering follows the convention established at the CRW site (Cannone et al., 2002) and Apis mellifera rRNA secondary structure (Gillespie et al., 2006) with minor modifications.

The A + T content of nucleotide sequences and relative synonymous codon usage (RSCU) were calculated using MEGA v7.0 (Kumar et al., 2016). Strand asymmetry was calculated using the formulae...
Phylogenetic analyses

Phylogenetic analyses were performed based on aligned sequences of the 13 PCGs and two rRNAs of the nearly complete mitochondrial genome of *A. bella* and 21 other sympatric mitochondrial genomes downloaded from GenBank (Table 1). These additional taxa represented five families: Tenthredinidae (Wei et al., 2015b, 2014; Song et al., 2015a, 2016), Cimbicidae (Song et al., 2016, Doğan and Korkmaz, 2017), Pergidae (Castro and Dowton, 2005), Orussidae (Dowton et al., 2009a), and Cephidae (Dowton et al., 2009a; Korkmaz et al., 2015, 2016, 2017). As the Symphyta is paraphyletic with respect to the suborder Apocrita, we also included the mitochondrial genomes of the apocritan species *Parapolybia crocea* (GenBank: KX679828) and *Taeniogonalos taihorina* (GenBank: NC027830) in the analysis. As outgroups, we included *Neopanorpa pulchra* (GenBank: FJ169955) from Mecoptera, *Anopheles gambiae* (GenBank: L20934) from Diptera, *Neochoaioideis parasparsus* (GenBank: KX821680) from Megaloptera and *Paroster microsturtensis* (GenBank: MG912997) from Coleoptera.

Nucleotide sequences of the 13 PCGs from the mitochondrial genomes of *A. bella* and the 27 other included species were translated into amino acid sequences and then aligned by MUSCLE in MEGA v7.0. Nucleotide sequences of two rRNAs from the included taxa were aligned by MAFFT (https://www.ebi.ac.uk/Tools/msa/mafft/). The amino acid alignments of the 13 PCGs and two rRNAs were concatenated using SequenceMatrix v1.7.8 (Vaidya et al., 2011) and used in phylogenetic analyses under the Maximum-likelihood (ML) criterion and Bayesian inference (BI). Partition schemes and substitution models were calculated simultaneously in PartitionFinder v1.1.1 (Lanfear et al., 2012). The branch lengths and search strategy of schemes were set as linked and greedy, and models were selected based on AICc and BIC. The GTR+I+G model was chosen as the best-fitting model for all partitions for both ML and BI analyses.

The ML analysis was performed using the IQ-TREE web server (http://iqtree.cibiv.univie.ac.at/) (Trifinopoulos et al., 2016), using default parameters except for 0.1 as the perturbation strength and 1000 as the IQ-TREE stopping rule. The BI analysis was performed using MrBayes v3.2.6 (Ronquist et al., 2012b) on the CIPRES Science Gateway (Miller et al., 2010). Rate and substitution-model parameters were unlinked across partitions. Two independent runs with four simultaneous Markov chains (one cold, three incrementally heated at T = 0.2) were run for five million generations, with sampling of parameters and trees occurring every 1000 generations. The maximum clade credibility tree showing all compatible groupings was calculated with a burn-in fraction of 10%, after confirming in Tracer v1.6.0 (Rambaut and Drummond, 2013) (Available at: http://beast.bio.ed.ac.uk/Tracer) that both runs had converged and that appropriate effective sample sizes were achieved for sampled parameters. Trees were edited in FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

RESULTS AND DISCUSSION

*Arge bella* Wei & Du sp. nov.

urn:lsid:zoobank.org:act:9AF00C3F-D5EE-474B-BD9E-4794BECACA4F

Etymology. This species is named after its beautiful body colour.

Holotype. Female. China: Hunan Province, Guidong County, Mt. Qiyun, Hydropower Station Valley, alt. 752m, 25°45.361′ N, 113°55.598′ E, April 4, 2015, Yuchen Yan, Ting Liu leg. (CSCS).

Paratypes. 2 Females. Locality and collecting time as the holotype, Hang Zhao, Mengmeng Liu leg. (CSCS).

Distribution. China (Hunan).

Remarks. This new species is somewhat similar to *Arge nigricrux* Malaise (1943) and *A. vitalisi* Turner (1919), but differs from these two species by the followings: the antennal flagellum entirely black; dorsum of mesonotum blue black except for posterior of mesoscutellum; abdomen yellow brown, terga 1-2 and 4-7 largely blue black; wings distinctly yellowish, without transverse macula; hind tarsus entirely blue black; mesepisternum blue black with upper fourth yellow brown. In *A. nigricrux* and *A. vitalisi*, the antennal flagellum and mesonotum in female entirely yellow; abdomen yellow brown, terga 4 largely black, terga 5-8 with short middle black maculae; wings weakly yellowish, with distinct transverse smoky macula just below pterostigma; hind tarsus entirely yellow brown; mesepisternum entirely black or entirely yellow brown.
Description

Female. Body length 13 mm (Figure 1). Body and leg yellow brown; apex of mandible, apical half of pedicel and flagellum entirely black, without bluish tinge; ocellar area, entire mesoscutal middle lobe, dorsum of mesoscutal lateral lobe, anterior triangular lobe of mesoscutellum, mesepisternum except for dorsal fourth, katepimeron except for margins, small central macula on metapleuron, large transversal band on tergum 1, dorsum of terga 2 and 4-7, small middle macula on tergum 3, apical 0.8 of middle and hind femora, apex of middle tibia, apex of middle basitarsus and tarsomeres 2-5, apical 0.4 of hind tibia and entire hind tarsus black with distinct bluish tinge, upper margin of black macula on mesepisternum convex (Figure 2c); most of body hairs yellow brown, hairs on flagellum, middle and hind femora, spines on inner sides of sheath mostly black, hairs on mesonotum dark brown. Wings hyaline, with distinct yellowish tinge, veins C, r1 and A largely pale brown, pterostigma and other veins black brown.

Head smooth, shiny; basal half of mandible with large and dense punctures; clypeus, face, lower half of inner orbit and frontal wall distinctly and rather densely punctured, temple densely and minutely punctured, postoccipital area hardly punctured, postorbital minutely and sparsely punctured, frontal basin smooth; pronotum finely and faintly punctured; mesonotum minutely and sparsely punctured, most of mesoscutellum smooth with some minute punctures, parapsis smooth, impunctate and without microsculptures; metanotum and mesepisternum smooth, hardly punctured; anepimeron minutely punctured, katepimeron finely microsculptured; center of mesosternum distinctly punctured; metapleuron impunctate, without microsculptures; dorsum of abdominal terga smooth, impunctate, terga 1-2 faintly microsculptured, sterna and sheath smooth.

Labrum about 2 times as broad as long, apex obtusely truncate; clypeus flat, anterior incision shallow and roundish; supraclypeal area rounded elevated, without middle ridge; lateral carinae between antennal toruli low and obtuse, almost parallel downwards, not merged together, largest breadth between lateral carinae about 1.5 times median diameter of median ocellus (Figure 2a); middle fovea round, distinct, shallowly open to frontal basin; frons small, center evenly concave, frontal wall distinct; malar space 0.6 times diameter of median ocellus; inner margin of eyes parallel, distance between eyes 1.1 times longest axis of eye; POL: OOL: OCL = 20: 27: 22; postocular area flat, breadth 1.7 times length; lateral furrows shallow and fine, slightly convergent backwards; postocular furrow fine and shallow, interocular furrow broad and shallow; in dorsal view (Figure 2d), head about 0.65 times as long as eye, hardly enlarged behind eyes; head in lateral view as in Figure 2i. Antenna slightly enlarged toward apex, hardly bent, longitudinal carina low and faint, pedicel as long as broad, flagellum 0.8 times as long as thorax and about 1.35 times head breadth (Figure 2i). Middle furrow on mesoscutal middle lobe faint, notauli distinct; mesoscutellum 1.2 times as broad as long, anterior 0.2 with a short middle furrow; distance between cencheri 0.2 times breadth of a cenchrus. Forewing: vein R short, about 0.7 times as long as vein and 0.6 times as long as free abscissa of vein Sc, vein R+M about half length of vein 1r-m, second abscissa of vein Rs clearly longer than third abscissa of Rs, third abscissa of Rs 1.4 times as long as fourth abscissa of Rs, cell 1Rs clearly longer than cell 2Rs, upper and lower margins of cell 2Rs equal in length; vein 2Rs roundly convex outwards, vein cu-a meeting cell 1M at basal 0.4, basal anal cell closed. Hind wing: cell Rs 1.25 times as long as cell M, cell M about 2.1 times as long as broad; anal cell closed; relative length of cells A, petiole of anal cell and cu-a about 80: 57: 27; outer margin of fore and hind wings naked (Figure 1). Middle and hind tibiae each with 1 preapical spur (Figure 2j); hind basitarsus slightly longer than following 3 tarsomeres together. Ovipositor sheath as long as hind femur, basal third distinctly concave in lateral view (Figure 2f); apex of sheath round in dorsal view (Figure 2m); subapical part of lance weakly narrowed (Figure 2i); lancet broad, annular spines very short, serrulae strongly protruding (Figure 2g), basal and middle serrulae as in Figure 2g.

Male. Unknown.

Architecture and nucleotide composition of A. bella mitochondrial genome

We sequenced the nearly complete mitochondrial genome of A. bella, which deposited in GenBank of NCBI under the accession number MF287761. The sequenced region is 15,576 bp in length, with 13 protein-coding, two rRNA genes and 22 tRNA genes. Of these, 23 genes (9 PCGs and 14 tRNAs) were encoded by the J strand, while the remaining ones were encoded by the N strand (Table 2).

Compared with the ancestral gene arrangement of insects, the mitochondrial genome of A. bella exhibited only few rearrangements: trnM and trnQ have swapped positions, and trnW has been translocated from the trnW-trnC-trnY cluster to downstream of trnL (Figure 3). We did not succeed in sequencing
a fragment spanning the A + T-rich region and genes flanking the A + T-rich region, and the same as
Orussus occidentalis and Cephus cinctus (Dowton et al., 2009a).

There were totally 23 overlapping nucleotides in six locations, and the length of the overlapping
sequences ranged from 1 to 7 bp (Table 2). Some overlapping nucleotides were conserved in the A.
bella mitochondrial genome: ATGATAA between ATP8 and ATP6, and ATGTTAA between ND4 and
ND4L, which are also common features of many other insect mitochondrial genomes (Chai et al., 2012).
There were totally 192 non-coding positions between neighboring genes in 15 locations, and the length of
non-coding sequences ranged from 1 to 44 bp (Table 2). There were five locations where the length was
over 15 bp: 33 bp between trnW and trnL, 30 bp between trnI and trnM, 25 bp between trnR and trnN,
and 44 bp between COX2 and trnK.

**Protein-coding genes and codon usage**
All PCGs were initiated by ATN codons: four genes (ND2, ATP8, ND3 and ND6) used ATA as start
codon, four genes (COX1, ND5, NDML and ND1) started with ATT; and five genes (COX2, ATP8, COX3,
ND4 and CYTB) were initiated with ATG (Table 2).

The stop codons of A. bella were generally TAA, except for ND6, which ended with TA, and COX1,
COX3 and ND4, which ended with T (Table 2). Incomplete stop codons have been reported for all
symphytan mitochondrial genomes sequenced to date.

The nucleotide composition of the mitochondrial genome of A. bella was A and T rich, with an 80.7%
A + T content (Table 3). In PCGs, the highest A + T content was observed in the third codon position, the
highest T content in the second codon position and the lowest G content in the third codon position. The
highest A + T content was observed in the ATP8 gene (89.7%).

It has been reported that the parental N strand remains as a single strand for a longer time during
replication of mitochondrial genomes, resulting in deamination of A and C (Reyes et al., 1998). This
leads to an A- and C-skew on the J strand and a T- and G-skew on the N strand. In the case of A. bella,
we observed that the AT skew was slightly positive (0.0706). On the contrary, GC skew was negative
(-0.2708) when considering the whole mitochondrial genome (Table 3), which shows that the occurrence
of A was higher than that of T, and the occurrence of C was higher than that of G, which is a general
phenomenon in symphytan mitochondrial genomes (Castro and Dowton, 2005; Dowton et al., 2009b; Wei
et al., 2015a). PCGs on the J strand were slightly T-skewed (-0.0218) and slightly C-skewed (-0.2273),
whereas PCGs encoded by the N strand were all slightly T-skewed (-0.2285) and moderately G-skewed
(0.3226).

Codon usage in the A. bella mitochondrial genome is presented in Table 4. As in other insect
mitochondrial genomes (Foster et al., 1997), a significant correlation between codon usage and nucleotide
composition was found. Leu, Ile, Phe, Met and Ser were most frequently used amino acids (Table 4).
UUA-Leu had the highest relative synonymous codon usage (4.83) (Table 4). A relationship between
the nucleotide compositions of codon usage and amino acid occurrence was noticed. The relationship
can be calculated by the ratio of G + C rich codons (Pro, Ala, Arg, and Gly) and A + T rich codons (Phe,
Ile, Met, Tyr, Asn, and Lys). The ratio found in A. bella (0.27) is similar to or lower than that of other
symphytan species (0.28–0.31) (Korkmaz et al., 2015).

**Transfer RNA genes**
The position and orientation of the predicted tRNAs and anticodon sequences were identical to most
of the hitherto reported symphytan mitochondrial genomes (Table 2). 14 tRNAs were encoded by the
J strand, and the others by the N strand. All tRNAs folded into a usual clover-leaf structure except for
trnS1 (AGN). Compared with other symphytan species, trnS1 (AGN) lacked a dihydrouridine (DHU) arm.

The size of tRNAs ranged from 64 bp (trnS1) to 74 bp (trnH and trnD) (Figure 4), placed well within
the observed ranges in insects. The observed size differences resulted from changes in the length of the
variable loop, dihydrouridine (DHU) arm and TΨC arm (Clary and Wolstenholme, 1985). Anticodon
sequences of the tRNA genes were identical with previously-reported symphytan mitogenomes (Table 2).
Pairing mismatches occurred mainly in the DHU arm, AA arm and AC arm, and sometimes in TΨC arm.
All of the 18 mismatches were G-U pairs.

**Ribosomal RNA genes**
In A. bella, rrnS was located downstream of trnV, and rrnL was located between trnL1 and trnV (Table
2). Both rRNAs were encoded by the N strand, and their lengths were 839 bp and 1,390 bp, and A + T
The rrnS secondary structure of *A. bella* contained four domains (Simon et al., 1994; Niehuis et al., 2006) and 27 helices (Figure 5). Previous studies have shown that the second half of the domain III sequence can be difficult to align precisely, even when information on secondary structure is considered. The rrnS domain III model of *A. bella* was very similar to the one described in Hickson et al. (1996). H821 was redundant compared with other symphytan species like *Cephus* (Korkmaz et al., 2015, 2016, 2017), which includes 26 helices. The predicted structure of H921 was well conserved in symphytan species, but loop size in H47 is variable. The predicted structures of H500, H769, H944 and H1047 were conserved in symphytan species. H1399 and H1506 helices were well conserved in *A. bella*, as well as in other insect species (Cameron and Whiting, 2008; Cannone et al., 2002; Gillespie et al., 2006; Misof and Fleck, 2003; Wei et al., 2009, 2010b).

The length of the rrnL gene was 1,390 bp (Table 1), with an 84.0% A + T content. The secondary structure of the rrnL gene in *A. bella* conformed to models proposed for other insects, with the 45 helices belonging to six domains (Figure 6) (Cameron and Whiting, 2008; Cannone et al., 2002; Gillespie et al., 2006; Misof and Fleck, 2003; Wei et al., 2009, 2010a,b). H563, H671, H1925 and H2043 were conserved, and H1775 almost with 3 pairs in symphytan species. H991 was different from those of *P. condei*, *O. occidentalis*, and *Monocellicampa pruni* with regards to helical length and loop size.

**Phylogenetic relationships**

We investigated the phylogenetic position of *A. bella* by combining our new mitochondrial genome sequences with previously-reported data from 21 species of Symphyta representing five other families (Table 1), as well as with sequences from two apocritan species and four non-hymenopteran outgroup taxa.

Both ML and BI analyses placed *A. bella* as sister to *Perga condei* with high support (Figure 7). This Pergidae + Argidae monophylum, as well as its placement as sister to the remaining non-blasticotomid tenthredinooids, which were consistent with the results from comprehensive morphological (Vilhelmsen, 1997; Schmidt et al., 2006) and molecular (Malm and Nyman, 2015) studies. The phylogenetic location of *A. bella* can be considered to constitute the basal branch of the normal Tenthredinoidea (Ross, 1937; Benson, 1938; Taeger et al., 2010), or the suborder Tenthredinomorpha (Wei and Nie, 1998). On a wider phylogenetic scale, our results supported a grouping of ((Cephidae, (Orussidae, Apocrita)), ((Argidae, Pergidae), (Cimicidae, Tenthredinidae))) within the Hymenoptera.

**CONCLUSIONS**

*Arge bella* Wei & Du sp. nov. is a new species belonging to *A. vitalisi* group. It is similar to *A. nigrircux* Malaise (1943) and *A. vitalisi* Turner (1919) from south Asia, but differs from them by the antennal flagellum entirely black, the dorsum of mesonotum mainly blue black, and the abdominal tergites 1, 3-6 each with a large and broad bluish black macula.

The nearly complete mitochondrial genome of *A. bella* (15,576 bp) displays a highly conserved structure and composition as compared to the mitochondrial genomes of other symphytans as well as insects in general. The main differences include minor rearrangements or translocations of three tRNAs, a non-clover-leaf-like structure of *trnS*1 (AGN), and redundancy of H821 of *rrnS* and H976 of *rrnL*. ML and BI phylogenetic analyses resulted in a hymenopteran tree with the structure ((Cephidae, (Orussidae, Apocrita)), ((Argidae, Pergidae), (Cimicidae, Tenthredinidae))) with high nodal supports. Hence, mitochondrial genome sequencing of additional symphytan taxa in the future can clearly produce useful data for resolving hymenopteran relationships.

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Table 1. General information of the mitochondrial genomes of Symphyta.

Table 2. Characteristics of the mitochondrial genome of *A. bella*.

Notes

1. Table 1. General information of the mitochondrial genomes of Symphyta.

2. Figure 1. *Arge bella* Wei & Du sp. nov. Adult female, dorsal view. Scale bar = 1 mm.

3. Figure 2. *Arge bella*.

4. Figures a–m. *Arge bella* Wei & Du sp. nov. a. Head of female, front view; b. Head of female, dorsal view; c. Mesopleuron of female, lateral view; d. Head of female, dorsal view; e. Sheath of female, lateral view; f. Sheath of female, ventral view; g. Serrula of female; h. Antenna of female; i. Head of female, lateral view; j. Tibia of hind leg; k. Lancet; l. Lance; m. Sheath of female, dorsal view.

5. Table 2. Characteristics of the mitochondrial genome of *A. bella*.

6. J and N refers to heavy and light strands, respectively; IGN refer to intergenic nucleotides. Minus indicates overlapping sequences between adjacent genes.

7. Figure 3. Mitochondrial genome organisation of *A. bella*.

8. Mitochondrial genome organisation of insects in general (above) and *A. bella* (below). Genes are transcribed from left to right, except for those underlined. PCGs are marked by white, the A + T-rich region by grey, rRNA genes by red, and tRNA genes by yellow and single-letter amino acid codes.
Gene rearrangements are shown with connecting lines that indicate the translocation of $trnW$ from the $trnW$-$trnC$-$trnY$ cluster to a position downstream of $trnI$, and the swapped position of $trnM$ and $trnQ$.

Table 3. Nucleotide composition of $A. bella$ mitochondrial genome.

Table 4. Codon usage of 13 PCGs in the mitochondrial genome of $A. bella$.

No., frequency of each codon; RSCU, relative synonymous codon usage.

Figure 4. $A. bella$ tRNAs.

Predicted secondary structures of the 22 tRNA genes of $A. bella$. Dashes indicate Watson–Crick base pairing and dots indicate G–U base pairing.

Figure 5. $A. bella$ rrnS.

The predicted secondary structure of $rrnS$ in the $A. bella$ mitochondrial genome. The numbering of helices follows Gillespie et al. (2006), and the domain names follow Niehuis et al. (2006), roman numbers refer to domain names.

Figure 6. $A. bella$ rrnL.

Predicted secondary structure of $rrnL$ in the $A. bella$ mitochondrial genome. The numbering of helices and domain names follows Gillespie et al. (2006), roman numbers refer to domain names.

Figure 7. Phylogenetic tree of Symphyta and selected apocritan and outgroup taxa, based on a Maximum-likelihood analysis of sequence data from 13 PCGs and 2 rRNA genes.

The numbers at the branches represent Maximum-likelihood bootstrap values/Bayesian posterior probabilities. 100/1.00 is denoted by an asterisk. The scale bar indicates the number of substitutions per site.
Table 1 (on next page)

General information of the mitochondrial genomes of Symphyta
| Species                | Length (bp) | Completeness | Family     | Subfamily | Accession number | Resources                     |
|------------------------|-------------|--------------|------------|-----------|------------------|-------------------------------|
| Perga condei           | 13,416bp    | partial      | Pergidae   | Perginae  | AY787816         | Castro and Dowton, 2005       |
| Orussus occidentalis   | 15,947bp    | complete     | Orussidae  | Orussinae | FJ478174         | Dowton et al, 2009a           |
| Trichiosoma anthracinum| 15,392bp    | partial      | Cimbicidae | Cimbicinae| KT921411         | Song et al, 2016              |
| Corynis lateralis      | 14,999bp    | partial      | Cimbicidae | Coryninae | KY063728         | Doğan and Korkmaz, 2017       |
| Monocellicampa pruni   | 15,169bp    | partial      | Tenthredinidae | Hoplocampinae | JX566509 | Wei et al, 2015b |
| Allantus luctifer      | 15,418bp    | complete     | Tenthredinidae | Allantinae | KJ713152 | Wei et al, 2014 |
| Asiemyphus rufoccephalus| 14,864bp | partial | Tenthredinidae | Allantinae | KR703582 | Song et al, 2016 |
| Tenthredo tienmushana  | 14,942bp    | partial      | Tenthredinidae | Tenthredininae | KR703581 | Song et al, 2015 |
| Cephus cinctus         | 19,339bp    | complete     | Cephidae   | Cephinae  | FJ478173         | Dowton et al, 2009a           |
| Cephus pygmeus         | 16,145bp    | partial      | Cephidae   | Cephinae  | KM377623         | Korkmaz et al, 2015           |
| Cephus sareptanus      | 15,212bp    | partial      | Cephidae   | Cephinae  | KM377624         | Korkmaz et al, 2015           |
| Calameuta filiformis   | 20,055bp    | complete     | Cephidae   | Cephinae  | KT260167         | Korkmaz et al, 2016           |
| Calameuta idolon       | 19,746bp    | complete     | Cephidae   | Cephinae  | KT260168         | Korkmaz et al, 2016           |
| Trachelus tudaicus     | 20,370bp    | complete     | Cephidae   | Cephinae  | KX257357         | Korkmaz et al, 2017           |
| Trachelus tabidus      | 18,539bp    | complete     | Cephidae   | Cephinae  | KX257358         | Korkmaz et al, 2017           |
| Hartigia linearis      | 20,116bp    | partial      | Cephidae   | Hartigiinae | KX907843 | Korkmaz et al, 2018 |
| Janus compressus       | 16,700bp    | partial      | Cephidae   | Hartigiinae | KX907844 | Korkmaz et al, 2018 |
| Pachycephus cruentatus | 14,568bp    | partial      | Cephidae   | Hartigiinae | KX907845 | Korkmaz et al, 2018 |
| Pachypephus smyrnensis | 15,203bp    | partial      | Cephidae   | Hartigiinae | KX907846 | Korkmaz et al, 2018 |
| Syrsta parreyssii      | 15,924bp    | partial      | Cephidae   | Hartigiinae | KX907847 | Korkmaz et al, 2018 |
| Characopygus scythicus | 10,558bp    | partial      | Cephidae   | Hartigiinae | KX907848 | Korkmaz et al, 2018 |
Figure 1

*Arge bella* Wei & Du sp. nov.

*Arge bella* Wei & Du sp. nov. Adult female, dorsal view. Scale bars = 1 mm.
Figure 2

Arge bella

Figures a-m. Arge bella Wei & Du sp. nov. a. Head of female, front view; b. Head of female, dorsal view; c. Mesopleuron of female, lateral view; d. Head of female, dorsal view; e. Sheath of female, lateral view; f. Sheath of female, ventral view; g. Serrula of female; h. Antenna of female; i. Head of female, lateral view; j. Tabia of hind leg; k. Lancet; l. Lance; m. Sheath of female, dorsal view.
### Table 2 (on next page)

Characteristics of the mitochondrial genome of *A. bella*

J and N refers to heavy and light strands, respectively; IGN refer to intergenic nucleotides. Minus indicates overlapping sequences between adjacent genes.
Mitochondrial genome characteristics of *A. bella*

| Gene | Strand | Start | Stop | Length(bp) | Start codon | Stop codon | Codon | IGN |
|------|--------|-------|------|-----------|-------------|------------|-------|-----|
| trnW | J      | 152   | 219  | 68        | UGA         |            |       |     |
| trnI | J      | 253   | 323  | 71        | AUC         |            |       | 33  |
| trnM | J      | 354   | 422  | 69        | AUG         |            |       | 30  |
| trnQ | N      | 420   | 488  | 69        | CAA         |            |       | -3  |
| ND2  | J      | 486   | 1,562| 1,077     | ATA         | TAA        | -3    |     |
| trnC | N      | 1,575 | 1,643| 69        | UGC         |            |       | 12  |
| trnY | N      | 1,651 | 1,722| 72        | UAC         |            |       | 7   |
| COX1 | J      | 1,723 | 3,271| 1,549     | ATT         | T          | 0     |     |
| trnL2| J      | 3,272 | 3,336| 65        |             |            |       |     |
| COX2 | J      | 3,337 | 4,017| 681       | ATG         | TAA        | 0     |     |
| trnK | J      | 4,062 | 4,133| 72        | AAG         |            | 44    |     |
| trnD | J      | 4,135 | 4,205| 71        | GAC         |            | 1     |     |
| ATP8 | J      | 4,207 | 4,380| 120       | ATA         | TAA        | 1     |     |
| ATP6 | J      | 4,374 | 5,048| 675       | ATG         | TAA        | -7    |     |
| COX3 | J      | 5,048 | 5,828| 781       | ATG         | T          | -1    |     |
| trnG | J      | 5,829 | 5,894| 66        | GGA         |            | 0     |     |
| ND3  | J      | 5,895 | 6,248| 354       | ATA         | TAA        | 0     |     |
| trnA | J      | 6,264 | 6,328| 65        | GCA         |            | 15    |     |
| trnR | J      | 6,329 | 6,393| 65        | CGA         |            | 0     |     |
| trnN | J      | 6,419 | 6,491| 73        | AAC         |            | 25    |     |
| trnS1| J      | 6,492 | 6,554| 63        | AGC         |            | 0     |     |
| trnE | J      | 6,556 | 6,622| 67        | GAA         |            | 1     |     |
| Gene          | Type | Location | Length | Start | Stop | Codon  | Stop Symbol | Codon Stop |
|--------------|------|----------|--------|-------|------|---------|-------------|------------|
| trnF         | N    | 6,621–6,688 | 68     | UUC   | -2   |         |             |            |
| ND5          | N    | 6,694–8,409 | 1,716  | ATT   | TAA  | 5       |             |            |
| trnH         | N    | 8,410–8,482 | 73     | CAC   | 0    |         |             |            |
| ND4          | N    | 8,483–9,821 | 1,339  | ATG   | T    | 0       |             |            |
| ND4L         | N    | 9,815–10,111| 297    | ATT   | TAA  | -7      |             |            |
| trnT         | J    | 10,114–10,179| 66     | ACA   | 2    |         |             |            |
| trnP         | N    | 10,180–10,246| 67     | CCA   | 0    |         |             |            |
| ND6          | J    | 10,248–10,777| 530    | ATA   | TA   | 1       |             |            |
| CYTB         | J    | 10,778–11,911| 1,134  | ATG   | TAA  | 0       |             |            |
| trnS2        | J    | 11,917–11,985| 69     | UCA   | 5    |         |             |            |
| ND1          | N    | 11,996–12,949| 954    | ATT   | TAA  | 10      |             |            |
| trnL1        | N    | 12,950–13,018| 69     | CUA   | 0    |         |             |            |
| rrnL         | N    | 13,019–14,408| 1,390  | GUA   | 0    |         |             |            |
| trnV         | N    | 14,409–14,475| 67     |         |      |         |             |            |
| rrnS         | N    | 14,476–15,314| 839    |         |      |         |             |            |
Figure 3

Mitochondrial genome organisation of *A. bella*

Mitochondrial genome organisation of insects in general (above) and *A. bella* (below). Genes are transcribed from left to right, except for those underlined. PCGs are marked by white, the A + T rich region by grey, rRNA genes by red, and tRNA genes by yellow and single-letter amino acid codes. Gene rearrangements are shown with connecting lines that indicate the translocation of *trnW* from the *trnW-trnC-trnY* cluster to a position downstream of *trnI* and the swapped position of *trnM* and *trnQ*. 
**Table 3** (on next page)

Nucleotide composition of *A. bella* mitochondrial genome
Nucleotide composition of *A. bella* mitochondrial genome

| Feature                        | Length(bp) | A%  | C%  | G%  | T%  | A+T% | AT-skew | GC-skew |
|--------------------------------|------------|-----|-----|-----|-----|------|--------|---------|
| Whole genome                   | 15,576     | 43.2| 12.2| 7.0 | 37.5| 80.7 | 0.0706 | -0.2708 |
| Protein coding genes           | 11,222     | 35.6| 10.7| 9.9 | 43.8| 79.4 | -0.1033| -0.0388 |
| First codon position           | 3,741      | 33.2| 11.7| 13.0| 42.1| 75.3 | -0.1182| 0.0526  |
| Second codon position          | 3,741      | 33.4| 11.9| 8.4 | 46.4| 79.8 | -0.1629| -0.1724 |
| Third codon position           | 3,741      | 40.2| 8.6 | 8.4 | 42.9| 83.1 | -0.0325| -0.0118 |
| Protein coding genes-J         | 6,928      | 38.2| 13.5| 8.5 | 39.9| 78.1 | -0.0218| -0.2273 |
| First codon position           | 2,310      | 39.8| 12.5| 7.4 | 40.3| 80.1 | -0.0062| -0.2563 |
| Second codon position          | 2,309      | 39.1| 14.1| 9.4 | 37.4| 76.5 | 0.0222 | -0.2000 |
| Third codon position           | 2,309      | 35.7| 13.8| 8.6 | 41.9| 77.6 | -0.0799| -0.2321 |
| Protein coding genes-N         | 4,294      | 31.4| 6.3 | 12.3| 50.0| 81.4 | -0.2285| 0.3226  |
| First codon position           | 1,432      | 34.4| 3.8 | 12.2| 49.6| 84.0 | -0.1810| 0.5250  |
| Second codon position          | 1,431      | 25.6| 9.4 | 15.2| 49.8| 75.4 | -0.3210| 0.2358  |
| Third codon position           | 1,431      | 34.0| 5.7 | 9.5 | 50.8| 84.8 | -0.1981| 0.2500  |
| *ATP6*                         | 675        | 39.1| 14.4| 7.0 | 39.6| 78.7 | -0.0064| -0.3458 |
| *ATP8*                         | 174        | 40.8| 9.2 | 1.1 | 48.9| 89.7 | -0.0903| -0.7864 |
| *COX1*                         | 1,549      | 34.9| 14.2| 12.0| 38.9| 73.8 | -0.0542| -0.0840 |
| *COX2*                         | 681        | 40.7| 13.7| 9.0 | 36.7| 77.4 | 0.0517 | -0.2070 |
| *COX3*                         | 781        | 36.4| 14.3| 10.9| 38.4| 74.8 | -0.0267| -0.1349 |
| *CYTB*                         | 1,134      | 36.1| 14.5| 9.3 | 40.1| 76.2 | -0.0525| -0.2185 |
| *ND1*                          | 954        | 49.9| 14.0| 6.5 | 29.6| 79.5 | 0.2553 | -0.3659 |
| *ND2*                          | 1,077      | 42.6| 11.6| 4.4 | 41.4| 84.0 | 0.0143 | -0.4500 |
| *ND3*                          | 354        | 37.6| 11.9| 8.2 | 42.4| 80.0 | -0.0600| -0.1841 |
| *ND4*                          | 1,339      | 50.0| 12.7| 6.0 | 31.3| 81.3 | 0.2300 | -0.3583 |
| Gene  | Count | Median Length | Interquantiles | Extremes | Nondiff | Extremes | Diff | Nondiff | Extremes |
|-------|-------|---------------|----------------|---------|---------|---------|------|---------|---------|
| ND4L  | 297   | 52.5          | 10.8           | 5.4     | 31.3    | 83.8    | 0.2530 | -0.3333 |
| ND5   | 1,716 | 49.8          | 11.2           | 6.5     | 32.5    | 82.3    | 0.2102 | -0.2655 |
| ND6   | 530   | 41.9          | 12.1           | 5.3     | 40.8    | 82.7    | 0.0133 | -0.3908 |
| rrnL  | 1,390 | 45.2          | 11.1           | 5.0     | 38.8    | 84.0    | 0.0762 | -0.3789 |
| rrnS  | 839   | 43.7          | 11.6           | 5.4     | 39.3    | 83.0    | 0.0530 | -0.3647 |
Table 4 (on next page)

Codon usage of 13 PCGs in the mitochondrial genome of *A. bella*

No., frequency of each codon; RSCU, relative synonymous codon usage.
### Codon usage of 13 PCGs in mitochondrial genome of *A. bella*

| Amino acid | Codon | NO.  | RSCU | Amino acid | Codon | NO.  | RSCU |
|------------|-------|------|------|------------|-------|------|------|
| Phe        | UUU   | 355  | 1.83 | Tyr        | UAU   | 147  | 1.76 |
|            | UUC   | 32   | 0.17 |            | UAC   | 20   | 0.24 |
| Leu        | UUA   | 459  | 4.85 | End        | UAA   | 0    | 0    |
|            | UUG   | 29   | 0.31 |            | UAG   | 0    | 0    |
| Leu        | CUU   | 31   | 0.33 | His        | CAU   | 53   | 1.66 |
|            | CUC   | 6    | 0.06 |            | CAC   | 11   | 0.34 |
|            | CUA   | 45   | 0.47 | Gln        | CAA   | 55   | 1.75 |
|            | CUG   | 0    | 0    |            | CAG   | 8    | 0.25 |
| Ile        | AUU   | 396  | 1.81 | Asn        | AAU   | 214  | 1.75 |
|            | AUC   | 42   | 0.19 |            | AAC   | 30   | 0.25 |
| Met        | AUA   | 308  | 1.9  | Lys        | AAA   | 128  | 1.83 |
|            | AUG   | 17   | 0.1  |            | AAG   | 12   | 0.17 |
| Val        | GUU   | 62   | 2.12 | Asp        | GAU   | 53   | 1.71 |
|            | GUC   | 0    | 0    |            | GAC   | 9    | 0.29 |
|            | GUA   | 52   | 1.78 | Glu        | GAA   | 66   | 1.74 |
|            | GUG   | 3    | 0.1  |            | GAG   | 10   | 0.26 |
| Ser        | UCU   | 87   | 2.21 | Cys        | UGU   | 39   | 1.9  |
|            | UCC   | 7    | 0.18 |            | UGC   | 2    | 0.1  |
|            | UCA   | 112  | 2.85 | Trp        | UGA   | 83   | 1.93 |
|            | UCG   | 3    | 0.08 |            | UGG   | 3    | 0.07 |
| Pro        | CCU   | 63   | 1.92 | Arg        | CGU   | 7    | 0.6  |
|            | CCC   | 16   | 0.49 |            | CGC   | 1    | 0.09 |
| Codon | Thr | Gly |
|-------|-----|-----|
| CCA   | 52  | 1.59 |
| CCG   | 0   | 0   |
| ACU   | 62  | 1.46 |
| ACC   | 13  | 0.31 |
| ACA   | 93  | 2.19 |
| ACG   | 2   | 0.05 |
| GCU   | 55  | 2.08 |
| GCC   | 8   | 0.3  |
| GCA   | 42  | 1.58 |
| GCG   | 1   | 0.04 |
| CGA   | 36  | 3.06 |
| CGG   | 3   | 0.26 |
| AGU   | 27  | 0.69 |
| AGC   | 2   | 0.05 |
| AGA   | 75  | 1.91 |
| AGG   | 1   | 0.03 |
| GGU   | 32  | 0.7  |
| GGC   | 2   | 0.04 |
| GGA   | 130 | 2.86 |
| GGG   | 18  | 0.4  |
Figure 4

*A. bella* tRNAs

Predicted secondary structures of the 22 tRNA genes of *A. bella*. Dashes indicate Watson–Crick base pairing and dots indicate G-U base pairing.
Figure 5

*A. bella rrnS*

The predicted secondary structure of *rrnS* in the *A. bella* mitochondrial genome. The numbering of helices follows Gillespie et al. (2006), and the domain names follows Niehuis et al. (2006), roman numbers refer to domain names.

*Note: Auto Gamma Correction was used for the image. This only affects the reviewing manuscript. See original source image if needed for review.*
Symbols Used In This Diagram:

- A-U - Canonical base pair (A-U, C-G)
- G-U - G-U base pair
- G-A - G-A base pair
- U-U - Non-canonical base pair (U-U, A-A, C-C, C-U)

Every 10th nucleotide is marked with a tick mark, and every 50th nucleotide is numbered.
Figure 6

*A. bella rrnL*

Predicted secondary structure of *rrnL* in the *A. bella* mitochondrial genome. The numbering of helices and domain names follows Gillespie et al. (2006), roman numbers refer to domain names.

*Note: Auto Gamma Correction was used for the image. This only affects the reviewing manuscript. See original source image if needed for review.*
Figure 7

Phylogenetic tree of Symphyta and selected apocritan and outgroup taxa, based on a Maximum-likelihood analysis of sequence data from 13 PCGs and 2 rRNA genes.

The numbers at the branches represent Maximum-likelihood bootstrap values/Bayesian posterior probabilities. 100/1.00 is denoted by an asterisk. The scale bar indicates the number of substitutions per site.