

An evaluation of ensign wasp classification (Hymenoptera: Evaniidae) based on molecular data and insights from ribosomal RNA secondary structure

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Supplementary Information

DNA Extraction

Prior to DNA extraction the propleura and front legs were separated from the mesothorax, and the head, metasoma, and right hind leg were removed from each specimen. The entire mesosoma, including legs and propleura, was then treated in one of two ways: 1) submerged in an extraction buffer (0.05 M Tris HCl, 0.01 M ethylene diamine tetra acetic acid, 0.5% sodium dodecyl sulphate, 50 mM NaCl) and incubated with 0.25-.40 μ l proteinase K for 4-12 hours at 40°C, followed by a standard phenol-chloroform extraction technique, or 2) processed using the QIAGEN (Valencia, CA, U.S.A.) DNeasy Tissue Kit protocol for animal tissues. The resulting DNA extracts were suspended in 15-40 μ l ddH₂O (for phenol-chloroform technique) or 50-150 μ l buffer AE (10 mM Tris·Cl; 0.5 mM EDTA; pH 9.0) depending on the size of the wasp/pellet.

PCR and Sequencing Protocols

Primers used to amplify and sequence COI, and the 16S and 28S rRNAs are listed in Table S-1. PCRs were performed using an Eppendorf Mastercycler (Eppendorf AG, Hamburg, Germany). Settings for amplification of 16S and 28S rRNA fragments were: 3 min at 94°C (initial denaturation), 36 cycles of 1 min at 94°C (denaturation), 1 min at 48°C (annealing), and 1 min at 68°C (elongation), followed by a final 5 min at 72°C for extension. Amplification settings for COI followed Hebert et al. (2003).

PCR products were purified using QIAquick PCR purification kit (QIAGEN, Inc., Valencia, CA, U.S.A.) and sequenced using the primers from Table 2 and BigDye version 3.1 (Applied Biosystems, Foster City, U.S.A.) The sequencing reactions were either purified using ethanol precipitation and run on an ABI 377 DNA sequencer (Applied Biosystems), or they were sent to the W. M. Keck Center for Comparative and Functional Genomics at the University of Illinois (UIUC) for purification and subsequent analysis on an ABI 3730XL sequencer.

The Folmer et al. (1994) primers amplified *Wolbachia* sequences in three taxa: *Decevania* 005, *Evaniella* 039, and *Zeuxevania* 028 (determined using the BLAST tool in GenBank). These fragments were excluded from our analyses and deposited in GenBank with the following accession numbers, respectively: AY800177, DQ192288, DQ192289.

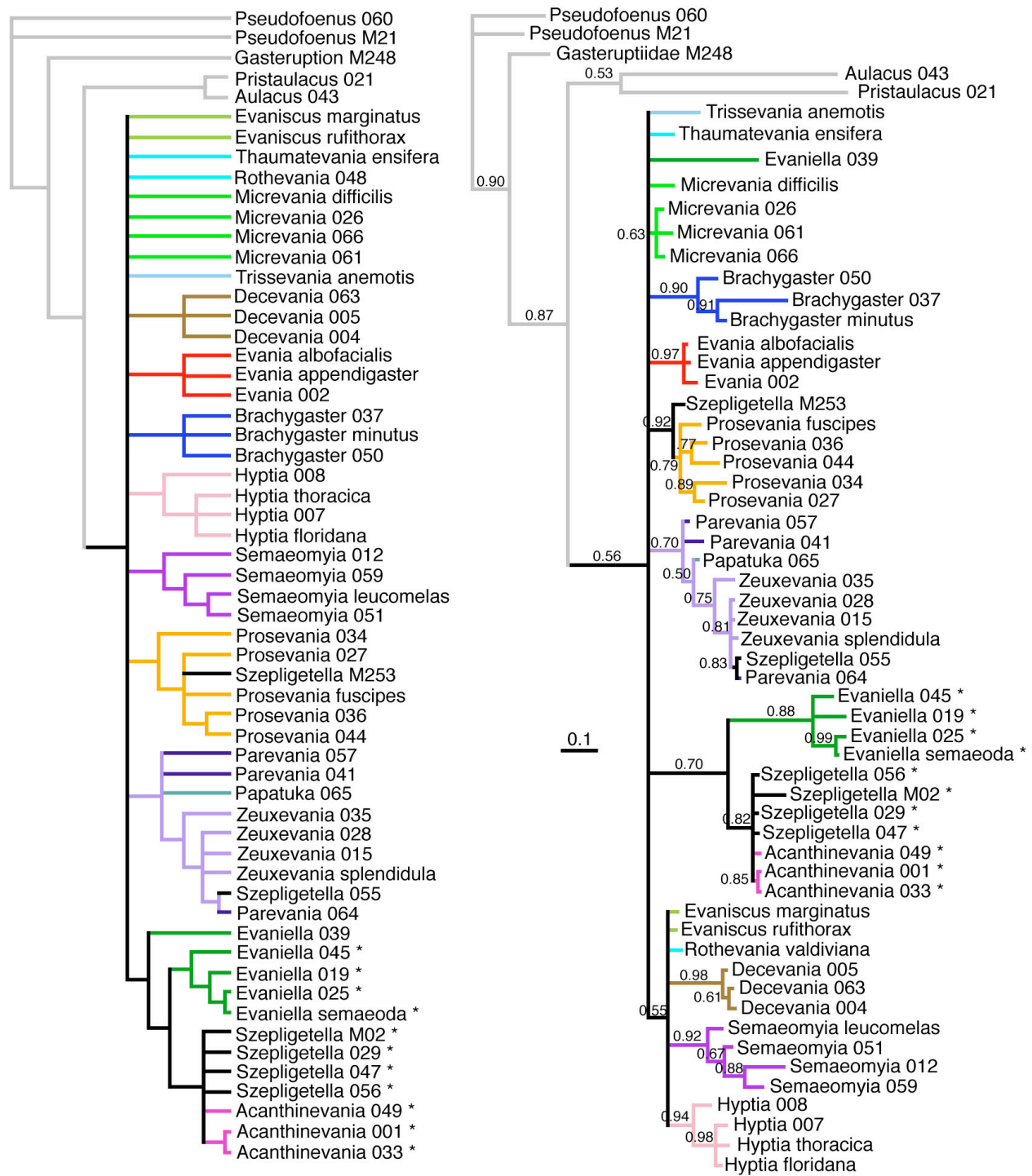
Sequence Editing

All sequences were edited using SeqEd (v. 1.0.3, Applied Biosystems, Inc., Foster City, CA, U.S.A.). During the editing of each strand, nucleotides that were readable but showed either irregular spacing between peaks or had some significant competing background peak were coded with IUPAC-IUB ambiguity codes. If these ambiguity codes dictated amino acid changes (in COI) or disruptions to conserved helices (in the rRNAs), we reevaluated the electropherograms and/or resequenced that taxon. We used MacClade 4.0 (Maddison & Maddison, 2000) to color-code the COI sequences for translated amino acid state to check for stop codons and gap-induced shifts to the reading frame.

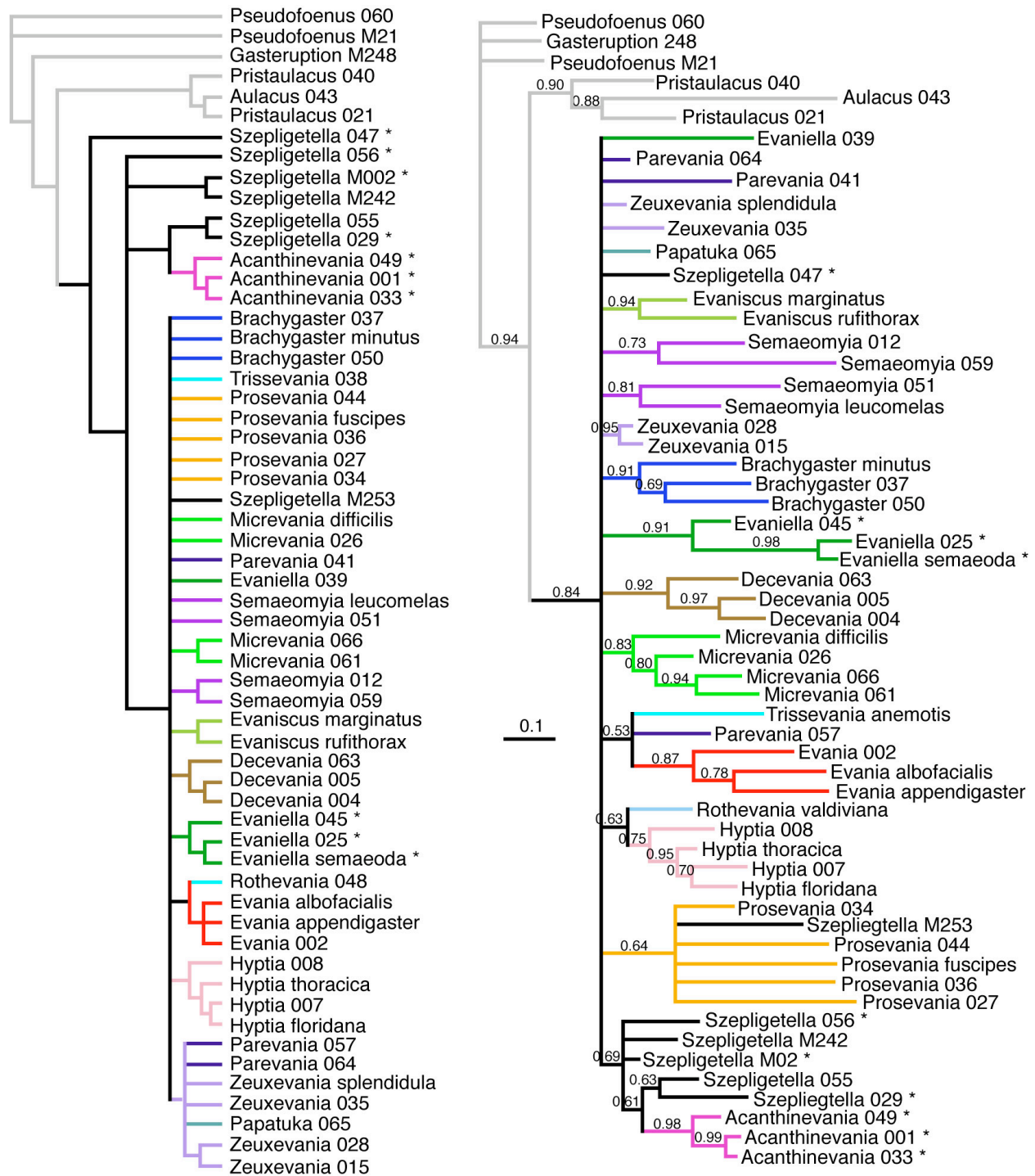
Table S-1. Primers used in this study with original references.

gene and primer sequence		reference
16S Wb:	5'-CACCTGTTTATCAAAAACAT-3'	Dowton and Austin 1994
16S outer:	5'-CTTATTCAACATCGAGGTC-3'	Whitfield 1997
16S-Dowton:	5'-CGTCGATTTGAACTCAAATC-3' *	Dowton and Austin 1994
28S-Dowton-F:	5'-AGAGAGAGTTCAAGAGTACGTG-3'	Dowton and Austin 2001
28S-Dowton-R:	5'-TTGGTCCGTGTTTCAAGACGGG-3'	Dowton and Austin 2001
COI-Dowton-F:	5'-TTTTTTGGTCATCCAGAAGT-3'	Dowton and Austin 2001
COI-Dowton-R:	5'-ATTGCAAATACTGCACCTAT-3'	Dowton and Austin 2001
COI-LCO1490:	5'-GGTCAACAAATCATAAAGATATTGG-3'	Folmer et al. 1994
COI-HCO2198:	5'-TAAACTTCAGGGTGACCAAAAAATCA-3'	Folmer et al. 1994

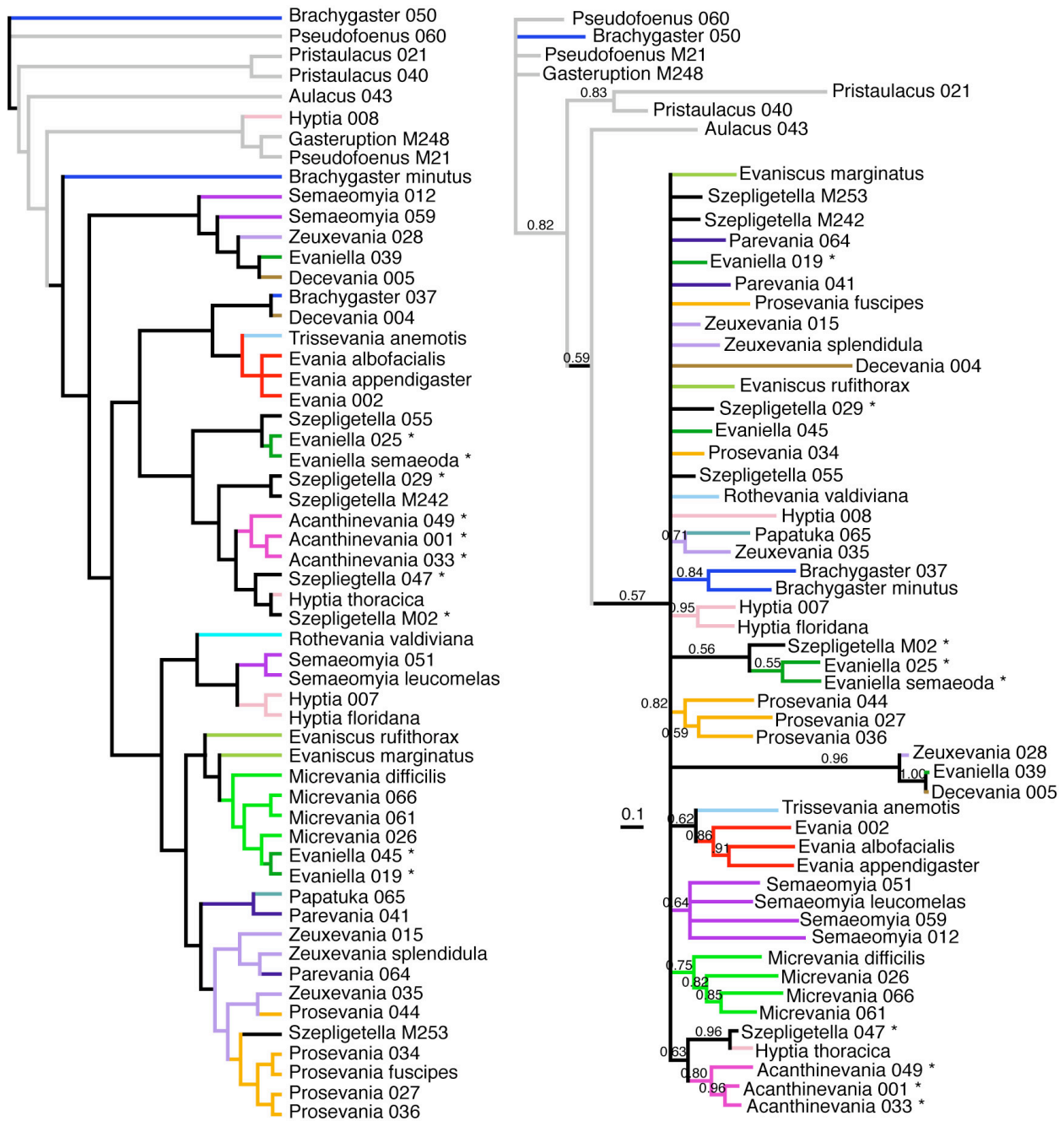
*used instead of 16S outer for some taxa



Figures S1-2. 28S rRNA phylogenies. Figure S1 (left) - 28S rRNA dataset analyzed in PAUP* using unweighted parsimony. Conditions: TBR branch swapping, 100 random sequence additions. Figure S2 (right) - 28S rRNA dataset analyzed in MrBayes under GTR+I+G model with helices and non-helices partitioned and unlinked. Conditions: nchains=10 temp=0.1 ngen=2000000. Numbers refer to posterior probabilities. Taxa with the 28S rRNA 2c-2 helix indicated with asterisks (*). 28S rRNA was not available for *Szepligetella* M242.



Figures S3-4. 16S rRNA phylogenies. Figure S3 (left) - 16S rRNA dataset analyzed in PAUP* using unweighted parsimony. Conditions: TBR branch swapping, 100 random sequence additions. Figure S4 (right) - 16S rRNA dataset analyzed in MrBayes under GTR+I+G model with helices and non-helices partitioned and unlinked. Conditions: nchains=10 temp=0.1 ngen=2000000. Numbers refer to posterior probabilities. Taxa with the 28S rRNA 2c-2 helix indicated with asterisks (*). 28S rRNA was not available for *Szepligetella* M242.



Figures S5-6. COI phylogenies. Figure S5 (left) - COI dataset analyzed in PAUP* using unweighted parsimony. Conditions: TBR branch swapping, 100 random sequence additions. Figure S6 (right) - COI dataset analyzed in MrBayes under GTR+I+G model with all three codon positions partitioned and unlinked. Conditions: nchains=10 temp=0.1 ngen=2000000. Numbers refer to posterior probabilities. Taxa with the 28S 2c-2 helix indicated with asterisks (*). 28S rRNA was not available for *Szepligetella* M242.