

Molecular Phylogenetics of the Cricket-Assassin Wasps (Hymenoptera: Rhopalosomatidae)

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Introduction

- Rhopalosomatid wasps are cricket ectoparasites. The family is composed of 4 extinct and 72 extant species found within 4 genera including: *Olixon*, *Liosphex*, *Rhopalosoma*, and *Paniscomima*.
- No family-level molecular analysis has ever been performed.

Objectives

- Create the first family-level molecular phylogeny of the family Rhopalosomatidae using the barcoding gene *COI* and nuclear-encoding gene *EF-1a*.
- Use phylogenetic trees to explore rhopalosomatid inter-generic relationships.



Figure 1. (a) Adult *Olixon*. Photo: Volker Lohrmann. (b) Cricket host with an attached rhopalosomatid larva. Photo: Paul Bertner.

Methods

- DNA Extraction using Qiagen® DNeasy Blood and Tissue Kit.
- PCR Amplification with 50 µL PCR reactions; pre-made and custom primers for *COI*; BIO-RAD T100™ Thermal Cycler; verification by gel electrophoresis.
- Bidirectional sequencing performed by GENEWIZ®.
- Editing/Aligning of nucleotide sequences using Geneious Prime 2021.0.1.
- Phylogenetic analyses using Maximum likelihood (ML), RAxML-HPC v.8 on XSEDE 8.2.12, and Bayesian analyses online via CIPRES portal using MrBayes on XSEDE 3.2.7a; performed using data sets from sequences generated and from GenBank® and BOLD Systems; visualized in Geneious Prime 2021.0.1.
- Bootstrap support (BS) values used for interpretation of statistical significance in Geneious Prime 2021.0.1.

Results

- Thirty-one new specimens were sequenced and recovered within Rhopalosomatidae with 100% BS (Figure 3).
- Olixon* was found to be monophyletic with 92% BS.
- The clade containing *Liosphex* was also recovered monophyletically with 100% BS.
- Paniscomima* was recovered within *Rhopalosoma* with 99% BS.
- Rhopalosoma* specimens from North America were recovered in two separate clades with 100% BS.
- The addition of *EF-1a* produced the same tree topology (Figure 4) while reducing the branch lengths within *Paniscomima*.

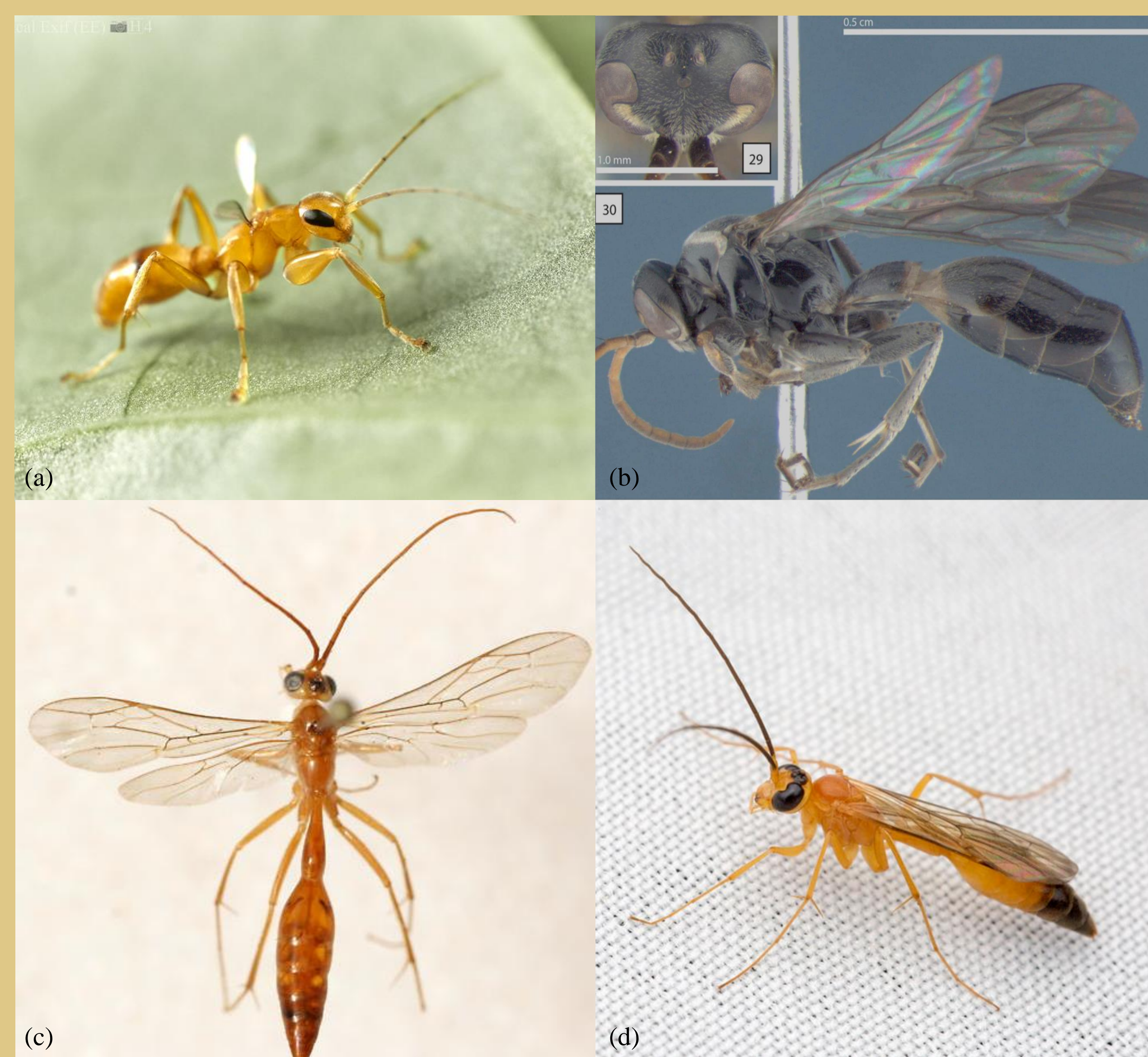


Figure 2. (a) Adult *Olixon* wasp. Photo: Paul Bertner. (b) Adult *Liosphex* wasp. Photo: Lohrmann and Ohl (2010). (c) Adult *Rhopalosoma* wasp. Photo: Ashley Bradford. (d) Adult *Paniscomima* wasp. Photo: budak.

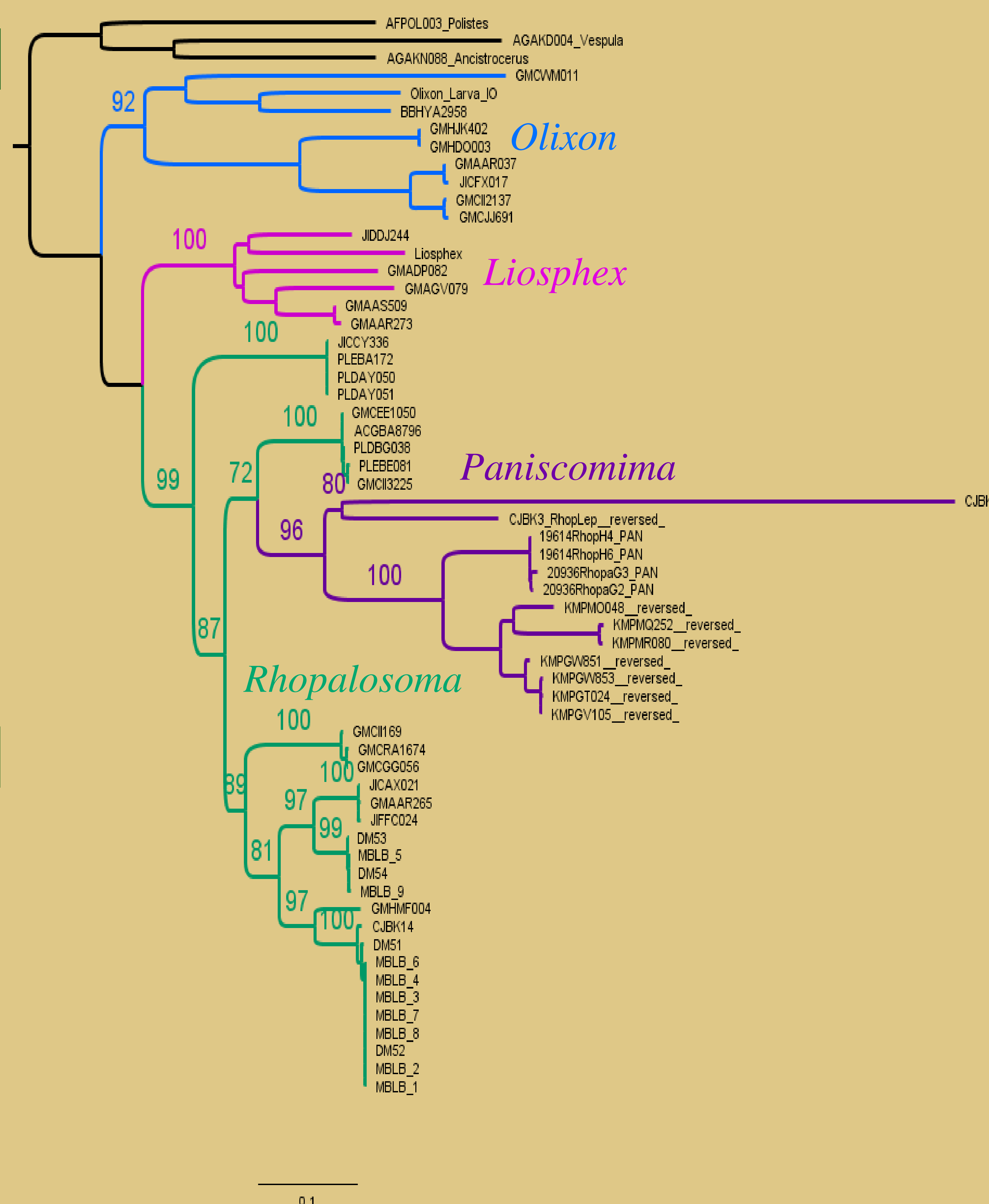


Figure 3. ML Phylogenetic tree made using *COI* showing the placement of each of the 4 genera within Rhopalosomatidae (BS values on nodes show statistical support).

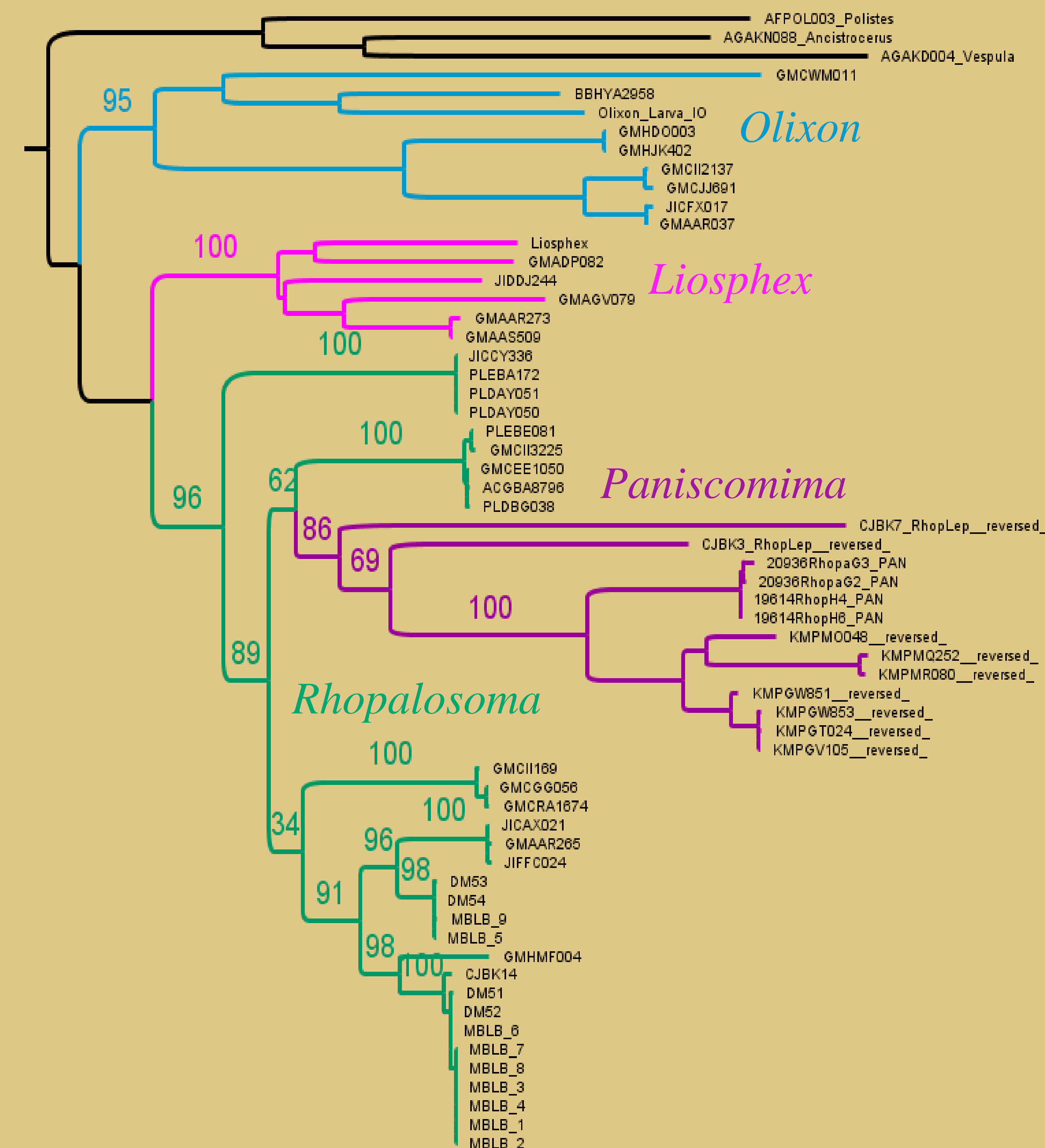


Figure 4. ML phylogenetic tree made using *COI* and *EF-1a* showing the placement of the 4 genera within Rhopalosomatidae when 2 phylogenetic signals are included (BS values on nodes show statistical support).

Discussion

- COI* and *EF-1a* proved to be useful genes for the analyses of Rhopalosomatidae despite incomplete amplification of *EF-1a*.
- For Maximum Likelihood, a BS score of 75 is needed to be statistically significant. Using 10,000 replicates of trees, significant statistical support can be seen throughout both phylogenetic trees (Figures 3 and 4).
- Each genus was recovered with robust support, as seen with *Olixon* and *Liosphex*.
- Olixon* was recovered monophyletic, confirming morphological hypotheses.
- The close relationship between *Paniscomima* and *Rhopalosoma* is apparent with *Rhopalosoma* being recovered paraphyletic in relation to *Paniscomima*.
 - This indicates that species of *Paniscomima* may not legitimately belong to a different genus.
 - Rhopalosoma* may represent new world species while *Paniscomima* includes old world species.
 - The phylogenetic signals present from two genes produced the exact same topology as the tree created using only *COI*, indicating that the use of *EF-1a* strengthens the need for synonymizing these two genera (Figure 4).
- Additional genes such as *POL* and *RSPS23* could be used to further explore the relationships within this family of wasps.