Investigating the Molecular Evolution of *COI* as Evidence of Potential Synonymy of *Paniscomima* and *Rhopalosoma* (Rhopalosomatidae)

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Introduction

- The cricket-assassin wasps of family Rhopalosomatidae are one of the few remaining taxa within Hymenoptera lacking a comprehensive family-level molecular analysis.
- The relatively small morphological distinctions between genera and species within Rhopalosomatidae make molecular analyses an important source of taxonomically informative data.
- The two closely related genera *Rhopalosoma* (New World) and *Paniscomima* (Old World) are separated by geography and the presence of small tooth on the tarsal claw (Figures 1 and 2).
- An initial phylogeny created with 73 *COI* sequences recovered *Paniscomima* within the clade of *Rhopalosoma* rendering the latter paraphyletic.
- When potential conflict occurs between molecular evidence and morphology-based classification, as is the case here, a thorough investigation of the quality of the molecular evidence is required.

Objectives

- Thoroughly explore evidence of *Paniscomima* and *Rhopalosoma* synonymy by investigating the molecular evolution of the *COI* gene.
- Locate potential conflicting signals within *COI* by comparing individual codon positions.
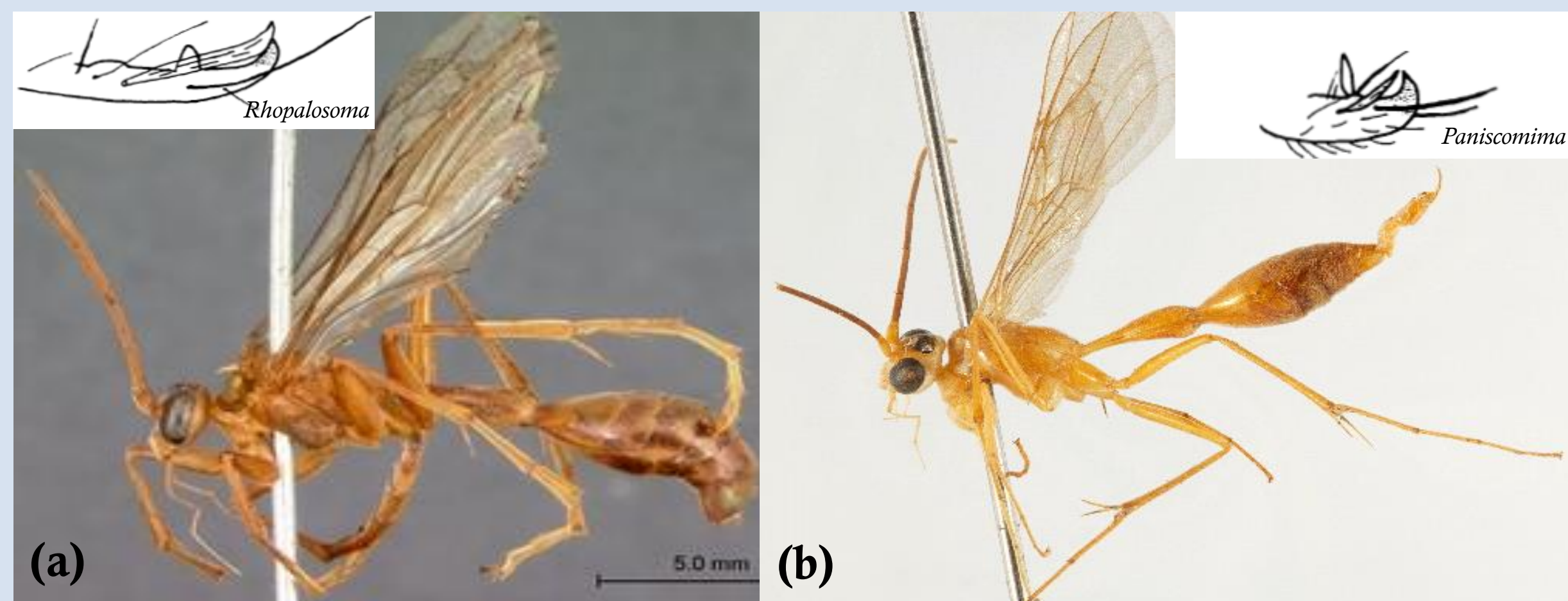- Test for base composition bias and mutational saturation of codon positions.

Figure 1. Two closely related rhopalosomatid genera that molecular evidence indicates may belong to a single genus (a) *Rhopalosoma* Photo: Volker Lohrmann; (b) *Paniscomima,* Photo: CBG Photography Group
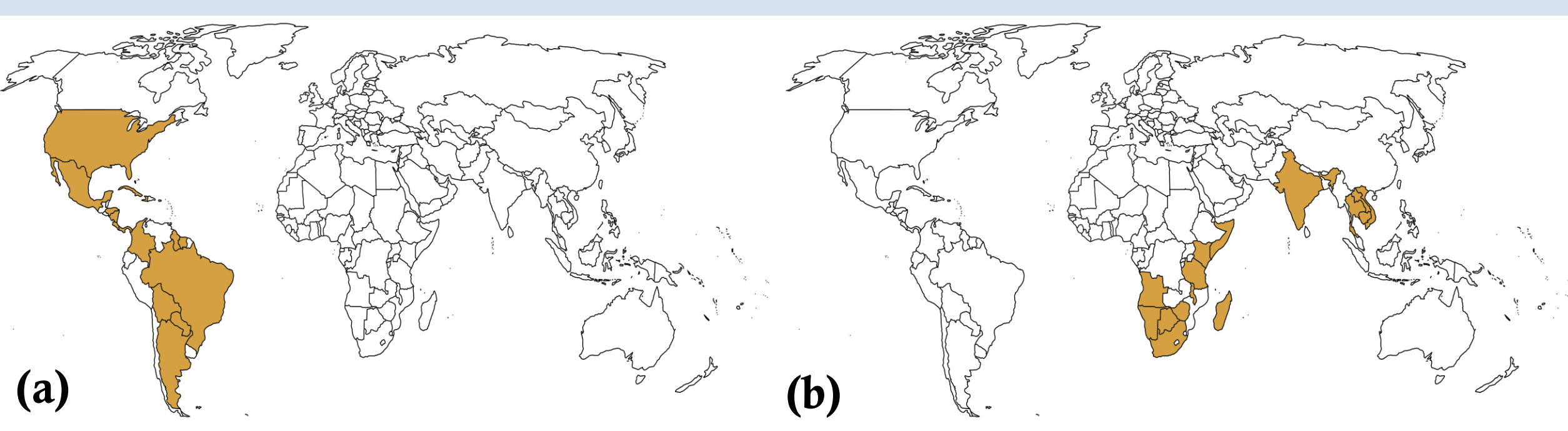

Figure 2. Biogeography maps showing (a) *Rhopalosoma* species, Map: Guidotti 1999; (b) *Paniscomima* species, Map: Guidotti 1999 and Lohrmann 2011
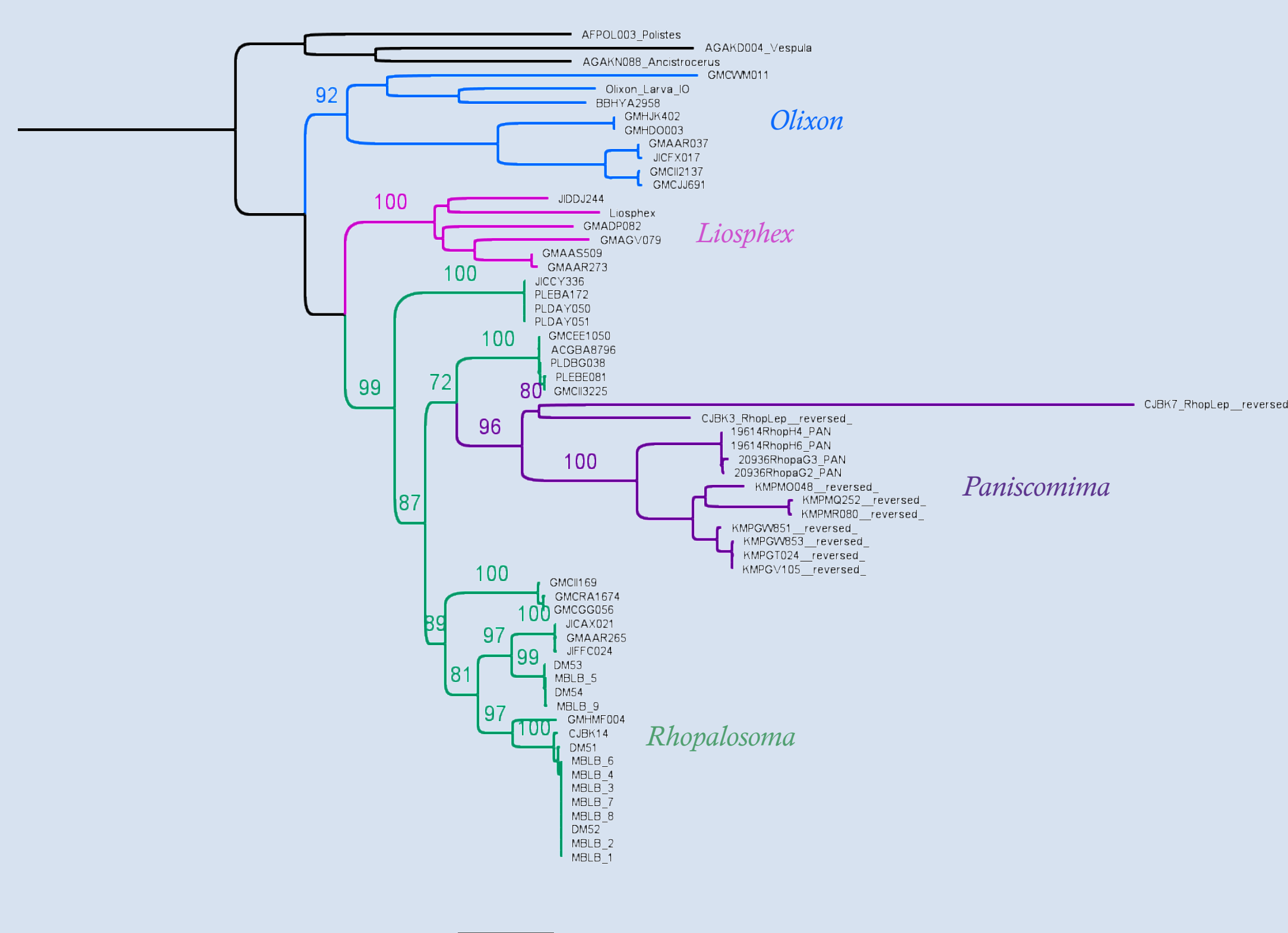

Figure 3. ML Phylogenetic tree with 73 *COI* sequences showing the placement of Rhopalosomatidae genera (BS values on nodes show statistical support).
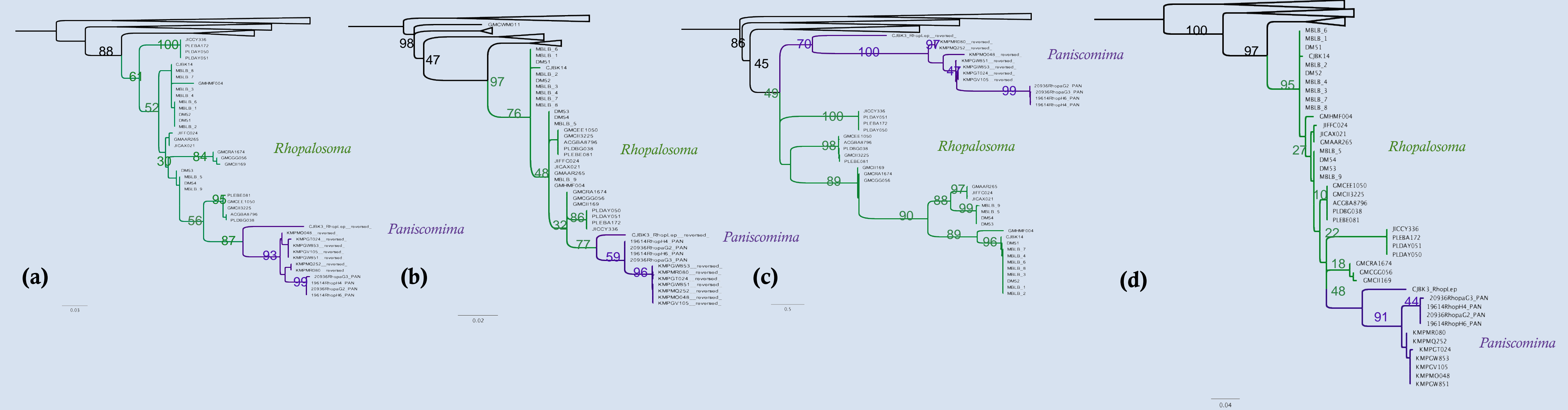

Figure 4. Phylogenetic trees including each codon position of *Rhopalosoma* and *Paniscomima COI* sequences; (a) Codon 1; (b) Codon 2; (c) Codon 3; (d) Codons 1 and 2

Methods

- DNA Extraction was performed using the Qiagen® DNeasy Blood and Tissue Kit.
- PCR Amplification involved 50 μL PCR reactions; with custom primers for *COI*; BIO-RAD T100™ Thermal Cycler; verification by gel electrophoresis.
- DNA Sequencing was performed with GENEWIZ®.
- DNA Editing/Aligning used Geneious Prime 2021.0.1.
- Creation of Phylogenetic trees was performed using Maximum likelihood (ML), RAxML-HPC v.8 on XSEDE 8.2.10, online via CIPRES portal; these were performed on data sets from sequences obtained and from GenBank® and BOLD Systems; visualized in Geneious Prime 2021.0.1.
- Phylogenies were interpreted with bootstrap scores showing intergeneric and interspecific distances, Geneious Prime 2021.0.1.
- To identify potential conflicting signals, phylogenies were constructed for each codon position independently and for codon positions 1 + 2 combined. A final tree was made with a 2:4:1 codon position weight.
- DAMBE® was used for identification of mutations, and calculating base composition bias and transition/transversion ratios (ti/tv) at each codon position.
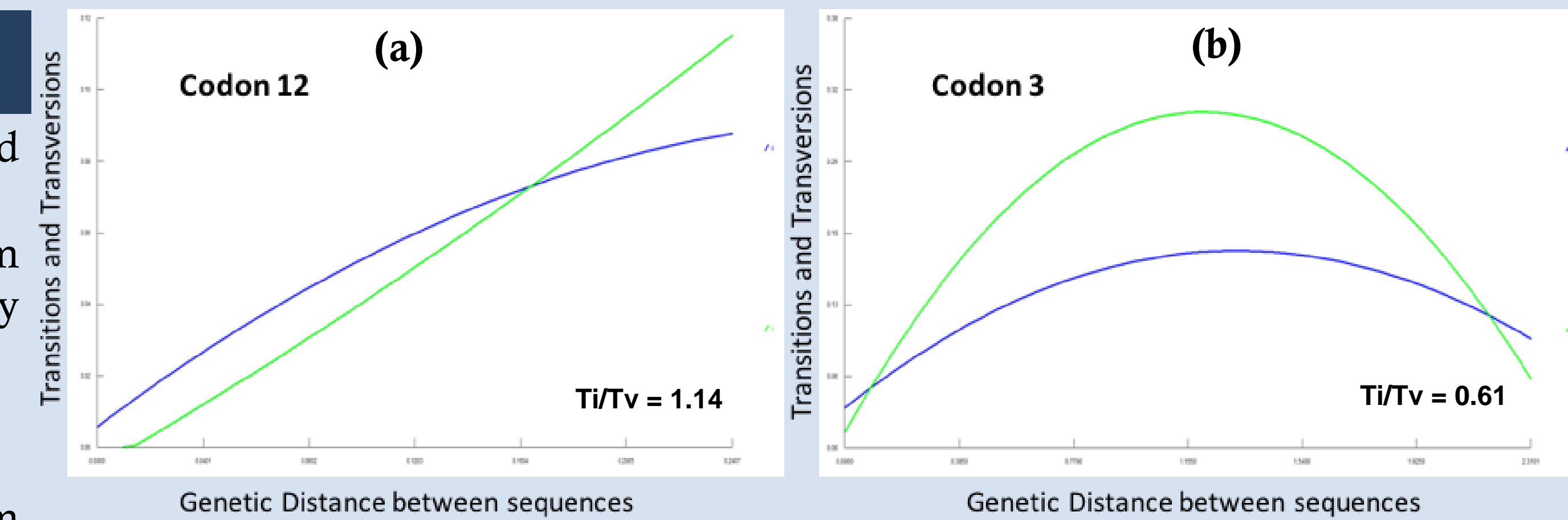

Figure 5. Saturation curves of (a) Codons 1 and 2 (b) Codon 3

Results

- The initial phylogenetic tree was created with 73 unique *COI* sequences from all 4 Rhopalosomatidae genera and including specimens from around the world (Figure 2).
- Unexpectedly, *Paniscomima* was recovered nested within *Rhopalosoma*.
- Codons 1 and 2 produced phylogenies showing *Paniscomima* as a highly derived lineage of *Rhopalosoma* wasps (Figure 4).
- Codon 3 produced a phylogeny with *Paniscomima* and *Rhopalosoma* as two monophyletic sister clades; agreeing with morphological evidence.
- However, evidence from base composition bias, ti/tv ratios, and mutational saturation curves strongly indicates almost complete saturation of the third codon position by redundant mutations (Figure 5).
- After the removal of codon position 3 due to saturation, the codon position 1 + 2 tree confirmed the paraphyly of *Rhopalosoma* with respect to *Paniscomima*. However, node support for *Rhopalosoma* species was low due to the missing information provided by codon position 3 (Figure 4).
- The final tree, constructed with a 2:4:1 weighted dataset for codon positions recovered *Paniscomima* nested within *Rhopalosoma* with high statistical support and all species of *Rhopalosoma* with high node support (Figure 6).
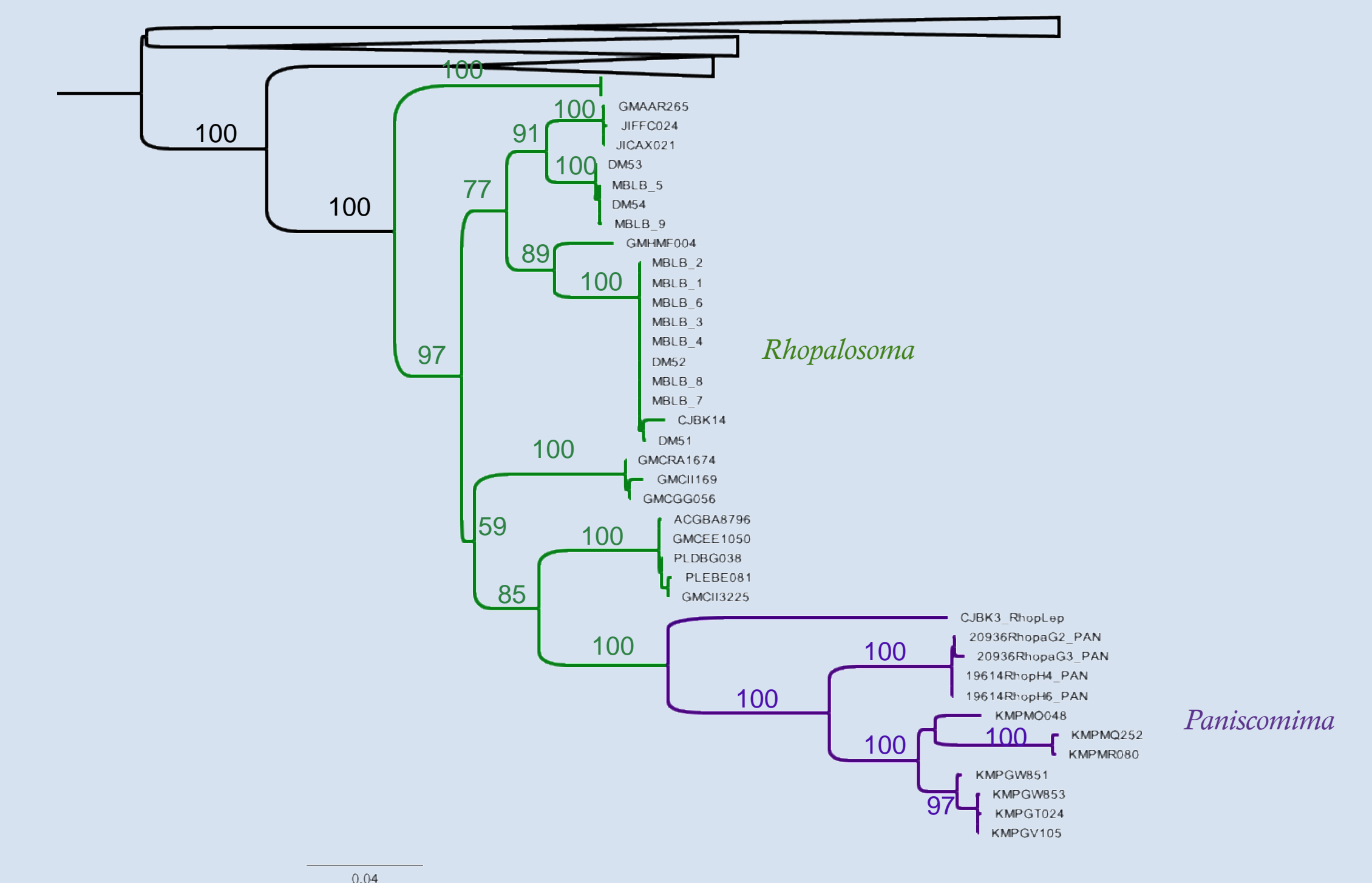

Figure 6. Phylogenetic tree with weighted codons positions 2:4:1 to show accurate evolutionary history.

Discussion

- Each codon position of *COI* carries a different phylogenetic signal.
- The third codon position has evidence of significant saturation from nucleotide bias and transversion bias.
- The third codon position is helpful for distinguishing between species, but obscures signal at deeper nodes.
- The phylogenetic signal coming from *COI* strongly suggests that *Paniscomima* is a highly derived lineage of *Rhopalosoma*, thus indicating the need to synonymize these two nominal genera.
- The final tree allows a biogeographical narrative of a *Rhopalosoma* species colonizing the Old World and diversifying into the "*Paniscomima*" lineage.

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