



UNCOVERING SPECIES BOUNDARIES IN THE NEOTROPICAL ANT COMPLEX *Ectatomma ruidum* (ECTATOMMINAE) UNDER THE PRESENCE OF NUCLEAR MITOCHONDRIAL PARALOGS



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Introduction

The use of taxonomic approaches that make use of information obtained from different sources of evidence represents a valuable option for the accurate delineation of species. Mitochondrial (mt) DNA sequences have been by far the most employed molecular markers for species delineation analyses due to its matrilineal inheritance and higher coalescence ratio in comparison to nuclear genes (Palumbi et al., 2001). Various phenomena, however, can obscure the accurate delineation of species using this locus, including incomplete lineage sorting (Funk and Omland, 2003), mt introgression (Bryson et al, 2014) and nuclear mt pseudogenes (numts; Song et al., 2014).

Ectatomma Smith (Ectatomminae) is one of the most common groups of foraging Neotropical ants, currently comprising 15 recognised species distributed from northern Mexico to central Argentina (Bolton, 2014; Fernández, 1991). *Ectatomma ruidum* (Roger) is, one of the species of the genus with widest geographic distribution, with records ranging from Mexico to Brazil (Bolton, 2014). We assessed the number of speciation events that could have occurred within *E. ruidum* based on specimens collected from a number of localities along its known geographic distribution. For this, we followed an integrative taxonomic criterion and employed two mitochondrial and one nuclear markers as well as external morphological features.

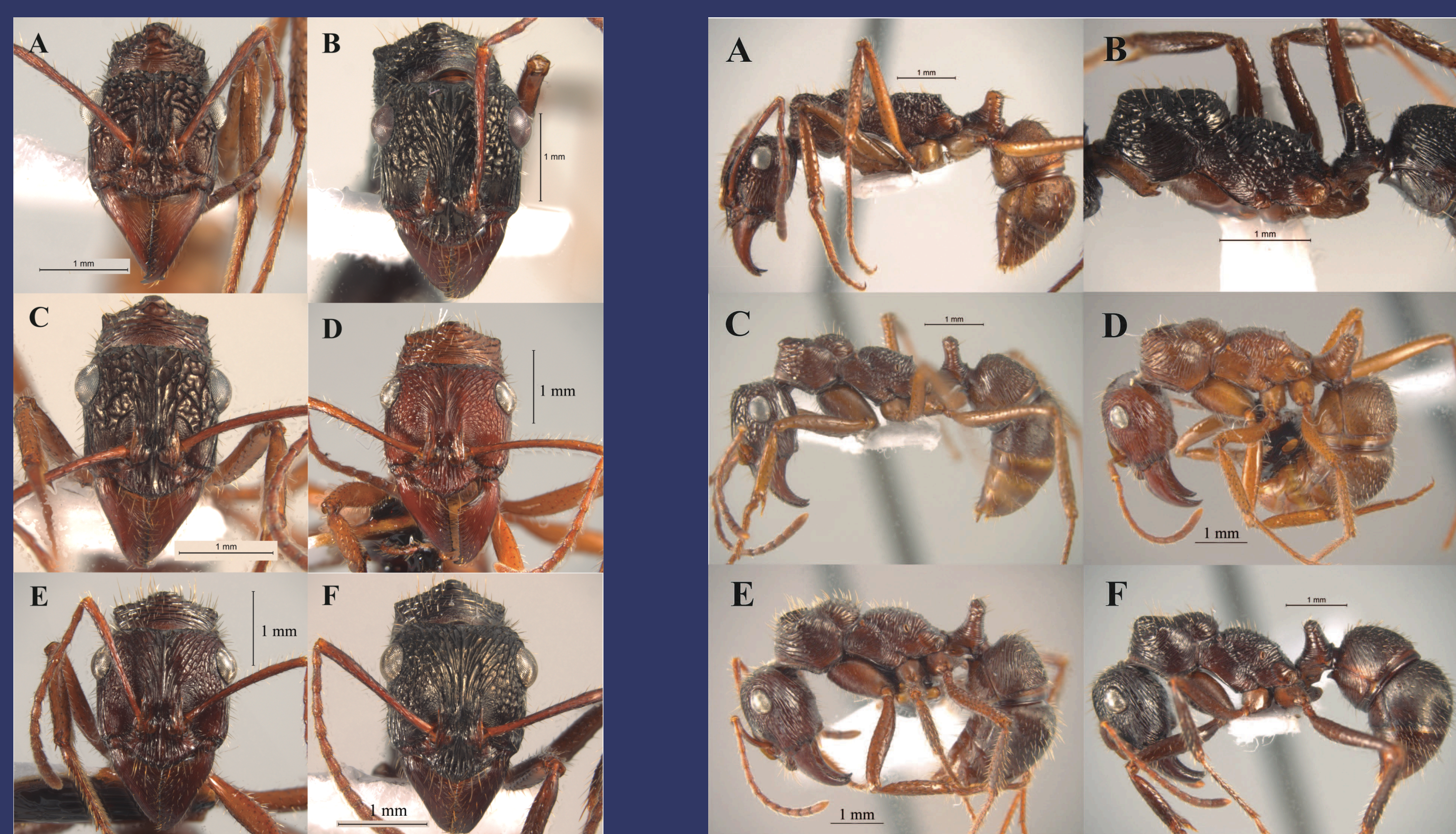


Figure 1: Digital pictures showing the head (full face), pronotal hump and lateral view of representative specimens belonging to the four delimited morphospecies originally assigned to *E. ruidum*: A) *E. gibbum*; B) *E. ruidum*; C) *E. sp. 'ruidum 2'*; D) *E. sp. 'ruidum 3'*; E) *E. sp. 'ruidum 4'*; F) *E. sp. 'ruidum 2 x sp. ruidum 3'*.

Materials and methods

We examined newly generated and previously published sequences of 138 specimens originally assigned to *E. ruidum* and *E. gibbum*. The taxon sampling included specimens collected from a number of localities along the Neotropics (Figure 2), with an emphasis on southeast Mexico. We markers included 632 bp fragment of the COI and 792 bp of the cytochrome b (cyt b) mtDNA genes, and 260 bp of the Histone H3 protein (H3) encoding nuclear gene. Nuclear mt paralogs were detected based on the presence of codon position substitution bias, distinct rates of evolution, internal stop codons, indels, apparent polymorphism in chromatograms and incongruent phylogenetic. The presence of recombination in the nuclear H3 gene was assessed using TOPALi version 2.5 (Milne et al., 2009).

Separate partitioned Bayesian analyses were performed both including the originally generated COI and cyt b sequences and those recovered with a pre-PCR dilution procedure. Additional Bayesian analyses were carried out for each mt marker and for a concatenated COI + cyt b matrix excising all sequences regarded as numts.

Species delimitation was assessed using Wiens and Penkrot's (2002) approach based on mtDNA haplotype phylogenies. In this approach, the discordance between haplotype clades and the geographic provenance of their individuals is assumed to represent evidence of gene flow. Therefore, the number of potential species can be assessed by the presence of lineages that are concordant with geography. Morphospecies were discriminated based on nine external morphological characters examined from a vast number of worker specimens belonging to all the populations sampled for the molecular analyses.

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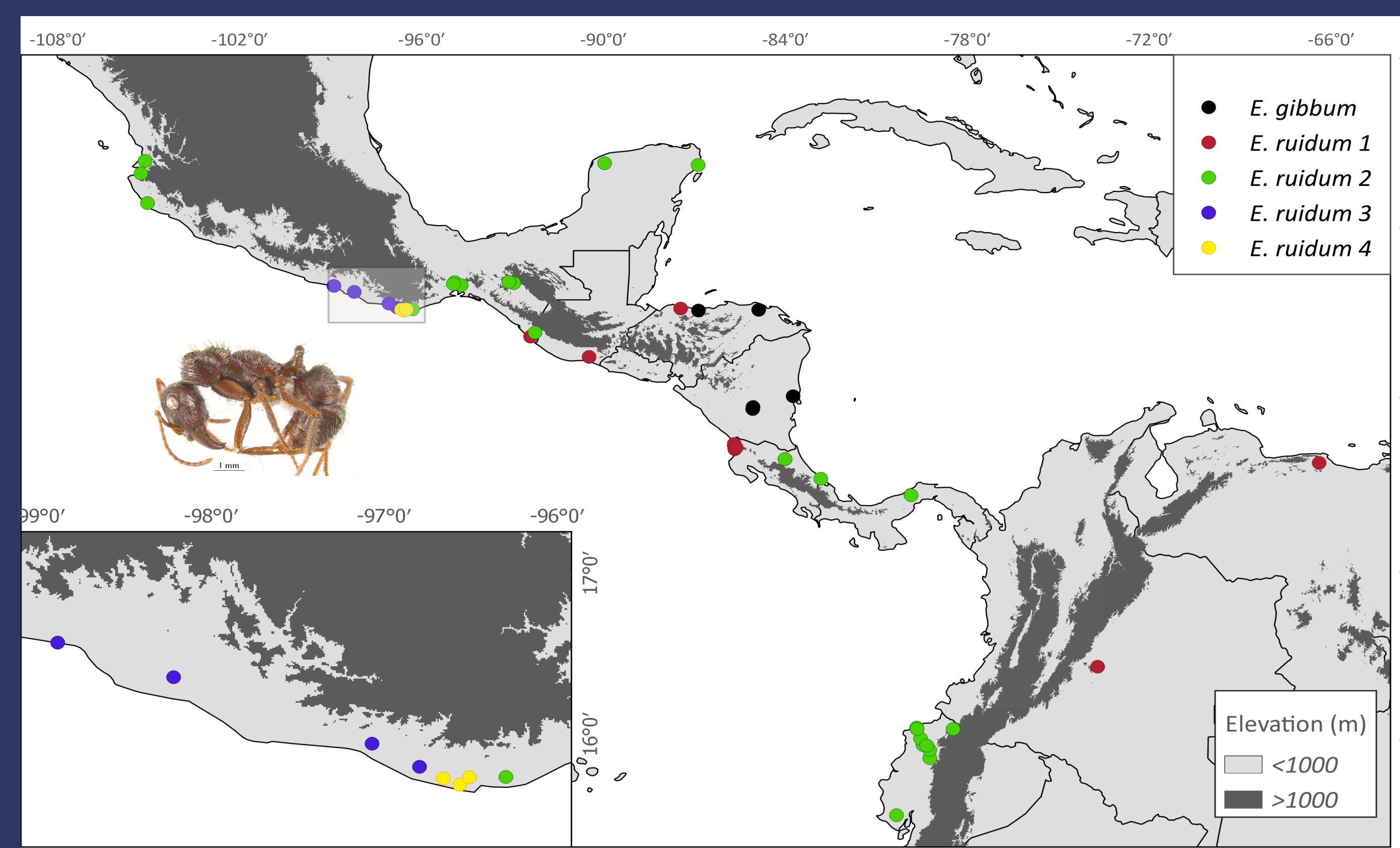


Figure 2: Map showing the sampled localities for the specimens assigned to *E. ruidum* and *E. gibbum*. The taxon names refer to the delimited morphospecies.

Results and discussion

Our concatenated and separate mt gene genealogies (Figure 3), revealed an incorrect phylogenetic placement for a number of the examined sequences. Based on the congruence found between the species delineation analyses carried out with our putative mt orthologs and the observed consistent morphological differences, we propose that *E. ruidum* represents a complex composed of three to four evolutionary units, two of which have wide geographic distribution along the Neotropics. The evidence obtained from the nuclear marker also suggests the existence of a hybrid zone between two of the proposed species in the Pacific coast of Oaxaca, southeast Mexico. This agrees with previous studies of other animal taxa, which have revealed a complex evolutionary history and overlooked species diversity along the Mexican Pacific coast.

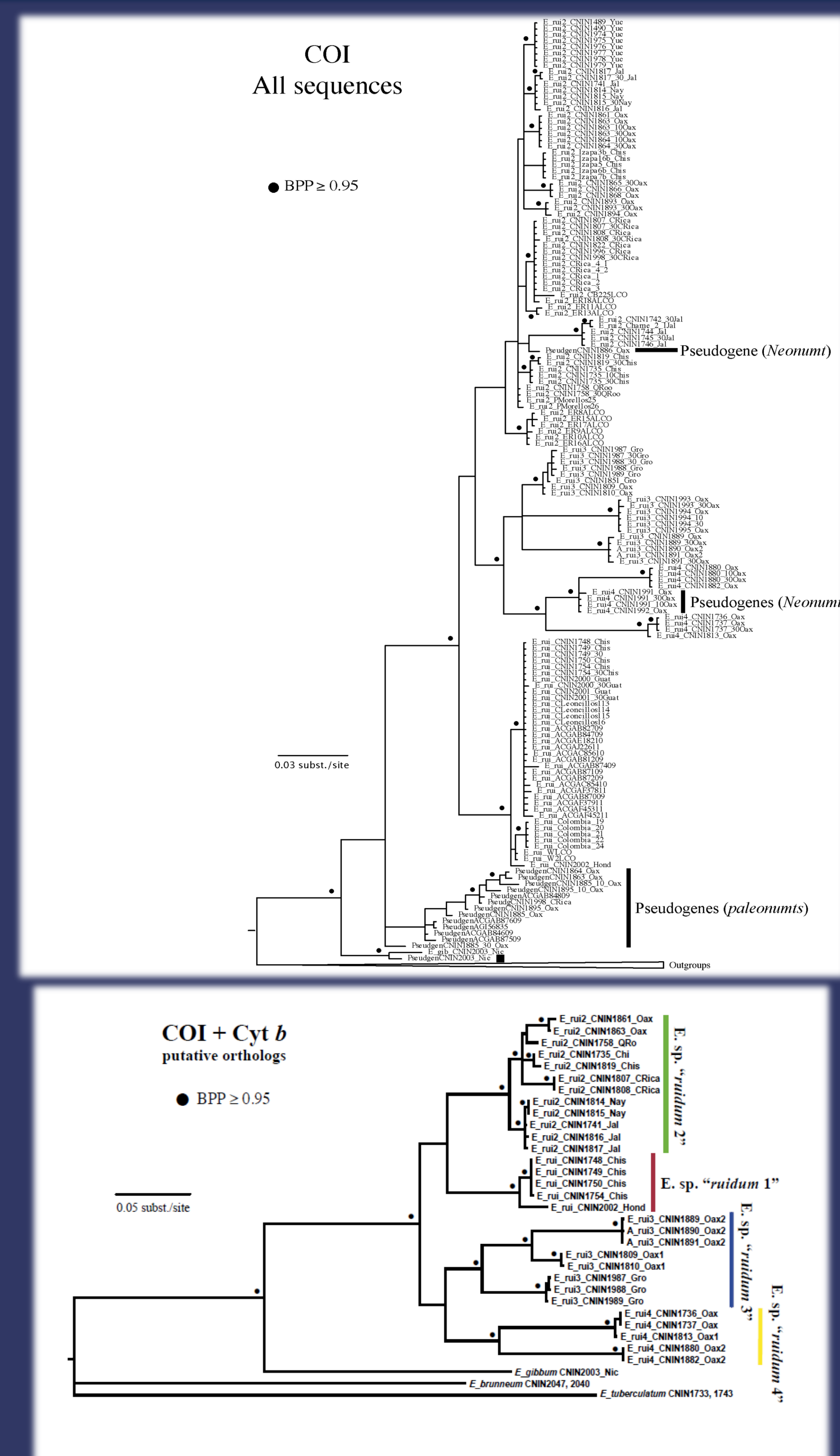


Figure 3: Phylogram derived from the Bayesian COI (including all the sequences) and concatenated (COI + cyt b) analyses excluding all potential numts and hybrids. Black circles near branches are Bayesian posterior probabilities ≥ 0.95 . Colour lines refer to the main lineages recovered. Taxon names refer to the delimited morphospecies.