

Biodiversity in Central Mexico: Assessment of Ants¹ in a Convergent Region

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Abstract. The occurrence of species is inadequately known for Morelos, one of the smallest states at the center of Mexico. Our research documented the occurrence of ant species in the State. Specimens were collected in four conserved areas and a range of disturbed habitats. Morphological traits and molecular sequences of the Cytochrome Oxidase subunit I (COI) were used as complementary tools to identify species. We identified 164 ant species of which 48 were new records for the State. Molecular analysis helped confirm and document the identity of species in groups of ants. The study could be used as a model to assess ecosystem health across extensive geographical regions.

Resumen. Morelos es uno de los estados más pequeños de México y está ubicado en el centro del país. El estado es poco explorado en términos de su mirmecofauna. En el presente trabajo, se documenta la presencia de especies de hormigas para el estado utilizando caracteres morfológicos y secuencias del gen Citocromo Oxidasa subunidad I (COI) como herramienta de apoyo. Los ejemplares fueron recolectados en áreas conservadas y en áreas perturbadas. Recopilamos 164 especies, de las cuales 48 representan nuevos registros para el estado. Los análisis moleculares ayudaron en la confirmación y documentación de algunas especies. Finalmente, el presente estudio puede usarse como modelo para evaluar el grado de disturbio y/o salud de los ecosistemas a lo largo de grandes extensiones geográficas.

Introduction

Ants are a taxonomically diverse and ecologically dominant group of insects inhabiting almost all terrestrial ecosystems (Folgarait 1998), with at least 13,674 species in the world (Bolton 2020). Geographically, some latitudes are important regions of ant diversity or "hot spots" especially important for species richness, levels of endemism or because they scarcely have been studied (Dunn et al. 2009, Economo et al. 2015).

Mexico is one of the most biodiverse countries, especially for ants, where recent national metanalysis of 21, 731 records reported 887 valid species (Dáttilo et al. 2019). Longino and Cox (2009) and García-Martínez et al. (2013), suggested

¹Hymenoptera: Formicidae

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richness in ant species of Mexico was concentrated in diverse tropical states such as Chiapas and Veracruz. However, ants have not been studied in many regions (Dáttilo et al. 2019), so data indicated that ant diversity in Mexico is not well documented (Ríos-Casanova 2013, Dáttilo et al. 2019). For example, the number of ant species reported for Veracruz in 2001 was 137 (Rojas 2001), increasing to 310 in 2013 (García-Martínez et al. 2013), while for Tlaxcala the number increased from two to three during the same period (Ríos-Casanova 2013). The number of species and sampling effort in particular regions of Mexico implies a gap in knowledge of general patterns of geographical distribution of ants.

The State of Morelos in Central Mexico is one of the smallest states, representing 0.3% of the territory of Mexico. However, much biodiversity, such as birds (33% of national bird occurrence per/diversity) of Mexico is in the State. Much diversity in different taxa is because of its geographical position and convergence of the Trans-Mexican Neovolcanic Belt, the unique depression of the Balsas River, combined with seasonally dry forests of the Pacific Slope (Contreras-MacBeath et al. 2004). However, the tropical dry forest of Morelos is very fragmented and disturbed by human activities (Noguera et al. 2002). Rapid assessment exploration/sampling efforts need to evaluate disturbance or conservation status in fragmented landscapes, as well as efficiently study diversity in scarcely studied regions undergoing anthropogenic modification.

Use of molecular techniques to aid assessment of biodiversity is well developed (Hebert et al. 2004). Mitochondrial Cytochrome Oxidase subunit I (COI) has been used for rapid identification of ant species (Smith and Fisher 2009, Smith et al. 2013) or to elucidate phylogenetic patterns in taxonomically complex groups (Narain et al. 2013). Some genera/species are notoriously challenging to identify by morphology (Bolton 2020).

The study had two goals to: update knowledge of ants of Morelos, Mexico, an essentially underexplored State, and combine morphological and molecular evidence to support the number of species in the State. Our methodology demonstrated morphological and molecular evidence necessary in unexplored regions, contributing to future studies of diversity of ants in Mexico.

Materials and Methods

Four habitats/regions were sampled for ants in the State of Morelos, Mexico, from 2017 to 2018. Two were conserved areas: Reserva Sierra de Montenegro in the municipality of Yautepec and Bosque de los Hongos Azules in the municipality of Cuernavaca. The other two areas were more disturbed and included the Archaeological Zone of Xochicalco in the municipality of Miacatlan and regions near the small town of Quilamula in the municipality of Tlaquiltenango. Ants also were collected in episodic surveys in randomly selected areas. Vegetation of the conserved and disturbed areas correlated with low stature dry tropical forest ("selva baja"). Ants were collected mostly by pitfall traps using the ALL protocol with Winkler traps and hand collecting. All material was deposited in the personal entomological collection of the first author at Universidad Autónoma del Estado de Morelos. All specimens were stored in ethanol (96%), and body parts of some specimens were used for genetic analysis.

Specimens or identification were observed and measured with the aid of an Olympus AZ binocular dissecting microscope. Keys for identification of ants to genera and species included specialized ant websites AntCat (Bolton 2020), AntWeb

(CAS 2020) and specific species: Ortiz-Sepúlveda et al. (2019) for *Brachymyrmex* (Mayr 1868); Cuzzo and Guerrero (2011) for *Dorymyrmex* (Mayr 1866), Watkins (1976) for *Labidus* (Jurine 1807); Kallal and LaPolla (2012) for *Nylanderia* (Jurine 1807), Pacheco and MacKay (2013) for *Solenopsis* (Westwood 1840) and Branstetter (2013) for *Stenammas* (Westwood 1839). *Pheidole* species were identified using Lucid (V. 3.3) for New World *Pheidole* available at <https://sites.google.com/site/newworldpheidole/>. We compared our measurements and species determination to Antweb images. Bolton (2020) was used for current anatomical description and nomenclature. To document species in the State we searched scientific literature and regional catalogs for ants from Mexico (e.g., Rojas 2001, Ríos-Casanova 2013, Vásquez-Bolaños 2015, Dáttilo et al. 2019) and databases from Internet-specialized websites such as AntWeb, California Academy of Sciences, Field Museum of Natural History, Museum of Comparative Zoology, Hymenoptera Online, and Global Biodiversity Information Facilities.

The standardized segment of cytochrome oxidase subunit I (COI) was used by the method of Smith and Fisher (2009) as a complementary tool to help identify ant species from the field. Total genomic DNA was extracted using Favorgen's kit following DNA extraction protocols and resuspended in 50 μ l of ddH₂O. PCR amplification used primers LepF1/LepR1. PCR reactions were in 10 μ l volumes containing 10 mM Tris HCl, 2.5 mM MgCl₂, 50 μ M dNTP's, 1.5 pmol of each primer and 0.3 unit of TaqDNA polymerase (Invitrogen) and 2 μ l of genomic DNA. PCR products were visualized on 1% agarose gel and bands were sequenced.

Chromatograms were analyzed using the FinchTV V. 1.4 program to ensure quality for each nucleotide and for editing sequences. Edited sequences were submitted for searching similar sequences in the Barcode of Life Data System (BOLD) database (Ratnasingham and Hebert 2007) in the Identification Interface to select two or three sequences and their respective specific names (sequences with a large percentage match but with no specific names were not used). The Neighbor-Joining statistical method was used with the Phylogeny Bootstrap method with 1000 replications to construct a dendrogram with the help of Kimura-2 parameters distance analysis (K2P) supported by MEGA X software to corroborate the morphological identity with the molecular data (Moulin et al. 2017).

Results and Discussion

In total, 164 species of ants were identified from the State of Morelos, which represented a 41.3% increase in reported species richness for the State. Thirteen new genera and one subfamily were added to the list of ants of Morelos. Subfamilies were Myrmicinae with 21 genera and 76 species, Formicinae with six genera and 30 species, Dorylinae with four genera and 15 species, Ponerinae with eight genera and 14 species, Pseudomyrmecinae with one genera and 13 species, Dolichoderinae with four genera and nine species, Ectatomminae with two genera and six species and Proceratiinae with one genera and one species (Table 1). Literature listed 116 species previously known to occur in Morelos. Forty-eight species were added after collecting in the field and searching databases on specialized Web sites not published formally.

Ant diversity of the State is emphasized by the fact that Morelos with just 0.3% of the total territory of Mexico increased to 48 genera (35 previously) representing 49.48% of all genera known for Mexico. Historically, Veracruz has been one of the most and best represented states of Mexico in terms of myrmecological fauna

Table 1. List of Species of the Ants of Morelos

***Dorymyrmex insanus* (Buckley, 1866)**
***Dorymyrmex pyramicus* (Roger, 1863)**
Forelius damiani Guerrero & Fernández, 2008
Forelius pruinosus (Roger, 1863)
Linepithema dispertitum (Forel, 1855)
Linepithema iniquum (Mayr, 1870)
Tapinoma melanocephalum (Fabricius, 1793)
Tapinoma ramulorum Emery, 1896
Tapinoma sessile (Say, 1836)
***Eciton vagans* (Olivier, 1792)**
Labidus coecus (Latreille, 1802)
Labidus praedator (Smith, 1858)
Neivamyrmex agilis Borgmeier, 1953
Neivamyrmex cornutus Watkins, 1975
Neivamyrmex fuscipennis (Smith, 1942)
Neivamyrmex graciellae (Mann, 1926)
Neivamyrmex impudens (Mann, 1922)
Neivamyrmex melanocephalus (Emery, 1895)
Neivamyrmex nigrescens (Cresson, 1872)
Neivamyrmex opacithorax (Emery, 1894)
Neivamyrmex pauxillus (Wheeler, 1903)
Neivamyrmex sumichrasti (Norton, 1868)
Neivamyrmex swainsonii (Shuckard, 1840)
Nomamyrmex esenbeckii (Westwood, 1842)
Ectatomma ruidum (Roger, 1860)
Ectatomma tuberculatum (Olivier, 1792)
Gnamptogenys regularis Mayr, 1870
Gnamptogenys striatula Mayr, 1884
Gnamptogenys strigata (Norton, 1868)
Gnamptogenys sulcata Smith, 1858
***Brachymyrmex heeri* Forel, 1874**
***Brachymyrmex minutus* Forel, 1893**
Brachymyrmex musculus Forel, 1899
Camponotus abscisus Roger, 1863
Camponotus andrei Forel, 1885
Camponotus atriceps (Smith, 1858)
Camponotus conspicuus zonatus Emery, 1894
***Camponotus fragilis* Pergande, 1893**
Camponotus fumidus toltecus Forel, 1899
Camponotus melanoticus flavopubens Emery, 1925
Camponotus melinus MacKay & MacKay, 1997
Camponotus mina Forel, 1879
Camponotus novogranadensis Mayr, 1870
Camponotus phytophilus Wheeler, 1934
***Camponotus picipes pilosulus* (Emery, 1925)**
Camponotus planatus Roger, 1863
***Camponotus pudorosus* Emery, 1925**
Camponotus pullatus Mayr, 1866
Camponotus rectangularis Emery, 1890

Camponotus rubrithorax Forel, 1899
Myrmecocystus mimicus Wheeler, 1908
Myrmelachista amicta Roger, 1863
Myrmelachista mexicana Wheeler, 1934
Myrmelachista skwarrae Wheeler, 1934
Myrmelachista skwarrae laeta Wheeler, 1934
Myrmelachista skwarrae picea Wheeler, 1934
***Nylanderia austroccidua* (Trager, 1984)**
Nylanderia* aff. *faisonensis
Nylanderia* aff. *caeciliae
Paratrechina longicornis (Latreille, 1802)
Acromyrmex octospinosus (Reich, 1793)
***Aphaenogaster huachucana* Creighton, 1934**
Atta mexicana (Smith, 1858)
***Cardiocondyla emeryi* Forel, 1881**
***Cardiocondyla minutior* Forel, 1899**
***Cardiocondyla obscurior* Wheeler, 1929**
Cephalotes bimaculatus (Smith, 1860)
Cephalotes hirsutus de Andrade, 1999
Cephalotes multispinosus (Norton, 1868)
Cephalotes scutulatus (Smith, 1867)
Cephalotes setulifer (Emery, 1894)
Cephalotes wheeleri (Forel, 1901)
Crematogaster ampla Forel, 1912
Crematogaster atra Mayr, 1870
Crematogaster crinosa Mayr, 1862
Crematogaster curvispinosa Mayr, 1862
Crematogaster distans Mayr, 1870
***Crematogaster opaca* Mayr, 1870**
Crematogaster rochai Forel, 1903
***Crematogaster torosa* Mayr, 1870**
Cyphomyrmex flavidus Pergande, 1896
Monomorium compressum Wheeler, 1914
Monomorium ebeninum Forel, 1891
Monomorium minimum (Buckley, 1867)
***Mycetomoellerius turrifex* (Wheeler, 1903)**
***Myrmica mexicana* Wheeler, 1914**
Nesomyrmex echinatinodis (Forel, 1886)
Nesomyrmex wilda (Smith, 1943)
***Octostruma balzani* (Emery, 1894)**
***Pheidole bilimeki* Mayr, 1870**
***Pheidole coracina* Wilson, 2003**
***Pheidole dwyeri* Gregg, 1969**
***Pheidole erethizon* Wilson, 2003**
***Pheidole granulata* Pergande, 1886**
Pheidole hirtula Forel, 1899
Pheidole hyatti Emery, 1895
Pheidole laevivertex Forel, 1901
***Pheidole lamancha* Longino, 2019**
Pheidole morelosana Wilson, 2003

***Pheidole nubicola* Wilson, 2003**
Pheidole optiva Forel, 1901
Pheidole obtusospinosa Pergande, 1896
***Pheidole punctatissima* Mayr, 1870**
Pheidole radoszkowskii Mayr, 1884
***Pheidole sciophila* Wheeler, 1908**
Pheidole skwarrae Wheeler, 1934
Pheidole spathicornis Wilson, 2003
Pheidole tepicana Pergande, 1896
***Pheidole tetra* Creighton, 1950**
Pheidole tolteca Forel, 1901
***Pheidole virago* Wheeler, 1915**
Pheidole* aff. *agricola
Pheidole* aff. *optiva
Pogonomyrmex barbatus (Smith, 1858)
***Pogonomyrmex desertorum* Wheeler, 1902**
Rhopalothrix weberi Brown & Kempf, 1960
***Rogeria creightoni* Snelling, 1973**
Solenopsis* aff. *azteca
Solenopsis geminata (Fabricius, 1804)
***Solenopsis xyloni* McCook, 1880**
Stenamma* aff. *maximon
***Stenamma manni* Wheeler, 1914**
***Strumigenys eggersi* (Emery, 1890)**
***Strumigenys elongata* Roger, 1863**
***Strumigenys nigrescens* Wheeler, 1911**
Temnothorax annexus (Baroni Urbani, 1978)
Temnothorax augusti (Baroni Urbani, 1978)
***Temnothorax goniops* (Baroni Urbani, 1978)**
Temnothorax manni (Wheeler, 1914)
***Temnothorax punctithorax* MacKay, 2000**
***Temnothorax silvestrii* (Santschi, 1911)**
Temnothorax skwarrae (Wheeler, 1931)
Temnothorax subditivus (Wheeler, 1903)
***Tetramorium simillimum* (Smith, 1851)**
***Tetramorium spinosum* (Pergande, 1896)**
Wasmannia auropunctata (Roger, 1863)
Anochetus mayri Emery, 1884
Belonopelta deletrix Mann, 1922
Hypoponera foeda (Forel, 1893)
Hypoponera opaciceps (Mayr, 1887)
Hypoponera punctatissima (Roger, 1859)
Leptogenys elongata (Buckley, 1866)
Leptogenys wheeleri Forel, 1901
Neoponera villosa (Fabricius, 1804)
Odontomachus bauri Emery, 1892
Odontomachus clarus Roger, 1861
Odontomachus haematodus (Linnaeus, 1758)
Odontomachus laticeps Roger, 1861
Platythyrea punctata (Smith, 1858)

Pseudoponera stigma (Fabricius, 1804)
***Proceratium mexicanum* de Andrade, 2003**
Pseudomyrmex championi (Forel, 1889)
Pseudomyrmex cubaensis (Forel, 1901)
Pseudomyrmex ejectus (Smith, 1858)
Pseudomyrmex elongatus (Mayr, 1870)
Pseudomyrmex gracilis (Fabricius, 1804)
Pseudomyrmex kuenckeli (Emery, 1890)
Pseudomyrmex major (Forel, 1899)
Pseudomyrmex pallidus (Smith, 1855)
Pseudomyrmex pupa (Forel, 1911)
Pseudomyrmex seminole Ward, 1985
Pseudomyrmex simplex (Smith, 1877)
Pseudomyrmex subater (Wheeler & Mann, 1914)
Pseudomyrmex veneficus (Wheeler, 1942)

Species in bold are new records obtained from collection databases and fieldwork.

(García-Martínez et al. 2013). Although Morelos is 50 times smaller than Veracruz, the myrmecological fauna at Morelos is about 50% of the latter.

Number of species might be correlated with a combination of geographical (regional) and climatic (local) factors that might explain the diversity. On the large scale of regional factors, an important element is geographic location of the State between Neotropical and Nearctic realms. At the level of biogeographic provinces of Mexico (Morrone et al. 2017), Morelos is split by the Neovolcanic Transitional Zone in the north and the Balsas depression in the south with distinct climate (Contreras-MacBeath et al. 2004).

As a consequence of the biogeographic intersection, a climatic pattern is derived where more temperate areas occur close to the Neovolcanic Transitional Zone and milder regions extend south of the State (Contreras-MacBeath et al. 2004). Combination of regional and local patterns might be important for some species. For example, according to Branstetter (2013) the *Stenammas manni* species has seven variants. The specimen we collected might represent one of four variations in an oak-pine forest in Bosque de los Hongos Azules. The variant occurs in more northern pine-oak forests at Pinal de Amoles, Queretaro. Our record potentially represents a southern extension of the variants.

Some species reported originally were thought to have restricted distribution, classically Nearctic distribution. For example, Wilson (2003) mentioned that *Pheidole granulata* was strictly Nearctic in distribution, but our data confirmed the species extends well into the Neotropics. Likewise, *Aphaeogaster huachucana* and *Pogonomyrmex desertorum* also were considered to be more restricted to the Nearctic. Bracamontes and Vásquez-Bolaños (2010) reported *A. huachucana* for the first time at Sonora, Mexico, but its presence at Morelos, like *P. granulata*, indicated a more southern distribution into the Nearctic-Neotropical interface. Distribution of various species of *Pheidole* were extended. For example, *Pheidole lamancha* (Longino 2019) was known to occur only at its type locality at Veracruz but was found at the Archaeological Zone of Xochicalco in this study.

Widely distributed species also were recorded in the State. *Octostruma balzani* is distributed from northern Mexico to Paraguay (Antweb). *O. balzani* belongs to the *O. balzani* complex in which the number of species is assumed to be underestimated (Longino 2013).

Combined morphological and molecular evidence with 23 DNA sequences analyzed supported the number of species for the State. Phylogenetic analysis of COI provided species sequences we recovered in defined clades with large bootstrap values when combined with those obtained from BOLD (Fig. 1).

The Neighbor-Joining dendrogram reinforced identity of *Crematogaster torosa* in a clade with other sequences of *C. torosa* with large bootstrap values (Fig. 1). *Crematogaster torosa* belongs to the *crinosa* complex and might not be easy to separate morphologically from other species such as *C. crinosa* or *C. rochai* (Longino (2003). The species varies morphologically across populations from Costa Rica to the United States. As with other widely distributed species, more detailed molecular analysis is needed to indicate if populations are reproductively isolated and considered a different species.

Cardiocondyla emeryi and *C. obscurior* were separated into two clades when analyzed with sequences from BOLD (Fig. 1). In the United States *C. nuda* probably is *C. minor* or *C. mauritanica* and most specimens of *C. wroughtonii* probably are *C. obscurior* (MacGown 2011). *C. obscurior* has not been reported in Mexico, but considering our specimen has postpetiolar sternites with anterolateral angular projections, a character that defines *C. obscurior*, and also that previously *C. wroughtonii* was misidentified with *C. obscurior*, we consider our specimens to be *C. obscurior*. *C. emeryi* and *C. obscurior* are distributed around the world. *C. emeryi* is an invasive species hypothesized to have been introduced to America with plant material transported from Africa 400 years ago (Seifert 2003). *C. obscurior* is native to Africa and Asia, but now dispersed all over the world.

We obtained 13 species of *Pheidole*, more than twice the number of species known to occur previously at Morelos. Three species of *Pheidole* identified through morphological characters were recognized as different species by sequence analysis (Fig. 1). The status of our specimen of *P. tepicana* was confirmed with other sequences from BOLD. *Pheidole* aff. *agricola* separated from *P. tepicana*, but COI only helped recognize it as a separate species. More detailed analysis is required to evaluate the species. *P. tolteca* separated from other species of *Pheidole* when analyzed with sequences from BOLD but combined with morphological data recognized its taxonomic status. Great diversity of the genus might be explained at least in part, because it is a dominant ecological and evolutionary group with most clades correlated with different biomes (Economo et al. 2015).

Sequence analysis allowed differentiation of polymorphic species such as *Solenopsis geminata* that forms a clade distinct from sequences of *S. xyloni* (Fig. 1). COI helped reveal the status of *S. geminata*, important from taxonomic and ecological perspectives. *S. geminata* is a polymorphic species that has been synonymized several times (e.g., as *S. xyloni*) and dominant in natural and disturbed ecosystems (Wetterer 2011).

Sequences of *Atta texana* were used to identify *Atta mexicana* where Neighbor-Joining corroborated two species supported by large bootstrap values (Fig. 1). Bacci Jr. et al. (2008) mentioned that *A. texana* + *A. insularis* was the sister group of *A. mexicana* that together formed a group once named Archeatta. The group is supposed to be the sister group of *Atta* s. str. where the Archeatta group is thought to have evolved mainly in Central and North America.

Molecular analysis helped separate three species of *Camponotus*, resulting in three clades well defined and supported by large bootstrap values (Fig. 1). The two correspond to *Camponotus novogranadensis* and *C. planatus* that are similar morphologically but differ in color. The entire body of *C. novogranadensis* is black, but *C. planatus* is reddish with a black gaster (Wetterer 2019). The other clade

belongs to a species in which our sequence matched another sequence in BOLD, but because of scattered or lack of identification keys for the group, its scientific name could not be determined without more detail.

In recent research, use of molecular analysis helped clarify two clades of tiny ants of the genus *Brachymyrmex*, one for *B. heeri* and the other *B. minutus* (Fig. 1). Morphologically, the species were separated by the absence or presence of pronotal hairs. Narain et al. (2013) mentioned utility of the barcode in identification of taxonomically complex tiny ants such as thief ants of the genus *Solenopsis*. *Brachymyrmex minutus* is widely distributed in the Neotropics, occurring in Bolivia, Brazil, Colombia, Costa Rica, Dominican Republic, Ecuador, French Guiana, Guatemala, Mexico, Panama, Peru, Surinam, and Venezuela (Ortiz-Sepulveda et al. 2019). In Mexico, it was reported for Chiapas (Vásquez-Bolaños 2015), but Ortiz-Sepulveda et al. (2019) mentioned it also was found at Oaxaca and Veracruz.

Identity of *Paratrechina longicornis* was supported by molecular analysis (Fig. 1). Among *Paratrechina* or *Nylanderia* species, *P. longicornis* is recognized besides its apparently erratic movements, by the presence of long scapes with no setae. According to Wetterer (2008), worldwide distribution of this tramp species makes it difficult to know its origin, but because of its presence in natural ecosystems in Thailand and Vietnam, Southeast Asia might be its origin.

An excellent example using COI is *Nylanderia*, a new genus that molecular analysis helped identify for the State. It has 123 species distributed worldwide. The genus belongs to the *Prenolepis* group and has several invasive species. However, the exact number is difficult to know because of taxonomic uncertainty of the group (LaPolla et al. 2010). In Neighbor-Joining analysis, *Nylanderia austroccidua* separated from other species of *Nylanderia* even when we could not join it with other *N. austroccidua* sequences because there are no sequences of the species in BOLD (Fig. 1). Morphologically, it is recognized by dense cephalic pubescence, sparse gastral pubescence, and bluish hue under microscopic examination. *N. austroccidua*, according to Kallal and LaPolla (2012) is the most widely distributed species, occurring mostly in *Quercus* L. (oak), *Acer* L. (maple) and *Pinus* L. (pine) forests from the United States to Costa Rica.

Tapinoma melanocephalum is supported as a clade in Neighbor-Joining analysis (Fig. 1). Morphological identification of *T. melanocephalum* was consistent with characters described by Smith (1965). The origin of this tramp species is purported to be Old World tropics (Wetterer 2009).

Pseudomyrmex elongatus was in a well-supported clade that corroborates the status of the species. A sequence of *P. elongatus* was interspersed with *P. simplex* but might be an effect of few samples analyzed. A larger number of sequences is expected to clarify and separate both species (Fig. 1). *P. elongatus* is a Neotropical generalist twig-nester ant from the United States (Florida) to Bolivia and Brazil (Ward 1999).

Bolton and Fisher (2011) considered *Hypoponera punctatissima* the most widely distributed ponerine tramp-species. Because of its polymorphic sex/caste system, many previous names have been synonymized. Molecular analysis indicated the specimen undoubtedly was *H. punctatissima* (Fig. 1). The species is considered of Afrotropical origin and separated from *H. parva* native to the Neotropics. Because of absence of autapomorphies and great morphological variation the genus needs taxonomic revision (Schmidt and Shattuck 2014).

In Neighbor-Joining analysis, *Proceratium mexicanum* was separated from *P. micrommatum* (Fig. 1). We did not find sequences of *P. mexicanum* for comparison

to determine if it joined the sequences as expected. However, Baroni-Urbani and de Andrade (2003) mentioned that specimens recognized as *P. mexicanum* were misidentified as *P. micrommatum*, so this last species has not been collected in Mexico instead, geographical distribution of *P. mexicanum* should be recognized.

Nomamyrmex esenbeckii is distinguished morphologically by a “highly positioned spiracle and lack of propodeal lobes, propodeum armed with cuticular projections, two-segmented waist and armed pretarsal claws” (Borowiec 2016). In molecular analysis, our sequence joined with sequences of *Neocerapchys esenbeckii* (Fig. 1). However, Borowiec (2016) described *Neocerapchys* gen. n. with two species, *N. neotropicus* and *N. splendens*. Because our sequence did not separate clearly from *Neocerapchys esenbeckii*, we concluded that all belonged to *Nomamyrmex esenbeckii* and the specimens originally were misidentified.

Labidus coecus is recognized among the subfamily Dorylinae mainly by the propodeum lacking spines or lamella, two-segmented waist and pretarsal claws with one tooth. Well supported values of bootstrap analysis corroborated our identified specimen as this species (Fig. 1). The species is widely distributed in Nearctic and Neotropical regions. The species level of the genus requires revision, preliminary analysis showed *L. praedator* was a complex of reproductively isolated species (Borowiec 2016), and this also could be the case for *L. coecus*.

In conclusion, ant diversity at Morelos represents almost 50% of the genera known for Mexico. Our methods, a combination of morphological and molecular data helped validate identity of ant species and has important biological and often economic connotations. We confirmed identity of 21 species with the barcode, although two (*Pheidole* aff. *agricola* and *Pheidole* aff. *optiva*) need more detailed analysis. Five were tramp species (*Cardiocondyla emeryi*, *Cardiocondyla obscurior*, *Hypoponera punctatissima*, *Paratrechina longicornis* and *Tapinoma melanocephalum*). *C. obscurior* is a new record for a tramp species for Morelos. Knowing this and other tramp or invader species will help in developing best strategies to control non-native ants and exploit natural resources for Mexico.

Acknowledgment

We thank Biotecfron for donating some laboratory equipment and most consumables and supplies. We also thank the Museum of Xochicalco and Bosque de los Hongos Azules for allowing collecting. We are grateful to Rubí Meza Ph.D. and Carlos Pedraza Ph.D. (researchers at UNAM) for comments and suggestions for molecular analysis and Gabriela Díaz M.S. for help in editing and analyzing sequences.

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