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Research

Ultra-Conserved Element Phylogenomics of New World *Ponera* (Hymenoptera: Formicidae) Illuminates the Origin and Phylogeographic History of the Endemic Exotic Ant *Ponera exotica*

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Abstract

The genus *Ponera* is a lineage of leaf litter ants, with a center of diversity in the Indo-Australian region. Two species occur in the New World; however, uncertainty exists with regard to their biogeographic origins and species limits, especially for isolated cloud forest populations in Middle America. We investigate the geographic distribution, phylogeny, and phylogeography of these two species to better characterize the American ant fauna and to gain insight into the biogeography of taxa that span hemispheres. Sequencing of Ultra-Conserved Element (UCE) loci was used to infer phylogenetic relationships, estimate divergence dates, and test species boundaries. The widespread native species *Ponera exotica* and *P. pennsylvanica* are each more closely related to Old World relatives than they are to each other, implying two independent colonizations of the New World. *Ponera pennsylvanica* is most closely related to the European species *P. coarctata*, while *P. exotica* is related to a clade of Indo-Australian species. *Ponera pennsylvanica* is abundant throughout the eastern United States, with scattered occurrences further west. *Ponera exotica* occurs from the southern United States to Nicaragua. Both species have alate and ergatoid queens. Sequenced specimens from multiple populations of *P. exotica* reveal a pectinate phylogeographic structure, from north to south, with the potential for multiple cryptic species. The southern United States to Middle America distribution pattern of *P. exotica* mirrors that of many plant and animal species and may be the result of climatic cooling in the Pliocene followed by repeated glacial cycles in the Pleistocene, which condensed and fragmented mesic forest habitat.

Key words: biogeography, Middle America, molecular systematics, ultra-conserved element, Ponerinae

The ant genus *Ponera* Latreille is globally distributed, with 57 described species (Schmidt and Shattuck 2014, Bolton 2018). The workers are small, nearly or completely eyeless, and are part of the 'cryptic' fauna found in soil, leaf litter, and rotting wood. The center of diversity is the Indo-Australian region, with low diversity representation in northern temperate zones. Only two species of *Ponera* are known to occur in the New World: *P. pennsylvanica* Buckley and *P. exotica* Smith. Here we provide a greatly improved understanding of the geography and evolutionary history of these American species, giving new insight into the nature of biotic exchange between eastern and western hemispheres and temperate and tropical America.

Ponera pennsylvanica has been known for a long time and is abundant in deciduous forests of eastern North America (Mackay and Anderson 1991, Mackay and Mackay 2002). It has presumably closely related European counterparts *P. coarctata* (Latreille) and *P. testacea* Emery (Taylor 1967, Csösz and Seifert 2003). *Ponera exotica* is known from the southeastern United States, from North Carolina to northern Florida and west to Oklahoma and Texas (Johnson 1987, Mackay and Anderson 1991). Smith described the species in 1962, and both Smith (1962) and Taylor (1967) thought that *P. exotica* might have been introduced because of its similarity to Indo-Australian species, (hence the name '*exotica*'). Johnson (1987) and Mackay and Anderson (1991) reported multiple collections from widespread native habitat, strongly suggesting that the species is native to North America. García-Martínez et al. (2016) reported *P. exotica* from a cloud forest site in the state of Veracruz, Mexico, the first report outside of the United States.

Ponera species are challenging to identify based on morphology alone (Taylor 1967). Workers have a very uniform habitus, and one of the main differentiating characters is size. Detailed

morphometrics were required to differentiate *P. coarctata* and *P. testacea* in Europe (Csösz and Seifert 2003). In North America, the two known species have been relatively easily differentiated based on size alone. The reported head width (HW) ranges are 0.53–0.63 mm for *P. pennsylvanica* and 0.36–0.41 for *P. exotica*.

During extensive ant survey work in Middle America (Mexico and Central America), we discovered that a species of *Ponera* occurred as a moderately abundant element of cloud forest ant communities throughout the region, ranging as far south as northern Nicaragua (www.antweb.org). In terms of size and general habitus, the material matched *P. exotica* from the southern United States. However, given the limited set of morphological characters, the potential for convergent evolution in these characters, and the allopatric geographic distribution, the specific identity of the Central American material was uncertain. Thus, to resolve species identities in the New World fauna, and to gain insight into geographic origins of species, molecular data are required. A few molecular studies have incorporated *Ponera* into their sampling (Brady et al. 2006, Moreau and Bell 2013, Schmidt 2013, Branstetter et al. 2017, Economo et al. 2018); however, none have specifically targeted the genus or included a broad sampling of New World species or populations.

Unlike the *Ponera* populations of the American temperate zone, which occur as potentially continuous populations over large areas of eastern deciduous forest, the Middle American *Ponera* occur as a series of separate cloud forest populations, island-like in their relationship to each other. This distributional pattern indicates the potential in Central America for varying degrees of genetic divergence among populations and possibly cryptic species. Understanding the structure of these populations and divergence times could yield insights into *Ponera* evolution and the biogeography of the region. If *P. exotica* is determined to be a single species (or a complex of closely related species) that occurs in the United States and Middle America, the distribution pattern would closely parallel the disjunct distributions reported in over 50 plant taxa (Graham 1999) and some animals (Martin and Harrell 1957). This pattern has interested biogeographers, sparking debate about historical drivers, especially as it relates to Pleistocene glaciation and refugia (Graham 1999, Morris et al. 2008, Cavender-Bares et al. 2011, Ruiz-Sanchez and Ornelas 2014).

In this study, we present a review of *Ponera* in the New World, relying heavily on phylogenomic data to resolve morphological uncertainties and to investigate evolutionary patterns. Specifically, we address several outstanding questions regarding New World *Ponera* taxonomy and evolution: 1) How many New World *Ponera* species are there? 2) What are the phylogenetic relationships between New and Old World species and what can this information tell us about geographic origins? and 3) Is there any evidence of geographic structure or cryptic species, especially among the cloud forest populations in Middle America? To answer these questions, we generated a phylogenomic dataset that included the two known species and many populations of New World *Ponera* and a selection of Old World congeners. To resolve relationships, we employed Ultra-Conserved Element (UCE) phylogenomics (Faircloth et al. 2012, Faircloth et al. 2015, Branstetter et al. 2017), a molecular tool that provides genome-scale data and that has the potential to resolve both inter- and intraspecific relationships. In light of the molecular results, we re-examine morphology and species limits to provide an update to the taxonomy of New World *Ponera*.

Materials and Methods

Material Examined

This study was based on 324 separate species occurrence records of *P. exotica* and *P. pennsylvanica*, plus small numbers of other species. Most of the examined material was from the Middle American corridor (Veracruz, Mexico to Nicaragua). Almost all the specimens were from Winkler or Berlese samples of sifted leaf litter and rotten wood from wet forest habitats. Most materials were from large-scale biodiversity inventory projects in Central America and southern Mexico, spanning 25 yrs (Projects ALAS, LLAMA, and ADMAC). All locality, collection, and specimen data are available in Supp. Table S1, as digital supplementary material to this article, at the journal's web pages (and also on AntWeb at www.antweb.org). Specimen collection data are derived from a specimen database and are not direct transcriptions of labels. Latitudes and longitudes, when present, are reported in decimal degrees, as a precise point (five decimal places) followed by an error term in meters. Distribution maps are augmented with unverified specimen records from AntWeb, BOLD (Ratnasingham and Hebert 2007) (www.boldsystems.org), and MacKay and Anderson 1991).

DNA Sequence Generation

We selected 34 *Ponera* and five outgroup specimens for DNA sequencing (Table 1). Species of *Cryptopone* Emery, *Diacamma* Mayr, *Parvaponera* Schmidt and Shattuck, and *Pseudoponera* Emery were used as outgroups. *Ponera* specimens included one individual each of eight Old World species, six specimens of *P. pennsylvanica*, and 20 specimens of *P. exotica*. Specimens of *P. pennsylvanica* and *P. exotica* were one individual each from widely separated populations, with the exception of one population of *P. exotica* from which one worker and one ergatoid queen were sequenced. All data were newly generated for this study, except for five samples, in which data were extracted from Branstetter et al. (2017) (Table 1).

We employed the UCE approach to phylogenomics (Faircloth et al. 2012, Faircloth et al. 2015, Branstetter et al. 2017), combining target enrichment of UCEs with multiplexed, next-generation sequencing. All UCE molecular work was performed following the UCE methodology described in Branstetter et al. (2017). In brief, the following steps were performed: DNA extraction, library preparation, UCE enrichment, sample pooling (100 total samples per sequencing pool), and sequencing on an Illumina HiSeq 2500 (PE125 v4) at the University of Utah genomics core facility. For UCE enrichment we used an ant-customized bait set ('ant-specific hym-v2') targeting 2,524 UCE loci common across Hymenoptera (Branstetter et al. 2017). The utility of this bait set to resolve relationships, both deep and shallow, in ants has been demonstrated in several studies (Branstetter et al. 2017, Pierce et al. 2017, Ward and Branstetter 2017, Blaimer et al. 2018).

UCE Matrix Assembly

After sequencing, the UCE data were demultiplexed by staff at the University of Utah bioinformatics core, and once received, the sequence data were cleaned, assembled and aligned using the PHYLUCE package v1.5 (Faircloth 2016) according to the process outlined in Branstetter et al. (2017). PHYLUCE includes a set of wrapper scripts that facilitates batch processing of large numbers of samples. Within the PHYLUCE environment, we used ILLUMIPROCESSOR (Faircloth 2013) and TRIMMOMATIC (Bolger et al. 2014) for quality trimming raw reads, TRINITY v2013-02-25 (Grabherr et al. 2011) for de novo assembly of reads into

Table 1. Voucher list of specimens used for DNA extraction and sequencing

Taxon	exID	Country	Admin1	Latitude	Longitude	VoucherID
Cryptopone butteli Forel[a]	EX1180	Malaysia	Sabah	4.74332	116.97303	CASENT0635385
Cryptopone cf. gilva	EX1549	Honduras	Ocotepeque	14.45775	−89.06814	CASENT0617514
Diacamma rugosum (Le Guillou)[a]	EX1574	Malaysia	Sabah	4.74000	116.97500	CASENT0634818
Parvaponera darwinii (Forel)[a]	EX1610	Malaysia	Sabah	4.96478	117.80465	CASENT0637361
Ponera clavicornis Emery	EX1624	Australia	Queensland	−13.72823	143.32997	CASENT0761218
Ponera coarctata[a]	EX1174	Italy	Liguria	44.44550	8.93850	LACM ENT 140941
Ponera exotica	EX1177	Nicaragua	Madriz	13.32912	−86.60834	CASENT0619992
Ponera exotica	EX1182	Mexico	Chiapas	16.15462	−93.60080	CASENT0603537
Ponera exotica	EX1183	Mexico	Chiapas	15.71569	−92.93849	JTLC000014287
Ponera exotica	EX1184	Guatemala	Zacapa	14.95335	−89.27576	CASENT0612538
Ponera exotica	EX1185	Guatemala	Suchitepéquez	14.54948	−91.19031	CASENT0612921
Ponera exotica	EX1186	Nicaragua	Nueva Segovia	13.98227	−86.18927	CASENT0629300
Ponera exotica	EX1187	Honduras	Olancho	15.09807	−86.72047	CASENT0633133
Ponera exotica	EX1188	Honduras	Olancho	15.09807	−86.72047	CASENT0633132
Ponera exotica	EX1192	Honduras	Francisco Morazán	14.34533	−86.86652	CASENT0616312
Ponera exotica	EX1193	Honduras	Olancho	14.94359	−85.91056	CASENT0616250
Ponera exotica	EX1552	Guatemala	Quiche	14.91852	−91.10458	CASENT0611162
Ponera exotica	EX1553	Guatemala	Guatemala	14.53349	−90.35941	CASENT0633037
Ponera exotica	EX1554	Guatemala	Jalapa	14.50476	−90.25613	CASENT0633038
Ponera exotica	EX1555	Guatemala	Santa Rosa	14.15115	−90.43123	CASENT0632976
Ponera exotica	EX1560	Mexico	Tamaulipas	23.10105	−99.19233	CASENT0603628
Ponera exotica	EX1603	Mexico	Veracruz	19.52172	−96.98908	CASENT0637775
Ponera exotica	EX1607	Honduras	Cortés	15.48510	−88.23627	CASENT0617727
Ponera exotica	EX1608	Guatemala	Izabal	15.51224	−88.86276	JTL-SV01241
Ponera exotica	EX1632	United States	Georgia	30.86139	−84.06750	CASENT0637287
Ponera exotica	EX1727	Mexico	Oaxaca	18.13949	−96.95869	CASENT0640742
Ponera leae Forel	EX1558	Australia	Queensland	−16.92145	145.58543	JTLC000006828
Ponera pennsylvanica	EX1178	United States	Utah	40.78808	−111.79634	CASENT0635669
Ponera pennsylvanica	EX1197	United States	Georgia	33.64550	−83.18401	CASENT0749269
Ponera pennsylvanica	EX1198	United States	Georgia	34.56826	−85.24176	CASENT0749270
Ponera pennsylvanica	EX1557	United States	West Virginia	39.03333	−79.31667	JTLC000006601
Ponera pennsylvanica	EX1559	United States	Maine	43.91957	−70.03943	JTLC000013858
Ponera pennsylvanica	EX1626	United States	Alabama	30.68333	−87.92000	CASENT0106719
Ponera petila Wilson	EX1630	Mauritius		−20.41883	57.73050	CASENT0637782
Ponera sc-sey	EX1629	Seychelles		−4.65121	55.45835	CASENT0159375
Ponera cf. sinensis	EX1625	China	Hong Kong	22.41595	114.12722	CASENT0761219
Ponera sp.	EX1179	Malaysia	Sabah	4.74332	116.97303	CASENT0635390
Ponera swezeyi (Wheeler)	EX1628	Madagascar	Antsiranana	−15.32331	50.30751	CASENT0637780
Pseudoponera stigma (Fab.)[a]	EX1576	Honduras	Gracias a Dios	15.70857	−84.86234	CASENT0613273

[a]Specimen data extracted from Branstetter et al. (2017).

contigs, and LASTZ v1.02 (Harris 2007) for identifying UCE contigs from all contigs. All optional PHYLUCE settings were left at default values for these steps. For the bait sequences file needed to identify UCE contigs, we used the ant-specific hym-v2 bait file. To calculate various assembly statics, including sequence coverage, we used scripts from the PHYLUCE package (*phyluce_assembly_get_trinity_coverage* and *phyluce_assembly_get_trinity_coverage_for_uce_loci*).

After extracting UCE contigs, we aligned each UCE locus using a stand-alone version of the program MAFFT v7.130b (Katoh and Standley 2013) and the L-INS-I algorithm. We then used a PHYLUCE script to trim flanking regions and poorly aligned internal regions using the program GBLOCKS (Talavera and Castresana 2007). The program was run with reduced stringency parameters (b1:0.5, b2:0.5, b3:12, b4:7), because the default settings are overly conservative. We then used another PHYLUCE script to filter the initial set of alignments so that each alignment was required to include data for 90% of taxa. This resulted in a final set of 1,782 alignments and 1,405,192 bp of sequence data for analysis. To calculate summary statistics for the final data matrix, we used a script from the PHYLUCE package (*phyluce_align_get_align_summary_data*).

Phylogenomic Analysis

To partition the UCE data for phylogenetic analysis, we used a recently developed method called SWSC-EN (Tagliacollo and Lanfear 2018). This stands for Sliding-Window Site Characteristics based on entropy and it uses a sliding window to partition UCE loci into three regions, corresponding to the right flank, core, and left flank. The theoretical underpinning of the approach comes from the observation that UCE core regions are conserved, while the flanking regions become increasingly more variable (Faircloth et al. 2012). Different methods can be used in the SWSC program to evaluate sites, but using entropy produced the best results (Tagliacollo and Lanfear 2018). After running the SWSC-EN algorithm, the resulting data subsets were analyzed using PARTITIONFINDER2 (Lanfear et al. 2012, Lanfear et al. 2017). For this analysis, we used the rclusterf algorithm, AICc model selection criterion, and the GTR+G model of sequence evolution. The resulting best-fit partitioning scheme included 1,122 data subsets and had a significantly better log-likelihood than alternative partitioning schemes (SWSC-EN: −6,352,081.6; By Locus: −6,582,034.0; Unpartitioned: −6,683,264.3).

Using the SWSC-EN partitioning scheme, we inferred phylogenetic relationships of *Ponera* species with the likelihood-based program IQ-TREE v1.5.5 (Nguyen et al. 2015). For the analysis, we selected the '-spp' option for partitioning and the GTR+G model of sequence evolution. To assess branch support, we performed 1,000 replicates of the ultrafast bootstrap approximation (UFP) (Minh et al. 2013, Hoang et al. 2018) and 1,000 replicates of the branch-based, SH-like approximate likelihood ratio test (Guindon et al. 2010). For these support measures, values ≥95% and ≥80%, respectively, signal that a clade is supported.

To get an alternative assessment of relationships and branch supports, we conducted a coalescent-based species tree analysis on the dataset using the summary program ASTRAL-III v5.5.9 (Zhang et al. 2017). We first created a set of gene trees for the set of 1,782 UCE loci using IQ-TREE v1.5.5. These analyses were performed in IQ-TREE using the MODELFINDER (Kalyaanamoorthy et al. 2017) option '-m MFP', the AICc model selection criterion, and 1,000 ultrafast bootstrap replicates. Once the gene trees were generated, we followed the recommendation of Zhang et al. (2017) and used NEWICK UTILITIES (Junier and Zdobnov 2010) to collapse branches with ≤10% bootstrap support. Using the modified gene trees, we performed a standard ASTRAL analysis, leaving all terminals as separate entities, and assessing support as local posterior probabilities.

Divergence Dating

We used BEAST2 v2.4.8 (Bouckaert et al. 2014) to estimate the timing of *Ponera* evolution in the New World. Due to computational constraints, we restricted our analysis to a subset of UCE loci and used a constraint tree. For the loci, we identified the 536 UCE loci that contained all samples (=100% taxon occupancy) and then randomly selected 200 of these loci for analysis using a PHYLUCE script (*phyluce_align_randomly_sample_and_concatenate*). For the constraint tree, we used the best tree from the IQ-TREE analysis described above, and before importing it into BEAST2, we made it ultrametric by rooting the tree on *Rasopone+Simopelta*, assigning the root node an age of 71.3 Ma, and performing a strict clock analysis using the *chronos* function in the R v3.4.4 (R Core Team 2018) package APE (Paradis et al. 2004). We calibrated the constraint tree in order to avoid initialization problems that often occur in BEAST2 when inputting a user-defined starting tree. To configure the BEAST2 analysis, we used the program BEUAti (included with BEAST2), and we applied two node-based calibration points. For the root node, we used a secondary calibration, extracting the age constraint from a comprehensive dating analysis of all ants (Economo et al. 2018), and we selected a normal distribution for the prior, assigning it a mean age of 71.3 Ma and a standard deviation of 6.1. For the other node calibration, we assigned two fossil species of *Ponera* from Baltic amber (Dlussky 2009) to the stem node of *Ponera*. For the prior, we selected a log-normal distribution and assigned a Priabonian age to Baltic amber (Perkovsky 2007) (BEAST2 settings: offset = 37.0, mean = 3.02, SD = 0.31). For the analysis, we used a GTR+G model of sequence evolution with 4 gamma rate categories, an uncorrelated log-normal clock, and a birth–death tree prior. The birth–death tree prior was chosen because our dataset includes inter- and intraspecific sampling and a recent simulation study showed that, with this type of sampling, the birth-death model more accurately recovers ages (Ritchie et al. 2017). In contrast, the commonly used yule model was found to inflate ages within species. We set an exponential distribution for the ucld mean prior (mean = 1.0, initial value = 0.01) and used default values for the remaining priors. We performed six independent runs, each for 200 million generations and sampling every 10 thousand generations. Run convergence was assessed using TRACER v1.7.1 (Rambaut et al. 2018) and runs were combined and summarized using LOGCOMBINER and TREEANNOTATOR, respectively, with node heights calculated as mean heights. All runs converged, and with the burn-in set at 25%, all parameter values had effective sample sizes (ESSs) above 200. All BEAST2 runs were performed using the CIPRES Science Gateway (Miller et al. 2010).

UCE Species Delimitation

As described below, our results indicate that populations of *P. exotica* show significant geographic structuring that could indicate the presence of cryptic species. Consequently, in addition to a morphological assessment of species boundaries, we performed an automated assessment of species boundaries within *P. exotica* using UCE data and the species delimitation program TR2 (Fujisawa et al. 2016). The program TR2 employs a rapid and scalable method that uses genealogical concordance among gene trees, broken down into rooted triples, and the multispecies coalescent, to determine species boundaries. For this analysis, we created a new dataset, consisting only of samples of *P. exotica*. This was carried out by extracting *P. exotica* sequences from the unfiltered set of UCE contigs, aligning and trimming the loci with MAFFT and GBLOCKS, respectively (as above), and then filtering the loci to have 100% taxon occupancy and at least 10 informative sites. This resulted in a set of 643 loci, with a mean number of informative sites of 15.6 (range: 10–96). Gene trees for each locus were estimated using IQ-TREE (as above) and all resulting trees were rooted on a population from Georgia (EX1632) using the program PHYX (Brown et al. 2017). For the TR2 analysis, we used all 643 rooted gene trees and a guide tree, which we selected as the IQ-TREEswsc tree (see above), pruned to include only *P. exotica* samples.

COI Barcode Analysis

Due to the high abundance of mitochondrial DNA in samples, *Cytochrome Oxidase I* (COI) sequence data are generated as a byproduct of the UCE sequencing process. To provide a separate assessment of species identities, possibly with more species/samples, we extracted COI sequences from our UCE enriched samples and combined them with *Ponera* COI sequences available from the BOLD database (Ratnasingham and Hebert 2007) (Accessed 22 July 2018). To extract COI from UCE data we downloaded a complete 658 bp barcode sequence of *P. exotica* from BOLD (Acc.#ASLAM754-11) and used this along with a PHYLUCE script (*phyluce_assembly_match_contigs_to_barcodes*) to extract COI from the bulk set of TRINITY contigs. We then downloaded all publicly accessible barcode sequences from BOLD matching the search term '*Ponera*' and filtered this initial set of sequences for obvious misidentifications and excessive population samples of Old World material. We also reduced the number of New World samples in cases where there were many samples from a single locality. The filtered set of BOLD samples were combined with the UCE samples and the sequences were aligned with MAFFT and then visually inspected using MESQUITE v3.2 (Maddison and Maddison 2018) for signs of pseudogenes or other anomalies. The final matrix was partitioned by codon position and analyzed with IQ-TREE using GTR+G, 1,000 ultrafast bootstrap replicates, and 1,000 SH-like replicates.

Morphological Measurements

Measurements were made with a dual-axis micrometer stage with output in increments of 0.001 mm. However, variation in specimen orientation, alignment of crosshairs with edges of structures,

and interpretation of structure boundaries resulted in measurement accuracy to the nearest 0.01 mm. All measurements are presented in mm. The only morphometric measurement used in this study is HW, defined as the maximum width of the head in full face view, not including the eyes.

Results

UCE Sequencing and Matrix Assembly

After sequencing, assembly, and the extraction of contigs representing UCE loci, we recovered an average per contig coverage of 35.2× (range: 15.4–48.4×) and a mean contig length of 801.5 bp (range: 369.2–972.4 bp). Following alignment, trimming, and filtering of the UCE contigs, our UCE matrix consisted of 1,782 loci and 1,405,192 bp of sequence data, of which 298,316 bp were informative. The mean alignment length post-trimming was 788.6 bp (range: 245–1,962 bp). The final matrix included only 14.9% missing data (including gaps). For additional assembly stats see Supp. Table S2.

Phylogenetics and Divergence Dating

For both the IQ-TREEswsc (Fig. 1) and ASTRAL (Supp. Fig. S1) results, *P. pennsylvanica* was recovered as sister to the European species *P. coarctata*, and the two together were sister to *P. leae*, a species from Australia (Fig. 1). *Ponera exotica* from the United States was found to be sister to a clade containing all Central American samples, confirming our tentative identification of this material. Circumscribed to include the Central American populations, *P. exotica* was recovered as sister to a clade that contained the remaining six *Ponera* species that we sampled, all with Old World native ranges. Thus, native New World *Ponera* do not form a monophyletic group and likely represent two separate dispersals (or range expansions followed by vicariance) from Old World ancestors.

The IQ-TREEswsc and ASTRAL trees differed slightly with regard to relationships among populations within *P. pennsylvanica* and *P. exotica*. However, given the uncertainties regarding species limits, we focus on the IQ-TREE result here. The six sequenced specimens of *P. pennsylvanica*, from a wide variety of sites in the United States, form a monophyletic group (Fig. 1). One specimen, from Oconee National Forest, GA, is sister to the other five. This specimen, and two other specimens we have examined from southern Mississippi and Florida, are somewhat lighter red-brown and are at the small end of the size range (HW 0.52–0.56). Thus, it is possible that there is a separate cryptic species in the southeastern portions of the range; however, the COI results are not entirely congruent (see below). The remaining five sequenced specimens are from sites in Maine, West Virginia, Georgia, Alabama, and Utah. These show no phylogeographic structure.

The 20 sequenced specimens of *P. exotica* show a pectinate relationship going from north to south (Fig. 3). The sequenced specimen from United States is sister to all the Mexican and Central American specimens. Specimens from the United States are the smallest and lightest-colored. Taylor (1967) reported HW of U.S. specimens to be 0.38–0.41. Mexican and Central American specimens have HW 0.42–0.53, and they tend to be darker colored. Members of different clades can be in close proximity. For example, in the Sierra de Chiapas, a specimen from the northern end of the range is in a northern clade, and a specimen from the central part of the range, only 80 km from the previous site, is in a southern clade. This phylogeographic structure, with some specimens in separate clades occurring relatively close to each other, reveals the potential for this

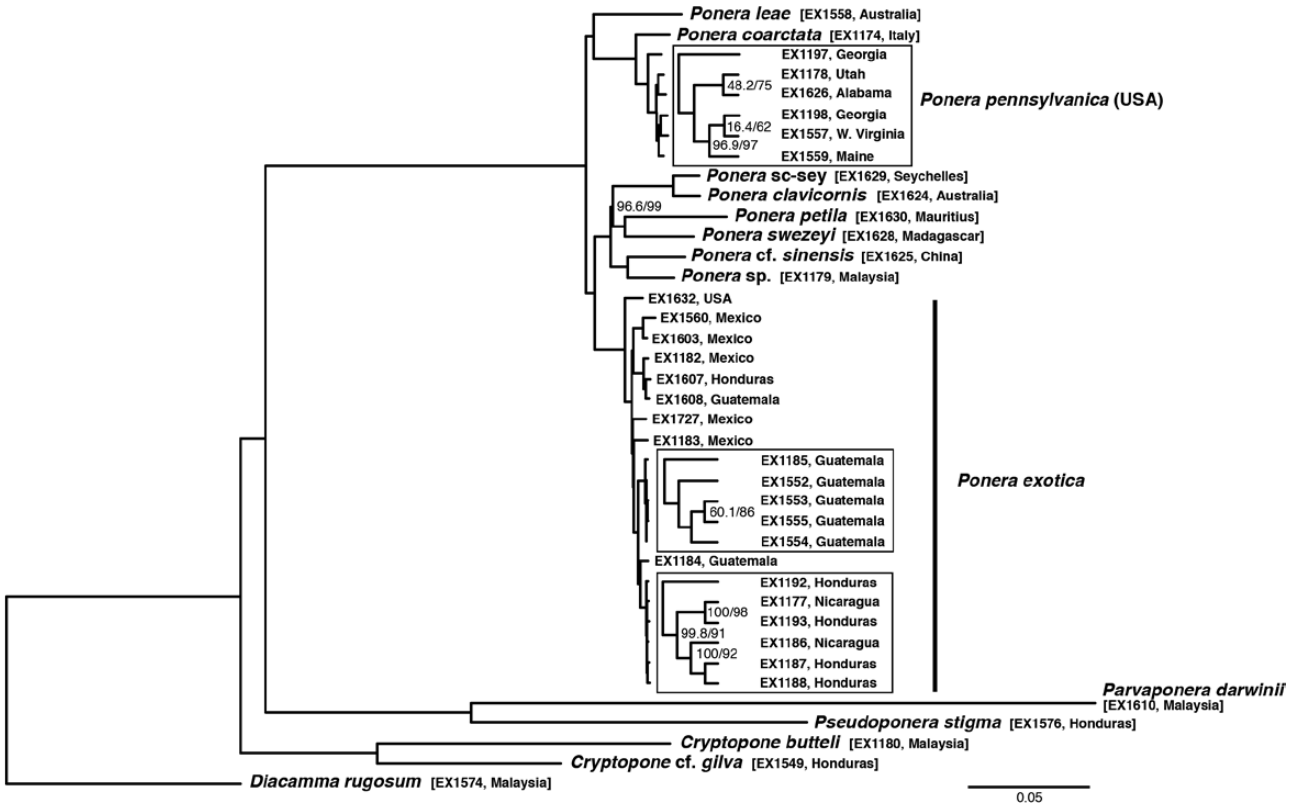

Fig. 1. Phylogeny of New World and selected Old World *Ponera*, inferred using the program IQ-TREE and 1,782 UCE loci. Node support values (ultrafast bootstrap/SH-like) are shown when <100/100. Insert boxes are portions of phylogram expanded as cladograms.

species to be composed of multiple cryptic species. All populations are closely related and indicate a relatively recent radiation. Based on UCE data, the greatest uncorrected p-distance between a pair of sequenced specimens is 1.28% (EX1632 from Georgia vs. EX1184 from Guatemala).

Crown-group ages from the BEAST analysis (Fig. 2; Supp. Fig. S2) suggest a Miocene origin for the genus as a whole (at least for the sampled species; 11.6 Ma, 95% highest posterior density [HPD] interval 8.2–15.5 Ma), a Pliocene origin for the *P. exotica* populations (3.1 Ma, 95% HPD 2.0–4.4 Ma), and a Pleistocene origin for the *P. pennsylvanica* populations (1.6 Ma, 95% HPD 0.9–2.3 Ma).

Molecular Species Delimitation

The TR2 analysis of species boundaries within *P. exotica*, delimited 11 putative species (Fig. 3). Six of these species comprise single populations only (including the population from Georgia), four comprise two populations, and one includes four populations. Considering mean ages, all sister species splits within Middle America occurred less than 2 Ma and the youngest occurred less than 0.2 Ma, suggesting very recent speciation events. The split with the putative species from the United States occurred 3.1 Ma.

COI Analysis

We recovered complete or nearly complete COI fragments from all 39 UCE samples and these ranged in length from 617 to 658 bp. This was combined with 79 COI fragments from BOLD, ranging in length from 438 to 658 bp (Supp. Table S3). For the BOLD sequences, all of the included Old World material clustered appropriately with conspecific UCE samples, confirming the approach of extracting COI sequence from UCE data (Fig. 4; see also Pierce et al. 2017). However, note that the clade containing *P. petila* Wilson, 1957 and *P. bableti* Perrault, 1993 comprises a single species, reflecting likely misidentification or synonymy in BOLD. For New World *Ponera* the BOLD data included 70 sequences of *P. pennsylvanica*, and only four sequences of *P. exotica*. The latter samples represented sequences submitted for COI sequencing by one of the authors (Longino), from populations also sampled for UCE data.

The *P. pennsylvanica* sequences included many populations not sampled by us, mostly from Canada (Ontario, Quebec, New Brunswick), but also the United States (Massachusetts, Tennessee, New York). The COI tree for *P. pennsylvanica* showed some sequence variability, with a maximum uncorrected p-distance of 7.4%, but no obvious geographic structuring. All of the UCE samples were found to be nested inside the more voluminous samples from Canada. Thus, contrary to the UCE results, there was no clear pattern indicating the presence of cryptic species. For *P. exotica*, the divergence among samples was significantly greater, with a maximum p-distance of 17.5%. Although phylogenetic relationships differed slightly from the UCE tree at deeper levels, geographically proximal populations clustered similarly to the UCE results.

Considering the broader COI phylogeny, closely related species clustered similarly to the UCE tree, but, not surprisingly, most relationships above the species level were not well supported. Consequently, these relationships are not discussed.

Discussion

Ponera is a taxonomically challenging group of ants whose species diversity and phylogeny are difficult to determine using morphology alone. Here we used UCE phylogenomic data and morphology to examine species boundaries in the New World and to investigate phylogenetic relationships, particularly as it concerns the origins of the New World fauna and the phylogeography of Central American populations.

Based on multiple lines of evidence (UCEs, COI, morphology, geography), we choose to recognize two native species of *Ponera* in the Americas (full taxonomy below). *Ponera exotica* occurs from the southeastern United States south to Nicaragua, and it is phylogenetically related to several other Asian and Indo-Australian species. Smith (1962) surmised that *P. exotica* was related to Indo-Australian

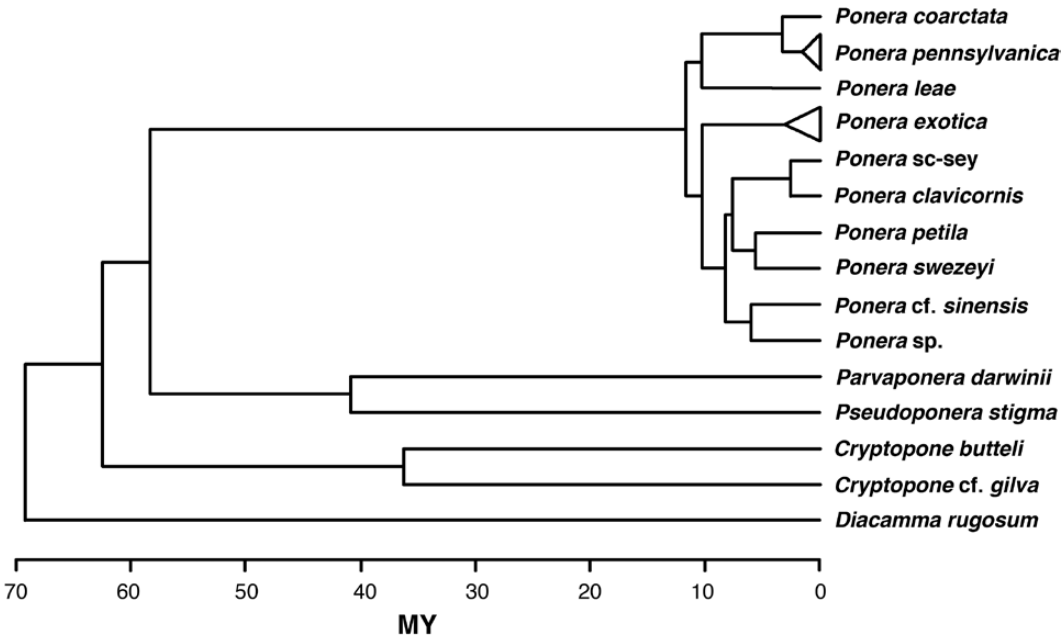

Fig. 2. Chronogram of American and selected Old World species of *Ponera* inferred with BEAST2 and a subset of 200 UCE loci (topology constrained). The tree shows interspecific relationships, with multiple population samples of *P. pennsylvanica* and *P. exotica* collapsed. Estimated crown ages are 11.6 Ma for sampled *Ponera* species, 1.6 Ma for *P. pennsylvanica* populations, and 3.1 Ma for *P. exotica* populations. See Supp. Fig. S2 for all divergence dates and error bars.

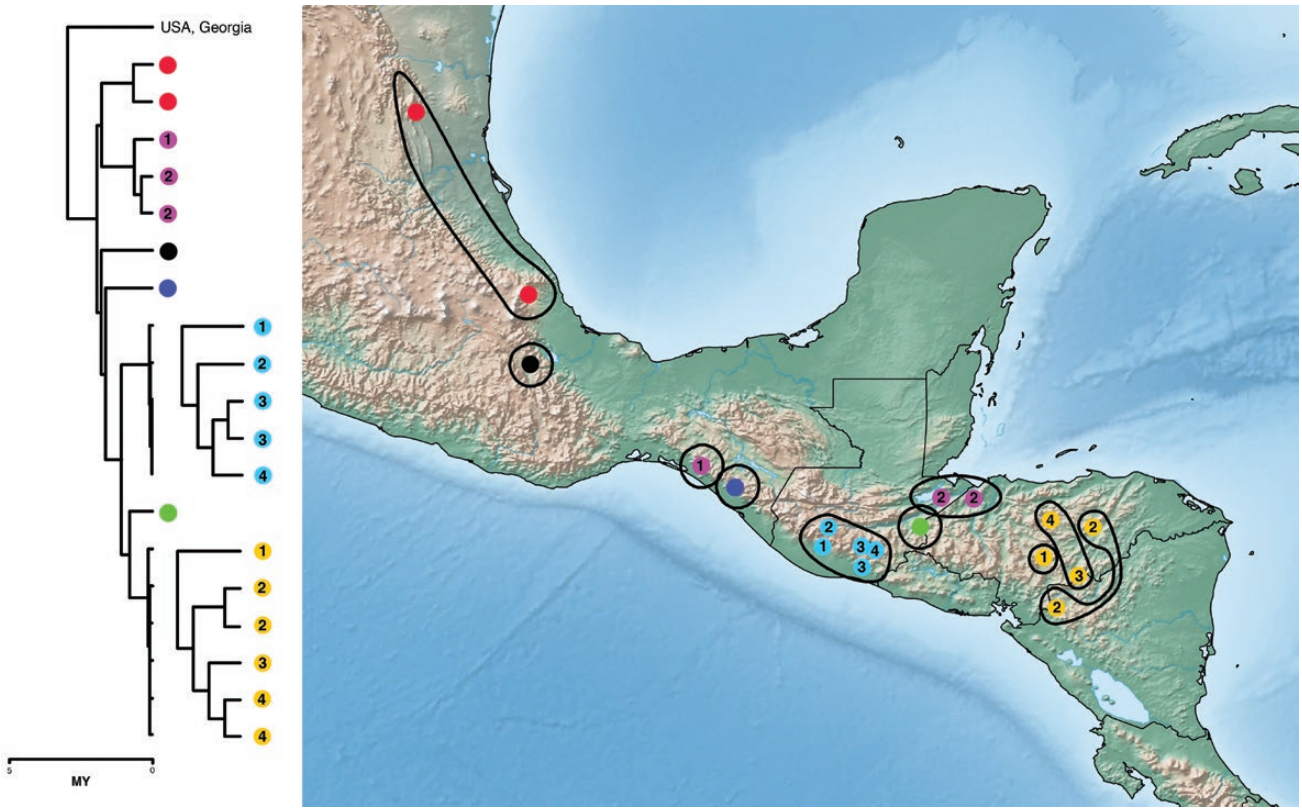

Fig. 3. Phylogeography and species delimitation of *Ponera exotica.* The chronogram on the left shows relationships and divergence times of populations estimated by BEAST2, with points matching localities on the map (the United States, Georgia specimen is not shown). Terminals are colored by clade and numbers are used to match terminals with map points. Two groups of populations with very shallow divergence times are expanded as cladograms. The circles around map points show the putative species delimited by the program TR2.

species, which we confirm, but also that it was recently introduced, which our divergence dating analysis firmly rejects (3.1 Ma crown age). *Ponera pennsylvanica* is widespread in eastern North America. Our phylogenetic results confirm the expected sister taxon relationship between *P. pennsylvanica* and *P coarctata*, a similar European species. *Ponera coarctata* has a sympatric sister species, *P. testacea* (Csösz and Seifert 2003), thus the three form a Holarctic *P. coarctata* clade. This clade is phylogenetically closer to the Australasian species *P. leae* than it is to *P. exotica*.

These phylogenetic results indicate that *P. exotica* and *P. pennsylvanica* most likely represent two independent colonizations of the Americas by Old World ancestors. Considering stem- and crown-group ages, *P. exotica* dispersed to the New World between 10.2–3.1 Ma and *P. pennsylvanica* between 3.4 and 1.6 Ma. Although chance long-distance dispersal is a remote possibility, it is more likely that both species dispersed across the Bering Land Bridge sometime over the last 13 Ma, given that this route has provided the most recent access to the New World (Sanmartín et al. 2001, Milne 2006). *Ponera exotica*, with a more tropical climate affinity relative to *P. pennsylvanica*, may have dispersed first and spread across North America during the warmer conditions of the Pliocene, and then slowly dispersed southward into Central America. In contrast, *P. pennsylvanica*, with more temperate climate affinities, remains restricted to temperate habitats. Its younger crown age may indicate that it arrived later, during the Pleistocene, or that population processes of expansion and contraction during the Pleistocene have erased evidence of earlier arrival.

By confirming that *P. exotica* represents a single species, or a species complex (see below), it can be added to the list of taxa that have a phylogeographic disjunction between the eastern United States and Middle America. This pattern has been documented in over 50 plant species (Graham 1999), but significantly fewer animals (Martin and Harrell 1957, Carlton 1990, Arbogast 2007). In ants, the most similar case that we know of is *Cryptopone gilva* (Roger), another ponerine ant species that occurs in the southeastern United States and Central America (Guatemala to Costa Rica). The Central American populations, however, appear to represent a sister clade comprised of several distinct species (Longino and Branstetter, in prep). The ant genus *Stenamma* Westwood, a cloud forest specialist, also has separate Holarctic and Middle American clades, but this separation involves multiple species and is older (Branstetter 2012, Branstetter 2013). To explain United States–Middle America phylogeographic disjunction, the most commonly proposed causal factor is Pliocene-Pleistocene cooling and glaciation, which began between 3.2 and 2.4 Ma (Raymo 1994, Ehlers and Gibbard 2007). For *P. exotica*, this explanation seems plausible, given that we inferred a divergence date between United States and Middle American populations of 3.1 Ma (95% HPD 2.04–4.35 Ma). A possible scenario is that *P. exotica* arrived to the New World during the late Miocene and then separated into distinct United States and Middle American clades as the climate cooled, with complete genetic isolation starting at the beginning of the Pleistocene.

Another intriguing pattern in *P. exotica* is the pectinate phylogenetic structure of its populations. The U.S. population is sister to the Middle American populations and there is a trend of north to south dispersal within Middle America. Different scenarios could explain this pattern, but the most intuitive one is that *P. exotica* has been slowly dispersing southward over the last 3.1 Ma. The rapid glacial

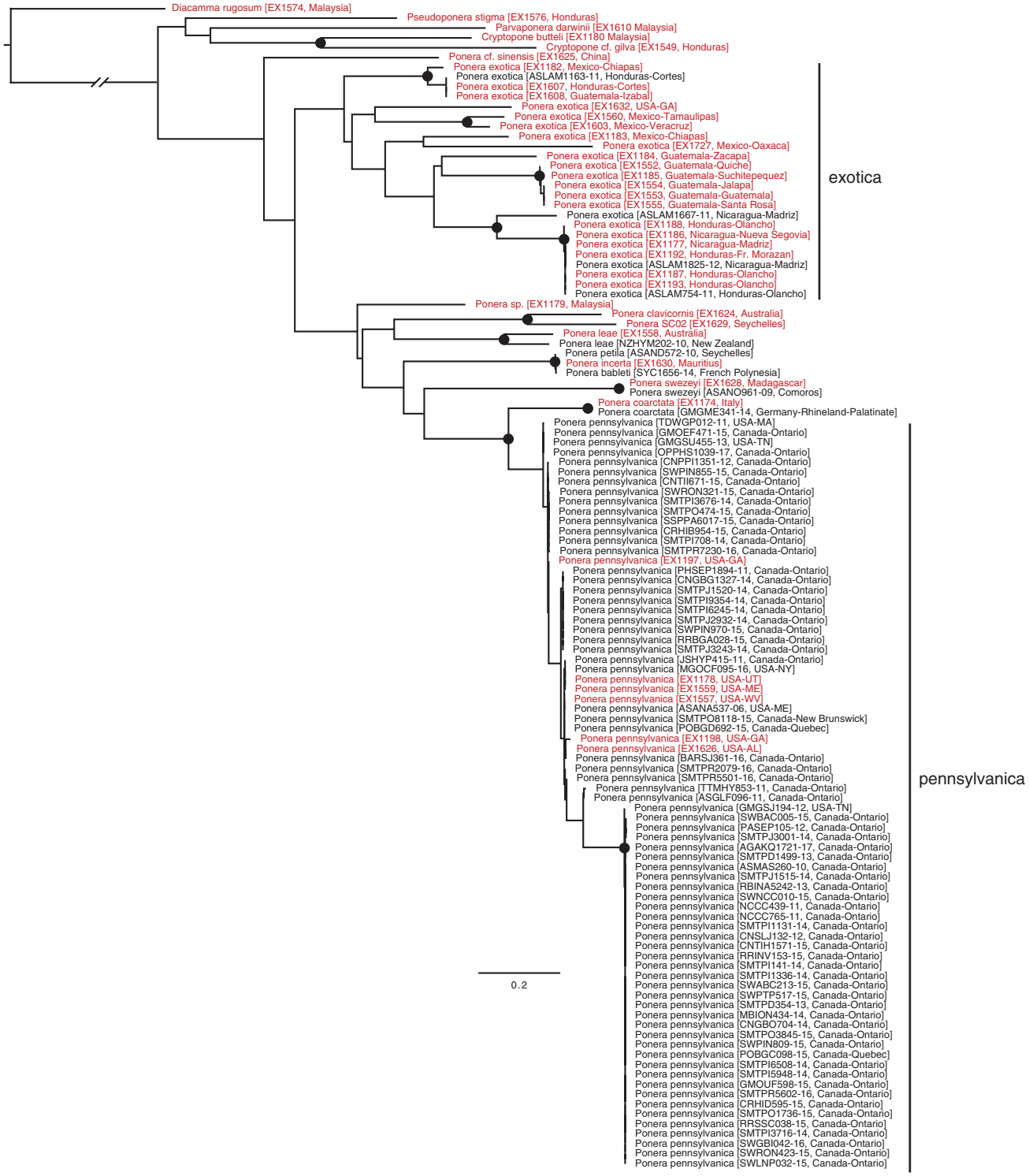

Fig. 4. Phylogenetic relationships among COI barcode sequences for *Ponera*. Red samples were sequenced for UCEs. Black samples were downloaded from the BOLD database. The tree was inferred using IQ-TREE with the data partitioned by codon position. Black circles on nodes indicate high support, which we define as ≥95% ultrafast bootstrap support and ≥95% SH-like branch support.

cycling of the Pleistocene is known to have caused cloud forest habitats to oscillate up and down mountains (Bush and Hooghiemstra 2005), opening and closing dispersal pathways. This may have created a stepping-stone advance into Central American cloud forest, with each advance followed by genetic isolation and divergence. We acknowledge, however, that our sampling within the United States is limited to a single population, so additional sampling in this area could reveal more nuanced patterns. Specifically, it would be interesting to see if additional sampling confirmed the existence of a sister clade relationships between the United States and Middle America, or revealed that the pectinate phylogenetic structure extended across U.S. populations. Sampling populations from Florida should be of

high priority given the proximity of Florida to the Caribbean/Middle America and the observation that Florida has been an important refugium during Pleistocene glacial events (Soltis et al. 2006, Morris et al. 2008).

Although we recognize only two native species of *Ponera* in the New World, the molecular data indicate that *P. exotica* might include cryptic species or at least has limited gene flow among populations. The delimitation of *P. exotica* into 11 putative species by the program TR2 was surprising. Given recent evidence that delimitation methods based upon the multispecies coalescent model tend to delimit populations rather than species (Sukumaran and Knowles 2017), we view this result as very tentative, especially since no obvious cases of sympatry have been observed (based on morphology) and there is little morphological variation among populations. The COI results showing deep splits among samples and up to 17.5% sequence divergence also indicate the possibility of cryptic species, but high sequence divergence does not necessarily mean reproductive isolation. It could just be the consequence of time, limited dispersal, and lack of selective sweeps within mtDNA. Additional within-population sampling is needed to examine population genetic structure and gene flow within *P. exotica*.

This work sheds light on the taxonomy of New World *Ponera* and identifies some intriguing evolutionary patterns that should be investigated further with additional sampling. One area that could be improved upon is our sampling of Old World taxa. We sampled only 8 out of 55 species. Although our sampling is sufficient to show that New World *Ponera* represent distinct lineages, including additional material would help identify the geographic origin of the genus and could identify different taxa as the closest relatives of the New World species. Also, sampling additional populations of *P. exotica* could help verify the presence of cryptic species and confirm the pectinate phylogeographic structuring. Furthermore, sampling multiple individuals per population would make it possible to estimate rates of gene flow and other population genetics parameters among populations. Such sampling would make *P. exotica* an animal version of such taxa as the plant *Liquidambar styraciflua* L., which has become a focal taxon for examining biogeographic disjunction between the eastern United States and Middle America (Morris et al. 2008, Ruiz-Sanchez and Ornelas 2014).

Taxonomy

Ponera Latreille, 1804

Identification of American Species

Ponera pennsylvanica and *P. exotica* can be differentiated on the basis of size and sculpture. From the measurements in Taylor (1967) and our new measurements, *P. pennsylvanica* HW is 0.53–0.63, *P. exotica* HW is 0.36–0.53. The larger *P. exotica* are in Central America, where they are not sympatric with *P. pennsylvanica*. In the zone of sympatry, *P. exotica* are smaller, with HW no greater than 0.41 according to Taylor (1967). *Ponera pennsylvanica* has the pronotal dorsum more densely and distinctly punctate, with puncta diameters greater than distances between them. *Ponera exotica* is shinier, with smaller, more widely spaced puncta on the pronotum.

Ergatoid Queens (Sensu Peeters 2012)

Both *P. pennsylvanica* and *P. exotica* have ergatoid queens that are morphologically intermediate between workers and fully alate queens. Ergatoid queens occur repeatedly within species, are relatively uniform in structure, and appear fully functional. Thus they are likely to be functional queens rather than 'intercastes' (Sensu Peeters 2012), which are occasional developmental abnormalities.

Potential Introduction

We received a collection of *Ponera* from an urban area in Portland, Oregon, in the northwestern United States. The collection contained two workers from a pitfall trap, collected by K. A. Larsen in 2010. The workers key to *P. testacea* based on the key of Taylor (1967) and the morphometric criteria of Csősz and Seifert (2003). Two species of European *Myrmica* have become established in the Pacific Northwest (Jansen and Radchenko 2009, Wetterer 2011), so it would not be surprising to discover that *P. testacea* has as well.

Ponera exotica Smith (Figs. 5A, 5B, 6)

Ponera exotica Smith 1962:378, Fig. 1. Holotype worker, paratype queen: United States, North Carolina, Craven Co., Croatan National Forest, 2mi E Croatan, 20 August 1960 (W. G. Carger #11; National Museum of Natural History, Washington, DC; not examined).

Geographic Range

Eastern United States west to Texas, south to Nicaragua.

Measurements

Worker HW 0.38–0.53 (n = 18); *Ergatoid queen* HW 0.45–0.54 (n = 7); *Queen* HW 0.50–0.54 (n = 4).

Comments

A few biological notes on this species are reported by Smith (1962), Taylor (1967), Johnson (1987), and Mackay and Anderson (1991). In the U.S. portion of its range, it occurs in dry to mesic woodlands and is found in soil and leaf litter. In the southeastern United States it occurs in the coastal plain and piedmont region. In the Mexican and Central American portion of its range, it is a cloud forest specialist and does not occur down to sea level. It is most often collected in Winkler or Berlese samples of litter and rotten wood. We have also observed specimens under epiphytes in a treefall and in the base of a treefern trunk.

Ergatoid queens are present in most populations we have sampled. At a minimum they have ocelli and all other characters are worker-like. Compound eyes may be very small, composed of a few fused ommatidia and only slightly larger than workers, or up to about half the size of eyes in alate queens. The mesosoma may be essentially worker-like, or with a small mesoscutellar sclerite. In one specimen the anepisternum and katepisternum are divided by a faint sulcus. Ergatoid and alate queens may occur together in the same population, and occasionally in the same litter sample, but populations vary in the frequency of the two forms. Areas with exclusively or mostly alate queens are the volcanic ranges of southern Guatemala, the Sierra de Los Tuxtlas in the state of Veracruz, Mexico, and nearby portions of the mountain ranges in Oaxaca. Populations in Nicaragua and Honduras, at the southern edge of the range, appear to have only ergatoid queens.

Ponera pennsylvanica Buckley (Figs. 5C, 5D, 6)

Ponera pennsylvanica Buckley 1866:171. Based on the worker caste, but no types are known to exist (Taylor 1967). Emery 1895a:267: description of queen, male. Wheeler and Wheeler 1952:631: description of larva. Emery 1895:267, Dennis 1938:227, Creighton 1950:48: as subspecies of *P. coarctata*. Taylor 1967:29: revived status as species.

Fig. 5. Images of face and profile views of New World *Ponera*. (A) *P. exotica* worker (CASENT0603539, Mexico, Chiapas). (B) *P. exotica* ergatoid queen (CASENT0603538, Mexico, Chiapas). (C) *P. pennsylvanica* worker (CASENT0003322, United States, North Carolina). (D) *P. pennsylvanica* ergatoid queen (CASENT0645791, United States, Virginia). All face views are to same scale, and all profile views are to same scale. Images for A, B, and C from www.antweb.org (A and B by April Noble).

Geographic Range

Eastern United States and Canada, west to Washington state.

Measurements

Worker HW 0.52–0.63 (n = 16); *Ergatoid queen* HW 0.65–0.66 (n = 2); *Queen* HW 0.66–0.69 (n = 3).

Comments

Taylor (1967) reviewed the biology of this species. It appears to be coextensive with the eastern deciduous forests of eastern North America, where it can be relatively abundant. Nests are typically in and under rotten wood. In drier, more open habitats it nests under stones in moist soil (Gregg 1963). Mackay and Anderson (1991)

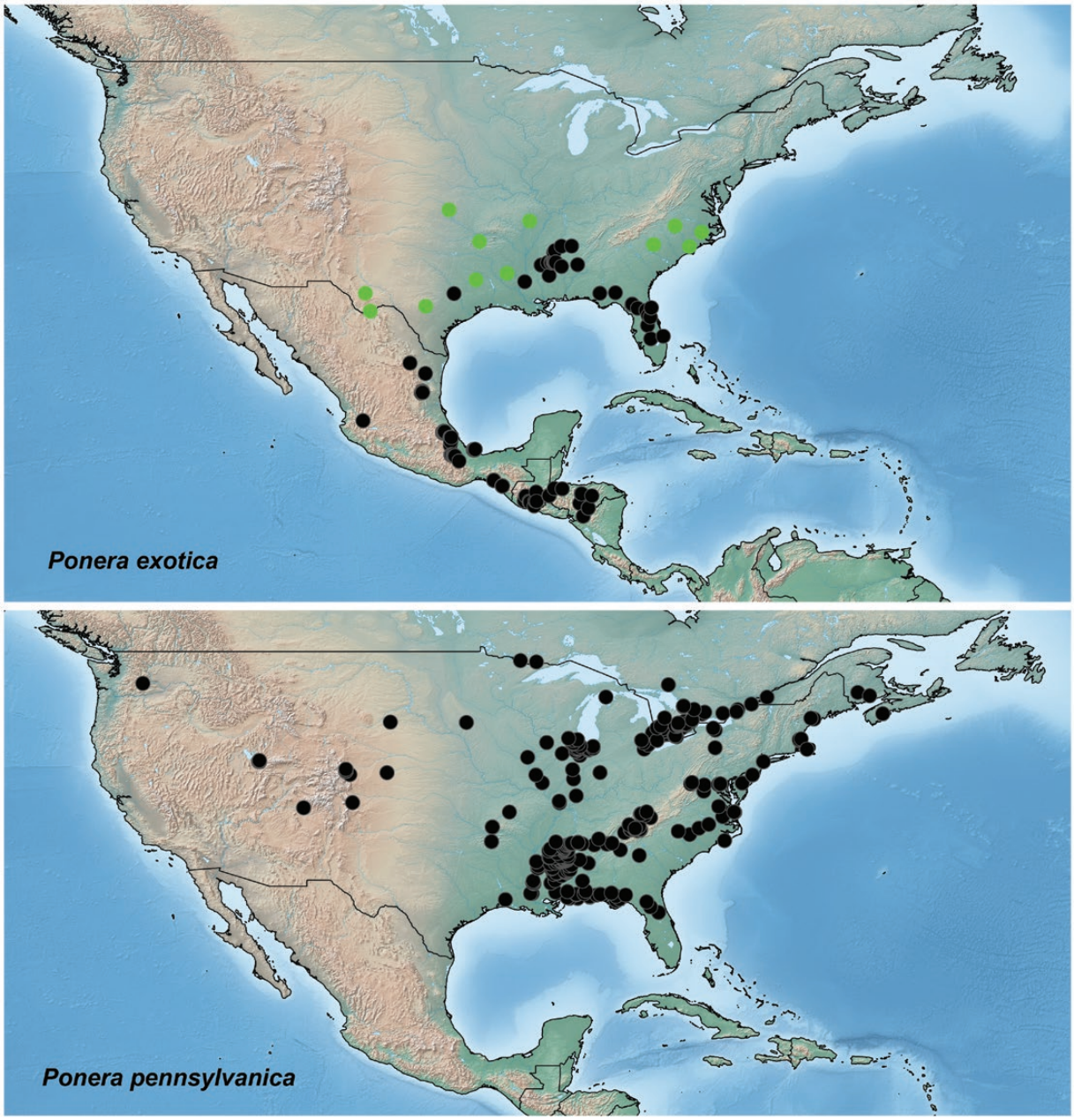

Fig. 6. Distribution of New World *Ponera*. Occurrence records (black dots) are a combination of specimens examined by the authors, records on AntWeb (www.antweb.org), and records on BOLD (www.boldsystems.org). Green dots are additional records of *P. exotica* from MacKay and Anderson (1991) that extend the range, but were not examined by us.

reported additional western localities and depicted a western range limit from the Dakotas south through Colorado and New Mexico We extend the western range limit to Utah and Washington state. In Salt Lake City, Utah, the species is moderately abundant under lawns in urban areas and under stones in nearby native gambel oak scrub habitat. Matthew Prebus collected workers in Yakima, Washington, in a suburban area under stones. Given the habitat preference, the species is possibly non-native west of the Rocky Mountains and has recently colonized urban habitats, which are artificial deciduous forest habitats in an otherwise arid landscape. Mackay and Anderson (1991) also reported an isolated record, based on one worker, from Michoacan, Mexico. We have not examined the Michoacan specimen and its identity should be verified because there is the potential for it to be a large specimen of *P. exotica*. We have examined an ergatoid queen of *P. exotica* collected in the nearby state of Guadalajara.

We have observed two instances of ergatoid queens, from localities in Maryland and Virginia. In each case, they were in Winkler samples of sifted leaf litter and rotten wood, and there were workers and alate queens in the same samples. The ergatoid queens have compound eyes nearly as large as the eyes of alate queens, ocelli are present, and the mesosoma is enlarged with mesoscutellar and metanotal sclerites present. Wing scars and the small sclerites associated with wings are absent.

Data Availability

Raw Illumina reads and contigs representing UCE loci have been deposited at the NCBI Sequence Read Archive and GenBank, respectively (BioProject# PRJNA513200 for new data and PRJNA360290

for previously sequenced samples). All newly generated COI sequences have been deposited at GenBank (MK381276-381314). A complete list of NCBI accession numbers are available in Table S4. The concatenated UCE matrix, the COI matrix, all Trinity contigs, all tree files, and additional data analysis files have been deposited at Dryad (https://doi.org/10.5061/dryad.jd1kn44). The Phyluce package and associated programs can be downloaded from github (github.com/faircloth-lab/phyluce). The ant-specific baits used to enrich UCE loci can be purchased from Arbor Biosciences (arborbiosci.com/products/uces/) and the bait sequence file is available at figshare (figshare.com/authors/brant-faircloth/97201).

Supplementary Data

Supplementary data are available at *Insect Systematics and Diversity* online.

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