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ORIGINAL ARTICLE

Phylogeography of the parthenogenic ant *Platythyrea punctata*: highly successful colonization of the West Indies by a poor disperser

Jon N. Seal*, Katrin Kellner†, Andreas Trindl and Juergen Heinze

Universität Regensburg, Universitätsstrasse 31, Lehrstuhl Biologie I, D-93040 Regensburg, Germany

ABSTRACT

Aim We investigated the spatio-temporal patterns of genetic diversity in West Indian and mainland populations of a widespread parthenogenic ant (*Platythyrea punctata* F. Smith) to infer source populations and subsequent colonizations across its geographic range.

Location Central America, Texas and the West Indies (Florida, the Bahamas, Greater and Lesser Antilles).

Methods We employed phylogeographic reconstruction based on 1451 bp of mitochondrial DNA (cytochrome *c* oxidase subunits I and II) sequenced from 91 individuals of *P. punctata*. We employed standard population genetic analyses, Bayesian phylogenetic analyses, haplotype networks and molecular dating methods as performed by BEAST. We also employed phylogenetic analysis using two nuclear markers (970 bp) to understand the placement of *P. punctata* in the globally distributed genus *Platythyrea*.

Results Based on highly reduced haplotypic variation and temporal estimates, rapid expansion and dispersal from Central America best explains the observed distribution of haplotypes. *Platythyrea punctata* successfully invaded the West Indies very few times. One haplotype occurred on every island surveyed from the Bahamas and Florida in the north to Barbados at the southern edge of its range. Haplotype diversity in the West Indies is quite low, despite a larger sample size relative to the mainland. Most mainland colonies collected each possessed a unique haplotype, whereas only Florida and the larger islands (the Dominican Republic, Puerto Rico and Guadeloupe) contained more than one haplotype. Island haplotypes were most similar to haplotypes collected in northern Mexico and southern Texas, but genetic distances were nevertheless high. The putative sister species of *P. punctata* appears to be an endemic of Hispaniola (*P. strenua* Wheeler & Mann), even though older, mainland populations of *P. punctata* are sympatric with at least two other congenerics.

Main conclusions Dispersal seems very limited on the mainland, with well-defined clades corresponding to geographical regions. Colonization of the islands from the mainland was extremely rare, but once successful there were very few barriers to expansion to nearly every island in the West Indies. We hypothesize that this invasion occurred during the late Pleistocene as the climate became warmer and less arid.

Keywords

Ants, Caribbean, Central America, climate change, Formicidae, parthenogenesis, phylogeography, Pleistocene, sexual reproduction, thelytoky.

*Correspondence: Jon N. Seal, Integrative Biology, University of Texas at Austin, 1 University Station #C0930, Austin, TX 78712, USA.
E-mail: trachymyrmex@googlemail.com
†Present address: Katrin Kellner, Integrative Biology, University of Texas at Austin, University Station #C0930, Austin, TX 78712, USA.

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INTRODUCTION

The West Indies, with their profound endemism and affinities with North and South America, have long been a focus of historical biogeographers (Liebherr, 1988a; Ricklefs & Bermingham, 2008; Losos & Ricklefs, 2009). The central goal has been to understand which mechanisms have been responsible for the emergence of the current flora and fauna (Liebherr, 1988a). The problem is that the islands exhibit vastly different geological histories, making it difficult to draw general and universal conclusions, especially regarding the relative importance of dispersal and vicariance (Crother & Guyer, 1996; Hedges, 1996b, 2006; de Queiroz, 2005; Crisci & Katinas, 2009). Solving these puzzles has been greatly facilitated by recent advances in molecular techniques (Hedges, 2006), especially those involving the use of non-recombining molecular markers to infer founder effects, population expansion, and bottlenecks within populations (Freeland, 2006; Avise, 2009).

The West Indies consist of the Greater Antilles (Cuba, Hispaniola, Jamaica and Puerto Rico), the Lesser Antilles (an island arc extending south-east of the Greater Antilles to near South America), and the Florida and Bahamian platforms in the north. The Greater Antilles are old continental land fragments, which prior to occupying their present position migrated into the region from the eastern Pacific after passing between North and South America (Graham, 2003; Iturralde-Vinent, 2006). This has led many to conclude support for vicariance under a scenario whereby the biota of the Greater Antilles was obtained from North and South America and then transported to its present position by plate tectonics (Rosen, 1975; Crother & Guyer, 1996). The Lesser Antilles are a much younger chain of primarily volcanic islands that occur along a fault between the Caribbean and North and South American plates. Biota probably reached these islands by dispersal, as deep water has long separated these islands from other main landmasses. However, many have argued for a land bridge with South America via the currently submerged Aves Ridge (Iturralde-Vinent, 2006), located just west of the main island arc of the Lesser Antilles. Florida and the Bahamas are part of the North American plate and, although they have been tectonically stable since the Cretaceous, the relative amounts of exposed and inundated areas have changed throughout the Cenozoic as a result of fluctuations in sea level and climatic conditions (Webb, 1990; Hedges, 1996a; Hearty & Kaufman, 2000). Therefore, vicariance and/or dispersal could reasonably be expected to have a role in the formation of their biota.

Most information on the roles of vicariance versus dispersal in the West Indies comes from studies on vertebrates, with most support at the molecular level indicating a strong role for dispersal over vicariance (Hedges, 1996a, 2006). Hedges (1996a, 2006) suggested that species capable of dispersal by flying or swimming (freshwater fish, birds or bats) colonized the islands from nearby North and Central America. Non-volant vertebrates (amphibians and reptiles) on Caribbean islands appear to have a South American origin and colonized by rafting from northern South America. This process was presumably helped by prevailing winds and currents that flow from east to west (Hedges, 1996a, 2006; Hedges & Heinicke, 2007; Camargo *et al.*, 2009). Other vertebrates may have reached the West Indies by rafting from Europe and Africa (Vidal *et al.*, 2007). In contrast to these studies on vertebrates, most studies addressing the biogeography of West Indian arthropods have been taxonomic works emphasizing endemism (e.g. Liebherr, 1988a,b; Miller & Miller, 1989). Other than a small handful of studies incorporating molecular markers, which have found evidence for vicariance in flightless crickets (Oneal *et al.*, 2009) and dispersal from multiple origins in heliconiine butterflies (Davies & Bermingham, 2002), genetic information is largely lacking on the phylogeography of West Indian arthropods.

Ants are widely considered to be important members of many low- to mid-latitude ecosystems on account of their occupying keystone and ecosystem engineering roles, but we lack an overview of ant biogeography in portions of North, Central and South America and the West Indies. Wilson (1988) postulated that Caribbean ants fall into two categories with respect to their origin. He proposed that the vast majority of Caribbean ants reached the West Indies from South America by first colonizing the Lesser Antilles before reaching the Greater Antilles (Fig. 1). On the other hand, a minority of the ant fauna seemed to be older species endemic to the larger islands and showed affinities with the mainland, especially North and Central America (Wilson, 1985, 1988). Wilson supposed that this indicated that the Greater Antilles were once much closer to the mainland than they are now, although it was unclear whether this was a consequence of continental drift or of the emergence of a larger Antillean landmass during

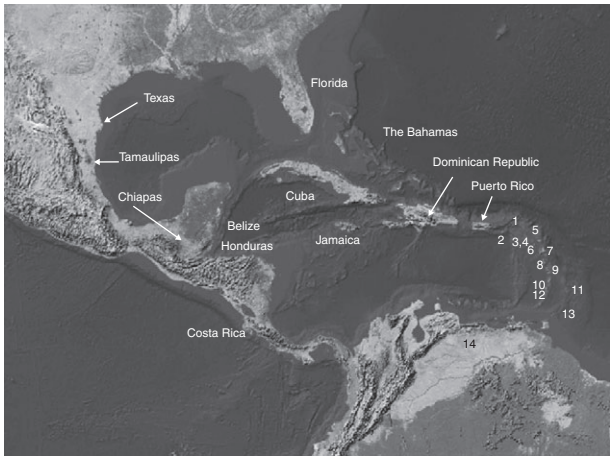

Figure 1 Map of the West Indies and nearby Central America. Named areas indicate islands or countries where specimens used in this study were collected. Numbers indicate the following locations: 1, Vieques island (Puerto Rico); 2, St Croix, US Virgin Islands; 3, St Kitts; 4, Nevis; 5, Antigua; 6, Montserrat; 7, Guadeloupe; 8, Dominica; 9, Martinique; 10, St Lucia; 11, Barbados; 12, Grenada; 13, Trinidad and Tobago; 14, Venezuela. Map produced with permission from ESRI (http://www.arcgis.com).

periods of lower sea levels. An examination of the Dominican amber ant fauna revealed a possible close connection to North and Central America: fossil army ants, for example, were more similar to contemporary species in Mexico and the USA than to those in Central or South America (Wilson, 1985, 1988). Generally, evaluations of these hypotheses require phylogeographic examination of colonization patterns to fully determine whether the patterns exhibited by ants indeed fall into Wilson's categories.

The parthenogenic ant *Platythyrea punctata* F. Smith is of general interest biogeographically because it occupies a very large range that extends from south Texas to Costa Rica and from Florida to most islands in the West Indies and the Bahamas. It is not known to occur in Panama, South America or Trinidad and Tobago. Throughout its range it is found in relatively undisturbed, wooded areas and is probably a native species (Deyrup *et al.*, 1988; Deyrup, 2000). A seeming contradiction to this wide distribution is that it would seem to be a very poor disperser. Winged queens have only been found in Florida, the Bahamas and the Dominican Republic (Wheeler, 1905; K. Kellner, unpublished data). Moreover, queens presumably do not fly, because they lack ocelli, possess poorly developed thoraces and wing muscles (Schilder *et al.*, 1999a) and do not appear to be collected in malaise traps (Deyrup & Trager, 1986; http://www.antweb.org). Colonies are presumed to reproduce mainly by splitting or fragmenting (fission or budding) and the ants subsequently disperse by walking over land.

The biogeography of Neotropical *Platythyrea* remains poorly understood, especially with regard to the origin of the West Indian species. The West Indies harbour only two *Platythyrea* species – *P. punctata* and *P. strenua* Wheeler & Mann – the latter an endemic only known to occur on Hispaniola (Dominican Republic and Haiti) (Wheeler & Mann, 1914). Wilson (1988) thought that West Indian *Platythyrea* had a South American origin because all other Neotropical *Platythyrea* species seemed to occur primarily on that continent, with northern range extensions into Central America (Brown, 1975). *Platythyrea angusta* Forel occurs from South America north to Trinidad and Tobago (Starr & Hook, 2003). *Platythyrea pilosula* F. Smith occurs from Costa Rica into South America, with some overlap with the distribution of *P. punctata* (Brown, 1975). *Platythyrea prizo* Kugler and the morphologically similar *P. lenca* De Andrade (both have serrate mandibles) appear to have similar distributions (Kugler, 1977; de Andrade, 2004). In addition to these five species, four others are known from fossilized Dominican amber deposits, indicating the current West Indian fauna to be comparatively depauperate (Lattke, 2003; de Andrade, 2004). This was also noticed by Wilson (1985, 1988), who left open the possibility that *Platythyrea* is an old taxon and has been present in the Caribbean for a very long time. Recent ant phylogenies suggest that *Platythyrea* occupies an early branch in the evolution of its subfamily (Ponerinae) (Brady *et al.*, 2006; Moreau *et al.*, 2006). The distribution of contemporary *Platythyrea* could therefore be a result of vicariance and subsequent extinction over long geological time-scales.

This study was motivated by the finding that *P. punctata* is one of the few ants known to exhibit thelytokous parthenogenesis (the production of diploid female offspring from unfertilized eggs) (Heinze & Hölldobler, 1995; Schilder *et al.*, 1999a,b; Hartmann *et al.*, 2005). A preliminary phylogeny by Hartmann *et al.* (2005) suggested that parthenogenesis was associated with the colonization of the West Indies by *P. punctata*, because parthenogenesis could only be confirmed in the island populations. This conclusion is plausible because parthenogenesis and its analogues (e.g. clonal growth) are often associated with younger and expanding populations (Vandel, 1928; Maynard Smith, 1972; Williams, 1975) and with climate change (Kearny, 2005). Hartmann also suggested that *P. punctata* arrived in the West Indies *c.* 1.5 Ma. However, these conclusions were limited because they were based on the collection of only one colony on the mainland, and that colony was incapable of parthenogenesis (Hartmann *et al.*, 2005). Their phylogeny was also limited in scope – only a small segment of mitochondrial DNA (mtDNA) was sequenced, and consequently there was poor resolution among Neotropical outgroups and within *P. punctata* sequences. Since then we have discovered that thelytoky is actually widespread on the American mainland and in older lineages (K. Kellner, unpublished data). Thus, the evolution of thelytoky appears to have occurred prior to the colonization of the West Indies. As the evolution of parthenogenesis is frequently associated with hybridization and polyploidy in insects (White *et al.*, 1982; Strasburg & Kearney, 2005; Gómez-Zurita *et al.*, 2009), an understanding of the evolution of asexuality in this species may involve comparisons with closely related species. This would require the identification of its closest living relatives, if not sister species.

The following study addresses these limitations by conducting a more thorough phylogeographic analysis that investigates spatio-temporal patterns of genetic diversity in West Indian and mainland populations of *P. punctata*. We use these to infer source populations and subsequent colonizations across its geographic range. Specifically, we test the following five hypotheses that could explain the current distribution of *P. punctata* (Table 1). (1) *Platythyrea punctata* might have arrived from sources on the North or Central American mainland. Even though taxa generally colonize islands from mainland areas, *P. punctata* could have colonized the mainland from origins in the West Indies, as reported for a number of terrestrial animals (Bellemain & Ricklefs, 2008). Regardless of the source, the invasion could be associated with traits typical of a recent invasion, with higher genetic diversity found in the core population and with reduced genetic diversity at the invasion front (Nichols & Hewitt, 1994; Hewitt, 2000, 2004). (2) Dispersal could be frequent and happen over longer periods. Invasions, subsequent extinctions and bottlenecks would produce a mixture of ancestral and derived haplotypes on the islands. (3) At the other extreme, this species could be very old and have been subject to vicariant events as continental drift split an ancestral population when the Caribbean plate drifted away from the mainland. Vicariance

Table 1 The five phylogeographic hypotheses and predictions tested in this study in an attempt to explain the current distribution of *Platythyrea punctata*.

	Description	Predictions
1	Rare, recent dispersal	Derived haplotypes and reduced variation in colonized range (islands or mainland)
2	Multiple dispersal over long time periods	Derived and ancestral haplotypes present in both regions
3	Ancient (plate tectonic) vicariance	Deep divergence between West Indian and mainland haplotypes
4	Dispersal and subsequent (sea level-induced) vicariance	Shallow divergence within West Indian populations
5	Human-mediated dispersal	No clear phylogeographic pattern

would be supported by deep divergence between the Greater Antilles and mainland populations, and both lineages would be millions of years old. Subsequent dispersal events could explain the presence of *P. punctata* on other islands (e.g. the Lesser Antilles, Bahamas, Florida, etc.). (4) On the other hand, the current distribution could be explained by a dispersal event to the West Indies and then subsequent vicariance as sea levels rose and closed off land connections among the islands of the Greater Antilles, or at least increased over-water distances. Evidence for this would come in the form of significant divergence among the islands and groups of islands, but this divergence would be relatively shallow compared with that in older populations found on the mainland. (5) The final possibility is that this species could be associated with human commerce (i.e. a tramp species) and thus have had multiple introductions across its West Indian and American mainland ranges. In this case, there should be no clear phylogeographic pattern.

MATERIALS AND METHODS

Taxon sampling

Approximately 250 colonies of *P. punctata* were collected over the period 2005–09 (see Appendix S1 in the Supporting Information). Individuals were preserved in 100% ethanol for DNA sequencing. With the exception of colonies from the mainland, for which an individual from each colony was sequenced ($n = 20$), individual colonies from Florida and the islands were selected from colonies that represented certain regions within islands or entire islands (Appendix S1). Sequences obtained from these individuals were augmented with sequences acquired by Schilder *et al.* (1999a) and Hartmann *et al.* (2005) (Appendix S2). In total, 62 sequences were obtained from West Indian populations and 29 sequences from the mainland.

Sequences were also obtained from an additional nine *Platythyrea* species, including four more from the northern Neotropics (*P. angusta, P. pilosula*, *P. prizo* and *P. strenua*) (Appendix S2). We used sequences available from *P. strenua* in GenBank: wingless, accession number EU155479; longwave rhodopsin, EU155460; and cytochrome *c* oxidase subunit I (COI), EU155441. Of Neotropical *Platythyrea*, there are only two species lacking from our dataset, both of which occur in southern South America (*P. exigua* and *P. zodion*) (http://www.antweb.org; Brown, 1975).

DNA sequencing

Total DNA was extracted from whole individual ants using the CTAB (cetyl trimethyl ammonium bromide) method (Doyle & Doyle, 1990). A 1451-bp sequence was obtained from the COI/COII region of mtDNA. This piece was sequenced from three overlapping sections using two published primers, C1-J-2195 (alias CO1-RLR) (5′-TTGATTTTTTGGTCATCCAGAAGT-3′) and C2-N-3661 (alias Barbara) (5′-CCACAAATTTCTGAACATTGACCA-3′) (Crozier & Crozier, 1993; Simon *et al.*, 1994). The third segment was amplified by a primer designed specifically for this study that occupied a central region of the entire segment, *c.* 386 bp into the COI-RLR sequence (CO1W-386for, 5′-ATTATGTRGTAGGTCATTTTCACT-3′). All of these segments typically ranged from 450 to 750 bp long. Nuclear sequences were obtained from the wingless (WG, 410 bp) and longwave rhodopsin (LW, 562 bp) genes. WG was obtained using the primers WGF (5′-GAACTTCCGCGTGGTCGGCGAC-3′) and WGR (5′-GGTGCAGGAGCACCTCTCGA-3′). LW was obtained by using the primers LR143F (5′-GACAAGTKCCACCRGARATGCT-3′) and LR639ER (5′-YTTACCGRTTCCATCCRAACA-3′). Visual inspection of polymerase chain reaction (PCR) product bands on gels prior to extraction indicated that these two nuclear genes were single-copy because only one band was observed.

Polymerase chain reaction mixtures for the amplification of mtDNA resembled the protocol of Brandt *et al.* (2007) and contained 1 μL of DNA, 2.5 μL of *Taq* 10× reaction buffer (Fermentas, St Leon-Rot, Germany), 2.5 μL of 1 mM dNTP, 2.8 μL of $MgCl_2$, 1.4 μL of primer, 12.4 μL of ddH_20 and 1 μL of *Taq* (1 U). The procedure for the PCRs was as follows: initial denaturation for 2 min at 94 °C, then 38 cycles of 94 °C for 1 min, 50 °C for 1 min, 68 °C for 2 min, and then a final extension at 72 °C for 5 min.

Amplification of nuclear genes was accomplished using the Bio-X-Act kit (self-contained PCR buffer and polymerase) (BioLine, Luckenwalde, Germany) in which 1 μL of DNA was reacted with 0.25 μL of $MgCl_2$ (50 mM), 8 μL of Bio-X-Act solution and 5.75 μL of ddH_20. PCRs for wingless were as follows: initial denaturation for 3 min at 95 °C, then 40 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, and then a final extension at 72 °C for 7 min. PCRs for longwave rhodopsin were as follows: initial denaturation for 3 min at 94 °C, then 39 cycles of 94 °C for 1 min, 54 °C for 1 min, 72 °C for 1 min, and then a final extension at 72 °C for 10 min.

All PCR products were extracted and cleaned using NucleoSpin Gel Extraction Kits (Macherey-Nagel, Düren, Germany) and *c.* 3 μL of the gel-extracted product was used for sequencing with BigDye Terminator Cycle Sequencing Kit 3.1 (Applied Biosystems, Darmstadt, Germany). Reactions were conducted as follows: initial denaturation at 95 °C for 1 min, then 36 cycles of 95 °C for 10 s, 50 °C for 10 s, and then a final extension of 60 °C for 4 min. Reactions were precipitated in 100% ethanol and analysed in an ABI Prism 310 Genetic Analyser (Applied Biosystems).

Singleton and doubleton haplotypes were re-sequenced, and, when available, different individuals from the same colony were sequenced. In all cases, the initial identity of these haplotypes was confirmed – therefore these haplotypes are unlikely to be mistakes arising during PCRs, sequencing or alignment. All sequences were deposited in GenBank (accession numbers HQ440132–HQ440173 and HQ439100–HQ439115).

Interspecific phylogeny

Despite numerous attempts using different primers, buffer solutions and PCR protocols, the amplicons of the mtDNA sequences from *P. angusta* and *P. mocquerysi* were always very short (*c.* 200 bp) – if sequences were present at all. Analysis of the full dataset with gaps present for the mtDNA sections of these two species indicated that the mtDNA provided very little phylogenetic information at the interspecific level. The topology was virtually identical with that produced from just the nuclear gene sequences. Consequently, the phylogeny reported here was constructed exclusively from the nuclear gene sequences.

The nuclear genes were analysed in a fully partitioned dataset. The rhodopsin gene contained an intron, the length of which varied for each species in the number of indels but roughly corresponded to the intron of *P. strenua* supplied to us by Chris Schmidt (University of Arizona) and was *c.* 100 bp in length. This intron was analysed as a separate partition in a full dataset that contained four partitions (wingless, the intron and the two sections of expressed longwave rhodopsin). Each partition of the dataset was analysed by jModelTest (Posada, 2008) for an appropriate model of sequence evolution. The HKY+G model was suitable for the exon of longwave rhodopsin, HKY for the intron LW, and HKY+I for Wg. Bayesian analysis was conducted using MrBayes 3.1 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). The standard deviation of split frequencies fell below 0.01 after *c.* 200,000 generations; consequently, the analysis was run for 250,000 generations with a sampling frequency of 100 (burn-in = 625). Maximum likelihood (ML) analyses were conducted using garli 0.96 (Zwickl, 2006) (http://garli.nescent.org). Initially, five runs with five replicates each were executed in the fully partitioned dataset as described above, and each resulted in an identical topology. Two additional analyses containing 100 bootstrap replicates each and a third containing 500 bootstraps produced the same topology.

Intraspecific phylogeny and population genetics

Analysis of haplotype variation was accomplished by a combination of phylogenetic and population genetic techniques appropriate for intraspecific genealogies (Posada & Crandall, 2001; Freeland, 2006). As a first step, a haplotype network was constructed. We constructed a network produced by the statistical parsimony method as implemented in tcs (Clement *et al.*, 2000). This topology was then compared visually with a network produced by the median-joining method using network (Bandelt *et al.*, 1999). In all cases, topologies were identical.

Initial network analysis yielded several networks that could not be connected with an acceptable level of statistical confidence (> 0.90) (see Results). Therefore, to identify ancestral and derived populations, traditional phylogenetic analysis was employed using the Bayesian algorithm implemented in MrBayes (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). jModelTest analyses indicated the best model of sequence evolution to be HKY+I+G (a proportion of invariant sites and gamma distribution of rates). The analysis was conducted as a single partition; separate analyses from each sequence produced similar models of sequence evolution and identical topologies. In this analysis, the standard deviation of split frequencies fell below 0.01 after *c.* 13.9 million generations; consequently, the analysis was run for 25 million generations with a sampling frequency of 10,000 (burn-in = 625).

Population genetic (haplotype) analysis was conducted using Arlequin 3.1 (Excoffier *et al.*, 2005). Haplotype and nucleotide diversity and tests for population expansion (Tajima's *D* neutrality test and Harpending's raggedness statistic *h*) were calculated. These tests were chosen because haplotype frequencies of expanding populations typically differ from neutrality and leave unimodal haplotype mismatch distributions. A significant Tajima's *D* statistic indicates significant deviance from neutrality, whereas a significant Harpending's *h* statistic indicates deviance from the expected null hypothesis of a unimodal distribution of haplotype pairwise differences or a star-like phylogeny. Thus an expanding population will have a significant Tajima's *D* statistic and a non-significant Harpending's *h* statistic.

Estimation of divergence dates

To assign temporal estimates to the main clades in the intraspecific phylogeny, the software package beast (Drummond & Rambaut, 2007) was utilized. This was accomplished in two steps. The first estimated a mutation rate for the genus *Platythyrea* from the COI sequences from the species-level phylogeny. In this analysis, fossils and published age estimates were used as calibration points. We used an age of 17.5 ± 2.5 (mean ± 1 SD) Ma for the node corresponding to a common ancestor of *P. punctata*, *P. strenua* and *P. pilosula*. This age approximates the age of the Dominican amber deposits (15–20 Ma) (Iturralde-Vinent & MacPhee, 1996); therefore it

is likely that these lineages have been present in North America and the West Indies for at least this long. *Platythyrea prizo* was excluded from this clade because it appeared to be quite unrelated to the rest of the Neotropical *Platythyrea* fauna (see Results). As a second calibration point, which corresponded to an estimated age of the genus *Platythyrea*, a value of 50 ± 25 Ma was used. This approximation is younger than the estimated age of the subfamily Ponerinae (*c.* 80–100 Ma: Brady *et al.*, 2006; Moreau *et al.*, 2006). Both ages were assumed to have a normal distribution. Mutation rate estimation was conducted under the GTR+G model of sequence evolution and a tree prior of speciation/Yule process. Sequence evolution was determined to be not clock-like using PAUP* (Swofford, 2003) because the strict- or fixed-clock model was rejected ($\chi^2 = 2461$, d.f. = 9, $P < 0.0001$). Consequently, mutation rates were estimated using a log-normal relaxed-clock model for 10 million generations with a burn-in of 1000 (Drummond *et al.*, 2006). Simulations were conducted until the effective sample sizes (ESSs) exceeded 200. BEAST analyses were conducted on only the COI portion (*c.* 805 bp) because mutation rates are readily available for this gene in other species and sequences from the COII region were unavailable from *P. strenua*.

The second analysis used the mutation rate calculated above on a dataset consisting of only *P. punctata* COI sequences. This rate was used to calculate divergence dates of the major clades of *P. punctata* in a fixed-clock framework because the pattern of sequence evolution did not differ from that expected from a clock-like model ($\chi^2 = 103.2$, d.f. = 89, $P = 0.15$). Fixed clocks assume a similar mutation rate across all lineages. As the algorithm implemented in BEAST has difficulties with datasets containing many identical sequences (Drummond *et al.*, 2006; Simon Ho, University of Sydney, pers. comm.), 26 identical sequences were removed from the alignment file prior to analysis. Both analyses were otherwise treated in an identical manner, and were conducted for 15 million generations (burn-in = 1000; ESSs exceeded 200). BEAST analyses were conducted under the under HKY+I+G model of sequence evolution, and, as the data were entirely intraspecific, a tree prior of coalescence (constant size) was employed.

RESULTS

Interspecific phylogeny of northern Neotropical species of *Platythyrea*

Both Bayesian and ML methods produced topologies showing that four northern Neotropical *Platythyrea* species (*P. angusta*, *P. pilosula*, *P. strenua* and *P. punctata*) comprise a monophyletic group that is closely related to a clade containing

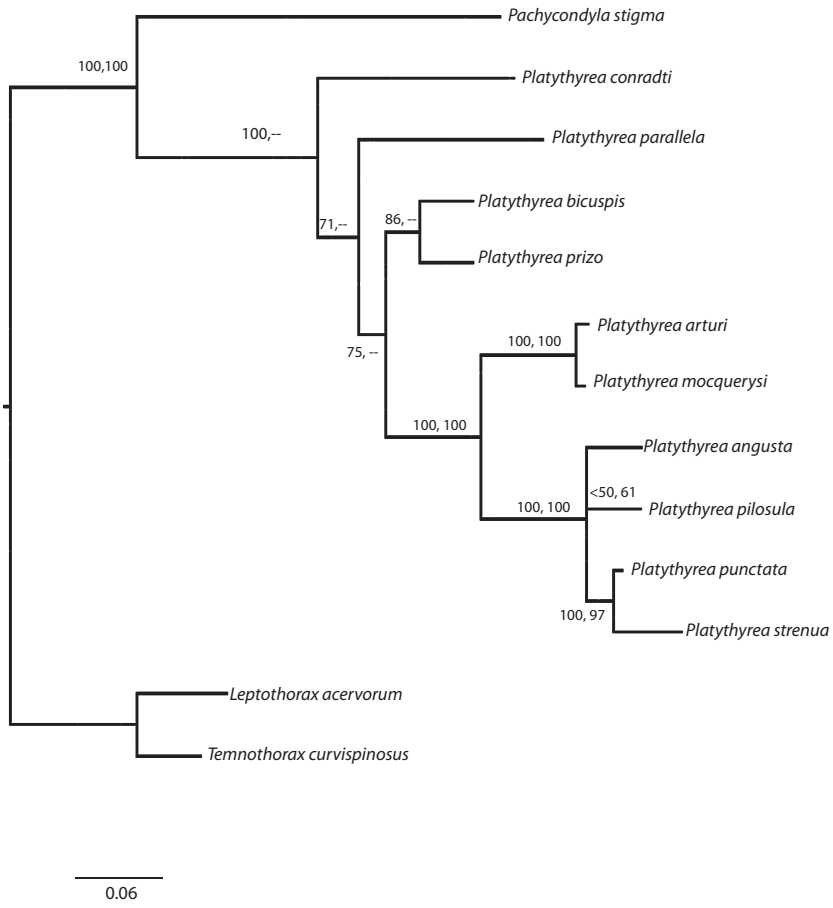

Figure 2 Phylogram based on a 972-bp sequence of wingless and longwave rhodopsin of 10 *Platythyrea* species. Numbers at each node respectively indicate Bayesian posterior probabilities and bootstrap values obtained from maximum likelihood (ML) analysis. The version depicted is Bayesian. Under ML, *P. conradti* and *P. prizo* switch their positions; therefore, the bootstrap values have been omitted. The scale bar indicates the number of substitutions per site.

P. arthuri Forel and *P. mocquerysi* Emery (Fig. 2), both of which occur in Madagascar and on other nearby islands. *Platythyrea prizo* appears unrelated to the four other Neotropical species and is allied with congenerics in Africa and Asia. Moreover, *P. punctata* appears to be closely related to *P. strenua*, the endemic to Hispaniola. *Platythyrea punctata* also appears to have a comparatively shorter branch length than *P. strenua*, suggesting a lower rate of molecular evolution. It is unlikely that *P. strenua* is simply a subset of *P. punctata*, as a phylogeny of the COI gene does not place *P. strenua* inside the *P. punctata* haplotypes (J. N. Seal, unpublished data). Nuclear sequences obtained from several individuals of *P. punctata*, *P. prizo*, *P. mocquerysi* and *P. bicuspis* show no variation within species (J. N. Seal, unpublished data). Nuclear sequences of *P. strenua* and *P. punctata* are also clearly distinct, making it unlikely that these two species are in fact subspecies of a larger complex. Although Bayesian support for *P. conradti* Emery occupying a basal node in the phylogeny was high, posterior probabilities for the locations of *P. parallela* F. Smith, *P. bicuspis* Emery and *P. prizo* were rather low. The ML method, moreover, failed to resolve the placement of *P. conradti*, *P. parallela* and *P. bicuspis*, and indicated that *P. prizo* represented the sister to all other *Platythyrea* species (bootstrap support > 0.99; Fig. 2).

Intraspecific phylogeny of *Platythyrea punctata*

Unlike the nuclear sequences, in which *P. punctata* specimens from mainland and island populations exhibited little if any variation, considerable variation was found in the 1451-bp section of COI/COII. Over this entire segment, of the 91 individuals analysed, 43 unique haplotypes were obtained. Furthermore, 264 nucleotide positions were variable, and 139 of these were parsimony-informative.

On the mainland, haplotypes appear to belong to one of three major clades, corresponding to Texas and northern Mexico, northern Belize and southern Mexico (Chiapas), and southern Belize and Honduras, respectively (Fig. 3). The basal lineages of *P. punctata* appear to occur in southern Mexico, because the sister haplotype to all other haplotypes was obtained from a single individual collected in Chiapas, Mexico. Sister to the remaining haplotypes appears to be a large clade containing all haplotypes collected in Honduras and southern Belize (Fig. 3).

The statistical parsimony network analysis produced a network for each of the four main clades described above for all confidence settings between 90% and 99%. In other words, these four networks are not interconnected. Forcing a network to include all haplotypes required that the maximum number of connections exceeded 100 base-pair differences, which is well below a statistical limit of 90%. Consequently, the genetic differences were large enough to prevent adequate determination of where the West Indian haplotypes came from on the mainland. In terms of genetic distances, West Indian haplotypes were most similar to those collected in northern Mexico and southern Texas (Table 2), which translated into *c.* 60 base-pair differences. The Honduran haplotypes were slightly more divergent from their West Indian counterparts, at about 70 base-pair differences (Table 2).

Haplotype and nucleotide diversities in *P. punctata* were much higher on the mainland than in the West Indies (Table 3). Although some colonies encountered on the mainland shared haplotypes, sharing occurred only among colonies in close proximity. For example, in Belize, haplotype BZ-2 was shared among three colonies that were collected along the same road (site BZ IA; Fig. 4a; Appendix S1). At this site, two other haplotypes were collected (BZ-1 and BZ-3). Two additional haplotypes were collected in Guanacaste National Park, located about 20 km to the north-west, one of which was shared between two colonies (haplotypes BZ-4 and BZ-5; Fig. 4a; Appendix S1). Haplotype BZ-6, collected in Mexico (Chiapas 2; *c.* 250 km to the north), showed affinity with Belizean haplotypes and was also unique. Two colonies in Texas and three in Honduras also shared haplotypes.

In contrast, all populations surveyed in the West Indies were characterized by nine haplotypes, only two of which were abundant. The most abundant was found on every island surveyed and in Florida (haplotype WI-2; $n = 40$; Fig. 4) over a distance exceeding 2700 km (central Florida–Barbados linear distance). The second most abundant was collected only in the Dominican Republic, Puerto Rico and Guadeloupe (the largest island in the Lesser Antilles) (haplotype WI-1; $n = 13$). The only haplotypes that were not collected on these three islands were a haplotype collected at two locations in Florida [haplotype WI-3; Matheson Hammock (near Miami) and Crane Point Nature Center (Vaca Key)] and a haplotype collected on St Croix (haplotype WI-6; US Virgin Islands) (Fig. 4). Five additional haplotypes were each collected once in the Dominican Republic, Puerto Rico and Guadeloupe (haplotypes WI-4–5, 7–9) (Fig. 4).

Statistical signatures of an expanding population were also found. Haplotype frequencies from the mainland do not differ from that expected from a neutral population (Tajima's *D* statistic), and haplotype mismatch distributions (Harpending's raggedness *h* statistic) differ from a pattern consistent with population expansion (Table 3). Exactly the opposite patterns are observed among the West Indian haplotypes, whose haplotype frequencies differ from neutrality but not from a haplotype mismatch distribution consistent with population expansion (Table 3).

Haplotype clades were congruent with certain geographical regions (related haplotypes were found in close proximity) (Figs 3 & 4; Table 3). The single exception was a colony collected from Curry Hammock, Florida, which had a haplotype that clustered in the clade of Honduras. Two individuals from this colony shared this haplotype; there was thus no mistake during sequencing. Other than this example there were no cases of clear dispersal among the mainland regions, from the mainland to the West Indies or from the West Indies to the mainland.

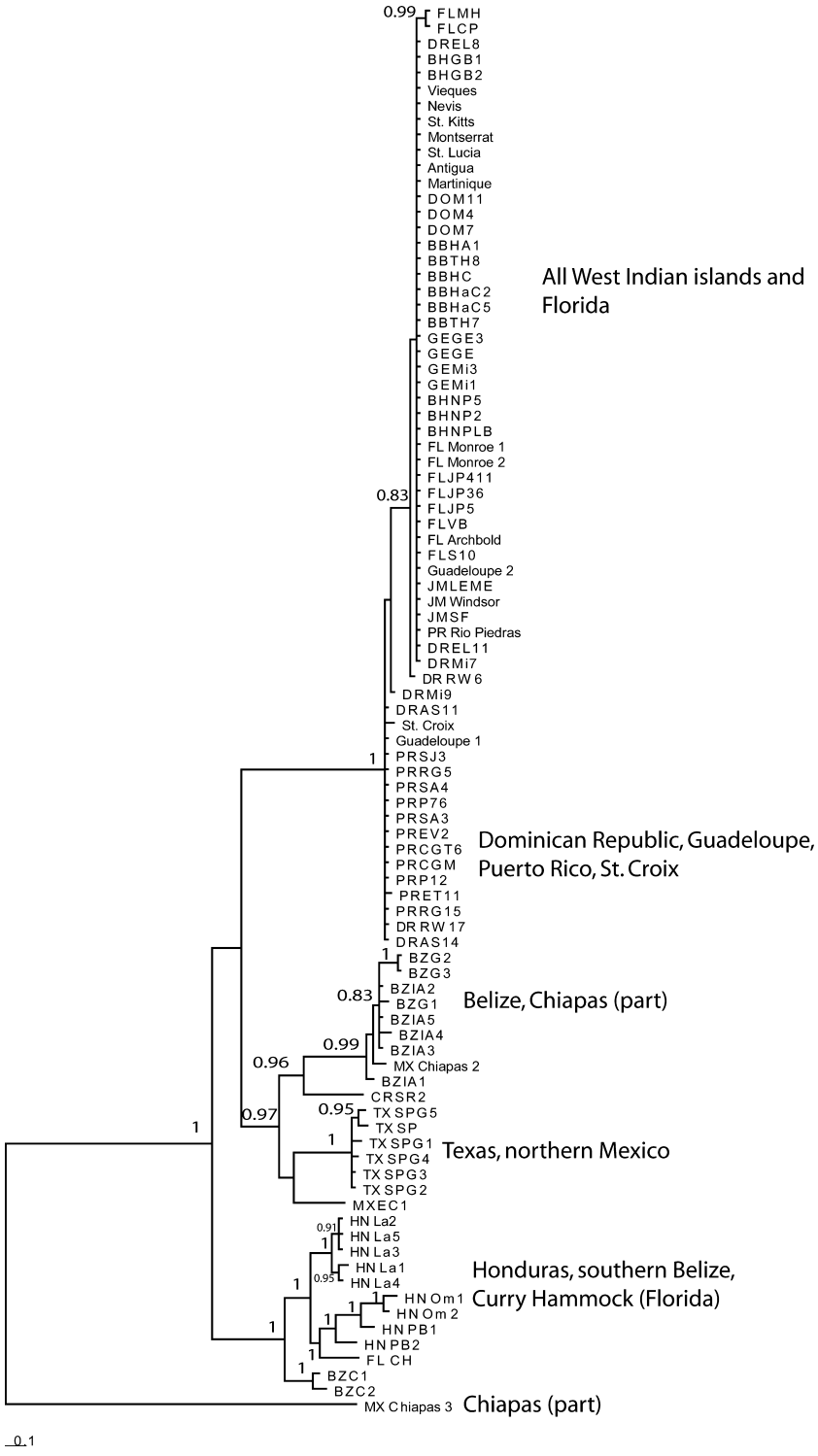

Figure 3 A Bayesian consensus phylogram based on a 1451-bp sequence of COI/COII mitochondrial DNA from *Platythyrea punctata*. Numbers at each node indicate Bayesian posterior probabilities. For clarity, posterior probabilities < 0.80 and those inside major clades are not shown. The outgroup (*P. parallela*) has likewise been omitted for clarity. The scale bar indicates the number of substitutions per site. Locality abbreviations are detailed in Appendices S1 and S2.

Molecular dating

Calibrations obtained from the genus-wide phylogeny of COI indicate that the substitution rate in the genus *Platythyrea* is *c.* 0.0133 ± 0.0004 (mean ± SE) bp Myr^{-1}, or similar to the published 0.015 bp Myr^{-1} reported for *Drosophila* (DeSalle *et al.*, 1987), land crabs (Schubart *et al.*, 1998) and *Crematogaster* ants (Quek *et al.*, 2004). Age estimates indicate that the mainland radiations are the oldest. At *c.* 1.17 Ma, populations radiated in Belize, Texas and Mexico, whereas the radiation in Honduras and southern Belize seems to have occurred later (at *c.* 750 ka) (Table 4). Radiations in the West Indies as well as the crown lineages of Texas and northern Belize occurred much more recently (146–252 ka) (Table 4). The analysis also indicates that haplotype WI-1 (found only in the Dominican Republic, Puerto Rico and Guadeloupe) is younger than the

Table 2 Genetic distances (average number of pairwise differences between populations) of individuals from the main clades of *Platythyrea punctata*. The diagonal represents genetic distances within each clade, whereas the sub-diagonal represents distances between clades. All comparisons were statistically significant ($P < 0.0001$).

	Honduras	Belize	Texas	Greater Antilles	Lesser Antilles
Honduras	17.1				
Belize	67.6	34.04			
Texas	57.1	51.6	10.7		
Greater Antilles	71.3	71.1	60.7	3.5	
Lesser Antilles	69	70.4	61.04	6.4	4.6

other class of haplotypes found on all islands and in Florida (161 vs. 200 ka, respectively) (Table 4).

DISCUSSION

The primary purpose of this paper was to describe the spatio-temporal patterns of genetic diversity of *P. punctata* in order to infer source populations and subsequent colonizations across its very wide geographic range. Colonization of the West Indies probably occurred within the past 150 kyr and involved the introduction of one or a very few haplotypes. It is tempting to conclude that parthenogenesis had a major role in the relatively rapid later radiations, as speculated by Hartmann *et al.* (2005). However, nearly all basal and all derived lineages examined in this paper are capable of parthenogenesis (K. Kellner, unpublished data). It is not known if other species of *Platythyrea* are capable of parthenogenesis. If one assumes they are not, then the evolution of parthenogenesis probably occurred at a point between the divergence of *P. punctata* from its sister species (*P. strenua*) and the radiation of populations in Honduras, southern Belize and at least part of Chiapas, which are in turn sisters to the remaining populations in Central American and the West Indies. Thus, efforts to understand the evolution of parthenogenesis should focus on populations of *P. punctata* in Central America and of *P. strenua* on Hispaniola.

The discovery that *P. punctata* colonized the West Indies from origins in Central America contrasts with the views of Hedges (1996a, 2006), who proposed (for vertebrates) that non-volant species arrived in the West Indies by drifting from northern South America. Our result is similar to results from the only other molecular dataset on a non-invasive West Indian ant, namely that the closest mainland relatives of the Cuban endemic leaf-cutting ant *Atta insularis* are species found in Mexico and Texas (*A. mexicana* and *A. texana*), and not species found further to the south (Bacci *et al.*, 2009). Whether this represents a wider trend of West Indian ant colonization patterns is pure speculation with such a small sample size, but it is an idea worth further investigation.

Our data are best explained by dispersal to the islands followed by expansion (Hypothesis 1). Our age estimates for the West Indian populations in particular are supported by coalescent theory, which predicts that the most common haplotypes should be the oldest (i.e. WI-2) and the less common ones are relatively younger and have had less time to colonize (i.e. WI-1) (Avise, 2000, 2009; Freeland, 2006). The relatively low haplotypic diversity in the West Indies indicates that getting to the islands was a rare event, but once this did occur, populations expanded quite rapidly to every island in the archipelago. A role for plate-tectonic vicariance is not supported, because the estimated age of the West Indian population of *P. punctata* is of the order of thousands of years, whereas for the vicariance hypothesis to be supported ages would have to be millions of years (*c.* 40–60 Ma, when the Caribbean plate was much closer to the mainland). An initial dispersal event followed by vicariance induced by sea-level rise cannot explain finding the same haplotype on Puerto Rico, in the Dominican Republic and on Guadeloupe, because these three islands are not thought to have had a land connection in the past (Liebherr, 1988a; Hedges, 2006).

A small number of dispersal events from Central America is therefore the most salient mechanism to explain the haplotype diversity in the West Indies. It is also unlikely that *P. punctata* is a tramp species aided by human movements and commerce, because island haplotypes were never found on the mainland and only in one case was a mainland haplotype found in the West Indies (Fig. 3). There is no obvious reason why human traffic would have preferred one haplotype over another and why this haplotype was found only in the West Indies and not on the mainland. Laboratory observations do not indicate that the colonies possessing the island haplotype exhibit higher per-capital growth rates than those from the mainland (J. N. Seal, unpublished data), making it unlikely that island haplotypes are radically different from those from the mainland.

Haplotype mismatch distributions and low diversity of haplotypes on the islands suggest a fairly recent invasion, because the patterns are similar to those observed in taxa in

Table 3 Diversity indices and tests of neutrality for the populations of *Platythyrea punctata* studied.

Region	N (n)	Haplotype diversity (H_d) ± SD	Nucleotide diversity (π) ± SD	Tajima's D	Harpending's raggedness h
Central America	29 (26)	0.99 ± 0.013	0.034 ± 0.005	$0.879^{n.s.}$	**0.016, $P = 0.04$**
West Indies	61 (9)	0.668 ± 0.06	0.00402 ± 0.0015	**−2.46***	0.4, $P = 0.4$

N = sample size, n = number of haplotypes. Bold text indicates significant differences ($\alpha = 0.05$). *$P < 0.01$, n.s. = $P > 0.05$.

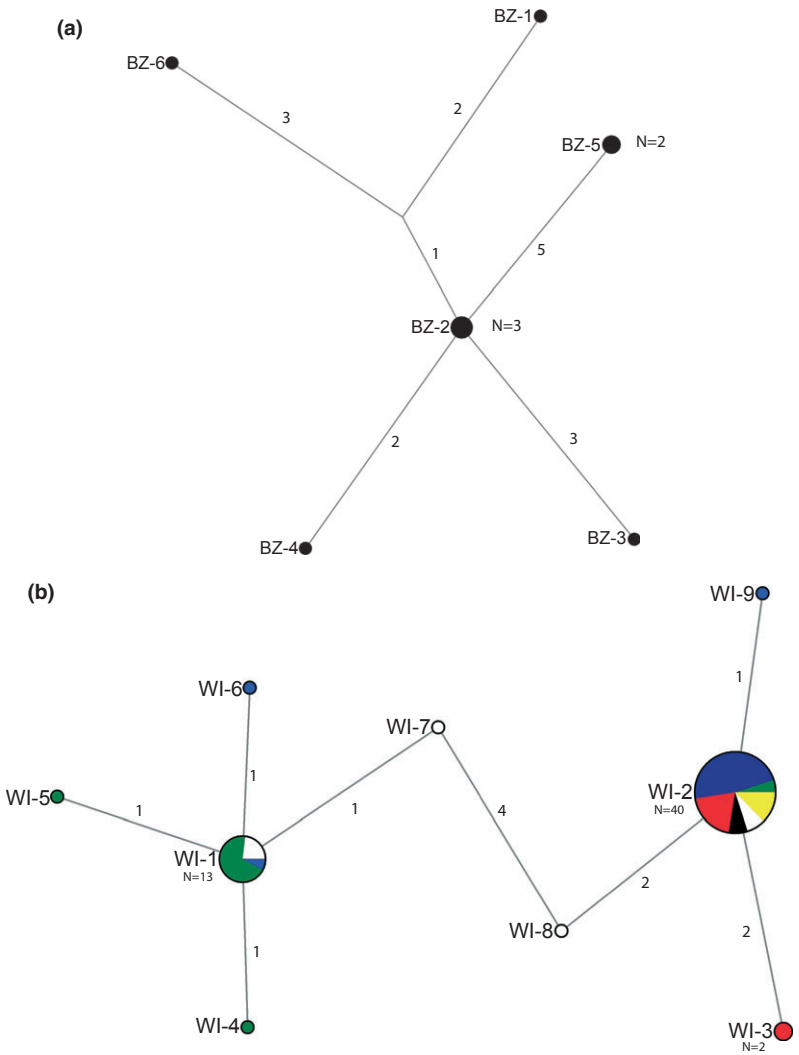

Figure 4 Parsimony haplotype networks of (a) a representative mainland population (the Belize clade) and (b) the West Indian population of *Platythyrea punctata*. Numbers indicate the number of base-pair differences between haplotypes. Except where indicated, each node corresponds to a single haplotype. Colours in the West Indian haplotype network (b) indicate islands, groups of islands, or Florida: green, Puerto Rico; blue, the Lesser Antilles; red, Florida; black, Jamaica; white, the Dominican Republic; and yellow, the Bahamas. There appear to be two broad classes of haplotypes in the West Indies. The size of the coloured slice corresponds to the number of individuals collected. One group was found only in Puerto Rico, the Dominican Republic and Guadeloupe (WI-1), whereas the other broad type was found on all islands and in Florida (WI-2). Except where indicated, each node corresponds to a single haplotype.

North America and Europe that expanded their ranges northwards at the end of the Pleistocene (i.e. *c.* 12 ka) (Hewitt, 2000, 2004). However, age estimates suggest that divergence occurred in the mid- to late Pleistocene (750–150 ka, respectively); that is, during the preceding interglacial period. This is problematic because sea levels during the latter may have been much higher than they are today. Much of south Florida and the Bahamian islands are thought to have been inundated (Webb, 1990; Hearty & Neumann, 2001). A similar discordance between the timing of divergences and Pleistocene inundations has also been reported for West Indian *Anolis* lizards (Glor *et al.*, 2005). Considering the general lack of genetic structure in the West Indian population, it is likely that *P. punctata* invaded in the current interglacial. Caution must be exercised in the interpretation of these age estimates, because age calibrations using fossils may overestimate ages by an order of magnitude or more (Pulquérioa & Nichols, 2007). On the other hand, the age estimates could be correct – haplotypes similar to those on the islands might exist somewhere on the mainland and have invaded the islands more recently. Unfortunately, our surveys did not include Cuba or most of Mexico, especially the central portion, which genetic distances indicate to be a possible source of the West Indian populations. We also cannot determine whether the transitional haplotypes separating these two regions are unsampled or extinct.

Several lines of evidence support the notion that *P. punctata* colonized the West Indies under conditions currently not experienced in the region. As for findings from studies on other flightless insects (Trewick, 2000; Oneal *et al.*, 2009), open ocean does not seem to represent an insurmountable barrier. Except for the Honduran haplotype found in Curry Hammock, which could have reached Florida by rafting, the general west-to-east pattern of population expansion in this species runs counter to the prevailing east-to-west flow of oceanic and wind currents in this region. One possibility is that the counterclockwise flow of tropical storms can cause temporary movements in the opposite direction, a point made by others (Hedges, 2006; Oneal *et al.*, 2009). However, this would also seem to allow for the movement of haplotypes from Honduras to the Greater Antilles, for example, but this was not observed in our data. Although many ant species have been

Table 4 Divergence times (time to the most recent common ancestor) in years (mean ± SE) of each clade of *Platythyrea punctata*. The West Indies WI-2 refers to the clade of haplotypes occurring on all West Indian islands and Florida, whereas WI-1 refers to the haplotype occurring only in Puerto Rico, the Dominican Republic and Guadeloupe. Estimated sample size (ESS) is provided in parentheses. Estimates are based on a 1.3% substitution rate (0.013 bp Myr^{-1}).

Clade	Age (ka)
Honduras/southern Belize	757 ± 0.2 (4723)
Texas/Belize (stem)	1170 ± 0.003 (4672)
Belize/Chiapas (crown)	252 ± 0.1 (4317)
Texas (crown)	146 ± 0.09 (4354)
West Indies (WI-2)	200 ± 0.1 (2067)
West Indies (WI-1)	161 ± 0.09 (3644)

noticed on floating trees and other flotsam, along with other animal taxa (Wheeler, 1916; Heatwole & Levins, 1972; Schoener & Schoener, 1984; Losos, 2007), no *Platythyrea* species has been observed to do this. *Platythyrea punctata* also seems absent along coastal areas in the West Indies, especially on the smaller islands such as the Florida Keys, Barbados and the Bahamas, where relief is low and conditions more saline and somewhat arid. On these islands, *P. punctata* seems restricted to hardwood forests in the island interiors, and especially to near to freshwater springs (J. Seal & K. Kellner, unpublished data). This may be why a comprehensive study on the biogeography of Bahamian ants reported *P. punctata* only from the larger islands (Morrison, 2002; Morrison, 2006).

Propagule pressure would thus seem to be very low in the contemporary West Indies. Certainly during glacial maxima, over-water distances between the islands and the mainland were much shorter owing to lower sea levels (Graham, 2003; Iturralde-Vinent, 2006), and this may have been crucial for taxa with limited dispersal capability. Curiously, winged queens are largely restricted to Florida, with the exception of a queen collected in the Dominican Republic [one colony out of 57 colonies (< 2%) collected] and Wheeler's report of a queen from the northern Bahamas (Wheeler, 1905). Approximately 30% of the colonies collected in Florida by Schilder *et al.* (1999a) and in the current study contained queens (J. Seal & K. Kellner, unpublished data). Of all West Indian populations, Florida, being at the edge of the tropics, experiences the most pronounced weather fluctuations in contemporary times, in terms of both freezing temperatures and dry–wet seasonality (Chen & Gerber, 1990). During the late Pleistocene/early Holocene the climate generally became wetter and warmer throughout the region from Central America (Lachniet & Seltzer, 2002; Horn *et al.*, 2003; Hodell *et al.*, 2005; Gischler *et al.*, 2008) to the West Indies and Florida (Bonatti & Gartner, 1973; Watts, 1980; Pregill & Olson, 1981; Grimm *et al.*, 1993; Higuera-Gundy *et al.*, 1999; Hearty & Kaufman, 2000). Whether the production of a volant, winged queen phenotype has any role with climate is currently unknown, but it is nevertheless an extremely intriguing idea.

One of the more surprising findings in this study was that the putative sister species of *P. punctata* seems to be *P. strenua*. Based on earlier morphological studies, a candidate was *P. pilosula*, because this species is thought to form a species complex with *P. punctata* (Brown, 1975). Very little is known about the biology of *P. strenua*, especially regarding whether it is also capable of parthenogenesis. Published records are restricted to the original description by Wheeler & Mann (1914), subsequent comments by Kugler (1977) and Brown (1975), and the collection of the specimen in 2001 used in this study (Chris Schmidt, pers. comm.). Morphologically, *P. strenua* seems to be 'larger and more robust' and 'lacking the course punctuation' (Wheeler & Mann, 1914; Brown, 1975) characteristic of *P. punctata*. It may be premature, however, to conclude that *P. strenua* does not occur on the mainland – given uncertainties concerning Neotropical biodiversity. For example, *P. prizo* was described only recently as a species (Kugler, 1977), and the record in our study was the first report of this species as far north as Mexico (J. Longino, Evergreen State College, pers. comm.). Although our data also support the notion that *P. strenua* is indeed a distinct species and not a form of *P. punctata*, our conclusions would be more robust with the inclusion of more sequences from *P. strenua* and *P. punctata* haplotypes from southern Mexico (Chiapas) and with some from *P. pilosula* and *P. angusta*. Understanding the phylogenetic relationships between an asexual (*P. punctata*) and its sexual relatives would provide much insight into the evolution of parthenogenesis, in addition to explaining the biogeography of Neotropical *Platythyrea*.

CONCLUSIONS

Phylogeographic analysis of *P. punctata* indicates a recent, one-way colonization from Central America to the West Indies. This ant species exhibits a pattern quite different from those revealed from the few available molecular studies on other West Indian arthropods, which show varying degrees of dispersal limitation, endemism and vicariance (Schubart *et al.*, 1998; Davies & Bermingham, 2002; Oneal *et al.*, 2009). This study highlights major deficiencies in our understanding of northern Neotropical biodiversity concerning not only the genus *Platythyrea*, but also most ant and arthropod species in the West Indies. Determination of source populations and patterns of colonization of other West Indian ants would be a worthwhile endeavour, especially considering the ecological importance of ants and conservation concerns in the region.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 List and GPS coordinates of collection localities of *Platythyrea punctata*.

Appendix S2 List and GPS coordinates of *Platythyrea* species used in this study.

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BIOSKETCH

Jon Seal is currently a postdoctoral fellow in the Section of Integrative Biology at the University of Texas at Austin. He is an organismal biologist with interests in the functional and molecular ecology of social insects and symbioses. He obtained his PhD at Florida State University and then spent two and a half years working on the enigma of parthenogenesis in *Platythyrea punctata* in the Department of Evolution, Behavior and Genetics at the University of Regensburg (http://www-evolution.uni-regensburg.de/index_en.html).

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