MOLECULAR ECOLOGY

Molecular Ecology (2011) **20**, 114–130 doi: 10.1111/j.1365-294X.2010.04916.x

# Biogeography and morphological evolution in a Pacific island ant radiation

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## Abstract

**While insular radiations are documented for many terrestrial arthropods, few examples are known for eusocial insects. This study seeks to ascertain whether the spinescence observed among Fijian *Pheidole* ants was inherited from an oversea ancestor or is evidence of ecological release from interspecific competitors. We broaden our understanding of morphological convergence, insular radiation and Pacific biogeography by testing three hypotheses proposed previously for the Fijian *Pheidole roosevelti* group: (i) the group is monophyletic; (ii) spinescence is a plesiomorphic trait inherited from an overseas ancestor; and (iii) the group is closely related to spinescent New Guinean relatives. The analysis included the fragments of two mitochondrial genes (COI, cytb) and two nuclear genes (H3, EF1α-F2) from 66 taxa, including all members of the *roosevelti* group, representatives from the spinescent subgenus *Pheidolacanthinus*, Fijian congeners and widespread Pacific congeners. Our results yield new insights into the biogeographic history of Fiji, reveal a fascinating example of convergent evolution and serve as a novel example of ecological release occurring within an insular eusocial insect lineage. These findings recover the history of a presumably unremarkable ant species that colonized a remote oceanic archipelago in the Miocene (17–10 Ma) and radiated across the emerging islands into niche-space occupied elsewhere in the Pacific by distantly related spinescent congeners. We propose the radiation of Fijian *Pheidole* into spinescent morphotypes was the consequence of ecological opportunities afforded by the absence of competing ant lineages with conspicuous epigaeic foraging strategies.**

*Keywords*: convergent evolution, ecological release, Fiji, Formicidae, *Pheidole*, spinescence

*Received 11 May 2010; revision received 1 October 2010; accepted 6 October 2010*

## Introduction

Island archipelagos are among Earth's greatest natural laboratories because they allow us to distill evolutionary processes often muddied by continental complexity. Since Darwin's (1859) landfall on the Galapagos and Wallace's (1869) sojourn in Malaysia, insular systems have been pushing the frontiers of evolutionary and ecological knowledge. The islands of the Pacific have proved particularly fertile testing grounds, inspiring such notable achievements as the modern concepts of biological speciation (Mayr 1942), adaptive radiation, community assembly (Diamond 1975), island biogeography (MacArthur & Wilson 1967) and taxon cycles (Wilson 1959a, 1961).

It is ironic that the theoretical foundations of both the taxon cycle and island biogeography were built largely upon distributional data of Pacific ants, as native ants are conspicuously absent from many Pacific islands (Wilson & Taylor 1967). While insular radiations are documented for many terrestrial arthropods (Gillespie & Roderick 2002; Liebherr 2005; Bickel 2006; Monaghan *et al.* 2006; Balke *et al.* 2007b; Joy *et al.* 2007), few examples can be drawn from the literature of eusocial insects (Wilson 1988; Fisher 1997, 2010; Lucky & Sarnat 2009). In fact, the *absence* of eusocial Hymenoptera (ants, bees and wasps) from eastern Pacific islands is implicated as a cause for the ecological release of other arthropods (Gillespie & Roderick 2002; Krushelnycky *et al.* 2005).

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If insular radiations are to be found among eusocial insects, the Fijian archipelago is a natural place to search. Insular radiation, as used here, includes adaptive (Schluter 2000; Losos 2010) and nonadaptive (Gittenberger 1991; Kozak *et al.* 2006) diversifications of island endemics descended from a recent common ancestor. Ants, despite their global ubiquity, are poor colonizers of oceanic islands. Based on distribution records, the islands east of Tokelau and Samoa are among earth's only terrestrial ecosystems, besides Antarctica, believed to be absent of endemic ants (Wilson & Taylor 1967; Fisher 2010). Fiji, just west of this 'ant line', supports a rich native ant fauna with high taxonomic diversity (188 spp., 43 genera) and endemism (>68% spp.) (Sarnat 2006, 2008; Ward & Wetterer 2006). Fiji's size, status as a 'Darwinian' island (Gillespie 2002) and proximity to the effective eastern dispersal limit of Old World ants predict high diversification rates for the select formicid lineages that manage to colonize the archipelago (MacArthur & Wilson 1967; Whittaker *et al.* 2008).

The extent to which high Fijian ant endemism is the consequence of multiple colonization events vs. *in situ* radiations remains a provocative question (Lucky & Sarnat 2009). Moreover, no support for the ecological opportunity hypothesis, which proposes that organisms freed from competitive constraint will experience a 'release' characterized by bursts of phenotypic evolution (Schluter 2000; Losos 2010), has been published for insular eusocial lineages in the Pacific.

The *Pheidole roosevelti* group is among the strongest candidates for insular radiation of a Fijian ant lineage and, more broadly, ecological release of an insular eusocial insect in the Pacific. The hyperdiverse genus *Pheidole* (Formicidae: Myrmicinae) is the most speciose of all ant genera. With 1400 described species (and potentially hundreds more undescribed), *Pheidole* includes 9.5% of the known world ant fauna (Bolton *et al.* 2006). *Pheidole* ants exhibit extreme ecological, behavioural and morphological diversity and are dominant components of nearly every terrestrial ecosystem on earth (Wilson 2003; Moreau 2008). A recent large-scale analysis of *Pheidole* presents strong support for generic monophyly and suggests that all the Old World taxa may have descended from a single ancestor nested within New World lineages (Moreau 2008).

One of the most striking morphological differences between extant New World and Old World *Pheidole* is the propensity of the latter to exhibit a wide diversity of spinescent phenotypes. Spinescence in ants (as defined by the number and length of spines) is commonly thought to serve as defence against predators and enemies, but rigorous testing of these hypotheses is severely lacking (Dornhaus & Powell 2010). Ornate examples of spinescent morphology, such as antler-like propodeal spines and acutely angled mesonotae, are found among all but one (*Pheidole simplispinosa*) of the seven Fijian *roosevelti* group species (Fig. 1).

The purpose of this study is to ascertain whether the spinescent morphology characteristic of the Fijian *P. roosevelti* group was derived from an *in situ* insular radiation or was inherited from a spinescent overseas ancestor. Since the first Fijian species were described nearly a century ago by W.M. Mann (1921), myrmecologists have predicted the *roosevelti* group to belong to the spinescent subgenus *Pheidole* (*Pheidolacanthinus*) and to be closely related to the morphologically similar *Pheidole cervicornis* group from New Guinea (Mann 1921; Wilson 1959b, 2003; Sarnat 2008). However, if the *roosevelti* group species derived their spinescence independently from other Pacific *Pheidole*, the case may serve as a novel example of ecological release occurring within an insular eusocial insect lineage.

To determine whether the spinescence observed among Fijian *Pheidole* was inherited from overseas ancestors or is evidence of ecological release, we test three hypotheses proposed in Sarnat's (2008) taxonomic revision: (i) the *roosevelti* group is monophyletic; (ii) spinescence is a plesiomorphic trait inherited from an overseas ancestor; and (iii) the *roosevelti* group is most closely related to the spinescent New Guinean *P. cervicornis* group.

Support for hypothesis (i) is required for the *roosevelti* group to be considered an insular radiation, regardless of the adaptive value or origin of spinescence. Support for hypothesis (ii) would suggest that spinescence was not the consequence of ecological release upon reaching Fiji. Moreover, the reduced spinescence of *P. simplispinosa* would suggest the ecological pressures promoting development and maintenance of elaborate spines are more relaxed in Fiji than in the native environment of its overseas ancestor. If, instead, hypothesis (ii) is rejected and spinescence is found to be a derived trait, it would suggest that the evolution of elaborate spines occurred within Fiji and may have released a radiation of species into niche-space occupied elsewhere in Melanesia by the distantly related *Pheidolacanthinus*.

Support for hypothesis (iii), while rejecting the notion that the evolution of spinescence among Fijian *Pheidole* was the consequence of ecological release, would provide important insights into the biogeographic history of the southwestern Pacific (Cook & Crisp 2005; Cowie & Holland 2006; Clark *et al.* 2008; Keppel *et al.* 2009). Further evidence for the primacy of New Guinea as a source for Fijian biota would substantiate the claim that Fijian endemics derive not from relictual Gondwanan lineages, as asserted by many botanists (Raven & Axelrod 1972; Whitemore & Page 1980; De Laubenfels 1996;

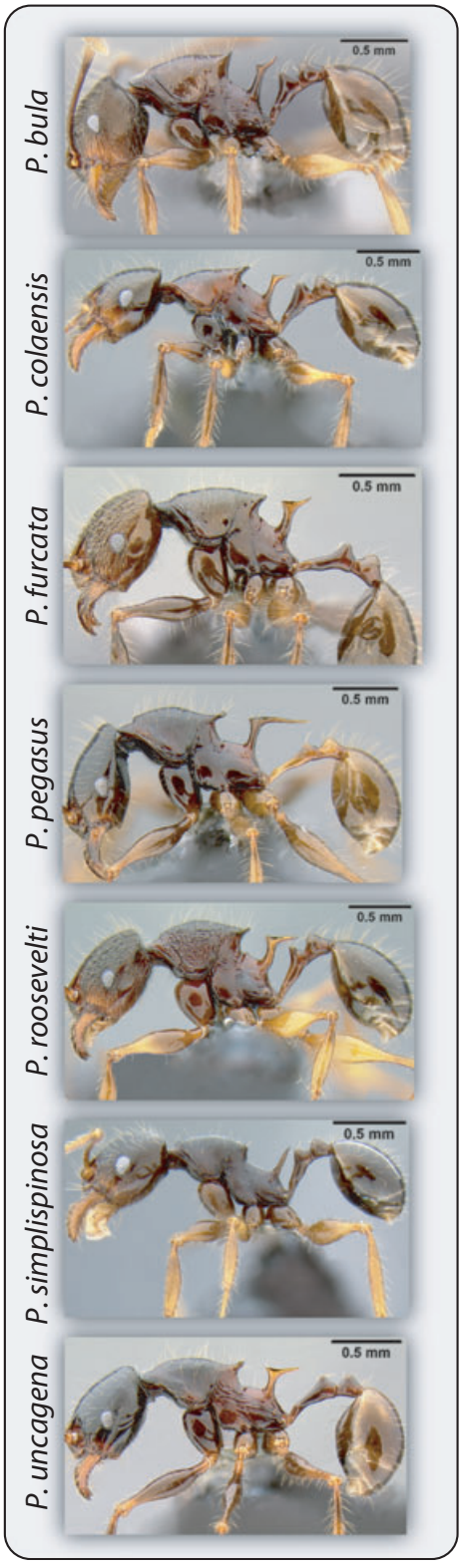



**Fig. 1** Profile photographs of the seven *Pheidole roosevelti* group species arranged in alphabetical order. Adapted from Sarnat (2008).

Weston & Crisp 1996), but rather from more recent Laurasian lineages, as asserted by entomologists (Duffels & Turner 2002; Monaghan *et al.* 2006; Balke *et al.* 2007a,b; Lucky & Sarnat 2009).

If hypothesis (iii) is rejected, the independent origin of a spinescent morphotype so similar to the *P. cervicornis* group would suggest a remarkable case of convergent evolution. The question then becomes, what ecological opportunities exist in Fiji and New Guinea that would allow this unique morphotype to originate twice among Melanesian archipelagos, but nowhere else in the pan-global range of *Pheidole*?

## Materials and methods

### *Taxon sampling*

Thirty-four species (66 specimens) of *Pheidole* (Hymenoptera: Formicidae) were sampled to help recover the biogeographic origins of the seven species assigned to the *Pheidole roosevelti* group (Mann 1921; Sarnat 2008) (Table 1). Of the species sampled, 14 are Fijian endemics, 17 are endemic to regions of Melanesia, two are pan-Pacific natives and one (*P. hyatti*) is native to North America. One-third of the sampled specimens were represented by *roosevelti* group species collected from Fiji's seven largest islands. Sequences have been deposited in GenBank (accession numbers: HM144140 – HM144384). The aligned data set for this study is available from TreeBASE (Study ID: 10445; http://www.treebase.org, 2005) or by request from the authors. The full collection data for each specimen may be obtained from the first author.

To determine the closest relatives of the *roosevelti* group, we included all available *Pheidole* species native to Fiji together with species native to Australia, New Guinea, the Solomon Islands and New Caledonia. The non-Fijian taxa were biased towards representative species of the spinescent subgenus *Pheidolacanthinus* Smith (including the *cervicornis* group, the *quadricuspis* group and the *quadrispinosa* group), to which the *roosevelti* group is hypothesized to belong (Mann 1921; Wilson 1959b, 2003; Sarnat 2008). The North American species, *P. hyatti*, was included on the basis of previous phylogenetic work (Moreau 2008) that found support for all Old World *Pheidole* forming a monophyletic group nested within New World *Pheidole*.

Taxa were identified by the first author to species where possible using Mann (1921), Wilson & Taylor (1967) and Sarnat (2008). Undescribed species were given a taxon code combining an abbreviation of the country they were collected from and a two-digit number. Voucher specimens are deposited at the National Museum of Natural History, Washington D.C., USA.

**Table 1** Specimen collection data sorted by *Pheidole* species and collection number

| Species | Collection no. | Species group | Country Code | Island/Province | Elev. (m) | Latitude | Longitude |
|---|---|---|---|---|---|---|---|
| *P.* AU01 | PSW15649 | *quadricuspis* | AU | Queensland | 70 | −6.81667 | 145.68333 |
| *P.* AU02 | PSW15722.11 | *cervicornis* | AU | Queensland | 170 | −11.68333 | 142.70000 |
| *P.* AU03 | PSW15666 | *quadricuspis* | AU | Queensland | 40 | −16.08333 | 145.46667 |
| *P.* AU04 | PSW15690.18 | *quadrispinosa* | AU | Queensland | 60 | −10.75000 | 142.51667 |
| *P.* BP04 | EMS2677 | — | BP | Makira | 724 | −10.40838 | 161.90633 |
| *P. bula* Sarnat | EMS1787 | *roosevelti* | FJ | Viti Levu | 1300 | −17.61480 | 178.01762 |
| — | EMS1789 | *roosevelti* | FJ | Viti Levu | 1300 | −17.61480 | 178.01762 |
| — | EMS1791 | *roosevelti* | FJ | Viti Levu | 863 | −17.61960 | 177.99046 |
| *P. colaensis* Sarnat | EMS1784 | *roosevelti* | FJ | Viti Levu | 1105 | −17.51850 | 178.00680 |
| — | EMS1823 | *roosevelti* | FJ | Viti Levu | 1023 | −17.62415 | 178.00558 |
| — | EMS2332 | *roosevelti* | FJ | Viti Levu | 1125 | −17.68658 | 177.54392 |
| — | EMS2365 | *roosevelti* | FJ | Viti Levu | 850 | −17.67035 | 177.99375 |
| *P.* FJ05 | EMS2094 | *knowlesi* | FJ | Koro | 440 | −17.29528 | 179.40433 |
| — | EPE267.18 | *knowlesi* | FJ | Gau | 408 | −17.97957 | 179.27633 |
| *P.* FJ09 | EMS1388 | *knowlesi* | FJ | Viti Levu | 150 | −18.05000 | 178.36667 |
| — | EMS1927 | *knowlesi* | FJ | Taveuni | 235 | −16.85472 | −179.88833 |
| — | EMS2012 | *knowlesi* | FJ | Vanua Levu | 152 | −16.80639 | 178.98643 |
| — | EMS2074 | *knowlesi* | FJ | Koro | 470 | −17.29083 | 179.40183 |
| — | EPE266.14 | *knowlesi* | FJ | Gau | 505 | −17.98270 | 179.27635 |
| — | EPE285.09 | *knowlesi* | FJ | Gau | 408 | −17.97957 | 179.27633 |
| *P. furcata* Sarnat | EMS2407 | *roosevelti* | FJ | Kadavu | 760 | −19.11806 | 177.98750 |
| *P. hyatti* Emery | SPC6331 | *fallax* | USA | Arizona | 1676 | 31.88833 | −109.20916 |
| *P. knowlesi* Mann | EMS1971 | *knowlesi* | FJ | Vanua Levu | 342 | −16.59286 | 179.77057 |
| — | EMS2055 | *knowlesi* | FJ | Taveuni | 100 | −16.82833 | −179.88400 |
| — | EMS2088 | *knowlesi* | FJ | Koro | 440 | −17.29528 | 179.40433 |
| — | EMS2144 | *knowlesi* | FJ | Viti Levu | 1050 | −17.56944 | 177.97000 |
| — | EMS2182 | *knowlesi* | FJ | Viti Levu | 575 | −17.74333 | 177.66817 |
| — | EPE266.12 | *knowlesi* | FJ | Gau | 505 | −17.98270 | 179.27635 |
| *P.* NC02 | NC02 | — | NC | Nord | N/A | −20.45000 | 164.21667 |
| *P.* NC03 | NC03 | — | NC | Nord | N/A | −20.45000 | 164.21667 |
| *P. oceanica* Mayr | EMS2062 | — | FJ | Taveuni | 160 | −16.82972 | −179.88717 |
| *P. onifera* Mann | EMS2472 | — | FJ | Ovalau | 500 | −17.68205 | 178.81317 |
| *P. pegasus* Sarnat | EMS2370 | *roosevelti* | FJ | Vanua Levu | 910 | −16.59028 | 179.31581 |
| *P.* PG01 | EMS680 | *quadricuspis* | PG | Chimbu | 720 | −6.75000 | 145.01667 |
| *P.* PG02 | EMS729 | *quadrispinosa* | PG | Chimbu | 1100 | −6.71667 | 145.10000 |
| *P.* PG03 | EMS793 | *cervicornis* | PG | Gulf | 700 | −6.80000 | 145.01667 |
| *P.* PG05 | EMS934 | *cervicornis* | PG | Madang | 200 | −5.23333 | 145.68333 |
| *P.* PG06 | EMS960.1 | *quadricuspis* | PG | Madang | 100 | −5.13333 | 145.76667 |
| *P.* PG07 | EMS687 | *cervicornis* | PG | Chimbu | 900 | −6.71667 | 145.10000 |
| *P.* PG08 | EMS813 | *quadrispinosa* | PG | Chimbu | 800 | −6.80000 | 145.01667 |
| *P.* PG09 | EMS804.29 | *cervicornis* | PG | Gulf | 700 | −6.80000 | 145.01667 |
| *P.* PG10 | EMS955 | *quadrispinosa* | PG | Madang | 200 | −5.25000 | 145.01667 |
| *P. roosevelti* Sarnat | EMS1832 | *roosevelti* | FJ | Viti Levu | 912 | −17.56973 | 177.95987 |
| — | EMS1968 | *roosevelti* | FJ | Viti Levu | 304 | −18.08803 | 178.37603 |
| — | EMS2331 | *roosevelti* | FJ | Viti Levu | 840 | −17.67939 | 177.54194 |
| — | EMS2343 | *roosevelti* | FJ | Viti Levu | 850 | −17.67593 | 177.55015 |
| — | EMS2458.01 | *roosevelti* | FJ | Ovalau | 400 | −17.68710 | 178.82350 |
| — | EPE236 | *roosevelti* | FJ | Gau | 717 | −17.99110 | 179.28100 |
| *P. simplispinosa* Sarnat | EMS1906 | *roosevelti* | FJ | Koro | 520 | −17.31250 | 179.38617 |
| — | EMS1949 | *roosevelti* | FJ | Taveuni | 734 | −16.83056 | −179.97433 |
| — | EMS2084 | *roosevelti* | FJ | Koro | 470 | −17.29083 | 179.40183 |
| — | EMS2097 | *roosevelti* | FJ | Koro | 440 | −17.29528 | 179.40433 |
| — | EMS2375 | *roosevelti* | FJ | Vanua Levu | 699 | −16.57525 | 179.31638 |
| — | EMS2396 | *roosevelti* | FJ | Vanua Levu | 570 | −16.62905 | 179.21103 |
| *P. umbonata* Mayr | EMS1962 | — | FJ | Viti Levu | 432 | −18.08578 | 178.37588 |
| — | EMS2049 | — | FJ | Taveuni | 100 | −16.82833 | −179.88400 |

**Table 1** (Continued)

| Species | Collection no. | Species group | Country Code | Island/Province | Elev. (m) | Latitude | Longitude |
|---|---|---|---|---|---|---|---|
| — | EMS2104 | — | FJ | Koro | 115 | −17.26583 | 179.37000 |
| *P. uncagena* Sarnat | EMS2372 | *roosevelti* | FJ | Vanua Levu | 910 | −16.59028 | 179.31581 |
| *P. vatu* Mann | EMS1841.9 | *knowlesi* | FJ | Viti Levu | 186 | −18.06661 | 178.44325 |
| — | EMS1886 | *knowlesi* | FJ | Koro | 420 | −17.29033 | 179.40500 |
| *P. wilsoni* Mann | EMS2067 | *knowlesi* | FJ | Taveuni | 160 | −16.82972 | −179.88717 |
| — | EMS2109 | *knowlesi* | FJ | Viti Levu | 1050 | −17.56944 | 177.97000 |
| — | EMS2153.11 | *knowlesi* | FJ | Viti Levu | 950 | −17.61806 | 178.00550 |
| — | EMS2187 | *knowlesi* | FJ | Viti Levu | 575 | −17.74333 | 177.66817 |
| — | EMS2413 | *knowlesi* | FJ | Kadavu | 700 | −19.11833 | 177.99028 |
| — | EMS2459.1 | *knowlesi* | FJ | Ovalau | 400 | −17.68710 | 178.82350 |

AU, Australia; BP, Solomon Islands; FJ, Fiji; NC, New Caledonia; PG, New Guinea.
Latitude and longitude given in decimal degrees.

### *DNA isolation*

Field collections were made in 95% EtOH and kept in the laboratory until the time of DNA extraction. Total genomic DNA was isolated for one individual worker except when only one individual was available and a noninvasive extraction protocol was used (P.S. Ward, personal communication) in lysis buffer with a Teflon grinding implement, followed by purification using the DNeasy™ tissue kit (Qiagen Inc., Valencia, CA, USA) following the manufacturer's protocols.

### *Polymerase chain reaction (PCR) amplification*

For most specimens, four fragments were amplified via PCR (Mullis *et al.* 1987; Saiki *et al.* 1988) using specific primers for each gene region following the protocols of Brady *et al.* (2006), Moreau *et al.* (2006) and Moreau (2008): *Cytochrome Oxidase* I (COI) protein-encoding mitochondrial molecular marker, *Cytochrome b* (cytb) protein-encoding mitochondrial molecular marker, *Histone* H3 protein-encoding nuclear marker and *Elongation factor 1α F2* (EF1α-F2) protein-encoding nuclear marker.

### *Sequencing*

All sequencing was carried out using dye terminator cycle sequencing following the protocol specified by the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit (Revision B, August 1995; Perkin-Elmer, Norwalk, CT, USA). Primers used for amplification served as sequencing primers. Additional internal primers were used for the COI protein-encoding mitochondrial gene to provide overlapping sequence coverage for the entire region following Moreau *et al.* (2006). All samples were sequenced in both directions again following the protocol of Moreau *et al.* (2006).

### *Sequence alignment*

After sequences were collected, they were analysed and initially aligned using the computer programs Sequencing Analysis 3.7 (ABI Prism™ 2001) and Sequencher 4.8 (GeneCodes 2005), respectively. Inferred amino acid sequences were used for all genes, allowing for comparatively uncomplicated alignment using MacClade 4.06 (Maddison & Maddison 2003).

### *Phylogenetic analysis*

To infer relationships among the species of *Pheidole*, several phylogenetic analyses were performed using PAUP*4.0b10 (Swofford 2001), GARLI v0.96b8 (Zwickl 2006), RAxML v7.2.6 (Stamatakis *et al.* 2008) and MrBayes v3.1.1 (Huelsenbeck & Ronquist 2001). A variety of model-based methods, in addition to maximum parsimony (MP), were employed to infer phylogenetic relationships. Parsimony searches were performed on the complete concatenated data set using the random stepwise addition option of the heuristic search for 500 replicates with tree bisection-reconnection (TBR) branch swapping, collapse of zero-length branches and equal weighting of all characters. If searches produced more than one tree, a strict consensus was performed to summarize data analyses. To measure the robustness of branching patterns of the parsimony trees, bootstrap analyses (bs) (Felsenstein 1985; Hillis & Bull 1993) were executed by using the closest stepwise addition of the heuristic search for 500 replicates.

To evaluate the fit of the data, likelihood analyses were conducted using the complete concatenated data set with GARLI v0.96b8 (Zwickl 2006), RAxML v7.2.6 (Stamatakis *et al.* 2008) and MrBayes v3.1.1(Huelsenbeck & Ronquist 2001). Modeltest 3.06 (Posada & Crandall 1998) was used to determine the most appropriate

nucleotide substitution model (GTR+Γ+I). A maximum likelihood (ML) search was implemented in GARLI v0.96b8 with model parameters being estimated during the run, with genthreshfortopoterm = 10 000 000; scorethreshforterm = 0.05; significanttopochange = 0.05; stopgen = 10 000 000; and stoptime = 10 000 000. This process was implemented several times to ensure the topology converged on the same ML tree. A single GTR+Γ+I model of sequence evolution was assumed to underlie all genes. To test the robustness of the final ML tree, a bootstrap analysis was performed in GARLI v0.96b8 for 500 pseudoreplicates. In addition, a ML analysis was implemented on the RAxML Web-Servers (Stamatakis *et al.* 2008) that allowed each gene region to have a separate GTR+Γ+I model (GTRGAMMAI) with parameters unlinked and 500 bootstrap pseudoreplicates.

Analyses were also performed using MrBayes v3.1.1, with model parameters being estimated during the run, and using the default value of four Markov chains. A 'temperature' parameter of 0.2 was implemented to produce incremental heating of each chain. The Markov chain Monte Carlo (MCMC) length was 20 000 000 generations, with the chain sampled every 100 generations. Bayesian posterior probabilities (bpp) were estimated as the proportion of trees sampled after burn-in that contained each of the observed bipartitions (Rannala & Yang 1996; Larget & Simon 1999). A single GTR+Γ+I model of sequence evolution was assumed to underlie all gene regions. Convergence of runs was confirmed in all Bayesian analyses by examination of the average standard deviation of split frequencies.

In addition, a partitioned analysis was conducted for 20 000 000 generations with MrBayes v3.1.1, with model parameters being estimated during the run and using the default value of four Markov chains. In this analysis, each separate gene region was assigned its own GTR+Γ+I model with parameters unlinked.

### *Dating constraints*

To calibrate a molecular clock, one or more calibration points must be linked to a particular geological or phylogeographic event to permit scaling of rates and times to absolute times. The use of this information in concert with molecular data can take two forms: (i) fossils or geological events can serve as fixed 'calibration' points used to calculate absolute branching times and (ii) they can serve as maximum or minimum age 'constraints' (Sanderson 1997). For this study, we used fossil information as a minimum age constraint to calibrate divergence times of crown-group endemic Fijian *Pheidole*.

### *Fossil constraints*

Two fossils [*Pheidole tethepa* (Wilson 2007) and *Pheidole primigenia* Baroni Urbani] known from Dominican Republic amber were used to constrain a single node in the phylogeny to a minimum date of 15 Ma. The pronotal spines present on both fossil species are unique among New World *Pheidole* and are argued to be derived from an ancestor common to extant members of the *P. quadricuspis* group (Baroni Urbani 1995). The Dominican Republic amber was formed 15–20 Ma, and the conservative date of 15 Ma is therefore used as a minimum age constraint for the origin of pronotal spines in *Pheidole* and is assigned to the clade that includes all *Pheidole* with pronotal spines.

### *Molecular clock analyses*

Dates of divergence for specific most recent common ancestors (MRCA) were estimated using the software package BEAST v1.4.8 (Drummond & Rambaut 2007). The model of molecular evolution was set to GTR+Γ+I with the molecular clock model set as an uncorrelated lognormal relaxed clock with a Yule process, speciation tree prior. For the fossil calibration, a lognormal prior distribution was implemented with an offset of 15.0 (corresponding to the minimum fossil age) and Log (Stdev) of 1.0 with a median of 16.0 Ma and 95% maximum of 20.23 Ma. The MCMC length was 30 000 000. A burn-in of 5000 was implemented in TreeAnnotator after analysis in Tracer, and the tree and confidence intervals were visualized in FigTree v1.2 (Rambaut 2008).

### *Hypothesis testing*

To test alternative hypotheses for the evolution of the species based on previous taxonomic definitions of the species groups and biogeographic hypotheses (Mann 1921; Wilson 1959b, 2003; Sarnat 2008), constraint tree searches were implemented in GARLI v0.94 (Zwickl 2006) and the Shimodaira–Hasegawa test (Shimodaira, 1999) #30 was executed to investigate significant differences in tree lengths. This test was performed using RELL with 10 000 bootstrap replicates, and the results were evaluated as a one-tailed test.

## Results

### *Simple sequence statistics*

This study produced a final aligned 2328-bp fragment with most taxa sequenced for the following four genes regions: a fragment spanning the mitochondrial COI

(1054-bp) gene, a portion of the mitochondrial gene *Cytochrome b* (cytb) (433 bp), a fragment of the nuclear protein-encoding gene *Histone* (H3) (324 bp) and a fragment of the nuclear protein-encoding gene *Elongation factor 1α F2* (EF1α-F2) (517 bp). The aligned fragment contained 1644 constant sites (70.6%), 134 variable sites (5.8%) and 550 parsimoniously informative sites (23.6%). Examinations of base composition of the entire data set resulted in the following: A: 0.27485; C: 0.21777; G: 0.18685; T: 0.32053. Sequence characters for individual genes are the following: COI (656 constant; 61 variable; 337 parsimony informative), cytb (227 constant; 39 variable; 167 parsimony informative), H3 (286 constant; 16 variable; 22 parsimony informative) and EF1α-F2 (475 constant; 18 variable; 24 parsimony informative).

### *Phylogenetic analyses*

The MP analysis of all characters resulted in 2057 most parsimonious trees (L = 2944) with a CI of 0.335 and a RI of 0.663. The bootstrap values recovered with the MP criterion (MP bs) are included in Fig. 2.

A ML search in GARLI v0.96b8 and RAxML v7.2.6 using the GTR+Γ+I model of sequence evolution resulted in one ML tree each with a −ln L = 16213.74821 for the GARLI concatenated single model analysis and one ML tree with a −ln L = −15633.045216 for the RAxML analysis where each gene was allowed to have independent model parameters. The bootstrap values recovered with the RAxML ML criterion (ML bs) for 500 pseudoreplicates are included in Fig. 2. Neither of the inferred topologies conformed to a strict molecular clock.

### *Bayesian inference phylogenetic analyses*

The likelihood analysis of the partitioned data in MrBayes v3.1.1 in which each gene region was assigned its own GTR+Γ+I model of sequence evolution run for 20 000 000 generations resulted in a sample of trees with a mean likelihood score of −ln L = −15992.159 as calculated in PAUP*. The average standard deviation of split frequencies of the runs after 20 000 000 generations was 0.0035, suggesting that the independent runs had reached convergence. The Bayesian posterior probabilities (bpp) for the partitioned model analysis are included in Fig. 2.

The likelihood analysis in MrBayes v3.1.1 using the single model of sequence evolution for the concatenated data resulted in a sample of trees with a mean likelihood score of −ln L = 16782.69122. The average standard deviation of split frequencies of the runs after 20 000 000 generations was 0.0135, suggesting that the independent runs had reached convergence. Overall, topology and posterior probabilities recovered for the single model analysis tended to agree with those of the partitioned data Bayesian analysis (results not shown).

### *Molecular dating*

Placement of the minimum fossil calibration and age estimations using the fossil data as a minimum calibration age in BEAST v1.4.8 are reported in Fig. 3 for the stem Fijian endemics, the stem *quadricuspis* and *quadrispinosa* groups, the stem *cervicornis* group, the crown *roosevelti* group and the stem *roosevelti* group southern clade. The BEAST searches achieved adequate mixing as assessed by the high effective sample size (ESS) values for all parameters.

### *Phylogenetic relationships*

The ML and Bayesian inference tree topologies show moderate to strong support (84% ML bs; 100% bpp) for the monophyly of the *roosevelti* group. The closest living relatives of the *roosevelti* group include a weakly supported clade of three species related to *P. knowlesi* Mann, several species of Fijian endemic ants related to *P. vatu* Mann and the single species (*Pheidole* BP04) from the Solomon Islands. The *roosevelti* group is more distantly related to the Fijian endemic *P. onifera*, the two pan-Pacific natives (*P. umbonata* and *P. oceanica*), the two New Caledonian species, and all sampled *quadrispinosa*, *quadricuspis* and *cervicornis* group species in the subgenus *Pheidole* (*Pheidolacanthinus*).

### *Hypothesis testing*

The spinescent *Pheidole* groups included in this study (the *roosevelti*, *quadrispinosa*, *quadricuspis* and *cervicornis* groups) were not recovered as a monophyletic lineage (Fig. 4). Tree topologies were compared using the Shimodaira–Hasegawa test (Shimodaira & Hasegawa, 1999) to test for significant differences in tree lengths. In this analysis, tree topologies obtained when all the spinescent species were constrained as a monophyletic lineage (−ln L = 16381.35513) were compared with the ML tree (−ln L = 16213.74821). Constraining the spinescent species groups as monophyletic was significantly different at the ≥0.05 level (difference −ln L = 167.60692; *P*-value = 0.000), from the ML topology.

Our most ML tree found the southern *roosevelti* species group polyphyletic and nested within the northern clade. To test the potential reciprocal monophyly of the northern and southern clades, competing tree topologies were again compared using the Shimodaira–Hasegawa test (Shimodaira & Hasegawa, 1999) to test for

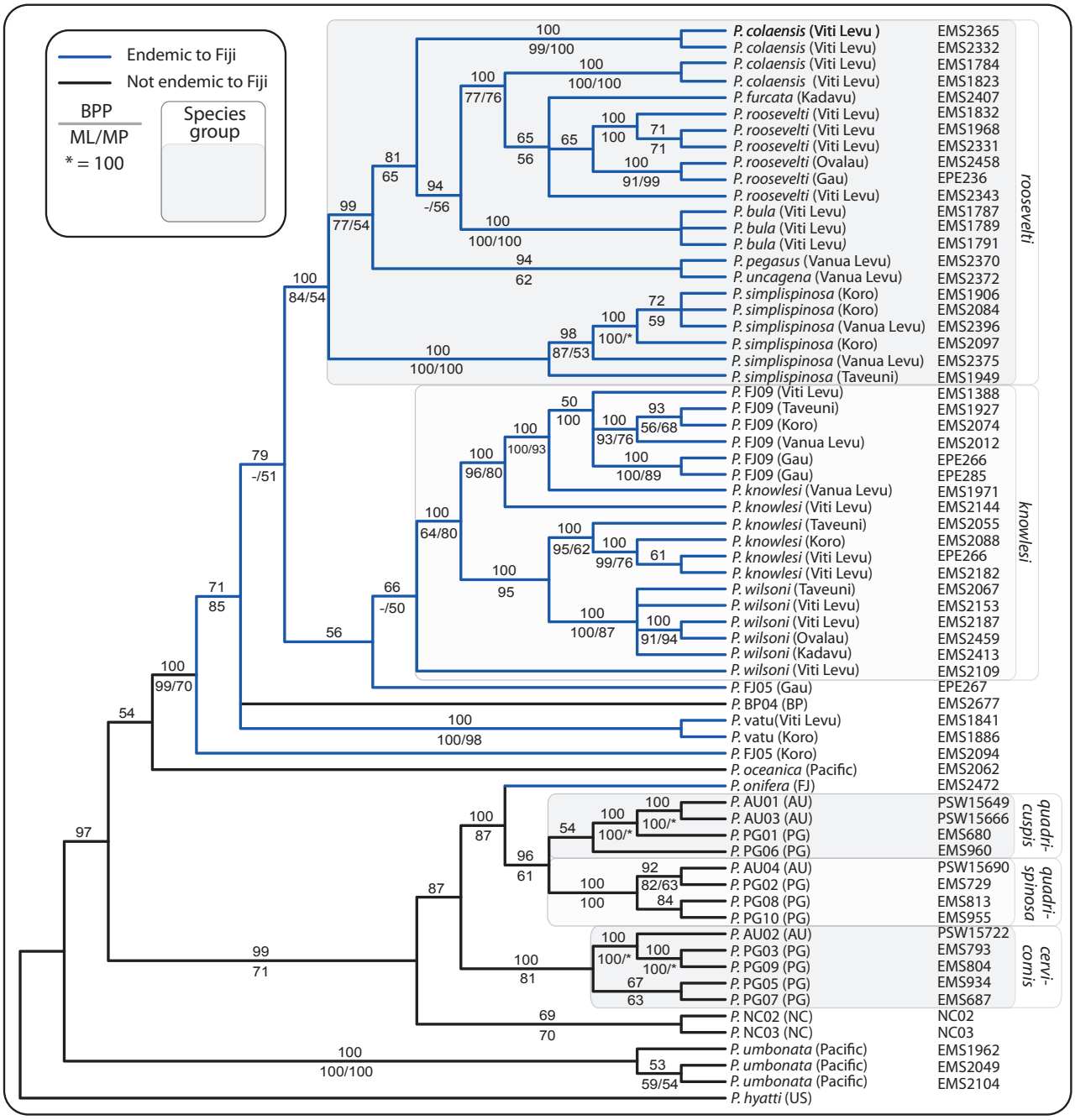



**Fig. 2** Cladogram of the *Pheidole roosevelti* group inferred by a Bayesian partitioned model based on the analysis of 66 taxa and four genes. Bayesian posterior probabilities (bpp) appear above the branches, maximum likelihood bootstrap (ML bs) values appear below the branches to the left and maximum parsimony bootstrap (MP bs) values to the right. Blue branches indicate lineages endemic to Fiji. For Fijian endemics, the island name from which the specimen was sampled appears in parentheses (see Fig. 5). For non-Fijian taxa, the country name from which the specimen was sampled appears in parentheses (AU, Australia; BP, Solomon Islands; NC, New Caledonia; PG, Papua New Guinea; US, United States; Pacific, widespread Pacific species). Collection codes appear to the right of the taxa.

significant differences in tree lengths. In this analysis, tree topologies obtained when all the northern species were constrained as a monophyletic lineage and sister to a monophyletic southern lineage (−lnL 16214.76806) were compared with the ML tree (−lnL 16213.74821). Constraining each of these clades as monophyletic was not significantly different at the ≥0.05 level (difference −ln L = 1.01976; $P$ = 0.466), from the ML topology.

### *Phylogenetic origins of spinescent* Pheidole

Our aforementioned findings yield strong support for two independent origins of spinescence in the Old World *Pheidole* (Fig. 4). One origin occurred within the Fijian endemic *Pheidole roosevelti* group and is characterized by a single transition from taxa with simple propodeal spines and obtuse mesosomae (i.e. *P. simplispinosa*) to species with angulate propodeal spines and acute mesosomal projections.

Spinescence originated in at least one other *Pheidole* lineage, in addition to the *roosevelti* group. The spinescent *quadrispinosa* and *quadricuspis* groups form a moderately well-supported clade. The most parsimonious explanation for the extended propodeal spines and pronotal spines in these groups is that both characters were derived from a single common ancestor. Moreover, it is uncertain whether spinescence was inherited from a common ancestor of the New World fossils *P. tethepa* and *P. primigenia* or was derived independently. This result is further tempered by the bias of our study towards sampling of spinescent species. Inclusion of more *Pheidole* with conservative morphologies could affect the topology and support values for the *quadrispinosa* and *quadricuspis* groups.

The weakly supported position of the *cervicornis* group relative to the other two *Pheidolacanthinus* groups casts doubt on the monophyly of this subgenus and suggests that the *cervicornis* group's spinescent morphology could also be independently derived.

### *Biogeographic origin of Fijian* Pheidole

At least two ancestral lineages of *Pheidole* independently colonized Fiji prior to human arrival. One putative clade includes the *roosevelti* group, the *knowlesi* group, *P. vatu* and an undescribed species (*Pheidole* FJ05). However, the inclusion of a species from the Solomon Islands (*Pheidole* BP04) and the sparse sampling of *Pheidole* species from other Pacific regions leaves open the possibility that the monophyly of the clade is an artefact of poor taxon sampling. If, in fact, the species from the Solomons is derived from Fijian ancestors, it would serve as a rare example of upstream colonization (Filardi & Moyle 2005). The second conclusive independent colonization of Fiji by an endemic lineage is represented by *P. onifera* Mann. The specimens of *P. umbonata* and *P. oceanica* were taken from Fiji, but their area of origin is obscured by their pan-Pacific distribution.

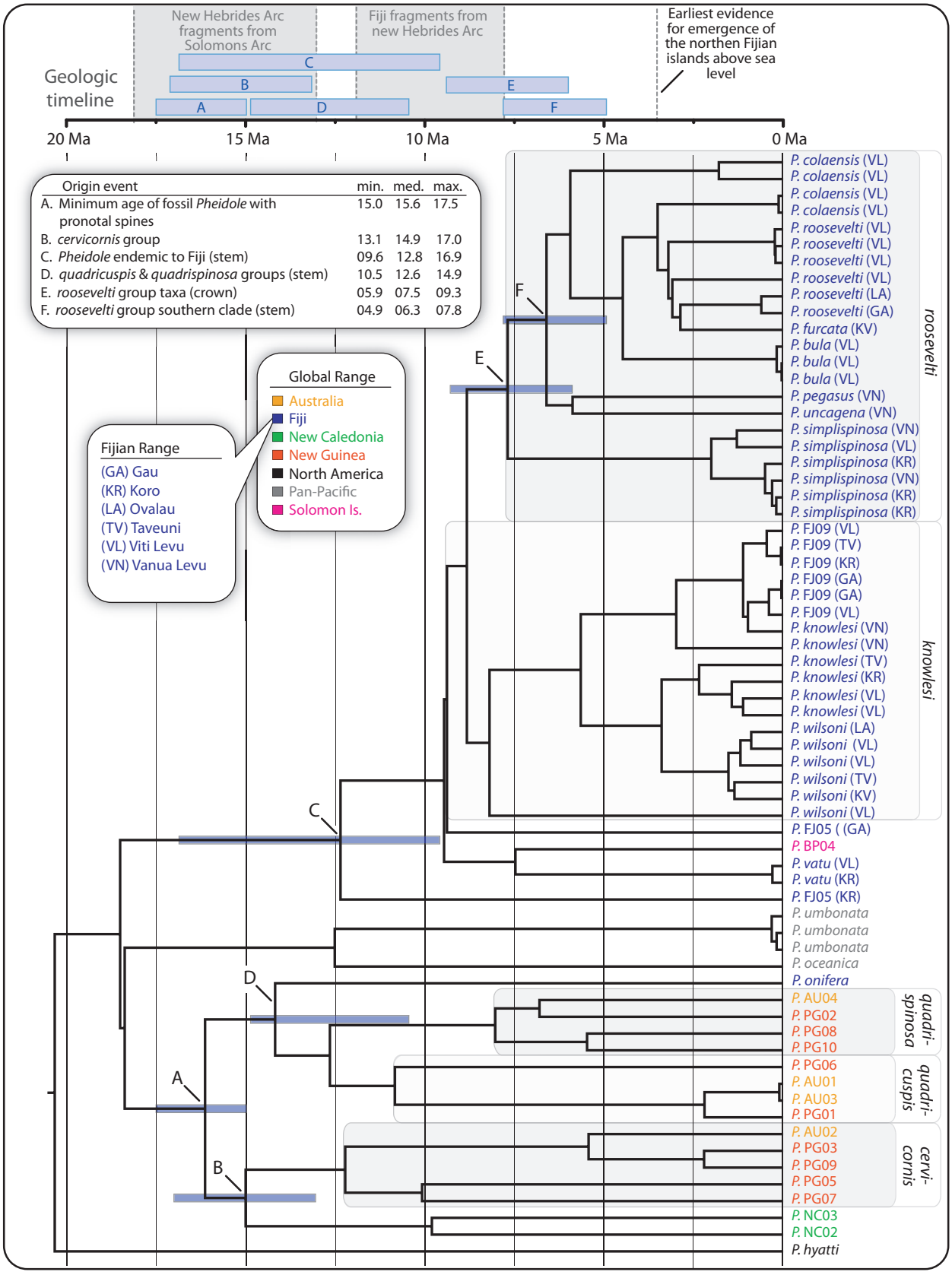
The most striking biogeographic pattern to emerge within the Fijian archipelago involves the split between the northern and southern *roosevelti* lineages. The three basal-most species of the *roosevelti* group (*P. simplispinosa*, *P. pegasus*, *P. uncagena*) are all restricted to the northern islands of Vanua Levu, Taveuni and Koro (Fig. 5), and it is within these northern lineages that the southern clade is nested. However, based on the results of our hypothesis testing elsewhere, we cannot reject reciprocal monophyly of each of the northern and southern clades.

## Discussion

Our four-gene phylogenetic analysis of the *Pheidole roosevelti* group and a sampling of its Pacific congeners yields new insights into the biogeographic history of Fiji, reveals a fascinating example of convergent evolution and serves as a novel example of ecological release occurring within an insular eusocial insect lineage. Taken together, these findings recover the history of a presumably unremarkable ant species that colonized a small, oceanic island in the Miocene (Fig. 3) and proceeded to radiate across the emerging islands of the Fiji archipelago into niche-space occupied elsewhere in the Pacific by distantly related spinescent congeners.

That the descendants of this pioneering lineage are today among Fiji's rarest species, restricted to its highest mountain peaks, may suggest an evolutionary trajectory towards increased specialization. Alternatively, the species may have occupied historically wider geographical and elevational ranges that have only recently been circumscribed by anthropogenic modifications that began 3000 kya with the first human colonization of the archipelago. Regardless of their historic range, the Fijian origins of this remarkable group of ants reveal the archipelago's often underestimated potential for *in situ* evolution.

**Fig. 3** Dated chronogram of *Pheidole roosevelti* group and congeners in relation the geologic history of the Fijian archipelago. Topology and dates inferred by Bayesian methods using BEAST. All dates in millions of years. Node A corresponds to the inferred fossil age which was calibrated to a minimum of 15 Ma. Nodes B–F correspond to the geologic timeline and origin event table. Origin event table gives minimum, median and maximum dates within 95% highest posterior density (HPD) when node was recovered in ≥50% of topologies. Timeline above and chronogram below show HPD bars that correspond to dates in origin event table. For non-Fijian taxa, the country name from which the specimen was sampled appears in parentheses (AU, Australia; BP, Solomon Islands; NC, New Caledonia; PG, Papua New Guinea; US, United States; Pacific, widespread Pacific species).



### *Biogeographic history*

What can our results, when filtered through the lens of Fiji's geologic history, tell us about the diversification of the *P. roosevelti* group over evolutionary time? Although the phylogeny does not provide strong evidence for where the group's ancestor emigrated from, the event most likely occurred in the Miocene. The colonization date (17–10 Ma) of the group's first Fijian ancestor corresponds to divergence of the clade that includes all sampled Fijian taxa excluding *P. onifera*. This result offers the earliest evidence of colonization for Fijian terrestrial arthropod lineages. Colonization by *Copelatus* diving beetles (Monaghan *et al.* 2006), *Rhantus* diving beetles (Balke *et al.* 2007b) and *Lordomyrma* ants (Lucky & Sarnat 2009) were all reported to have occurred between 13 and 5 Ma. Together, these findings suggest Fiji's oldest insect lineages are not ancient Gondwanan relicts as suggested from botanical studies (Raven & Axelrod 1972; Whitemore & Page 1980; De Laubenfels 1996; Weston & Crisp 1996), but relatively recent Laurasian colonists. This result adds to a growing number of findings supporting Laurasian origins for other Fijian, and even New Caledonian, insect groups (Duffels & Turner 2002; Monaghan *et al.* 2006; Balke *et al.* 2007a,b; Guilbert *et al.* 2008; Lucky & Sarnat 2009).

All of these colonization events are inferred to have occurred after Viti Levu's initial emergence 20–25 Ma, but before the island's significant uplift 5 Ma (Whelan *et al.* 1985). Miocene island hopping across the western Melanesian islands could have been facilitated by the fragmenting Vitiaz Arc, which formed a nearly continuous chain of archipelagos stretching from New Guinea to Fiji (Hall 2002).

By approximately 9 Ma, the *roosevelti* group had diverged from the *knowlesi* group, and the ancestor of the crown *roosevelti* group had diverged by 7.5 Ma. Where on the archipelago this common ancestor was derived presents a paradox. The most basal three extant species of the *roosevelti* group (*P. simplispinosa*, *P. uncagena*, *P. pegasus*) are restricted to the northern islands of Vanua Levu, Taveuni and Koro (Fig. 5). However, none of these islands had emerged above sea level before 4 Ma. While the oldest known rocks from Vanua Levu are dated to 8–6.5 Ma (Rodda & Kroenke 1984; Kroenke & Yan 1993; Rodda 1994), the first evidence of a subaerial volcanic eruption is dated to approximately 4 Ma (Rodda 1994). The first known subaerial deposition of Taveuni is dated at 3 Ma, and the bulk of the dry was formed in the last 8 00 000 years (Rodda & Kroenke 1984; Rodda 1994). Koro was formed during the early Pliocene (Rodda 1994), but little is known about when it emerged above sea level.

Regardless of the timing of divergence, the observed pattern of the southern *roosevelti* group clade being nested within the northern species (Fig. 5) is unexpected, although not statistically significant. Viti Levu is the largest and oldest of the archipelago's islands and is often presumed to be the source from which the other island populations are derived. Based on our hypothesis testing, we cannot reject a sister relationship between the northern and southern species. It will be interesting to see whether further phylogenetic work produces additional examples of lineages from peripheral islands invading the southern Fijian island of Viti Levu.

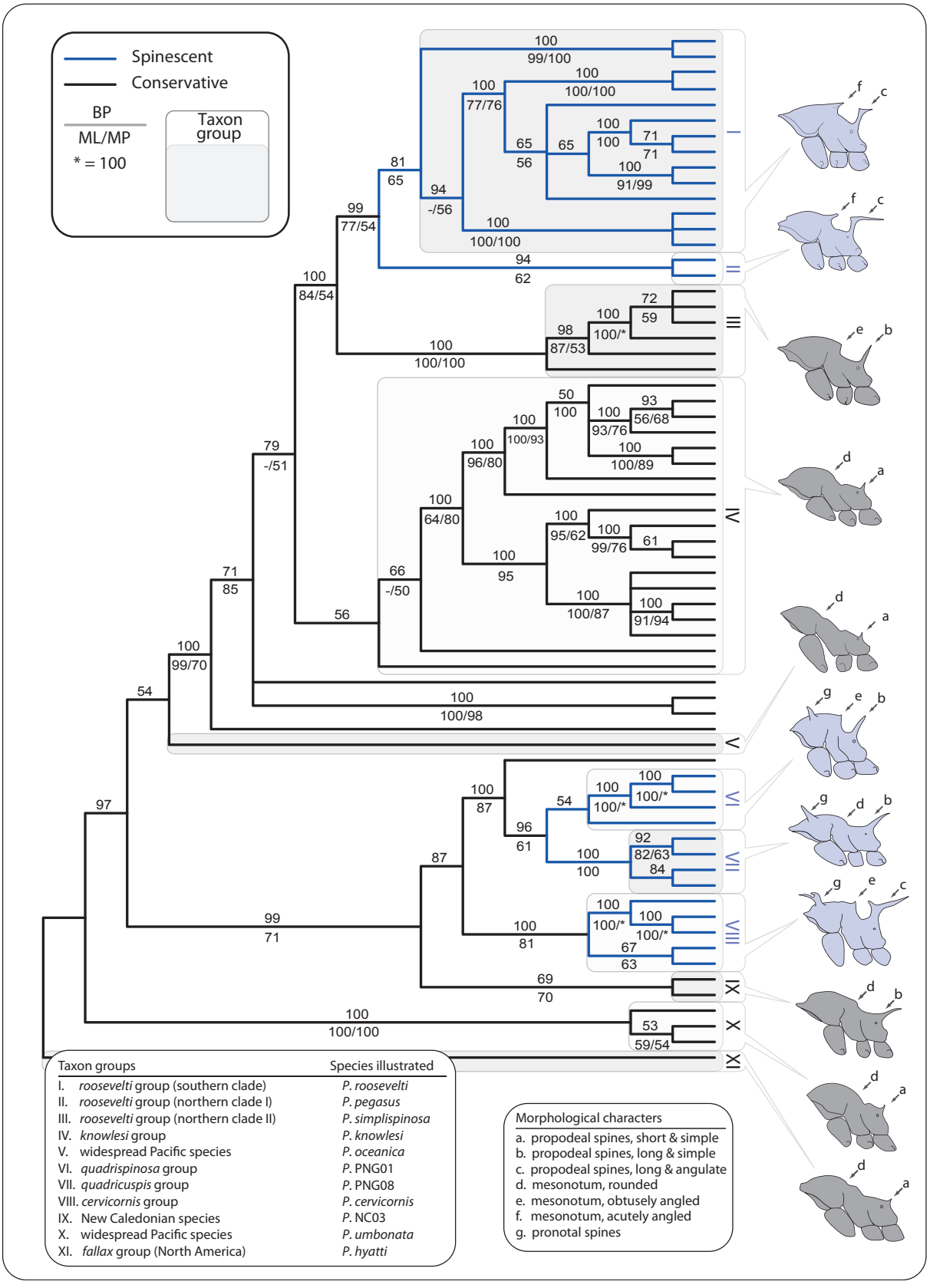
### *Convergent evolution of spinescence*

*Pheidole* is one of the most successful, diverse and ubiquitous lineages on earth (Wilson 2003; Moreau 2008), and it should not be surprising to discover morphological homoplasy among its ranks. As first erected to house spinescent *Pheidole* species collected by Alfred Wallace, the subgenus *Pheidolacanthinus* (Smith 1865) has been the recipient of all spinescent *Pheidole*.

A half century later, Emery (1921) demarcated *Pheidolacanthinus* into the *quadrispinosa*, *quadricuspis* and *cervicornis* groups. He postulated that the subgenus was an artificial assemblage based more on the convergent evolution of multiple spines than on phylogenetic relationship. In his treatment of the fossils *P. tethepa* and *P. primagenia*, Baroni Urbani (1995) discusses the validity of *Pheidolacanthinus* as a subgenus while submitting arguments for and against a single evolutionary origin of pronotal spines in *Pheidole*.

The results of our analysis provide moderate support for Emery's postulation that the *cervicornis* group is distantly related to both the *quadrispinosa* group and the *quadricuspis* group and that the development of pronotal spines and modified propodeal spines is of homoplastic origin. Although our analysis supports the monophyly of the *quadrispinosa* and *quadricuspis* groups, our limited out-group sampling (especially with regard to taxa without spinescent morphology) tempers the suggestion

**Fig. 4** Distribution of morphological spinescence across the *Pheidole roosevelti* group and sampled congeners. Cladogram and support values identical to Fig. 2. Taxon groups are represented by Roman numerals. Exemplar species are illustrated for each taxon group and pertinent morphological characters are labelled (a–g). Taxon groups with spinescent morphology are represented by blue branches and illustrations. [Correction added after online publication 7 December 2010: the figure was updated with the text for taxon groups VIII and IX switched with each other].



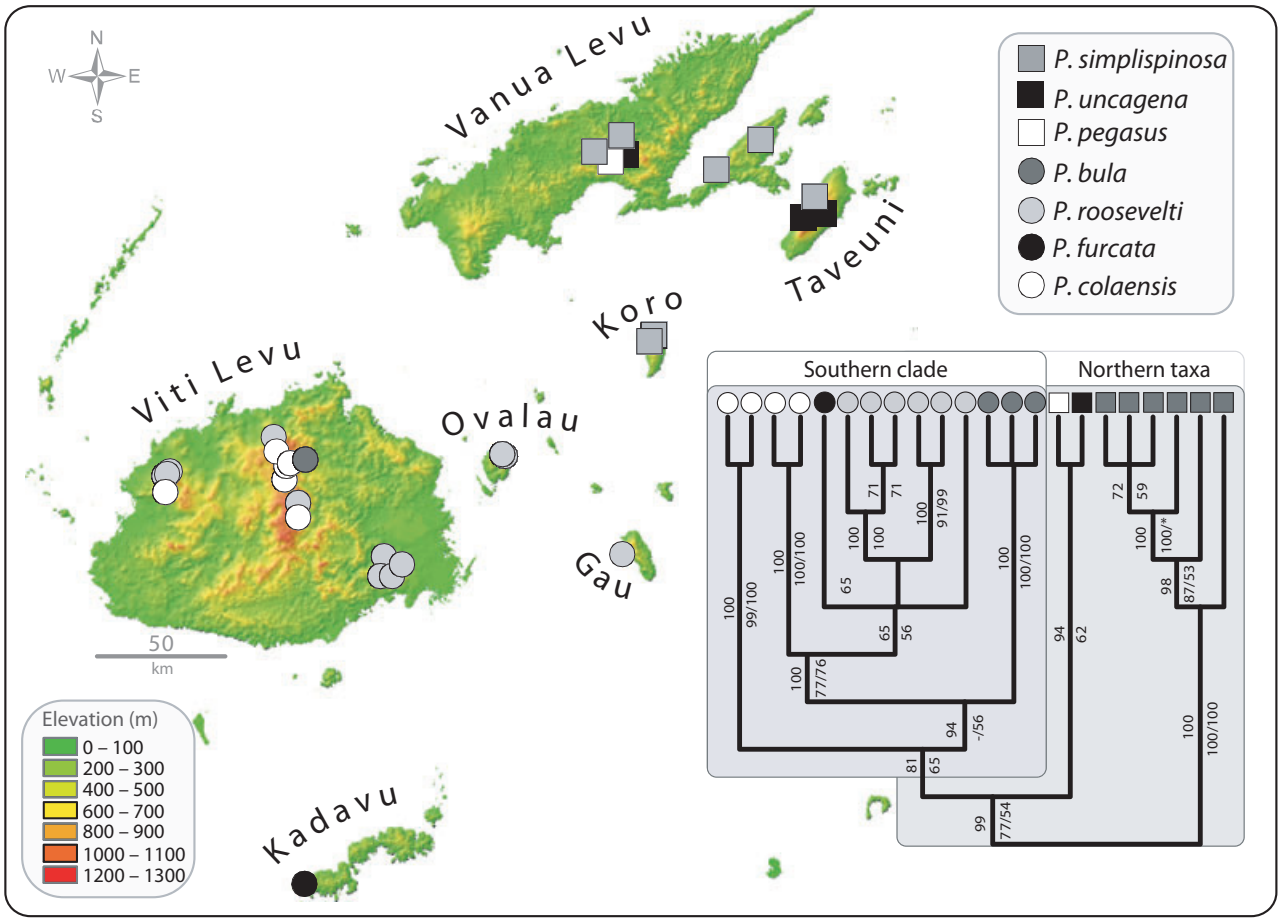



**Fig. 5** Map of Fiji archipelago with biogeographic affiliation of samples used in this study. Locality overlaid on cladogram of *Pheidole roosevelti* group. Northern islands include Vanua Levu, Koro and Taveuni. Southern islands include Viti Levu, Ovalau, Gau and Kadavu. All species are represented by symbols. Support values and cladogram are identical to corresponding branches in Fig. 2.

that the pronotal spines of the two groups derive from a common ancestor.

More striking is the considerable evolutionary distance separating the *cervicornis* and *roosevelti* groups. Mann (1921) erected the subgenus *Pheidole* (*Electropheidole*) for *P. roosevelti* and *P. colaensis* but acknowledged the similarities between these species and *P. cervicornis* from New Guinea. Wilson (1959b), in discussing the ecology of *P. cervicornis* in New Guinea, considered the species as belonging to *Electropheidole*. Most recently, Sarnat (2008), based on a preliminary morphological analysis, also proposed close relationship between the *roosevelti* and *cervicornis* groups and hypothesized that *P. simplispinosa* may represent a secondary loss of the lineage's spinescent morphology.

That the morphologically conservative *P. simplispinosa* represents ancestral traits of the *roosevelti* group and that the spinescent morphology of the other group members is convergent with the *cervicornis* group is a testament to the capacity of molecular analysis to reveal relationships obscured by homoplasy.

### *Ecological release of an insular ant radiation*

Our findings reveal that the spinescent morphology of the *roosevelti* group did not derive from one of the Pacific's ancestrally spinescent lineages, but from a morphologically conservative Fijian lineage. That such elaborate structures evolved *in situ* suggests them to have resulted from the ecological release of a distant ancestor upon reaching an island system depauperate of ants. The expansion of these Fijian *Pheidole* into morphospace occupied elsewhere by distantly related spinescent congeners is an example of Darwin's (1859) observation that 'oceanic islands are sometimes deficient in certain classes and their places are apparently occupied by other inhabitants'.

While additional study of the ecological function of spines in ants is required before this example meets Schluter's (2000) strict definition of adaptive radiation, the prevalence of spinescence among Melanesian *Pheidole* suggests it confers some degree of selection advantage. It is our hypothesis that the same selective

pressures that maintain lineages of spinescent *Pheidole* elsewhere in the Pacific precipitated the independent evolution of spinescent *Pheidole* in Fiji.

What selective pressures are responsible for the phenotypic transition from plesiomorphic morphology retained by the *knowlesi* group and *P. simplispinosa* to spinescent morphology of the more derived *roosevelti* species? We predict that the radiation of Fijian *Pheidole* into spinescent morphotypes was the consequence of ecological opportunities afforded by the absence of competing ant lineages with conspicuous epigaeic (above-ground) foraging strategies.

It is somewhat surprising, considering the spectacular diversity of spines among ants and the considerable literature devoted to ant ecology (Hölldobler & Wilson 1990; Lach *et al.* 2010), that no study has tested the ecological significance of spinescence. A recent review of ant defensive strategies suggests erect spines might deter vertebrate predators and are most commonly associated with large taxa that forage on vegetation in the understory or canopy in the tropics (Dornhaus & Powell 2010). A descriptive study of New Guinea ants found spinescence to be associated with species that forage in the open, either on the ground or on the lower arboreal strata, where they may be vulnerable to vertebrate predation (Wilson 1959b). It is likely that of all vertebrates, birds exert the strongest predation pressure on Fijian ants, as this remote oceanic island supports a very depauperate fauna of reptiles, amphibians and mammals.

Members of the *roosevelti* group fit the syndrome of conspicuous foragers. Compared to other Fijian *Pheidole*, they are substantially larger and are more easily observed foraging on the ground and in low vegetation (Sarnat 2008). Moreover, unlike other Fijian *Pheidole* that nest in rotting logs and hollow twigs or between flat surfaces such as leaves or stones, all *roosevelti* group species excavate large nest chambers directly in stable soil substrates. While elaborate armature might be cumbersome for ants nesting in rotting logs, in twigs or between leaves, ants that nest in stable soils (such as the spinescent Neotropical *Atta* and *Acromyrmex* leaf-cutting ants) can tailor their tunnel diameters and chamber dimensions to accommodate morphological specializations.

*Pheidole simplispinosa* is a living link between the plesiomorphic state of the *roosevelti* group and its transition to spinescence. *P. simplispinosa* has transitioned from nesting in soil chambers but has not evolved the degree of spinescence observed in the group's more derived species. A more detailed study comparing the ecologies of *P. simplispinosa* to the spinescent *roosevelti* species could provide insights into the evolutionary advantage of spinescent morphology.

Equally important as what selective pressures existed in the *roosevelti* group's evolutionary past are what selective pressures were absent. Competition has been described as the 'hallmark of ant ecology' (Hölldobler & Wilson 1990), and interspecific competition is a significant factor and is the structuring of ant communities (Parr & Gibb 2010). The first ant colonists of Fiji likely experienced a significant release from the historic constraints of competition exerted by their previous community.

We propose that the failure of both army ants and spinescent *Pheidole* lineages to colonize Fiji left open ecological opportunities that the *roosevelti* group's ancestor exploited. Army ants, arguably the most dominant of all epigaeic foragers, are absent from Fiji. The absence of army ants has been invoked by several authors as a promoting factor for insular ant radiations (Ward & Wetterer 2006; Fisher 2010). The only other record of a spinescent *Pheidole* outside the *roosevelti* group from Fiji is a single specimen of the wide-ranging *P. sexspinosa* (*quadricuspis* group) collected once from the southern coastline of Viti Levu.

That no other lineages of spinescent *Pheidole* are known from Fiji suggests several possibilities. First, if spinescence has evolved multiple times within Melanesian *Pheidole*, it suggests that occupation of that morphospace allows for the exploitation of a particular ecological niche, specifically that of the conspicuous epigaeic forager. If no ancestrally spinescent *Pheidole* lineage was present on Fiji, the ecological niche would be available for another lineage to occupy. The transition to soil nesting by the *roosevelti* group may have allowed it to be the first Fijian ant lineage to fill the niche. Second, the failure of the other three groups of Pacific spinescent *Pheidole* to colonize Fiji may, in fact, be caused by the *roosevelti* group's prior occupation of the niches those lineages have adapted to exploit.

Unfortunately, the unique opportunity afforded by these Fijian endemics to study the ecological causes and consequences of an evolutionary shift towards spinescence may be short lived. The undisturbed primary forest, upon which all *roosevelti* group species depend, is under increasing threat from timber harvest, expanding cultivation and invasive species (Olson *et al.* 2006). While all members of the group are restricted to high-elevation habitats, *P. pegasus* is known from a single locality on the highest mountain of Vanua Levu and *P. bula* is known from a single locality on the highest mountain of Viti Levu. The slightest increase in climatic temperatures could well push these species to extinction and with them their fascinating story of evolution on a remote island archipelago.

## Acknowledgements

We thank the following individuals for donating specimens to this study: Julien LeBreton, Evan P. Economo, Stefan P. Cover and Philip S. Ward. Thank you to Evan P. Economo, Philip S. Ward, Stefan P. Cover and three anonymous reviewers for comments, which greatly improved this manuscript. This research was supported by the National Science Foundation (NSF) grant DEB-0425970 (Fiji Terrestrial Arthropod Survey), an NSF Graduate Research Fellowship to EMS, UC Davis Department of Entomology and the Field Museum Department of Zoology.

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