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Out of the Neotropics: newly discovered relictual species sheds light on the biogeographical history of spider ants (*Leptomyrmex*, Dolichoderinae, Formicidae)

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Abstract. Spider ants of the genus *Leptomyrmex* Mayr (Hymenoptera: Formicidae: Dolichoderinae) are conspicuous species of Australasian rainforests, with putative fossil relatives in the Neotropics and Europe. There is longstanding debate over the biogeographical history of the genus, with the Palaearctic and Neotropical regions proposed as alternate centres of origin. We propose a resolution of this debate with the recent discovery and analysis of an extant species from central Brazil, *L. relictus* **sp.n.**, which we describe from workers, males and brood. We sequence ten nuclear genes in the new species and in several Australian *Leptomyrmex* species, and append these data to a 54-taxon, 10-gene data matrix previously generated for the subfamily Dolichoderinae. We conduct phylogenetic and divergence dating analyses, and re-evaluate the fossil record of the group. We recover *Leptomyrmex relictus* **sp.n.** as a member of the *Leptomyrmex* clade with high support. It is sister to the Australasian species, and the genus *Leptomyrmex* is, in turn, sister to a pair of Neotropical genera, *Forelius* and *Dorymyrmex*. We infer a Neotropical origin for the genus and estimate a mid-Eocene (46 Ma, 95% CI 56 to 36 Ma) origin for the crown genus and an Oligocene origin for the Australasian clade (29 Ma, 95% CI 40 to 19 Ma). We confirm placement of the Dominican amber species †*L. neotropicus* Baroni Urbani in the genus but reject a close relationship with the Palaearctic fossil taxa †*Leptomyrmula* Emery and †*Usomyrma* Dlussky, Radchenko & Dubovikoff, considering them *incertae sedis* in the subfamily (Dolichoderinae). In contrast to the mesophilic preferences of the Australasian species of *Leptomyrmex*, the new Brazilian species inhabits cerrado (dry savannah). Our results support a Neotropical origin for spider ants with dispersal to Australia. Rafting on west-bound currents and/or a historical diversity imbalance between Australia and South America are proposed as alternate hypotheses to explain a pattern of biased E–W mid-Tertiary dispersal for ants with austral distributions. This pattern is suggested by our results in conjunction with observations of other ant clades. Overall, our findings highlight the value of integrated taxonomy, critical interpretation of morphology, and a comparative phylogenetic framework when conducting palaeontological and biogeographical studies of insect species.

This published work has been registered in ZooBank, http://zoobank.org/urn:lsid:zoobank.org:pub:6E9E6617-6E53-40B8-82C7-67F89A83C553.

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Introduction

Spider ants (*Leptomyrmex* Mayr, Formicidae, Hymenoptera; Fig. 1) are gracile, often brightly coloured species belonging to the chemically-defended subfamily Dolichoderinae. With species distributed in the mesic forests of eastern Australia, New Caledonia, New Guinea, and the nearby islands of Aru and Seram (Lucky, 2011; Fig. 2), spider ants are divided unevenly between two clades: the 'micro-' and 'macro-' *Leptomyrmex*, with 6 and 21 species, respectively (Lucky, 2011; Fig. 1B, C, F, G). The most conspicuous members of the genus belong to the macro-*Leptomyrmex*, which is characterized by ergatoid (worker-like and permanently wingless) queens (Wheeler, 1915, 1934), replete nutrient-storing subcastes (Wheeler, 1910), highly derived males (Lucky & Ward, 2010; B.E. Boudinot, unpublished data) and the habit of holding the gaster over the mesosoma. These remarkable ants also serve as Batesian mimicry models for phasmid insects (Key, 1970) and spiders (Rainbow, 1898; Turner, 1898), and are highly sensitive to habitat disturbance, making them useful bioindicator species (Sinclair & New, 2004).

Three fossil taxa have been attributed to *Leptomyrmex* or inferred to be very closely related to the genus: †*L. neotropicus* Baroni Urbani (Dominican amber, 19 to 16 Ma; Fig. 1D; Iturralde-Vinent, 2001; Penney, 2010), †*Leptomyrmula maravignae* (Emery) (Sicilian amber, 34 to 14 Ma; Ragazzi & Roghi, 2014) and †*Usomyrma mirabilis* Dlussky *et al.* (Danish/Baltic amber, 48 to 41 Ma; Ritzkowski, 1997). Morphology-based studies of these fossils have postulated the historical distribution of spider ants to be cosmopolitan or tropicopolitan (Baroni Urbani, 1980; Dlussky *et al.*, 2014), and the region of origin variously interpreted as Australian (Wheeler, 1915), Palaearctic (Emery, 1913; Dlussky *et al.*, 2014) or Neotropical (Baroni Urbani, 1980). Most recently Dlussky *et al.* (2014) posed two alternative hypotheses explaining the apparent extreme disjunct distribution of these groups: *Leptomyrmex* originated in the Palaearctic and dispersed to Australia via (i) Southeast Asia, or (ii) North America and subsequently Australia through South America. A third alternate hypothesis – that of a Neotropical origin with dispersal to Australia – was proposed by Ward *et al.* (2010) based on a molecular phylogeny and dispersal-vicariance (DIVA) analysis. This study, however, did not explicitly address the fossil record of putative *Leptomyrmex* relatives. We investigate this problem by integrating palaeontology, morphology and phylogenetic systematics based on the surprising discovery of an extant species from Brazil, *L. relictus* **sp.n.** (Fig. 1A).

Materials and methods

Sequencing and alignment

We chose a single worker of *L. relictus* **sp.n.** (CASENT0730400, paratype) and specimens of four Australian *Leptomyrmex* species for sequencing. These sequences were appended to an existing 54-taxon data matrix developed by Ward *et al.* (2010) for study of the ant subfamily Dolichoderinae. This matrix already contained two other *Leptomyrmex* species, giving a total of six Australian species and the new taxon from Brazil. DNA was extracted nondestructively using the Qiagen DNeasy Blood & Tissue Kit (Qiagen Inc., Valencia, CA, U.S.A.). We amplified and sequenced fragments of the same ten nuclear genes employed by Ward *et al.* (2010): *18S*, *28S*, Abdominal (*AbdA*), Arginine Kinase (*ArgK*), Carbamoyl-Phosphate Synthetase 2 (*CAD*), Elongation Factor 1 alpha-F1 (*EF1aF1*) and F2 (*EF1aF2*), Enolase (*EL*), Long Wavelength Rhodopsin (*LW Rh*) and Wingless (*Wg*). The new sequences have been deposited in GenBank (accession numbers KU215798–KU215835; Table S1). Sequence characteristics are reported in Table S1; matrix completeness was computed using AMAS (Borowiec, 2016). Sequencing procedures were similar to those described in Ward & Downie (2005), Brady *et al.* (2006) and Ward *et al.* (2010). Sequences were edited using Sequencher v5.0 (Gene Codes Corporation, Ann Arbor, MI), and appended to the dolichoderine data matrix of Ward *et al.* (2010) using MacClade v4.08 (Maddison & Maddison, 2000). The new 59-taxon dataset comprises 8915 bp of aligned sequence, excluding introns and hypervariable regions of *28S*. The matrix has no missing fragments, is 99.94% complete, and has been deposited in TreeBase (matrix accession number S18604).

Phylogenetic analyses

Data partitions.
The 59-taxon 10-gene data matrix was initially partitioned into 26 blocks, corresponding to the three codon positions of each of the eight protein-coding genes and one block each for *18S* rDNA and *28S* rDNA. We then employed Partition Finder v1.1.1 (Lanfear *et al.*, 2012) to choose among-partition schemes and models, with models = mrbayes, model_selection = bic and search = greedy. This yielded a 12-partition scheme (Table S2), which was used in subsequent Bayesian and maximum-likelihood analyses.

Bayesian analysis.
We carried out a partitioned Bayesian analysis using MrBayes 3.2.3 (Ronquist *et al.*, 2012a), with all parameters unlinked across the twelve partitions except branch lengths and topology. We set the branch length prior, brlenspr, to unconstrained:exponential(100). This setting corresponds to a mean branch length prior of 0.01, as opposed to the MrBayes default of 0.1; this better matches the shorter branch lengths in this dataset (Ward *et al.*, 2010). We ran the analysis on the CIPRES Science Gateway (Miller *et al.*, 2010; http://www.phylo.org/) for 40 million generations, with nchains = 4, nruns = 2, sample freq = 1000 and the default 25% burn-in. Indications that stationarity had been achieved included standard deviations of split frequencies less than 0.002 in the post-burn-in samples; PSRF (Potential Scale Reductions Factor) values of 1.000 for all parameters; minimum estimated sample size (ESS) values >4000; and similar mean marginal likelihoods for the two runs. The more heavily partitioned models employed in Ward *et al.*

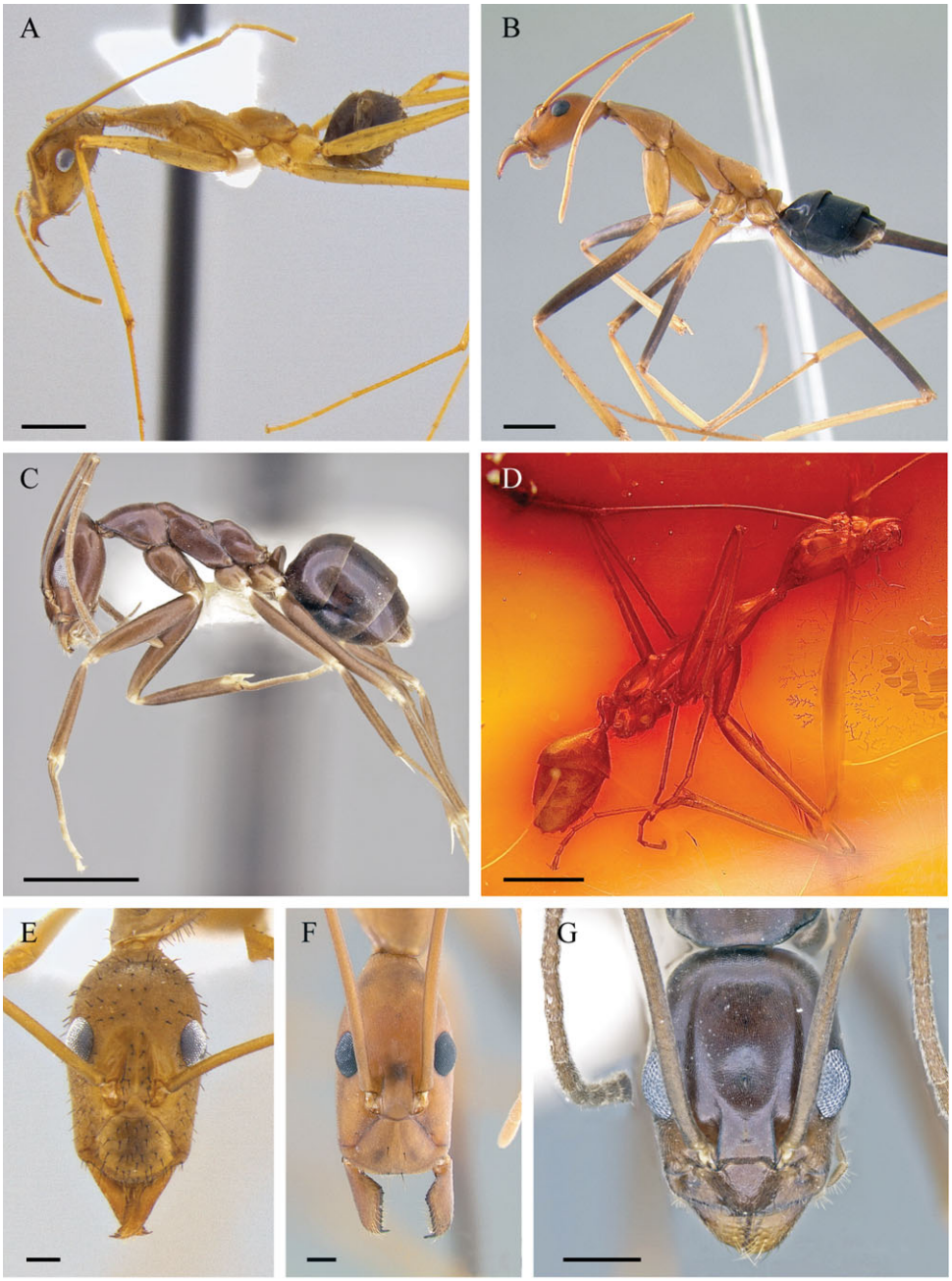

Fig. 1. Representative workers of each *Leptomyrmex* clade. A, E, *L. relictus* **sp.n.**; B, F, macro- clade, *L. cnemidatus* (CASENT0012219); C, G, micro-clade, *L. aitchisoni* (CASENT0012033); D, †*L. neotropicus* (PSWC). A–D, Profile view; E–G, full-face (dorsal) view. Scale bars: A–D = 1.0 mm, E–G = 0.25 mm. (B and F imaged by A. Lucky, C and G by Estella Ortega; these images from AntWeb.org.)

(2010) were associated with some difficulties in achieving convergence but we did not experience this with the 12-partition scheme chosen by Partition Finder.

Maximum-likelihood (ML).

For ML analyses we employed GARLI 2.0 (Zwickl, 2006), as implemented on the CIPRES Science Gateway. We used the same partitions as in MrBayes, and the following modifications from GARLI defaults: topoweight = 0.01, brlenweight = 0.002, modweight = 0.0065, and genthreshfortopoterm = 2 000 000. We carried out one round of eight search replicates in estimating the ML tree. Separately we ran a bootstrap analysis with 100 replicates (one search replicate per bootstrap replicate) and the same settings as the ML search except that genthreshfortopoterm = 1 000 000.

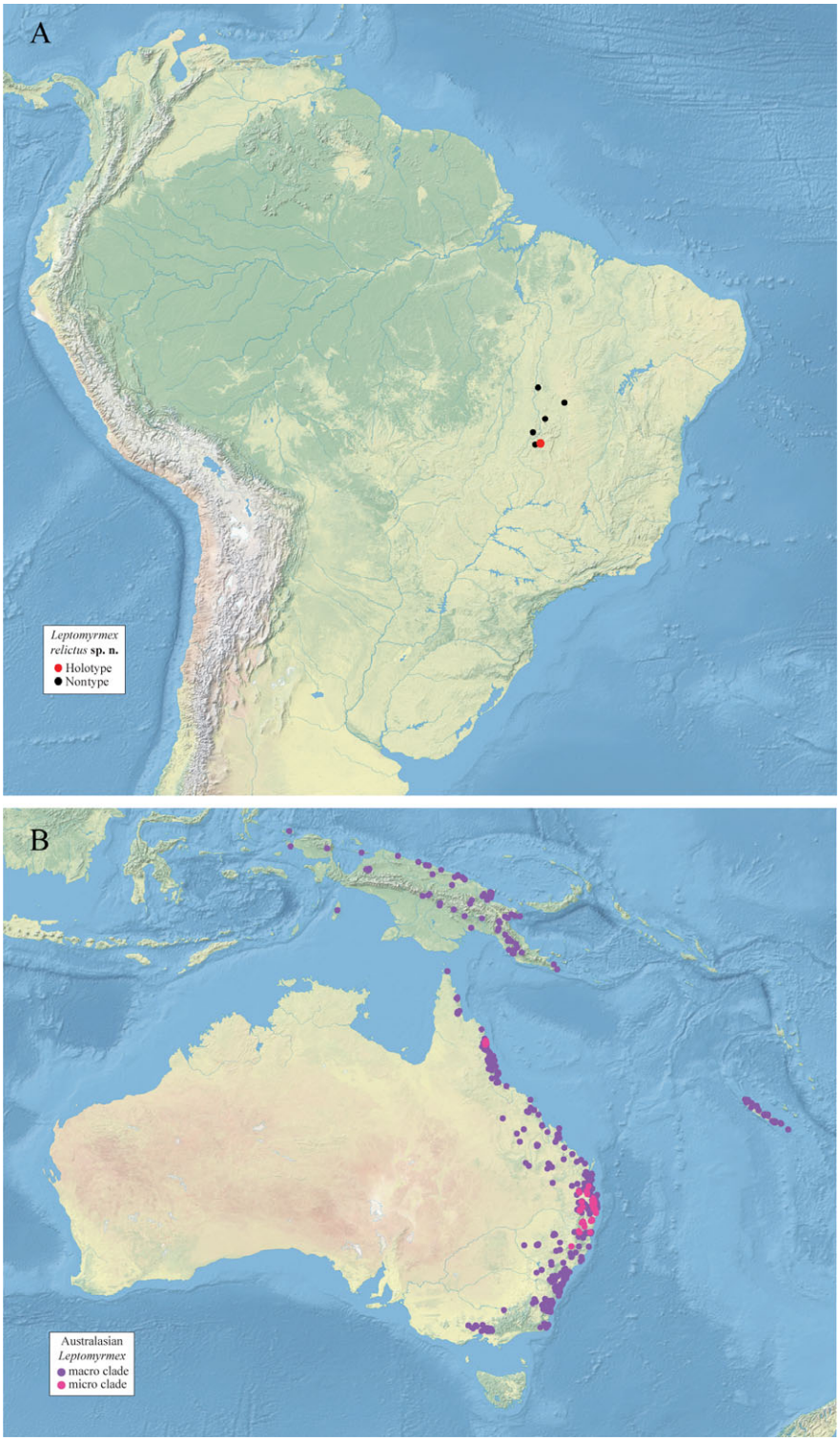

Fig. 2. Distribution maps of extant *Leptomyrmex*. A, South America, part; B Australasia, part. Georeferenced specimen records: red dots, *L. relictus* **sp.n.** holotype; black dots, *L. relictus* **sp.n.** nontype collections; violet dots, macro-*Leptomyrmex* clade; pink dots, micro-*Leptomyrmex* clade. Australasian records from the AntWeb (2015) and CSIRO (2015) databases, and Lucky & Ward (2010). Basemaps ('Natural Earth 1') from Natural Earth (2015). [Colour figure available on the online version on Wiley Online Library.]

Table 1. BEAST divergence dating analysis node calibrations. Prior ranges (ages) in Ma in the following order: maximum-minimum-median. BEAST settings: mean, standard deviation, offset (except Dolichoderomorph root calibration which has no offset).

Node calibrated	Prior range	BEAST settings	Fossil	Deposit
Dolichoderus quadripunctatus group stem	72-59-42	2.8, 0.4, 42.0	*Dolichoderus* spp.	Baltic amber[a]
Liometopum–*Tapinoma* clade crown	77-59-42	2.9, 0.4, 42.0	*Liometopum*, *Tapinoma*	Baltic amber[a b]
Azteca–*Gracilidris* crown	77-44-15	3.4, 0.45, 15.0	*Azteca*, *Gracilidris* Wild & Cuezzo	Dominican amber[c, d]
Technomyrmex stem	77-55-30	3.2, 0.4, 30.0	†*Technomyrmex deletus* Emery	Sicilian amber[e]
Leptomyrmex crown or stem	70-45-15	3.4, 0.4, 15.0	†*L. neotropicus*	Dominican amber[f]
Tapinomini crown	88-72-55	2.8, 0.4, 55.0	*Technomyrmex*, *Tapinoma*	Canadian Hat Creek amber[g]
Dolichoderinae stem	110-95-78	2.5, 0.6, 78.0	†*Chronomyrmex*	Canadian Grassy Lake amber[h]
Dolichoderomorph root	120-100-90	103, 7.0	–	–
Myrmeciomorph crown	110-90-52	3.6, 0.3, 52.0	*Tetraponera* (Smith, F.), †*Ypresiomyrma* Archibald, Cover & Moreau	Oise amber[i], Mo-Clay[j]

[a]Dlussky & Perkovsky (2002).
[b]Dlussky (1997).
[c]Wilson (1985).
[d]Wild & Cuezzo (2006).
[e]Emery (1891).
[f]Baroni Urbani (1980).
[g]Ward *et al.* (2010).
[h]McKellar *et al.* (2013).
[i]Aria *et al.* (2011).
[j]Archibald *et al.* (2006).

Divergence date estimation.

BEAST v2.3.0 (Bouckaert *et al.*, 2014) was used for divergence dating analyses. XML files for analysis were created in BEAUti and edited manually using a text editor. Clock and tree models were linked across the 12 partitions, using default settings. The Yule birth process was applied with an uncorrelated lognormal relaxed-clock model (Drummond *et al.*, 2006). Site models were unlinked and set as in Table S2, and four categories were used for gamma distributed partitions. Node calibrations (Table 1) were modified from Ward *et al.* (2010) to be more diffuse, reflecting discovery of †*Chronomyrmex* McKellar, Glasier & Engel in Campanian Canadian amber (79 to 78 Ma; McKellar *et al.*, 2013), and uncertainty of the placement of †*Burmomyrma* Dlussky from early Cenomanian Burmese amber (99 to 98 Ma, LaPolla *et al.*, 2013; Boudinot, 2015) which had been used previously to calibrate the dolichoderomorph root node (Ward *et al.*, 2010, 2015). For this node a normal distribution was provided with soft maximum and minimum ages bracketed by previously published estimates (Brady *et al.*, 2006). The following nondated monophyly constraints were implemented following Ward *et al.* (2010) based on a reading of their XML files: (i) *Azteca* Forel, (ii) Bothriomyrmecini, (iii) *Doleromyrma* Forel – *Iridomyrmex* Mayr clade, (iv) Dolichoderinae, (v) Dolichoderini and (vi) Leptomyrmecini. †*Leptomyrmex neotropicus* was used to calibrate either the stem or crown node of *Leptomyrmex*. Two independent runs of each alternate calibration were analysed for 25 million generations, sampling trees every 25 000 and logging every 1000 generations. A data-free prior-sampling run for each treatment was conducted to evaluate whether the data informed the posteriors. After assessing mixing and convergence in TRACER v1.6 (Rambaut *et al.*, 2014) the independent runs for each treatment were combined with 15–25% burn-in in LogCombiner. Consensus chronograms were visualized and annotated with FigTree v1.4.2 (Rambaut & Drummond, 2014). To facilitate future divergence dating analyses of the Dolichoderinae, our XML, LOG and TREE files have been archived on DRYAD (http://www.datadryad.org).

Biogeographical inference.

A coarse classification of biogeographical regions was used for inferring the biogeographical history of the Dolichoderinae following Ward *et al.* (2015): Neotropical (T), Nearctic (N), Palaearctic (P), Afrotropical (E), Indomalayan (O) and Australasian (A) regions. Wallace's line was used to divide the latter two areas. The likelihood-based program LAGRANGE v20130526 (Ree *et al.*, 2005; Ree & Smith, 2008) was used to estimate ancestral areas. The post-burn-in consensus chronogram from BEAST and a matrix of distributions for all terminal taxa excluding nondolichoderomorph outgroups were used as input. Due to the coarseness of taxon sampling (53 of ∼700 dolichoderine species), some terminal taxa were coded for multiple ranges given known or inferred relationships with other species. These 'artificially polymorphic' taxa are: *Iridomyrmex sanguineus* Forel, coded A and O for the related species *I. anceps* (Roger); *Liometopum* Mayr species, coded N and P to account for related Palaearctic species; *Ochetellus* cf. *clarithorax* (Forel), coded A and O for whole distribution of genus; *Technomyrmex voeltzkowi* (Forel), coded E and T as this species is the closest sampled relative of the Neotropical *Technomyrmex gorgona* Fernández & Guerrero (P.S. Ward, unpublished data); *Tapinoma melanocephalum* (Fabricius) and MG03, coded E, O and A due to uncertain relationships with other species; and

Tapinoma sessile (Say), coded T, N and P due to uncertain relationships with other species. The maximum ancestral range size was set to three areas, and dispersal constraints were set for three periods, 99.9 to 66.6, 66.6 to 33.3 and 33.3 to 0 Ma, reflecting palaeogeographical change inferred from Scotese (2010).

Morphological analysis

Specimens of fossil and extant Formicidae were examined from ten collections and from an online database of high-quality micrographs (AntWeb, 2015). Reproductives of all dolichoderine genera were examined in addition to male *Leptomyrmex* specimens of 20 of 21 macro- clade and one of five microclade species. A critical and detailed description of the new species, including morphometrics, is provided in Appendix S2, with additional details of the methods in Appendix S1.

Other taxa examined.
In order to evaluate morphological characters of *Leptomyrmex*, in addition to males and/or queens of all dolichoderine genera, the following taxa were examined (w = worker, m = male):

Macro-*Leptomyrmex* (21): *L. cnemidatus* Wheeler [w, m], *L. darlingtoni* Wheeler [w, m], *L. erythrocephalus* (Fabricius) [w, m], *L. flavitarsus* (Smith, F.) [w, m], *L. fragilis* (Smith, F.) [w, m], *L. geniculatus* Emery [w], *L. melanoticus* Wheeler [w, m], *L. mjobergi* Forel [w, m], *L. niger* Emery [w, m], *L. nigriceps* Emery [w, m], *L. nigriventris* (Guérin-Méneville) [w, m], *L. pallens* Emery [w, m], *L. puberulus* Wheeler [w, m], *L. rothneyi* Forel [w, m], *L. ruficeps* Emery[w, m], *L. rufipes* Emery [w, m], *L. rufithorax* Forel [w, m], *L. tibialis* Emery [w, m], *L. unicolor* Emery [w, m], *L. varians* Emery [w, m], *L. wiburdi* Wheeler [w, m].

Micro-*Leptomyrmex* (5): *L. aitchisoni* Smith & Shattuck [w], *L. burwelli* Smith & Shattuck [w, m], *L. dolichoscapus* Smith & Shattuck [w], *L. garretti* Smith & Shattuck [w], *L. ramorniensis* Smith & Shattuck [w].

Fossils: †*Leptomyrmex neotropicus* [5w, 1m], †*Usomyrma mirabilis* [1m paratype].

Specimen repositories and reference collections.
BEBC, Brendon E. Boudinot personal collection, Davis, CA, U.S.A.; CASC, California Academy of Sciences, San Francisco, CA, U.S.A.; DZUP, Coleção Entomológica Pe. Jesus Santiago Moure, Universidade Federal do Paraná, Curitiba, Brazil; JTLC, John T. Longino personal collection, Salt Lake City, UT, U.S.A.; LACM, Los Angeles County Museum of Natural History, Los Angeles, CA, U.S.A.; MZSP, Museu de Zoologia da Universidade de São Paulo, Brazil; PSWC, Philip S. Ward personal collection, Davis, CA, U.S.A.; UCDC, University of California, Davis, Bohart Museum of Entomology, Davis, CA, U.S.A.; UFU, Universidade Federal de Uberlândia, Brazil; ZMUC, Zoological Museum of the University of Copenhagen, Denmark.

Taxonomy and nomenclature

The new species described herein has been registered with ZooBank (LSID in Taxonomy in the Results section below).

Results

Taxonomy

***Leptomyrmex relictus* sp.n.**
http://zoobank.org/urn:lsid:zoobank.org:act:936F5440-9B94-43C0-95ED-2099FD380471
(Figs. 1–5, Figures S1, S2)

Type material. Holotype worker. BRAZIL, Goiás: Niquelândia, Serra da Mesa, 14°20′39.5″S, 48°10′21.1″W, 16.xi.2013, cerrado, inside nest of *Cornitermes silvestrii* (*D. E. Oliveira, R. G. Santos & T. Carrijo*) (sample code DEO2013-030; specimen code CASENT0106501) [MZSP].

Paratype workers (w) *and males* (m). Same data and colony as holotype; sample code R06 [1 w MZSP, 1 w (CASENT0730400) 1 m UCDC]; sample code TC08 [1 w DZUP, 1 w MZSP]; sample code DEO2013-030 [1 w LACM, 1 m MZSP].

Additional material examined (nontype): 12 w, 6 m, 3 larvae, and 2 pupae from 6 localities in Goiás and Tocantins, Brazil (Fig. 2).

Diagnosis. With diagnostic characters of genus from Shattuck (1992), notably with medial hypostomal notch (synapomorphy of genus) and lacking pterostigmal appendage in male (synapomorphy of macro-*Leptomyrmex*). Distinguished from all *Leptomyrmex* by the following characters: (i) short, thick, erect setae present on the head capsule, mesosoma (legs included) and metasoma (Figs 1A, 3A, B); (ii) clypeal setae comparatively more numerous (Figs 1E, 3B); (iii) anterior clypeal margin strongly convex (Figs 1E, 3B); (iv) lateral hypostoma weakly flanged; (v) worker with convexity subtending subapical mandibular tooth (Fig. 1E); (vi) worker petiole lateromedially narrow; (vii) worker gaster strongly lateromedially compressed; (viii) male scape elongate (SI 1.26–1.40 versus 0.19–1.05 in other *Leptomyrmex*); (ix) male wing with five closed cells (costal, basal, subbasal, submarginal 1, marginal 1) (Fig. 5E); (x) male petiole dorsal margin concave (Fig. 3A); (xi) male petiolar spiracles situated on tubercles (Fig. 3A); (xii) several features of male genitalia (Fig. 3C–K; see Appendix S2 for description). Characters 1–5, 9–11 and those of 12 are unique in the genus.

Biology. Although males of *L. relictus* have been collected as far back as 1956 – remaining unidentified for over half a century – workers were only recently discovered in 2013 from nest samples of *Cornitermes silvestrii* Emerson and in 2012 from epigaeic and arboreal pitfall traps in typical cerrado (Brazilian savannah) habitat. It is unknown whether workers are nocturnal or diurnal, but hypothetical nocturnal habits and/or their *Camponotus*-like habitus may account for the low

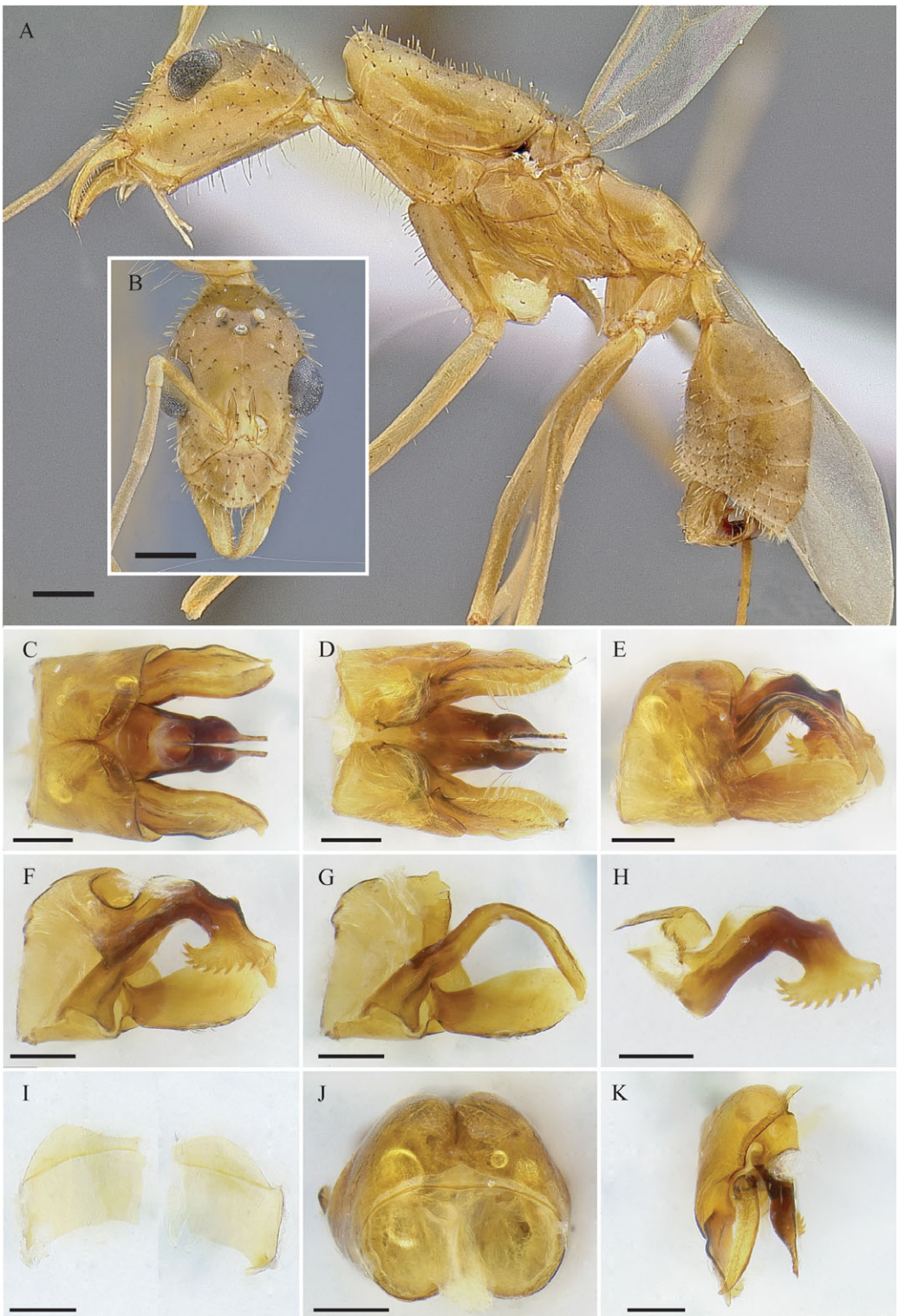

Fig. 3. *Leptomyrmex relictus* **sp.n.** male; head and body A, B, genitalia C–K. A, body, profile view; B, head, full-face (dorsal) view; C, genital capsule, dorsal view; D, genital capsule, ventral view; E, genital capsule, lateral view; F, right half of genital capsule, mesal (medial) view; G, right paramere and attached volsella, mesal view; H, left penisvalva with fragment of basimere attached to sclerotized penisvalvar membrane, ectal (lateral) view; I, abdominal sternum IX (note: broken along sagittal plane), mesal (dorsal) view; J, genital capsule, viewed anterior to posterior; K, left half of genital capsule, viewed posterior to anterior. Scale bars: A, B = 0.5 mm, C–K = 0.2 mm.

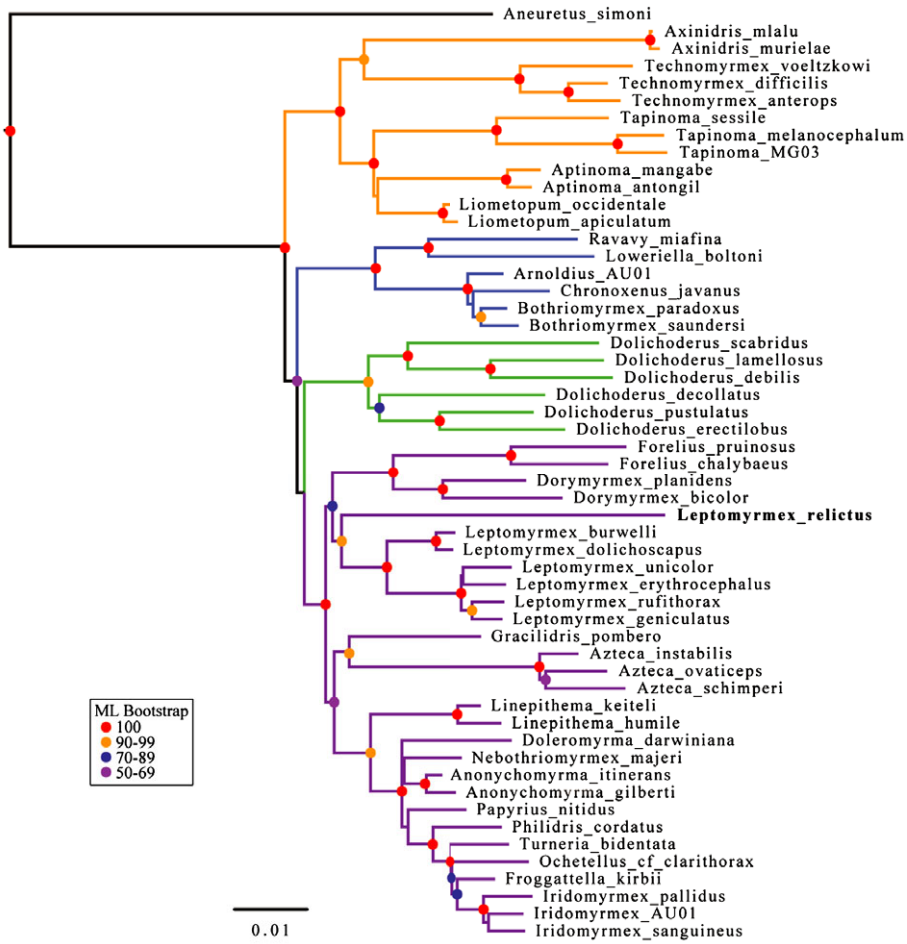

Fig. 4. Maximum-likelihood (ML) phylogeny of the Dolichoderinae, based on partitioned analysis of a 59-taxon 10-gene data matrix (−lnL = −53297.7). Tribes indicated by branch colour: orange, Tapinomini; blue, Bothriomyrmecini; green, Dolichoderini; purple, Leptomyrmecini. Support values (ML bootstrap percentages) indicated at nodes with coloured dots: red = 100, orange = 90–99, blue = 70–89, purple = 50–69, no dot = <50. [Colour figure available on the online version on Wiley Online Library.]

encounter rate of this species. It is interesting to note that at least three of the specimens were captured in arboreal pitfall traps, suggesting that workers may forage both on the soil and in the vegetation strata. The collection of workers (Fig. 1A, E) and brood (Figure S1) inside termitaria suggests that *L. relictus* may live in association with *Cornitermes silvestrii.* One termite nest measuring 1.30 m wide and about 40–50 cm tall inhabited by *L. relictus* was found in northeast Goiás state under a tree, and was also occupied by another ant species, *Camponotus blandus* (Smith, F.), which is known to be a facultative termite inquiline (Gallego-Ropero & Feitosa, 2014). The other termite nest with *L. relictus* specimens was found in southeast Tocantins state, along a trail edge. Unfortunately, no data are available on how the *Leptomyrmex* occupy the termitaria or if they were sharing galleries with the termites. A potential association with *C. silvestrii* is compelling as the termitaria of *C. silvestrii* are large, environmentally-stable clay mounds and may house several other ant and termite species (see Appendix S2). In addition to describing and comparing the sociometric and sociogenic patterns (Tschinkel, 1991, 2011) of *L. relictus* with the Australian species, future studies should test the association of the species with *C. silvestrii*.

Phylogeny. Phylogenetic analyses of the 10-gene dolichoderine data matrix yielded results congruent with those of Ward *et al.* (2010) (Fig. 4). In particular, we consistently recovered all four dolichoderine tribes, with Tapinomini as sister to all other Dolichoderinae. *Leptomyrmex relictus* is positioned as sister to the six Australian *Leptomyrmex* with strong support: Bayesian posterior probability (PP) of 0.96, and ML bootstrap support (MLBS) of 93%. There is maximum support (PP 1.00, MLBS 100%) for the Australian *Leptomyrmex* and for the micro- and macro- clades within it. Our results differ from those of Lucky (2011) for the internal topology of *Leptomyrmex* in that, with our sampling, *L. unicolor* is sister to *L. erythrocephalus*, rather than *L. geniculatus* + *L. rufithorax*. A sister-group relationship between *Leptomyrmex* and the (*Forelius* Emery + *Dorymyrmex* Mayr) clade is also sustained (PP 1.00, MLBS 74%). Finally

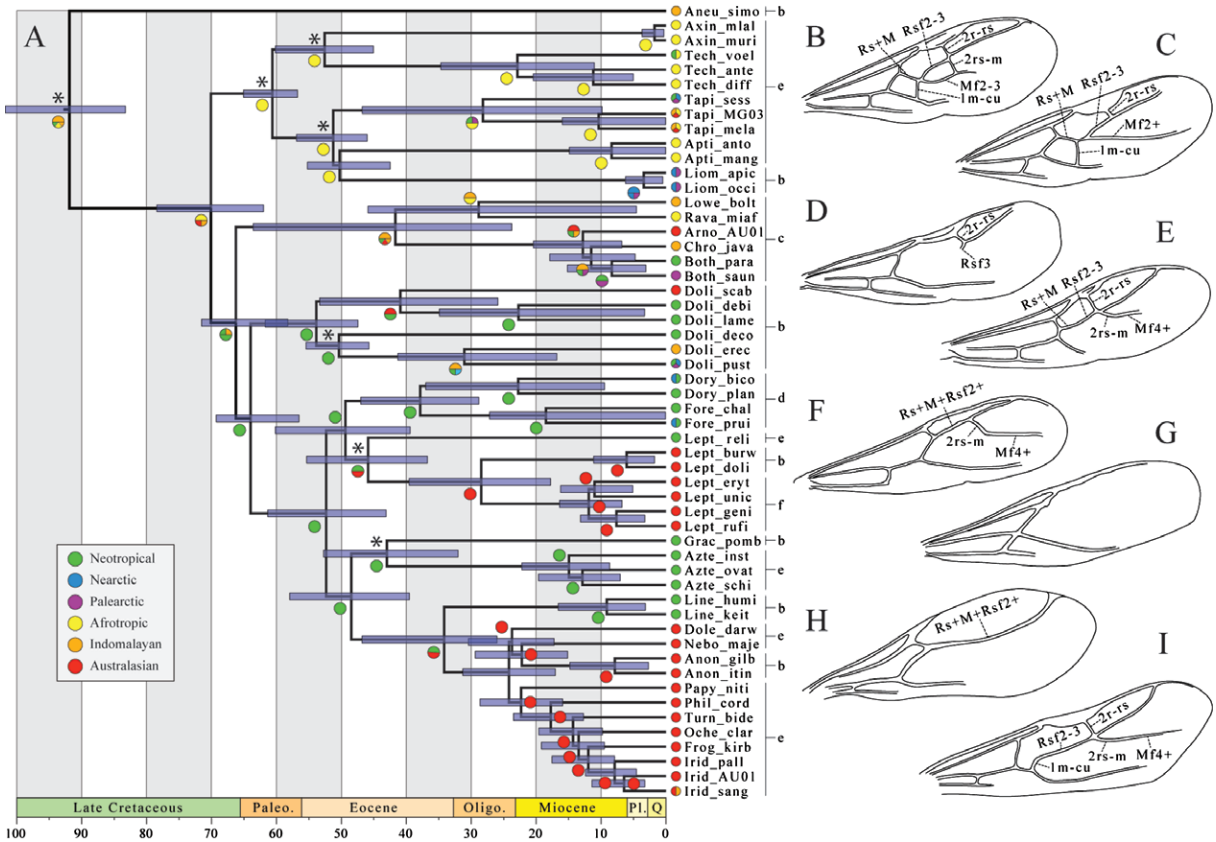

Fig. 5. Results of BEAST and LAGRANGE analyses, and comparative morphology of dolichoderine wing venation. A, Chronogram of the Dolichoderinae, *Leptomyrmex relictus* **sp.n.** bolded; B, *L. burwelli*: complete (unreduced) wing venation; C, *Chronoxenus javanus*: loss of crossvein 2rs-m with retention of other veins; D, *Dorymyrmex insanus* (male): loss of Rsf2-3 in addition to loss of other abscissae; E, *L. relictus* **sp.n.**: loss of Mf2-3; F, *L. fragilis*: loss of Mf2-3, 2r-rs (note: pterostigmal appendage not illustrated); G, †*L. neotropicus*: loss of Rs + M, Mf2-3, 2rs-m, Mf4+, 2r-rs; H, †*Leptomyrmula maravignae*: loss of Mf2+, 2rs-m, 2r-rs; I, †*Usomyrma mirabilis*: loss of Rs + M with retention of 1m-cu and loss of Mf2-3. In part A blue bars are 95% confidence intervals; coloured circles near nodes are inferred inherited ranges, with the top half corresponding to the upper branch and bottom half with the lower branch; coloured circles next to terminals represent biogeographical coding; asterisks indicate calibrated nodes; bracketed letters to the right of terminal names indicate wing venation reduction pattern occurrence, with letters corresponding to the wing patterns B–F. Note that 1m-cu presence is variable for most taxa displaying loss pattern E, and some *Linepithema* species have lost Mf2-3. Figures H and I redrawn from Emery (1891) and Dlussky *et al.* (2014), respectively. Palaeo., Palaeocene; Oligo., Oligocene; Pl., Pliocene; Q, Quaternary.

this larger clade of (*Leptomyrmex* + (*Forelius* + *Dorymyrmex*)) is sister to all other Leptomyrmecini, as in Ward *et al.* (2010). Notably, *L. relictus* is a long-branched taxon; because its divergence from the most recent common ancestor of the crown *Leptomyrmex*, it is apparent that *L. relictus* has undergone considerable morphological (especially genitalic) and molecular evolution.

Divergence dating. Two alternate analyses were run treating †*Chronomyrmex* as a stem dolichoderine, reflecting uncertainty of stem or crown placement of †*L. neotropicus* with respect to extant *Leptomyrmex*. Dating results obtained were robust to alternate placements of †*L. neotropicus*. The chronogram from the stem *Leptomyrmex* treatment is presented in Fig. 5. Mean ages between the two treatments for the clades listed in Table S3 differed by an average of 0.21 Ma, and 95% highest probability densities (hpd) differed by an average of 0.26 Ma at the older and 0.53 Ma at the younger ends, respectively. These findings are in accord with results of Ward *et al.* (2010) and Lucky (2011) (Table S3), although less so with the latter which incorporated fewer outgroups and loci. Only the dolichoderomorph crown age differed notably from Ward *et al.* (2010), being 11 Ma younger. This difference is attributable to the modified dolichoderomorph root node calibration used herein which is a diffuse normal distribution, not restricting the minimum age of the node to 100 Ma with the unplaceable yet putative aneuretine fossil †*Burmomyrma*.

Biogeographical history. The LAGRANGE analysis was run with a single outgroup taxon (*Aneuretus simoni* Emery) (Fig. 5), and all results are presented in Table S4. A Neotropical origin was recovered with high support (relative probabilities > 0.80) for the Leptomyrmecini, the *Leptomyrmex* + (*Dorymyrmex* + *Forelius*) clade and *Leptomyrmex*

(Fig. 5A). The crown ages of *Leptomyrmex* and the Australasian clade are estimated to be 46 Ma (95% hpd 53–37 Ma) and 28 Ma (95% hpd 40 to 18 Ma), respectively (Table S3, calibration 2). We therefore infer dispersal to Australia between those two time periods. This range expansion probably happened before the breakup of the Antarctic land bridge, estimated to have occurred about 35 Ma for Australia and Antarctica, and about 30 to 28 Ma for Antarctica and South America (Lawver *et al.*, 1992; Sanmartín & Ronquist, 2004). Another notable result is an inferred Neotropical origin for the Dolichoderini (comprising solely *Dolichoderus* Lund) (Fig. 5A). Only four nodes had relative probabilities <0.33 for inferred ancestral ranges: the crown and root Dolichoderinae, the BDL (Bothriomyrmecini + Dolichoderini + Leptomyrmecini) clade, and the Bothriomyrmecini. All other relative probabilities were >0.66, except the *Liometopum* + *Aptinoma* Fisher + *Tapinoma* Foerster clade, which had a relative probability between 0.33 and 0.66. Further sampling within the Bothriomyrmecini is expected to increase the relative probabilities of inferred ancestral ranges for the tribe as well as the BDL clade.

Results and evaluation of fossil record. Dlussky *et al.* (2014) claimed that †*Usomyrma*, †*Leptomyrmula* and *Leptomyrmex* form a natural group based on the putatively synapomorphic 'reduction of the central vein sections and cells.' This is refuted by the retention of complete wing venation in the species of micro-*Leptomyrmex*, which is nested well within the genus. Moreover, the venation reduction patterns of †*Usomyrma*, *Dorymyrmex* + *Forelius* and *Leptomyrmex* are not homologous. †*Usomyrma* has lost Rs + M and Mf2-3; the former is autapomorphic and the latter has occurred at least five times in the Dolichoderinae (Fig. 5). At least two reductions have occurred in *Leptomyrmex* (Fig. 5) as the micro- clade retains complete wing venation, whereas *L. relictus*, the macro-*Leptomyrmex* and †*L. neotropicus* have differing reduction patterns. *Leptomyrmex relictus* has lost 1 m-cu and Mf2-3, †*L. neotropicus* has lost Rsf2+, Mf2 + m, 2rs-m, 1m-cu and 2r-rs, whereas macro-*Leptomyrmex* have lost Mf2-3, 2r-rs (variable) and 1m-cu, and retained Rs + M, Rsf2+ and Mf4+. Other characters claimed by Dlussky *et al.* (2014) to support a relationship between †*Usomyrma* and †*Leptomyrmex* are, by their explicit statement, either plesiomorphic or autapomorphic; these are thus uninformative of relationships by definition. Furthermore, based on examination of a paratype male, †*Usomyrma* lacks a newly discovered genitalic synapomorphy of the Leptomyrmecini: a dorsal sclerotic bridge between the penisvalvar bases. The sclerotic bridge is anteroposteriorly broad and fused with the basimeres in *L. relictus* (Fig. 2C, F), and is narrowed anteroposteriorly in the Australasian clade. The strap- or thread-like condition of the bridge in the Australasian clade is a synapomorphy, whereas fusion of the bridge to the basimeres in *L. relictus* is autapomorphic.

Regarding †*Leptomyrmula* specifically, the only known specimen may be lost (G.M. Bargossi, University of Bologna, Italy, personal communication), but it has fortunately been illustrated (Emery, 1891) (reproduced in Fig. 5H). The pattern of vein abscissa loss is most similar between †*Leptomyrmula* and the macro- clade (Fig. 5F), with the venation being clearly different from the micro-*Leptomyrmex* (Fig. 5A) and *L. relictus* (Fig. 5E), which have complete or relatively complete wing venation, respectively. Homology of the loss pattern between †*Leptomyrmula* and the macro-*Leptomyrmex* is unlikely, however, given the distinctions of the venational patterns. Forewing characters of †*Leptomyrmula* differ as follows from the macro-*Leptomyrmex* clade: costal cell broad and short (vs. absent, with secondary sclerotization); free R lacking along the anterior wing margin (vs. continuing almost to wing apex); pterostigmal appendage absent (vs. present); 2rs-m + Mf4+ absent (vs. usually present); basal cell narrow and Mf1 subparallel to Sc + R + Rs (vs. broad, Mf1 and Sc + R + Rs clearly converging); free Cu short and diverging from M + Cu in the basal third of the wing (vs. about midlength); and anal vein elongated after crossvein cu-a (vs. anal vein strongly reduced or absent). Moreover, the somatic morphology of †*Leptomyrmula* is considerably divergent, including the following differences: head capsule dorsoventrally compressed; compound eyes small and anteriorly-situated; mandibles small; flagellomeres short; pronotum dorsoventrally tall; mesonotum flat; and limbs short (metatibia not exceeding metasomal apex). Neither †*Leptomyrmula* nor †*Usomyrma* have the key macro-*Leptomyrmex* clade forewing synapomorphies of the pterostigmal appendage (Lucky & Ward, 2010) and the absent costal vein with secondary sclerotization. We therefore consider †*Leptomyrmula* and †*Usomyrma incertae sedis* in the Dolichoderinae. The placement of †*Leptomyrmula* in the Dolichoderinae itself could be questioned because the genitalia, as illustrated in Emery (1891), do not conclusively demonstrate a synapomorphic condition of dolichoderine genitalia: enlarged basimere and reduced telomere (Yoshimura & Fisher, 2011; Boudinot, 2015).

Study of worker and male †*L. neotropicus* specimens revealed presence of the hypostomal notch, a synapomorphy of the genus almost completely unique in the Dolichoderinae, except for the enigmatic leptomyrmecine *Anillidris* Santschi (B.E. Boudinot, personal observation), confirming placement of the fossil within the genus. Presence of the notch could not be evaluated in either †*Leptomyrmula* or †*Usomyrma*; the ventral head surface of the paratype †*Usomyrma* examined is obscured by a fracture within the amber. It is plausible that †*L. neotropicus* is more closely related to *L. relictus* than to the Australasian clade based on genitalic characters, although the placement of the Dominican amber fossil should be tested in a 'total-evidence' framework (Ronquist *et al.*, 2012b).

Discussion

The argument for a non-Neotropical origin for *Leptomyrmex* was predicated on the century-old belief that the male-based †*Leptomyrmula,* from Sicilian amber, is a direct ancestor (Emery, 1913; Wheeler, 1915, 1934) or stem lineage (Baroni Urbani, 1980) of the genus; the recent discovery of †*Usomyrma* in Danish amber was adduced as further support for this scenario (Dlussky *et al.*, 2014). These claims are based primarily on the interpretation of wing venation – a generally neglected

character set in ants. We conclude, given our evolutionary morphological study of dolichoderine and *Leptomyrmex* wing venation, that the observed patterns of vein reduction in †*Usomyrma* are not homologous with the reductions which occurred in *Leptomyrmex*, and that aspects of the particular pattern are prone to homoplasy (Fig. 5). Thus, this central support for a relationship of †*Usomyrma* with *Leptomyrmex* dissolves, leaving the fossil unplaced in the subfamily. The venation of †*Leptomyrmula* is more similar to that of *Leptomyrmex* due to a relatively higher degree of modification. We find that this similarity is only superficial, however, because the exact pattern is not equivalent to that seen in any of the *Leptomyrmex* clades. Furthermore, a close relationship with the macro-*Leptomyrmex* clade is rejected based on several divergent traits of †*Leptomyrmula* and due to the absence of key macro-*Leptomyrmex* synapomorphies. Of the fossil taxa assumed to be allied with *Leptomyrmex*, we are left with †*L. neotropicus*, which we confirm as a member of the *Leptomyrmex* clade.

With the West Palaearctic fossils determined to be unrelated to *Leptomyrmex*, our LAGRANGE analysis unequivocally supports a Neotropical origin for *Leptomyrmex*, rejecting the scenario of origination in the West Palaearctic or Asia. Not only is the Neotropical *L. relictus* sister to the Australasian clade, but the sister group of *Leptomyrmex* is the Neotropical (*Forelius* + *Dorymyrmex*) clade, and these taxa, in turn, are sister to other Neotropical leptomyrmecines. If *Leptomyrmex* or stem *Leptomyrmex* were present in the West Palaearctic, such a distribution could only be explained by either: (i) dispersal to Australia, then through the Indomalayan region to Europe, or (ii) long-distance dispersal across the Atlantic from the Americas. There is no evidence for either scenario; the claim of a *Leptomyrmex* in Indian amber (Rust *et al.*, 2010) is unsubstantiated and is almost certainly a misidentification, whereas trans-Atlantic dispersal is especially unlikely. Additionally, the current distribution of Australasian clade species does not support these hypotheses, because the greatest diversity is in the east-central region of Australia, with just a few species of a particular clade (Lucky, 2011) on New Guinea and nearby islands (Fig. 2). Thus, the distribution of *Leptomyrmex* – given a Neotropical origin for the genus – is best explained by a single mid-Tertiary dispersal event from South America to southeastern Australia.

In addition to *Leptomyrmex*, seven other ant clades with apparent trans-Antarctic dispersals have been studied: *Heteroponera* Mayr (R.M. Feitosa, unpublished data); a subclade of the Amblyoponinae (Ward & Fisher, 2016); the tribe Melophorini (Blaimer *et al.*, 2015); the '*Chelaner* group' of *Monomorium* Mayr, the *Daceton* genus group and the *Rhopalothrix* genus group (Ward *et al.*, 2015); and a large and successful radiation of Australian dolichoderine ants which are sister to the Neotropical genus *Linepithema* Mayr (Ward *et al.*, 2010; Fig. 5A). For all but *Heteroponera*, the splits between these South American and Australian lineages have been dated, and fall in the range of 50 to 25 Ma. Moreover, in seven of the eight studied ant taxa – all except the Melophorini – the Australian species form a clade that is embedded within a paraphyletic assemblage of Neotropical taxa, suggesting a biased E–W direction of movement, similar to that observed for plants (Sanmartín & Ronquist, 2004). This contrasts with the pattern for animals in general, which have had comparable frequencies of E–W and W–E dispersal events (Sanmartín & Ronquist, 2004). Two alternative hypotheses may then explain the E–W dispersal bias for ants: (i) passive dispersal via rafting of colonies or foundresses on plant matter along the warm, westward-bound South Pacific Equatorial current, or (ii) historically unequal pools of ant species capable of dispersal between Australia and South America.

Rafting is supported circumstantially by the tropical-mesic ecological requirements of most of the austral ant clades considered. Warm tropical rainforests did not extend to Antarctica even during the Eocene climatic optimum (Segovia & Armesto, 2015), thus any geodispersing austral species must have been able to tolerate cooler environments. The only W–E dispersal observed here, *Lasiophanes*, fits this profile, as the genus is restricted to temperate South America. Geodispersal cannot be rejected outright for *Leptomyrmex*, however, given the ecological flexibility evidenced by *L. relictus*. This species is found in the cerrado in the dry diagonal of Brazil, a transition which presumably took place after the grasses rose to prominence in South America <18.5 Ma (Strömberg *et al.*, 2013). It should also be noted that the Australasian *Leptomyrmex* are not restricted to tropical regions either. Although most species occur in the subtropical–temperate transition zone, a few species do occur in the temperate southeast of Australia. Furthermore, over-land dispersal with subsequent Antarctic extinction is roughly corroborated by similar minimum estimated ages for trans-Antarctic interchange of other arthropod groups (~30 Ma; e.g. chironomid midges, Krosch *et al.* 2011; collectid bees, Almeida *et al.* 2012; euophryine spiders, Zhang & Maddison, 2013; window flies, Winterton & Ware, 2015; and various stoneflies, McCulloch *et al.*, 2016).

Overall, our findings highlight the value of integrated taxonomy, critical evolutionary interpretation of morphology, and a comparative phylogenetic framework when conducting palaeontological and biogeographical studies of insect species. Further study of the biology *L. relictus* may clarify the historical ecology of the genus, notably as the only colonies of this species are known from the shelter of termitaria. In general, relictual species provide insight into evolutionary and historical processes related to past changes in species' distributions (Hewitt, 2000) and evolutionary transitions (e.g. *Tricholepidion* Wygodzinski; Blanke *et al.*, 2014; Misof *et al.*, 2014), because these species often represent isolated and early branching lineages of particular clades (Pie & Feitosa, 2016). In this context, comparative biological studies of *L. relictus* and species of the micro- and macro- clades of *Leptomyrmex* will be illuminating. Moreover, relictual species are important for comparative ecological and evolutionary studies (Lesica & Allendorf, 1995; Grandcolas *et al.*, 2014) and are valuable for improving estimates of extinction rates (Nee *et al.*, 1994). The discovery of *L. relictus* in Brazilian cerrado provides a concrete example for the conservation of this underappreciated and threatened habitat which is undergoing a major extinction crisis (Klink & Machado, 2005).

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/syen.12181

Appendix S1. Supplementary materials and methods.

Appendix S2. Supplementary results and discussion.

Figure S1. *Leptomyrmex relictus* **sp.n.** young larva and pupa. A, Larva, lateral view; B, larva head, profile view; C, ventral larva spinules; D, larval prothoracic seta; E, larval anal aperture; F, pupa, lateral view. Scale bars: A = 0.50 mm, B, E = 0.10 mm, C (inset) and D = 0.01 mm, F = 2 mm.

Figure S2. *Leptomyrmex relictus* **sp.n.** hypostoma and †*Usomyrma mirabilis* genitalia. A, Head, ventrolateral view; B, head, ventral view; C, genitalia, dorsolateral oblique view. Scale bars = 0.25 mm.

Table S1. Sequence characteristics for individual loci and GenBank accession numbers.

Table S2. Partitions and evolutionary models for phylogenetic analysis.

Table S3. Divergence dating results and comparison to prior studies.

Table S4. Results of LAGRANGE biogeographical reconstruction analysis.

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References

Almeida, E.A.B., Pie, M.R., Brady, S.G. & Danforth, B.N. (2012) Biogeography and diversification of colletid bees (Hymenoptera: Colletidae): emerging patterns from the southern end of the world. *Journal of Biogeography*, **39**, 526–544.

AntWeb (2015) *AntWeb v5.34.3* [WWW document]. URL http://www.antweb.org/ [accessed on 15 September 2015].

Archibald, S.B., Cover, S.P. & Moreau, C.S. (2006) Bulldog ants of the Eocene Okanagan Highlands and history of the subfamily (Hymenoptera: Formicidae: Myrmeciinae). *Annals of the Entomological Society of America*, **99**, 487–523.

Aria, C., Perrichot, V. & Nel, A. (2011) Fossil Ponerinae (Hymenoptera: Formicidae) in Early Eocene amber of France. *Zootaxa*, **2870**, 53–62.

Baroni Urbani, C. (1980) The first fossil species of the Australian ant genus *Leptomyrmex* in amber from the Dominican Republic. *Stuttgarter Beiträge zur Naturkunde, Serie B*, **62**, 1–10.

Blaimer, B.B., Brady, S.G., Schultz, T.R., Lloyd, M.W., Fisher, B.L. & Ward, P.S. (2015) Phylogenomic methods outperform traditional multi-locus approaches in resolving deep evolutionary history: a case study of formicine ants. *BMC Evolutionary Biology*, **15**, 271.

Blanke, A., Kock, M., Wipfler, B., Wilde, F. & Misof, B. (2014) Head morphology of *Tricholepidion gertschi* indicates monophyletic Zygentoma. *Frontiers in Zoology*, **11**, 16.

Borowiec, M.L. (2016) AMAS: a fast tool for large alignment manipulation and computing of summary statistics. *PeerJ*, **4**, e1660. DOI: 10.7717/peerj.1660.

Bouckaert, R., Heled, J., Kühnert, D. *et al.* (2014) BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, **10**, e1003537. DOI: 10.1371/journal.pcbi.1003537.

Boudinot, B.E. (2015) Contributions to the knowledge of Formicidae (Hymenoptera, Aculeata): a new diagnosis of the family, the first global male-based key to subfamilies, and a treatment of early branching lineages. *European Journal of Taxonomy*, **120**, 1–62.

Brady, S.G., Schultz, T.R., Fisher, B.L. & Ward, P.S. (2006) Evaluating alternative hypotheses for the early evolution and diversification of ants. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 18172–18177.

CSIRO (2015) *Australian National Insect Collection* [WWW document]. URL http://www.csiro.au/en/Research/Collections/ANIC/ [accessed on 15 September 2015].

Dlussky, G.M. (1997) [Genera of ants (Hymenoptera: Formicidae) from Baltic amber.] *Paleontologicheskii Zhurnal*, **1997**, 50–62 (in Russian).

Dlussky, G.M. & Perkovsky, E.E. (2002) [Ants (Hymenoptera, Formicidae) from the Rovno Amber.] *Vestnik Zoologii*, **36**, 3–20 (in Russian).

Dlussky, G., Radchenko, A. & Dubovikoff, D. (2014) A new enigmatic ant genus from late Eocene Danish amber and its evolutionary and zoogeographic significance. *Acta Palaeontologica Polonica*, **59**, 931–939.

Drummond, A.J., Ho, S.Y.W., Phillips, M.J. & Rambaut, A. (2006) Relaxed phylogenetics and dating with confidence. *PLoS Biology*, **4**, e88.

Emery, C. (1891) Le formiche dell'ambra siciliana nel museo mineralogico dell'Università di Bologna. *Memorie della Reale Accademia delle Scienze dell'Istituto di Bologna*, **1**, 141–165.

Emery, C. (1913) Hymenoptera. Fam. Formicidae. Subfam. Dolichoderinae. *Genera Insectorum*, **137**, 1–50.

Gallego-Ropero, M.C. & Feitosa, R.M. (2014) Evidences of Batesian mimicry and parabiosis in ants of the Brazilian savanna. *Sociobiology*, **61**, 281–285.

Grandcolas, P., Nattier, R. & Trewick, S. (2014) Relict species: a relict concept? *Trends in Ecology and Evolution*, **29**, 655–663.

Hewitt, G. (2000) The genetic legacy of the Quaternary ice ages. *Nature*, **405**, 907–913.

Iturralde-Vinent, M.A. (2001) Geology of the amber-bearing deposits of the Greater Antilles. *Caribbean Journal of Science*, **37**, 141–167.

Key, J.H.L. (1970) Phasmatodea. *The Insects of Australia* (ed. by CSIRO), pp. 348–359. Melbourne University Press, Melbourne.

Klink, C.A. & Machado, R.B. (2005) Conservation of the Brazilian cerrado. *Conservation Biology*, **19**, 707–713.

Krosch, M.N., Baker, A.M., Mather, P.B. & Cranston, P.S. (2011) Systematics and biogeography of the Gondwanan Orthocladiinae (Diptera: Chironomidae). *Molecular Phylogenetics and Evolution*, **59**, 458–468.

Lanfear, R., Calcott, B., Ho, S.Y.W. & Guindon, S. (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution*, **29**, 1695–1701.

LaPolla, J.S., Dlussky, G.M. & Perrichot, V. (2013) Ants and the fossil record. *Annual Review of Entomology*, **58**, 609–630.

Lawver, L.A., Gahagan, L.M. & Coffin, M.F. (1992) The development of paleoseaways around Antarctica. *The Antarctic Paleoenvironment: A Perspective on Global Change: Part One* (ed. by J.P. Kennett and D.A. Warkne), pp. 7–30. American Geophysical Union, Washington, D.C.

Lesica, P. & Allendorf, F.W. (1995) When are peripheral populations valuable for conservation? *Conservation Biology*, **9**, 753–760.

Lucky, A. (2011) Molecular phylogeny and biogeography of the spider ants, genus *Leptomyrmex* Mayr (Hymenoptera: Formicidae). *Molecular Phylogenetics and Evolution*, **59**, 281–292.

Lucky, A. & Ward, P.S. (2010) Taxonomic revision of the ant genus *Leptomyrmex* Mayr (Hymenoptera: Formicidae). *Zootaxa*, **2688**, 1–67.

Maddison, D.R. & Maddison, W.P. (2000) *MacClade 4. Analysis of Phylogeny and Character Evolution*. Sinauer Associates, Sunderland, Massachusetts.

McCulloch, G.A., Wallis, G.P. & Waters, J.M. (2016) A time-calibrated phylogeny of southern hemisphere stoneflies: Testing for Gondwanan origins. *Molecular Phylogenetics and Evolution*, **96**, 150–160.

McKellar, R.C., Glasier, J.R.N. & Engel, M.S. (2013) New ants (Hymenoptera: Formicidae: Dolichoderinae) from Canadian Late Cretaceous amber. *Bulletin of Geosciences*, **88**, 583–594.

Miller, M., Schwartz, T. & Pfeiffer, W. (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshops* (*GCE*). New Orleans, Louisiana, 14 November 2010, pp. 1–8.

Misof, B., Liu, S., Meusemann, K. *et al.* (2014) Phylogenomics resolves the timing and pattern of insect evolution. *Science*, **346**, 763–767.

Natural Earth (2015) *Natural Earth* [WWW document]. URL http://www.naturalearthdata.org [accessed on 11 December 2015].

Nee, S., Holmes, E.C., May, R.M. & Harvey, P.H. (1994) Extinction rates can be estimated from molecular phylogenies. *Philosophical Transactions of the Royal Society B*, **344**, 77–82.

Penney, D. (2010) Dominican amber. *Biodiversity of Fossils in Amber from the Major World Deposits* (ed. by D. Penney), pp. 22–41. Siri Scientific Press, Manchester.

Pie, M.R. & Feitosa, R.M. (2016) Relictual ant lineages (Hymenoptera: Formicidae) and their evolutionary implications. *Myrmecological News*, **22**, 55–58.

Ragazzi, E. & Roghi, G. (2014) La simetite, ambra della Sicilia. Simetite, the Sicilian amber. *La storia delle piante fossili in Italia*, No. 9, Chapter 9.2 (ed. by E. Kustatscher, G. Roghi, A. Bertini and A. Miola), pp. 232–237. Pubblicazione del Museo di Scienze Naturali dell'Alto Adige, Bolzano, Italy.

Rainbow, H.W. (1898) Descriptions of some new Araneidae of New South Wales. No. 8. *Proceedings of the Linnean Society of New South Wales*, **22**, 514–533.

Rambaut, A. & Drummond, A. (2014) *FigTree Version 1.4.2*. [WWW document]. URL http://beast.bio.ed.ac.uk/software/figree [accessed on 2 February 2015].

Rambaut, A., Suchard, M.A., Xie, D. & Drummond, A.J. (2014) *Tracer v1.6* [WWW document]. URL http://beast.bio.ed.ac.uk/Tracer [accessed on 2 February 2015].

Ree, R.H. & Smith, S.A. (2008) Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology*, **57**, 4–14.

Ree, R.H., Moore, B.R., Webb, C.O. & Donoghue, M.J. (2005) A likelihood framework for inferring the evolution of geographic range on phylogenetic trees. *Evolution*, **59**, 2299–2311.

Ritzkowski, S. (1997) K-Ar-Altersbestimmungen der bernsteinführenden sediment des Samlandes (Paläogen, Bezirk Kaliningrad). *Metalla (Sonderheft)*, **66**, 19–23.

Ronquist, F., Teslenko, M., van der Mark, P. *et al.* (2012a) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, **61**, 539–542.

Ronquist, F., Klopfstein, S., Vilhelmsen, L., Schulmeister, S., Murray, D.L. & Rasnitsyn, A.P. (2012b) A total-evidence approach to dating with fossils, applied to the early radiation of the hymenoptera. *Systematic Biology*, **61**, 973–999.

Rust, J., Singh, H., Rana, R.S. *et al.* (2010) Biogeographic and evolutionary implications of a diverse paleobiota in amber from the early Eocene of India. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 18360–18365.

Sanmartín, I. & Ronquist, F. (2004) Southern hemisphere biogeography inferred by event-based models: plant versus animal patterns. *Systematic Biology*, **53**, 216–243.

Scotese, C.R. (2010) *PALEOMAP Project* [WWW document]. URL http://www.scotese.com/ [accessed on 2 July 2015].

Segovia, R.A. & Armesto, J.J. (2015) The Gondwanan legacy in South American biogeography. *Journal of Biogeography*, **42**, 209–217.

Shattuck, S.O. (1992) Generic revision of the ant subfamily Dolichoderinae (Hymenoptera, Formicidae). *Sociobiology*, **21**, 1–176.

Sinclair, J.E. & New, T.R. (2004) Pine plantations in south eastern Australia support highly impoverished ant assemblages (Hymenoptera: Formicidae). *Journal of Insect Conservation*, **8**, 277–876.

Strömberg, C.A.E., Dunn, R.E., Madden, R.H., Kohn, M.J. & Carlini, A.A. (2013) Decoupling the spread of grasslands from the evolution of grazer-type herbivores in South America. *Nature Communications*, **4**, 1478. DOI: 10.1038/ncomms2508.

Tschinkel, W.R. (1991) Insect sociometry, a field in search of data. *Insect Sociaux*, **38**, 77–82.

Tschinkel, W.R. (2011) Back to basics: sociometry and sociogenesis of ant societies (Hymenoptera: Formicidae). *Myrmecological News*, **14**, 49–54.

Turner, G. (1898) Notes upon the Formicidae of Mackay, Queensland. *Proceedings of the Linnean Society of New South Wales*, **22**, 129–144.

Ward, P.S. & Downie, D.A. (2005) The ant subfamily Pseudomyrmecinae (Hymenoptera: Formicidae): phylogeny and evolution of big-eyed arboreal ants. *Systematic Entomology*, **30**, 310–335.

Ward, P.S. & Fisher, B.L. (2016) Tales of dracula ants: the evolutionary history of the ant subfamily Amblyoponinae (Hymenoptera: Formicidae). *Systematic Entomology*, DOI: 10.1111/syen.12186.

Ward, P.S., Brady, S.G., Fisher, B.L. & Schultz, T.R. (2010) Phylogeny and biogeography of dolichoderine ants: effects of data partitioning and relict taxa on historical inference. *Systematic Biology*, **59**, 342–362.

Ward, P.S., Brady, S.G., Fisher, B.L. & Schultz, T.R. (2015) The evolution of myrmicine ants: phylogeny and biogeography of a hyperdiverse ant clade (Hymenoptera: Formicidae). *Systematic Entomology*, **40**, 61–81.

Wheeler, W.M. (1910) *Ants: Their Structure, Development and Behavior*. Columbia University Press, New York.

Wheeler, W.M. (1915) Australian honey-ants of the genus *Leptomyrmex* Mayr. *Proceedings of the American Academy of Arts and Sciences*, **51**, 255–286.

Wheeler, W.M. (1934) A second revision of the ants of the genus *Leptomyrmex* Mayr. *Bulletin of the Museum of Comparative Zoology*, **77**, 69–118.

Wild, A.L. & Cuezzo, F. (2006) Rediscovery of a fossil dolichoderine ant lineage (Hymenoptera: Formicidae: Dolichoderinae) and a description of a new genus from South America. *Zootaxa*, **1142**, 57–68.

Wilson, E.O. (1985) Ants of the Dominican amber (Hymenoptera: Formicidae) 3. The subfamily Dolichoderinae. *Psyche*, **92**, 17–38.

Winterton, S.L. & Ware, J.L. (2015) Phylogeny, divergence times and biogeography of window flies (Scenopinidae) and the therevoid clade (Diptera: Asiloidea). *Systematic Entomology*, **40**, 491–519.

Yoshimura, M. & Fisher, B.L. (2011) A revision of male ants of the Malagasy region (Hymenoptera: Formicidae): key to genera of the subfamily Dolichoderinae. *Zootaxa*, **2794**, 1–34.

Zhang, J.-X. & Maddison, W.P. (2013) Molecular phylogeny, divergence times and biogeography of spiders of the subfamily Euophryinae (Araneae: Salticidae). *Molecular Phylogenetics and Evolution*, **68**, 81–92.

Zwickl, D.J. (2006) *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. PhD Thesis, The University of Texas at Austin, Austin, TX.

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