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Out of South-East Asia: phylogeny and biogeography of the spiny ant genus *Polyrhachis* Smith (Hymenoptera: Formicidae)

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Abstract. Spiny ants (*Polyrhachis* Smith) are a hyper-diverse genus of ants distributed throughout the Palaeotropics and the temperate zones of Australia. To investigate the evolution and biogeographic history of the group, we reconstructed their phylogeny and biogeography using molecular data from 209 taxa and seven genes. Our molecular data support the monophyly of *Polyrhachis* at the generic level and several of the 13 recognized subgenera, but not all are recovered as monophyletic. We found that *Campomyrma* Wheeler consists of two distinct clades that follow biogeographic affinities, that the boundaries of *Hagiomyrma* Wheeler are unclear depending on the analysis, that *Myrma* Billberg might be treated as one or two clades, and that *Myrmhopla* Forel is not monophyletic, as previously proposed. Our biogeographic ancestral range analyses suggest that the evolution of *Polyrhachis* originated in South-East Asia, with an age of the modern crown-group *Polyrhachis* of 58 Ma. Spiny ants dispersed out of South-East Asia to Australia several times, but only once to mainland Africa around 26 Ma.

Introduction

Ants are among the most ecologically important and abundant arthropods (Lach *et al.*, 2010). These social insects are especially common in tropical forest ecosystems, where they act as predators, scavengers and herbivores (Pfeiffer *et al.*, 2014). One hypothesis for their abundance in tropical ecosystems is the ability of many species to rely on plant-derived nutrition sources (Davidson *et al.*, 2003), in many cases with the help of bacteria, which permit the ants to rely on diets consisting mostly on carbohydrates (Russell *et al.*, 2009). Their ecological importance is not limited to the present day. The ants appeared in the Jurassic period around 139–158 Ma, but only truly began to diversify in the Cretaceous around 100 Ma (Brady *et al.*, 2006; Moreau *et al.*, 2006; Moreau, 2009; Moreau & Bell, 2013; Ward *et al.*, 2015); the first fossil records are 100–112 million years old (LaPolla *et al.,* 2013). According to the earliest fossil records, the evolution of the formicoid clade, which includes some of the most common and biologically diverse subfamilies such as Formicinae, began at *c*. 92 Ma (Moreau *et al.*, 2006; Hölldobler & Wilson, 2009). One of the formicine tribes, Camponotini, includes eight genera, most notably the genera *Camponotus* Mayr (carpenter ants) and *Polyrhachis* Smith (spiny ants) (Fig. 1) (Bolton, 2003, 2013). These genera are very common and widespread, as well as very conspicuous ants due to their above-ground foraging activities. *Polyrhachis* is the second most species-rich genus in this tribe, currently comprising 697 valid species (Bolton, 2015). Spiny ants have an Old World distribution, ranging from the tropical regions of Africa and Asia to Australia and a few Pacific islands, but being oddly absent from Madagascar (Dorow, 1995; Fisher, 1997; Guénard *et al.*, 2014). Their highest species richness and diversity are in Oriental and Australasian regions. *Polyrhachis* is currently divided into 13 subgenera: *Aulacomyrma* Emery, *Campomyrma* Wheeler, *Chariomyrma* Forel, *Cyrtomyrma* Forel, *Hagiomyrma* Wheeler, *Hedomyrma* Forel, *Hemioptica* Roger, *Hirtomyrma* Kohout, *Myrma* Billberg, *Myrmatopa* Forel, *Myrmhopla* Forel, *Myrmothrinax* Forel and *Polyrhachis* s.s. (Dorow, 1995; Kohout, 2010; Bolton, 2013). The taxonomy of this genus is an active area of research and is well established for many of the subgenera (Kohout, 2006, 2007a,2007b, 2008, 2010, 2012, 2013, 2014). Robson *et al.* (2015) confirmed the monophyly of *Polyrhachis* at the generic level based on molecular data and representatives from all 13 subgenera. Almost all *Polyrhachis* species are diurnal, foraging on the vegetation layer, which makes them very conspicuous and showy ants in the ecosystems

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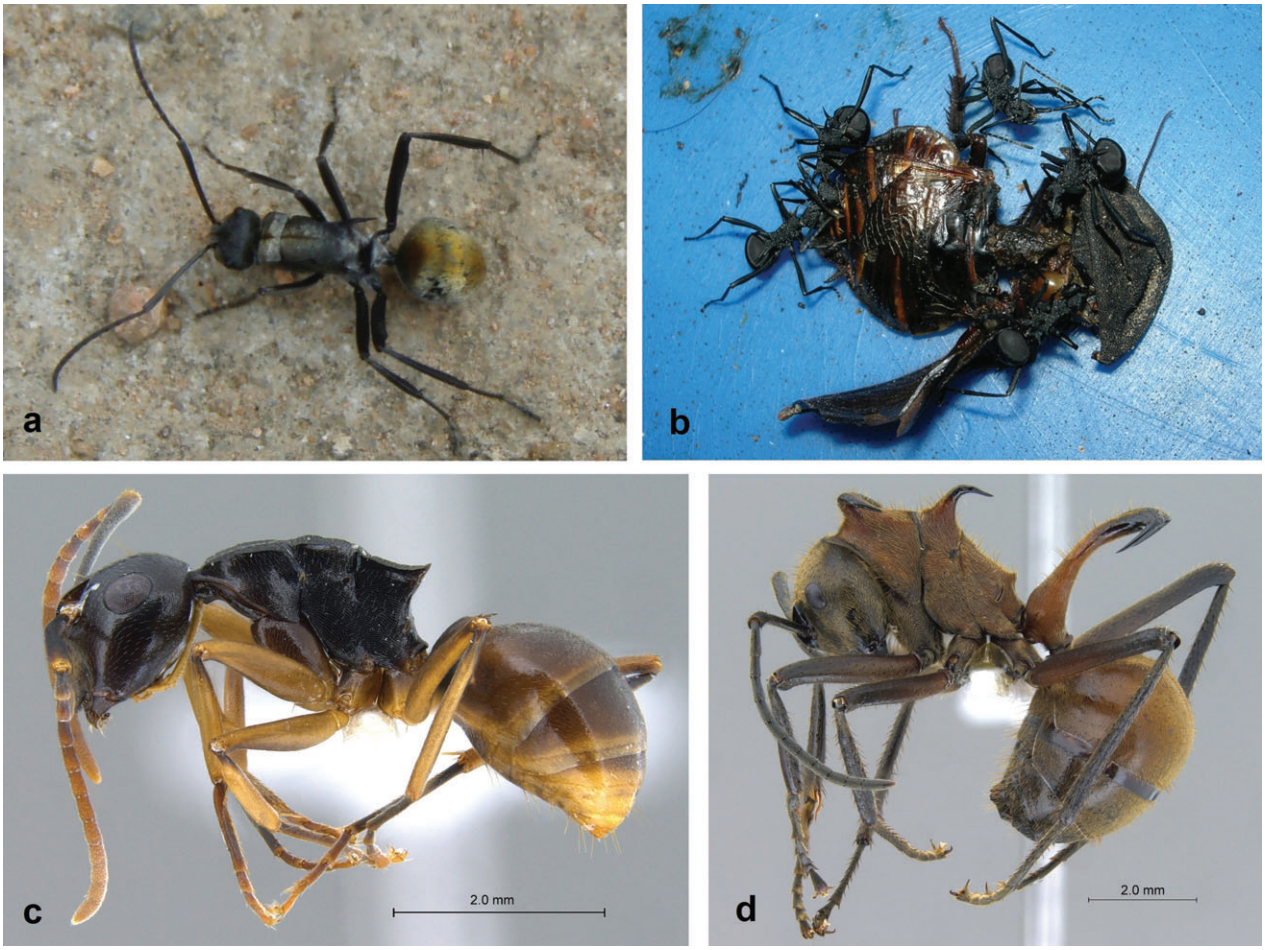

Fig. 1. Four species of *Polyrhachis*. (a) *Polyrhachis* (*Hagiomyrma*) *ammon*, Queensland, Australia. (b) *Polyrhachis* (*Myrmhopla*) *abdominalis*, Borneo, Malaysia, showing unusual feeding behaviour of *Polyrhachis* by eating parts of a dead cockroach. (c) *Polyrhachis* (*Campomyrma*) *equina*, Borneo, Malaysia. (d) *Polyrhachis* s.s. *bihamata*, Borneo, Brunei. (Photographs: (a) Corrie S. Moreau; (b) Dirk Mezger; (c, d) Martin Pfeiffer and Hans-Peter Katzmann, ©www.antbase.net.)

in which they are found. Their diet consists of hemipteran exudates (Blüthgen *et al.*, 2006), extrafloral nectaries and, to a lesser extent, arthropod prey obtained by opportunistic foraging (Liefke *et al.*, 2001). In contrast to their similar dietary preferences (Blüthgen & Feldhaar, 2010; but see Pfeiffer *et al.*, 2014), *Polyrhachis* species have a diversity of nesting habits; nests can be subterranean, in the leaf litter, attached to stones, lignicolous, inside hollow bamboo nodes or between leaves (Robson & Kohout, 2007; Robson *et al.*, 2015). Some groups of *Polyrhachis* use their larvae for silk production and weaving, which is used for nest-building (Robson & Kohout, 2007). Weaving is a behavioural trait limited to only a few other ant genera (Robson *et al.*, 2015). Species of the subgenus *Hirtomyrma* have a social parasitic life-history strategy and inhabit the nests of ant species from Ectatomminae and Ponerinae (Maschwitz *et al.*, 2003). The colony structure ranges from small monogynous (single queen) colonies with a few hundred worker ants to polydomous (multiple nests), often polygynous (multiple queens) colonies with several 10 000 workers (Robson & Kohout, 2007). The most important and most obvious morphological character of *Polyrhachis* is their spinescence (Fig. 1), as almost all *Polyrhachis* species have one to four pairs of spines on their integument, which vary in length and shape (Dorow, 1995). Spinescence is a characteristic morphological trait shared by many diurnal and vegetation foraging ants, such as *Dolichoderus* Lund (Dill *et al.*, 2002), *Pheidole* Westwood (Sarnat & Moreau, 2011) and a few Neotropical *Camponotus* species (Dorow, 1995). This spinosity is hypothesized to provide protection against diurnal vertebrate predators such as birds and reptiles (Feldhaar, 2011).

Tropical forests are thought to be the origin of ants' diversity (Moreau *et al.*, 2006), with many taxa originating in the Neotropics (Moreau & Bell, 2013) and then spreading to the Old World, such as *Pheidole* (Moreau, 2008), but South-East Asia has also been shown to be the origin of hyper-diverse genera, with cosmopolitan distributions like *Crematogaster* Lund (Blaimer, 2012). Little is known about the evolution, origin and biogeographic history of *Polyrhachis*, with only a single, recent

Fig. 2. Shaded areas show the current distribution range of *Polyrhachis* (Guénard *et al.*, 2014), stars indicate where samples used in this study were collected, and the solid black lines delimit the biogeographic ranges used for ancestral range reconstruction (Lomolino *et al.*, 2005): A, Australasia; S, Oriental; E, Afrotropis.

fossil species described belonging to the subgenus *Myrmatopa* discovered from Late Miocene deposits of the island of Crete (Greece) and dated to 18 Ma (Wappler *et al.*, 2009). The greatest subgeneric diversity of *Polyrhachis* is found in South-East Asia and Australia, which suggests that one of these regions could be their origin. In this study we address the following questions: (i) are the subgenera of Polyrhachis monophyletic; (ii) what are the phylogenetic relationships among these ants; (iii) where is the geographic origin of *Polyrhachis*; and (iv) what is the ancestral biogeographical pattern in *Polyrhachis* giving rise to their current distribution?

Material and methods

Taxon sampling

The taxa used in these analyses were selected to represent the diversity of the genus *Polyrhachis*, as well as representing samples from across the geographic distribution range of these ants. For this study, 206 taxa containing at least 84 described species from 12 of the 13 subgenera were included (only *Aulacomyrma* was not included). Table S1 contains a list of the total species within each subgenus and the number of taxa used for these analyses. A geographic overview of the genus is presented in Fig. 2. Three samples of other ant genera from the subfamily Formicinae were included as outgroups based on previously published phylogenies of the ants (Brady *et al.*, 2006; Moreau *et al.*, 2006; Moreau & Bell, 2013). We included *Camponotus* and *Echinopla* Smith as closely related members of the tribe Camponotini, and *Oecophylla* Smith as a more distantly related genus from a different tribe. Voucher specimens have been deposited at the entomological collections of the Field Museum of Natural History, Chicago, USA or at the preferred collections of the sample donors. A list of all samples used for analysis including collection accession numbers and depository is presented at Table S2 and detailed collection information in Table S3.

DNA isolation and sequencing parameters

Samples used for analyses were stored in 70–95% ethanol and kept in laboratory conditions until the start of the DNA extraction. We also used some dry-mounted specimens; these samples were dismounted prior to extraction and treated in the same way as wet samples afterwards. Laboratory work was done according to Moreau (2014). Total genomic DNA was isolated from one complete worker ant. For samples where only singletons where available, we used either three legs for destructive DNA extraction or conducted an extraction according to a non-destructive protocol, with the cuticle being pierced before the extraction (P.S. Ward, personal communication). The material used for destructive analyses was ground with a metal bead

before starting the purification with the DNeasy Tissue Kit (Qiagen Inc., Valencia, CA, USA), but eluting the extracted DNA into half the volume of the recommended amount of buffer AL according to Moreau (2014).

Up to seven protein-encoding genes were amplified for each sample by polymerase chain reaction (PCR) using fragments of the following genetic loci: *cytochrome oxidase 1* gene (CO1) (660 bp), *arginine kinase* (ArgK) (355 bp), *elongation factor 1 alpha F1* (Ef1aF1) (358 bp), *elongation factor 1 alpha F2* (Ef1aF2) (519 bp), *long-wavelength rhodopsin* (LR) (548 bp), *RNA polymerase II* (RNA pol II) (766 bp) and *wingless* (Wg) (400 bp). Region-specific primers for each gene were as follows: Wg1032R, Wg578F, AK720Er, AK244f, AK346Ef, F1-1829R, F1-1424F, F2-1118R, F2-557F, LR639Er, LR143f, RNAp2r_t2, RNAp2f_t3, HCO2198modAntR and LCO1490 (Table S4). We amplified double-stranded DNA in 25 μL volume reactions: 2.0–5.0 μL of extracted DNA (according to the quality of the DNA), 0.0–1.0 μL of $MgCl_2$, 2.5 μL of buffer (10×), 2.5 μL of dNTPs (0.8 mmol), 1.2 μL of each of the two primers, 1.0 μL of bovine serum albumin and 0.2 μL of Taq DNA Polymerase (Roche, Indianapolis, IN, U.S.A.). Ultrapure water [high-performance liquid chromatography (HPLC) quality] was added to the 25 μL reaction volume. Samples that did not amplify after the first or second try were further amplified using PCR beads (Illustra PuReTaq Ready-To-Go; GE Healthcare, Chalfont St Giles, U.K.) – to each bead we added 4.0–8.0 μL of extracted DNA, 1.0 μL of $MgCl_2$ and 1.2 μL of the forward and reverse primer, and ultrapure water (HPLC quality) was added to reach 25 μL reaction volume. All reactions were initially denatured for 2 min at 94°C in an MJ Dyad Thermal Cycler (MJ Research, Waltham, MA, USA). Next, 22–30 cycles were run involving 1 min denaturation, annealing temperature 45–54°C for 1–2 min (depending on gene), and extension temperature 72°C for 1–2 min. A final extension at 72°C for 10 min was included. Successful PCR products were cleaned using 2 μL of EXOSAP (Affymetrix, Cleveland, OH, U.S.A.) for 25 μL reaction volume. The reaction was run in a thermal cycler with a temperature of 37°C for 15 min and 80°C for 10 min.

We used the same primers for cycle sequencing reactions as we used for PCR amplification. We sequenced all samples in both directions. For these reactions a volume of 10.0 μL was used. We added 1.5–3.0 μL of DNA, 2.0 μL of buffer (5×), 0.5 μL of primer, 4.25–5.75 μL of ultrapure water and 0.25 μL of big dye. Cycle sequencing reactions were performed with the following parameters: an initial step of 94°C for 2 min, followed by 25 cycles of 10 s at 94°C denaturation, 5 s at an annealing temperature of 50°C, and 4 min at 60°C in an MJ Dyad Thermal Cycler (MJ Research). The sequencing was performed using an ABI 3730 DNA Analyzer (Applied Biosystems, Waltham, MA, USA).

Sequence alignment

Sequences were aligned with the program GENEIOUS 6.1.6. (Kearse *et al.*, 2012) using the CLUSTALW alignment function (Larkin *et al.*, 2007). A further step of alignment was done with the program MAFFT, version 7 (Katoh & Standley, 2013). For subsequent viewing and manual alignment adjustments, we used the program MEQUITE, version 2.75 (Maddison & Maddison, 2011). Alignments were confirmed using the appropriate amino acid reading frame. Introns were excluded from analyses. All sequences generated by this study have been deposited in GenBank, and the accession numbers are presented in Table S2. The aligned matrix and the Bayesian tree have been deposited at TreeBase (ID:TB2:S16268; http://purl.org/phylo/treebase/phylows/study/TB2:S16268?xaccesscode=b6a7e1df96052e5e859b3ab54ff4f1e8&format=html).

Phylogenetic analyses

To infer the phylogenetic relationships among *Polyrhachis*, several model-based phylogenetic analyses were performed via XSEDE (version 3.2.3.) on the CIPRES Science Gateway version 3.3 (Miller *et al.*, 2010) using RAXML v7.3.2 (Stamatakis *et al.*, 2005) and MRBAYES v3.1.2 (Huelsenbeck & Ronquist, 2001). All of these analyses were implemented on a concatenated matrix of all seven genes, with each gene region having a separate general time reversible (GTR) + gamma model with parameters unlinked. For the maximum likelihood (ML) search in RAXML, 500 bootstrap pseudoreplicates were performed. For the Bayesian inference (BI) analyses, we used default settings with a Markov chain Monte Carlo of 50 000 000 generations, with the chain sampled every 10 000 generations and a burn-in period of the first 5 000 000 generations (Posada & Crandall, 1998). Independence of runs was ensured by only accepting analyses where the average standard deviation of the split frequencies was below 0.01.

To test the possible monophyly of several subgenera that were not reconstructed as monophyletic, we constrained them as monophyletic and implemented the likelihood-based Shimodaira approximately unbiased (AU) test (Shimodaira, 2002) in PAUP* ver 4.0a146 (Swofford, 2002) with 100 000 resampling estimated log-likelihood (RELL) bootstrap pseudoreplicates. We compared our BI likelihood topology with two alternative scenarios, each of which constrained the monophyly of the subgenera in question.

Divergence time estimation and biogeographic interference

We estimated divergence dating using BEAST version 1.8.0 (Drummond *et al.*, 2012) on the CIPRES Science Gateway. We used an uncorrelated log-normal relaxed clock model and Yule process, speciation tree prior (Reid & Carstens, 2012). We constrained the calibration points as monophyletic and used as a starting point the tree inferred by MRBAYES. For this analysis we used the GTR substitution model and a site frequency model gamma with four categories. The chain length was 100 000 000 generations with a log of parameters every 1000 steps. We summarized our trees as maximum clade credibility trees in TREEANNOTATOR version 1.8.0. We calibrated two nodes in our

phylogenetic tree with data from fossils in order to estimate divergence times. Minimum calibration points were assigned a lognormal distribution with the minimum age of the fossil as the offset, log(mean) of 1.0, and log(SD) of 1.0. To ensure stationarity among independent runs and determine burn-in, only runs with high (>200) effective sample size (ESS) values were accepted. The following fossils were assigned as minimum age constraints to the following monophyletic clades: 1, *Camponotus* sp. (44.1 Ma) (Dlussky, 1997) for *Camponotus* + *Echinopla*; 2, *Polyrhachis (Myrmatopa) annosa* (18 Ma) (Wappler *et al.*, 2009) for all species in the subgenus *Myrmatopa*.

To infer the ancestral biogeographic origin and range evolution of *Polyrhachis*, we implemented the likelihood-based program package LAGRANGE v2.0130526 (Ree *et al.*, 2005; Ree & Smith, 2008). We performed two analyses: one with equal distribution constraints and one with a lower probability (0.1) of long-distance distribution over water for the potential pathway from Australia to Africa compared with a distribution from South-East Asia to Australia and to Africa (1.0). This analysis is based on the topology inferred in the BEAST analysis. To model this analysis, we defined the geographic ranges of *Polyrhachis* as Australasian, Oriental, or Afrotropical (Lomolino *et al.*, 2005); for the separation of the Australasian and Oriental region, we referred to the Wallace line. As LAGRANGE also calculates combined biogeographic origins, four possible origins are proposed as results: Australasian, Australasian/Oriental, Oriental and Oriental/Afrotropical. For visualizing our results, we used the software RASP (Yu *et al.*, 2015).

Results

Sequence alignment details

For this study, a final alignment of a fragment of 3606 bp was produced for the 209 taxa; for each taxon, up to seven genes were aligned (Table S2). For the total aligned data matrix, 2146 bp (59.5%) are constant, 513 bp (14.2%) are variable and 947 bp (26.3%) are parsimony-informative (Table S5). Base compositions of the alignment matrix were as follows: mitochondrial genes ($n = 1$) – A, 0.29140; C, 0.19013; G, 0.12393; T, 0.39454; nuclear genes ($n = 6$) – A, 0.24758; C, 0.27076; G, 0.27768; T, 0.20398.

Phylogeny

All analyses (RAXML, MRBAYES and BEAST) recovered *Polyrhachis* as monophyletic and group species into four large clades: I, the Asian species of *Campomyrma* [ML = 100%; Bayesian posterior probability (BPP) = 1.0]; II, a clade consisting of the various species groups of *Myrmhopla* and the subgenera *Cyrtomyrma* and *Myrmatopa* (BPP =0.85); III, a clade of mostly Australian subgenera *Hedomyrma*, *Hagiomyrma*, *Chariomyrma* and *Hirtomyrma*, and the Australian species of *Campomyrma* and *Myrmothrinax* (BPP = 0.81); and IV, a clade consisting of *Hemioptica*, *Myrma* and the subgenus *Polyrhachis* s.s. (ML = 89%; BPP = 0.99) (Figs 2, 3). The position of *Myrmothrinax* varied among different phylogenies; according to RAXML and MRBAYES analyses, it was part of clade II, but the BEAST analysis recovered this subgenus as part of clade III where it was sister group to all other taxa. All analyses reconstructed the Asian species of *Campomyrma* as the sister clade to all other *Polyrhachis*. The subgenus *Myrmhopla* was always recovered as nonmonophyletic, with the subgenera *Cyrtomyrma* and *Myrmatopa* nested within the different clades of *Myrmhopla*. The *Myrmhopla sexspinosa* species group was the sister taxon of all clades within this group. For the fourth clade, there were differences among the topologies for the relationships of the African and the Asian-Australian species of *Myrma*. While RAXML and BEAST recovered *Polyrhachis* s.s. and the Asian *Myrma* species in one clade and the African species of *Myrma* as a different clade (ML = 80), MRBAYES recovered all *Myrma* as belonging to a single clade (BPP = 0.7088). The placement of the subgenera *Hedomyrma* and *Hagiomyrma* differed between topologies. The ML tree had *P.* (*Hagiomyrma) anderseni* as sister to *Hedomyrma* species, and the remaining *Hagiomyrma* species and *Hedomyrma* were two clades. According to the BI analysis, both subgenera were inferred as monophyletic. The results of our tests of monophyly for the *Campomyrma* and *Myrmhopla* subgenera using the likelihood-based Shimodaira AU test with 100 000 RELL bootstrap pseudoreplicates resulted in the following: BI topology (best tree), $-\ln L = 46\,212.912\,87$; (i) *Campomyrma* constrained as monophyletic (significantly different), $-\ln L = 46\,231.129\,83$, difference $-\ln L = 18.216\,96$, $P = 0.0033$; (ii) *Myrmhopla* constrained as monophyletic (significantly different), $-\ln L = 46260.15348$, difference $-\ln L = 47.240\,61$, $P = 0.0006$. These results suggested no support for the monophyly for these two subgenera as currently defined. Despite presenting the results of three analyses, we considered the results of the MRBAYES as our main hypothesis for the phylogeny and the findings of the BEAST analysis as our main hypothesis of biogeography and referred to this one if not stated otherwise.

Molecular dating

Based on our ML age estimation we recovered an estimated age of 58 Ma for the age of the modern crown-group *Polyrhachis*. Most of the clades of subgenera had age estimations from 35 to 20 Ma. The African clade of *Myrma* had an estimated age of 26 Ma (Fig. 4; Table S6).

Biogeographical history

The biogeographical origin of *Polyrhachis* seemed to be tropical Asia (Fig. 4 and Table S4). The various clades of *Myrmhopla* also originated in South-East Asia and dispersed to Australia. One clade of this subgenus, the *Myrmhopla*-dives group barely reached the northernmost areas of the Afrotropical Region. The subgenus *Cyrtomyrma* had a

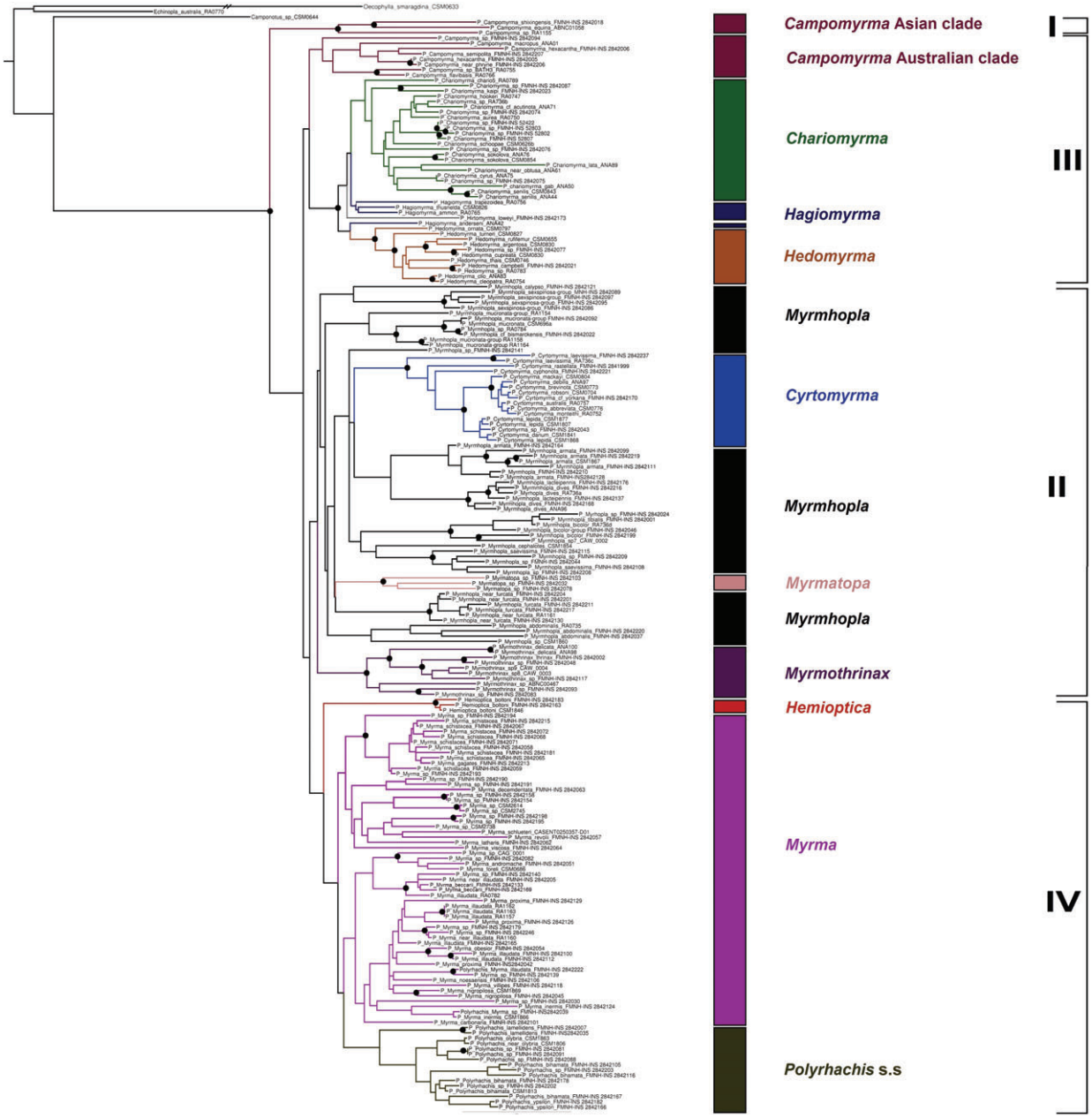

Fig. 3. Phylogeny of *Polyrhachis* reconstructed by Bayesian inference including 209 taxa; each black dot at a node indicates a Bayesian posterior probability above 0.95. I, II, III and IV refer to four major clades of Polyrhachis (described in the Results section).

similar origin in South-East Asia and also reached Australia. *Myrmothrinax* possibly emerged in the Australian region and dispersed from there to South-East Asia. Except for a few species, which dispersed from Australia to South-East Asia, the clades of the Australian group are mostly restricted to the Australian region. Of the fourth group, consisting of *Hemioptica*, *Polyrhachis* s.s. and *Myrma*, one clade of *Myrma* dispersed once to Africa, while some species of the Asian *Myrma* clade and *Polyrhachis* s.s. dispersed into the Australian region.

Discussion

Our analyses of DNA sequence data strongly supported the monophyly of the genus *Polyrhachis*, which was similar to the result by Robson *et al.* (2015) using an overlapping set of DNA loci. This was in contrast to the result of Dorow (1995), based on morphological data, who suggested that the genus was not monophyletic. Dorow (1995) also postulated that all the subgenera were monophyletic except for *Myrmhopla*. According to our results, *Myrmhopla* as well as *Campomyrma* were not

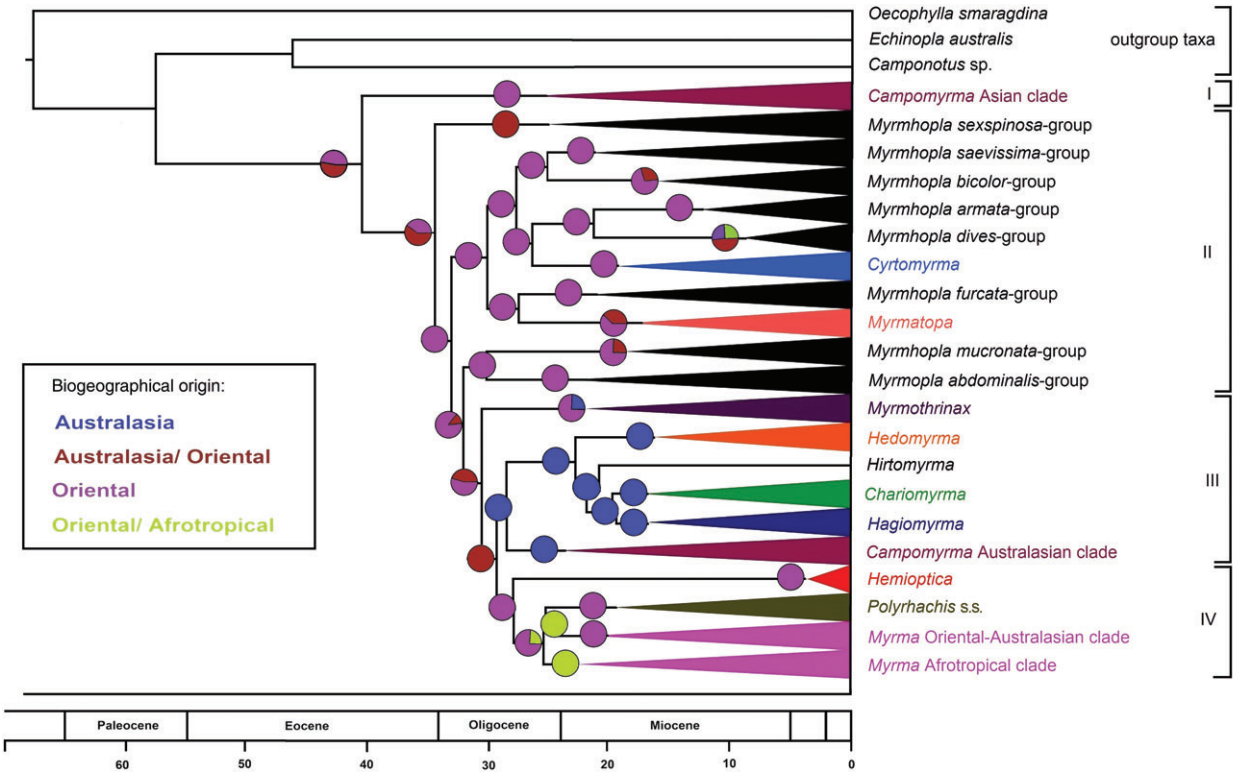

Fig. 4. Results of the divergence dating analyses using BEAST (pruned to major clades), showing the minimum ages of the studied clades. The circles display information on their biogeographic range reconstructions according to the ancestral range analyses presented in Table S4. I, II, III and IV refer to four major clades of Polyrhachis (described in the Results section).

monophyletic. *Myrmhopla* was a large paraphyletic grade, while the polyphyletic *Campomyrma* consisted of two distantly related separated clades with a superficially similar morphology, but distinct biogeographic history with the genus separated into a South-East Asian and an Australian clade. As the type species of *Campomyrma* is from the Indian subcontinent (Wheeler, 1911), the Asian clade should probably be considered as the true *Campomyrma* and the Australian clade as a separate taxonomic group. In the case of *Myrmhopla*, which was defined by Emery (1925) mostly by the presence of a rounded, emarginated thorax with pronotal spines shorter than the propodeal spines, our analysis supported the earlier view of this group as a taxonomic 'storage bin' of unrelated taxa (Dorow, 1995). The Australian subgenus *Hagiomyrma* needs a critical review for the true subgeneric boundaries as one species, *Polyrhachis* (*Hagiomyrma*) *anderseni*, is not part of *Hagiomyrma* clade, but grouped as a sister taxon to *Hedomyrma*. While a formal revision of the subgeneric classification of *Polyrhachis* is not presented here, this paper does present specific areas on which future studies should focus.

Robson *et al.* (2015) found *Myrmatopa* as the sister group to the rest of *Polyrhachis*, while here we found the Asian clade of *Campomyrma* as sister to all other species in the genus. This difference was probably because Robson *et al.* (2015) did not include any *Campomyrma* species from the South-East Asian clade in their study. Otherwise, the framework of the phylogeny of Robson *et al.* (2015) was mostly confirmed by our study. Both studies found the mostly Australian subgenera *Chariomyrma*, *Hedomyrma* and *Hagiomyrma* topologically grouped together, in this case also grouped with *Hirtomyrma*. Robson *et al.* (2015) also found *Cyrtomyrma* nested within *Myrmhopla*. Due to the limited number of included species of *Myrmhopla*, Robson *et al.* (2015) did not resolve the complex relationships of the species of this subgenus.

Ants have several different dispersal mechanisms: spreading over land (bridges), oceanic rafting of nests, and airborne dispersal by winged sexuals (Fisher, 2010). The many nesting types of *Polyrhachis* differ in their suitability for oceanic dispersal. Nests of soil-nesting species might not experience oceanic dispersal at all; leaf nests are more suitable while species nesting in plant cavities have the best chances of surviving oceanic dispersal on a fallen tree acting as a raft (Blaimer, 2012). As both sexes are winged in the ant subfamily Formicinae, *Polyrhachis* also has the potential of limited distance dispersal by air. The unspecialized nutrition habits of spiny ants are very favourable for successful colonization after a long-distance dispersal event; a diet based on plant-derived carbohydrates makes it easy for them to find suitable food resources in a new colonized habitat.

According to our ancestral range scenario, *Polyrhachis* started with its diversification in South-East Asia approximately 40 Ma, with the Asian species of *Campomyrma* being the earliest

modern lineage of this ant genus; this corresponds with the age postulated by Moreau & Bell (2013). As this clade appears to be mostly soil-nesting (D. Mezger, personal observation), dispersal opportunities may have been limited, resulting in the current restriction of this clade to South-East Asia. More derived clades, which included arboreal and plant cavity-nesting species, were able to disperse from South-East Asia to Australia, probably via the Indonesian island archipelago. Many of the subgenera with an Asian origin dispersed to Australia, while few lineages dispersed from Australia back to South-East Asia. These multiple dispersal events suggest that spiny ants are effective dispersers and colonizers, but as they have not colonized more distant islands of the Polynesian Region, they are not as successful at long-distance dispersal as other ant genera that have reached islands in the Central Pacific, such as Fiji (Sarnat & Economo, 2012). All *Polyrhachis* species found in Africa belong to a single subgenus, *Myrma*. Only *Polyrhachis (Myrmhopla) lacteipennis* reaches the northernmost stretches of the Afrotropical area, but this seems to be a quite recent dispersal. Species of the subgenus *Myrma* are distributed from Asia to Africa and inhabit both open habitats and forested areas (Dorow, 1995), which makes a long-distance distribution over areas with different degrees of forest cover easier. *Myrma* dispersed from Asia to Africa around 26 Ma.

For the myrmicine ant genus *Crematogaster*, Blaimer (2012) also found a South-East Asian origin and a similar age as the spiny ants, and inferred how these ants reached their contemporary cosmopolitan distribution, which is complementary to *Polyrhachis* because of their similar origin and divergence time. But these genera differ in their current distribution, with *Polyrhachis* being largely restricted to the Palaeotropics and temperate parts of Australia, while *Crematagaster* can be found on all continents. A possible reason for the restriction of spiny ants to the Old World could be their late arrival to Africa, which potentially did not permit further dispersal to the New World as the continents had already drifted far apart. A similar pattern could be the reason for their absence in Madagascar. *Polyrhachis* arrived in Africa when the ocean currents were less suitable for dispersal from Africa to Madagascar during the mid-Miocene; dispersal-friendly eastwards-directed currents were replaced by the trade winds blowing in the opposite direction (Ali & Huber, 2010). Another potential reason why *Polyrhachis* has not reached the New World is that spiny ants are more restricted to tropical latitudes, with no species being largely distributed in the northern temperate zone like *Crematogaster* or *Camponotus*. This excludes potential distribution routes via Beringia to North America, as has been proposed for other ant genera such as *Myrmica* (Jansen *et al.*, 2010).

Conclusion

By leveraging molecular data for the species-rich formicine ant genus *Polyrhachis* from across the distribution range, we have investigated how these ants became distributed across the Palaeotropics with their centre of diversity and origin in South-East Asia. From this biogeographic region, *Polyrhachis* dispersed from South-East Asia several times to Australia and only once to Africa and is entirely absent from the New World and Madagascar. These findings contribute to our understanding of the distribution and diversification process of this diverse and ecologically important group of ants of the warmer parts of the Old World.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/syen.12163

Figure S1. Phylogeny of *Polyrhachis* reconstructed by maximum likelihood, including 209 taxa; each black dot marks node support above 95%. I, II, III and IV: four major clades of *Polyrhachis* described in the results section.

Table S1. List of species numbers for the subgenera included in these analyses and the total species numbers for each respective subgenus (numbers according to R. Kohout and S. Robson, personal communication). Biogeographic origin refers to the sample used in the analyses.

Table S2. Collection numbers, specimen depository and GenBank accession numbers for the sequenced taxa and genes. X indicates genes for which no sequence is available. Specimen depository is designated by the first letters of the collection number: CSM, RA & FMNH, Field Museum of Natural History, Chicago, USA; ABNC, Antbase.Net collection, housed at the University of Landau, Germany; ANA, CSIRO Tropical Ecosystems Research Centre (TERC) in Darwin, Australia; CAG & CAW, Department of Crop Sciences, Agroecology, University of Göttingen, Germany; CASENT, Entomological Collection of the California Academy of Sciences, San Francisco, USA.

Table S3. Detailed information to each collection assesion number: given are country, region and locality of collection as well as geographic coordinates. NP, National Park.

Table S4. Primers used for amplification and sequencing. For the amplification of the *arginine kinase* gene, a nested design was applied. First, this gene was amplified by using AK244f and AK720Er, and the resulting PCR product was reamplified with the primer combination of AK346Ef and AK720Er.

Table S5. Sequence characteristics for the included genes and total aligned data matrix. Given are the total number of base pairs, and the number of constant, variable and parsimony-informative base pairs.

Table S6. Ancestral range reconstructions for subgenera and major clades (LaGrange analyses); rP, relative probability. Abbreviations of distribution range: A, Australasia; S, Oriental and E, Afrotropis.

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