Tales of dracula ants: the evolutionary history of the ant subfamily Amblyoponinae (Hymenoptera: Formicidae)

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Abstract. The ants in the subfamily Amblyoponinae are an old, relictual group with an unusual suite of morphological and behavioural features. Adult workers pierce the integument of their larvae to imbibe haemolymph, earning them the vernacular name 'dracula ants'. We investigate the phylogeny of this group with a data set based on 54 ingroup taxa, 23 outgroups and 11 nuclear gene fragments (7.4 kb). We find that the genus *Opamyrma* has been misplaced in this subfamily: it is a member of the leptanilline clade and sister to all other extant Leptanillinae. Transfer of *Opamyrma* to Leptanillinae renders the Amblyoponinae monophyletic. The enigmatic Afrotropical genus *Apomyrma* is sister to all other amblyoponines, and the latter cleave into two distinct and well-supported clades, here termed POA and XMMAS. The POA clade, containing *Prionopelta*, *Onychomyrmex* and *Amblyopone*, is well resolved internally, and its structure supports synonymy of the genus *Concoctio* under *Prionopelta* (**syn.n.**). The XMMAS clade comprises two well-supported groups: (i) a predominantly Neotropical clade, for which we resurrect the genus name *Fulakora* (**stat.r., stat.n.**), with junior synonyms *Paraprionopelta* (**syn.n.**) and *Ericapelta* (**syn.n.**); and (ii) the remaining taxa, or 'core XMMAS', which are manifested in our study as a poorly resolved bush of about a dozen lineages, suggesting rapid radiation at the time of their origin. Most of these XMMAS lineages have been assigned to the catch-all genus *Stigmatomma*, but the more distinctive elements have been treated as separate genera (*Xymmer*, *Mystrium*, *Myopopone* and *Adetomyrma*). Resolution of basal relationships in the core XMMAS clade and reconfiguration of '*Stigmatomma*' to restore monophyly of all named genera will require more extensive genetic data and additional morphological analysis. However, the genus *Bannapone* can be synonymized under *Stigmatomma* (**syn.n.**) because it is embedded within a clade that contains *S. denticulatum*, the type species of *Stigmatomma*. Divergence dating analysis indicates that crown Amblyoponinae arose in the mid-Cretaceous, about 107 Ma (95% highest probability density: 93–121 Ma). The POA and XMMAS clades have estimated crown ages of 47 and 73 Ma, respectively. The initial burst of diversification in the core XMMAS clade occurred in the Late Paleocene/Early Eocene (50–60 Ma). Ancestral range reconstruction suggests that amblyoponines originated in the Afrotropics, and dispersed to the Indo-Malayan region and to the New World. During none of these dispersal events did the ants break out of their cryptobiotic lifestyle.

Introduction

Biological diversity is far from evenly distributed among different kinds of organisms. Evolutionarily successful groups,

containing hundreds or thousands of species, often stand in contrast to their depauperate sister lineages (Donoghue & Sanderson, 2015). Biologists have long been intrigued by 'relictual taxa', i.e. groups that are old and yet contain relatively few extant species compared with their relatives (Habel & Assmann, 2010). Typically these relicts retain characteristics considered ancestral in comparison to related clades, and they often live in habitats

that shield them from competition with their more derived relatives. Study of such organisms can be hampered by their occurrence in cryptic or inaccessible locations (Naskrecki, 2011).

Ants (Hymenoptera: Formicidae) are the most successful and ecologically dominant group of eusocial insects (Hölldobler & Wilson, 1990; Lach *et al.,* 2009), but different clades of ants vary markedly in dominance and diversity and they include a considerable number of taxonomic relics (Wilson *et al.,* 1956; Rabeling *et al.,* 2008; Yamane *et al.,* 2008; Pie & Feitosa, 2016). The ant subfamily Amblyoponinae falls under this rubric: its species live and forage almost exclusively underground in the soil or undercover in leaf litter and rotten logs, where they hunt specialized prey such as geophilomorph centipedes (Brown, 1960). Occurring in all major biogeographic regions but usually infrequently encountered, amblyoponine ants currently number about 130 described species, apportioned among 13 genera (AntCat, 2016). Although there are additional undescribed (or unidentifiable) amblyoponine specimens in collections, the group is clearly dwarfed in size by other, more prominent ant subfamilies of equivalent (or younger) age, such as Ponerinae, Dorylinae, Formicinae and Myrmicinae (Ward, 2014).

Amblyoponine ants are often considered to be 'primitive' in morphology and social behaviour (Haskins, 1970; Traniello, 1982; Masuko, 1986; Ito, 1993; Peeters, 1997; Thorne & Traniello, 2003). The members of this subfamily are typically slow and lumbering in their movements (Wilson, 1958) and do not exhibit some of the more complex forms of communication and social organization seen in other ants. At the same time, they have a number of obviously derived characteristics such as small (or nonexistent) worker eyes, reduction/fusion of sclerites, and a narrow prey spectrum. Other intriguing features of uncertain polarity include the specialized, peg-like setae on the anterior margin of the clypeus (Ward, 1994); the broad attachment of the petiole to the rest of the metasoma (Bolton, 2003); and the habit of adult ants of biting larvae with their mandibles and consuming the exuding haemolymph (Masuko, 1986; Ito & Billen, 1998; Wild, 2005; Ito, 2010). This last behaviour has earned amblyoponines the sobriquet 'dracula ants' (Saux *et al.,* 2004), although larval haemolymph feeding is known to occur in a few other ant taxa, including *Leptanilla* and *Proceratium* (Masuko, 1986, 1989).

Despite limited species diversity, the Amblyoponinae show notable phenotypic variation (Fig. 1). Some species in the genus *Mystrium* have bizarre miniature queens smaller in size than the workers and differing markedly in colour (Molet *et al.,* 2009). The genus *Adetomyrma* is characterized by highly modified metasomal morphology in workers, with apparent reversal of tergosternal fusion (Ward, 1994). Ants in the genus *Onychomyrmex* have army ant-like lifestyles, with nomadic colonies and modified wingless queens (Wheeler, 1916; Miyata *et al.,* 2003). The robustly built workers of *Myopopone castanea* paralyse large cerambycid larvae in rotten logs, and apparently transport their larvae to the prey in some instances (Wilson, 1958), although retrieval of food to the nest has also been reported (Ito, 2010).

For most of their taxonomic history, these ants were treated as a tribe, Amblyoponini, within the subfamily Ponerinae (Emery,

1911; Brown, 1954, 1960; Bolton, 1995). The composition of Amblyoponini varied somewhat but was stabilized in a landmark generic revision by Brown (1960). Bolton (2003) noted the heterogeneous nature of the subfamily Ponerinae (as then defined) and, among other actions, he reinstated Amblyoponinae as its own subfamily. This decision has been validated by subsequent molecular phylogenetic studies showing that other taxa are interpolated between these two subfamilies, within a larger assemblage of ants known as the poneroid group (Brady *et al.,* 2006; Moreau *et al.,* 2006; Rabeling *et al.,* 2008; Schmidt, 2013).

The relationships of amblyoponine ants to one another and to other poneroids have not been fully resolved. Inferences based on morphology (Yoshimura & Fisher, 2012) and DNA sequence data (Saux *et al.,* 2004; Brady *et al.,* 2006; Moreau & Bell, 2013) have suggested the existence of two major amblyoponine clades, here termed POA and XMMAS (cf. Yoshimura & Fisher, 2012). But there are two other enigmatic ant genera, currently placed in Amblyoponinae, whose affinities are unclear. *Apomyrma* is an Afrotropical genus, known from one described and several undescribed species, whose peripatetic taxonomic history has seen it assigned to Amblyoponinae, Leptanillinae and its own subfamily, Apomyrminae (Bolton, 2003). *Opamyrma* is a monotypic genus known only from two workers collected in Vietnam; it evinces a curious combination of leptanilline-like and amblyoponine-like features (Yamane *et al.,* 2008). These two taxa, both subterranean and with pale, blind workers, are superficially similar to one another, but whether this is due to close relationship or convergence remains unclear (Ward, 2014).

In this study we investigate the phylogenetic relationships of Amblyoponinae using a comprehensive set of taxa and 11 nuclear gene fragments. We confirm the division of the subfamily into two major clades and we clarify the positions of *Apomyrma* and *Opamyrma*. We also propose several taxonomic changes to bring the classification in line with new phylogenetic findings.

Materials and methods

Taxon sampling and gene sequencing

Our 77-taxon data set consists of 54 amblyoponines, including *Opamyrma hungvuong* and three *Apomyrma* species, as well as 23 outgroups: 19 other ants, and four non-ant aculeate Hymenoptera (Table S1, Supporting Information). Among the latter, two taxa (*Scolia*, *Apterogyna*) were used as 'outer outgroups' to root the tree, based on inferred aculeate relationships (Johnson *et al.,* 2013). The sequenced amblyoponines include representatives of all genera, with multiple species of most of them, and 31 species of *Stigmatomma*, a group suspected of being nonmonophyletic (Esteves & Fisher, 2015).

We sequenced fragments of 11 nuclear genes: abdominal-A (abdA), elongation factor 1-alpha F2 copy (EF1aF2), long wavelength rhodopsin (LW Rh), arginine kinase (argK), DNA topoisomerase I (Top1), ultrabithorax (Ubx), DNApol-delta (POLD1), NaK ATPase (NaK), Antennapedia (Antp), wingless (wg) and 28S ribosomal DNA (28S). The data matrix has
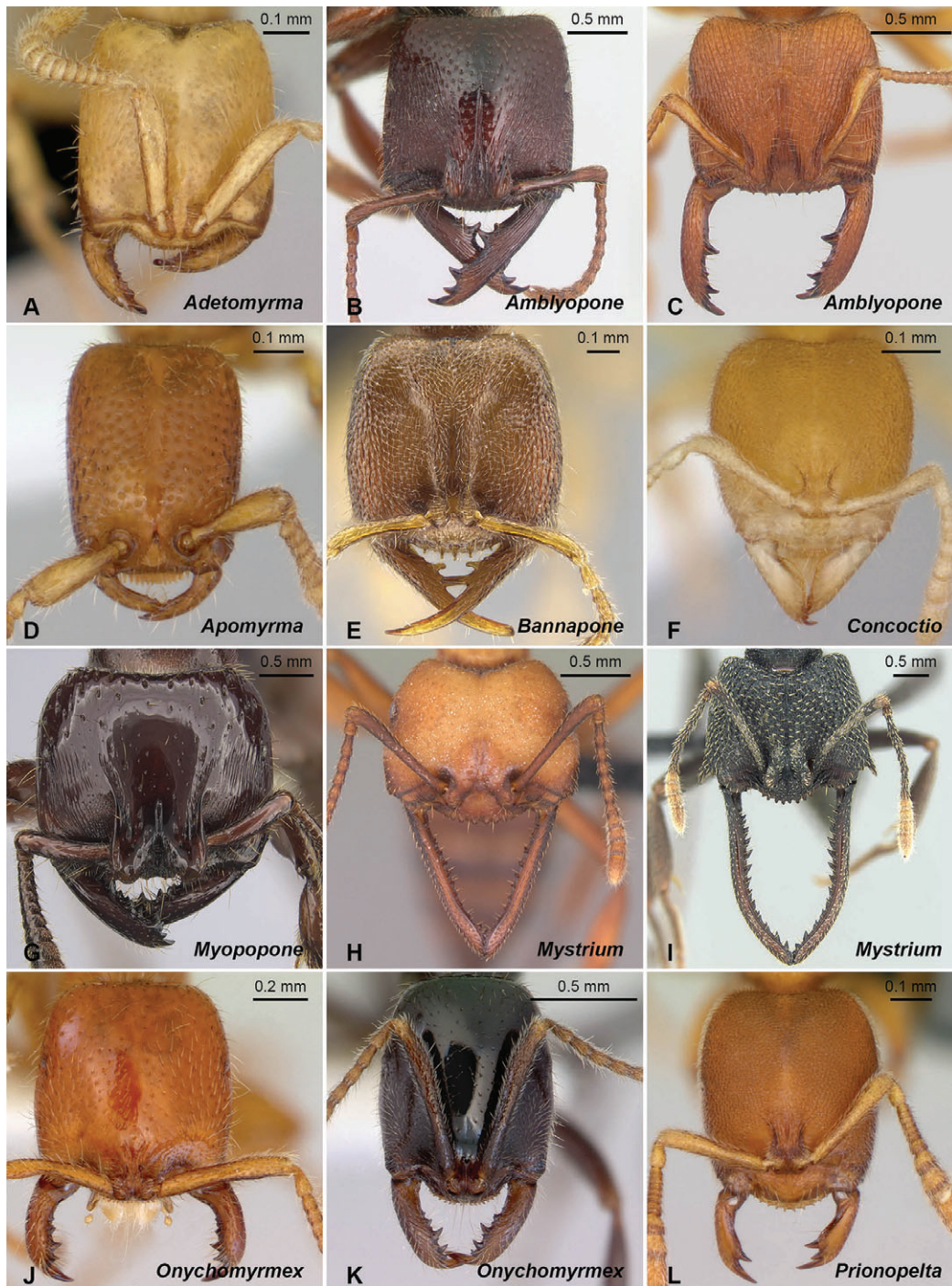
Fig. 1. Morphological diversity in Amblyoponinae. (A–R) Full-face view of worker head; (S–V): lateral view of worker body. (A) *Adetomyrma venatrix* (CASENT0489808); (B) *Amblyopone australis* (CASENT0434465); (C) *Amblyopone longidens* (CASENT0100485); (D) *Apomyrma stygia* (CASENT0000077); (E) *Bannapone scrobiceps* (CASENT0339957); (F) *Concoctio concenta* (CASENT0004306); (G) *Myopopone castanea* (CASENT0260455); (H) *Mystrium mirror* (CASENT0492109); (I) *Mystrium shadow* (CASENT0077648); (J) *Onychomyrmex mjobergi* (CASENT0172295); (K) *Onychomyrmex* AU03 (CASENT0172301); (L) *Prionopelta subtilis* (CASENT0494610); (M) *Stigmatomma rothneyi* (CASENT0914940); (N) *Stigmatomma saundersi* (CASENT0006813); (O) *Stigmatomma zwaluwenburgi* (CASENT0173925); (P) *Stigmatomma mystriops* (CASENT0178868); (Q) *Xymmer* MG04 (CASENT0151732); (R) *Xymmer* MG01 (CASENT0033448); (S) *Amblyopone australis* (CASENT0260444); (T) *Myopopone castanea* (CASENT0260455); (U) *Mystrium janovitzi* (CASENT0006082); (V) *Onychomyrmex doddi* (CASENT0172297). In this study, *S. saundersi* (N) and *S. mystriops* (P) are transferred to the newly resurrected genus *Fulakora* (see text). Images from AntWeb (http://www.antweb.org).

Fig. 1. Continued.

no missing fragments (Table S1, Supporting Information). Of the 847 sequences, 675 were newly generated for this study (GenBank accession numbers KU671399-KU672073).

Methods of DNA extraction, amplification and sequencing are detailed elsewhere (Ward & Downie, 2005; Brady *et al.,* 2006; Ward *et al.,* 2010). For most samples we carried out two rounds of amplification, with the second amplification employing a nested or hemi-nested pair of primers. The primers for three genes new to this study (POLD1, NaK, and Antp) are given in Table S2, Supporting Information. Sequences were assembled with SEQUENCER v5.2.2 (Gene Codes Corporation, Ann Arbor, MI, U.S.A.), aligned with CLUSTAL X v2.1 (Thompson *et al.,* 1997), and manually edited and concatenated with MACCLADE v4.08 (Maddison & Maddison, 2005). We excluded introns of

Table 1. Partition scheme selected by PARTITIONFINDER.

Blocks	Partition	Model
AA_pos1, DP_pos2, UB_pos1	p1	HKY + I + G
AA_pos2, UB_pos2	p2	GTR + I + G
AA_pos3	p3	HKY + I + G
F2_pos3	p4	K80 + I + G
F2_pos1, NK_pos1, TP_pos1	p5	GTR + I + G
AK_pos2, F2_pos2	p6	GTR + I + G
AK_pos3, LR_pos3, NK_pos3	p7	GTR + I + G
LR_pos1	p8	SYM + I + G
LR_pos2	p9	GTR + I + G
AK_pos1, DP_pos1	p10	SYM + I + G
TP_pos3	p11	K80 + I + G
TP_pos2	p12	HKY + I + G
AP_pos3, UB_pos3	p13	K80 + I + G
DP_pos3	p14	HKY + I + G
NK_pos2	p15	K80 + I
AP_pos1	p16	GTR + I + G
AP_pos2	p17	GTR + I + G
Wg_pos3	p18	GTR + I + G
28S, Wg_pos1, Wg_pos2	p19	GTR + I + G

Codes for each gene are as follows: AA, abdominal-A; F2, elongation factor 1-alpha F2 copy; LR, long-wavelength rhodopsin; AK, arginine kinase; TP, DNA topoisomerase 1; UB, ultrabithorax; DP, DNApol-delta; NK, NaK ATPase; AP, Antennapedia; Wg, wingless; and 28S, 28S ribosomal DNA.

LW Rh (544 bp in the alignment) and ArgK (1422 bp), as well as hypervariable regions of 28S (157 bp) and autapomorphic indels in the exons of abdA (3 bp), Antp (66 bp) and Wg (18 bp). After these exclusions the data matrix comprised 7426 bp, of which 3523 sites were variable and 2945 were parsimony-informative. This matrix has been deposited in TreeBase (S18847).

Data analysis: partitioning

We partitioned the sequence data into 31 blocks, corresponding to 28S and the three codon positions of each of the ten protein-coding genes. We then ran PARTITIONFINDER v1.1.1 (Lanfear *et al.,* 2012) to choose among partition schemes and models, with models = mrbayes, model_selection = bic, and search = greedy. This resulted in the 19-partition scheme and models indicated in Table 1.

Phylogenetic analyses

For phylogenetic inference under maximum likelihood (ML) we used GARLI 2.0 (Zwickl, 2006), as implemented on the CIPRES Science Gateway V. 3.3 (http://www.phylo.org/portal2), and employed the 19-partition scheme selected by PARTITIONFINDER. Departures from GARLI default settings were as follows: genthreshfortopoterm was set to 200 000, as opposed to the default of 20 000; and modweight was set to 0.01, i.e. 0.0005 × (#partitions + 1), as opposed to the default of 0.05. Forty search replicates were undertaken in the search for the ML tree. A bootstrap analysis was also conducted using 100 bootstrap replicates and genthreshfortopoterm set to 100 000.

Bayesian analysis was carried out with MRBAYES 3.2.3 (Ronquist *et al.,* 2012), as implemented on CIPRES, using the same 19-partition scheme. We set brlenspr to unconstrained:exponential (100), corresponding to a mean length prior of 0.01 (as opposed to the MRBAYES default of 0.1), which better matches the shorter branch lengths in this data set (see also Ward *et al.,* 2010). We ran MRBAYES 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: (i) standard deviation of split frequencies values of ∼0.01 or less; (ii) potential scale reduction factor values of 1.000 for all parameters; (iii) minimum effective sample size (ESS) values > 2000; and (iv) similar harmonic mean likelihoods between the two runs.

We also ran a second MRBAYES analysis, similar to the above, except that rather than assigning a fixed substitution model to each partition we sampled across model space (lset nst = mixed) for each partition. This yielded results almost identical to the first analysis, with respect to tree topology and node support, so we present the results of the first (fixed model) analysis only.

Divergence dating

We conducted divergence dating with BEAST 2.3.0 (Bouckaert *et al.,* 2014) on a data matrix from which outgroups had been excluded. Because of concerns that partitioned analyses can result in spurious precision of divergence time estimates (Dos Reis *et al.,* 2014; Zhu *et al.,* 2015), we treated this as a single partition to which we applied the GTR + G model. We chose an uncorrelated lognormal relaxed-clock model with a Yule birth process. Three fossil-calibrated nodes were assigned lognormal priors on age (Table 2), while the root node (Amblyoponinae) was assigned a diffuse normal prior with a mean of 90 Ma and a 95% highest probability density (hpd) of 70–110 Ma, to capture the range of prior estimates appearing in the literature (Brady *et al.,* 2006; Moreau & Bell, 2013; Schmidt, 2013). Based on the results of previous ML and Bayesian analyses *Apomyrma* was constrained to be sister to all other amblyoponines. We ran BEAST for 50 million generations, sampling every 5000 generations. Examination of log files with TRACER v1.6 (Rambaut *et al.,* 2014) indicated that stationarity had been achieved by five million generations (all ESS values > 700), so we employed a burn-in of 10%.

Biogeographic inference

We investigated range evolution in amblyoponine ants with the dispersal–extinction–cladogenesis model of LAGRANGE v20130526 (Ree & Smith, 2008). We coded each terminal taxon according to its occurrence in six major biogeographic areas: Neotropical (T), Nearctic (N), Palearctic (P), Afrotropical (E), Indomalayan (O), and Australasian regions (A) (Cox, 2001). The input for this analysis was the ultrametric tree generated by BEAST, with branch lengths in units of time (Ma), and a matrix of distribution ranges of the terminal taxa (Table S3, Supporting

Table 2. Prior age distributions applied to three fossil-calibrated nodes for BEAST dating analysis.

Taxon (stem-group)	Offset	Median	95% quantile	Log (mean)	Log (SD)	Comments
Stigmatomma denticulatum group	42	55	80	2.57	0.65	*Stigmatomma groehni*, in Baltic amber, said to be member of *S. denticulatum* group (Dlussky, 2009)
Stigmatomma mystriops group	15	40	60	3.22	0.36	Undescribed species from Dominican amber, similar to *S. mystriops* (AntWeb CASENT0906275)
Prionopelta	15	40	60	3.22	0.36	Undescribed *Prionopelta* species from Dominican amber (Wilson, 1988)

Information). The adjacency matrix and dispersal rates (over two time periods: 0–50 and 50–110 Ma) were the same as those used in Ward *et al.* (2015), and the maximum ancestral range size was set to three areas.

Results

Phylogenetic relationships

Maximum likelihood and Bayesian analyses yielded similar tree topologies, with comparable branch support (Fig. 2). The Amblyponinae are part of a poneroid clade that is sister to the formicoids. Leptanillinae and *Martialis* fall outside these two groups. One of the more striking findings concerns the placement of *Opamyrma*. It is recovered as sister to Leptanillinae and the clade comprising these two taxa has very strong support: Bayesian posterior probability (PP) of 1.00 and ML bootstrap support (MLBS) of 100%. The (Leptanillinae + *Opamyrma*) clade is well separated from the Amblyponinae, with the latter being more closely related to other poneroids and even to the formicoid clade than to leptanillines (Fig. 2). With *Opamyrma* removed, the Amblyponinae are monophyletic (PP = 1.00, MLBS = 92%), with *Apomyrma* (PP = 1.00; MLBS = 100%) sister to the remaining taxa (PP = 1.00, MLBS = 65%). These remaining taxa are further divided into two distinct clades, POA and XMMAS, each with maximum support (PP = 1.00, MLBS = 100%).

The POA clade comprises *Prionopelta*, *Concoctio*, *Onychomyrmex* and *Amblyopone*. The last two genera, both confined to the Australian biogeographic region, are recovered as sister taxa (PP = 1.00, MLBS = 94% for the combined clade). *Concoctio*, a monotypic African genus, appears to be nested within, or at least closely related to, the tropicopolitan genus *Prionopelta*. This result motivates synonymy of *Concoctio* under *Prionopelta* (see later).

Most amblyoponine diversity resides within the XMMAS clade, containing species assigned to the genera *Xymmer*, *Myopopone*, *Mystrium*, *Adetomyrma* and *Stigmatomma*, as well as *Bannapone* and *Paraprionopelta*. Most of these are monophyletic (PP 1.00, MLBS 100%) or monotypic, but the genus *Stigmatomma*, as currently defined, is highly artificial. It essentially encompasses all of the less derived members of XMMAS. The type species, *S. denticulatum*, is part of a well-supported group (PP = 1.00, MLBS = 100%) of Palearctic and Southeast Asian species that includes *Bannapone*. The XMMAS clade as a whole is divided into two very well-supported groups (PP = 1.00, MLBS = 100%): (i) a predominantly Neotropical clade, for which we resurrect the genus name *Fulakora* (see later); and (ii) all remaining taxa, here referred to as 'core XMMAS'. In contrast to the other parts of the amblyoponine phylogeny, the base of core XMMAS is very poorly resolved, manifested as a bush-like radiation of a dozen lineages – seven well-supported clades (PP = 1.00, MLBS = 100%) and five isolated species. This limits our ability to reconfigure the genus-level classification of the subfamily.

Nevertheless, the XMMAS clade does include a number of well-supported subgroups with distinct biogeographic affinities. *Fulakora* appears to be largely restricted to the Neotropics, with a few species in New Zealand, eastern Australia, New Guinea and the Solomon Islands. Within core XMMAS there are subgroups of species occurring in the Afrotropics (*Adetomyrma*), Southeast Asia (*Myopopone*) or both (*Mystrium*, *Xymmer*).

Divergence dates and biogeographic history

The chronogram resulting from the BEAST analysis (Fig. 3) suggests that the subfamily Amblyoponinae is indeed an old clade, with a crown-group origin in the mid-Cretaceous, about 107 Ma (95% hpd, 93–121 Ma). Discounting the *Apomyrma* lineage, the remaining amblyoponines have an estimated crown age of 101 Ma (95% hpd, 88–115 Ma). The two sister groups, the XMMAS clade and the POA clade, have estimated crown origins of 73 Ma (95% hpd, 64–83 Ma) and 47 Ma (95% hpd, 38–57 Ma), respectively, indicating more extensive pruning of early lineages in the latter. Initial diversification of the 'core XMMAS' clade took place 50–60 Ma, in the late Paleocene/early Eocene.

Age estimates for individual genera are mostly in the range 15–20 Ma, bracketed by *Xymmer* (29 Ma; 95% hpd, 23–36 Ma) and *Adetomyrma* (10 Ma; 95% hpd, 6–14 Ma). The newly resurrected *Fulakora* is older, at 52 Ma (95% hpd, 43–62 Ma), while the remaining, paraphyletic assemblage of *Stigmatomma* species dates to 59 Ma (95% hpd, 53–66 Ma).

Ancestral range inferences suggest that the Paleotropics have been the predominant arena for amblyoponine diversification (Fig. 3), with an origin in the Afrotropical region. *Apomyrma*
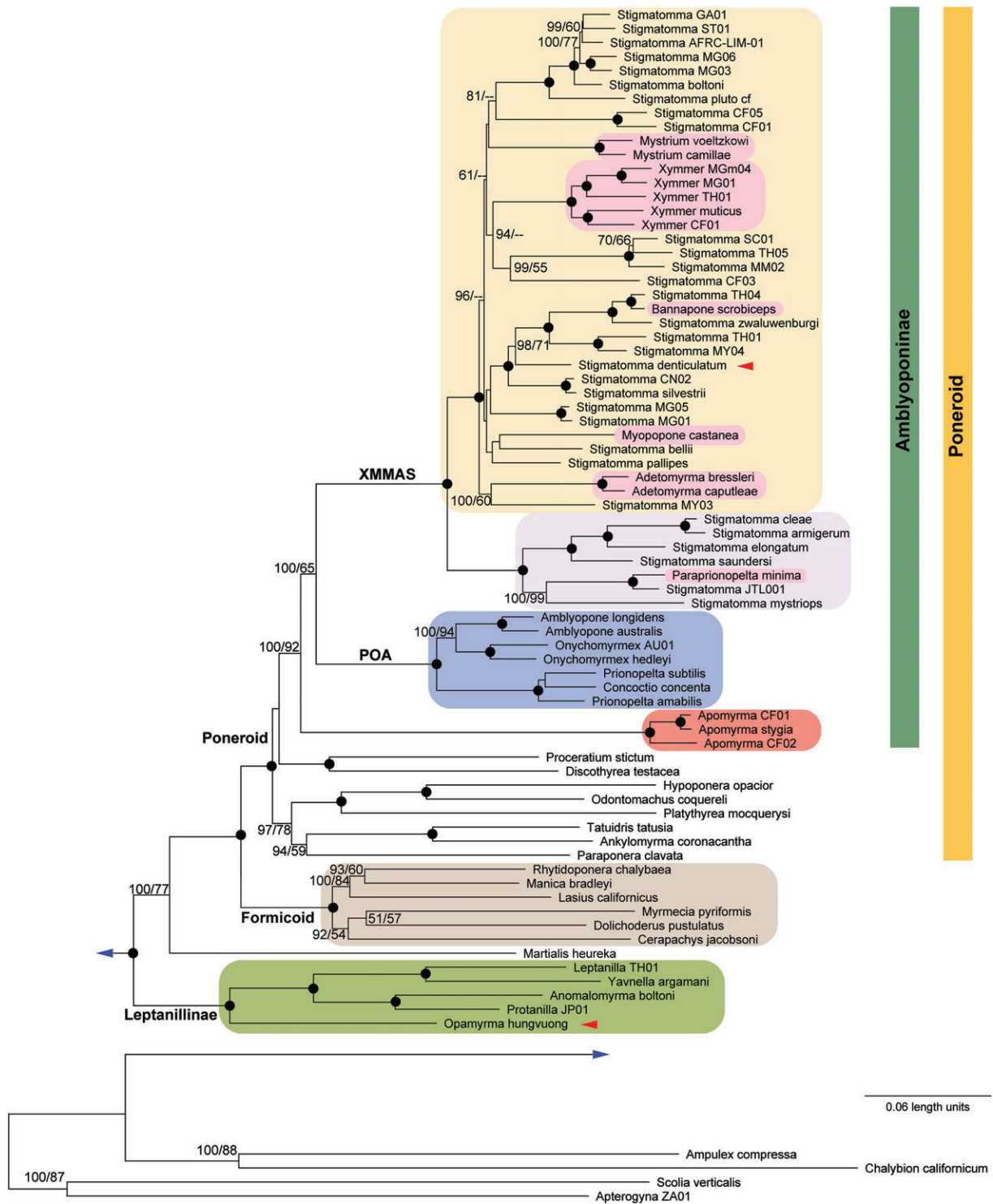
Fig. 2. Phylogeny of the ant subfamily Amblyoponinae and related taxa, based on 11 nuclear genes. This is the maximum likelihood (ML) tree found by GARLI (−ln L 113531.8202), with node support derived from separate Bayesian and ML bootstrap analyses. Support values >50 are indicated at each node as Bayesian posterior probability (PP) × 100, followed by ML bootstrap percentage (MLBS). Black dots signify support values of 100/100. Two taxa of particular interest are highlighted with red arrows: *Stigmatomma denticulatum*, the type species of *Stigmatomma*; and *Opamyrma hungvuong*, a species whose phylogenetic placement has been unclear. 'POA' and 'XMMAS' signify two major clades of Amblyoponinae. The subclade highlighted in grey, which is sister to all other XMMAS, corresponds to the genus *Fulakora*, newly resurrected in this study (see text). 'Poneroid' and 'Formicoid' refer to two major groups of ants, here both recovered as monophyletic.
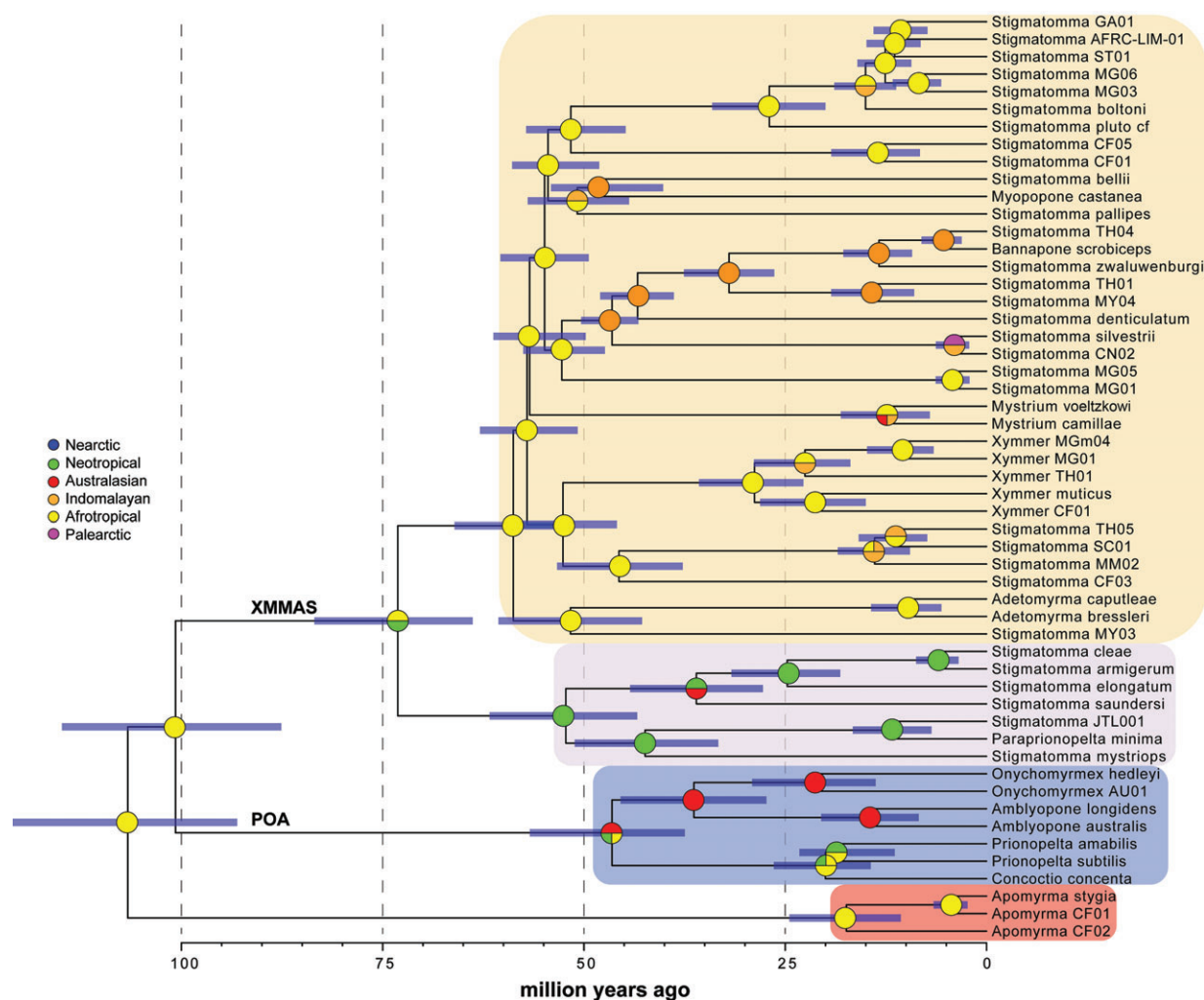
Fig. 3. Chronogram and biogeographic history of the Amblyoponinae based on BEAST2 and LAGRANGE analyses. Horizontal blue bars depict the 95% highest probability density of age estimates. Biogeographical analysis employed a dispersal–extinction–cladogenesis model, and six regions. The ancestral range inheritance scenario with the highest relative probability is indicated for the two descending branches of each node; the lower half of the sphere refers to the lower descending branch; the upper half to the upper descending branch. Most (75%) of the relative probabilities are greater than 0.60, but for some deeper nodes the most probable scenario has weak support and there are alternative scenarios of only slightly lower relative probability. Table S4, Supporting Information, provides a more detailed account of range inheritance scenarios for the major lineages of Amblyoponinae.

has remained confined to the African continent but the other amblyoponines dispersed (at a slow rate) to Indomalaya, the Palearctic, and the Neotropics. Such reconstructions have high probability for most branches, but there remains greater uncertainty in deeper parts of the tree (Table S4, Supporting Information).

Taxonomic changes

The new phylogeny presented here provides the basis for several taxonomic changes. We are conservative in these actions, proposing only modifications that are supported by results with strong statistical support in the tree (Fig. 2). For all generic and species names cited, the author, year, and original citation can be found in AntCat (2016). Taxonomic changes are given in tabular format in Table S5, Supporting Information.

Opamyrma is here transferred from Amblyoponinae to the subfamily Leptanillinae. Its position as sister to other Leptanillinae was recovered in all analytical treatments, with maximum support. In the original description of this genus (Yamane *et al.*, 2008), the authors noted similarities to Leptanillinae (shape of clypeus, configuration of metapleural gland orifice, and hypertrophied pygidium) and expressed some uncertainty about its placement in Amblyoponinae. Those reservations now appear to have been fully justified. The definition of subfamily Leptanillinae is hereby expanded to include *Opamyrma*, whose workers have a single-segmented waist (the petiole), in contrast to other Leptanillinae whose workers have a two-segmented waist composed of the petiole (A2) and postpetiole (A3).

Opamyrma (Leptanillinae) is separable from *Apomyrma* (Amblyoponinae), which also has a one-segmented nodiform waist, by the placement of the helcium. In *Apomyrma*, the helcium is attached low down on the anterior face of the first gastral segment (A3), while in *Opamyrma* the helcium is attached at mid-height, as in other Leptanillinae.

Based on its placement within *Prionopelta* in the ML tree (Fig. 2), the genus *Concoctio* is synonymized (**syn.n.**) under *Prionopelta*, and its sole species, *concenta*, is transferred to the latter genus (**comb.n.**).

Fulakora **stat.r., stat.n.** is resurrected from synonymy under *Stigmatomma* and elevated to genus (it was previously a subgenus) to contain the species in the predominantly Neotropical clade that is sister to all other XMMAS taxa (see earlier). There are only two XMMAS lineages in the New World, and they have complementary distributions: *Stigmatomma pallipes* and two related species in North America, and *Fulakora* in Central and South America. *Fulakora* is also present in Australia and adjacent islands, where it overlaps marginally with other XMMAS species. *Paraprionopelta* and *Ericapelta*, both described from Argentina, are newly synonymized (**syn.n.**) under *Fulakora*.

Fulakora can be distinguished from *Stigmatomma* as follows. In workers of New World *Stigmatomma* (i.e. *S. pallipes* and relatives), the frontal lobes and antennal sockets are widely separated by a broadly rounded or triangular section of clypeus, and the teeth along the mid-length of the mandible are fused at the base. In contrast, in New World *Fulakora* workers, the frontal lobes are usually fused or separated by a very narrow extension of the clypeus. If the frontal lobes are widely separated (e.g. *F. mystriops*), then the inner surface of the mandible has two separate rows of teeth. In Australasian *Fulakora*, the frontal lobes are fused, the anterior subpetiolar process (keel) has a distinct fenestra, and the mesonotum is generally separated from the propodeum by a metanotal groove. In Australasian *Stigmatomma*, the subpetiolar process lacks a fenestra and the mesonotum is not separated from the propodeum by a metanotal groove.

The following are new combinations (except where noted otherwise) in *Fulakora*: *agostii*, *armigera*, *bierigi* (**comb.r.**), *celata* (**comb.r.**), *chilensis* (**comb.r.**), *cleae*, *degenerata*, *egregia*, *elongata* (**comb.r.**) (including synonyms *elongata barretoi*, *elongata minor*, *elongata paranensis*), *exigua*, *falcata*, *gnoma*, *gracilis* (**comb.r.**), *heraldoi*, *lucida* (**comb.r.**), *lurilabes*, *minima*, *monrosi*, *mystriops*, *orizabana* (including synonym *tropicalis*), *papuana*, *punctulata* (**comb.r.**), *saundersi* (**comb.r.**), *smithi*, and *wilsoni*. All transfers are from *Stigmatomma*, except *minima*, which is transferred from the newly synonymized *Paraprionopelta*. The revived combinations refer to previous combinations with *Fulakora* as a subgenus.

During examination of Australasian *Stigmatomma* species, two species were found to belong to the POA clade. *Stigmatomma ferrugineum* (including synonym *mandibularis*) is reassigned to *Amblyopone* (**comb.r.**) based on the shape of clypeus, mandibular dentition and frontal lobes. *Stigmatomma glauerti* is transferred to the genus *Onychomyrmex* (**comb.n.**) based on the presence in the male of two apical mandibular teeth and in the worker of proximal frontal lobes and an elongate apical tooth on the mandible. As a consequence, the erstwhile monotypic genus *Lithomyrmex* (of which *glauerti* is the type species), which was previously a junior synonym of *Stigmatomma*, becomes a junior synonym (**syn.n.**) of *Onychomyrmex*.

Bannapone is newly synonymized (**syn.n.**) under *Stigmatomma*, on the basis that it is embedded within the clade that contains the type species of that genus, namely *S. denticulatum*. We cannot fully resolve the 'Stigmatomma problem' here, but no matter how the genus is ultimately redefined, it will almost certainly have to encompass *Bannapone* to avoid paraphyly of *Stigmatomma* (Fig. 2). Thus, although *Bannapone* has distinctive dentition (Eguchi *et al.,* 2015), it is properly interpreted as a specialized subset of taxa, nested within *Stigmatomma* (sensu stricto). This new synonymy results in the following transfers to *Stigmatomma*, all revived combinations unless otherwise noted: *caliginosum*, *crypticum* (**comb.n.**), *fulvidum*, *mulanae* (**comb.n.**), *pertinax*, *scrobiceps* (**comb.n.**), and *zwaluwenburgi*.

Discussion

Phylogenetic analyses based on 54 ingroup taxa and 11 nuclear genes reveal the principal divergence events within Amblyoponinae. *Opamyrma* is definitively excluded from the subfamily; the genus *Apomyrma* is sister to all other amblyoponines; and the latter split into the distinct and well-supported POA and XMMAS clades. Crown Amblyoponinae arose in the mid-Cretaceous, about 107 Ma, while the POA and XMMAS clades have estimated origins 47 and 73 Ma, respectively. The XMMAS clade comprises two well-supported groups: a predominantly Neotropical clade (*Fulakora*) and a poorly resolved bush of about a dozen lineages (core XMMAS). Diversification of the clades within this bush took place 50–60 Ma, in the late Paleocene/early Eocene. Morphologically distinctive elements in core XMMAS have been treated as separate genera (*Xymmer*, *Mystrium*, *Myopopone* and *Adetomyrma*), but many of the less divergent species have been placed in the catch-all paraphyletic genus, *Stigmatomma*. Our data are insufficient to allow resolution of basal relationships in core XMMAS and this also prevents full-scale reconfiguration of 'Stigmatomma' to ensure monophyly of all recognized genera in the subfamily. This will require more comprehensive taxon and gene sampling, as well as additional morphological analyses.

Phylogenomic data offer the promise of better resolution of the more intractable parts of the ant tree-of-life (Blaimer *et al.,* 2015). This includes the 'core XMMAS' radiation in the amblyoponine tree, and the sequence of branching events separating Amblyoponinae from other poneroids. Our ML tree recovers Proceratiinae as the sister group of Amblyoponinae, but without convincing support (Fig. 2). These poneroid divergences are old (mid-Cretaceous) and subtended by short branches; they may prove to be challenging to resolve, even with genome-scale data.

Ancestral range reconstruction indicates that amblyoponines originated in the Afrotropics, and dispersed to the Indomalayan,

Australasian and Neotropical regions (Fig. 3). For some of the more prominent, species-rich ant subfamilies, such as Formicinae and Myrmicinae, a New World origin is considered more likely (Moreau & Bell, 2013; Blaimer *et al.,* 2015; Ward *et al.,* 2015), although the subfamily Dolichoderinae appears to have arisen in the Paleotropics (Ward *et al.,* 2010). All such groups of dominant ants dispersed widely, however, and radiated extensively in other continental areas. This occurred throughout the Tertiary, accompanied by major innovations in nesting behavior, feeding biology and social organization. In contrast, there is no evidence that at any time in their long evolutionary history amblyoponines broke out of their cryptobiotic lifestyle, even during the period of rapid divergence of the 'core XMMAS' lineages 50–60 Ma. Retention of subterranean habits may have buffered these ants from above-ground drivers of extinction, especially competition with more aggressive ant clades. For the last 100 million years, it would seem, 'dracula ants' have stayed under the radar screen, nesting in cryptic locations, hunting specialized prey, and feeding on the haemolymph of their food-digesting larvae. Because of their elusive habits, many details of the biology and taxonomy of amblyoponines remain to be elucidated.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/syen.12186

Table S1. List of taxa sampled, voucher specimen codes, and GenBank accession numbers.

Table S2. Primers of three genes, DNApol-delta (POLD1), NaK ATPase (NaK) and Antennapedia (Antp), newly designed and employed in this study.

Table S3. Taxon distribution matrix, based on six biogeographic areas: Neotropical (T), Nearctic (N), Palearctic (P), Afrotropical (E), Indomalayan (O) and Australasian (A) regions.

Table S4. Range inheritance inferences for major clades of Amblyoponinae, based on LAGRANGE analysis.

Table S5. Summary of taxonomic changes in Amblyoponinae.

Acknowledgements

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