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Molecular phylogenetics and diversification of trap-jaw ants in the genera *Anochetus* and *Odontomachus* (Hymenoptera: Formicidae)

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ABSTRACT

Ants in the genera *Anochetus* and *Odontomachus* belong to one of the largest clades in the subfamily Ponerinae, and are one of four lineages of ants possessing spring-loaded "trap-jaws." Here we present results from the first global species-level molecular phylogenetic analysis of these trap-jaw ants, reconstructed from one mitochondrial, one ribosomal RNA, and three nuclear protein-coding genes. Bayesian and likelihood analyses strongly support reciprocal monophyly for the genera *Anochetus* and *Odontomachus*. Additionally, we found strong support for seven trap-jaw ant clades (four in *Anochetus* and three in *Odontomachus*) mostly concordant with geographic distribution. Ambiguity remains concerning the closest living non-trap-jaw ant relative of the *Anochetus* + *Odontomachus* clade, but Bayes factor hypothesis testing strongly suggests that trap-jaw ants evolved from a short mandible ancestor. Ponerine trap-jaw ants originated in the early Eocene (52.5 Mya) in either South America or Southeast Asia, where they have radiated rapidly in the last 30 million years, and subsequently dispersed multiple times to Africa and Australia. These results will guide future taxonomic work on the group and act as a phylogenetic framework to study the macroevolution of extreme ant mouthpart specialization.

1. Introduction

Ants are one of the most abundant insect groups on the planet and occupy virtually every niche in terrestrial ecosystems (Hölldobler and Wilson, 1990; Lach et al., 2010). The evolutionary success of ants has been accompanied by the morphological diversification and specialization of their mandibles, which, along with other adaptations, allowed the family to utilize a wide variety of environmental resources. Some examples of ant mandible specialization are elaborate, including pitchforks, sickles, hooks, grinders, shears, and fangs (Wheeler, 1927; Gotwald, 1969; Hölldobler and Wilson, 1990), despite being constrained by social functions such as nest excavation and brood care. This diversity in mandible morphology makes ants an excellent model for studying the evolution of specialized structures in eusocial organisms, but their usefulness depends on a stable classification and an accurate understanding of their evolutionary relationships. In recent years, molecular phylogenetics has led to major revisions of ant classification at many taxonomic ranks (Brady et al., 2006; Moreau et al., 2006; Wild, 2009; Ward et al., 2010, 2015; Branstetter, 2012; Blaimer, 2012; Moreau and Bell, 2013; Schmidt, 2013). In this study we use molecular phylogenetics to better understand the evolution of trap-jaw ants in the genera *Anochetus* and *Odontomachus*, whose spring-loaded mandibles are one of the most extreme examples of mandible specialization in insects.

Trap-jaw mandibles are characterized by a catapult mechanism that enables them to achieve record speeds and accelerations (Gronenberg, 1995; Gronenberg and Ehmer, 1996; Patek et al., 2006). They are long, linear, and insert close to the midline of the head, opening to an angle of 180° or more. A latch keeps the

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mandibles locked open even when the large mandible adductor muscle contracts, causing potential energy to be stored in the head and mandible. When a specialized "trigger muscle" unlocks the latch, this energy is released and the mandibles rapidly accelerate shut. Trap-jaw ants use these rapid jaw strikes for prey capture, nest defense, and even escape from predators.

Spring-loaded mandibles have independently evolved at least four times in the subfamilies Myrmicinae, Formicinae, and Ponerinae (reviewed in Larabee and Suarez, 2014), but ponerine trap-jaw ants are particularly interesting because of their species richness and ecological diversity. The genera *Anochetus* and *Odontomachus* are the second largest group of trap-jaw ants (114 and 67 described species, respectively (Bolton, 2014)) and are distributed in tropical and subtropical regions around the world. Nesting preferences vary widely within the clade, and include leaf litter, soil, rotten logs, and even arboreal nests (Raimundo et al., 2009; Cerquera and Tschinkel, 2010; Hart and Tschinkel, 2011; Camargo and Oliveira, 2012; Shattuck and Slipinska, 2012). Across the clade, there is considerable variation in head and mandible morphology, and body mass spans two orders of magnitude (Larabee, unpublished data). Like other trap-jaw ants, they are active predators (De La Mora et al., 2008; Dejean and Bashingwa, 1985), but they also have been observed tending hemipterans, collecting seeds, and scavenging (Ehmer and Hölldobler, 1995; Evans and Leston, 1971). In addition to using their rapid mandible strikes during predatory interactions, some species of *Odontomachus* also use their strikes for defense by "bouncing" intruders away from nest entrances (Carlin and Gladstein, 1989), and for escape by striking the substrate and jumping away from predators (Spagna et al., 2009; Larabee and Suarez, 2015). This ecological and morphological variation makes ponerine trap-jaw ants a good system in which to study the causes and consequences of mandible specialization.

Despite the attention that the behavioral ecology and biomechanics of *Anochetus* and *Odontomachus* have received, much about their evolutionary history remains unclear. The two genera clearly form a clade in the *Odontomachus* Genus Group (*sensu* Schmidt, 2013), being united by their synapomorphic spring-loaded mandibles. Numerous phylogenetic studies based on morphological and molecular data have also confirmed this grouping (Moreau et al., 2006; Brady et al., 2006; Spagna et al., 2008; Keller, 2011; Schmidt, 2013), but the relationship between and within each genus is still unclear. A preliminary unpublished molecular phylogeny of the two genera was unable to distinguish between a scenario where both genera are reciprocally monophyletic, and a scenario where *Odontomachus* is paraphyletic with respect to *Anochetus* (Schmidt, 2009). Contrasting with those results, morphological and karyotype data have suggested that *Anochetus* is paraphyletic with respect to *Odontomachus* (Brown, 1976; Santos et al., 2010). Additionally, several multi-gene phylogenetic analyses focusing on ant genus relationships each recovered four different sister groups to the *Odontomachus* + *Anochetus* clade (Brady et al., 2006; Moreau et al., 2006; Spagna et al., 2008; Keller, 2011; Moreau and Bell, 2013). Therefore, reconstructing the phylogenetic relationships of trap-jaw ants is a necessary first step in stabilizing their taxonomy and in exploring the origin and subsequent evolution of trap-jaw mandibles.

The goals of the present study are to use molecular phylogenetics to build a framework for understanding the evolution of trap-jaw mandibles in the genera *Anochetus* and *Odontomachus*. Specifically, we address whether *Anochetus* and *Odontomachus* are sister monophyletic genera, and clarify the species-level relationships within each genus. Also, to better understand how the specialized, spring-loaded mandible evolved from unspecialized mandibles, we attempt to identify the closest living non-trap-jaw ant relative in the *Odontomachus* Genus Group. Finally, using fossil calibrations, we estimate the timing and biogeography of trap-jaw ant diversification.

2. Materials and methods

2.1. Taxon sampling

Samples for phylogenetic analysis were selected to include as many of the *Anochetus* and *Odontomachus* species groups defined by Brown (1976, 1978) as were available, enabling coverage of as much of the morphological diversity as possible. Ethanol-preserved or point-mounted specimens were obtained from field collections, collaborators, and museum collections. Table A1 (see Appendix A) summarizes the identities and distribution of the sampled ingroup taxa, and includes 49 specimens of *Anochetus* and 43 of *Odontomachus*. This sample set comes from across the clade's worldwide distribution and represents approximately half of the named species in the group. Duplicates of several species were included (*A. mayri*, *A. graeffei*, *O. rixosus*, and *O. tyrannicus*) based on preliminary analyses that suggested they were not monophyletic (data not shown). Ants were identified primarily using the keys of Brown (1976, 1977, 1978), Sorger and Zettel (2011), and Shattuck and Slipinska (2012), and by comparison to a reference collection (Smithsonian Institution National Museum of Natural History, Harvard Museum of Comparative Zoology). In several cases, species identity could not be determined from existing keys and samples were either designated with "cf" for their morphological resemblance to described species or given a unique identifier.

Taxa for outgroup comparison were selected based on recent phylogenetic studies and a revision of the subfamily Ponerinae, with special attention to species within the *Odontomachus* Genus Group (Keller, 2011; Schmidt, 2013; Schmidt and Shattuck, 2014) (see Appendix A Table A2). This large clade includes 20 genera and displays a wide range of mandible morphology. The genus *Leptogenys*, for example, includes species with long linear mandibles that could reflect the ancestral condition of trap-jaw ants. Other genera, such as *Phrynoponera*, *Odontoponera*, or *Pseudoneoponera*, have more generalized triangular mandibles of varying lengths. In total, 42 non-trap-jaw ant species were included in the analyses as outgroups, with most sequences coming from previous studies (Schmidt, 2013). Specimens that were sequenced in this study have been deposited at the Smithsonian Institution National Museum of Natural History, or in the collection of their owner.

2.2. Gene sampling and molecular techniques

Using a strategy similar to Schmidt (2013), sequence data were generated for five gene fragments: the mitochondrial gene *cytochrome oxidase I* (COI); the three nuclear protein-coding genes *wingless* (Wg), *long-wavelength rhodopsin* (LWR, including introns), and *rudimentary* (CAD); and the nuclear large subunit ribosomal RNA gene (28S). These five genes were considered the CORE_DATA for subsequent phylogenetic analyses. To find the sister group of trap-jaw ants, sequences from three additional nuclear gene fragments were obtained from GenBank and included in the ALL_DATA matrix: *abdominal-A* (Abd-a), *elongation factor 1-alpha F1 copy* (EF1αF1), and *elongation factor 1-alpha F2 copy* (EF1αF2). All of these genes were selected to include both rapid (COI, introns) and slowly (28S) evolving sequences (Simon et al., 1994), and were also chosen based on their usefulness in resolving relationships in previous ant phylogenetic studies (Brady et al., 2006; Moreau et al., 2006; Wild, 2009; Ward et al., 2010, 2015; Branstetter, 2012; Blaimer, 2012; Moreau and Bell, 2013; Schmidt, 2013). The aligned and concatenated matrix of the CORE_DATA was 4812 bp in length

and 80% complete for the ingroup taxa. Sequence characteristics for each gene are listed in Appendix A (Table A3).

Genomic DNA was extracted from one leg of an adult worker using a DNEasy Blood and Tissue Kit (Qiagen Inc., Velencia, California) according to the manufacturer's protocol, and diluted in 150 μl nuclease-free water. Sequence fragments were amplified using polymerase chain reaction (PCR) using the primers listed in Table A4 (see Appendix A). PCR was performed in reaction volumes of 50 μl and contained 1 μl genomic DNA template, 100 nM primer (Integrated DNA Technologies, Coralville, IA), and 1× GoTaq DNA Polymerase Master Mix (Promega, Madison, WI). PCR conditions for all genes started with an initial melting step at 94 °C (5 min), followed by 10 cycles of 94° (30 s), 60 °C (30 s, decreasing by 1 °C per cycle), and 72 °C (30 s), followed by 30 cycles with the same conditions but an annealing temperature of 50 °C, and a final extension of 72 °C (5 min). PCR products were cleaned with ExoSAP-IT (USB Corporation, Cleveland, OH), and amplicon size was verified using gel electrophoresis and GelRed DNA Stain (Biotium, Hayward, CA). Cycle-sequencing reactions were performed using PCR primers and BigDye Terminator ver 3.1, and were analyzed on an ABI 3730 Sequencer (Life Technologies, Grand Island, NY) at the Laboratories of Analytical Biology at NMNH.

Sequences were assembled and edited in GENEIOUS v7.1 (Biomatters, Auckland, New Zealand) and aligned in MUSCLE v3.8 (Edgar, 2004). Ambiguously aligned regions of 28S and introns from CAD and LWR for outgroup taxa were removed from the data analysis. The sequences generated for this study are deposited in GenBank, and the aligned data matrix is deposited in TreeBase (ID18504 http://purl.org/phylo/treebase/phylows/study/TB2:S18504).

2.3. Phylogenetic analysis

Three methods were used to infer the molecular phylogeny of trap-jaw ants: partitioned Bayesian analyses (BI) in MrBayes 3.2.2 (Ronquist et al., 2012), fossilized birth-death model divergence dating (FBD) in BEAST 2.3.0 (Bouckaert et al., 2014), and partitioned maximum likelihood (ML) in RAxML 8.1.24 (Stamatakis, 2014). Analyses were run either on the CIPRES Science Gateway (Miller et al., 2010) or on the Smithsonian NMNH Topaz computing cluster.

The partitioning scheme and model of nucleotide substitution for the DNA alignment were simultaneously selected using PartitionFinder 1.1.1 (Lanfear et al., 2012). The concatenated alignment of 8 genes was first divided into 24 subsets by gene, expression pattern (exon, intron), and codon position. Bayesian Information Criterion was then used to select the optimal scheme of 11 partitions for the CORE_DATA matrix, and 15 partitions for the ALL_DATA (Table A5). The third codon position of COI (p3) was excluded from analysis because of saturation. Individual genes partitioned by codon position were also analyzed with BI to examine conflicts among gene trees.

MrBayes analyses were run with default priors, with the exception of the branch-length prior. To prevent artificially long branch-length posterior distributions, a compound Dirichlet prior was used (brlenspr = Unconstrained:GammaDir(1.0, 0.10, 1.0, 1.0)) (Brown et al., 2010; Zhang et al., 2012). Substitution models for each partition were set according to the results of the PartitionFinder analysis (Table A5). Base frequencies, substitution rates, and gamma shape parameters were unlinked across partitions, but topology and branch lengths were linked. Each analysis consisted of two simultaneous Markov Chain Monte Carlo (MCMC) runs with four chains per run for 10–40 million generations. The first 25% of sampled trees were discarded as burn-in. Several methods were used to confirm that the runs had converged: (1) MCMC analyses were run until the average split frequencies of standard deviation were below 0.01, (2) the potential scale reduction factor of all parameters were close to 1.00, (3) the effective sample sizes of all parameters were above 200, and (4) the likelihood and sample parameters were checked with Tracer 1.6 to confirm they had reached stationarity (Rambaut et al., 2014). All analyses were also run under the prior without sequence data to check for overly influential priors on the posterior probability distribution. Bayesian analyses were also run without substitution models selected *a priori* using reversible jump MCMC (rjMCMC) in MrBayes. This approach samples the posterior probability of all models in the GTR substitution family (Huelsenbeck et al., 2004). Trees were summarized as majority rule consensus trees in R 3.1.3 using the "ape" and "phytools" packages (Paradis et al., 2004; Revell, 2012; R Core Team, 2015).

Maximum likelihood analyses were conducted using the rapid bootstrapping algorithm (1000 replicates) combined with a ML tree search RAxML (Stamatakis, 2014). Both unpartitioned and partitioned analyses were performed on the CORE_DATA and ALL_DATA matrices, each with a GTR + Γ model of nucleotide substitution.

2.4. Hypothesis testing

To test support for the monophyly of trap-jaw ants and for different trap-jaw ant sister-group relationships, we used Bayes factors (BF) to compare the relative support for different topological models (Bergsten et al., 2013). In MrBayes, groups under question were constrained to be monophyletic with topology priors, then the marginal likelihood of each model was estimated using the stepping-stone sampling method (Xie et al., 2011). Two runs with four chains each were sampled for 20 million generations, with 2 million generations discarded as burn-in. The $\log_e$ BF statistics were calculated as:

$$\log_e \mathrm{BF}(M_0, M_1) = \log_e P(X|M_0) - \log_e P(X|M_1)$$

where $\log_e P(X|M_i)$ is the marginal $\log_e$-likelihood estimate for the model M_i. The strength of support for a given model was based on the interpretation of BF suggested by Kass and Raftery (1995). Values of $2\log_e$ BF between 0 and 2 were interpreted as no evidence for the alternative model, M_1, over the null model, M_0. When $2\log_e$ BF values were above 2, the alternative model M_1 was supported over the null model M_0, and values over 10 were considered very strong support for the alternative model.

2.5. Divergence time estimation

The timing of trap-jaw ant diversification was estimated by generating fossil calibrated trees in BEAST 2.3.0 (Bouckaert et al., 2012). We used an uncorrelated lognormal relaxed clock model with a fossilized birth-death (FBD) model tree prior to analyze both the ALL_DATA and CORE_DATA sequence matrices. Unlike more common node-calibration models that use influential *ad hoc* probability distributions for node age priors, the FBD process incorporates speciation rate (λ), extinction rate (μ), fossil recovery rate (ψ), and the proportion of sampled extant species (ρ), as parameters in a single comprehensive model for estimating node ages (Stadler, 2010; Heath et al., 2014). This provides a way to integrate fossil dates into the tree estimation process and potentially provides more accurate dating estimates than node-calibration methods.

Sixteen fossil species were incorporated into the BEAST analyses to calibrate the tree, including four *Anochetus* and three *Odontomachus* species (Table A6). Six fossils from four other extant genera were also included, as well as fossils from three putative stem ponerine genera (*Archiponera*, *Cephalopone*, and *Protopone*). Taxonomic sets were created for strongly supported clades found in the MrBayes analyses and fossils were placed in stem clades

to which they could be confidently assigned based on morphology. Prior distributions on age of monophyletic clades were not set (as is done in node calibration methods) because fossil ages are directly used in the FBD model to estimate the diversification, extinction, and fossilization process. The model was conditioned on the time of origin, or the start of the diversification process that produced the observed tree. Previous estimates of the age of stem Ponerinae range from 94 to 111 My (Schmidt, 2013). We used a Lognormal (3.66, 0.5) distribution with an offset of 59 (the age of the oldest fossil in our dataset), for the prior on time of origin.

Two clock models were used, one linking the mitochondrial gene partitions (p1 and p2) and a second linking the nuclear gene partitions (p4–p14). The hyperpriors estimating the mean and standard deviation for both clock rates were given Exponential(1) and Exponential(0.333) distributions, respectively. Other parameters in the fossilized birth-death process (λ, μ, ψ) were reparameterized as net diversification prior (λ-μ), turnover (μ/λ), and sampling proportion ($\psi/(\mu+\psi)$). The prior on turnover was set to a Uniform(0, 1) distribution, and a Beta(2, 2) distribution was used for sampling proportion prior. The net diversification prior was set to a Normal(0.06, 0.01) based on previous estimates of diversification rate (Moreau and Bell, 2013) and our analyses of taxon sampling (see below and Fig. C2). Sampling proportion, ρ, was set to 0.5 based on the number of described species of *Anochetus* and *Odontomachus* included in our analysis (Bolton, 2014).

Two independent analyses were run, each composed of four MCMC chains. Each run had a length of 200 million generations and parameters were sampled every 5000 generations. Convergence was assessed by confirming that parameter estimated sample sizes were above 200 and that parameter posterior probabilities had reached plateau in Tracer 1.6. The first 100 million generations were discarded as burn-in, and the maximum clade credibility tree was summarized in TreeAnnotator 2.2.1 (Drummond et al., 2012).

We investigated the effect of taxon sampling on the estimation of FBD model parameters. The ALL_DATA matrix was analyzed with four outgroup taxon sampling strategies in addition to the full set of 134 extant taxa and 16 fossil species (Table 4): Tribe Ponerini (131 extant taxa, 10 fossil species), Odontomachus Genus Group (122 extant species, 8 fossils), the Odontomachus Genus Group without *Leptogenys* + *Myopias* (110 extant species, 7 fossils), and just *Odontomachus* + *Anochetus* (93 extant species, 7 fossils). The priors for these analyses were identical to above with the exception of net diversification rate, which was given an Exponential (1) distribution.

Divergence date estimates from the FBD model were also compared with analyses using node calibration models. For these analyses, an uncorrelated lognormal relaxed clock model with a birth-death tree prior was used. The prior on birthrate was set to Uniform(0, 1000) and the relative death rate prior was set to Uniform(0, 1). Fossils from extant genera were used to calibrate the minimum ages of the stem (total) clades to which they could be assigned (Table A6). Exponential(2) distributions were assigned to each node with offsets set to the age of each fossil. The root node (Crown Ponerinae) was given a Normal(94, 10) prior which spans the 95% CI of the estimated age by Schmidt (2013).

Lineage-through time plots were constructed for a preliminary examination of trap-jaw ant diversification. We used the R package APE to generate LTT plots for our trap-jaw tree and simulate 1000 trees under a pure birth model for 95% confidence intervals of branching times.

2.6. Ancestral range reconstruction

The ancestral biogeography of trap-jaw ants was estimated using the likelihood-based program Lagrange v20130526, which implements a dispersal, extinction, and cladogenesis model (Ree and Smith, 2008; Ree et al., 2005). Because identical tree topology was recovered from multiple BEAST analyses, we used the maximum clade credibility tree from the FBD analysis with all outgroup species removed as input for the biogeography analysis. Each species of *Anochetus* and *Odontomachus* was assigned to one or more of five previously defined biogeographic regions (Cox, 2001): Neotropics, Nearctic, Afrotropics, Indomalaya (including southeast Asia and the Pacific Islands west of Wallace's Line), and Australasia (including New Zealand and New Guinea). The Palearctic biogeographic region was not considered in this analysis because only a few species of *Anochetus* are known from this region, none of which were sampled in our dataset. Historic migration between biogeographic regions was weighted with an adjacency matrix, which modeled the instantaneous transition between geographic ranges (Table A7). Dispersal between neighboring regions was weighted more favorably (1.0) than non-neighboring regions (0.5) or those separated by oceans (0).

3. Results and discussion

3.1. Phylogenetics

All analyses resulted in trees with essentially the same phylogenetic relationships, especially within the trap-jaw ant genera *Anochetus* and *Odontomachus* (Fig. 1, Table 1). The broad-scale topological features were retained across different phylogenetic inference methods, partitioning schemes, and gene sampling (see Appendix B). In the following discussion (unless otherwise noted), support values refer to the Bayesian posterior probabilities (BPP) of the MrBayes analysis of the CORE_DATA with an *a priori* specified substitution model and the bootstrap support (BS) values of the RAxML analysis of the CORE_DATA matrix.

Separate Bayesian analyses of the individual genes LWR, Wg, and CAD yielded results similar to the concatenated analyses, but with much lower support values (Appendix Fig. B4). Trees based on individual analyses of COI and 28S, however, had very different topologies from the concatenated alignment. Trees for 28S consisted mostly of polytomies with very low support values, especially within trap-jaw ants, consistent with this gene's slower mutation rate. The faster-evolving COI, on the other hand, produced trees with poor resolution among outgroup genera, but relatively similar species relationships within trap-jaw ant genera. In the analysis of COI, *Odontomachus* was found to be paraphyletic with respect to *Anochetus*, but this node had weak support (BPP = 0.59). Analyses of matrices that omitted either 28S or COI had much weaker resolution and support values (data not shown).

3.2. Monophyly and placement of trap-jaw ants

We found support for the *Odontomachus* Genus Group (BPP = 1.0, BS = 88) consistent with other previous molecular analyses (Moreau and Bell, 2013; Schmidt, 2013). Additional genes in the ALL_DATA set increased the support of this clade (BPP = 1.0, BS = 98), indicating an effect of gene sampling on support at deeper nodes. All genera were strongly inferred to be monophyletic. As in previous studies, relationships within the *Odontomachus* Genus Group were poorly supported at the genus level and consisted mostly of polytomies (Fig. 1).

Trap-jaw ants (*Odontomachus* and *Anochetus*) form a clade within the *Odontomachus* Genus Group with very strong support (BPP = 1.0, BS = 100). This clade is also strongly supported by previous morphological and molecular studies (Brady et al., 2006; Moreau et al., 2006; Spagna et al., 2009; Keller, 2011; Schmidt, 2013; Moreau and Bell, 2013). The sister-group relationship and reciprocal monophyly of *Odontomachus* and *Anochetus* are also

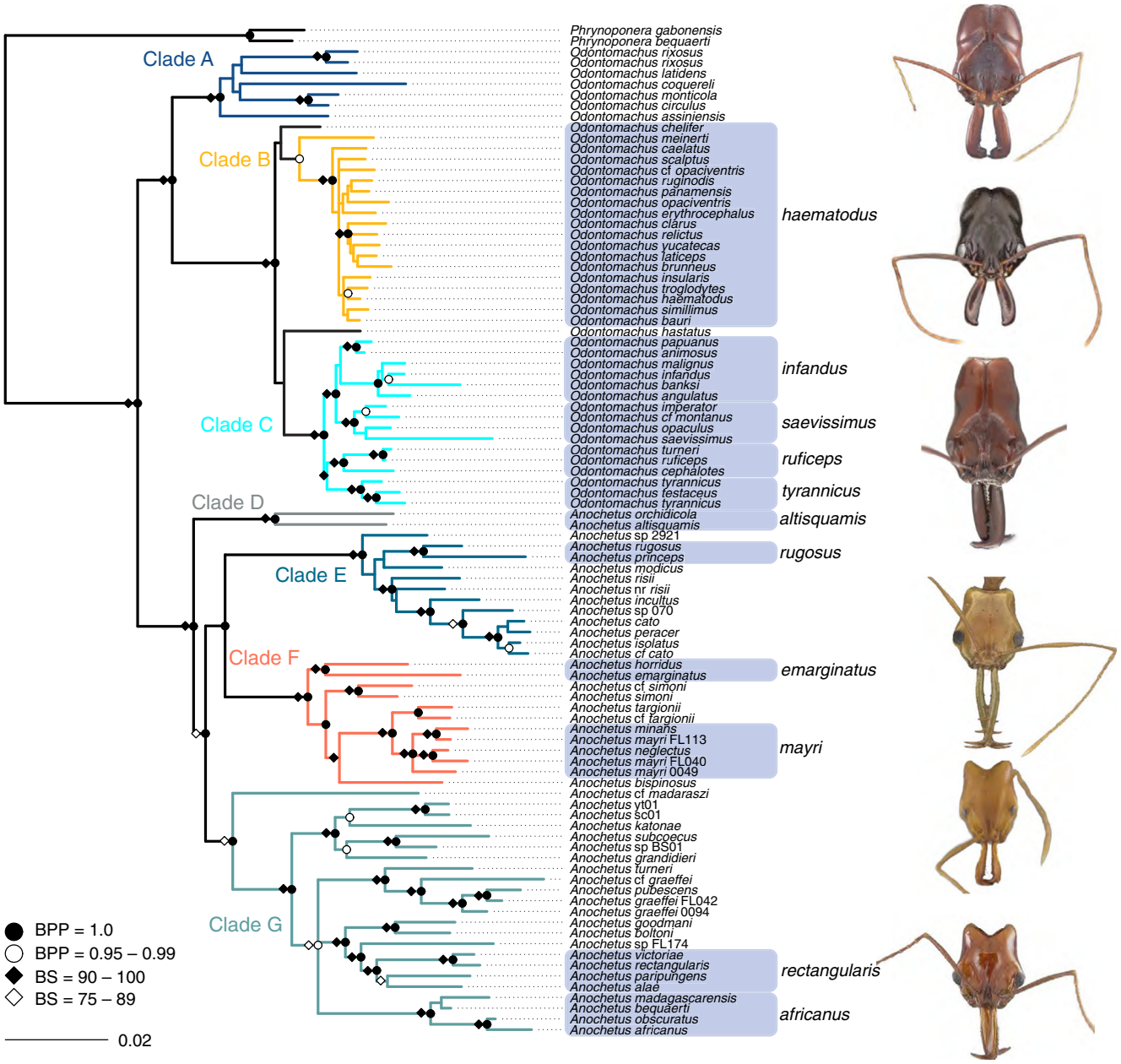

Fig. 1. Phylogeny of trap-jaw ants reconstructed from MrBayes. Majority-rule consensus tree for *Anochetus* and *Odontomachus* from a partitioned Bayesian analysis of the CORE_DATA matrix. Outgroup taxa have been removed with the except of *Phrynoponera*. Nodal support is given in both Bayesian Posterior Probabilities (BPP) and Maximum Likelihood Bootstrap Percentages (BS). Clades as described in text are indicated by branch color and letter (A–G). Morphologically inferred species groups from Brown (1976, 1978) that were recovered as monophyletic are marked with blue boxes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

strongly supported in all analyses (*Odontomachus*: BPP = 1.0, BS = 98; *Anochetus*: BPP = 1.0, BS = 99) except for those of the individual genes COI and 28S (Appendix A).

Hypothesis testing with Bayes Factors also strongly supported reciprocal monophyly of *Anochetus* and *Odontomachus* (Table 2). Constrained models that force monophyletic *Anochetus* and *Odontomachus* genera to be nested in a monophyletic *Anochetus* + *Odontomachus* clade had an estimated marginal $\log_e$-likelihood of −47148.98. Past analyses suggested that *O. coquereli* and close relatives (Clade A) may be sister to *Anochetus* (Schmidt, 2009), rendering *Odontomachus* paraphyletic. The marginal $\log_e$ -likelihoods of this constrained model and a model that unconstrained the relationship between Clade A, *Anochetus*, and the rest of *Odontomachus* were −47159.05 and −47210.44, respectively. The resulting $2\log_e$ BF comparing the M_0 ((Anochetus) + (Odontomachus)) to the alternative M_1 ((*Anochetus* + Clade A) + (Clade B + Clade C)) or ((*Anochetus*) + (Clade A) + (Clade B + Clade C)) were 20.14 and 122.92, respectively, strongly supporting the models constraining trap-jaw ant genera monophyly.

Our analysis failed to resolve the closest extant non-trap-jaw ant relative, and the inferred sister group relationships varied depending on gene sampling and analysis. Bayesian analysis of both CORE_DATA and ALL_DATA resolved *Phrynoponera* as sister to trap-jaw ants, but with weak support (BPP = 0.53, BPP = 0.58, respectively). The RAxML analysis of CORE_DATA resulted in a polytomy with trap-jaw ants as sister to all the other genera in the *Odontomachus* Genus Group (Fig. B3). The RAxML analysis of ALL_DATA, however, recovered *Pseudoneoponera* as sister to

Table 1
Summary of statistical support for different phylogenetic analyses. For MrBayes and rjMCMC analyses, support is reported in Bayesing posterior probability. For RAxML analyses, support is reported as Bootstrap Support.

Clade	MrBayes CORE_GENES	MrBayes ALL_GENES	rjMCMC CORE_GENES	rjMCMC ALL_GENES	raxml CORE_GENES	raxml ALL_GENES
Anochetus	1.00	1.00	1.00	1.00	99	100
Odontomachus	1.00	1.00	1.00	1.00	98	98
Anochetus + *Odontomachus*	1.00	1.00	1.00	1.00	99	100
Clade A	1.00	1.00	1.00	1.00	100	100
Clade B + *O. chelifer*	0.82	0.79	0.90	0.76	58	66
Clade B	0.99	0.98	0.97	0.98	100	100
Clade C + *O. hastatus*	0.94	0.93	0.99	0.82	69	66
Clade C	1.00	1.00	1.0	1.0	100	100
Clade D	1.00	1.00	1.00	1.00	100	100
Clade E	1.00	1.00	1.00	1.00	100	100
Clade F	1.00	1.00	1.00	1.00	100	100
Clade G	1.00	1.00	1.00	1.00	88	89

Table 2
Constraint models used in hypothesis testing. Clades constrained to be monophyletic are set in parentheses. Marginal log-likelihoods were estimated with stepping-stone MCMC in MrBayes and used to calculate Bayes factor.

Constraint model	Marginal $\log_e$ likelihood
((*Anochetus*) + (Clade A + (Clade B + Clade C))	−47148.98
((*Anochetus* + Clade A) + (Clade B + Clade C))	−47159.05
((*Anochetus*) + (Clade A) + (Clade B + Clade C))	−47210.44
((*Anochetus*) + ((Clade A) + (Clade B + *O. chelifer*) + (Clade C)))	−47152.46
((*Anochetus*) + ((Clade A) + (Clade B) + (Clade C + *O. chelifer*)))	−47158.67
((*Anochetus*) + ((Clade A) + ((Clade B) + (Clade C)) + *O. chelifer*))	−47162.00
((*Anochetus*) + ((Clade A) + (Clade B) + (Clade C + *O. hastatus*)))	−47155.44
((*Anochetus*) + ((Clade A) + (Clade B + *O. hastatus*) + (Clade C)))	−47156.11
((*Anochetus*) + ((Clade A) + ((Clade B) + (Clade C)) + *O. hastatus*))	−47158.58
(*Anochetus* + *Odontomachus*) + (*Phrynoponera*)	−47154.91
(*Anochetus* + *Odontomachus*) + (*Pseudoneoponera*)	−47161.06
(*Anochetus* + *Odontomachus*) + (*Leptogenys*)	−47179.83

trap-jaw ants (BS = 21, Fig. B3) with very weak support. When these two topological hypotheses were constrained during independent stepping-stone MCMC analyses (Table 2), the estimated marginal log-likelihood of the model constraining (*Odontomachus* + *Anochetus*) + *Pseudoneoponera* was −47161.06 and the model constraining (*Odontomachus* + *Anochetus*) + *Phrynoponera* was −47154.91. The $2\log_e$ BF test statistic comparing these two models was 12.3, favoring *Phrynoponera* as sister to the trap-jaw ants. When these two models were compared with the model constraining the long-mandible genus *Leptogenys* as sister to trap-jaw ants, the BF still supported *Phrynoponera* as sister to trap-jaw ants (Table 2). Additional taxa in the *Odontomachus* Genus Group and possibly additional molecular markers will be required to resolve these relationships with high confidence.

3.3. Genus Odontomachus

Odontomachus is a monophyletic genus with three strongly supported clades generally corresponding to their biogeography: (1) Clade A (BPP = 1.0, BS = 100) consisting of species in the Afrotropics and Southeast Asia, (2) Clade B (BPP = 0.99, BS = 100) consisting of species almost entirely found in Central and South America, and (3) Clade C (BPP = 1.0, BS = 100), with species located in Australasia. Two additional species, *O. chelifer* and *O. hastatus*, were weakly associated with Clades B and C, respectively (see below). Analyses of all concatenated datasets found Clade A to be sister to Clade B + Clade C. Many of the species groups defined by Brown (1976) were recovered as monophyletic, in particular the *tyrannicus*, *saevissimus*, *ruficeps*, and *haematodus* groups (Fig. 1).

Clade A was recovered as six species found in Africa, Madagascar, and continental Asia displaying significant morphological variation. The Malagasy species *O. coquereli*, for example, is particularly distinctive having well-developed subapical teeth, conical head lacking any temporal prominences, and long petiole. Other species, such as *O. assiniensis* or *O. rixosus*, are more typical of the genus, with finely serrate teeth along the mandibular border, well-developed temporal prominences, and more node-like petioles. Despite Clade A having very strong support in this analysis, the relationships among species in the clade were not well resolved. The *rixosus* species group, for example, which Brown defined as *O. rixosus*, *O. monticola*, and *O. latidens* and suggested might be geographical variants of the same species, was found to be polyphyletic (Fig. 1), although with low support.

Clade B corresponds to the large *haematodus* species group complex (Brown, 1976). These species are joined morphologically by well-developed temporal prominences, relatively short mandibles with blunt apical teeth, node-like petioles, and labial palps with only 3 segments. The large species *O. chelifer* was recovered as sister to the rest of Clade B, consistent with Brown's (1976) morphological classification, but with relatively weak support (BPP = 0.82, BS = 58). Bayes factor testing favored *O. chelifer* being in Clade B over the alternative model of being grouped with Clade C ($2\log_e$ BF = 12.4, Table 2). Interestingly, the predominately Neotropical *haematodus* group also included the Afrotropical species *O. troglodytes* and the widespread Asian/South Pacific species *O. simillimus*. This grouping is consistent with Brown's morphological analysis and also suggests a complicated dispersal history (see below). Many of the relationships among the *haematodus* group species were poorly resolved by the analysis of the CORE_DATA, ALL_DATA and COI alone, possibly as a result of a rapid radiation or slow substitution rates in these markers.

Clade C is strongly supported (BPP = 1.0, BS = 100), and consists of several well-supported clades including the *ruficeps* (BPP = 1.0, BS = 97), *saevissimus* (BPP = 1.0, BS = 99), and *tyrannicus* species groups (BPP = 1.0, BS = 100). These species are distributed in Australia and Melanesia, and include some of the most morphologically diverse species in the genus (Fig. 1). Species in the *tyrannicus* group, for example, have relatively short heads and lack the temporal prominences that are characteristic of other species in the genus. The *saevissimus* group, on the other hand, have temporal prominences on their long conical heads, but have distinctive elongated petioles. This clade also includes the Australasian species in the *ruficeps* group (*O. cephalotes*, *O. ruficeps*, and *O. turneri*), which are the only species of *Odontomachus* to display significant worker body size polymorphism. *Odontomachus hastatus*, which

is widespread throughout the Neotropics, was consistently recovered as sister to Clade C, but with relatively low support (BPP = 0.94, BS = 69). The Bayes factor test did not support a sister relationship between *O. hastatus* and Clade C over Clade B (2 $\log_e$ BF = 1.2, Table 2), leaving the placement of *O. hastatus* still unresolved. The morphology of the head, petiole, and labial palps of *O. hastatus* closely resemble the *saevissimus* group, and Brown (1976) postulated a close relationship between *O. hastatus* and Old World *Odontomachus*. Inclusion of the Neotropical species *O. mormo* or *O. bradleyi*, which have 4-merous labial palps and head morphology similar to *O. hastatus* and the *saevissimus* group, could clarify the relationship between Clade C and other Neotropical *Odontomachus*.

In his revision of the genus, Brown (1976) considered ancestral mandibular traits of *Odontomachus* to consist of mandibles that are long relative to the head and long, sharp, apical teeth. These traits were also associated with the lack of well-defined temporal prominences. Species fitting this description include *O. coquereli*, *O. hastatus*, and members of the *O. tyrannicus* species group, ants found in both Clade A and Clade C. This may be evidence of parallel evolution in the two clades of more derived mouthpart and head traits.

3.4. Genus Anochetus

The genus *Anochetus* was also found to be monophyletic, and contained four well-supported deep divergent clades: (1) Clade D (BPP = 1.0, BS = 100), (2) Clade E (BPP = 1.0, BS = 91), a Neotropical Clade F (BPP = 1.0, BS = 100), and Clade G (BPP = 1.0, BS = 88). Similar to *Odontomachus*, many of the *Anochetus* species groups that had been inferred by morphology were recovered as monophyletic. Additionally, most species-level relationships were recovered with very high support.

All analyses of CORE_DATA recovered Clade D as sister to the rest of the *Anochetus*. This group contains just two species, *A. altisquamis* and *A. orchidocola* (the *altisquamis* species group), which range from southern Mexico to northern Argentina. These species are typical of the genus as a whole, being relatively small, robust ants that nest in leaf litter. Brown (1976) hypothesized that the most basal lineage of *Anochetus* were large epigaeic ants in the *gladiator* group, which he thought gave rise to the genus *Odontomachus.* Our taxonomic sample did not include any *gladiator* group species, but it is likely that large bodied *Anochetus* evolved multiple times independently in the genus. Among the remaining *Anochetus* clades, there was strong support for Clade G being sister to Clade E + Clade F.

Species found in Clade E are medium-sized ants that are found mostly in tropical forest habitats throughout the islands of Southeast Asia and Melanesia. It is comprised of members of the *risii, rugosus*, and *cato* species groups. Although the *rugosus* group was recovered as monophyletic, the sister relationship between *A. cato* and *A. peracer* renders the *cato* group paraphyletic. At the root of this clade is the undescribed species *Anochetus* sp. 2921, which is notable for having a large medial tooth on the inner margin of its mandible. The trait is uncommon in the genus and is not found in the rest of Clade E.

Clade F is a neotropical group consisting of the *emarginatus*, *mayri*, *inermis*, and *bispinosus* species groups. The clade displays a great deal of morphological and ecological variation, with large arboreal species such as *A. horridus* and *A. emarginatus*, and also tiny, hypogaeic species such as *A. minans* or *A. mayri*. The *inermis* species group was found to be polyphyletic, with a sister relationship between *A. targionii* and the *mayri* species group. The placement of *A. neglectus* and *A. minans* renders the species *A. mayri* paraphyletic. However, *A. mayri* displays a great deal of morphological variation, and has been hypothesized to contain two or more sibling species (Brown, 1978).

Like Clade F, Clade G is incredibly variable in terms of morphology; however, it is spread across the Afrotropics, Australasia, and throughout Southeast Asia. The Australian *rectangularis* and African *africanus* species groups were the only morphological groups identified by Brown recovered as monophyletic. Our analysis included several undescribed species, emphasizing the need for more collecting and revisionary work on the clade.

3.5. Divergence estimation and biogeography

Dating analyses of the ALL_DATA and CORE_DATA alignments in BEAST recovered the same phylogenetic relationships as the analysis in MrBayes (Fig. 2 and Appendix C), but arrived at different age estimates depending on gene sampling and analysis method (Tables 3 and 4). For the FBD analyses, the ALL_DATA matrix resulted in slightly older median age estimates than the CORE_GENES, but with significant overlap in the 95% highest posterior density (HPD). Node calibration (NC) analyses, in contrast, estimated very similar median ages, with completely overlapping 95% HPD. Analyses using FBD tree models were consistently younger than those using NC with little or no overlap in 95% HPD at deep nodes. For example, the FBD analysis of ALL_DATA found an age of 74.9 My (HPD: 61.1–88.9) for the *Odontomachus* Genus Group and 52.5 My (HPD 39.4–62.5) for the trap-jaw ant clade. The NC analysis of the same data, in contrast, recovered an age of 100.4 My (86.9–114.6) for the *Odontomachus* Genus Group and 72.1 (60.6–84.3) My for trap-jaw ants. The younger age estimates under the FBD model contrast with other studies that found that FBD models estimated *older* ages than NC analyses (Arcila et al., 2015). However, many factors influence diversification estimation including net diversification priors (Appendix C), extent of fossil sampling (Arcila et al., 2015; Hug and Roger, 2007), and change in model parameters over time (Zhang et al., 2016). The divergence dates estimated in our analysis of the ALL_DATA matrix under the FBD model closely match those found in Schmidt (2013). This is the tree that is discussed below unless otherwise noted.

Taxon sampling also influenced the estimation of FBD model parameters (Table 4). Decreasing the number of outgroup clades increased estimates of net diversification rate and sampling proportion, and decreased turnover. When only the *Anochetus* + *Odontomachus* clade was analyzed, the median net diversification rate was estimated to be 0.064, which is similar to the estimate found for the trap-jaw ant clade in a study of diversification rates across the entire family (Moreau and Bell, 2013). Variance in parameter estimates across analyses is not particularly surprising, as diversification, extinction rate would be expected to be heterogenous across the tree (Zhang et al., 2016), but the FBD model assumes the rates of these parameters are constant (Heath et al., 2014). The recovered age of trap-jaw ants varied among analyses, but not with any correlation to outgroups sampled. Predictably, when the net diversification rate parameter was fixed, age estimates negatively correlated with diversification rate (Appendix C).

The most recent common ancestor of *Anochetus* and *Odontomachus* originated in the early Eocene (median: 52.5 Mya; 95% HPD: 39.4–62.7 Mya), and the median crown group ages were estimated to be 45 My for *Anochetus* and 40 My for *Odontomachus*. The infrageneric clades are all much younger, having diversified between 11 and 49 Mya. The very short branch lengths of the *haematodus* species group of *Odontomachus*, in particular, suggests that it may have undergone a rapid radiation within the last 20 million years. The lineage-through time (LTT) analysis plot is shown in Fig. 3. Diversification in the trap-jaw ant clade fit a null pure-birth model of diversification with a constant birth rate of 0.06, but there is evidence of a shift in diversification rate approximately 25 Mya, coinciding with the radiation of most of the major trap-jaw ant clades (Fig. 2). However, LTT plots should be

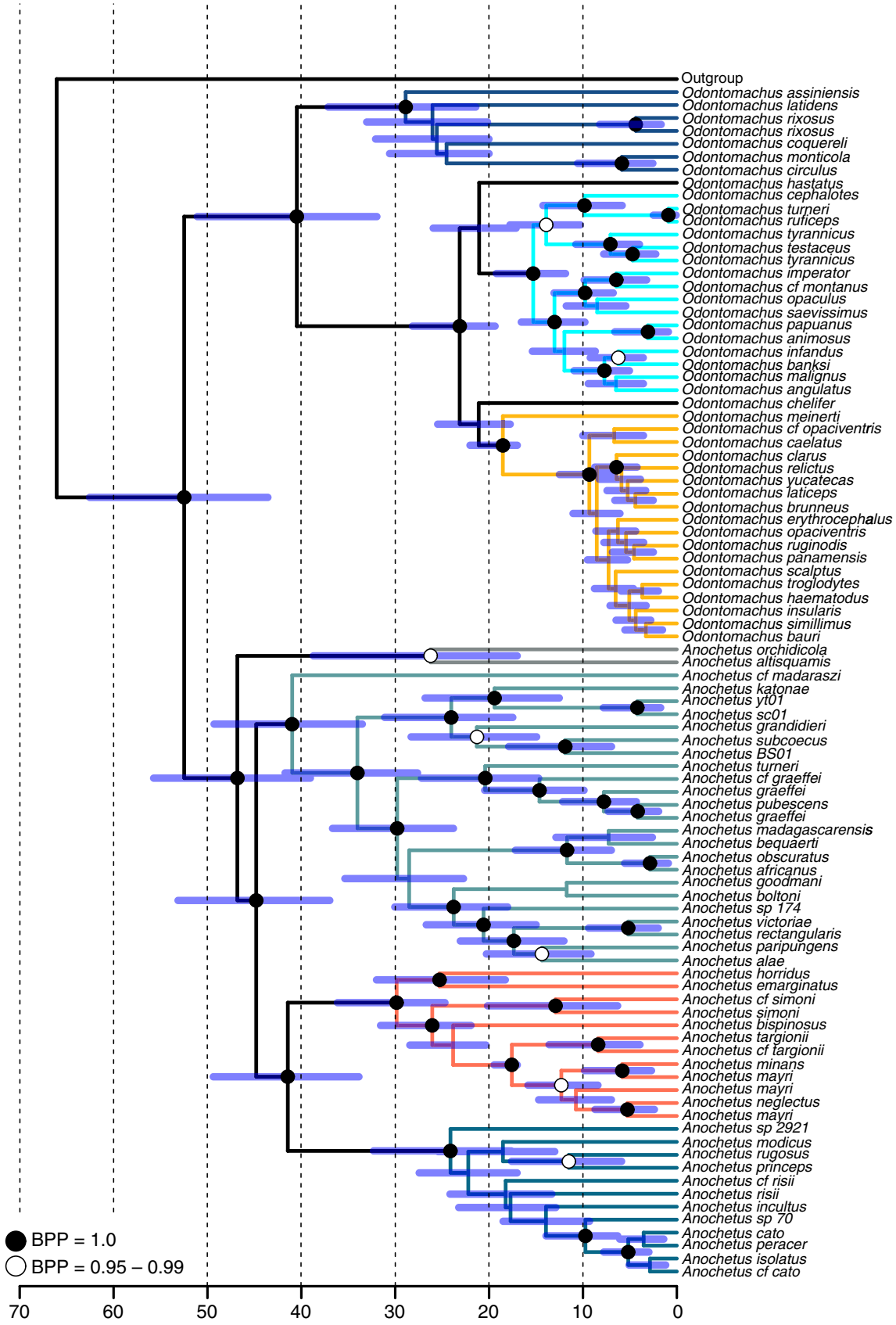

Fig. 2. Dated phylogeny of trap-jaw ants. Maximum clade credibility tree of *Anochetus* and *Odontomachus* resulting from the Fossilized Birth-Death analysis of the ALL_DATA matrix in BEAST. Mean node ages are illustrated with 95% highest density probability (blue bars). Node support is indicated with circles, with black circles having BPP $\geqslant$ 0.99 and white circles having 0.95 $\leqslant$ BPP < 0.99. Each highly supported clade is colored as in Fig 1. X-axis represents millions of years before present. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Crown-group age estimates for major clades in divergence dating analyses. FBD = Fossilized Birth Death model. HPD = Highest Posterior Density. Ages in millions of years (My).

	FBD ALL_DATA	FBD CORE_GENES	NC ALL_DATA	NC CORE_GENES
Crown Ponerinae	111.8 (90.7–136.7)	103.9 (84.5–125.5)	117.0 (103–130)	116 (102.6–131)
Odontomachus Genus Group	74.9 (61.1–88.9)	65.7 (54.4–78.7))	100.4 (86.9–114.6)	103.3 (88.8–118.9)
Trap-jaw ants	52.5 (39.4–62.7)	44.9 (37.3–53.3)	72.1 (60.6–84.3)	73.9 (61.3–86.3)
Odontomachus	40.5 (32.0–51.1)	35.6 (28.7–43.8)	54.8 (41.8–68.8)	55.5 (42.3–68.7)
Clade A	28.9 (21.4–37.1)	25.5 (20.1–31.9)	36.2 (24.0–50.1)	36.5 (43.1–36.2)
Clade B	18.5 (17.0–22.0)	13.1 (10.9–17.0)	25.5 (19.8–32.0)	25.6 (19.6–32.2)
Clade C	15.3 (11.9–19.2)	12.5 (10.2–16.5)	21.9 (17.2–27.2)	22.1 (16.9–27.9)
Anochetus	44.8 (39.0–55.7)	39.7 (33.9–47.2)	65.5 (55.6–77.1)	66.9 (55.1–78.6)
Clade D	26.2 (17.0–38.7)	23.3 (17–34.2)	34.0 (16.6–51.9)	34.6 (17.2–52.8)
Clade E	24.1 (17.6–32.3)	20.8 (17.0–29.2)	38.7 (31.2–46.8)	38.9 (30.8–47.5)
Clade F	29.8 (24.7–36.2)	24.1 (18.8–31.5)	39.7 (30.6–48.7)	40.1 (31.3–48.9)
Clade G	41.0 (33.5–49.3)	34.7 (28.3–41.7)	57.0 (46.9–67.4)	58.3 (47.5–69.9)

Table 4
Effect of taxon sampling on FBD parameter estimates. All values presented as medians and 95% highest probability density. Age of the most recent common ancestor of trap-jaw ants is given in millions of years.

Taxon sampling	Extant taxa	Fossil taxa	Net diversification	Turnover	Sampling proportion	Trap-jaw ant mrca
Subfamily Ponerinae	134	16	0.032 (0.019–0.046)	0.507 (0.213–0.772)	0.084 (0.015–0.200)	53 (40–62)
Tribe Ponerini	131	10	0.038 (0.022, 0.054)	0.228 (0.010–0.513)	0.221 (0.021–0.575)	62 (42–89)
Odontomachus Genus Group	122	8	0.044 (0.026–0.062)	0.137 (0.004–0.358)	0.291 (0.026–0.655)	63 (46–95)
Odontomachus Genus Group w/o *Leptogenys* + *Myopias*	110	7	0.052 (0.030–0.074)	0.111 (0.004–0.300)	0.322 (0.042–0.674)	72 (48–108)
Anochetus + *Odontomachus*	92	7	0.064 (0.040–0.089)	0.095 (0.003–0.242)	0.400 (0.075–0.746)	63 (45–88)

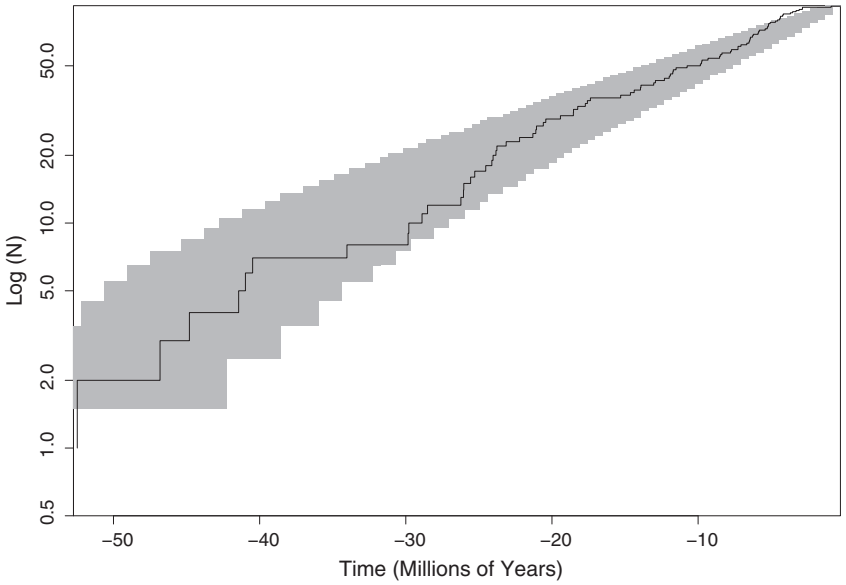

Fig. 3. Lineage diversification of *Anochetus* and *Odontomachus* clade. Lineage through time plot was generated with the dated tree from the BEAST analysis. The solid line represents the accumulation of trap-jaw ant species over time. The shaded area represents the 95% confidence interval of lineage diversification under a pure birth model with a $\lambda = 0.06$.

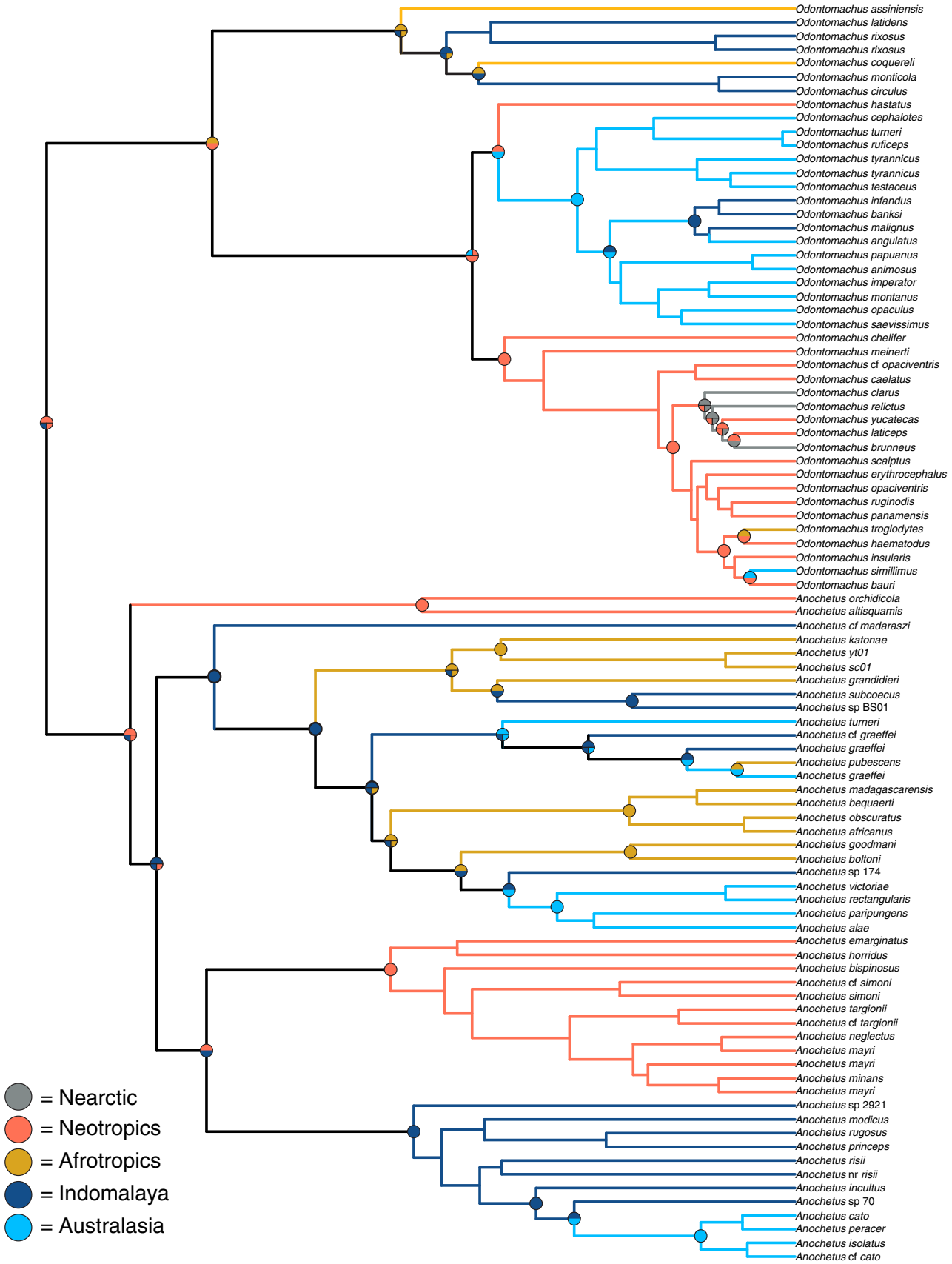

Fig. 4. Biogeography of trap-jaw ants inferred with lagrange. Maximum clade credibility tree from divergence estimation is presented with inferred ancestral nodes labeled. The upper and lower half of the colored circle indicates the inherited range for the upper and lower branch of the descending branch, respectively. Split halves, indicate that the descending branch inherits a range spanning multiple biogeographic regions. The x-axis is in millions of years before present. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 5
Ancestral range reconstruction from lagrange analysis. Inheritances are displayed as left|right splits where "left" and "right" are the ranges inherited by descendant upper and lower branches, respectively, on the dated phylogeny (Fig. 2). T = Neotropical, N = Nearctic, E = Afrotropical, O = Indomalayan, and A = Australasian.

Clade	Split	Rel. prob.
Odontoamchus + Anochetus	TO|T	0.814
Odontomachus	E|T	0.771
Clade A	EO|E	0.488
Clade A	E|E	0.423
Clade B	T|T	0.999
Clade C	A|T	0.956
Anochetus	TO|T	0.999
Clade D	T|T	0.998
Clade E	O|O	0.959
Clade F	T|T	0.996
Clade G	O|O	0.943

considered a preliminary examination of patterns of trap-jaw ant diversification because extant taxa have been sampled incompletely (Pybus and Harvey, 2000).

Results from the ancestral range reconstruction analysis lagrange are shown in Fig. 4 and Table 5. The majority of extant trap-jaw ant clades originated in regions that reflect their current distributions, mostly in the Neotropics or Indomalaya. The descendant branches of crown *Anochetus* are reconstructed as having inherited a Neotropical or Indomalayan distribution. Multiple lineages subsequently migrated to the Afrotropics and Australasia. Clade G, in particular, likely colonized Australia, Africa, and Madagascar multiple times. The ancestral range at the root of *Odontomachus* was ambiguous (no scenario had more than 0.77 relative probability), but the genus likely originated in the Neotropics or Afrotropics. There was at least one migration to Australasia and Indomalaya from the Neotropics, where Clade C radiated over the last 17 My. At least two subsequent dispersals to the Afrotropics and Indomalaya from the Neotropics occurred in Clade B with the lineages that lead to *O. troglodytes* and *O. similimus*. Resolving the relationship of *O. hastatus* to Clade B and C will be critical for correctly understanding the dispersal history of *Odontomachus* between the Old and New World. The range of the most recent common ancestor of all ponerine trap-jaw ants was also ambiguous, but was probably either Southeast Asia or the Neotropics, both of which have been shown to be important in the evolution of other members of the *Odontomachus* Genus Group (Schmidt, 2013). The New World Tropics, in particular, have been suggested to be essential to generating and maintaining ant biodiversity, in part because of the high diversity of plants in this region (Pie, 2016; Moreau and Bell, 2013). Increased sampling of extant trap-jaw ant taxa and closely related outgroups would likely clarify the relative importance of the New and Old World Tropics in trap-jaw ant diversification.

4. Conclusions

Molecular phylogenetic analyses confirmed that *Anochetus* and *Odontomachus* are monophyletic sister groups that evolved in the middle Eocene predominantly in Southeast Asia or the Neotropics. *Anochetus* and *Odontomachus* are composed of four and three well-supported clades, respectively, that colonized the rest of the world-wide tropics and subtropics multiple times. There is evidence that both genera have radiated rapidly in the past 15–20 My, especially in the Neotropics and Australasia. Of the 21 species groups that were previously defined morphologically (Brown, 1976, 1978), only 10 were recovered as monophyletic in our phylogenetic analyses. This emphasizes the need for additional phylogenetic analyses and taxonomic revisions for this and other ponerine genera. Although there is still some ambiguity about the identity of the closest living non-trap-jaw ant relative, it is most likely not a genus with long linear mandibles such as those in *Leptogenys*. Uncertainty therefore remains about the sequence of morphological changes that led to the evolution of trap-jaw ants.

This study is the first species-level molecular phylogenetic analysis of the trap-jaw ant genera *Anochetus* and *Odontomacus*. Genera in the subfamily Ponerinae have received much less attention from molecular systematists than the other three large ant subfamilies (Myrmicinae, Formicinae, and Dolichoderinae). Due to their combination of ancestral and derived traits, ponerine ant genera are excellent systems in which to study the evolution of eusociality and understand how specialized traits have contributed to the evolutionary success of ants. Trap-jaw ants, in particular, are a useful system to study patterns of morphological macroevolution because of their highly specialized, spring-loaded mandibles. Future studies should be able to use this phylogenetic hypothesis as a framework to answer questions about trap-jaw ant evolution in a phylogenetic context.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2016.07.024.

References

Arcila, D., Alexander Pyron, R., Tyler, J.C., Orti, G., Betancur-R, R., 2015. An evaluation of fossil tip-dating versus node-age calibrations in tetraodontiform fishes (Teleostei: Percomorphaceae). Mol. Phylogenet. Evol. 82 Pt A, 131–145.

Bergsten, J., Nilsson, A.N., Ronquist, F., 2013. Bayesian tests of topology hypotheses with an example from diving beetles. Syst. Biol. 5, 660–673.

Blaimer, B.B., 2012. Acrobat ants go global – origin, evolution and systematics of the genus *Crematogaster* (Hymenoptera: Formicidae). Mol. Phylogenet. Evol. 65, 421–436.

Bolton, B., 2014. An Online Catalog of the Ants of the World http://antcat.org (Retrieved 15 August 2015).

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C., Xie, D., Suchard, M.A., Rambaut, A., Drummond, A.J., 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. PLoS Comput. Biol. 10, e1003537.

Brady, S.G., Schultz, T.R., Fisher, B.L., Ward, P.S., 2006. Evaluating alternative hypotheses for the early evolution and diversification of ants. Proc. Natl. Acad. Sci., USA 103, 18172–18177.
Branstetter, M.G., 2012. Origin and diversification of the cryptic ant genus *Stenamma* Westwood (Hymenoptera: Formicidae), inferred from multilocus molecular data, biogeography, and natural history. Syst. Entomol. 37, 478–496.
Brown, J.M., Hedtke, S.M., Lemmon, A.R., Lemmon, E.M., 2010. When trees grow too long: investigating the causes of highly inaccurate Bayesian branch-length estimates. Syst. Biol. 59, 145–161.
Brown Jr., W.L., 1978. Contributions toward a reclassification of the Formicidae. Part VI. Ponerinae, tribe Ponerini, subtribe Odontomachiti. Section B. Genus Anochetus and bibliography. Stud. Entomol. 20, 549–652.
Brown Jr., W.L., 1977. A supplement to the world revision of *Odontomachus* (Hymenoptera: Formicidae). Psyche 84, 281–285.
Brown Jr., W.L., 1976. Contributions toward a reclassification of the Formicidae. Part VI. Ponerinae, tribe Ponerini, subtribe Odontomachiti. Section A. Introduction, subtribal characters. Genus Odontomachus. Stud. Entomol. 19, 67–171.
Carlin, N.F., Gladstein, D.S., 1989. The "bouncer" defense of *Odontomachus ruginodis* and other odontomachine ants (Hymenoptera: Formicidae). Psyche 96, 1–19.
Camargo, R.X., Oliveira, P.S., 2012. Natural history of the Neotropical arboreal ant, *Odontomachus hastatus*: nest sites, foraging schedule, and diet. J. Insect Sci. 12, 1–9.
Cerquera, L.M., Tschinkel, W.R., 2010. The nest architecture of the ant *Odontomachus brunneus*. J. Insect Sci. 10, 1–12.
Cox, C.B., 2001. The biogeographic regions reconsidered. J. Biogeogr. 28, 511–523.
De la Mora, A., Pérez-Lachaud, G., Lachaud, J., 2008. Mandible strike: the lethal weapon of *Odontomachus opaciventris* against small prey. Behav. Process. 78, 64–75.
Dejean, A., Bashingwa, E.P., 1985. La prédation chez *Odontomachus troglodytes* Santschi (Formicidae-Ponerinae). Insectes Soc. 32, 23–42.
Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29, 1969–1973.
Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucl. Acids Res. 32, 1792–1797.
Ehmer, B., Hölldobler, B., 1995. Foraging behavior of *Odontomachus bauri* on Barro Colorado Island, Panama. Psyche 102, 215–233.
Evans, H.C., Leston, D., 1971. A ponerine ant (Hym., Formicidae) associated with Homoptera on cocoa in Ghana. Bull. Entomol. Res. 61, 357–362.
Gronenberg, W., 1995. The fast mandible strike in the trap-jaw ant *Odontomachus*. I. Temporal properties and morphological characteristics. J. Comp. Physiol. A 176, 391–398.
Gronenberg, W., Ehmer, B., 1996. The mandible mechanism of the ant genus *Anochetus* (Hymenoptera, Formicidae) and the possible evolution of trap-jaws. Zoology 99, 153–162.
Gotwald Jr., W.H., 1969. Comparative morphological studies of the ants, with particular reference to the mouthparts (Hymenoptera: Formicidae). Cornell Univ. Agric. Exp. Station Mem. 408, 1–150.
Hart, L.M., Tschinkel, W.R., 2011. A seasonal natural history of the ant, Odontomachus brunneus. Insectes Soc. 59, 45–54.
Heath, T.A., Huelsenbeck, J.P., Stadler, T., 2014. The fossilized birth-death process for coherent calibration of divergence-time estimates. Proc. Natl. Acad. Sci. USA 29, E2957–E2966.
Hölldobler, B., Wilson, E.O., 1990. The Ants. The Belknap Press of Harvard University Press, Cambridge, MA, p. 732.
Huelsenbeck, J.P., Larget, B., Alfaro, M.E., 2004. Bayesian phylogenetic model selection using reversible jump markov chain Monte Carlo. Mol. Biol. Evol. 21, 1123–1133.
Hug, L.A., Roger, A.J., 2007. The impact of fossils and taxon sampling on ancient molecular dating analyses. Mol. Biol. Evol. 24, 1889–1897.
Kass, R.E., Raftery, A.E., 1995. Bayes factors. J. Am. Stat. Assoc. 90, 773–795.
Keller, R.A., 2011. A phylogenetic analysis of ant morphology (Hymenoptera: Formicidae) with special reference to the poneromorph subfamilies. Bull. Am. Museum Nat. History 355, 1–90.
Lach, L., Parr, C., Abbott, K. (Eds.), 2010. Ant Ecology. Oxford University Press, Oxford, p. 424.
Lanfear, R., Calcott, B., Ho, S.Y., Guindon, S., 2012. Partitionfinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol. Biol. Evol. 29, 1695–1701.
Larabee, F.J., Suarez, A.V., 2014. The evolution and functional morphology of trap-jaw ants (Hymenoptera: Formicidae). Myrmecol. News 20, 25–36.
Larabee, F.J., Suarez, A.V., 2015. Mandible-powered escape jumps in trap-jaw ants increase survival rates during predator-prey encounters. PLoS ONE 10, e0124871.
Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE) IEEE, pp. 1–8.
Moreau, C.S., Bell, C.D., 2013. Testing the museum versus cradle tropical biological diversity hypothesis: phylogeny, diversification, and ancestral biogeographic range evolution of the ants. Evolution 67, 2240–2257.
Moreau, C.S., Bell, C.D., Vila, R., Archibald, S.B., Pierce, N.E., 2006. Phylogeny of the ants: diversification in the age of angiosperms. Science 312, 101–104.
Paradis, E., Claude, J., Strimmer, K., 2004. APE: analysis of phylogenetics and evolution in R language. Bioinformatics 20, 289–290.
Patek, S.N., Baio, J.E., Fisher, B.L., Suarez, A.V., 2006. Multifunctionality and mechanical origins: ballistic jaw propulsion in trap-jaw ants. Proc. Natl. Acad. Sci., USA 103, 12787–12792.
Pie, M.R., 2016. The macroevolution of climatic niches and its role in ant diversification. Ecol. Entomol. 41, 301–307.
Pybus, O.G., Harvey, P.H., 2000. Testing macro-evolutionary models using incomplete molecular phylogenies. Proc. Roy. Soc. Lond. B: Biol. Sci. 267, 2267–2272.
R Core Team, 2015. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Australia.
Raimundo, R.L.G., Freitas, A.V.L., Oliveira, P.S., 2009. Seasonal patterns in activity rhythm and foraging ecology in the neotropical forest-dwelling ant, *Odontomachus chelifer* (Formicidae: Ponerinae). Ann. Entomol. Soc. Am. 102, 1151–1157.
Rambaut, A., Suchard, M.A., Xie, D., Drummond, A.J., 2014. Tracer v1.6. <http://beast.bio.ed.ac.uk/Tracer>.
Ree, R.H., Smith, S.A., 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and clodogenesis. Syst. Biol. 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.
Revell, L.J., 2012. Phytools: an R package for phylogenetic comparative biology (and other things). Methods Ecol. Evol. 3, 217–223.
Ronquist, R., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–542.
Santos, I.S., Costa, M.A., Mariano, C.S.F., Delabie, J.H.C., Andrade-Souza, V., Silva, J.G., 2010. A cytogenetic approach to the study of neotropical *Odontomachus* and *Anochetus* ants (Hymenoptera: Formicidae). Ann. Entomol. Soc. Am. 103, 424–429.
Schmidt, C., 2009. Molecular Phylogenetics and Taxonomic Revision of Ponerine Ants (Hymenoptera: Formicidae: Ponerinae) Dissertation. University of Arizona, p. 279.
Schmidt, C., 2013. Molecular phylogenetics of ponerine ants (Hymenoptera: Formicidae: Ponerinae). Zootaxa 3647, 201–250.
Schmidt, C.A., Shattuck, C.O., 2014. The higher classification of the ant subfamily Ponerinae (Hymenoptera: Formicidae), with a review of ponerine ecology and behavior. Zootaxa 3817, 1–242.
Shattuck, S.O., Slipinska, E., 2012. Revision of the Australian species of the ant genus *Anochetus* (Hymenoptera: Formicidae). Zootaxa 3426, 1–28.
Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., Flook, P., 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. 87, 651–701.
Sorger, D.M., Zettel, H., 2011. On the ants (Hymenoptera: Formicidae) of the Philippine Islands: V. The genus Odontomachus Latreille, 1804. Myrmecol. News 14, 141–163.
Spagna, J.C., Vakis, A.I., Schmidt, C.A., Patek, S.N., Zhang, X., Tsutsui, N.D., Suarez, A. V., 2008. Phylogeny, scaling, and the generation of extreme forces in trap-jaw ants. J. Exp. Biol. 211, 2358–2368.
Spagna, J.C., Schelkopf, A., Carillo, T., Suarez, A.V., 2009. Evidence of behavioral co-option from context-dependent variation in mandible use in trap-jaw ants (*Odontomachus* spp.). Naturwissenschaften 96, 243–250.
Stadler, T., 2010. Sampling-through-time in birth-death trees. J. Theor. Biol. 267, 396–404.
Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313.
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. Syst. Biol. 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). Syst. Entomol. 40, 61–81.
Wheeler, W.M., 1927. The physiognomy of insects. Q. Rev. Biol. 2, 1–36.
Wild, A.L., 2009. Evolution of the Neotropical ant genus *Linepithema*. Syst. Entomol. 34, 49–62.
Xie, W., Lewis, P.O., Fan, Y., Kuo, L., Chen, M., 2011. Improving marginal likelihood estimation for Bayesian phylogenetic model selection. Syst. Biol. 60, 150–160.
Zhang, C., Rannala, B., Ziheng, Y., 2012. Robustness of compound Dirichlet priors for Bayesian inference of branch lengths. Syst. Biol. 61, 779–784.
Zhang, C., Stadler, T., Klopfstein, S., Heath, T.A., Ronquist, F., 2016. Total-evidence dating under the fossilized birth-death process. Syst. Biol. 65, 228–249.