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Neotropical ant parasitoids (Hymenoptera: Eucharitidae): interpreting taxonomy, phylogeny and divergent morphologies

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Abstract. The Neotropical 'Kapala Clade' is a group of 13 described genera of ant parasitoids that includes some of the most morphologically bizarre members in the family Eucharitidae (Chalcidoidea). Across the Kapala Clade, various conspicuous morphological modifications – notably the long mesoscutellar spines – have led to the description of numerous genera. Monophyly of the clade has been supported in morphological and molecular analyses; however, relationships among genera within the clade are not easily resolved. Within this clade, there is one especially problematic taxon: *Kapala* Cameron is a common, diverse and easily recognizable genus, with species distributed across the Neotropical region north into the southern Nearctic, and one disjunct species found in the Afrotropical region. However, *Kapala* is defined by pleisiomorphic features – lacking the derived characters of the other Kapala Clade genera – with previous molecular evidence showing this genus to be paraphyletic. To evaluate relationships among Kapala Clade genera, we use a comprehensive sampling of 195 taxa and a molecular dataset of five gene regions. A morphological matrix of 52 characters was analysed both separately and combined with molecular data, which allowed rare genera to be incorporated into the phylogeny. Molecular results were at odds with the historic generic relationships, and the genus *Kapala* was rendered polyphyletic by two distinct clades of other kapaline genera. We find that there were bursts of morphological change clustered in the phylogeny; however, these are not correlated with higher rates of diversification. We discuss the timing of divergence events, analyse diversification patterns, and evaluate life-history information within a phylogenetic context.

Introduction

Eucharitidae (Hymenoptera: Chalcidoidea) is a unique family of insects in which all members are ant parasitoids (Heraty, 2002; Lachaud & Pérez-Lachaud, 2012). About one-quarter of the described genera are found in one monophyletic New World group termed the 'Kapala Clade' (Eucharitinae: Eucharitini) (Heraty, 2002; Murray *et al.*, 2013; Torréns, 2013). These are typically eye-catching specimens because adults in the Kapala Clade possess some of the most distinctive structures within Chalcidoidea – most prominent being the paired mesoscutellar processes (spines) projecting from the mesosoma (Fig. 1). The processes take on a variety of forms within the clade, and can be short (Fig. 1B), long and cylindrical (Fig. 1C, D, E, J, K, M, O), long and flattened (Fig. 1I, N), or long and forming a carapace (Fig. 1F, L). Most genera are distinguished based on spine morphology, although there are other, often major, phenotypic differences. Mesoscutellar spines are found in other members of Eucharitini, but in each case they are independently derived in these other generic groups (Murray *et al.*, 2013).

Eucharitids are parasitoids that essentially need to utilize two hosts: a plant host for oviposition, and an immature ant as host for larval development. Female eucharitids do not enter the ant nest; eggs are laid on appropriate host plants where they hatch into active, heavily sclerotized first-instar larvae called planidia.

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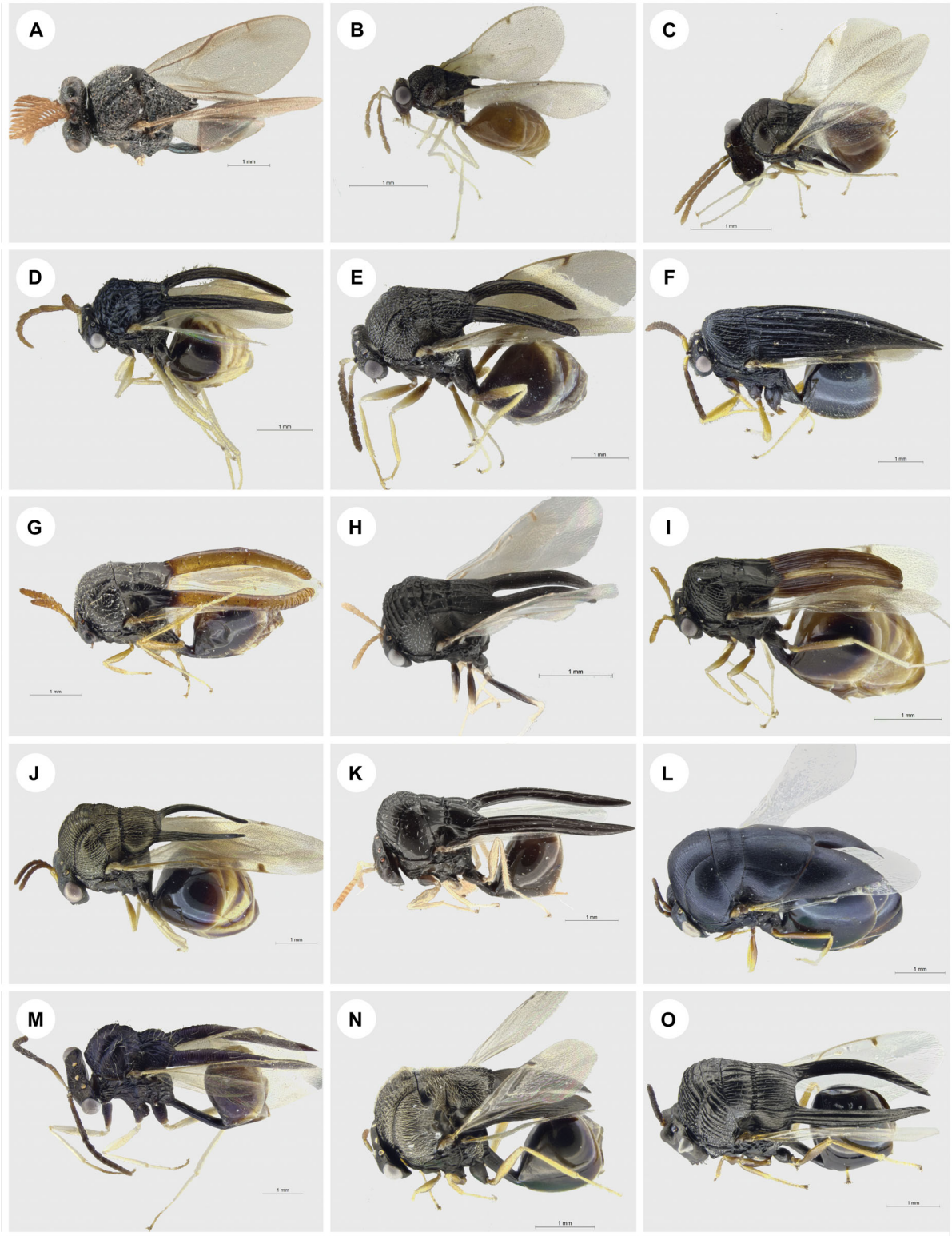

Fig. 1. Habitus images of the Kapala Clade genera and *Carletonia*, the related New World genus. All specimens are females except *Carletonia*, where only males are known. (A) *Carletonia*; (B, C) group 1: (B) *Colocharis*, (C) New Genus; (D–I) group 2, clade C: (D) *Neolirata*, (E) *Latina*, (F) *Thoracantha*, (G) *Lasiokapala*, (H) *Parakapala*, (I) *Dicoelothorax*; (J) *Kapala*, with *K. furcata* representing genus; (K–N) group 3, clade F: (K) *Liratella*, (L) *Galearia*, (M) *Lirata*, (N) *Dilocantha*; (O) *Isomerala*. All bars, 1 mm. [Colour figure can be viewed at wileyonlinelibrary.com].

The planidia must gain access to the host nest, either by phoresy on ants or on their prey items (Clausen, 1923; Clausen, 1940a, 1940b; Das, 1963; Wilson & Cooley, 1972; Carey *et al.*, 2012; Herreid & Heraty, 2017). Development is completed on the ant pupa within the nest (Lachaud & Pérez-Lachaud, 2012). Within the Kapala Clade, eggs can be laid in masses into flower buds or onto the undersurface of leaves, with a wide variety of host plant associations known (Torréns, 2013). Only two ant subfamilies, Ectatomminae and Ponerinae, serve as host ants for this clade (Lachaud & Pérez-Lachaud, 2012). Neither plant host nor ant host traits have been interpreted within a phylogenetic context. Trait reconstruction has not been feasible, in part, because relationships of genera within the Kapala Clade are uncertain.

Heraty (2002) used 88 morphological characters in an analysis of the genera of Eucharitidae and recovered a monophyletic New World Kapala Clade (Fig. 4, inset). Diagnostic characters of the clade include distinct anteclypeus, paired mesoscutellar processes (habitus images; Fig. 1), small lateral axillar lobes, frenal line demarking a lateral shelf, emarginate propodeal spiracles, and marginal vein of hindwing incomplete (Heraty, 1998, 2002). The New World genus *Carletonia* Heraty was recovered as the sister group to the Kapala Clade, based on what were recognized as homoplastic characters (Heraty, 2002). Overall, *Carletonia* shares little similarity with members of the Kapala Clade. Murray *et al.* (2013) found the Kapala Clade to be monophyletic using molecular data, and estimated that the Kapala Clade diverged *c*. 35 Ma (26–46 Ma) from its Old World sister group. The Kapala Clade is nested within a monophyletic group of poneromorph ant parasitoids (i.e. utilizing Ponerinae, Ectatomminae and Myrmeciinae ants) that includes the Chalcura and Schizaspidia Clades, both of which are restricted to the Old World. This topological relationship and monophyly of the Kapala Clade was again recovered in a parsimony analysis of molecular data by Heraty *et al.* (2015).

The Kapala Clade includes 13 genera, with *Kapala* Cameron being the most numerically abundant and diverse member. In the New World, 17 described species have a distribution that ranges from the southern U.S. to northern Argentina. A single species, *Kapala ivorensis* Risbec, occurs in the Afrotropical region, with its distribution considered to be the result of a recent dispersal event from the New World (Heraty, 2002; Murray & Heraty, 2016). While *Kapala* is easily recognizable, it cannot be defined by synapomorphies. It has been circumscribed based only on similarity in morphology (Heraty, 2002), and has never been recovered as monophyletic in molecular analyses of Eucharitidae (Heraty *et al.*, 2004; Murray *et al.*, 2013).

Across the Kapala Clade, extreme morphological modifications have led to the description of numerous genera. Head shape, antennal morphology, sculpture patterns, and spine morphology are particularly variable across the clade (Fig. 1). The extreme phenotypes are best exemplified by the paired spines that originated within the Kapala Clade. These can vary from the spines in *Kapala*, which are slightly curved and linearly carinate (Fig. 1J), to forms in other genera that vary from circularly striate to smooth in different species (*Lasiokapala* Ashmead and some *Lirata* Cameron), dorsoventrally flattened and narrowly separated (*Dicoelothorax* Ashmead and *Dilocantha* Shipp), to broadly arched and forming a carapace over the gaster (*Galearia* Brullé and *Thoracantha* Latreille). Sexual dimorphism is mostly confined to morphological differences in the antenna, metasoma and scutellar spine shape, with spines of the males much more reduced and more slender than those of females (*Parakapala* Gemignani, *Thoracantha*, *Dicoelothorax*, *Galearia* and *Dilocantha*) (Torréns *et al.*, 2016). Another of the more unusual nonsex-specific modifications is found in *Isomerala* Shipp, in which the eyes are conical-shaped and the frons has swollen protuberances. Within Hymenoptera, similar modifications of the eye are extremely rare, being found only in one extant member of Platygastroidea and one extinct member of Evanioidea (Jennings *et al.*, 2018). While many Kapala Clade genera are defined by unique features of the spines, the genus *Kapala* is recognized by its consistent phenotype across species and relatively invariable morphology in comparison to most other genera.

Using five gene regions and multiple analytical methods, we elucidate the relationships of the Kapala Clade genera using nearly 200 specimens spanning the entire geographic and taxonomic range of the clade. Disagreement in the phylogeny under morphological-based and molecular-based hypotheses motivated us to explore relationships using both types of data. All 13 genera are available for morphological coding, and 11 genera are available for molecular analyses. Understanding generic relationships in this clade is the foundation for interpreting biological data and morphological transformations across the group. We found that the monophyly of each of the genera of the Kapala Clade is supported, except for the paraphyletic *Kapala*. We also explore patterns of diversification to determine if specific clades which exhibited bizarre morphological phenotypes also had a higher rate of diversification. Molecular phylogenetic results are compared with morphology-based hypotheses of relationships, and we review life-history traits and behaviour in a phylogenetic context.

Materials and methods

Specimens and sequencing

The molecular matrix consists of a total of 195 specimens, with 189 from the Kapala Clade, representing an estimated 100 species (Table S1). Outgroup taxa are from the nearest eucharitid relatives in the New and Old World. Eleven of the 13 Kapala Clade genera are included in the molecular matrix with only *Parakapala* and *Liratella* Girault having no molecular data. We also lack molecular data for the putative Neotropical sister genus to the Kapala Clade, *Carletonia*. These three genera were included in the morphological character matrix.

Both alcohol and dried specimens were used for DNA extraction. Specimens were nondestructively extracted using a chelex-proteinase-K protocol (see SI of Murray *et al.*, 2013) or the DNeasy Blood and Tissue Kit (Qiagen, Germantown, MD, U.S.A.). We sequenced three nuclear ribosomal regions (18S, 28S-D2 and 28S-D3-D5) and two mitochondrial regions (COI and COII) with primers and PCR protocols following Murray

et al. (2013). Amplified DNA was purified using GeneClean (MP Biomedicals, Salon, OH, U.S.A.) and sequenced at the IIGB Genomics Core Facility at UC Riverside. SEQUENCHER 4.8 (Gene Codes Corp) was used to verify and edit chromatograms. Individual genes were aligned using the MAFFT v.7 online server (Katoh & Standley, 2013) under default settings. For nuclear ribosomal genes, the E-INS-i algorithmic strategy was applied, and for mitochondrial genes, the G-INS-i strategy was applied, with post-alignment manual modification if needed. Mitochondrial genes were translated to amino acids to verify that they were functional copies. Across the five gene regions, 473 sequences were deposited in GenBank, under the accession numbers KT336826–KT337298 (Table S2).

Molecular data matrices

Two datasets were used: 'all taxa' and 'full data' (Table S2). The 'all taxa' dataset (2942 nucleotides) includes 195 taxa – 189 Kapala Clade individuals and six outgroups. We included all genetic information that was available for all genes. The 'full data' dataset includes 96 taxa (2350 nucleotides) – 92 Kapala Clade individuals and four outgroups – with the target of minimizing gaps in the molecular matrix. Of the 96 taxa, 94 specimens had data for all five gene regions, and two genera had near-complete data (*Thoracantha*, no COI; all three *Lirata*, no COI or COII). We needed to use the *Thoracantha* as the only genus representative and wanted at least two *Lirata* to calculate a crown age for the genus. Additionally, the 18S and COI gene regions were trimmed on the 5′ and 3′ ends to further minimize gaps, because the sequences had been varying lengths. Utilizing incompletely sequenced genes may be detrimental to an analysis (Roure *et al.*, 2013), so our aim was to use the portion that was present for all taxa. All ribosomal genes and individual codon positions passed a test for base homogeneity using the χ^2 metric in PAUP* v.4.0a164 (Swofford, 2003).

Morphological character coding

Morphological characters were coded for use alone and in a combined matrix with the taxa with complete genes. The characters had three origins: (i) directly from Heraty (2002) or Heraty & Woolley (1993); (ii) modified from Heraty (2002); or (iii) developed here for the Kapala Clade. Of the 52 characters, 20 are from the head and antenna, 17 are mesosomal, six are of the gaster, and nine are of the leg and wing (Appendix S1; Table S3). Most terminals were coded by species to allow for polymorphism and to incorporate male and female characters. Several terminals were coded at the genus level [*Carletonia* (New World, unplaced outgroup to Kapala Clade), *Liratella*, and *Parakapala* (New World Kapala Clade), and outgroups *Austeucharis* and *Chalcura* (Old World Chalcura Clade), and *Ancylotropus* and *Schizaspidia* (Old World Schizaspidia Clade)] to account for polymorphisms and encompass a larger amount of outgroup morphological variation. There are a total of 99 taxa in the combined analyses.

Additionally, we have examined thousands of specimens over the course of this study and have digitized records in the internal Heraty laboratory database. These Kapala Clade specimen localities are mapped by genus in Fig. S1.

Phylogenetic analyses

Parsimony. TNT v.1.1 (Goloboff *et al.*, 2008a) was used for parsimony reconstruction using the New Technology Search. Gaps were treated as missing data. For all analyses, sectorial search, ratchet, tree drifting and tree fusing (10 rounds) were used. TNT was used for molecular, morphological and the combined molecular and morphological data. Implied weighting was used in the morphological and combined analyses to down-weight the most homoplasious characters using the concavity function ($k = 3$ or $k = 15$) (Goloboff *et al.*, 2008b). Extended implied weighting was used for the combined analysis to partition the data into two subsets (DNA and morphology) and apply the function separately to each (Goloboff, 2014). Branch support values are based on 1000 bootstrap replicates.

Maximum likelihood. For maximum likelihood analyses, on molecular data only, RAXML v.8.0.24 (Stamatakis *et al.*, 2008) was employed through the CIPRES interface (Miller *et al.*, 2010). Data were partitioned by gene region, and COI and COII were additionally split into positions 1 + 2 and 3, resulting in biologically-relevant subsets. Branch support was computed using 1000 rapid bootstrap replicates.

Bayesian additive. Bayesian phylogenetic inference was performed in MRBAYES (Ronquist *et al.*, 2012), using v.3.2.2 through CIPRES. An exponential distribution with a mean of 0.01 [exp(100)] was used instead of the default mean of 0.1 [exp (10)]. Individual gene trees from the 96-taxon dataset were estimated using reversible-jump Markov chain Monte Carlo (MCMC), so no substitution model needed to be designated *a priori*. Concatenated molecular analyses were run under a seven-partition scheme with the data subset by gene region, and COI and COII were additionally split into positions 1 + 2 and 3. For a combined analysis with morphological data, the latter scheme was used with the addition of a morphological block under the Markov *k*-state one-parameter (Lewis, 2001) with a gamma distribution to account for rate heterogeneity. Convergence of Bayesian analyses was assessed by confirming that the average standard deviation of split frequency of the two runs was <0.01 at completion and that the effective sample size of the posterior parameter distributions was >200 in Tracer v1.6 (Rambaut *et al.*, 2013).

Based on preliminary results, we aimed to identify taxa that potentially contribute to low branch support and reduce resolution in the tree. ROGUENAROK (accessed 2014) (Aberer *et al.*, 2013) was used to identify 'rogue' taxa that are placed in contradictory positions over multiple topologies. We used 10 000 trees from the 96-taxon dataset (taxa had all five genes), with a threshold majority-rule consensus tree and a maximum dropped set of two taxa.

Bayesian dating. There are no fossils within the Kapala Clade to calibrate internal nodes, so we used an estimate for the

stem age of the entire Kapala Clade from a prior study (Murray *et al.*, 2013). The previous fossil-calibrated dating analysis incorporated over 230 taxa across Eucharitidae and its sister group Perilampidae. For the current analyses, a normal distribution was set on the stem of the Kapala Clade based on the node age posterior probability density of previous results, using a mean at 35.6 Ma, and a sigma of 6 [to overlap the previous 95% highest posterior density (HPD) range]. The genes were partitioned with the trees and clock rates linked and the nucleotide substitution rates unlinked. The ribosomal data were partitioned by gene, and mitochondrial genes were partitioned into positions 1 + 2 and position 3 in the all taxa dataset. In the 'full data' dataset, the COI and COII were not split due to issues of convergence. The mutation rate of one gene, D2, was fixed (Drummond & Bouckaert, 2015), because analyses did not converge if this step was omitted. The add-on RBS v.1.1 (Bouckaert *et al.*, 2013) was loaded in BEAUTI v.2.2 (Bouckaert *et al.*, 2014) to allow for a reversible-jump MCMC in lieu of choosing each subset nucleotide substitution model. Taxon D2782 was removed from the analysis, because it had data for only one gene and appeared to be contributing to topologies not consistent with previous analyses. The Kapala Clade was constrained as monophyletic, with the tree built under a Yule process, and a diffuse gamma distribution set on the birth rate and the UCLD clock mean (alpha = 0.001, beta = 1000). BEAST v.2.2 (Bouckaert *et al.*, 2014) was run on a desktop computer, in conjunction with BEAGLE v.2.1 (Ayres *et al.*, 2012). Two chains were run to 100 million generations, sampling every 10 000. TRACER v.1.6 (Rambaut *et al.*, 2013) was used to confirm the ESS of the posterior probability distributions and was instrumental in diagnosing early problems of convergence. TREEANNOTATOR v.1.8 was used to obtain a maximum clade credibility tree (MCC) after removing burn-in.

Diversification, biogeography and life history

BAMM (Rabosky, 2014) was used estimate diversification rates. This program identifies configurations of rate shifts under both constant and time-varying diversification models, allowing the user to explore the most likely suite of rate changes across a phylogeny. There is ongoing discussion of the utility of BAMM for empirical data (Rabosky, 2016; Meyer *et al.*, 2018; Meyer & Wiens, 2018; Rabosky, 2018; Title *et al.*, 2019). Because there is not yet a resolution for the BAMM controversy, we proceeded with using the program, and suggest caution in interpreting the results. BAMMTOOLS (Rabosky *et al.*, 2014) was used for data interpretation and visualization.

Outgroup taxa were removed from the dated phylogeny of 194 taxa. The tree was then pruned to represent the number of species across the clade (e.g. only one tip was kept from 11 *K. ivorensis* tips). When trimming genera besides *Kapala*, values were obtained from current species counts (Heraty, 2017) and estimated species values (Heraty, 2002), with a consideration of the amount of phylogenetic diversity in the dated tree. For the many undescribed species of *Kapala*, we mainly relied on identifying tentative morphospecies while taking into account the age of the putative species. After pruning, 99 tips remained, and each was assigned a portion of the estimated species diversity of the clade. We used a clade-specific sampling fraction for all analyses (Table S4). We ran the analysis assuming 100% global sampling and 80% global sampling.

The normalized Colless index of imbalance (Colless, 1982) was used to test for tree balance, using the R package APTREESHAPE (Bortolussi *et al.*, 2006). The balance of a tree is the extent to which nodes define subgroups of equal size (Mooers & Heard, 1997) and imbalance potentially can indicate biased speciation (Blum & Francois, 2005).

We estimated ancestral geographic areas of the Kapala Clade. BIOGEOBEARS v.1.1.2 (Matzke, 2013; Matzke, 2018) was used to calculate optimal scenarios using the dispersal–extinction–cladogenesis model (DEC). The optional jump parameter for founder events was not used, due to recent evidence that it is incorrectly modelled (Ree & Sanmartín, 2018). Four areas were coded (Table S5): Nearctic, Central American (including southern Mexico and the Caribbean), Neotropical and Ethiopian (Murray & Heraty, 2016). Species were not allowed to occupy more than two areas at a time; no other constraints were set.

Additionally, MESQUITE (Maddison & Maddison, 2018) was used to trace trait histories onto a reduced phylogeny via parsimony. The combined analysis phylogeny was pruned to just the major groups of the Kapala Clade and outgroups, for genus-level representation (24 tips total). We used supraordinal plant groups of the APG IV system of classification (The Angiosperm Phylogeny Group *et al.*, 2016) to code host plants, and then we mapped associations, having data for eight of the 16 tips in the Kapala Clade. Additionally, the location of female oviposition on the plant was mapped to the phylogeny (Table S6). Ant host was also coded by subfamily and mapped (Table S7).

Results

Broad patterns across datasets. The Kapala Clade topology is distributed in three groups (Figs 2–4, S2–S9). Group 1 is sister to the rest of the Kapala Clade but recovered as alternatively paraphyletic or monophyletic, depending on analytical method. A sister-group relationship of group 2 and group 3 has support under most analyses. A summary of relationships is reported in the following, and support values across major clades from different analyses are in Table S8. We use letters to designate major clades of interest found consistently across trees.

Molecular. In all data permutations and analyses, the genus *Kapala* is rendered paraphyletic by all genera but the two in group 1. Gene trees each also clearly support a nonmonophyletic *Kapala* (Fig. S10). Individual genes have little phylogenetic signal overall, and we focus on the concatenated results. All non-*Kapala* genera having multiple taxa sampled are recovered as monophyletic under all concatenated molecular analyses and sampling regimes. Deeper divergences often have short internodes, typically with low support.

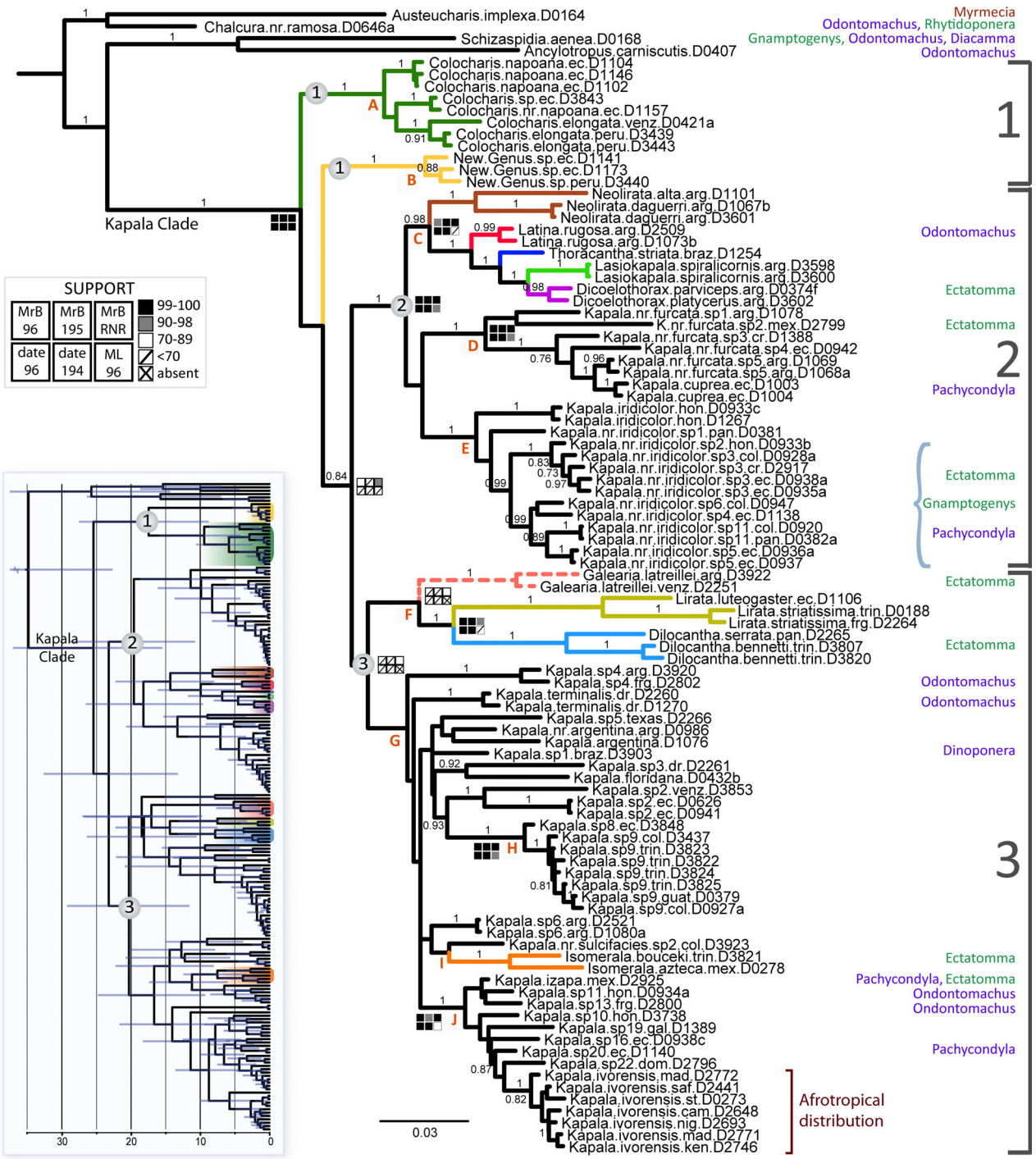

Fig. 2. Bayesian maximum clade credibility phylogeny from 96 taxa and five gene regions. Posterior probabilities are given above branches. Major clades have a summary of support shown from various analyses; with full phylogenies found in Figs S2–S9 and Table S8. The three major groups are labelled as 1, 2 and 3, and notable clades are identified by bold red letters. *Kapala* have black branches, and all other genera have coloured branches. Ant hosts are identified to the right of the tree. Hosts coloured green are Ectatomminae, and hosts coloured blue are Ponerinae. *Galearia* is shown with a dotted line due to uncertain placement. (Inset) Dated BEAST phylogeny using 194 taxa (complete phylogeny in Fig. S2). [Colour figure can be viewed at wileyonlinelibrary.com].

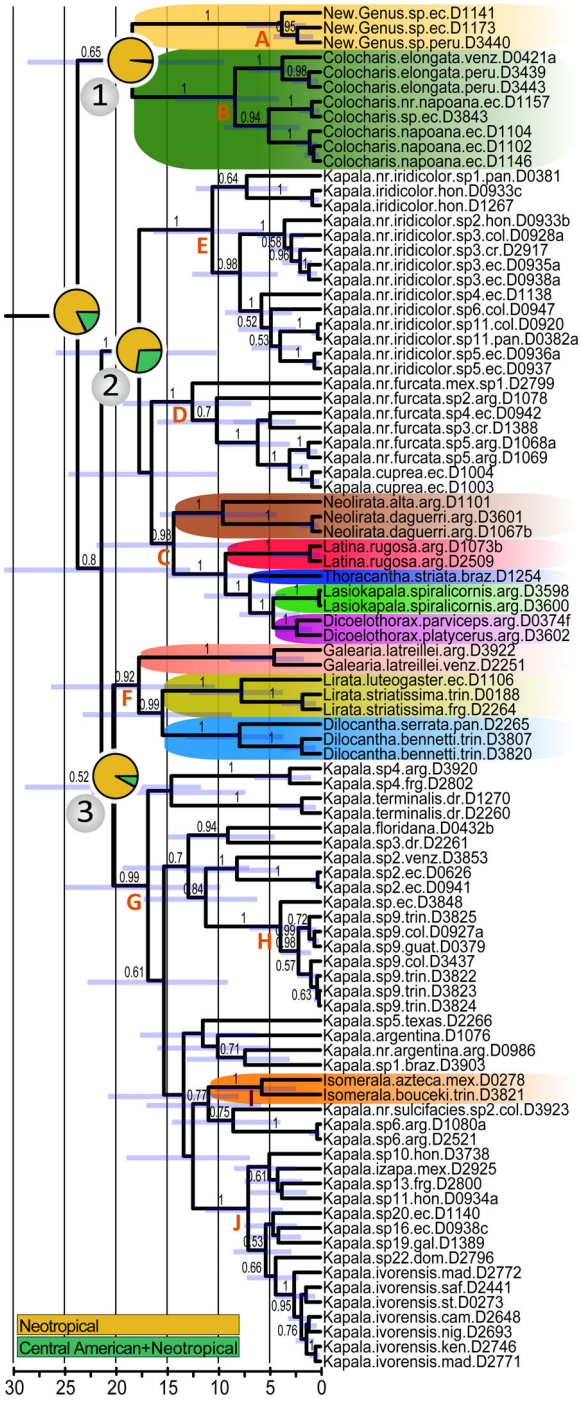

Fig. 3. Dated phylogeny of the 96 taxa with complete gene data. Non-*Kapala* genera are highlighted, and major clades are designated by letter with the three major groups labelled as 1, 2 and 3. Posterior probability values > 0.50 are shown. Node heights are median ages (in Ma); blue bars indicate the 95% highest posterior density values for the nodes. Ancestral geographic areas were calculated using a more species-rich dated phylogeny and are summarized here for the Kapala Clade and each major group, with the legend at the bottom of the tree (full historical biogeography results; Fig. S11). [Colour figure can be viewed at wileyonlinelibrary.com].

Most of the following discussion will focus on results from the Bayesian maximum clade credibility tree from the 'full data' matrix of 96 taxa (Fig. 2) and the 'all taxa' 194-taxon timetree of (Figs 3, S2). A previous analysis using three fossil-calibrated nodes gave an estimate of the crown age of the Kapala Clade of 23.3 Ma (16.8–30.9) (Murray *et al.*, 2013), and here, using a secondary calibration point for dating, we recover the crown age of the Kapala Clade at 23.6 Ma (95% HPD = 13.5–34.3) from the 'full data' 96-taxon dataset, or 25.4 Ma (15.5–37.1) from the 194-taxon 'all taxa' dataset. Node ages for major clades below will be reported from the results of the all taxa dataset (ages summarized in Fig. S12). Analyzing the dataset with the 10 rogue taxa removed resulted in increased support of most of the major clades (Fig. 2 node support grids, Fig. S9; Table S8). The ML trees tended to have variable topologies and low support for major clades (Figs S4, S5), so results and phylogenetic discussions will be centred on the Bayesian and parsimony topologies.

Morphological. A parsimony analysis of 52 morphological characters under implied weighting $k = 3$ resulted in three shortest trees of 331 steps, which are summarized as a strict consensus tree (Fig. S7). The retention index (RI), used to measure the character fit to the tree, was 0.75. In the five shortest unweighted trees, there were 323 steps (not shown). The enigmatic New World genus, *Carletonia*, is recovered within the Old World outgroup and is not recovered as the sister to the Kapala Clade. Group 1 is a grade to the rest of the Kapala Clade. The non-*Kapala* genera from groups 2 and 3 cluster together. Most of the *Kapala* form a monophyletic unit, composed of group 2 and 3 *Kapala* species, which is not what we accept as the correct topology. The morphology-only tree is unresolved or conflicts with DNA data in many areas.

Combined data. The parsimony analysis of the morphology + DNA matrix resulted in one tree (Fig. 4). The phylogeny of the combined analysis is dominated by the molecular data and follows the general topology of the parsimony-based molecular tree. Bayesian inference gave a different topology from the parsimony analysis in that the Lirata Clade (*Galearia*, (*Dilocantha*, (*Liratella*, *Lirata*))) is recovered as sister to the rest of group 2 + 3 [posterior probability (PP) = 0.75] (Fig. S8). All but three of the morphological characters exhibit homoplasy across the Kapala Clade, and 49 of the characters are parsimony-informative (RI = 0.76). The RI of each character as optimized onto the combined tree is given in Table S3. The RI is 1.0 for only three characters: eye shape (character 2; *Isomerala*), male number of flagellomeres (character 17; 10 flagellomeres in all but *Carletonia* and *Colocharis* Heraty), and mesoscutal flange over tegula (character 28; *Lirata* and *Dilocantha*).

Kapala Clade phylogenetic relationships

There are two synapomorphic characters for the Kapala Clade: cylindrical or flat mesoscutellar processes (character 21), and male antennal flagellomeres uniformly branched, with F_2 and F_3 of similar length (character 20). Under a paraphyletic group 1, all of the Kapala Clade except *Colocharis* have the synapomorphy

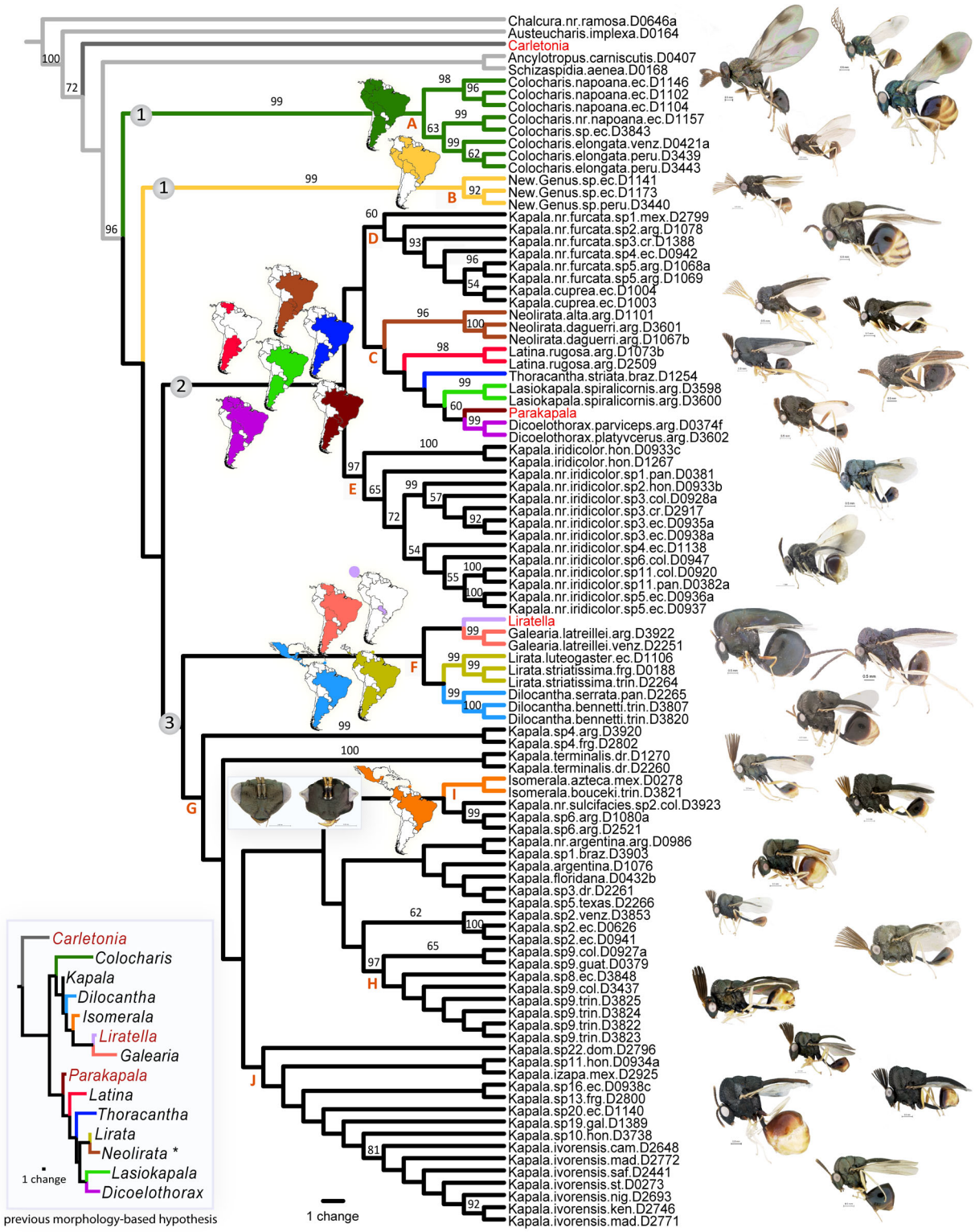

Fig. 4. Combined morphological and molecular hypothesis, using parsimony. Best tree under implied weighting ($k = 3$) in TNT. Retention index = 0.57, consistency index = 0.28. Groups 1, 2 and 3 are indicated at the nodes, and major clades are designated by letter. Bootstrap support is shown above branches. Known distributions of genera are shown by maps coloured by country. On the right are images of taxa. (Inset tree) Kapala Clade relationships from Heraty (2002). (Inset images) *Kapala* and *Isomerala* faces. *Isomerala* is easily recognized by its facial characteristics and is the only genus that is nested alone in a group of *Kapala*. [Colour figure can be viewed at wileyonlinelibrary.com].

of a round propodeal spiracle having an incision of the ventral margin (character 37). No synapomorphies were found for only *Kapala*.

Two short-spined genera are sister to the rest of the clade. Except for *Isomerala* (clade I, group 3), all other non-*Kapala* genera group together in two clades: in a cluster of six genera in group 2 (clade C) and a cluster of four genera in group 3 (clade F). Within these two 'multi-genera' clusters are where the especially large or broad scutellar spines originate independently on the tree.

Group 1. Group 1 comprises two genera: *Colocharis* (clade A) and a newly identified genus (clade B), hereafter 'New Genus', with a crown age of 17.5 Ma (95% HPD = 8.8–27.5). Group 1 is recovered as monophyletic in both dated analyses (PP = 0.88 for 194 taxa and PP = 0.65 for 96 taxa), and in the 195-taxon Bayesian analysis (PP = 0.32). Conversely, group 1 is paraphyletic – with *Colocharis* sister to the rest of the Kapala Clade – in the 96-taxon dataset (Fig. 2) and in some other analyses (Figs S4, S8, S9), although there is generally low support for this pattern. There is only one gene (28S D2, Fig. S10) where the two group 1 genera have > 0.5 PP support as a monophyletic group. It is unclear what is driving these differing results. Except for the two dated analyses, recovery of the monophyletic relationship is not ostensibly associated with analysis type or taxon sampling regime.

Group 1 diagnostic characters include a smooth face, six to seven labral digits, females with six to eight flagellomeres, and presence of a marginal fringe on the forewing. Members are distributed across northern and central South America, but do not extend below the northern part of Argentina (Fig. 4). *Colocharis* are easily recognized by their having very short paired mesoscutellar spines (shorter than the mesoscutellar disc), which is unique in the Kapala Clade (Fig. 1B). Other diagnostic characters include a circular propodeal spiracle and males with only eight flagellomeres (both unique within the Kapala Clade). Females have six or seven flagellomeres. The new genus has a smooth face and relatively short spines (as compared with most *Kapala*), not longer than around twice the length of the mesoscutellar disc. It is often identified as *Kapala* near *iridicolor* in collections; however, the New Genus females have eight flagellomeres (instead of nine or 10, as in *K. iridicolor*). This new genus will be described elsewhere.

Group 2. Group 2 is supported with a Bayesian PP of 0.98–1.0 in all molecular results (Figs 2,3, S2–S6, S8, S9), but is not recovered using morphology only (Fig. S7). It originated 19.6 Ma (10.8–28.8) and comprises three clades: Neolirata Clade (clade C), *K. furcata* complex (clade D) and K. iridicolour complex (clade E). Each clade is strongly supported; however, the relationship among the three is unclear, with very little branch support.

The Neolirata Clade (PP = 1.0; Fig. 2, 'C') is a monophyletic group of six genera, with an estimated crown age of 14.3 Ma (8.0–22.3). Five genera have molecular data (*Dicoelothorax*, *Lasiokapala*, *Latina* Koçak & Kemal, *Neolirata* Torréns & Heraty and *Thoracantha*) and one has morphological data only (*Parakapala*). *Lasiokapala* females are the only members of the Kapala Clade to have pectinate antennae. The six genera, including *Lirata*, are diagnosed by four synapomorphies by Heraty (2002). Aided by information from molecular phylogenies, *Neolirata* was recently split from *Lirata* (Torréns & Heraty, 2013), and is distinguished by a sharp carina separating the ocelli. Genera of the Neolirata Clade are found across much of South America and do not reach north to Central America.

The *K. furcata* complex (PP = 1.0; Fig. 2, 'D') has a crown age estimated at 13.3 Ma (6.6–20.7) and contains the designated type species of the genus, *K. furcata* (Fabricius). Many of the specimens in this clade have thick longitudinally carinate spines and robust bodies. The holotype of *K. furcata* is a female from Brazil, and although we have no molecular data from *K. furcata* from that country, the specimens from Argentina and Paraguay (*K*. nr *furcata* sp5) are very similar morphologically. The specimen D1078 is the only one that may be considered *K. furcata* sensu stricto. The K. iridicolor complex (PP = 1.0; Fig. 2, 'E') has a crown age estimated at 11.4 Ma (6.3–18.8) and is consistently well supported in all analyses. Most of these species are found in northern South America.

Group 3. Group 3 (PP = 0.47; Fig. 2) is the most speciose clade and has low support at the base of the clade across different analyses; removing rogue taxa does not remedy the uncertainty (Fig. S9). Group 3 is difficult to circumscribe but is made up of many *Kapala* typically identified as *K. sulcifacies* or 'near *K. sulcifacies*'. All group 3 *Kapala* females have eight flagellomeres, as opposed to nine flagellomeres in group 2. It has an estimated crown age of 20.3 Ma (11.6–29.3). The lack of phylogenetic support may be due to a radiation that was too rapid for our genetic markers to recover nodes accurately. Explosive species diversifications are often characterized by short internodes with low support (Whitfield & Kjer, 2008), a pattern which is exhibited in all of the basal bipartitions in group 3. There are many undescribed *Kapala* species of uncertain placement and low support. Group 3 has the largest geographic range of the three main groups, with members from Argentina to the southern U.S., including Arizona, Texas and Florida, and one species (*K. ivorensis*) found in Africa and Madagascar.

The Lirata Clade (PP = 0.67; Fig. 2, 'F'), usually recovered in group 3 (Table S8), is estimated at 17.1 Ma (9.8–27.1). It comprises *Dilocantha* and *Lirata* as sister taxa (PP = 1.0; Fig. 2), with *Liratella* and the problematic *Galearia* included in the clade under most reconstructions. The placement of *Galearia* is not always stable across replicate analyses of the same parameters, though the genus itself is always monophyletic (PP = 1.0). It is recovered as sister to *Lirata* + *Dilocantha* using the 96-taxa dataset in MRBAYES and BEAST. However, in some analyses *Galearia* is recovered with *Colocharis* (not shown) or, in the 96-taxon RAXML analysis, it is sister to groups 2 + 3 (Fig. S4). In the combined analysis using Bayesian inference (Fig. S8), *Galearia* retains its relationship with *Lirata* and *Dilocantha*, but the entire clade is recovered as sister to group 2 + the remainder of group 3, instead of within group 3.

In the combined morphology and molecule parsimony analyses, *Liratella* is recovered in the Lirata Clade using implied weights parsimony analysis when the concavity constant is $k = 3$ (Fig. 4). However, when $k = 15$, *Liratella* is sister to the Neolirata Clade (group 2) (not shown). In the Bayesian

combined analysis (Fig. S8), *Liratella* is also found in the Lirata Clade. *Liratella* shares a greater number of characters with *Galearia*, which was recovered as its sister genus in Heraty (2002), than with *Lirata* and *Dilocantha*. There is geographic support for *Liratella* belonging to the Lirata Clade because of its distribution into Central America. While the Neolirata Clade ('C') is found only in South America, the Lirata Clade ('F') has species reaching into Central America (*Dilocantha* ranges as far north as Mexico).

The rest of the group 3 members are often recovered as one clade, but with very low support (PP = 0.45; Fig. 2, 'G'). There is one non-*Kapala* genus in this clade. *Isomerala* (PP = 1.0; Fig. 2, 'I') lacks clear sister-group support but is always embedded in group 3 in molecular analyses [both in this study and in Murray *et al.* (2013)]. Morphologically, it appears similar to *Kapala*, but has a modified head shape with conical eyes. The crown age of *Isomerala* is estimated at 7.5 Ma (3.4–12.5). *Isomerala* is found in Central America and northern South America.

The K. sulcifacies complex (PP = 1.0; Fig. 2, 'H') has an estimated crown age of 7.9 Ma (3.7–13.3). The clade is always recovered with high support, but the intraclade relationships are not. Most of the individuals sampled are from Trinidad, Colombia and Costa Rica, with two from Ecuador. The males have relatively long antennae (F_2 branch 1.3–1.8 × the head height) and typically have serrated scutellar spines.

The Kapala izapa-ivorensis complex (PP = 1.0; Fig. 2, 'J') is always recovered and usually with high support (Table S8). The estimated crown age is 11.4 Ma (7.7–21.7). There is very little internal branch support, and morphological variation obscures species boundaries. This clade includes the only kapaline species found in the Old World, *K. ivorensis* (Murray & Heraty, 2016).

Diversification, biogeography and life history

Despite the bursts of morphological change within the Kapala Clade, there is no evidence of increased rates of diversification of any of the genera. Using BAMM, there were no rate shifts detected across the Kapala Clade phylogeny using either 100% or 80% global sampling fraction. The background rate of diversification was similar in each, 0.1418 and 0.1417, respectively. Additionally, the Colless test indicated that the Kapala Clade is balanced; we cannot accept the alternative hypothesis that branching events were uneven across lineages (standardized statistic = 0.097, *P*-value = 0.405).

Groups 1, 2, and 3 of the Kapala Clade each have a high probability of their ancestral biogeographic area being Neotropical under the DEC model (Figs 3, S11). Each clade has a smaller probability of being found ancestrally in the Central American + Neotropical regions combined (the Central American region includes southern Mexico and the Caribbean). No major clades have a high probability of an ancestral distribution being solely Central American, although in a few clades there is a strong probability of a Central American + Neotropical ancestral distribution, e.g. in the Kapala iridicolor complex (clade 'E') or the Kapala izapa-ivorensis complex ('J') (Fig. S11).

Three biologically relevant traits were mapped onto a genus-level phylogeny: ant host, plant host and oviposition behaviour (Tables S6, S7; Fig. S13). When ant subfamily host use is mapped onto the reduced-to-genus tree, there is equal support for either Ponerinae or Ectatomminae being the ancestral host of the Kapala Clade (Figs 2, S13A). Unfortunately, we do not know the ant host of the only poneromorph clade relative in the New World, *Carletonia*. The Kapala Clade members oviposit on a variety of plant species; but most of the recorded plant hosts are in the APG IV clades of lamiids and malvids (Fig. S13B). We found that within the Neolirata Clade ('C'), members generally utilize malvids (in the group rosids) – with only *Thoracantha* on lamiids (group asterids). Species in the Kapala Clade are known to oviposit either onto the underside of leaves, with eggs deposited parallel to the leaf surface and usually between leaf spicules, or into young flower buds, with emergence of planidia from the eggs timed to match the opening of the flower bud (Clausen, 1940b; Berry, 1947; Heraty & Darling, 1984; Pérez-Lachaud *et al.*, 2006; Torréns, 2013). Species in the Neolirata Clade all oviposit onto the undersides of leaves (Fig. S13C) (Torréns, 2013; Torréns & Heraty, 2013). This behaviour is not unique, with some group 3 members, including *Kapala terminalis* (Clausen, 1940b), also doing so.

Discussion

Phylogeny

We now recognize 14 genera within the Kapala Clade, which includes a new genus (to be described separately) that is differentiated based on both molecules and diagnostic morphological characters. *Colocharis* + New Genus (group 1) are supported in most analyses as sister to the rest of the Kapala Clade, but it is unclear if they form a grade or a clade. If a grade, then this would demonstrate a transition from the very short mesoscutellar spines in *Colocharis* (Fig. 1B) to the longer spines of the remaining members of the clade. The topology is different from Murray *et al.* (2013), where the Lirata Clade formed a grade to the rest of the Kapala Clade; however, a partitioned maximum likelihood analysis of the 2013 data did not show the Lirata Clade as sister to the rest of the Kapala Clade (E. A. Murray *et al.*, unpublished data). Using the current Kapala Clade data matrix, the Lirata Clade (clade 'F') is still not stable, as it comes out as sister to groups 2 and 3 in our combined morphological and molecular data Bayesian inference result (Fig. S8). These alternate topologies may be due to a difference in outgroup sampling, incorrect model fit or different alignments and missing data, with the Murray *et al.* (2013) alignment having a larger proportion of gaps due to a broader phylogenetic scope. Future phylogenomic work may help to strengthen confidence in placing some difficult taxa, which will aid in making taxonomic decisions. We find that *Kapala* are always paraphyletic, and find support for a monophyletic group 2 + 3 across most analyses. Based on our molecular results and our survey of museum

collections, group 3 has the most species and species complexes (mostly undescribed) within the Kapala Clade.

Our results suggest that the genus *Kapala* will need to be split into at least three different genera, while retaining currently recognized genera. A more radical solution is to sink all of the genera in groups 2 and 3 into a single genus, *Kapala*. However, the distinct morphologies of the genera combined with the molecular divergence and age of the groups lend strong opposition to this approach. The placement of *Galearia* and its relationship to *Lirata* and *Dilocantha* are also a concern for defining the limits of *Kapala*, as variability across analyses leaves ambiguity in the placement of the Lirata Clade. None of these genera were identified as rogue taxa (Fig. S9). Due to the low branch support across the backbone of the tree, we feel that circumscription of the genera needs to be subject to further analyses, using more molecular data, before implementing major taxonomic changes.

Our combined analyses (Figs 4, S8) include three genera that lack molecular data: *Carletonia* (outgroup), *Parakapala* and *Liratella*. We do not recover *Carletonia* as the nearest relative to the Kapala Clade as did Heraty (2002). This may be a product of our reduced outgroup sampling or the fact that is the only outgroup that lacks molecular data. *Carletonia* is morphologically similar to the Old World genera *Eucharissa* Westwood and *Saccharissa* Kirby (in the Schizaspidia Clade), based on their lack of mesoscutellar spines, a 13-segmented antennal flagellum (vs 10 or fewer in the Kapala Clade), and the male antennae double-branched on the funicle (Heraty, 2002). Our representatives of the Schizaspidia Clade (*Ancylotropus* and *Schizaspidia*) are sister to the Kapala Clade in all analyses, as proposed by Murray *et al.* (2013). Our groups 2 and 3 were recovered by Heraty (2002) in differing configurations (Fig. 4, inset), although *Lirata* and *Neolirata* at that time were treated as a single genus (*Lirata*). Previous studies have not proposed *Colocharis* and the New Genus as the sister groups to the remainder of the Kapala Clade (Heraty, 2002; Murray *et al.*, 2013). As in the morphology-only results from Heraty (2002), *Liratella* here is placed as the sister group of *Galearia*, and *Parakapala* is included as part of the Neolirata Clade.

It has been shown that missing data can affect accurate placement on the tree (Wiens, 2006). To address concerns of missing data affecting topology, we used two datasets. The 'all taxa' matrix (195 tips) had 34.75% missing data, while the 'full data' (96 tips) had 14.95% missing data. Our preference is towards topologies using the most complete DNA sampling with fewer taxa (Figs 2,3); the 195-taxon dataset with more missing data more often produced variable topologies and had lower branch support (Table S8).

Clade ages, geographic distributions and diversification

The Kapala Clade crown age is estimated at 23.4 Ma, and groups 1, 2 and 3 were all established by *c*. 17 Ma. We find a span of 2.8–9.6 Ma (median node age) for the origin of genera having two or more species included (Fig. S12). Much of the morphological differentiation and generic origins within the Kapala Clade took place during the Miocene (5.3–23 Ma) – a time period that has been hypothesized to have facilitated diversification of some insect groups due to global cycles of warming and cooling opening new habitats (Condamine *et al.*, 2012) or indirectly through host plant diversification (Winkler *et al.*, 2009). Some insect groups exhibiting increased diversification at this time include a genus of braconid wasps (*c*. 16–24 Ma; Ceccarelli & Zaldivar-Riveron, 2013), a tribe of noctuid moths (*c*. 29 Ma; Toussaint *et al.*, 2012), and two genera of clearwing butterfies (*c*. 13–14 Ma; Elias *et al.*, 2009), all before a general trend in cooling starting *c*. 14 Ma (Potter & Szatmari, 2009). Andean uplift events (at 23, 12, and 4.5 Ma) had a sizeable effect on all of South America, including via rainfall, soil nutrient levels and landscape evolution (Hoorn *et al.*, 2010), which influenced biotic diversification. The Andes may have served as a dispersal barrier for eucharitids, because there are no Kapala Clade members – or their ant hosts – in Chile, west of the mountains.

South America was analytically estimated to be the ancestral area of the Kapala Clade by Murray *et al.* (2013). Likewise the Neotropical region is found here as having the highest probability of being the ancestral area of both the Kapala Clade and each of the three major groups (Figs 3, S11). Excluding the genus *Kapala*, groups 1 and 2 genera are found primarily in South America (with *Colocharis* also distributed in the southern part of Central America). The two groups' geographic restriction occurs even though South America has been connected to Central America via the complete closure of the Isthmus of Panama since 3.5 Ma (Potter & Szatmari, 2009). Conversely, the non-*Kapala* genera of group 3 do have a range that extends more northerly into Central America – only one of these five genera is found solely in South America (*Galearia*) (Figs 4, S11). Only *Kapala* within group 3 extend into the southern United States (Arizona, Florida and Texas).

A distantly related genus of Eucharitidae, *Orasema* Cameron (Oraseminae), provides a comparison to the Kapala Clade. There are parallels in these two eucharitid groups in that the *Orasema* dispersed to the New World during a similar time (*c*. 24–33 Ma for the *Orasema*) and in that they subsequently expanded their host range to novel ant genera (Baker *et al.*, 2019). *Orasema* probably arrived via a Beringian route, which is also a possibility for the Kapala Clade due to the similar timing and its Old World Schizaspidia Clade relatives being presently found as far north as Japan. The two eucharitid groups are similar in terms of host generic diversity, with New World *Orasema* commonly parasitizing three genera (*Pheidole*, *Solenopsis* and *Wasmannia*) (Baker *et al.*, 2019, Table S1) and the Kapala Clade commonly parasitizing four genera (*Ectatomma*, *Gnamptogenys*, *Odontomachus* and *Pachycondyla*; Table S7). We use 'commonly' as instances of genera where two or more species have been documented as hosts.

Bursts of morphological evolution are seen across the Kapala Clade phylogeny, yet we do not find signs of uneven diversification based on the phylogeny of the 14 genera. There is no shift in diversification rates on the tree, even for the clades of morphologically divergent genera. According to the Colless index, the topology is balanced, which supports an expectation of even

diversification across the tree. In opposition to the Kapala Clade pattern, after arriving in the New World all species of *Orasema* shared an increased rate of diversification, but did not noticeably diversify morphologically. Today all *Orasema* are phenotypically similar, as are other members of Oraseminae, which is in stark contrast to the varied phenotypes of the Kapala Clade. *Orasema* attack a different subfamily of ants (Myrmecinae) than the Kapala Clade and may use a different mode of chemical mimicry (Vander Meer *et al.*, 1989; Howard *et al.*, 2001). Myrmicinae lack a cocoon, and immature *Orasema* are proposed to be protected by sequestering cuticular hydrocarbons from their ant host. Adults emerging in the nest are either ignored or protected by their ant host (Wheeler, 1907; Chien & Heraty, 2018). By contrast, all Eucharitinae are parasitoids of pupae within their cocoons (isolated from the host ant adults as they develop), and in species of *Kapala*, chemical mimicry has been proposed as a means of eliciting ant behaviours that removed adult *Kapala* from the nest; these behaviours can be relatively aggressive and the spines may be useful in protecting the wings during ejection (Heraty *et al.*, 2015; Pérez-Lachaud *et al.*, 2015). These factors have potentially influenced morphological stasis in *Orasema* (and Oraseminae) as compared with morphological diversification in the Kapala Clade (and many other Eucharitinae).

Molecular and morphological patterns

Molecules and morphology are the most common data used to interpret phylogenetic relationships, and may be used independently or combined. However, the two often produce dissimilar topologies. Disjunction between morphological and molecular phylogenies is potentially due to our inability both to identify *a priori* the phylogenetically important characters amidst all of the phenotypic variation and to effectively translate continuous morphological change into character states and discretize nuanced differences into phenetic gaps.

Two evolutionary processes can obscure phylogenetic signal as assessed from morphology, giving general instances where molecules are preferable for recovering evolutionary relationships: convergent evolution, and retention of ancestral similarities by some taxa within a larger diversifying clade (Ward, 2011). In the Kapala Clade, both of these processes are apparent. *Lirata* (within group 3) and *Neolirata* (within group 2) phenotypes are convergent, according to molecules. These two genera were originally grouped as one because they have similar body and spine shapes and are also the only two genera in the Kapala Clade to possess an antennal scape reaching beyond the median ocellus (Appendix S1, character 11, state 1). An example of retention of ancestral characters is the genus *Kapala*, which is nearly morphologically static in comparison to the intervening genera.

This pattern of apomorphic morphology nested within a group exhibiting a pleisiomorphic phenotype has been described as a progenitor-derivative evolutionary event (Crawford, 2010). As a progenitor, *Kapala* retains its pleisiomorphic character states while numerous other phenotypically divergent groups branch out of it. For instance, *Isomerala* is nested within other *Kapala* species in group 3 and is distinguished by a modification of head morphology – displaying a bulging frons (Heraty, 2002) and conical eyes (character 2) (Fig. 3 inset). This situation has also been termed 'budding', which describes the origin of a new taxon that does not affect the existence or characters of the original stem group (Hörandl & Stuessy, 2010).

Our tree supports the hypothesis that *Kapala* is a genus that gave rise to morphologically divergent taxa, rendering it paraphyletic. A comparable pattern has been observed in a related chalcidoid, the genus *Perilampus* Latreille (Perilampidae), which was shown via molecular data to be three (Murray *et al.*, 2013) or four (Munro *et al.*, 2011) lineages, and in *Orasema* sensu lato (Eucharitidae), which has now been split into nine genera to reflect natural groups (Burks *et al.*, 2017). Similar to *Kapala*, *Perilampus* and *Orasema* sensu lato each appear to be phenotypically cohesive groups, but molecules reveal a different evolutionary history. This pattern is also noted in the ant genus *Cerapachys* Smith (Dorylinae), which had been recognized based on the collective retention of a generalized morphology while other ant groups nested within (e.g. the army ants) exhibit derived phenotypes (Ward, 2011; Brady *et al.*, 2014). The discovery of nonmonophyly in a molecular phylogeny may be a common theme for morphologically conserved genera.

Life history

The association of described species with their behaviour and life-history traits is still in its early stages in the Kapala Clade. For *Kapala*, the matching of historical trait records to species is confounded by the fact that it is a paraphyletic genus with dozens of undescribed species of uncertain circumscription. Due to the importance of interpreting biological traits within a phylogenetic framework, we mapped three characters having satisfactory genus-level data onto the Kapala Clade, to gain an understanding of the evolutionary history of important behaviours (Tables S6, S7; Fig. S13).

The plant host associations are most complete for group 2. The Neolirata Clade has a strong pattern of host plant utilization on malvids (Fig. S13B) and a shared behaviour of female oviposition in the host plants (Fig. S13C). Where known, species in the Neolirata Clade ('C') oviposit on the undersides of leaves, usually depositing eggs under the spicules on the leaf surface (Torréns, 2013; Torréns & Heraty, 2013). This type of oviposition is known for *Dicoelothorax*, *Lasiokapala*, *Latina*, *Neolirata* and *Thoracantha* (Torréns, 2013; Torréns & Heraty, 2013; Torréns *et al.*, 2016). This oviposition type is not exclusive to this group, and other Kapala Clade species also oviposit onto the surface leaves (Clausen, 1940b; Berry, 1947; Torréns *et al.*, 2007; Torréns & Heraty, 2012; Torréns & Heraty, 2013; Torréns *et al.*, 2016). Specifically, Kapala Clade members usually oviposit on the ventral sides of the leaf with the eggs tucked under the plant spicules or pubescence, with the eggs parallel to the surface. Interestingly, the two *Kapala* species complexes in group 2 (furcata, clade 'D'; iridicolor, clade 'E') oviposit on and into flower buds or bracts, which sets

up a biological split to the Neolirata Clade. More field records will be needed to substantiate this finding and augment group 3 records, of which there are too few to interpret definitive patterns.

The Kapala Clade and the Old World Chalcura Clade and Schizaspidia Clade – termed the PEM parasitoids (Murray *et al.*, 2013) – attack only Ponerinae and Ectatomminae (excepting a host switch to Myrmeciinae in the Chalcura Clade's *Austeucharis*). Two genera, *Gnamptogenys* (Ectatomminae) and *Odontomachus* (Ponerinae), are shared between the Kapala Clade and the nearest Old World outgroup (Schizaspidia Clade). In mapping the host ant subfamily to the phylogeny, both ponerines and ectatommines are reconstructed as equally likely to be the ancestral host of the Kapala Clade (Figs 2, S13), though we do not have host data for the two short-spined genera (group 1). Group 3 has more clades attacking Ectatomminae; however overall, there are more Ponerinae recorded as hosts. Host patterns suggest a lack of strict specialization within the Kapala Clade and perhaps an opportunistic use of suitable ants near to or associated with the plant hosts.

The antiquity of the Kapala Clade does not conflict with ages of their ant hosts. Kapala Clade groups 2 and 3 split *c*. 21 Ma (96 taxa) or 23 Ma (194 taxa). There are three ant genera attacked by both groups 2 and 3: *Odontomachus* is estimated to be aged *c*. 40 Ma (Larabee *et al.*, 2016), which is slightly older than the stem age of the Kapala Clade (*c*. 35 Ma); the Neotropical genus *Ectatomma* (Ectatomminae), which has been estimated to be *c*. 19 Ma old (Nettel-Hernanz *et al.*, 2015); and the *Pachycondyla* (Ponerinae) genus group, recently revised (Schmidt & Shattuck, 2014), which has a common ancestor *c*. 60 Ma (Schmidt, 2013). Hence, the ant host genera are older than the wasps parasitizing them. Ponerinae and Ectatomminae are not closely related subfamilies, and it appears that eucharitids are able to be successful on ants that share a similar ecological niche, not an immediate evolutionary past (Murray *et al.*, 2013).

The Kapala Clade genera exhibit a distinctive and rather exceptional phenotype in their long, paired mesoscutellar spines. It has been hypothesized that the spines aid in safe transport by ants out of the nest (Heraty *et al.*, 2015; Pérez-Lachaud *et al.*, 2015). *Galearia* (Fig. 1L) is a good example of what looks like a strategy of extreme protectionism. Although eucharitids outside of the Kapala Clade also have spines, those are probably convergent phenotypes that always arise from an ancestral form that lacks spines (Heraty, 2002; Murray *et al.*, 2013; Heraty *et al.*, 2015). We have not tested for spine selection that might be associated with the aggressiveness of the ant host. However, it is not apparent from our data that spine phenotype confers an increase in overall diversification or differential association with the ponerine and ectatommine ant hosts.

Notably, another subfamily of eucharitid wasps, Oraseminae, attacking a different subfamily of ants, had a similar biogeographic history, but had a nearly opposite response to a new host resource – showing greater diversification but without the correlated morphological explosion. We propose that this difference lies in the within-nest interactions between the parasitoids and their different ant host subfamilies.

Conclusions

The Kapala Clade contains a remarkable amount of morphological diversity and is a group where evolutionary signal is obscured by bursts of phenotypic change, morphological convergence and retention of pleisiomorphic characters. Phenotypic change and the recognition of genera across the tree are unbalanced, and discrete genera in the Kapala Clade have been described based on novel morphologies and gaps in phenotypic variation. This method was successful for delimiting divergent genera, but molecular tools were necessary to identify the paraphyly of the morphologically static genus *Kapala*. We now have a comprehensive overview of Kapala Clade relationships. The incorporation of different data types and methods provides a concrete depiction of those areas of the phylogeny that need additional attention and those that are well resolved, which should aid future systematic and life-history research on the group.

The Kapala Clade exemplifies a successful application of Hennig's principle of reciprocal illumination, in that the molecular phylogeny was invaluable to guide the reinterpretation of the morphological characters. In turn, mesoscutellar morphologies of the genera gave evidence to indicate that the most commonly recovered topology was more credible than alternate placements. Morphological data and DNA each have value for phylogenetic reconstructions, with taxonomic level and clade characteristics determining their suitability and the weight of their relative contributions.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Databased localities of the Kapala Clade. A total of 5384 specimens are represented on the maps, of which 5046 are the genus *Kapala*. The 13 other genera have from three to 110 specimen records each. (A) All *Kapala* records, from both group 2 and group 3 (see Figs 2–4, S2–S4 for group members); (B) nearest New World outgroup to Kapala Clade, *Carletonia*, and group 1 genera *Colocharis* and 'New Genus'; (C) group 2 genera (excluding *Kapala*); (D) group 3 genera (excluding *Kapala*).

Figure S2. Dated phylogeny of 194 taxa. Non-*Kapala* genera are highlighted. Blue bars indicate the 95% highest posterior density values for the nodes. Node heights are median ages (in Ma). The three major groups are labelled as 1, 2 and 3.

Figure S3. Bayesian phylogeny of all 195 taxa. Maximum clade credibility tree shown, with posterior probability support values shown above branches. The three major groups are labelled as 1, 2 and 3.

Figure S4. RAXML phylogeny of 96 taxa, with rapid bootstrap support values. The three major groups are labelled as 1, 2 and 3.

Figure S5. RAXML phylogeny of 195 taxa, with rapid bootstrap support values. The three major groups are labelled as 1, 2 and 3.

Figure S6. Parsimony analysis of 96-taxon molecular dataset under equal weights. Results shown as a 50% majority-rule tree, from four shortest trees of 1764 steps. Retention index = 0.68, consistency index = 0.36. The three major groups are labelled as 1, 2 and 3.

Figure S7. Morphological parsimony phylogeny of 99 taxa, built from 52 characters. Strict consensus topology of three trees under implied weights ($k = 3$). Retention index = 0.75, consistency index = 0.31, with 331 steps. Bootstrap values from 1000 replicates are shown above branches, and the three major groups (as recovered by molecules) are labelled across the tree. *Kapala* sp4 D3920 had mostly missing data, and is indicated by a dotted terminal branch. Non-*Kapala* genera have coloured branches. The three major groups are labelled as 1, 2 and 3. Inset tree: Kapala Clade relationships from Heraty (2002). Inset images: *Kapala* and *Isomerala* faces. *Isomerala* is easily recognized by its facial characteristics and is the only genus that is nested alone in a group of *Kapala*.

Figure S8. Combined molecular and morphological tree of 99 taxa using Bayesian inference, with posterior probability support values. The three major groups are labelled as 1, 2 and 3.

Figure S9. The 96-taxon dataset trimmed of the 10 taxa suggested by ROGUENAROK, leaving 86 taxa. Bayesian maximum clade credibility phylogeny, with posterior probabilities. The three major groups are labelled at the interior nodes as 1, 2 and 3.

Figure S10. Individual gene trees from the 96-taxon matrix; four of the taxa are outgroups to the Kapala Clade. Majority rule consensus trees are from reversible-jump MRBAYES analyses. Top (left to right): 18S, 28S D2 and 28S D3–D5; bottom: COI and COII.

Figure S11. Ancestral biogeographic analysis using the dispersal–extinction–cladogenesis model in BIOGEOBEARS, with no jump parameter. The 194-taxon dated phylogeny (Fig. S2) was pruned to include one representative from each species or morphospecies, for a total of 99 tips. (This tree topology was also used for the BAMM diversification analysis.) Each tip was coded by present-day distribution (Table S5), and colours of the pie charts correspond to the region colours shown at the branch tips. The axis shows time (in Ma). Large clades are denoted by letters. The three major groups are labelled as 1, 2 and 3. Each group has a high probability of having its ancestral area in the Neotropical region.

Figure S12. Ages of major clades, as estimated in BEAST2 from the 194-taxon dataset and the 96-taxon dataset. For each clade, stem ages of each dataset are shown by the top two bars, and crown ages (if applicable) are the bottom two.

Figure S13. Trait ancestral reconstruction, using parsimony. Three traits were mapped on a phylogeny (pruned from the parsimony combined analysis of main body Fig. 4). Colours are described in legends; each state was unordered and was coded starting with 0 at the top of the legend list (Tables S6, S7). The colour grey on a terminal branch indicates that the state is unknown. Boxes next to tip labels show the tip state coding. A, ant host, by subfamily; B, plant host, by the APG IV system classification; C, oviposition site on plant.

Table S1. Specimen data for molecular matrix.

Table S2. GenBank accession numbers. Those in greyed cells and starting with 'KT' were newly sequenced for this study (472 total). This comprises 67 18S sequences, 102 D2, 114 D3–5, 89 COI and 100 COII sequences.

Table S3. Character coding table. Characters and states are defined in Appendix S1.

Table S4. BAMM sampling fractions.

Table S5. Geographic areas and coding used for the historical biogeographic analysis.

Table S6. Plant host data and oviposition information for trait matrix coding. Plant hosts were grouped using APG IV classifications.

Table S7. Ant host data and trait matrix coding.

Table S8. Support values for major clades across all four analytical programs. An 'n/a' indicates the clade was not recovered in the topology.

Appendix S1. Characters and states used in the morphological matrix.

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