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Cite this article: Murray EA, Carmichael AE, Heraty JM. 2013 Ancient host shifts followed by host conservatism in a group of ant parasitoids. *Proc R Soc B* 280: 20130495. <http://dx.doi.org/10.1098/rspb.2013.0495>

Received: 24 February 2013

Accepted: 8 March 2013

Subject Areas:

taxonomy and systematics, evolution

Keywords:

Eucharitidae, molecular dating, cophylogenetic reconstruction, diversification

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Electronic supplementary material is available at <http://dx.doi.org/10.1098/rspb.2013.0495> or via <http://rspb.royalsocietypublishing.org>.

Ancient host shifts followed by host conservatism in a group of ant parasitoids

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While ant colonies serve as host to a diverse array of myrmecophiles, few parasitoids are able to exploit this vast resource. A notable exception is the wasp family Eucharitidae, which is the only family of insects known to exclusively parasitize ants. Worldwide, approximately 700 Eucharitidae species attack five subfamilies across the ant phylogeny. Our goal is to uncover the pattern of eucharitid diversification, including timing of key evolutionary events, biogeographic patterns and potential cophylogeny with ant hosts. We present the most comprehensive molecular phylogeny of Eucharitidae to date, including 44 of the 53 genera and fossil-calibrated estimates of divergence dates. Eucharitidae arose approximately 50 Ma after their hosts, during the time when the major ant lineages were already established and diversifying. We incorporate host association data to test for congruence between eucharitid and ant phylogenies and find that their evolutionary histories are more similar than expected at random. After a series of initial host shifts, clades within Eucharitidae maintained their host affinity. Even after multiple dispersal events to the New World and extensive speciation within biogeographic regions, eucharitids remain parasitic on the same ant subfamilies as their Old World relatives, suggesting host conservatism despite access to a diverse novel ant fauna.

1. Introduction

Intimate ecological associations of parasites and their hosts have been considered important in shaping species evolution [1–3], an idea tracing at least back to Darwin who stressed the co-dependency of these organisms [4]. Host diversity, host shifts and niche diversification are significant influences in the radiation of parasitoids [5–8], which are a specialized group of parasites that develop in or on a single host, eventually killing it [9]. Studies of host–parasitoid relationships conclude that host range is often dependent on behavioural or ecological characteristics of the host [5,10] rather than being limited to taxonomic relatedness of host. To contribute to the understanding of host–parasitoid interactions, we elucidate the evolutionary history of an association between ants and a specialized group of parasitoid wasps, the Eucharitidae (Hymenoptera: Chalcidoidea).

Ants represent one of the most successful radiations within the insects, and in terms of available biomass, distribution and diversity, they offer a tremendous resource for a variety of nest associates [11]. The roughly 15 000 ant taxa (antweb.org, AntWeb (accessed September 2012). Hosted by the California Academy of Sciences) support at least 17 orders of arthropod myrmecophiles, including specialized predators, scavengers, commensals, parasites and trophobionts [12,13]. Ant parasitoids are known from three insect orders: Diptera, Strepsiptera and Hymenoptera [13–15]. Although roughly 77 000 species of parasitoids are described, and more than 600 000 are estimated [16], only a fraction attack ants and even fewer can gain access inside the nest [15,17].

Via a complex suite of behavioural, morphological and chemical adaptations, Eucharitidae are one of the few groups to successfully circumvent the formidable colony defence mechanisms and attack immature ants [18–22]. Females deposit their eggs away from the ant nest on a host plant [18,23,24]. The eucharitid planidia (active, first instar larvae) enter the ant nest via phoresy, either directly on worker ants or on prey items carried by ants [23,25–27], and

Table 1. Comparison of relevant ant clade estimated ages from three previous studies, along with taxon appearance in the fossil record. Age and range given in millions of years.

	Moreau <i>et al.</i> [37] min. age fossils	Brady <i>et al.</i> [38] 145 Myr root	Schmidt [39] 155 Myr root	oldest known fossils
Formicidae w/Martialinae	n.a.	n.a.	123 (116–130)	100 [46]
Formicidae w/o Martialinae	140.6 (132.6–148.6)	116 (112.2–119.8)	118 (112–124)	
poneroid clade	128.2 (122.3–134.1)	100 (103.9–116.1)	107 (99–115)	
Ponerinae	110.7 (104.4–117)	79 (72.7–85.3)	94 (85–104)	88.6–92 [47]
formicoid clade	124.7 (118.2–131.2)	105 (101.5–108.5)	104 (98–111)	
Myrmeciinae	108.3 (105.3–111.3)	47 (41.6–52.4)	n.a.	54.5 [48]
Ectatomminae	79.5 (78.6–80.4)	56 (51.9–61.1)	n.a.	79 [49]
Formicinae	92.0 (91.8–92.2)	77 (73.5–80.5)	66 (56–76)	88.6–92 [50]
Myrmicinae	99.8 (95.6–104)	82 (77.7–86.3)	76 (66–85)	52 [51]

eventually develop as an ectoparasitoid of the ant pupae [28,29]. Within the nest, both adults and immature stages are generally accepted by the ants, being groomed, carried or protected if the colony is under attack [19,22,30]. This intimate interaction is based on semiochemical recognition involving similar hydrocarbon profiles between eucharitid parasitoids and their host ants [20,21].

An examination of the nearest relatives of Eucharitidae is necessary to understand how this life cycle might have evolved. Their paraphyletic sister group, Perilampidae [24, 31–33], parasitize a diverse array of species, including several Hymenoptera [34], but never attack ants. Both families possess planidial larvae and oviposit away from the host, which are shared life-history traits exhibited by no other Chalcidoidea [25,35].

Eucharitidae are known to attack five of the 21 subfamilies of ants: Ectatomminae, Formicinae, Myrmeciinae, Myrmicinae and Ponerinae (as referenced in [36]; electronic supplementary material, table S1). Along with recent ant phylogenies [37–39], a comprehensive, dated phylogenetic analysis of Eucharitidae makes possible an investigation of the historical evolutionary relationships of the two families, one where congruence of parasitoids to their host could be expected owing to the close dependent association. There are few studies evaluating ants and their myrmecophiles within a phylogenetic framework [40–43], thus this large-scale examination is a significant contribution to our understanding of ant-associates.

Fossil data place the origin of ants at 110–120 Ma [44,45] which is in general agreement with molecular divergence dating analyses that estimate an age of 115–140 Ma ([37–39]; table 1). Ant fossils are rare in the Cretaceous, but show a gradual increase in representation from 5 per cent of the total Baltic amber insects to 36 per cent of the Dominican amber insect fossils [52]. While the major ant lineages were established and had diversified by the end of the Cretaceous, it was not until the Eocene that ants attained ecological dominance [37,38,45,53].

Fossil evidence suggests that the superfamily Chalcidoidea arose in the Early Cretaceous [44,54], yet most chalcidoid families do not appear until the Eocene [31,33]. The sole eucharitid fossil dates to the Middle Eocene [31], which coincides with the rise in dominance of ants. Previous taxonomic analysis of Baltic amber fossils of both Eucharitidae

(*Palaeocharis rex*) and their nearest relative Perilampidae (*Perilampus pisticus*) indicates a relatively derived phylogenetic placement of these extinct species, leading to the conclusion that the two families diverged considerably earlier than the approximate 45 Myr age of the fossils [31].

To examine the evolutionary history of this ant–eucharitid association, we first present a molecular phylogeny of the Eucharitidae (237 taxa) that includes calibrated divergence time estimates. We then use this phylogeny combined with cophylogenetic analysis, ancestral host reconstruction and biogeographic analysis to address three objectives: (i) establish if there is evidence for cophylogeny between eucharitids and ants, (ii) determine if diversification rates of eucharitids coincide with novel host colonization, and (iii) resolve the parasitoid biogeographic history and consider its overlap with ant distribution. We find that despite the evidence for ancient ant host shifts, there is high host affinity and apparent ecological constraints maintaining host-specificity in the Eucharitidae.

2. Results and discussion

(a) Eucharitid dated phylogeny

The monophyly of Eucharitidae is strongly supported with a posterior probability (pp) of 1.0 (see figure 1a and electronic supplementary material, S1). The subfamilies Gollumiellinae, Oraseminae and Eucharitinae are each recovered as monophyletic, and relationships among them are strongly supported, with Gollumiellinae sister to Oraseminae + Eucharitinae. These results are in general agreement with phylogenetic relationships based on morphology [55] and analyses of molecular data [27,32]. Previous analyses have suggested both *Akapala* (Akapalinae; [33]) and *Jambiya* (Perilampidae; [32]) as sister to Eucharitidae, but with low support; we found high support for *Akapala* as the sister group of the remaining Eucharitidae (0.98 pp; figure 1b; electronic supplementary material, S1). Eucharitidae diverged from the perilampid non-ant parasitoids approximately 85.7 Ma (95% highest posterior density interval = 63.4–110.2 Ma) and began diversifying by 72.0 Ma (53.9–92.6 Ma; figure 1c; electronic supplementary material, table S3). Our study and others support a relationship in which Eucharitidae renders Perilampidae paraphyletic [32,33]. Perilampids and eucharitids are

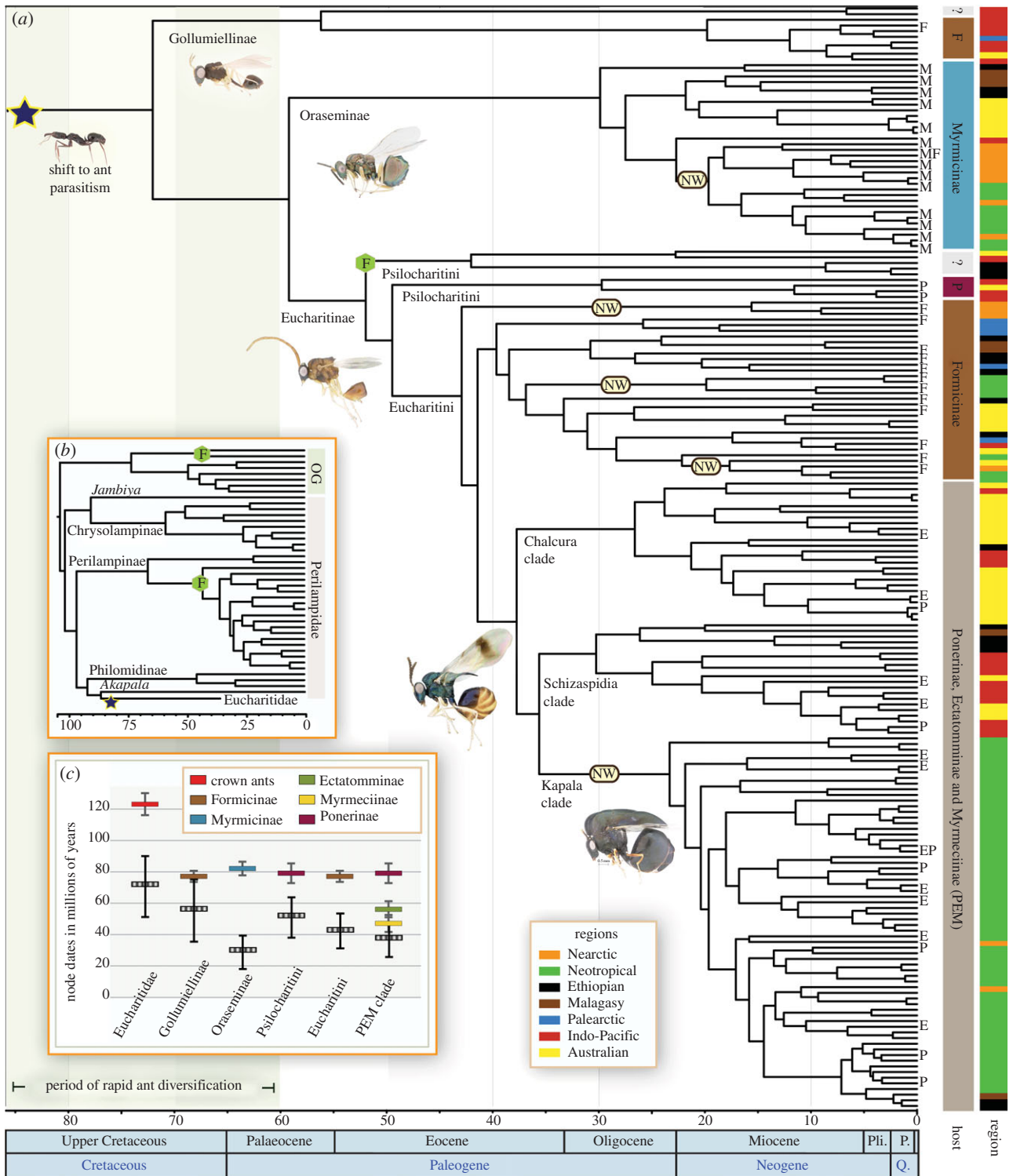


Figure 1. (a) Fossil-calibrated phylogeny of Eucharitidae. Two hundred and thirty-seven taxa were analysed. Terminal labels, posterior probabilities and error range of node ages found in the electronic supplementary material, figure S1. Blue star signifies eucharitid origin (stem node). Green shading on left indicates the major period of ant diversification, which coincides with the origin of their eucharitid parasitoids. 'F' symbols indicate the three fossil constraints, and 'NW' indicates that the subtending clade members are found in the New World, whereas ancestral eucharitids are Old World. Bars to the right indicate ant hosts and biogeography, with the specific ant-subfamily host indicated by abbreviation at tree terminals: E, Ectatomminae; F, Formicinae; Me, Myrmecinae; M, Myrmecinae; P, Ponerinae. Ant image (adapted from [11]). (b) Portion of tree showing age and relationships of the paraphyletic Perilampidae relative to Eucharitidae. (c) Ages of major eucharitid groups and their respective ant host subfamilies (crown ant age from Schmidt [39], remaining from Brady *et al.* [38]).

united by their common strategy of host accession via planidia, but the host association preceding Formicidae in the eucharitid ancestor remains elusive because perilampids attack a wide range of insect orders, including Coleoptera, Diptera and Hymenoptera; the hosts for *Akapala* and *Jambiya* are unknown.

Molecular dating techniques have been applied to only two chalcidoid families: Eucharitidae and the pollinating fig

wasps, Agaonidae. Our results indicate that Eucharitidae originated in the Late Cretaceous. Likewise, the most recent study from Agaonidae suggests an origin shortly prior to the Cretaceous boundary at 75.1 Ma (56.2–94.9 Ma), as inferred from 200 taxa and six genes [56]. Both Eucharitidae and Agaonidae belong to a derived clade of larger hard-bodied chalcidoid wasps [33]. Our dates provide evidence

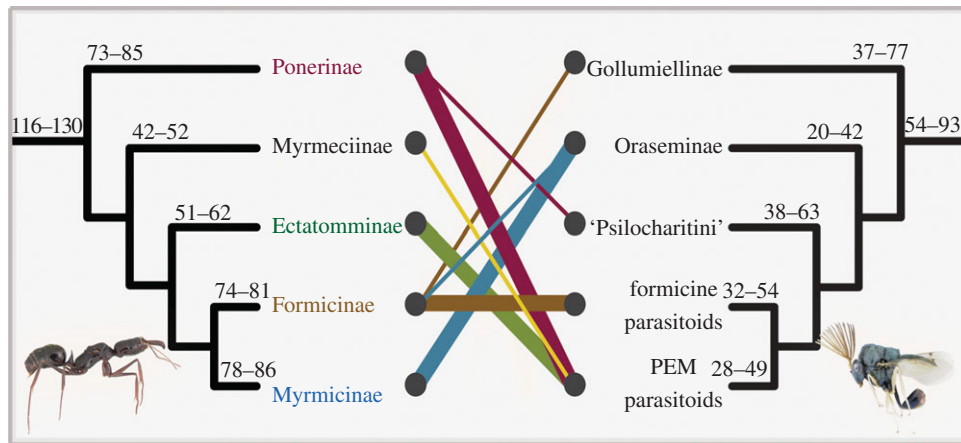


Figure 2. Host–parasitoid taxonomic associations. Tanglegram simplified from analysis of 29 eucharitid genera and 23 formicid genera. Ant cladogram on left (adapted from Moreau *et al.* [37]). Thin interaction lines indicate utilization of just one host genus, thick lines indicate multiple hosts. Psilocharitini and the formicine parasitoid groups are non-monophyletic. Estimated node age ranges above branches, as in figure 1c.

for a Late Cretaceous origin for this group of related families, despite the lack of described fossils for this time period.

(b) Cophylogeny test and ancestral host mapping

Host–parasitoid relationships at the subfamily and generic level show a conserved pattern of host use within major clades of Eucharitidae (see figures 2 and 3 and electronic supplementary material, table S1). We found that the eucharitid and ant host phylogeny were statistically more similar than expected by chance, under event-based reconstruction methods. Cophylogeny reconciliation in Jane [57] offers support for phylogenetic host tracking. Zero per cent of random sample solutions and of random parasitoid tree simulations (mean costs = 171 and 159) returned a lower cost than the original problem solution (minimum cost = 97). This provides high support ($p < 0.01$) for non-random patterns of the two phylogenies. There is topological congruence, but we were unable to incorporate temporal data owing to non-overlap in host and parasitoid node ages. By the time of eucharitid crown group diversification at 72.1 Ma, most ant subfamilies were established ([37–39]; figure 1c and table 1), and there is a lack of correspondence between dates for clades of Eucharitidae and their respective ant-subfamily hosts owing to older ant ages.

The eucharitid–ant association developed during a period of high ant diversification 60–100 Ma [37]. To reconstruct historical host associations, ancestral states were calculated over a distribution of trees using BAYESTRAITS [58]. General patterns across major clades in Eucharitidae indicate a series of host jumps to a new ant subfamily and then range expansion within each group, typically extending to several host ant genera. Diverse clades of Eucharitidae also exhibit a high degree of endemism, suggesting that much of the host diversification took place after major continental dispersal routes were closed. Under parsimony, Formicinae are mapped as the ancestral host (figure 3). Bayesian ancestral reconstruction indicates uncertainty but suggests that the ancestral host was likely to be Formicinae (58%), with other ant-subfamily hosts possible at a much lower probability (less than 14%). There are several major host colonizations throughout the history of Eucharitidae. All but one ant subfamily (Myrmeciinae) were colonized prior to approximately 30 Ma. Reconstruction shows a series of shifts away from the ancestral formicine host in three wasp groups: Oraseminae, Psilocharitini and the ‘Ponerinae–

Ectatomminae–Myrmeciinae’ (PEM) parasitoids (see figure 3 and electronic supplementary material, S2). In addition, there is a host-use shift within Formicinae, from Plagiolepidini (Gollumiellinae wasp host) to Lasiini, Formicini and Camponotini (Eucharitini wasp hosts). Also within Eucharitini, a major host shift occurs in the PEM parasitoids. The ancestral host in this clade is equally likely to be Ponerinae or Ectatomminae, with one recent jump to Myrmeciinae in the Australian genus *Austeucharis* 12.9 Ma (6.6–19.8 Ma; figure 3). Ectatomminae and Ponerinae were historically treated as one subfamily (Ponerinae; [59]), but are now known to be distantly related [37,38]; they are mid- to large-bodied, ‘socially primitive’ predators in a non-phylogenetic assemblage collectively referred to as the poneromorph ants [53,59]. Myrmeciinae are also ground-nesting generalist predators/scavengers with a simple social structure [52,60]. While Myrmeciinae (*Myrmecia*) is a unique host association for one eucharitid taxon, both Ectatomminae and Ponerinae are hosts for each of the three clades attacking the PEM ants (Chalcura, Schizaspidia and Kapala clades; figure 1). Although the host associations in this terminal PEM parasitoid group are phylogenetically diverse, the ant hosts share similar morphology and behaviour.

(c) Eucharitidae diversification and biogeography

Under a homogeneous birth–death model, there are potentially two rate shifts in Eucharitidae as compared with the background ($r = 0.0307$). Although not necessarily causative, life-history or geographical transitions can be correlated to diversification rate shifts. One rate increase occurs in Eucharitini, excluding *Pseudometagea* ($r = 0.0988$). The group encompassed in the rate transition includes both formicine and PEM parasitoids. These increases coincide with eucharitid expansion on speciose groups of ants, including the worldwide ponerine, ectatommine and camponotine ants (see figure 3 and electronic supplementary material, S2 and table S1). Members of the Eucharitini switched to attacking ants with their pupae in cocoons, and these wasps also exhibit an extraordinary amount of morphological variation [55] in characteristics of body size, antennal structure and thoracic spines. The second diversification rate increase is at the base of the New World *Orasema* ($r = 0.1902$). As in the Old World *Oraseminae* genera, New World *Orasema* are able to successfully exploit

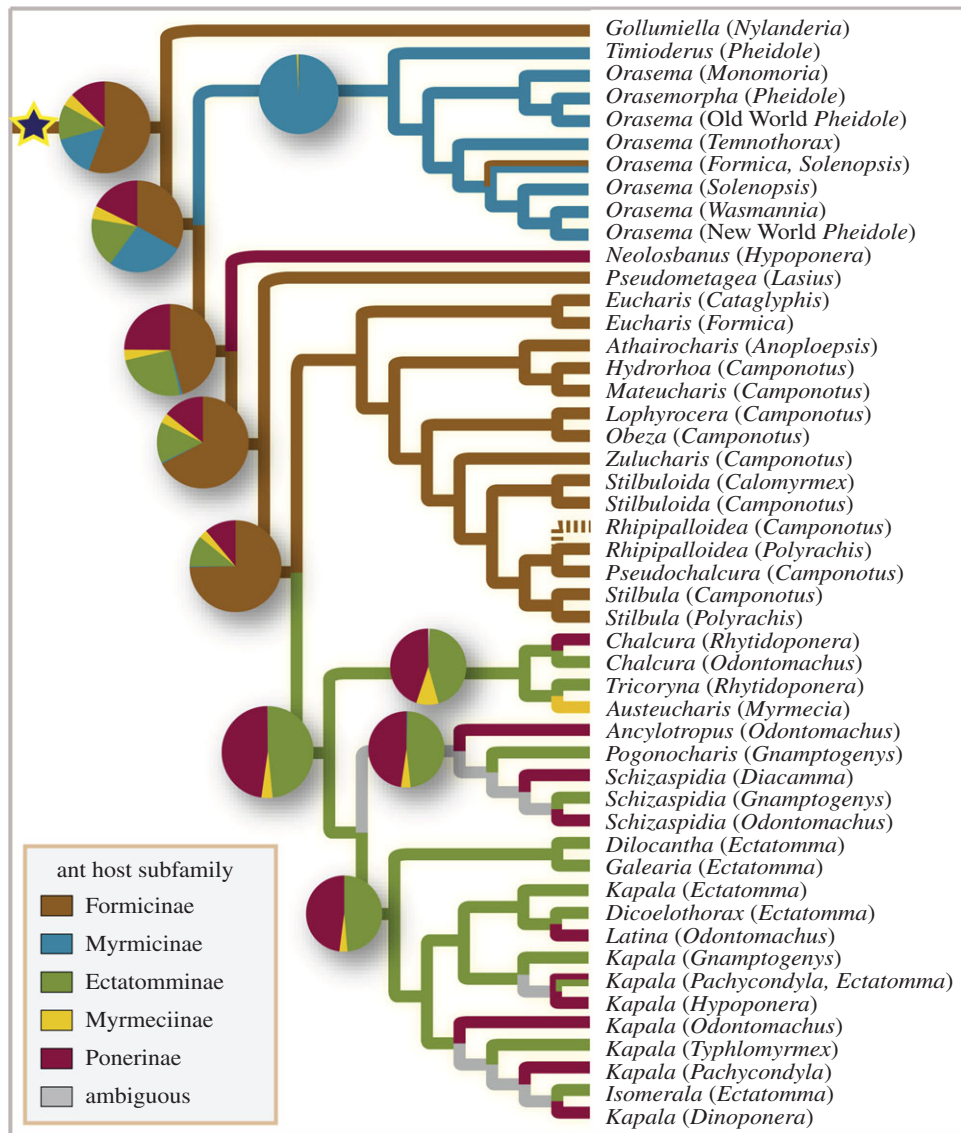


Figure 3. Ancestral state reconstruction. Forty-eight terminal taxa in analysis, with each genus of ant host represented by a parasitoid taxon (see the electronic supplementary material, table S1). Dashed line indicates the record was included for illustrative purposes (taxon not in data matrix). Pie charts at selected nodes display proportional probability under Bayesian inference. Coloured branches show parsimony reconstruction. Terminals labelled by eucharitid genus, with ant genera in parenthesis.

the hyperdiverse *Pheidole* [61], but they also parasitize five additional ant genera in the Nearctic and Neotropics, including the fire ants *Solenopsis* and *Wasmannia* [29,36,62].

Based on reconstruction of ancestral areas using the dispersal-extinction-cladogenesis model in Lagrange [63,64], our results support an origin of Eucharitidae in the Old World. Stem eucharitids have a relative probability of 20.2 per cent of originating in ancient Australia (locality of sister group *Akapala*), and the crown Eucharitidae have the highest probability of their ancestral area being the Indo-Pacific region, at 24.6 per cent. For each, there were multiple biogeographic areas included within the 2 log likelihood unit cut-off [64], indicating uncertainty in reconstruction. The major eucharitid clades, excluding the Old World Gollumiellinae, are distributed in both the Old and New World, and ancestral area reconstruction suggests members of the myrmecine, formicine and PEM parasitoid groups invaded the New World in five separate events (figure 1a). Adult eucharitids typically live only a few days outside the nest, and likely could not undergo long-distance dispersal [18]. The low probability of chance dispersal is supported by a high degree of

geographical endemism for most genera and clades [52], although we know of one case in the PEM parasitoids in which a single-derived species (*Kapala ivorensis*) of the diverse Neotropical *Kapala* clade colonized sub-Saharan Africa and Madagascar, presumably 1.4 Ma (0.5–2.6 Ma; figure 1a; electronic supplementary material, S1; bottom branches). This is the only instance of a dispersal event from the New to the Old World.

Ants are incredibly diverse in the Neotropics [65], and the New World ant groups evolved without parasitism pressure from eucharitids until approximately 43 Ma. At this point, we hypothesize multiple dispersals of eucharitid wasps from the Old World into the New World. Lagrange reconstruction points to South American ancestral areas for three New World clades (*Obeza* + *Lophyrocera*, *Pseudochalcura*, and the 13 genera comprising the *Kapala* Clade), whereas two (*Pseudometagea* and New World *Orasema*) exhibit a North American ancestral area.

Our evidence points to the possibility of multiple dispersal mechanisms and routes for different groups to colonize New World ants. The five dispersals potentially occurred throughout a time period greater than 20 Myr (approx. 20–43 Ma), as

global landmasses and climate were changing [66,67]. Although land routes were intermittently open for eucharitid passage, long-distance oceanic rafting cannot be ruled out. Both North and South American ancestral areas are hypothesized, indicating a possibility for both northern and southern dispersal. *Orasema* may have used a northern dispersal route. The age of the New World *Orasema* stem at approximately 20–23 Ma suggests this dispersal likely overlapped with the Late Oligocene warming, when arctic climate was temperate [66,68]. Remarkably, the major Old World Oraseminae ant host, *Pheidole*, may have moved in the opposing direction approximately 30 Ma, dispersing from the New to the Old World [61].

It has also been shown that ants were dispersing worldwide during the time of eucharitid diversification [61,69] and were likely using Northern Beringian routes to move from the Old World to New 10–30 Ma [70], and southern land routes to move from the New to Old World approximately 30 Ma [71]. Through each movement to the New World, despite the abundance of available ant taxa, eucharitids remained constrained to the same ant subfamilies as their Old World relatives. Thus, established host constraints remained in place despite the availability of new host niches.

(d) Ant–eucharitid associations

It has been postulated that the coevolution of ants and their associates follows a gradual progression from predaceous hostile invader to the eventual integration of the species into the ant colony [12], with parasitoids representing the ultimate nest symbionts [72]. In the case of Eucharitidae, however, they successfully colonized Formicidae directly as brood parasitoids via planidial larvae shared with perilampid relatives.

Eucharitidae exhibit a general trend of ant subfamily colonization (host-switching) occurring infrequently at an early time period, followed by high host conservatism (phylogenetic affinity) at the ant-subfamily level in extant lineages. In the PEM parasitoids attacking three different subfamilies, these eucharitids seem to be successful on ants with a similar ecological niche as opposed to success owing to a taxonomic affinity (figure 3). These findings are in agreement with previous research on arthropod host–parasitoid or parasite associations concluding that host use is not determined by host phylogeny [5,10,73] as had been hypothesized in various historical studies [1,2,10].

If ecological similarity rather than host phylogeny accounts for the high amount of host-switching within the PEM parasitoids, this leads to the hypothesis that parasitoid host range may be limited by ecological constraints [73], and host switches shaped by ecological fitting [74,75], where organisms can succeed in a novel environment owing to their suite of traits previously evolved. Eucharitids potentially have succeeded in diversifying on many ant taxa owing to the mechanism by which the planidia unite with the hosts and subsequently by how immatures and adults mimic host hydrocarbon profiles [20]. Evidence exists of other myrmecophiles that facilitate shifts among different ant hosts by exploiting communication codes [72].

3. Conclusions

Eucharitids colonized ants by approximately 72 Ma and have since proliferated worldwide and are known to parasitize 23 genera in 12 tribes. These wasps are able to break the

communication codes used in kin recognition among colony members to successfully escape harm as both immatures and adults while in the ant nest. Eucharitidae use ants across the phylogeny, yet there are still empty niches in speciose or resource-rich ant groups, namely the dolichoderine ants, fungus ants (attines) and the driver and army ants (dorylomorphs) which typically support diverse symbionts and myrmecophiles that need the ‘protection’ of large, long-lived colonies [13,17].

Eucharitidae are abundant and diverse but the ecological effects they have on their hosts are still not quantified, though adults and larvae have been recorded in nests year-round and can reach nest parasitism rates of greater than 25 per cent of pupae parasitized [18,76]. Their success suggests that they could form a promising model for the investigation of parasitoid impact on ant colonies [36]. The major eucharitid clades display phylogenetic conservatism through a pattern of ancient novel host colonization and subsequent host tracking; this lack of strict cophylogeny coincides with other documented host–parasitoid relationships [10]. Together, the evolutionary and biogeographic histories of ant and eucharitid have produced the unique association where hundreds of diverse parasitoid species have profited by proliferating on a eusocial host family.

4. Material and methods

(a) Taxon sampling

The molecular dataset includes 237 specimens, with dense taxonomic sampling across Eucharitidae comprising 44 of the 53 eucharitid genera from 41 countries. Eight taxa are outgroup Chalcidoidea, 34 are Perilampidae and 195 are Eucharitidae (see the electronic supplementary material, table S2). Five gene regions were sequenced: 18S, 28S-D2 and 28S-D3-D5 (nuclear), and COI and COII (mitochondrial; electronic supplementary material, text S1). Genbank accession nos. are given in the electronic supplementary material, table S2, and the aligned matrix is deposited in the Dryad Depository (datadryad.org; doi:10.5061/dryad.qn57t). Summary statistics and primers are compiled for each gene region in the electronic supplementary material, tables S4 and S5. Specimen images can be found on Morphbank (morphbank.net) under collection no. 816728.

(b) Phylogenetic analyses, divergence dating and rate diversification

Gene regions were partitioned into three unlinked groups: 18S, 28S-D2–D5 and COI + COII (electronic supplementary material, table S4). To streamline computation and tree-drawing, monophyly was enforced for Perilampidae + Eucharitidae because this has been supported in previous studies [27,32,33]. A birth–death process was used for the tree priors, using a starting tree generated from the same dataset under a Yule model. The trees were calculated under an uncorrelated lognormal relaxed clock.

Three Baltic amber fossils were used to calibrate nodes. (i) *Monodontomerus* sp. (Torymidae; [77]) was used to constrain the crown outgroup Torymidae. (ii) *Perilampus pisticus* (Perilampidae: Perilampinae; [31]) was used to calibrate the crown node at the base of the present-day paraphyletic *Perilampus*. (iii) *Palaeocharis rex* (Eucharitidae: Eucharitinae; [31]), sister to present-day *Psilocharis*, was used to constrain the node of the stem of *Psilocharis*. The mean date of the Baltic amber was estimated at 44.1 ± 1.1 Myr [78], corresponding to the age of the fossil-rich blue earth stratigraphic layer. To accommodate uncertainty in the date, the prior

for all three nodes was specified as a lognormal distribution at 44.1, mean 8.08, offset 39.2 (in real space), which translates to a 95% probability range of 40.2–64.6 Myr for the included fossils, with the highest prior probability at 44.1 Ma.

In BEAST v. 1.6.2 [79], two Markov chain Monte Carlo (MCMC) chains were run to 200 million generations, logging parameters every 20 000. We also ran an empty alignment to verify that the data were driving the pp distributions [80]. Subsequent to the phylogenetic analysis, TRACER v. 1.5.0 [81] confirmed the effective sample size (ESS) of the posterior and all major clades reached greater than 200. LOGCOMBINER v. 1.6.2 and TREEANNOTATOR v. 1.6.2 were used to combine the trees from the two runs and then obtain a single tree of highest clade probabilities. A total of 10 002 trees were removed as burnin, for a final distribution of 10 000 trees.

Eucharitidae clade diversification was analysed using turboMEDUSA [82] in R (v. 2.13.1, R Development Core Team 2011). MEDUSA (modelling evolutionary diversification using stepwise Akaike information criterion (AIC)) uncovers diversification rate shifts in the phylogeny by fitting alternative models to the input chronogram [83]. From an initial model specifying one rate across the phylogeny, rate change breakpoints are inserted successively at internal nodes until the optimal corrected AIC is reached. We included 68 genera in the eucharitid + perilampid chronogram and specified the estimated species richness of each genus; required if the tree is not completely sampled. The projected diversity values were from Heraty [55] and the Universal Chalcidoidea Database [84]. The input combination of phylogeny and taxonomy is used to reveal clades that deviate statistically from the number of species expected owing to age of the group.

(c) Cophylogenetic reconstruction, character mapping and biogeography

Cophylogeny reconstruction methods were used to explore the possibility of parallel patterns of phylogeny between host and parasitoid. We used Jane 4 [57] for reconstruction and statistical analysis. Jane 4 implements event-cost methods and a genetic algorithm to map the parasite tree to the host tree as based on the ant phylogeny of Moreau *et al.* [37]. We reduced the datasets of ants and eucharitids down to the genera that had a host record pairing it to the opposing family, resulting in 23 host genera and 29 parasitoid genera. The cost matrix used the following settings (cospeciation = 0, duplication, loss, failure to diverge = 1 and duplication + host switch = 2), and the analysis was run to 200 generations with a population size of 400. We could not implement timing capabilities for incorporating temporal congruence owing to the large gap in origin of host and parasitoid species; host switches for nodes in different time zones are not permitted in Jane. Statistical significance was assessed by randomly permutating the tree tip pairings and re-assessing the cost distribution to determine if the input pairings remain as

the lowest-cost scenario. Two statistical analyses were run to a sample size of 200: (i) 'random tip mapping' of the two phylogenies and (ii) 'random parasite tree' calculation at beta = -1.0. A result of less than 5 per cent of random solutions as better than the observed cost total is strong evidence for cophylogeny [85].

Ant host associations are available for 29 of the 44 eucharitid genera in the dataset (electronic supplementary material, table S1). BAYESTRAITS v.1.0 [58] MultiState analysis was used for reconstruction of an ancestral character state at specified nodes. A fully Bayesian implementation was used, with a distribution of 10 000 trees (from the dating analysis). The trees were pruned to 48 taxa, which represented the unique ant genera records for each wasp genus available in the molecular phylogeny. Each eucharitid terminal was coded by ant subfamily, for a total of five discrete states. Analyses were run to 200 million generations, sampling every 20 000, discarding the first 50 million generations. We used the reversible-jump MCMC option, using an exponentially distributed prior and a uniform hyperprior drawn from the interval [0,10], with an additional parameter of a rate deviation of 0.015 to ensure that acceptance rates were above 20 per cent, which did result in mean acceptance rate of 24.5 per cent of the 3000 post-burnin trees. TRACER v. 1.5.0 was used to confirm ESS greater than 200 and to obtain the mean output value for all five subfamily probabilities at each node of interest. In addition, MESQUITE v. 2.73 [86] was used to trace host associations on the topology using parsimony reconstruction.

For the reconstruction of ancestral areas, we used LAGRANGE v. 20120508 (likelihood analysis of geographical range evolution; [63,64]), which implements a stochastic model of range evolution, incorporating dispersal, extinction and cladogenesis. This program uses a given set of areas with their connections (dispersal routes) in conjunction with an input chronogram to estimate the ancestral area likelihoods at each node of the tree [63] with a script assembled via the online configurator. Seven areas were recognized: (i) North and Central America including Caribbean, (ii) South America and Lesser Antilles, (iii) Ethiopian, (iv) Malagasy, (v) Indo-Pacific and (vi) Australian, following Heraty [55]. However, Central America/Caribbean is here included with North America instead of with South America as in [55] owing to its historic connection with the northern landmass. We developed dispersal constraints for four time periods (electronic supplementary material, text S2). All 229 terminals of Perilampidae and Eucharitidae were coded for geographical range according to specimen collection locality.

The authors thank all institutions and individuals contributing or loaning collection material. We thank David Hawks for sequencing a portion of the specimens. We are grateful to Astrid Cruaud, John Hash, Jason Mottern, Greg Walker and Christiane Weirauch for helpful comments and discussion. We also thank two anonymous referees for their input. This work was supported through funding from NSF Advances in Bioinformatics DBI-0641076 to J.H., NSF PEET DEB-0730616 to J.H. and two UC van den Bosch Biocontrol scholarships to E.M.

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