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ORIGINAL ARTICLE

Phylogenetics of the allodapine bee genus *Braunsapis*: historical biogeography and long-range dispersal over water

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ABSTRACT

Aim A previous study of the allodapine bee genus *Braunsapis* suggested an African origin, with dispersal events into Madagascar and Asia, and from Asia into Australia. We re-examine the phylogeny of this genus, using an expanded set of taxa from Madagascar and Malawi and additional sequence data, in order to determine the number of dispersals and the timeframe over which they occurred.

Location Africa, Madagascar, Malawi, Asia and Australia.

Methods One nuclear (EF-1α F2) and two mitochondrial (CO1 and Cyt *b*) gene regions were sequenced for 36 allodapine bee species (including members of the genera *Braunsapis*, *Nasutapis*, *Allodape*, *Allodapula*, and *Macrogalea*) and one ceratinine species (*Ceratina japonica*). We used Bayesian analyses to examine phylogenetic structure and a penalized likelihood approach to estimate approximate ages for key divergences in our phylogeny.

Results Our analyses indicate a tropical African origin for *Braunsapis* in the early Miocene followed by very early dispersal into Asia and then a subsequent dispersal, following Asian diversification, into Australia during the late Miocene. There have also been two dispersals of *Braunsapis* from Africa to Madagascar and this result, when combined with phylogenetic and biogeographical data for other allodapines, suggests that these bees have the ability to cross moderately large ocean expanses. These dispersals may have been aided by the West Wind Drift, but rafting across the Mozambique Channel is also possible, and could be aided by the existence of developmental stages that require minimal or no feeding and by tolerance to sea water and spume. Accumulating evidence suggests that many biogeographical patterns in the southern hemisphere may be better explained by dispersal than by Gondwanan vicariance hypotheses. Our results add to this growing body of data and raise the possibility that some puzzling trans-Indian Ocean distributions may also be explained by historical dispersal events across oceanic barriers that now seem insuperable.

Keywords
Allodapine bees, dispersal, historical biogeography, Madagascar, Mozambique Channel, phylogenetics, vicariance.

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INTRODUCTION

As with many animal and plant groups, Madagascar has a high level of endemicity among its bee fauna (Pauly *et al.*, 2001). While it has been assumed that many Malagasy bee taxa have resulted from dispersal events across the Mozambique Channel (e.g. Michener, 1977), there have been no studies that have explicitly assessed how frequently bees have been able to cross this barrier or have assessed the likelihood of vicariance models, involving the rifting of Madagascar and India from Africa, in shaping the Malagasy fauna. Madagascar provides an excellent opportunity for contrasting vicariance and dispersal models because the time at which rifting occurred (*c.* 80–90 Ma) is well understood. The rapid separation of the

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Madagascan and Indian plate left a barrier of *c.* 450 km that has changed little since the initial separation (Rabinowitz *et al.*, 1983). This means that for clades where African and Malagasy elements diverged < 80 Ma, dispersal events are implicated, and these divergences provide opportunities to assess how frequently successful dispersal events have occurred. A recent study examining the historical biogeography of plant and animal groups in the southern hemisphere (Sanmartín & Ronquist, 2004) has suggested that dispersal events may have played a much more important role, compared to vicariance events, than had been assumed. The known palaeogeological relationships between the African, Madagascan and Indian plates, when combined with recent techniques for estimating divergence times from DNA sequence data (e.g. Thorne *et al.*, 1998; Sanderson, 2002), provide an excellent opportunity to assess dispersal abilities for taxa that span these areas. For allodapine bees, a Gondwanan vicariance origin is very unlikely as it would require the divergence of allodapines to have occurred shortly after the origin of bees *per se* in the Early Cretaceous (Engel, 2001), something that seems unlikely given the derived nature of allodapines in the family Apidae (Michener, 2000; Engel, 2001) which is itself a non-basal bee clade.

Allodapine bees have for a long time been used to explore hypotheses concerning the evolution of social behaviour (Schwarz *et al.*, 1998), but recent studies have shown that they also raise some interesting questions for historical biogeography. One of the most basal divergences in the tribe involves a split between the Australian 'exoneurine' genera (*Exoneura*, *Exoneurella*, *Brevineura* and *Inquilina*) and the key African genera of *Compsomelissa*, *Halterapis*, *Allodapula*, *Allodape* and *Braunsapis*. This ancient divergence is biogeographically puzzling because there are no intermediate lineages in the Asian region which could represent an early northern dispersal corridor. Furthermore, the exoneurine genera are exclusively non-tropical, again arguing against an Asian dispersal route. Bull *et al.* (2003) raised the possibility of a trans-Indian Ocean dispersal event, perhaps enabled by now-submerged elements of the Kerguelen Plateau.

The genus *Braunsapis* forms an interesting contrast to the puzzling basal split between the Australian and mostly African genera. While *Braunsapis* is most speciose in Africa, and appears to have a tropical African origin (Schwarz *et al.*, 2004), it also occurs in Madagascar, southern Asia and non-temperate regions of Australia. Phylogenetic analyses (Schwarz *et al.*, 2004) suggested an early dispersal into Asia from Africa, followed by a later dispersal into Australia. That study also indicated that the sole Malagasy species for which sequence data was available was nested within the African clade, indicating dispersal across the Mozambique Channel.

Apart from *Braunsapis*, Madagascar also contains three other allodapine genera: *Macrogalea*, *Effractapis*, and *Halterapis*. *Effractapis* is likely to have arisen from within *Braunsapis* (Michener, 1977), while molecular phylogenetic analyses indicate that *Macrogalea* dispersed from Africa to Madagascar (Smith, 2004; Tierney, 2004). The Malagasy species of *Halterapis* form a monophyletic group and this clade diverges basally from the non-*Macrogalea* African clades very early in allodapine evolution (Schwarz *et al.*, 2005a).

The ability of allodapine bees to traverse wide ocean expanses is important because it raises the possibility that some very broad biogeographical distributions of bees that appear to have Gondwanan distributions may reflect dispersal, rather than vicariance, events. However, the timing and frequency of such events has been unknown. Here we analyse an expanded set of *Braunsapis* from Madagascar and Malawi, including the parasitic genus *Nasutapis*, and show that *Braunsapis* has dispersed into Madagascar at least twice, raising the likely number of allodapine dispersals to Madagascar to four. We discuss the implications of our results for broader aspects of bee biogeography.

METHODS

Taxa and collecting localities

Schwarz *et al.* (2004) suggested that *Braunsapis trochanterata*, which is morphologically similar to *Allodape* but is a tropical species, was the sister clade to all other *Braunsapis* in their study, but did not have very strong support for this. In our analyses we wished to further explore the phylogenetic position of this species and we therefore include a wide representation of *Allodape* species to help resolve basal nodes in *Braunsapis*. We also included representatives of *Allodapula*, sister clade to *Braunsapis* and *Allodape*, and *Macrogalea*, sister group to all other allodapines. *Ceratina japonica* was used as the outgroup since the Ceratinini are the extant sister tribe to the Allodapini (Engel, 2001).

We included the same *Braunsapis* species used by Schwarz *et al.* (2004) and added a further five *Braunsapis* species as well as an undescribed species of *Nasutapis*, a social parasite of some *Braunsapis* species, and a Malagasy species of the genus *Macrogalea*, which is the sister group to all other allodapine genera. The locality data for these new taxa are given in Table 1. Of the additional putative *Braunsapis* species from Madagascar, two were from the island of Saint Marie, and although they differed in face markings, they may actually be the same species. The new *Braunsapis* taxa were collected from widely different regions of Madagascar, viz. the far north east (St. Marie), the far west (Toliara) and the far south (Taolagnaro). The species used by Schwarz *et al.* (2004), and tentatively identified here as *B. madecassa*, was from the central plateau near Antananarivo. The undescribed species of *Nasutapis* and its *Braunsapis* host were from Malawi. *Nasutapis* has only one described species, from Kwa-Zulu Natal in South Africa and it is likely that our species is different, given the very different climates in which they are found. Kwa-Zulu Natal has a subtropical climate, and although a number of tropical African bee species have been found there, it is likely that our *Nasutapis* species is different given the very large distance separating Malawi from Kwa-Zulu Natal.

Table 1 New taxa sequenced for this study, along with their collecting localities and GenBank accession numbers. N/A = no sequence obtained

Taxon	Collecting locality	Accession number
Braunsapis Toliara sp.	Toliara, Madagascar	CO1: DQ160165 Cytb: N/A EF-1a: DQ160173
Braunsapis Taolagnaro sp.	Taolagnaro, Madagascar	CO1: DQ160166 Cytb: N/A EF-1a: DQ160174
Braunsapis StMarie sp1.	St Marie, Madagascar	CO1: DQ160167 Cytb: DQ160171 EF-1a: DQ160175
Braunsapis StMarie sp2.	St Marie, Madagascar	CO1: DQ160168 Cytb: DQ160172 EF-1a: DQ160176
Braunsapis Nasutapis host	40 km NW Mangochi, Malawi	CO1: DQ160164 Cytb: DQ160170 EF-1a: DQ160177
Nasutapis sp.	40 km NW Mangochi, Malawi	CO1: DQ160163 Cytb: DQ160169 EF-1a: DQ160178
Macrogalea antanosy	Taolagnaro, Madagascar	CO1: DQ149680 Cytb: DQ149692 EF-1a: DQ149724

DNA sequencing

Specimen handling and DNA extraction techniques are described by Schwarz *et al.* (2003, 2004). One nuclear and two mitochondrial gene regions were amplified and sequenced (bi-directionally). The mitochondrial regions were from the protein coding genes cytochrome oxidase *b* (Cyt *b*; 428 bp sequenced) and cytochrome oxidase I (CO1; 1279 bp), and the nuclear exon region from the F2 copy of elongation factor 1α (EF-1α F2; 772 bp). The primers used for polymerase chain reaction (PCR) amplification of the Cyt *b* and COI regions, and amplification conditions for these regions are given in Schwarz *et al.* (2004). EF-1α occurs as two copies, EF-1α F1 and EF-1α F2, in bees, which are expressed at different stages of development (Danforth & Ji, 1998). The primers used for PCR amplification of the EF-1α F2 region included the F2-specific forward primer (HaF2For1: 5′-G GGY AAA GGW TCC TTC AAR TAT GC-3′) designed by B.N. Danforth (pers. comm.) and the F2-specific reverse primer (F2-Rev1) designed by Danforth & Ji (2001) for halactid bee species. Amplification conditions for the EF-1α F2 region included an initial hot start of 94 °C for 9 min, followed by 35 cycles of denaturation at 94 °C for 30 s; annealing at 54 °C for 45 s, extension at 72 °C for 1 min; and then a final extension step of 72 °C for 6 min.

PCR products were purified using Ultraclean PCR Clean-up columns (MO BIO Laboratories, Inc., Carlsbad, CA, USA), and *c.* 50 ng of product was sequenced in 20 μL reaction volumes using the Big Dye Sequencing Ready Reaction kit Version 3.1 (Applied Biosystems, Foster City, CA, USA), with the original PCR primers used as sequencing primers. Reaction products were purified by isopropanol precipitation and sequenced on a capillary DNA sequencer. Forward and reverse sequences were compared for each gene fragment, and sequences were manually edited and aligned using SeqEd 1.03 (Applied Biosystems). The intron region of EF-1α F2 was excluded from analyses because large sections of sequence were unalignable.

Phylogenetic analyses

Schwarz *et al.* (2004) showed that maximum parsimony (MP) analyses where mitochondrial 3rd codon positions are included can lead to spurious topologies because of long branch effects exacerbated by extreme AT richness. They also showed that single model maximum likelihood methods can also lead to spurious results, probably because single models are not appropriate for partitions which are highly heterogeneous in both base composition and substitutional dynamics (Bruno & Halpern, 1999). However, the same study showed that partitioned Bayesian analyses appear to give realistic topologies for allodapines because they allow more realistic fitting of separate models to heterogeneous partitions.

Here we undertake a MP analysis where mitochondrial 3rd codon positions are excluded. Heuristic and bootstrap analyses used 50 random sequence additions, holding five trees at each step, with stepwise addition and tree bisection and reconnection (TBR). Bootstrap analyses employed 500 pseudoreplicates. However, we also undertook a MP analysis including all codon positions, to explore the effect of including 3rd codon mitochondrial nucleotides.

We also undertook a partitioned Bayesian analysis using MrBayes (Huelsenbeck & Ronquist, 2001). We used the same methods as Schwarz *et al.* (2004, 2005b) and separate models were fitted to six partitions comprising the 1st, 2nd and 3rd positions across both the combined mitochondrial genes and EF-1α. Base composition, R matrices and gamma shapes were unlinked for all partitions. Preliminary analyses indicated non-zero proportions of invariant sites for 1st and 2nd codon positions, but effectively zero proportions of sites for 3rd positions. We therefore used the MrBayes 'invgamma' option for 1st and 2nd positions (a prior specifying a proportion of invariant sites with the remaining sites having gamma-distributed rate variation) and the 'gamma' option (all sites having gamma-distributed rates) for 3rd positions. Otherwise, default MrBayes priors were used.

Bayesian analyses were run for 3×10^6 generations, sampling every 500th generation to reduce autocorrelation among parameter values over sampled generations. Stationarity in model parameters was checked using graphs of log likelihood values and the values of model parameters against generations sampled. We used a burnin period 1.5×10^6 generations, which was well beyond the reaching of stationarity which occurred before 0.5×10^6 generations. Node support was examined using posterior probabilities. Three independent Bayesian runs were undertaken to investigate whether model parameters, branch lengths and posterior probability (PP) support for nodes converged on similar values.

Estimating relative divergence times

One aspect of our study requires broad comparison of relative divergence times for several clades to evaluate likely biogeographical dispersal possibilities. We therefore produced an ultrameric tree showing relative divergence times with a penalized likelihood (PL) approach using Sanderson's (2002) r8s program. Penalized likelihood is a semi-parametric method that allows branches to have different evolutionary rates at the expense of a roughness penalty (Sanderson, 2002). We used the truncated Newton algorithm for finding optima and estimated the smoothing parameter using cross validation, comparing χ^2 errors across a range of $\log_{10}$ smoothing values from 0 to 5.

Confidence intervals for node ages estimated using penalized likelihood are usually computed from maximum likelihood (ML) phylograms fitted to bootstrapped data. However, this was not possible for our data since the gene/codon position partitions in our data set were clearly too divergent for application of a single ML model (Schwarz *et al.*, 2004). Instead, we chose the following method. We ran the Bayesian analysis, described above, for 4×10^6 generations and with *Apis mellifera* and *Lasioglossum lanarium* added to the taxa set (see below). The post-burnin trees (sampled every 500th generation) were filtered using the post-burnin consensus phylogram, leading to a set of phylograms with identical topology but with varying branch lengths. These were then exported into r8s. The r8s 'profile' command is commonly used to estimate confidence in node ages by estimating mean node ages and their standard deviations using bootstrapped data. However, our Bayesian generation samples do not constitute bootstrap replicates, since each generation is based on all available sequence data, and these generations are unlikely to have the same statistical qualities as bootstrapped pseudoreplicates. We chose to estimate confidence in node ages by determining the central 95% age distribution of each node of interest using the r8s PL chronograms based only on the filtered Bayesian phylograms.

It is not possible to calibrate the allodapine phylogeny using internal nodes because there are no known allodapine fossils. However, the fossil tribe Boreallodapini is the sister tribe to the allodapines and three fossil *Boreallodape* species are reliably dated from Baltic amber 45 Ma. The extant tribe Ceratinini is the sister clade to Boreallapini + Allodapini (Engel, 2001), and therefore the divergence between Ceratinini and the Allodapini has occurred at least 45 Ma (through probably much earlier than this). We also added *Apis mellifera* to the taxa set because the corbiculate bees are likely to have evolved by *c.* 90 Ma (Engel, 2001). Because Sanderson's PL method requires that the outgroup be pruned from trees prior to analysis, we added a further taxon, *Lasioglossum lanarium* (Halictidae), to the data set in order to root the remaining apid species. Sequences for the two latter species were obtained from GenBank (*Apis mellifera*: U72278, AJ581105, AF015267; *Lasioglossum lanarium*: AF103956, AF264793).

Dispersal and vicariance analysis (DIVA)

We used the program DIVA 1.1 (Ronquist, 1996, 1997) to infer likely vicariance and dispersal events that shaped the current distribution of *Braunsapis*. Species were recorded as belonging to any of four regions, namely Africa, Madagascar, Asia and Australia; the sister clade, *Allodape*, and the next-most basal group in our analysis, *Allodapula*, were recorded as African. Although *Macrogalea* occurs in both Africa and Madagascar, it has only recently (*c.* 2 Ma) colonized this island via a single dispersal event (Tierney, 2004) and the Malagasy clade is nested within the African clade, so following the recommendation by Ronquist (1996) we recorded its presumed ancestral distribution as African. The outgroup, Ceratinini, occurs worldwide, so its distribution was recorded as including all regions occupied by *Braunsapis*. DIVA allows one to specify the maximum number of regions (henceforth 'maxareas') that could be simultaneously occupied by hypothetical ancestral lineages, ranging from two to the number of regions currently occupied by terminal taxa (Ronquist, 1996) and this will depend on assumptions about how widespread ancestral populations could have been in the past. We carried out three analyses with maxareas ranging from two to four.

RESULTS

We recovered 1282, 427 and 774 bp of COI, cytb and an exon region of EF-1α respectively, of which 406, 151 and 213 bp were parsimony informative.

The bootstrap consensus tree from the analysis where 3rd mitochondrial positions were excluded is given in Fig. 1. Relationships among genera were the same as for MP analyses by Schwarz *et al.* (2003, 2004). Bootstrap support for bifurcation order was generally low to very low, which was not surprising given the problems for MP analysis to handle the heterogeneity in partitions and high levels of homoplasic change in the mitochondrial genes. Importantly, the Malagasy *Braunsapis* species were recovered as two separate clades, one comprising *B. madecassa* and the other comprising the remaining species. However, bootstrap values for the nodes separating these clades were not very high, ranging from 73% to 86%. The *Nasutapis* species was recovered firmly within *Braunsapis*, supporting Michener's (1970) suggestion that it is derived from within that genus. *Nasutapis* was not placed as sister clade to its host (an undescribed *Braunsapis* species from Malawi), but as the sister group to a clade that contained both African and Malagasy species. Support for monophyly of the Asian + Australian species was weak and indeed the highest bootstrap value for nodes within this group was only 49%.

The MP bootstrap analysis where 3rd mitochondrial positions were included gave a broadly similar topology to that shown in Fig. 1 except for some differences where bootstrap values were very low. *Allodape* was placed as the sister group to *Allodapula* (48% bootstrap support), *B. trochanterata* was placed as the sister clade to all other *Braunsapis* species (27% support), *B. KoChang* sp. was placed as sister species to the

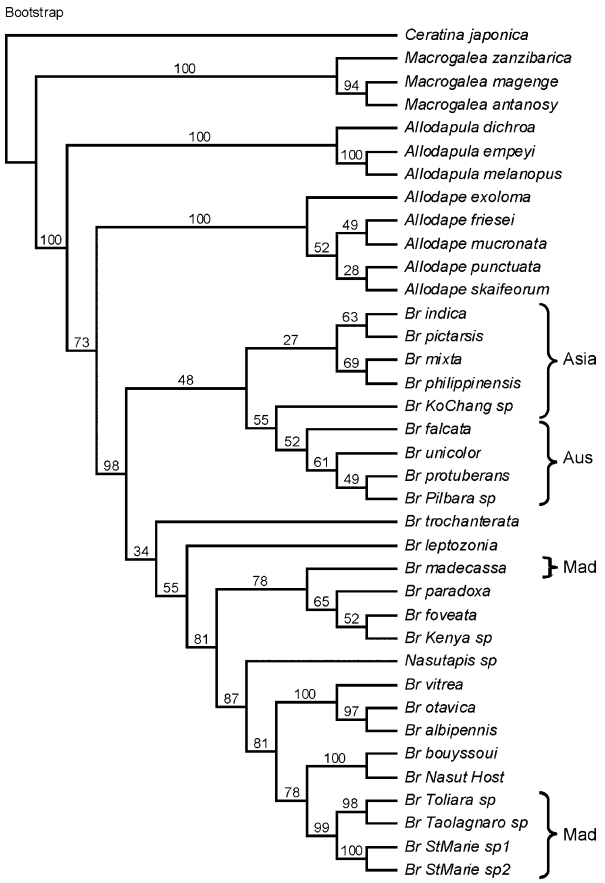

Figure 1 Bootstrap MP analysis with mitochondrial 3rd codon positions excluded. Bootstrap values, consistent with a 50% majority rule consensus, from 500 pseudoreplicates are shown for each node. Regional occurrence is indicated as Mad, Madagascar; Aus, Australia; Asia, southern Asia. All other ingroup taxa are African.

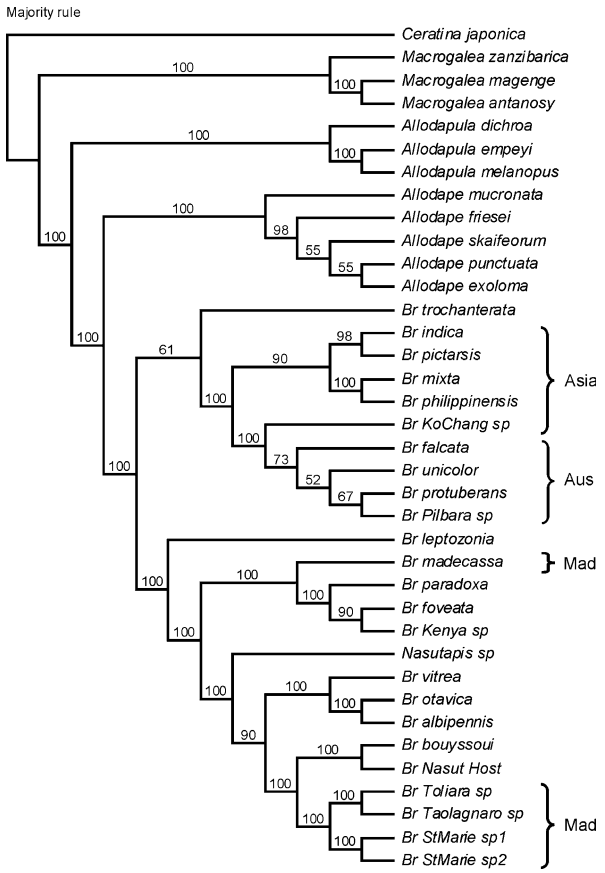

Figure 2 Consensus Bayesian tree with posterior probabilities shown for each node. Regional distributions are as in Fig. 1.

Australian species *B. unicolor* (65%), and *B. madecassa* was recovered as sister clade to the Australian + *B. KoChang* sp. clade (61%). Compared to the analysis where 3rd mt positions were excluded, there were higher bootstrap values for more distal and especially subterminal nodes, and lower values for more basal nodes, as would be expected when levels of homoplasy become higher and long branch effects more likely.

A consensus phylogram from the first Bayesian run is given in Fig. 2 where PP support is indicated for all nodes. The two other analyses converged on identical topologies and highly similar model parameters and PP support values. The Bayesian tree is almost identical in topology to the MP bootstrap tree and the only topological differences arise for nodes with low support in both analyses, involving some distal nodes in *Allodape* and the position of *B. trochanterata*. In the MP analysis *B. trochanterata* was the sister group to the remaining African species, whereas in the Bayesian analysis it was recovered as sister group to the Asian + Australian taxa. However, node support for the position of this species was very low for both analyses. This suggests that divergences shortly after the origin of *Braunsapis* may have been very rapid, with little informative signal being retained among current sequences.

Relative divergence times

Graphing of chi-square error terms against smoothing values (not shown here) indicated an optimal smoothing value of 1.45. This value was used in subsequent r8s analyses. The PL transformed tree, based on the Bayesian consensus phylogram, is given in Fig. 3, but with the outgroup, *Lasioglossum lanarium*, pruned. This tree assumes divergence between *Apis mellifera* and the outgroup as being set at 90 Ma with a minimum divergence between the Ceratinini and Allodapini of 45 Ma. The estimated divergence times, along with the central 95% age distributions for specific nodes, are also indicated on the figure.

Because there are no fossil allodapines with which the tree can be internally calibrated, relative divergence times need to be approached cautiously. However, estimated ages for some key nodes are concordant with expectations from palaeogeography: our estimated time for dispersal of *Braunsapis* from Africa to Asia coincides with the closing of the Tethys Sea in the early Miocene, and dispersal of *Braunsapis* from Asia to

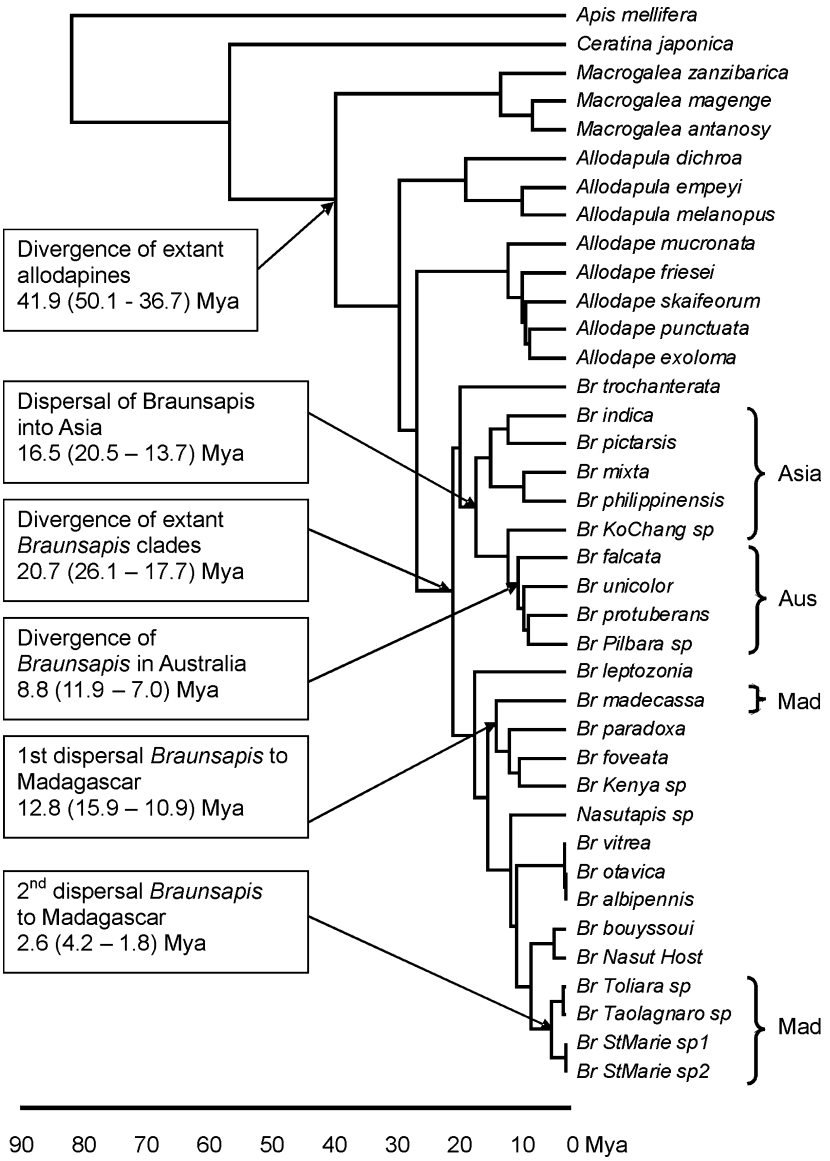

Figure 3 Chronogram based on penalized likelihood transformation of the Bayesian consensus phylogram. 95% central distribution limits, based on a sample of 120 post-burnin Bayesian generation samples filtered for topological concordance with the Bayesian consensus tree, are indicated for key nodes.

Australia coincides with the collision between the Australian and Laurasian plates in the late Miocene, when biotic interchange between these two landmasses became common. The estimated divergence time (60+ Ma) between the Ceratinini and Allodapini also appears feasible, given that the 45 Myr old intervening Boreallodapini is clearly distinct from both tribes and, given that three to four species are known from Baltic amber (Engel, 2001), was probably speciose at that time.

Dispersal and vicariance analysis (DIVA)

All three DIVA analyses ('maxareas' ranging from two to four) gave identical exact solutions for ingroup internodes, and all inferred Africa as the origin of *Braunsapis* as well as a solely African distribution for the ancestor to *Allodape* and *Braunsapis*, and the ancestor to *Allodapula* and *Braunsapis* + *Allodape*. The inferred vicariance events involving *Braunsapis* were: (1) separation of Africa from Madagascar resulting in the Malagasy *Braunsapis* St Marie clade and the *B. bouyssoui* clade; (2) separation of Africa from Madagascar resulting in the Malagasy *B. madecassa* clade and the African *B. foveata* clade; (3) separation of the African and Asian + Australian clades with *B. trochanterata* as sister clade to the Asian + Australian clade; and (4) divergence of the Australian clade from within the African clade. However, each of these inferred vicariance events is impossible: (1) Madagascar + India rifted from Africa *c.* 120 Ma (Rabinowitz *et al.*, 1983), long before the tribe Allodapini had evolved (Schwarz *et al.*, 2005b); (2) southern Asia gradually moved closer to Africa with the closing of the Tethys Sea, rather than rifting away; and (3) Asia and Australia have gradually moved closer together with the northwards movement of the Australian plate, and the most recent connection between the two regions was severed *c.* 120 Ma when Madagascar + India rifted from Africa. A study by Yuan *et al.* (2005) also found impossible vicariance events inferred

by DIVA and noted that this was because DIVA regarded wide distribution and vicariance as a default optimization, but one which was clearly precluded by their dating analyses. Therefore, the inferred vicariance events in our analyses must instead reflect dispersals, meaning that there have been two dispersals from Africa to Madagascar, one from Africa to Asia, and one from Asia to Australia.

DISCUSSION

Although the MP analysis gave a well resolved tree, bootstrap support for many nodes was very low, especially those involving bifurcation order among genera. This is similar to other MP studies on allodapines (e.g. Bull *et al.*, 2003; Schwarz *et al.*, 2003) and is probably due to the high AT richness of mitochondrial genes leading to high levels of homoplasic change. Furthermore, because bootstrap samples were generated through a process of sampling with replacement, low support values may result from having a reduced proportion of informative sites not affected by AT richness included in the analysis. In contrast, the Bayesian posterior probability support values were generally high, though tree topology was very similar to the MP tree. This is not surprising given a number of recent studies that suggest that bootstrapping leads to conservative estimates of support whereas PP values tend to be inflated (e.g. Leache & Reeder, 2002; Whittingham *et al.*, 2002; Wilcox *et al.*, 2002). However, the PP values of 100% for nodes that are related to multiple colonization events of Madagascar and the nesting of *Nasutapis* within *Braunsapis*, are sufficiently high to allow confidence in conclusions based on those nodes.

Phylogenetic relationship of *Nasutapis* to *Braunsapis*

Our analyses firmly placed *Nasutapis* within the *Braunsapis* clade, confirming Michener's (1970) suggestion that this parasitic genus is derived from within *Braunsapis* making that genus paraphyletic, and that it's remarkable morphology represents extreme adaptation to obligate social parasitism. Interestingly, our *Nasutapis* species was not closely allied with its host species, but was the sister group to a clade containing both African and Malagasy species.

Basal nodes and historical biogeography of *Braunsapis*

The expanded sequence data used in our study lead to the same broad relationships found by Schwarz *et al.* (2004) indicating that divergence between the African and Asian *Braunsapis* clades occurred early in the evolution of *Braunsapis*, and that the Australian species were nested within the Asian clade. All DIVA analyses indicated an origin of *Braunsapis* in Africa, which is not surprising given that *Braunsapis* is most abundant and diverse in Africa (Michener, 2000) and its sister genus, *Allodape*, is entirely African. Although the DIVA analyses inferred four vicariance events involving *Braunsapis*, these are geologically impossible and must instead reflect dispersal events. For example, the geological rifting of Madagascar + India from Africa *c.* 120 Ma (Rabinowitz *et al.*, 1983) occurred well before the origin of the Allodapini (Schwarz *et al.*, 2005b), and ever since the origin of the bees *per se* the relationships of southern Asia to both Africa and Australia involved a coming together rather than rifting apart. Consequently, our analyses suggest two dispersals from Africa to Madagascar, one dispersal from Africa to Asia, and one from Asia to Australia.

Our PL analysis suggests an origin of *Braunsapis* in the early Miocene with dispersal into southern Asia *c.* 17 Ma, which is concordant with the southern closing of the Tethys Sea in the early Miocene. The estimated timing of dispersal from southern Asia into Australia is also concordant with geographical events, corresponding to the collision between the Laurasian and Australian plates, with uplifting of the southern Alps, in the late Miocene. Like the Schwarz *et al.* (2004) study on the exoneurine clade, we found little support for bifurcation order among the Australian *Braunsapis* species, suggesting that radiation of species may have occurred very rapidly after colonization of a new continent.

One issue not resolved by Schwarz *et al.* (2004) was the position of the tropical species *B. trochanterata*. Their results suggested that this species may be the sister clade to all other *Braunsapis*. This species has strong larval and adult morphological affinities to *Allodape* and although *Allodape* is most diverse in southern Africa, a number of taxa occur in tropical Africa. Although our MP analysis provides little firm support for placing *B. trochanterata* among the more basal nodes, the Bayesian analysis places it firmly between the origin of *Braunsapis* and divergences of the remaining African species and the Asian + Australian clade. Although *B. trochanterata* was grouped with the Asian + Australian clade, support was very weak (61% PP support), but monophyly of the Asian + Australian and the remaining African clades was very strong (100% PP support). This supports the hypothesis of a tropical origin of *Braunsapis c.* 21 Ma, followed by early dispersal into southern Asia (*c.* 17 Ma).

Dispersal into Madagascar

Our results indicate that the Malagasy *Braunsapis* species form two clades (one being mono-specific) and both were firmly grouped within different African clades. This indicates that there have been at least two successful dispersals from Africa to Madagascar in this genus. The earlier dispersal occurred *c.* 13 Ma and resulted in one extant species (*B. madecassa*), and the later dispersal occurred *c.* 2–3 Ma, and has resulted in at least three putative species (*B.* Toliara sp., *B.* Taolagnaro sp., *B.* St Marie sp1 and 2). The distance between Africa and Madagascar has been more-or-less constant at *c.* 450 km since *c.* 80 Ma (Rabinowitz *et al.*, 1983). McCall (1997) suggested that a landbridge may have connected Africa to Madagascar between 45 and 26 Ma, although this is not supported by geological studies (Rogers

et al., 2000). Even if such a landbridge or, more likely, isolated subaerial seamounts (Rogers *et al.*, 2000) had existed, they subsided well before the first *Braunsapis* dispersal event suggested by our analyses and could not have aided this dispersal across the Mozambique Channel. Phylogenetic analyses of African and Malagasy species of *Macrogalea* indicate one dispersal event into Madagascar and dating analyses indicate this was recent (< 5 Ma, Smith, 2004; Tierney, 2004). Together these findings indicate three unambiguous dispersal events from Africa to Madagascar across an ocean barrier that was at least 450 km wide. Phylogenetic analyses of the Malagasy species of *Halterapis* (Schwarz *et al.*, 2005a) indicate that this group is the sister clade to the African non-*Macrogalea* taxa and that these two clades form the sister group to the Australian exoneurine genera (*Exoneura*, *Exoneurella*, *Inquilina* and *Brevineura)*. This suggests a fourth dispersal event between Africa and Madagascar, although the direction of dispersal cannot be determined from present data. However, our estimated time for the most basal allodapine divergence, that is between *Macrogalea* and the remaining allodapines (50.1–36.7 Ma) precludes a vicariance explanation for this early divergence between Malagasy *Halterapis* and the African non-*Macrogalea* genera.

It is possible that dispersals from Africa to Madagascar were aided by the West Wind Drift, but the possibility of rafting is also likely. Allodapines nest in dead stems and branches and it is conceivable that rafts of vegetation washed into the Mozambique Channel could have ferried allodapines across to Madagascar. A rafting hypothesis, from Africa to Madagascar, has been suggested for lemurs (Yoder *et al.*, 1996) and this possibility seems even more feasible for allodapines for several reasons: (1) allodapine immatures often show very protracted development and larvae can show arrested development over winter (Michener, 1965, 1971) so that lack of feeding during this time need not result in death; (2) having a pupal stage means that at least one immature instar does not require feeding even under warm conditions and would be immune to lack of food throughout its developmental duration. Finally, colonies of one Australian allodapine bee survive tidal inundation of nests (Neville *et al.*, 1998), and many allodapine genera, including Malagasy *Braunsapis*, nest in coastal dune vegetation where exposure to spume is common (M. Schwarz, pers. obs.), suggesting that some species may be able to tolerate exposure to sea water during oceanic rafting.

The likelihood of rafting vs. wind dispersal from Africa to Madagascar could be assessed by examining phylogenetic relationships among other bee taxa that occur in both these regions If wind dispersal is likely then we would expect to also see multiple dispersals for bee taxa that are ground nesters, but if rafting provides a better means of crossing ocean barriers we would expect to see a disproportionate rate of dispersal for stem and branch nesting species, such as megachilid, xylocopine and ceratinine bees. All of these latter groups, as well as many ground nesting bee taxa, are well represented in both Africa and Madagascar, so that a comparative investigation of rafting vs. wind dispersal is highly feasible.

Given the above considerations, it should be kept in mind that our results indicate only two successful dispersals of *Braunsapis* from Africa to Madagascar over the *c.* 20 Myr since the origin of *Braunsapis*. Our Madagascan samples of *Braunsapis* involved intensive collection of nests from the far north, the far south, the far west and the central highlands and it seems unlikely that we failed to sample clades resulting from additional dispersal events. Given the high number of species and abundance of *Braunsapis* in Africa (Michener, 1975), the two successful dispersals into Madagascar should be viewed as indicating that dispersal over such a distance is an extremely rare event.

Lastly, our results may be important for understanding the puzzling origin of the Australian exoneurine genera. Bull *et al.* (2003) showed that this group had a very early divergence from the African clades, and dispersal into Australia via a northern Asiatic route was very unlikely. They suggested dispersal across the Indian Ocean, via island stepping stones that may have been remnants of the now largely submerged Kerguelen Plateau (Sanmartín & Ronquist, 2004). Schwarz *et al.* (2005b) also argue that this divergence between the African and Australian clades is too early for a Laurasian dispersal route. Our results, indicating ability to cross ocean barriers of 450 km, suggest that dispersal across the Indian Ocean may indeed be a feasible hypothesis if periods of low sea level or volcanic uplifting lead to retention or emergence of Kerguelen elements that could provide stepping stones. However, the rarity of long-range dispersal events from Africa to Madagascar, indicated above, suggests that such stepping stones would have to have been common and not too widely dispersed. There is a clear need for better understanding of emergent land elements in the Indian Ocean over the last 50 Myr.

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