THE EVOLUTION AND DIVERSIFICATION OF THE ALLODAPINE BEES

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CONTENTS

SUMMARY

This thesis investigates the evolution and biogeographical history of the bee tribe Allodapini. Examinations of life history traits, nesting patterns, and morphology were the primary methods used to explore allodapine biology, whilst a molecular-based phylogenetic approach was used to explore patterns of speciation and diversification within the tribe. A primary feature of this research is the expansion and revision of the allodapine phylogeny, using DNA sequence techniques. The phylogenetic components of this thesis focus primarily on the earliest divergences and generic-level bifurcations within continentally and environmentally defined allodapine clades. These relationships were used to examine the nature of broader changes in sociality, nesting biology, morphology, and geographic distribution across the tribe as a whole.

Results indicate that the strong benefits social nesting affords in repelling enemies-at-the-nest is implicit in the universal retention of social behaviour within the Allodapini, and that this characteristic may also apply to other taxa that show a lack of reversions from social to solitary living. Results also suggest that some of the tribe's farthest-reaching radiations occurred rapidly and relatively early, with some of the foremost involving dispersal events that do not appear to fit with current palaeogeographical reconstructions. The infrequency of major transitions between different environmental biomes within the tribe is indicative of ecological constraints and niche conservatism that appears to have resulted in low adaptive radiation and diversification. These constraints appear ameliorated during periods of climatic and environmental instability; possibly by way of allopatric speciation promoted by habitat fragmentation. Allodapines play a fundamentally important role as pollinators within their ecosystems. As such, these findings highlight the impact that climatic and environmental change, as well as rare and poorly understood mechanisms of dispersal, can have on key components of a biome's constituent taxa and hence the course of an ecosystems future evolution.

DECLARATION

I declare that this thesis does not contain any material previously submitted for any diploma or degree in any university without acknowledgement, and that to the best of my knowledge it does not contain any material previously published by any other person except where due reference is made.

Luke Ballingall Chenoweth

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"In all things of nature there is something of the marvellous."

— Aristotle

GENERAL INTRODUCTION

For species that play pivotal roles in the function of terrestrial ecosystems, such as key pollinators, a better understanding of both biological and biogeographical history has the potential to provide important information about an ecosystem's evolution and development over time. Additionally, understanding how and why certain ecological constraints may restrict an organism's physiology, biology or behaviour can have important ramifications, as the taxonomic composition of an ecosystem is inevitably linked to the frequency or rarity of species' transitions between biomes.

The diversification of the bees is strongly tied in with the sudden and rapid diversification of the angiosperms during the early-mid Cretaceous (Engel 2001). Due to their role as the most diverse and specialised group of angiosperm pollinators (Michener 2007), bees play an integral role in the function of almost every terrestrial environment on Earth. Understanding the history of diversification, speciation, and adaptation of the bees is therefore crucial in better understanding the origins and evolutionary histories of many ecosystems across the globe.

At present, the extant bee lineages are divided into seven families: the "long-tongued" families Apidae and Megachilidae, and the "short-tongued" families Andrenidae, Colletidae, Halictidae, Melittidae, and Stenotritidae. Current understanding of the origins of these families point to the melittids *sensu stricto*, meganomiines and dasypodaiines as the most basal bee taxa (Danforth *et al.*, 2006), followed by the long-tongued lineages Megachilidae and Apidae. Among the Apidae, the subfamily Xylocopinae is situated as the possible sister group to the rest of the family (Roig-Alsina & Michener, 1993), suggesting a relatively early origin for this group amongst the bees. One group of xylocopine bees, the tribe Allodapini, has been the subject of several phylogenetic studies to date. The allodapine bees are largely restricted to the southern hemisphere, where they comprise a significant component of the African, Malagasy, and Australian bee fauna. Despite this relatively widespread intergeneric distribution and habitat range, the allodapine bees frequently show a high level of generic-level conservatism in habitat, nesting biology, life-history traits, and adult morphology (Schwarz *et al.*, 1998; Tierney *et al*., 2000). Perhaps most notably, social nesting strategies are seemingly present (albeit highly variable) in all but a single species; *Halterapis nigrinervis* (Michener 1969; 1974). The unique attributes this tribe displays, combined with their important functional role in many southern hemisphere ecosystems, means that important insight into the historical ecology of the

southern hemisphere may be gained from a better understanding of diversification and evolution within the allodapines. To date, the biology and phylogenetics of the allodapines is fairly well understood. However, in order to further investigate the history of this group, several key issues still need to be addressed.

This thesis endeavours to address several of these issues in order to better understand the biogeographical and evolutionary history of the allodapines. The components of this thesis are presented in the form of four multi-authored chapters. Versions of chapter I and II have been published in *BMC Evolutionary Biology* (**7:**246) and *Systematic Entomology* (**33(4):** 700-710) respectively, and these chapters are presented as copies of the final submitted drafts. Chapter III is currently accepted and awaiting publication in *Journal of Biogeography*, and is also presented as a copy of the final submitted draft. Chapter IV is in the final stages of preparation for submission to *Molecular Phylogenetics and Evolution*, and is presented as the first draft to be submitted to this journal. The following is a summary of each chapter.

CHAPTER I: Social complexity in bees is not sufficient to explain lack of reversions to solitary living over long time scales.

Chapter I investigates the causative factors of the development of social behaviour in the allodapines, focusing chiefly on its maintenance and alteration through speciation.

Michener (1971) described *Halterapis nigrinervis* as nesting in a subsocial manner with each nest containing a single inseminated female and any additional females described as newly emerged callows preparing to disperse. However, the possibility of alloparental care or the presence/absence of size-based reproductive hierarchies within nests has never been fully investigated. A study by Chenoweth (2005) was the first to show reproductive skew based loosely on size, strong female bias in sex ratio, and increased per capita brood production in multifemale nests, suggesting benefits of social nesting do exist in *H. nigrinervis*. As a result, no solitary allodapine species would be known to exist despite a potentially ancient origin of sociality. Unlike other taxonomic groups where social behaviour is ubiquitous such as ants and termites, the Allodapini do not display any of the key behavioural, physiological, or genetic traits which have been traditionally associated with highly social nesting strategies (Wilson & Hölldobler 2005). This leaves an intriguing avenue for further study.

CHAPTER II: *Hasinamelissa*: a new genus of allodapine bee from Madagascar revealed by larval morphology and DNA sequence data.

Chapter II examines the relationship between the African and Malagasy species of the allodapine genus *Halterapis*, and explores how this relationship fits with current hypotheses of biotic interchange between Africa and Madagascar. Recent studies into the social biology of the African+Malagasy genus *Halterapis* suggest the presence well-established sociality and highly unique brood rearing strategies restricted to the genus' Malagasy taxa (Schwarz *et al.* 2005; Chenoweth & Schwarz 2007.). Whilst the African and Malagasy taxa are currently assigned as congeneric, lack of information on larval characteristics or a well-resolved phylogeny renders the placement of the Malagasy and African taxa together unpersuasive. Madagascar has one of the highest levels of species endemism in the world, and this also applies to its bee fauna (Pauly *et al.* 2001). Determining the historical connection between the Malagasy *Halterapis* and the remainder of the tribe could provide important insight into not only the biogeographical history of the allodapines, but also the greater history of biotic interchange between Madagascar and Africa.

CHAPTER III: Biogeographical origins and diversification of the exoneurine allodapine bees of Australia (Hymenoptera, Apidae).

Chapter III explores the geographical hypothesis of the Australian 'exoneurine' clades' dispersal into Australia, and its subsequent history of diversification within the continent. Two distinct clades of allodapines are known to exist in Australia. The first are members of the largely African genus *Braunsapis* and provide a typical example of dispersal into Australia via the Indian Ocean Rim after the collision between the Australian and Laurasian plates (Fuller *et al.* 2005). The history of the second allodapine group in Australia is more puzzling. The Australian allodapine genera *Exoneura*, *Brevineura*, *Exoneurella*, and the parasitic *Inquilina* form an endemic, continentally defined clade that primarily inhabits the southern semiarid and temperate regions of Australia (Michener 1965; Schwarz *et al.* 2006). Schwarz *et al.* (2003) showed that the Australian clade is monophyletic among the allodapines and Bull *et al.* (2003) showed this divergence to be very early in the tribe's history. Current palaeogeographical models rebuke a Gondwanan origin for the clade, and Schwarz *et al.* (2006) approximated the initial radiation of the exoneurines dating back at least 25-30 My, making the notion of dispersal of the exoneurines into Australia through Asia also unlikely. Despite these findings, very little can be substantiated about either the means by which the clade reached Australia, or their

subsequent history of diversification and speciation. Australia has the most unusual compliment of bee fauna in the world (Michener 1965, 2007), of which the allodapines comprise a relatively archaic and basal group. A better understanding of the origin and diversification of the exoneurines can thus provide a greater understanding of the biogeographical history of what may be some of the oldest members of the Australian bee fauna.

CHAPTER IV: Biogeographical history of the African allodapine bees (Hymenoptera, Apidae).

Chapter IV looks at the major speciation events within the African allodapines and the factors that have influenced tribe's radiation both within and out of Africa. The continent of Africa holds an important position as the likely origin of the Allodapini (Bull et al. 2003; Schwarz et al. 2004) and the African taxa are rendered paraphyletic to both the Australian and Malagasy clades. The African taxa are composed of a basal '*Macrogalea*' clade and a more derived clade containing the remainder of the African taxa. This latter 'African' clade spans a broad range of habitats across the Afrotropic zone of sub-Saharan Africa, and is unique as it also contains two major dispersals into other regions of the world. One of these dispersals resulted in the genus *Exoneuridia*: a rare montane genus present in the Arabic peninsula, Iran, and southern Turkey (Terzo 1999). The second, involving *Braunsapis*, is thought to have its origins in tropical Africa and subsequently spread throughout Asia and into northern Australia (Schwarz *et al.* 2006). Whilst both these genera are thought to have diverged from the remainder of the African taxa at similar times, they differ radically in their current distribution and habitat ranges. At present the topological placement of these genera within the African clade remain poorly defined. This is primarily due to the fact that despite strong phylogenetic support in a majority of the clade, certain areas require a much larger taxonomic set in order to confidently infer phylogenetic relationships. A better understanding of the history of the African taxa is fundamental to understanding the broader trends of diversification within the Allodapini as a whole. Additional phylogenetic examination of the African allodapine taxa, using an expanded data set, is essential to more accurately infer these divergences and explore the earliest stages of diversification within the Allodapini.

Finally, the overall conclusions and broader implications of these studies, as well as important avenues for further research, are examined in a General Discussion following Chapter IV.

The candidate is primarily responsible for all data collection, analysis, laboratory work, interpretation/discussion of the results, as well as production and formatting of published material. The following contributions were made by various co-authors: Chapter I drew on data collection assisted by Jaclyn A Smith and the analytical expertise of Dr Steven JB Cooper. Chapters I and II drew from substantial data collection and sequence data assisted by Simon M Tierney. Chapter II also drew from sequence data obtained by Yung C Park and Susan Fuller. Chapter IV drew from the field collections and analytical expertise of Michael J McLeish. All four chapters were written with commentary from the candidate's supervisor, Associate Professor Michael P Schwarz. Funding from grants awarded to Michael Schwarz are also recognised by his inclusion as author.

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CHAPTER I

Social complexity in bees is not sufficient to explain lack of reversions to solitary living over long time scales

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Abstract

Background

The major lineages of eusocial insects, the ants, termites, stingless bees, honeybees and vespid wasps, all have ancient origins (≥ 65 mya) with no reversions to solitary behaviour. This has prompted the notion of a 'point of no return' whereby the evolutionary elaboration and integration of behavioural, genetic and morphological traits over a very long period of time leads to a situation where reversion to solitary living is no longer an evolutionary option.

Results

We show that in another group of social insects, the allodapine bees, there was a single origin of sociality > 40 mya. We also provide data on the biology of a key allodapine species, *Halterapis nigrinervis*, showing that it is truly social. *H. nigrinervis* was thought to be the only allodapine that was not social, and our findings therefore indicate that there have been no losses of sociality among extant allodapine clades. Allodapine colony sizes rarely exceed 10 females per nest and all females in virtually all species are capable of nesting and reproducing independently, so these bees clearly do not fit the 'point of no return' concept.

Conclusions

We argue that allodapine sociality has been maintained by ecological constraints and the benefits of alloparental care, as opposed to behavioural, genetic or morphological constraints to independent living. Allodapine brood are highly vulnerable to predation because they are progressively reared in an open nest (not in sealed brood cells), which provides potentially large benefits for alloparental care and incentives for reproductives to tolerate potential alloparents. We argue that similar vulnerabilities may also help explain the lack of reversions to solitary living in other taxa with ancient social origins.

Background

Highly social insect groups have had enormous ecological success [1], yet eusociality has evolved very infrequently [2], raising the question of what barriers there might be to its origin. Furthermore, soldier castes have been lost in some thrips [3] and aphids [4], resulting in the loss of eusocial nesting strategies. In halictine bees there have been three origins of sociality but as many as twelve losses, suggesting that in an evolutionary sense complex sociality may be difficult to gain, but easy to lose [5]. In contrast, there have been no losses of sociality in the ants, termites, vespid wasps and corbiculate bees, all of which evolved sociality > 65 mya [6].

In both thrips and halictid bees, sociality has evolved much more recently than the Cretaceous origins of sociality in ants, termites, corbiculate bees and vespid wasps. McLeish *et al.* [7] showed that sociality in gall forming thrips evolved less than 10 mya, and Brady *et al.* [6] showed that the three origins of sociality in halictines are also relatively recent (22 - 20 myBP). This raises the question of whether losses of sociality are more likely in lineages where sociality is relatively recent, compared to older social lineages that may have reached a 'point of no return'.

The notion of a 'point of no return' was first suggested in the early 1970s by Wilson [1] and proposes that, given suitable evolutionary time, the multiple and integrated adaptations associated with highly complex social behavior may preclude reversions to less complex or non-social life cycles. Wilson and Hölldobler [8, p. 13368] refer to this evolutionary point as one where it is either "impossible, or at least difficult and uncommon", for a eusocial species to revert back to a more primitively social or non-social form of organization, and speculate that it coincides with the evolution of an anatomically distinct worker caste. The conjecture is important because it proposes a degree of irreversibility in social evolution due to integration among adaptations in multiple traits, a situation akin to the idea of phylogenetic inertia arising from bauplan constraints [9].

Importantly, the 'point of no return' hypothesis seems to be the only one proposed for the lack of reversions to solitary life-cycles in the major social insect groups and as such is an almost default paradigm. This is surprising, given the amount of debate on other aspects of social evolution. Yet the point of no return hypothesis lacks a clearly stated underlying mechanism for why reversions are precluded and, as such, is not truly falsifiable. Nevertheless it is open to indirect assessments: in particular, demonstrating that very long term maintenance of sociality does not require social complexity or a distinct

worker caste would indicate the possibility of alternative explanations for long term maintenance of sociality.

Indirectly assessing whether points of no return depend on social complexity and distinct worker castes can be achieved by examining patterns of origins and losses in taxa where these two traits are absent or variable. Brady *et al.*'s [6] study of origins and losses in halictine bees provides one such assessment. Halictine bees provide special insights into social evolution because, unlike most other eusocial groups, adult females in all species are totipotent and capable of producing brood [10]. Thus, individual females are not constrained to group nesting, so that evolution is able to 'explore' non-social options. Only one other group of bees, tribe Allodapini (Apidae), is speciose, exhibits diverse range in forms of sociality and, in virtually all species, all females are totipotent [11].

Until recently it was thought that sociality in allodapines had arisen comparatively recently among the extant lineages [11] from a subsocial ancestor (i.e. a solitary ancestor displaying extended parental care), and this ancestral trait had been retained in a phylogenetically basal African allodapine, *Halterapis nigrinervis* [12]. At the same time, nesting biology of the speciose *Halterapis* group from Madagascar was unknown. Recent molecular analyses [13] show that the African *Halterapis* is not basally situated in the allodapine phylogeny, and that the African and Malagasy members of this genus are in fact paraphyletic, implying a need for future taxonomic revision. Using both Bayesian and penalized likelihood approaches, Schwarz *et al.* [14] indicated an origin of the allodapine tribe as being >40 mya but pointed out that this is likely to be a highly conservative estimate. Recent behavioral studies also show that the Malagasy *Halterapis* display complex social behavior [15, 16]. These findings indicate that sociality is plesiomorphic for the allodapines, and despite the relatively ancient origin of the tribe, the African *Halterapis* is the only allodapine to have potentially lost complex social behavior.

Results

The initial study of *H. nigrinervis* [12] was based on a sample size of only eleven nests and multifemale colonies comprised a single inseminated female along with one to several uninseminated females that were presumed to be recently eclosed adults and soon to disperse.

Our sample comprised a total of 52 *H. nigrinervis* nests. Seven nests were stored in ethanol and the remaining 45 in Kahle's solution for dissection. These samples (Table 1) indicate that *H. nigrinervis* is indeed social because: (i) approximately half (54%) of the nests collected were multifemale; (ii) multifemale nests were far more likely to contain brood (Table A1, Additional Files) and the number of young brood (eggs and larvae) increased with the number of adult females, indicating enhanced brood production when multiple adult females are present within a nest (Figure 1); (iii) sex ratios were strongly female-biased, consistent with positive kin interactions leading to local resource enhancement [17, 10]; and (iv) ovary sizes of nestmates were strongly influenced by intra-colony body-size rank (Figure 2) indicating the existence of reproductive hierarchies. These results mean that *H. nigrinervis* is social and that there are consequently now no known losses of sociality in allodapines.

Consensus phylogenies from three partitioned Bayesian analyses of sequence data all had identical topologies and almost identical branch lengths. The consensus cladogram with posterior support values is given in Figure A1 (Additional Files). Ingroup generic-level bifurcations were highly supported and consistent with other sequence-based studies of allodapines [14]. We also analysed sequence data with a maximum parsimony approach and that gave broadly consistent results to our Bayesian analyses and previous phylogenetic studies [14] (Figure A2, Additional Files). The only inconsistencies between our Bayesian, our MP results, and those of previous studies, involve nodes close to terminal taxa.

We used penalized likelihood transformation of the Bayesian phylograms to produce a chronogram (Figure 3), which also indicates the geographic distribution of the major clades. When the basal node (divergence of corbiculate bees from the xylocopine bees) is conservatively set at 90 myBP, the point estimate for the earliest divergence of allodapine clades is 47 myBP, with a lower (most recent) 95% limit of 40 myBP. Because sociality occurs in all the extant allodapine lineages, it must be a plesiomorphic trait and must therefore have originated by at least 40 myBP, and probably much earlier given the very conservative nature of all three calibration points. This age-estimate is similar to that of Schwarz *et al.* [14] and suggests that the age of the allodapine root node is approximately twice the ages for estimated origins of sociality in halictine bees [6].

As an independent assessment of approximate divergence ages, we compared pairwise maximum likelihood distances for allodapine species whose most recent common ancestor (MRCA) was at the root allodapine node with those for halictine species whose

MRCA was at one of the three origins of sociality in halictines [6], which were dated at 20-22 mya [6], using a sequenced fragment of EF-1α common to both groups. Substitutional parameters for this gene fragment are almost identical for halictines and allodapines (see supplementary material) indicating that evolutionary dynamics for this gene fragment are very similar for the two bee groups. If evolutionary rates are also comparable in the two groups, the resulting distances suggest that the root node of the allodapines is about twice the age of the origins of sociality suggested for the halictines (Figure A3, Additional Files), which concords very closely with our penalised likelihood analysis.

Discussion

Our results are important because they suggest that, compared to halictine bees, allodapines have a much older origin of sociality but show no losses of sociality. In this respect, they are more similar to eusocial lineages such as ants, corbiculate bees, vespid wasps and termites, which also have ancient social origins with no losses. This raises the question of what factors may prevent losses of sociality over very large time scales.

Lack of reversions to solitary living in allodapines cannot be explained by arguments that they are restricted to habitats or climatic regimes that favour sociality. The ecological diversification of allodapines covers habitats such arid gibber deserts, savannas, bushvelds, coastal heathlands, equatorial and subtropical rainforests, and sub-alpine regions [12, 18]. Colony phenology ranges from highly seasonal univoltine egg production schedules [18], all the way through to asynchronous development patterns where egg production, brood maturation, and foundress dispersal occur year round [10]. The lack of reversions to purely solitary living is even more notable because of the near-ubiquity of female totipotency in allodapines [10], which means that physiological factors do not preclude independent living. We argue that the absence of reversions to solitary living is due to ecological consequences of the way in which allodapines rear their brood, and this may also help explain some broader trends found in ants, termites and vespid wasps.

Unlike virtually all other bees, which rear their brood in fully provisioned sealed cells, allodapines rear brood in un-partitioned and unsealed tunnels. Because brood lack the physical protection of an enclosed cell, they are highly vulnerable to predation in the absence of an adult guard. Indeed, the major benefit of group living in allodapines is avoidance of total brood failure [18], and this benefit is greatest when comparing single-

with two-female colonies, because brood in the former colonies are unprotected when the sole adult is foraging [18]. This vulnerability of brood is heightened by orphaning, since not only will post-feeding brood be unprotected, but feeding-stage larvae will be unable to complete their development due to lack of food. In the event of orphaning, potential alloparents could reap large indirect fitness gains by simply protecting post-feeding brood or completing the feeding of partially reared larvae [19], as well as direct benefits from inheriting a nest along with a subsequent cohort of potential alloparents. In fact, alloparental rearing in the absence of possible mothers is recorded from diverse species [20, 21] indicating that it does not require maternal coercion. At the same time, mothers would gain benefits from permitting some daughters to remain in the nest as insurance against orphaning [22].

In contrast, the vulnerability of brood and the benefits of alloparental care in cell-provisioning insects are quite different. Mass provisioning of brood in cells means that mothers sequester their investments as completed units over time, and these cells provide brood with some physical protection from enemies at the nest. In addition, if orphaning does occur, immatures sealed in cells will have sufficient food for their complete development, further reducing the scope for benefits from alloparental care.

Whilst protecting the nest from both predation and parasitism is important for all bees [11, 23], allodapine brood are particularly vulnerable due to the lack of cells [10, 11]. Continuous protection from predation is impossible for a solitary nesting allodapine, as the nest is completely unguarded during the female's foraging trips. We suggest this vulnerability is a compelling explanation for why there have been no reversions to purely solitary living in allodapines but multiple losses in halictines.

It has been argued that the lack of reversions to solitary nesting in ants and termites may reflect the evolution of social complexity to a 'point of no return' [8], where a species is no longer able to live solitarily. However, there are numerous ant groups where newly founded colonies involve non-claustral queens rearing their first brood cohort to maturity without help from workers [24], suggesting that competency for solitary brood rearing *per se* exists in many taxa. For example, within many ant subfamilies (predominantly within in the poneroid clade but also some formicoid subfamilies [24, 25]), there are species where colony foundation almost exclusively involves a single brood-rearing foundress and very small ultimate colony sizes [24, 26]. Queens must forage in order to feed their first clutch of brood, indicating that solitary-founding females in these species are capable of foraging effectively enough to rear through their first generation of brood alone [26]. Many of the

aforementioned subfamilies also contain multiple independent losses of the queen caste, which has resulted in numerous species displaying ubiquitous female totipotency [26] but without any transitions to purely solitary living.

In social taxa where females can successfully rear brood to maturity in a non-claustral fashion without help of a worker caste, explanations for a lack of reversions to solitary living must involve something other than incompetence for independent brood rearing. Ants share one key life history trait with allodapines, namely that brood are progressively reared in unsealed communal tunnels. In social vespid wasps, larvae are also reared to pupation in unsealed cells, and while termite young are not 'provisioned' by adults, they also develop in unsealed chambers and are highly vulnerable in the absence of adults. In all these taxa, protection of brood depends heavily on adults, and this contrasts with halictines where nearly all species sequester their fully provisioned brood in closed cells. Losses of sociality would remove the brood protection that group living confers in the former groups, but the physical protection of cells in halictines would allow some protection of brood in solitary nesting halictines to be maintained.

Lastly, we have argued that the 'point of no return' paradigm has been framed in a way that does not readily allow falsification. We believe that our conjecture provides two predictions that permit empirical assessment: (i) that reversions from social to solitary brood rearing are more likely in clades where constraints to solitary living are low; and (ii) that reversions from social to solitary can occur even after specialised worker/soldier castes have evolved, provided that ecological constraints for independent reproduction are relaxed. Halictine bees and gall-forming thrips comprise two groups where these predictions could be tested, but there are likely to be many other taxa as well.

Conclusions

Our findings suggest a very different framework for understanding social evolution from that argued in some recent studies [27, 28] that emphasize the importance of mechanistic approaches involving physiology, regulatory circuits and genetic-networks. In particular, Hunt and Amdam [29] suggest "…that social evolution in insects can be fully – and finally – understood" by such mechanistic approaches. Although such approaches may help explain the ontogeny of worker-like behaviour, our results indicate that retention of such behaviour is likely to be due to ecological and life-history factors, and these have the

potential to determine very long term patterns of social evolution. The ecological dimensions of sociality cannot be ignored when trying to understand its origins and long-term maintenance.

Methods

Sociality

Colonies of *H. nigrinervis* were collected from Grahamstown, South Africa in 2005 from 20 to 23 February, when colonies were rearing brood. Colonies were preserved in ethanol for molecular studies and Kahle's solution for dissections. Ovarian indices of females were calculated as the summed lengths of the three largest oocytes divided by wing length (used as an indication of body size), and used when estimating ovarian enlargement to avoid body-size scaling effects. Colony productivity was measured as the number of eggs and larvae divided by the number of adult females.

Benefits of social nesting

If multifemale colonies contain only a single mother and other females are recently eclosed daughters who are soon to disperse, we would expect that the latter daughters would not have an effect on the number of brood being actively reared (larvae) or soon to be reared (eggs). We examined this statistically. Variation in the number of eggs + larvae among different colony sizes was assessed using Kruskal Wallis non-parametric test, rather than parametric ANOVA, because brood numbers were 1-truncated (colonies lacking any eggs or larvae were excluded). This test indicated a significant effect ($\chi^2_3 = 12.145$, $P = 0.007$) and Figure 1 below indicates an increasing function.

Molecular phylogenetics

Using molecular data, Schwarz *et al.* [14] argued that the Allodapini had an origin in the Eocene of at least 40 mya. Here, we re-examine the time of this origin using penalised likelihood transformation of a molecular phylogeny based on an expanded set of taxa that allows an additional fossil calibration point and better resolution of some internal nodes including the position of *Halterapis*. We also explore the effects of varying the date of the

basal node, namely the divergence of lineages leading to the allodapine and corbiculate bee clades.

We used two mitochondrial (COI and cyt b) gene regions and an exon region of one nuclear gene (F2 copy of EF-1α) comprising 1279, 428 and 772 nucleotides respectively. Our taxa comprised at least two species of each non-parasitic allodapine genus except *Exoneuridia* and *Compsomelissa* which are rare and for which we had only one species each. Most of the ceratinine and allodapine taxa used in our study have been used in previous phylogenetic analyses of allodapines by Schwarz *et al.* [13, 30, 15, 14] and Bull *et al.* [31] and GenBank accession numbers are provided in these manuscripts. In addition to these taxa, we used a Malagasy *Halterapis*, *Halterapis isaloensis* (EU254247, EU254248, EU254249) and an additional African *Halterapis*, *Halterapis nigrinervis* with a black metasoma (EU254250, EU254251). We used two halictid bees, *Lasioglossum lanarium* and *Agapostemon tyleri* [32] as the outgroup. *Apis mellifera*, as well as representatives of the genus *Bombus* (*Bombus terrrestris* and an unidentified *Bombus sp.* from Santiago, Chile (EU254244, EU254245, EU254246)) and an unidentifiable species of *Liotrigona* from Madagascar (EU 254241, EU254242, EU254243) were included to allow additional calibration points for determining the age of the allodapine root node (see below).

DNA extraction, amplification and sequencing methods used for the gene fragments used on our analyses have already been published, see Schwarz *et al.* [14] and references cited therein.

Phylogeny was inferred using both Maximum parsimony and Bayesian inference. We place greater reliance on Bayesian methods for allodapines because of greater ability of this approach to deal with heterogeneity in substitutional dynamics of different codon positions and gene fragments, as well as problems arising from signal degradation at third codon positions [32]. Bayesian inference was implemented with MrBayes v3.0.4 [33], with codon positions separately partitioned for the nuclear and combined mitochondrial genes, and all partitions unlinked for model parameters and using default priors, as described by Schwarz *et al.* [14]. Three Bayesian runs were undertaken in order to ensure runs were consistently converging on similar patterns. MCMC chains were run for $3x10^6$ generations, with a burnin of 1.5 million generations, and post-burnin trees combined across runs. Trees were sampled every 500th generation. All runs converged on identical consensus topologies and posterior probability values for nodes and branch lengths were based on a total of 9000 post-burnin sampled trees. The resulting consensus phylogram was

transformed into a chronogram using Sanderson's [34] penalised likelihood method. Bayesian methods using relaxed-clock models were not used because the estimated transition matrix clearly contradicted the most complex (F84) model that can be implemented using Thorne and Kishino's [35] Multidivtime software.

For our maximum parsimony (MP) analyses, we ran a heuristic search with 50 random sequence stepwise additions, with 5 trees held at each step, and TBR branch swapping with 500 bootstrap pseudoreplicates to estimate support. 3rd mitochondrial positions were removed from the analysis to prevent long-branch attraction between genera observed in previous studies [14, 30, 32].

To estimate the age of the allodapine root node (earliest divergence among extant clades) we employed three calibration points. We set a minimum divergence between Ceratinini and Allodapini at 45 myBP because of the existence of fossil Boreallodapini species from Baltic amber [36]. Boreallodapini is the extinct sister group to the extant tribe Allodapini and Ceratinini is the next-most basal tribe. This minimum age is therefore highly conservative [14] and the Allodapini+Boreallodapini is likely to have diverged from the Ceratinini much earlier than this. We also used the presence of the amber fossil stingless bee *Cretotrigona prisca*, dated at 70-65 myBP [37] to set a minimum time of divergence of 70 myBP between the Meliponini and Bombini, but as with the boreallodapine calibration point this is likely to be highly conservative, requiring that *C. prisca* had arisen as soon as the two clades diverged. The last calibration point was the node joining the xylocopine and the corbiculate lineages in our sample. Fossils of the plant family Clusiaceae, whose floral morphology is closely linked to corbiculate bees, are dated at 90 myBP [38] and so we fixed this node at 90 myBP. However, it is possible that corbiculates diverged from the clade leading to xylocopines well before this, so we also explored the effect of setting the date of this node to 100, 110 and 120 myBP. Confidence intervals for node ages were estimated as 95% central distribution limits using the method of Schwarz *et al.* [14], where all post-burnin Bayesian trees were filtered by the consensus Bayesian topology, then subjected to penalised likelihood transformation. The resulting chronograms were sorted for age of the allodapine root node and the resulting 2.5% upper and lower node ages removed.

As a further check of the antiquity of the allodapine root node we compared sequence divergences of EF-1α for species-pairs whose most recent common ancestor occurred at that node, with equivalent species-pairs for the origins of sociality in halictines (three origins, with estimated ages of 22-20 mya) where internal fossil calibration points

are available [6, 14], with an overlapping region of exon 772 bp. For both data sets, gene fragments were trimmed to the overlapping region and ModelTest 3.0.4 [39] was used to fit substitution models to both taxa. These analyses returned highly similar models (Table A2, Additional Files), suggesting that this gene region evolves with very similar dynamics in both halictines and allodapines. We then used these models to calculate maximum likelihood pairwise distances for both data sets. For allodapines, this involved pairwise distances between the *Macrogalea* species (forming the sister clade to all other allodapines) and representatives of all other known allodapine non-parasitic genera. For halictines, this involved all taxa pairs whose most recent common ancestor (MRCA) was the inferred node of origin of sociality. These pairwise distances are not independent of each other, since most taxa are included in the calculation of more than one pairwise distance in either bee group, so that mean and median values cannot be statistically compared between the two taxa.

Authors' contributions

LBC helped conceive the study, carried out the field work, was responsible for all work on sociality and sex allocation in *Halterapis*, obtained DNA sequence data, analyzed the data and prepared the manuscript. SMT contributed very substantial DNA sequence material and help with analyses. JAS helped with fieldwork and DNA sequencing. SJBC oversaw DNA work and helped conceive the study. MPS supervised and helped conceive the study as part of a broader program on allodapine social evolution and phylogenetics and helped with fieldwork and analyses. All authors contributed to interpretation of data and manuscript revisions.

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Table 1 - Colony contents data

Brood numbers and sex ratio parameters for 52 nests of *H. nigrinervis* collected from Grahamstown, South Africa in late summer (February).

Adult females/nest	Per capita eggs+larvae	Mean total brood (eggs, larvae prepupae and pupae)	Mean sex ratio of pupae (total male pupae: total female pupae)	Number of nests
1	0.292	0.75	0 (0:2, 2 nests)	24
2	0.60	2.13	0.22 (2:7, 9 nests)	15
3	0.503	2.50	0 (0:5, 5 nests)	8
4	0.313	3.50	0.33 (2:4, 3 nests)	4
5	0.60	3.00	- (0 nests)	1

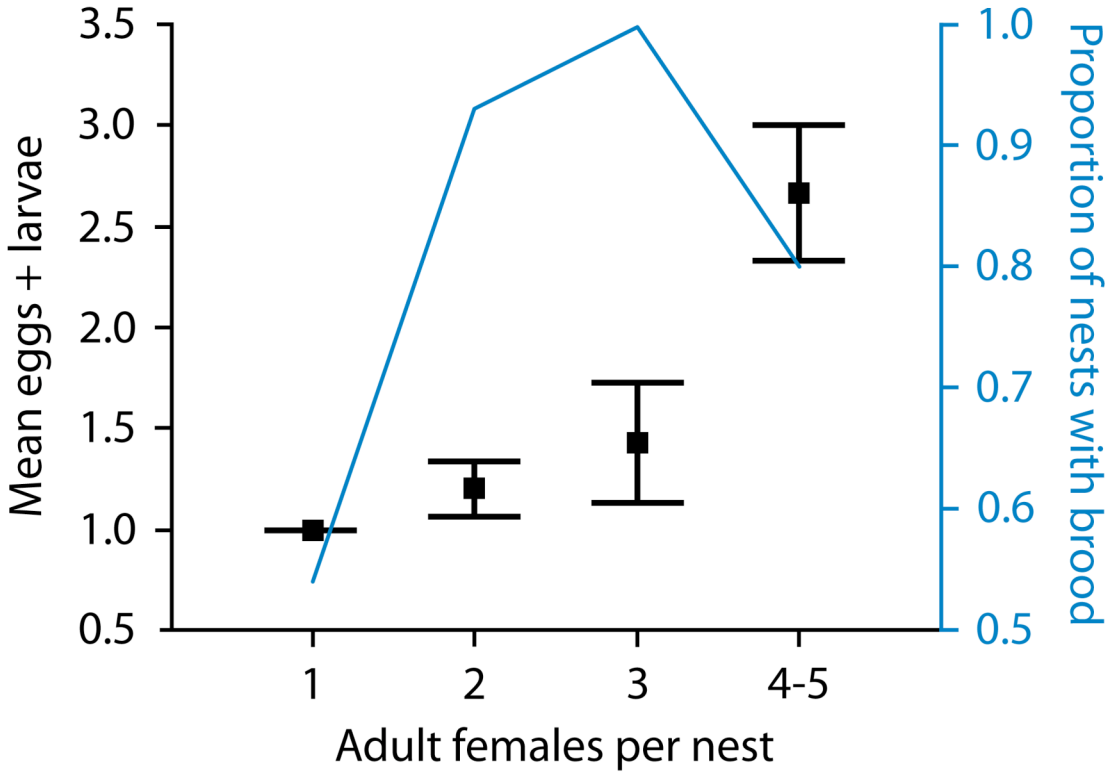

Figure 1 - Variation in the number of eggs + larvae among different colony sizes

Brood numbers were 1-truncated (colonies lacking any eggs or larvae were excluded). A Kruskal Wallis non-parametric test indicated a significant effect of the number of adult females on the number of eggs and larvae present within nests ($\chi^2_3 = 12.145$, $P = 0.007$). The blue line displays the proportion of nests containing brood for different colony sizes.

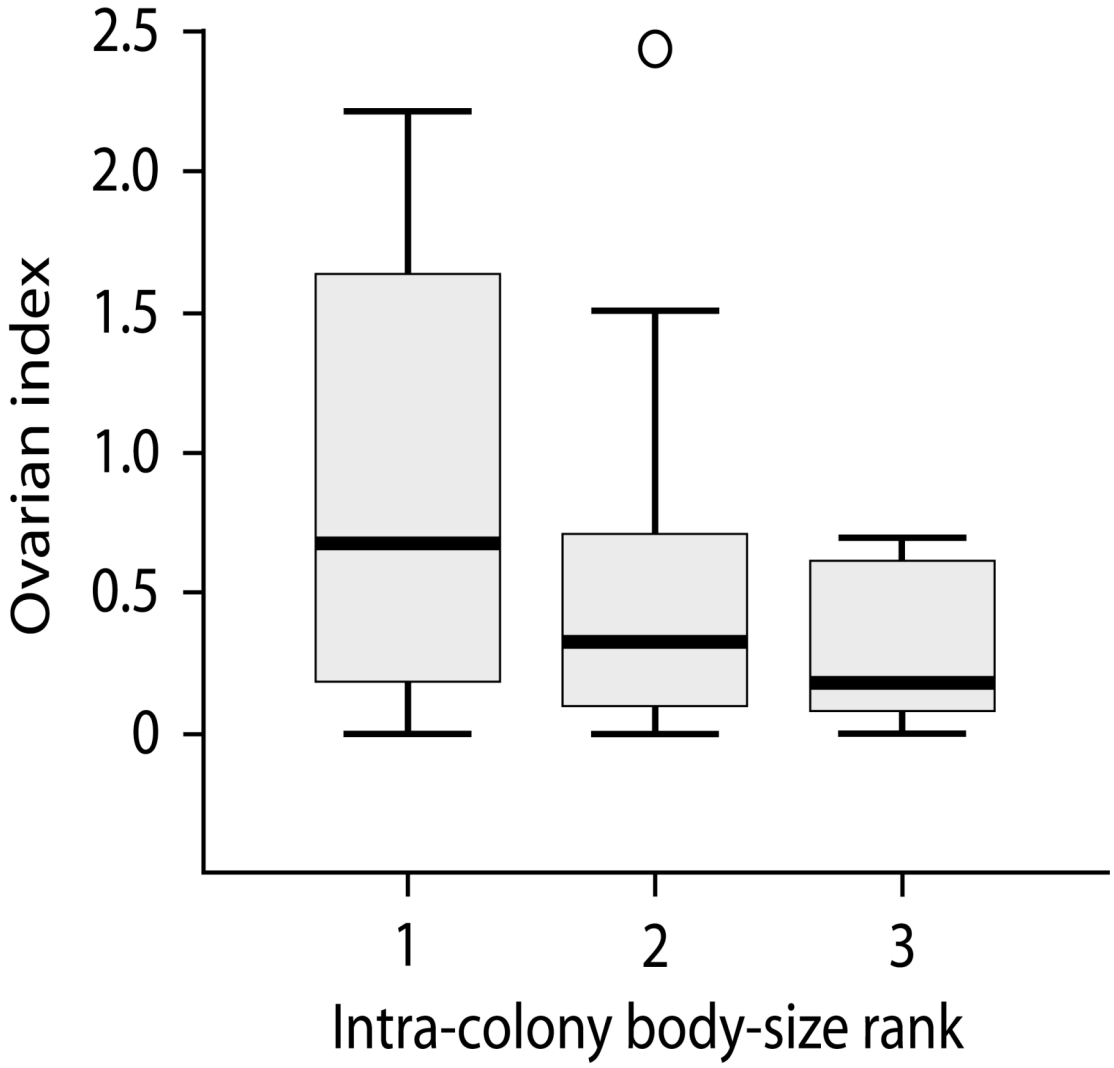

Figure 2 - Mean ovarian index versus intra-colony body size rank

Mean ovarian index (±1 S. E.) as a function of intra-colony body size rank for adult females from multifemale colonies of *H. nigrinervis*. Individuals with identical sizes were given the same sequential rank. By regressing the ovarian index of individuals within a nest on their residual body size, we found a significant decrease in ovarian index as relative female body size decreases within colonies (P= 0.008).

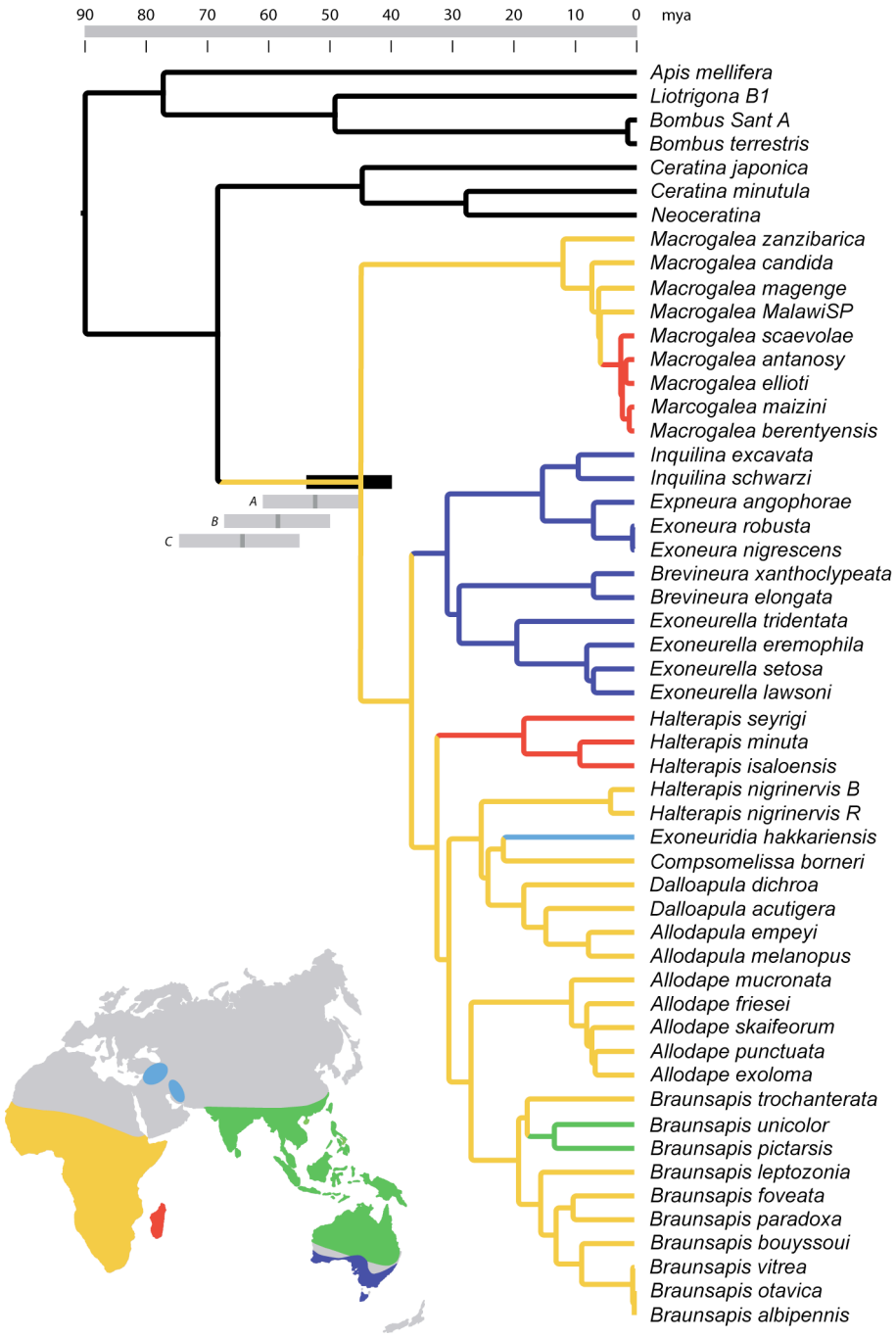

Figure 3 - Chronogram of the allodapines derived from penalized likelihood transformation of a consensus Bayesian phylogram

95% central distribution limits for the allodapine root node are indicated by the black bar, assuming a 90 myBP divergence between the xylocopine and corbiculate lineages, and by grey bars assuming divergence times of 100 (bar A), 110 (B) and 120 (C) myBP. Geographic distributions of clades are colour coded according to the map.

Chapter I: Supplementary Material

Reproductive hierarchies

The reproductive hierarchies suggested in Figure 2 (main article) for colonies with three or more females cannot be assessed using regression or correlation analyses because the independent variable is ranked body-size and ranks within a nest are not independent. Therefore, we calculated the residual body size for each female within a nest and then regressed both ovarian index and ovary size on the residuals separately for each nest. If ovary size (or ovarian index) is independent of within-nest relative body size, regression slopes should have equal likelihood of being positive or negative, and deviations from this were tested using a binomial test. Colonies where all females were tied for body size were excluded. The null hypothesis was that there would be an equal likelihood of positive and negative relationships. However, we found that all 12 regression slopes for ovary size were positive, 11 of the 12 coefficients for ovarian index were positive, with a two-tailed significances of $P < 0.001$ and $P = 0.008$ respectively. The positive slopes indicate that larger females (within colonies) tended to have larger ovaries.

Benefits of social nesting

Previous studies on allodapines have shown that a major benefit of social nesting is prevention of total brood loss in multifemale colonies. We examined this using a log likelihood test with presence/absence of brood crossed with the number of adult females per nest (ranging from 1 – 5, with 4 and 5-female colonies combined because of small samples sizes, Table A1 below). This test indicated significant dependence ($\chi^2_3 = 11.899$, $P = 0.008$).

DNA sequence distances

We used sequence divergences of the nuclear gene EF-1α to assess whether the allodapine root node (minimum origin for sociality in allodapines) was likely to be older than the three origins of sociality in halictines. Halictine sequences were obtained from Genbank (accession numbers in S1 and references therein). Pairwise distances were estimated using a maximum likelihood distance, and parameters for this were based on recommended parameters from ModelTest 3.0.4 [S2] using only that part of the gene fragment that was common to both the allodapine and halictine samples, comprising 778 basepairs and

excluding the intron in this region, which could not be aligned for most taxa. Evolutionary substitutional parameters were estimated by fitting sequence data to best-known trees for both groups: for halictines this was the topology given by Brady et al. [S1] and for allodapines it was the topology given in this paper (Table A2). A boxplot, giving medians and inter-quartile ranges for all taxa-pairs whose most recent ancestor is the inferred origin of sociality, are presented in Figure A3 below for both allodapines and halictines.

Pairwise distance estimates include the effects of divergences in both lineages descending from the most recent common ancestor to the two species being compared. If the amount of genetic change, modelled by the ML distance parameters, has a linear relationship to time, then relative ML distances for any pair of taxa should be proportional to (time since divergence)/2. That means that if distances are linearly related to time since divergence, then a four-fold difference in distances between two taxa-pairs should correspond, approximately, to a two-fold difference in the divergence ages of the two pairs of taxa being compared.

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S1. Brady SG, Sipes S, Pearson A, Danforth BN: **Recent and simultaneous origins of eusociality in halictid bees.** *Proc R Soc B* 2006, **273(54):** 1643-1649.

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Table A1 - Number of colonies with or without brood for different sized colonies

4 & 5- female colonies were pooled because of small sample sizes. Nests containing more than one female were far more likely to contain brood.

	Adult females/nest			
	1	2	3	4 - 5
Brood absent	13	1	0	1
Brood present	11	14	8	4

Table A2 - Substitution parameter matrices for allodapines and halictines

Parameter matrices for allodapines are based on the data and topology presented in this paper, and halictine parameter matrices are based on data and topology presented in Brady et al. [S1]. Parameters estimated with ModelTest 3.0.4 comprising base frequencies, substitution rates, proportion of invariant sites and α shapes.

Model parameters	Allodapines	Halictines
Base frequencies		
A	0.2604	0.2675
C	0.2079	0.1997
G	0.2456	0.2419
T	0.2861	0.2909
Rate matrix values		
A-C	1	1
A-G	5.7094	6.0159
A-T	1	1
C-G	1	1
C-T	11.9618	12.2367
G-T	1	1
Prop(invariant sites)	0.6256	0.6126
Gamma shape	1.3651	1.2578

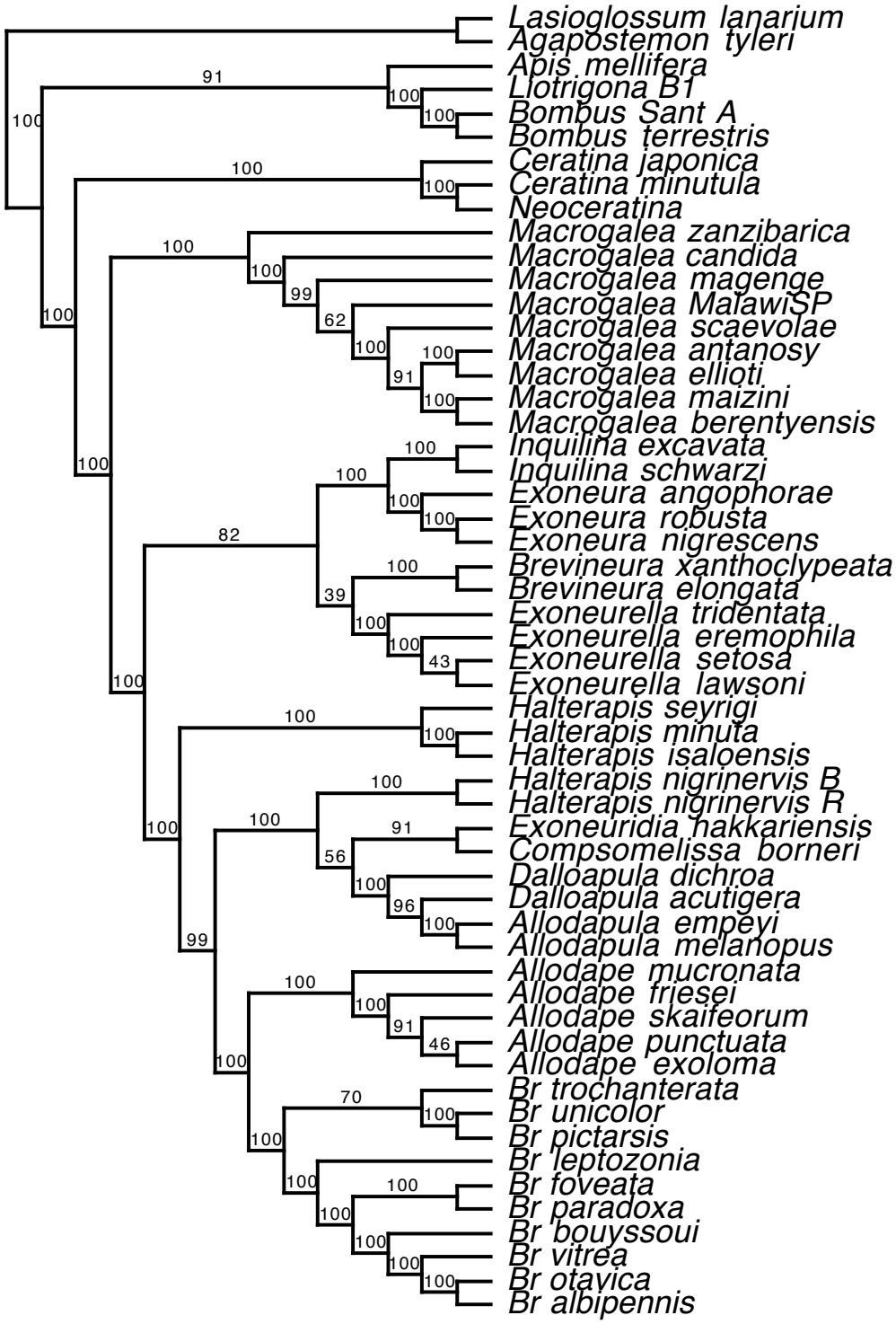

Figure A1 - Consensus cladogram of representatives of major allodapine genera.

Posterior probability values are provided as percentages above nodes. Taxa from the genus *Braunsapis* are abbreviated into the genus name "*Br*".

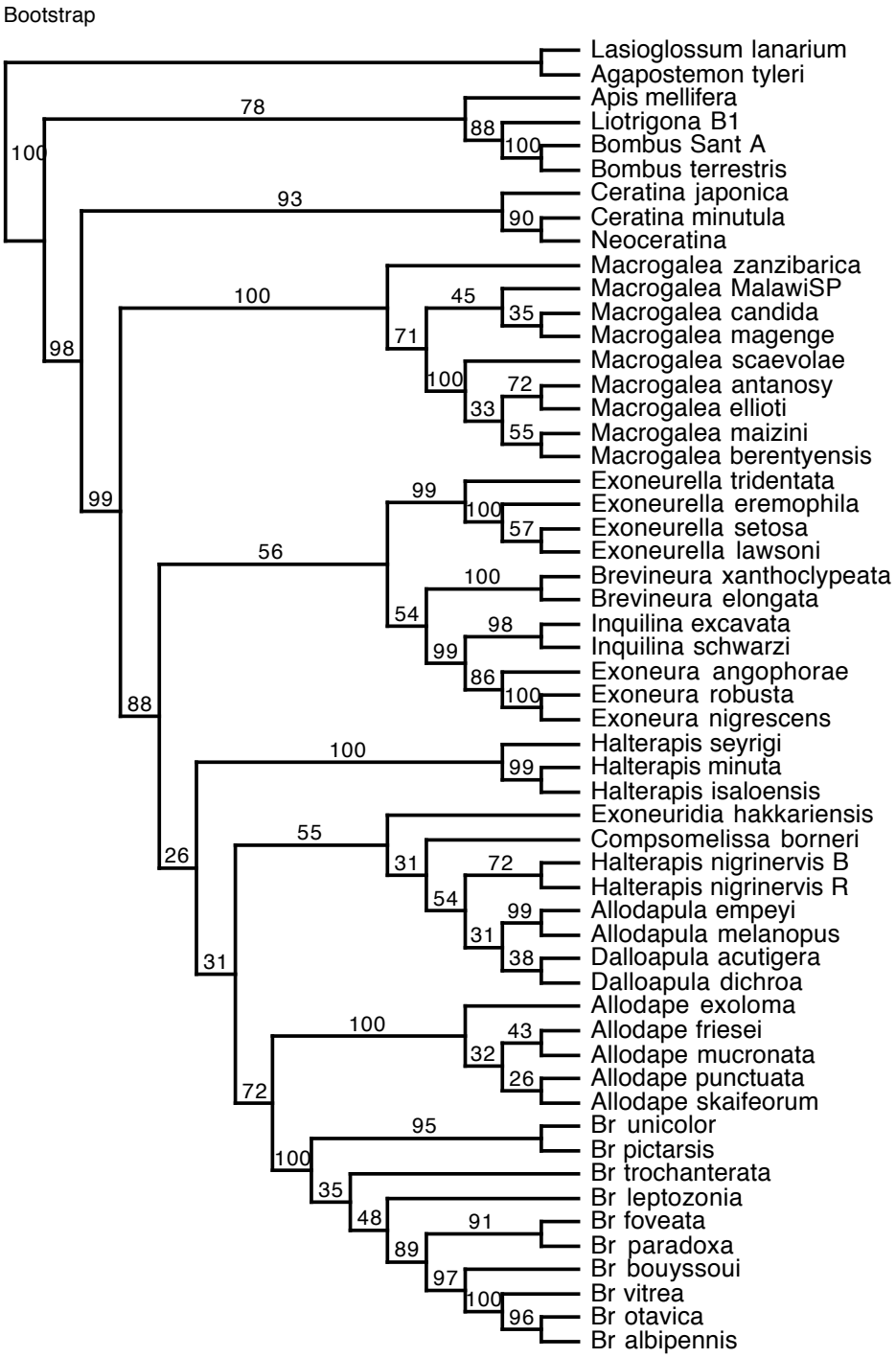

Figure A2 - Bootstrap tree from maximum parsimony analysis of EF-1 α and 1nt and 2nd mitochondrial nucleotides of COI and Cyt *b*. Bootstrap support values are provided as percentages above nodes. Taxa from the genus *Braunsapis* are abbreviated into the genus name "*Br*"

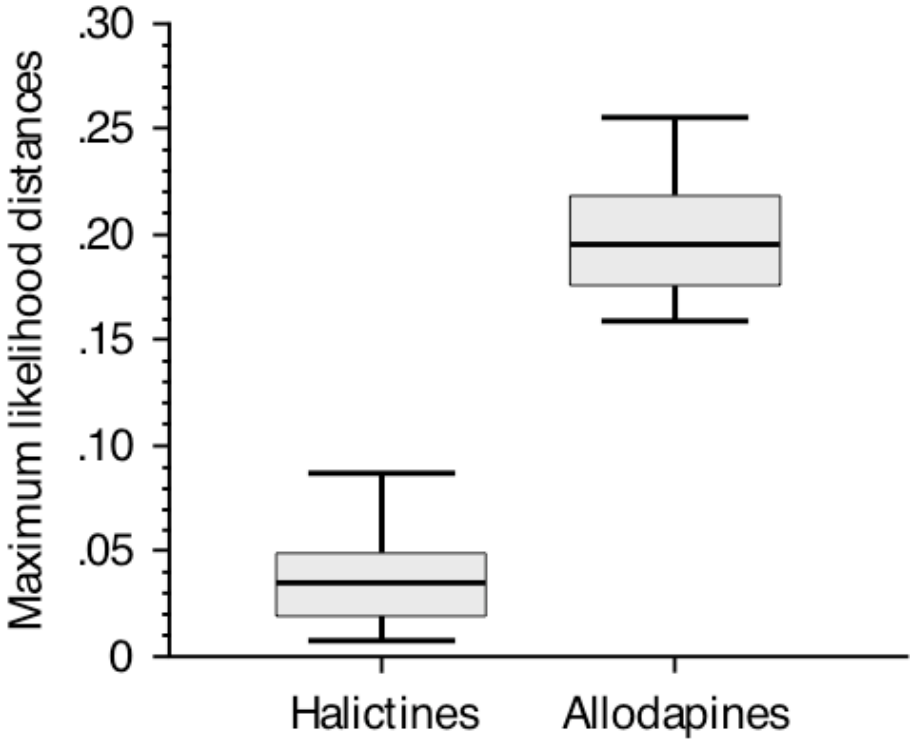

Figure A3 - Boxplot of pairwise maximum likelihood distances, calculated using PAUP with the parameters given in Table 3.

Allodapine distances are for pairwise taxa whose most recent common ancestor is the root allodapine node which is the minimal point for the inferred origin of sociality in allodapines. The same protocol is used for calculating halictine pairwise distances except that there are three inferred origins.

CHAPTER II

Hasinamelissa: a new genus of allodapine bee from Madagascar revealed by larval morphology and DNA sequence data

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Abstract. Allodapine bees have received attention because of their utility for studies of insect social evolution. The African genus *Halterapis* has been especially important because until very recently it was thought to contain non-social species. Due to the extreme diversity in allodapine larval morphology, which is much greater than variation in adult morphology, larvae have provided some of the most informative characters for allodapine taxonomy. The taxonomic position of Malagasy allodapine bees previously placed in the genera *Halterapis* has always been doubtful because the larval stages have been unknown. We provide the first description of larval morphology for three Malagasy allodapine species currently placed in the genus *Halterapis* that span the range in adult morphology for this island fauna. Larvae of all three species exhibit a variety of traits that are strikingly different from other allodapine genera. Phylogenetic analyses based on DNA sequence data from one nuclear and two mitochondrial gene fragments indicate that the Malagasy species form a strongly supported monophyletic group that is the sister group to the combined African allodapine clades excluding *Macrogalea*. This endemic Malagasy bee clade further highlights the distinctiveness of the Malagasy bee fauna and raises biogeographical questions about how, when, and why the Malagasy biota became so unique. We erect a new genus, *Hasinamelissa*, to contain the Malagasy species previously placed in *Halterapis* and transfer 18 Malagasy species into this new genus [*H. seyrigi* (Benoist) (type-species) **comb.n.**, *H. keiseri* (Benoist) **comb.n.**, *H. minuta* (Brooks & Pauly) **comb.n.**, *H. isaloensis* (Brooks & Pauly) **comb.n.**, *H. tulearensis* (Michener) **comb.n.**, *H. albigena* (Brooks & Pauly) **comb.n.**, *H. kraussi* (Michener) **comb.n.**, *H. didyensis* (Brooks & Pauly) **comb.n.**, *H. acaciae* (Brooks & Pauly) **comb.n.**, *H. ankaratrensis* (Brooks & Pauly) **comb.n.**, *H. platyprosoton* (Michener) **comb.n.**, *H. rufa* (Michener) **comb.n.**, *H. pentagonalis* (Brooks & Pauly) **comb.n.**, *H. benoisti* (Michener) **comb.n.**, *H. curtipilosa* (Brooks & Pauly) **comb.n.**, *H. personata* (Brooks & Pauly) **comb.n.**, *H. spatulata* (Brooks & Pauly) **comb.n.** and *H. spinipennis* (Brooks & Pauly) **comb.n.**].

Introduction

Madagascar has one of the highest levels of species endemism in the world, due to its size, geographical variation, and long separation from the African and Indian plates (> 88 Myr, Yoder and Nowak 2006). The high levels of endemicity extend to its bee fauna (Pauly et al. 2001). Determining the relationships of the Malagasy biota to that of other regions is important because of the insights that can be gained into historical biogeography, involving vicariance, dispersal, or both (Yoder and Nowak 2006). Allodapine bees from Madagascar have hitherto been placed in five genera: *Macrogalea*, *Braunsapis*, *Effractapis*, *Halterapis* and *Allodapula*. *Macrogalea* is the sister group to all other Allodapini (Schwarz et al. 2003), and the Malagasy species form a monophyletic group derived from an African ancestor (Tierney 2004; Smith et al. 2007). Analyses of DNA sequence data show that the Malagasy *Braunsapis* species are derived from two colonisation events from African *Braunsapis* ancestors (Fuller et al. 2005), and the socially parasitic genus *Effractapis* probably is derived from within *Braunsapis* rendering the latter paraphyletic (Michener 2000).

The most speciose group of Malagasy allodapines is placed currently in the genus *Halterapis* (Pauly et al. 2001). *Halterapis* has been treated both as a subgenus of *Compsomelissa* (eg. Michener 1975) and as a genus in its own right (Reyes 1998; Pauly et al. 2001). Initially, Malagasy *Halterapis* species had been placed in *Allodape* (Benoist 1962) and later either *Halterapis* or *Allodapula* (Michener 1975; 1977a), but the complete absence of information on larval morphology and the almost complete absence of information on male morphology created doubt about the affinities of this group with African genera (Michener 1977a). On the basis of subsequently-examined male specimens, Reyes and Michener (1992) argued that one Malagasy species assigned previously to *Allodapula, A. keiseri*, should be placed in *Halterapis*, and suggested that the remaining Malagasy *Allodapula* also were likely to belong to *Halterapis*. However, they noted that the absence of larval specimens made generic assignment problematic. More recently, Pauly et al. (2001) greatly expanded the number of described Malagasy taxa, describing males for many species, and transferring all species previously placed in *Allodapula* to *Halterapis*. However, Pauly et al.'s revision also was carried out in the absence of larval material and they noted that assignment to *Halterapis* still should be regarded as tentative.

There are likely to be only four or fewer African *Halterapis* species (Michener 1975) and they are morphologically very similar. In contrast, there are 17 described species from Madagascar in which adult females have been identified (Pauly et al. 2001) and these vary widely and sometimes strikingly in adult morphology, suggesting very different patterns of radiation between the African and Malagasy faunas.

The morphology of allodapine larvae has been extensively investigated, with multiple descriptions for every genus except *Exoneuridia* (Michener 1977b and references therein). Larval morphology of allodapines is highly variable, especially between genera, and variation within the tribe greatly exceeds variation in all other bee taxa combined (Michener 2000). Allodapines are unique among bees in that brood are reared within communal burrows in dead stems and branches, and are not enclosed within cells (Schwarz et al 2007). This method of brood rearing means that immatures have extended contact with each other and with adults, and it is likely that this environment has selected for morphological features not found in other bee taxa, where individual larvae are nearly always isolated within closed cells. The larvae of most allodapine genera have tubercles, branched or unbranched appendages, and/or variously developed setae that can differ enormously in size, density, form, and distribution over the body and head. Larval morphology has been an important factor in systematic treatments of allodapines (Michener 1975, 1976, 1977b, 2000) and used in some cases to delimit generic boundaries that are otherwise obscure in adult morphology (Michener 1975).

Based on a study of the African species *Halterapis nigrinervis* (Michener 1971), *Halterapis* was thought to be both non-social and a mass provisioner. In *H. nigrinervis*, after oviposition, each egg is provided with enough food for its entire development, and all nests (N = 11) that had been studied by Michener (1971) contained only a single fully-pigmented adult female along with brood and/or callows. Two recent studies of Malagasy *Halterapis* (*H. minuta*; Schwarz et al. 2005, *H. seyrigi*, and *H. isaloensis*; Chenoweth and Schwarz. 2007) have shown that all three produce clutches of eggs prior to commencement of provisioning in a manner similar to African *Allodapula* species, but that these eggs are provided with a single mass provision in the form of a large cylindrical mixture of pollen and nectar. While some African *Allodapula* species provide communal food masses for eggs and small larvae, these provisions are small in size and are supported as an irregular mass held in the centre of a group of eggs glued in a circular pattern to the nest lumen (Michener 1971). In terms of brood provisioning, all Malagasy *Halterapis* studied to date therefore share some features in common with African species of both *Halterapis* and

Allodapula, but differ from both genera in that clutches of eggs, rather than individual eggs, are provided with a large, communal mass provision.

Recent DNA-based phylogenetic studies (Schwarz et al. 2003; 2006) have greatly changed our understanding of the systematics of allodapines, and have shown that the African genus *Macrogalea* is the sister group to all other allodapine groups. Due largely to the belief that *H. nigrinervis* was a solitary mass-provisioner, it had previously been thought that *Halterapis* and its putative sister group *Compsomelissa* jointly formed the sister clade to the rest of the Allodapini. This principle in turn influenced theories on broad aspects of the evolution of social and life history traits in the Allodapini (Michener 1977b; Reyes 1998). Phylogenetic studies by Schwarz et al. (2003) has shown that *Halterapis* was not basally located within the Allodapini, and more recent phylogenetic analyses (Schwarz et al. 2005; Chenoweth et al. 2007) have suggested that the Malagasy *Halterapis* actually form a separate and distinct group from the African *Halterapis*. Here we present a systematic study of the Malagasy allodapine taxa currently assigned to *Halterapis* and erect a new genus, *Hasinamelissa*, to contain these species as distinct from the African *Halterapis*.

Methods

Taxa, collection localities and sample preservation

Species were sampled from six localities in Madagascar: 85km north west of Antananarivo (*Halterapis keiseri*); 5 km south of Anakao and in Ifaty (*H. minuta, H. seyrigi* and *H. isaloensis*), Taolagnaro (*H. seyrigi* and *H. isaloensis*), an undescribed species from Ramena village 20km east of Diego Suarez (referred to here as *Hasinamelissa* R43H), and two further undescribed species from Morondava (referred to as *Hasinamelissa* MorondavaA and *Hasinamelissa* MorondavaB). The site north west of Antananarivo (near Ambohitantely Reserve, 18°10′0″S 47°17′0″E) comprised small patches of remnant rainforest in steep valleys and adult females were collected from unidentified flowers. Anakao (23°40′0″S 43°39′0″E) and Ifaty (23°9′0″S 43°37′0″E) are small fishing villages in spiny forest habitats approximately 40 km to the south and north of Toliara (also called Tuléar) respectively, and specimens were collected from both flowers and from intact nests collected from several species of herbaceous shrubs and small trees. Specimens from Taolagnaro (25°2′0″S 46°59′0″E, also called Fort Dauphin)

were collected exclusively from nests in dead stems of *Scaevola* sp. (family *Goodeniaceae*) along coastal dunes bordering the township. Specimens from Ramena village (12°14′42″S 49°20′38″E) were collected from nests in kapok trees (*Ceiba pentandra*, family *Malvaceae*) and specimens from Morondava (20°17′5″S 44°19′3″E) were collected from nests in *Bougainvillea* sp. (family *Nyctaginaceae*). Larval descriptions were based on multiple larval specimens. The larvae used were always sampled from nests where species-diagnosable adults were present in order to avoid the false diagnosis of larva from factors such as cleptoparasitism, however no social parasites have been described for these particular allodapine taxa to date. Nest contents were preserved in both Kahle's solution and 100% ethanol. No differences in larval shape or morphology were evident between the two preservation methods at the time of examination.

DNA sequencing methods

DNA extraction. All DNA extractions were from mesosomal or metasomal sections of ethanol preserved specimens following a modified protocol of Doyle and Doyle's (1990) CTAB method or using DNAzol Genomic DNA Isolation Reagent (Chomczynski et al 1997, 1998). 10µl of Proteinase-K (20mg/ml) was added to the extraction prior to incubation (CTAB: 55^{o}C for 2 hours; DNAzol: 37^{o}C overnight). DNA pellets were resuspended in 50-100µl of TE buffer and stored frozen.

PCR primers. One nuclear and two mitochondrial gene regions were amplified and sequenced bi-directionally. The mitochondrial regions were from the protein coding genes cytochrome *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 PCR amplification of the Cyt *b* region were designed by Y. C. Crozier (Latrobe University, Melbourne, Australia, Schwarz et al. 2004): cb1: 5'-TAT GTA CTA CCA TGA GGA CAA ATA TC-3' and cb2: 5'-ATT ACA CCT CCT AAT TTA TTA GGA AT-3'. Two sets of primers were used to sequence the COI region. The first set were designed by Lunt et al. (1996) and comprised of 620 bp at the 3' end of COI: UEA7: 5'-TAC AGT TGG AAT AGA CGT TGA TAC-3' and UEA10: 5'-TCC AAT GCA CTA ATC TGC CAT ATT A-3'. The second set of primers were designed by Folmer et al. (1994), M414 (LC01490): 5'-CCT TTT ATA ATT GGA GGA

TTT GG-3' and from bee sequences by S. Cooper (Schwarz et al. 2004) M399: 5'-TCA TCT AAA AAC TTT AAT TCC TG-3', providing a 759bp upstream of, and overlapping 91 bp of, the region amplified by the primers of Lunt et al. (1996). EF-1α occurs as two copies, EF-1α F1 and EF-1α F2, in bees, which are expressed at different stages of development (Danforth and 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 Danforth (pers. comm.) and the F2-specific reverse primer designed by Danforth and Ji (2001) for halictid bee species (EF1F2rev1: 5'-AAT CAG CAG CAC CTT TAG GTG G-3').

PCR amplification and sequencing. PCR reactions were carried out in 25µl reaction volumes containing 11.9µl of mqH_2O, 2.5µl TAQ polymerase buffer, 4.0µl of 25mM $MgCl_2$, 2.0µl dNTPs, 1µl forward primer, 1µl reverse primer and 2.5µl diluted DNA. Additionally, 0.1µl of Amplitaq Gold (Applied Biosystems) was added to each sample. Amplification conditions included an initial hot start of 94^{o}C for 9 min, followed by 35 cycles of denaturation at 94^{o}C for 30 sec; annealing at 54^{o}C for 45 sec, extension at 72^{o}C for 1 min; and then a final extension step of 72^{o}C for 6 min. PCR products were purified using Ultraclean PCR Clean-up columns (MO BIO Laboratories, Inc.), and ~50 ng of product was sequenced in 20 µl reaction volumes using the Big Dye Sequencing Ready Reaction kit Version 3.1 (Applied Biosystems), with the original PCR primers used as sequencing primers. PCR products were then sequenced in 20µl reaction volumes using the BigDye Sequencing Ready Reaction kit (ABI-Perkin-Elmer. The sequencing primers used were the corresponding PCR primers used. The amount of PCR product used in the reaction was dependant on the concentration of the product. Reaction products were purified by isopropanol precipitation and sequenced on a capillary DNA sequencer.

Specimens included

Our analyses included seven Malagasy putative species for which we had multiple specimens in our samples. It is important to note that three undescribed taxa (*H.* MorondavaA, *H.* MorondavaB and *H.* R43H) are used here due to the uniqueness of their localities as well as genetic distinctiveness. Because very few were collected, nothing about their nesting habits or social biology is known. As such, we do not formally

recognise them as described species per se but rather as undescribed representatives of the genus. The remaining 35 allodapine species included in our analyses were chosen to ensure inclusion of all major allodapine clades (Schwarz et al. 2003, 2006). A majority of the taxa used in this study have been used in previous phylogenetic studies (Schwarz et al. 2003; Schwarz et al. 2004; Tierney 2004; Schwarz et al. 2005; Bull et al. 2003; Fuller et al. 2005; Chenoweth *et al.* 2007) and GenBank accession numbers are provided in these manuscripts. In addition to three Malagasy taxa used in prior studies, we also incorporated sequence data for *H. keiseri* (EU814530, EU814523), *H.* R43H (EU814533, EU814528, EU814529), *H.* MorondavaA (EU814531, EU814524, EU814525), and *H.* MorondavaB (EU814532, EU814526, EU814527), four Malagasy taxa for which sequence data were previously unavailable. Where possible, two specimens of each taxon were sequenced however due to small sample sizes, only a single specimen could be sequenced for *H.* MorondavaA, *H.* MorondavaB and *H. keiseri*. Despite this, the use of bi-directional sequencing for all samples provided an additional means to check for sequence ambiguities resulting from sequencing or amplification errors. Where possible we included more than one species of each genus to minimize possible long-branch effects. We only have sequence data for one species each of *Compsomelissa* and the middle eastern genus *Exoneuridia,* but included three species of *Allodapula s. s.* and both species of *Allodapula (Dalloapula)* because of the superficial similarity in some aspects of larval morphology between these groups and several of the Malagasy species (see larval descriptions below). We included both Malagasy and African species of *Macrogalea* and used *Ceratina japonica* (tribe Ceratinini) as the outgroup. The Ceratinini form the sister tribe to the Allodapini among the extant Xylocopinae (Engel 2001).

Phylogenetic analyses

Forward and reverse sequences were compared for each gene fragment, and sequences were edited and aligned manually using SeqEd 1.03 (Applied Biosystems). The intron region of EF-1α F2 was excluded from the phylogenetic analyses because large sections of sequence were unalignable.

Like other Hymenoptera, allodapine bees exhibit extreme AT bias in mitochondrial genes, especially for 3rd codon positions, and this can lead to signal erosion for more ancient phylogenetic divergences. In a recent study of the allodapine genus *Braunsapis*, Schwarz et al. (2004) showed that maximum parsimony and single-model maximum

likelihood approaches (using PAUP*) to allodapine data can produce spurious results, especially for older clade divergences where homoplasic changes at fast evolving sites become very likely. Spurious topologies also can result from applying a single model to gene partitions (such as 1st, 2nd and 3rd codon positions) when these clearly show different substitutional dynamics, leading to misleading topologies even for relatively recent allodapine divergences (Schwarz et al. 2003, 2004). Therefore we follow Schwarz et al. (2004) in employing separate models for clearly different partitions, performed in MrBayes version 3.0.4 (Huelsenbeck and Ronquist 2001), which uses a Metropolis-Coupled Markov Chain Monte Carlo method (henceforth MC3). Further, we performed a maximum parsimony analyses and compared this with results from the Bayesian analysis to see whether broad topological features were robust to widely varying analytic approaches.

For Bayesian analyses, we followed Schwarz et al. (2006) by using six partitions (1st, 2nd and 3rd codon positions for the two mitochondrial genes and 1st, 2nd and 3rd codon positions for the F2 copy of EF-1α). We used default priors and unlinked all substitutional parameters for the analyses, which employed a MC3 procedure with three hot chains and one cold chain. We ran analyses for three million generations, sampling every 500th generation, and inspected log likelihoods and the average standard deviation of split frequencies for parallel runs to assess stationarity over generation number. Posterior probabilities for nodes were based on the last 3000 samples (i.e. > 1.5 million generations). Therefore, our analyses were based on very large generation numbers, sampled well after stationarity was reached. Runs were performed three times to check that solutions converged on identical topologies and similar branch lengths.

For the maximum parsimony analysis we excluded 3rd codon positions for the mitochondrial genes because of the high degree of homoplasy and consequent long branch attraction problems (Schwarz et al. 2003, 2004). We used 50 random sequence stepwise additions, keeping five trees at each step and using TBR branch swapping. Confidence in nodes was estimated by bootstrapping, with the same addition and TBR procedure as above, and support values were based on 1000 bootstrap pseudo-replicates.

Results

Hasinamelissa, **gen. nov.** Chenoweth and Schwarz.

Type species. *Hasinamelissa seyrigi* (Benoist) [Selected as the type species, as it is the only species of the three first described for which male, female, and larval samples are known].

Generic Diagnosis. Larval descriptions are based on multiple nests of *H. minuta*, *H. isaloensis* and *H. seyrigi*. Larvae used were always taken from a nest where at least one fully identifiable adult female was present.

Larvae. Body slightly curled, more so than *Allodapula* species, but less curled than other allodapine genera. All species are characterized by the presence of two or more longitudinal ventrolateral rows of protuberances or tubercles, decreasing posteriorly in size with the first two lateral-most thoracic protuberances each with one to three large and irregularly shaped, posteriorly-curved, stout setae terminating in blunt, clubbed or spatulate ends. Further diagnostic characters include sparse but stout setae on the dorsomedial ridges of most or all body segments, becoming larger and clubbed or blunt anteriorly, but body otherwise hairless. Head hairless and antennae short and blunt, arising from slightly elevated area. Labrum markedly constricted at base, widening anteriorly and strongly bilobed. Anterior margins of maxillae equidistant with labium when viewed anteriorly. All of the above features are absent in all other allodapine genera for which larval specimens have been recorded.

Adults. Adult morphology has been described by Michener (1977a), Reyes and Michener (1992) and Pauly et al. (2001) and is much more variable than for African *Halterapis*. Except for *Hasinamelissa minuta* and *Hasinamelissa pentagonalis*, females differ from African *Halterapis* in the presence of paraocular cream or white bands or, in *Hasinamelissa tulearensis,* a cream spot. Females of all species have orange metasomal coloration, except in *H. minuta*, and in some specimens of *Hasinamelissa isaloensis* and *Hasinamelissa seyrigi*. These latter species are distinguished from red-coloured African *Halterapis* by the presence of an acute apicolateral angle in T6 separating the dorsal and

ventrally reflexed portion of the tergum, which is a synapomorphic character of the genus. The males of the majority of species are unknown, having never been collected.

Description of mature larvae. *Hasinamelissa seyrigi* (Figs 1A, 2A). Body slightly curled as in Fig. 1A. Two ventro-lateral rows of protuberances, with the more lateral protuberances larger, and both rows becoming smaller posteriorly. Anterior-most lateral protuberance simple and next four lateral protuberances bidentate. First two lateral protuberances each with three stout, clubbed and posteriorly curving setae. One row of small tubercles between the two rows of protuberances and a second row of similar size located more medially. Body segments dorsally raised forming obtuse angles when viewed laterally. First three body segments each with two sets three dorso-ventrally projecting stout setae with a small hook at the end. Fourth body segment dorsally hairless and remaining segments with sparse dorso-medial setae, fine and tapering at the tip. Body otherwise hairless. Labrum narrowed at base and strongly bilobed with each lobe distally rounded and medial part of labrum strongly concave. Maxillae produced anteriorly, extending as far as the labium. Presence of two elevated palpae on the labium and another one on each maxilla. Mandibles simple (Fig. 2A).

Hasinamelissa isaloensis (Figs 1B, 2B). Similar to *H. seyrigi* except for the following: ventrolateral protuberances of the more medial row are relatively smaller than those of *H. seyrigi*, and longitudinal rows of tubercles are completely absent. Only two stout setae from first pair of lateral protuberances and a single seta on second pair. Dorso-medial areas of body segments more produced and for the anterior part of body forward sloping, forming conspicuous ridges. First body segment with two clusters of five dorso-ventrally projecting setae. Dorsal setae blunt and not as stout or long as in *H. seyrigi*. Second and third body segments with two pairs of short and stout setae, projecting dorso-ventrally and clubbed apically. Remaining segments with sparse, knob-like dorsomedial setae, shorter and stouter than those of *H. seyrigi*. Labrum bilobed as in *H. seyrigi* but lobes more produced apically, leading to marked angularities. Presence of two elevated palpae on the labium and another two on each maxilla (Fig. 2B).

Hasinamelissa minuta (Figs 1C(i), (ii); 2C) Body as in Fig. 1C(i) and (ii), and not as posteriorly curled as *H. seyrigi*. Two ventrolateral rows of protuberances, with the first two lateral pairs well developed but remaining protuberances small and nipple-like, and no

protuberances bidentate. First two lateral protuberances produced into very long, irregular, spatulate setae, almost as long as body width. Dorsal body segments medially produced, leading to well-defined ridges, most with sparse medial setae directed dorsally and dorso-laterally, setae blunt or weakly clubbed on anterior segments, becoming tapering on posterior segments. Anterior-most setae larger than following ones, but not as large as those of *H. isaloensis*. Head capsule tapering anteriorly, appearing slightly convex when viewed anteriorly. Labrum constricted at base as in *H. isaloensis* and strongly bilobed, produced into apical angularities (Fig. 2C). No elevated palpae apparent on labium or maxillae.

Etymology. *Hasina* is a Malagasy word that refers to a spiritual notion of a force that derives from the land, and moves through the ancestors into the society of the living, and this seems to resonate with the endemicity of this Malagasy clade and the notion that habitat has influenced extant forms by means of selection on ancestral lineages. *Melissa* is Greek for bee.

Included species.

Hasinamelissa seyrigi (Benoist) **comb. n.**

Allodape seyrigi Benoist, 1962, p 141. Holotype ♀, Beckily, Madagascar (Toliara province). [Museum National d'Histoire Naturelle, Paris, France].

Allodapula seyrigi (Benoist): Michener 1977a, p 10.

Halterapis seyrigi (Benoist): Pauly et al. 2001, p 325.

Hasinamelissa keiseri (Benoist) **comb. n.**

Allodape keiseri Benoist, 1962, p 139. Type ♀, Vohiparara, Madagascar (Fianarantsoa province). [Naturhistorisches Museum, Basel, Switzerland].

Allodapula keiseri (Benoist): Michener 1977a, p 11.

Halterapis keiseri (Benoist): Pauly et al. 2001, p 327.

Hasinamelissa minuta (Brooks & Pauly) **comb. n.**

Halterapis minuta Brooks & Pauly: Pauly et al. 2001, p 318. Type ♀, Ifaty, Madagascar (Toliara province). [United States National Museum of Natural History, Smithsonian Institution, Washington D.C.].

Hasinamelissa isaloensis (Brooks & Pauly) **comb. n.**

Halterapis isaloensis Brooks & Pauly: Pauly et al. 2001, p 320. Type ♀, Isalo National Park, Madagascar (Toliara province). [Faculté Universitaire des Sciences Agronomiques, Gembloux, Belgique.].

Hasinamelissa tulearensis (Michener) **comb. n.**

Halterapis tulearensis Michener, 1997a, p 17. Type ♀, Lavanono, Madagascar (Toliara province). [Natural History Museum, London, United Kingdom].

Hasinamelissa albigena (Brooks & Pauly) **comb. n.**

Halterapis albigena Brooks & Pauly: Pauly et al. 2001, p 320. Type ♀, Didy, Madagascar (Toamasina province). [Faculté Universitaire des Sciences Agronomiques, Gembloux, Belgique.].

Hasinamelissa kraussi (Michener) **comb. n.**

Halterapis kraussi Michener, 1977a, p 15. Type ♀, Nosy-Komba, Madagascar (Antsiranana province). [Museum National d'Histoire Naturelle, Paris, France].

Hasinamelissa didyensis (Brooks & Pauly) **comb. n.**

Halterapis didyensis Brooks & Pauly: Pauly et al. 2001, p 321. Type ♀, Didy, Madagascar (Toamasina province). [Faculté Universitaire des Sciences Agronomiques, Gembloux, Belgique.].

Hasinamelissa acaciae (Brooks & Pauly) **comb. n.**

Halterapis acaciae Brooks & Pauly: Pauly et al. 2001, p 322. Type ♀, Didy, Madagascar (Toamasina province). [Faculté Universitaire des Sciences Agronomiques, Gembloux, Belgique.].

Hasinamelissa ankaratrensis (Brooks & Pauly) **comb. n.**

Halterapis ankaratrensis Brooks & Pauly: Pauly et al. 2001, p 323. Type ♀, Didy, Madagascar (Toamasina province). [Faculté Universitaire des Sciences Agronomiques, Gembloux, Belgique.].

Hasinamelissa platyprosoton (Michener) **comb. n.**

Allodapula platyprosoton Michener, 1977a, p 14. Type ♀, Nosy-Komba, Madagascar (Antsiranana province). [Museum National d'Histoire Naturelle, Paris, France].
Halterapis platyprosoton (Michener): Pauly et al. 2001, p 323.

Hasinamelissa rufa (Michener) **comb. n.**

Allodapula rufa Michener, 1977a, p 14. Type ♀, Isaka-Ivondro, Madagascar (Toliara province). [Museum National d'Histoire Naturelle, Paris, France].
Halterapis rufa (Michener): Pauly et al. 2001, p 324.

Hasinamelissa pentagonalis (Brooks & Pauly) **comb. n.**

Halterapis pentagonalis Brooks & Pauly: Pauly et al. 2001, p 324. Type ♀, Morarano-Chrome, Madagascar (Toamasina province). [Faculté Universitaire des Sciences Agronomiques, Gembloux, Belgique.].

Hasinamelissa benoisti (Michener) **comb. n.**

Allodape longula Benoist, 1962, p 140. Type ♀, Ambalamanakana, Madagascar (Fianarantsoa province). [Naturhistorisches Museum, Basel, Switzerland].
Allodape benoisti Michener 1977a, p 11.
Halterapis benoisti (Michener): Pauly et al. 2001, p 325.

Hasinamelissa curtipilosa (Brooks & Pauly) **comb. n.**

Halterapis curtipilosa Brooks & Pauly: Pauly et al. 2001, p 326. Type ♀, Mororano-Chrome, Madagascar (Toamasina province). [Faculté Universitaire des Sciences Agronomiques, Gembloux, Belgique.].

Hasinamelissa personata (Brooks & Pauly) **comb. n.**

Halterapis personata Brooks & Pauly: Pauly et al. 2001, p 327. Type ♀, Mororano-Chrome, Madagascar (Toamasina province). [Faculté Universitaire des Sciences Agronomiques, Gembloux, Belgique.].

Hasinamelissa spatulata (Brooks & Pauly) **comb. n.**

Halterapis spatulata Brooks & Pauly: Pauly et al. 2001, p 328. Type ♀, Mororano-Chrome, Madagascar (Toamasina province). [Faculté Universitaire des Sciences Agronomiques, Gembloux, Belgique.].

Hasinamelissa spinipennis (Brooks & Pauly) **comb. n.**

Halterapis spinipennis Brooks & Pauly: Pauly et al. 2001, p328. Type ♂, Antsiranana, Madagascar (Antsiranana province). [Bohart Museum, Davis campus, University of California, U.S.A]. Note that Pauly et al. (2001) suggest this specimen may in fact be a male of the species *H. spatulata.*

Phylogenetic analyses

Bayesian inference. All three BI runs converged on identical topologies with highly similar branch lengths among consensus phylograms. A randomly chosen 50% majority rule consensus phylogram with posterior probabilities for each node is given in Fig. 3 in which the Malagasy species are denoted by the generic nomen *Hasinamelissa*. Only three nodes in the tree had support ≤ 50%; one involving a bifurcation among the Australian exoneurine genera. Lack of support for generic-level bifurcations for the exoneurines have been found in previous maximum parsimony and maximum likelihood analyses and probably results from rapid divergence following colonisation of Australia (Bull et al. 2003; Schwarz et al. 2006). The remaining two nodes with low support

involved bifurcations between species in the genera *Exoneurella* (separating *E. lawsoni* and *E. eremophila*), and another between species in *Hasinamelissa*, with low support for the position of *H. keiseri*. Our analyses indicate that the Malagasy species form a strongly supported (100%) monophyletic group. The undescribed taxa referred to as *H.* MorondavaB and *H.* R43H are likely to be conspecific, given the short branch lengths separating them, though such determination cannot be made until males and larvae are available. The remaining, largely African, taxa were recovered as a single sister clade to *Hasinamelissa*, with relatively strong support for monophyly of that group (98%). There was very strong support (100%) for the monophyly of *Braunsapis* and *Allodape* (as found by Schwarz et al. 2004) and monophyly of *Compsomelissa*, *Halterapis* and *Allodapula* (100%; as found by Tierney 2004). The Australian exoneurine genera (*Exoneura*, *Exoneurella, Inquilina* and *Brevineura*) were recovered as a sister group to the remaining allodapines, including *Hasinamelissa* but excluding the basal clade *Macrogalea*.

Maximum parsimony. Our MP bootstrap analysis recovered a single best tree from 3946 steps, and results from the analysis are summarised in Fig. 4 as a 50% majority rule tree, with bootstrap values indicated for each node where support was 50% or greater. The topology of this tree is largely concordant with the Bayesian analysis but with different bifurcation orders among the Australian genera, as well as differing bifurcation orders among both *Hasinamelissa* and *Braunsapis* species. In general, poor concordance between the BI and MP trees appears to occur where Bayesian posterior probabilities were low (<90%), and hence differences in topology in these regions is not unexpected. Despite these differences in topology, both MP and Bayesian analyses indicate that the Malagasy species form a monophyletic group (100% bootsrap and posterior probability support respectively) and that this group is clearly not allied with *Halterapis nigrinervis*.

Discussion

Our BI and MP phylogenetic analyses based on DNA sequence data show that the seven Malagasy specimens for which we have sequence data form a very strongly supported monophyletic group. Furthermore, this group is not nested within the African clade that contains *Braunsapis, Allodape, Halterapis*, *Compsomelissa* and *Allodapula*, but instead appears to be the sister group to the clade comprising all those groups. Studies of adult morphology (Michener 1977a, Reyes and Michener 1992) have not suggested that any members of the Malagasy *Halterapis* species show stronger affinities to African species than to other Malagasy species. Additionally, the species included in our study span the range in adult morphology for the Malagasy species formerly under *Halterapis* (ranging from the black, gracile and very small *H. minuta* which lacks paraocular and genal marks, through to the large and robust *Hasinamelissa keiseri* with orange metasoma and extensive paraocular markings). As such, it seems highly likely that all of the other Malagasy species placed formerly in *Halterapis* and *Allodapula* comprise a single clade. Larval morphology of the three species examined here supports the distinctiveness of the Malagasy group, and when the larvae of the three species are considered together, they are as distinctive as those of any other allodapine genus (Michener 1975). All species are characterized by the presence of ventrolateral rows of protuberances and the presence of long, posteriorly curving setae with blunt, spatulate, hooked, or clubbed ends, protruding from the first two thoracic tubercles. Examples of larvae from all other allodapine genera excluding the rare genus *Exoneuridia* have been sampled to date (Michener 1977b), and the above features are absent in all of these genera. Furthermore, the labrum differs from other genera in being strongly bilobed and widening markedly from its base. While the multiple ventro-lateral rows of protuberances and tubercles are superficially similar to those of *Allodapula*, that genus lacks the unique stout setae on the first two appendages, the dorsal body setae, and the strongly bilobed labrum. There is almost no resemblance between the Malagasy species and the African *Halterapis* species, which lack larval setae and rows of body protuberances and which have a quadrate labrum that is not constricted at their base.

For *H. minuta*, *H. seyrigi* and *H. isaloensis*, in which nesting biology has been studied (Schwarz et al. 2005; Chenoweth & Schwarz 2007), smaller larvae gradually move up the cylindrical mass provisions as they eat, and the larval appendages and setae probably are used for this movement. It is likely that the marked differences in larval

morphology between *Hasinamelissa*, *Allodapula* and *Halterapis* are related to the very different methods in larval provisioning.

The strong Bayesian and Maximum Parsimony support for a sister group relationship between the Malagasy species and the African genera (excluding *Macrogalea*) indicates that the origin of the Malagasy clade predates divergence of the latter genera. This means that if the Malagasy species were to be included in *Halterapis*, that genus would be polyphyletic. Alternatively, broadening *Halterapis* to include all clades that intervene between the African *Halterapis* species and the Malagasy species would result in a single genus encompassing >200 species that are currently placed in *Allodapula*, *Allodape*, *Braunsapis* and *Compsomelissa* and for which generic-level divergences have been dated as greater than 20 Myr BP (Schwarz et al. 2007).

Phylogenetic analyses of divergence dates in allodapine bees (Chenoweth et al. 2007), which included *H. minuta*, *H. seyrigi* and *H. isaloensis* indicate that the divergence of this Malagasy clade from its sister African clade is ancient, > 30 Myr BP. This may help explain the wide variation in adult morphology found within *Hasinamelissa* adult females (Pauly et al. 2001), which is high compared to levels of variation in female morphology found in most other allodapine genera (Michener 1977a). Three of the taxa in our study do not appear to belong to any described species and our collections were based on very limited fieldwork in close proximity to only five major regional towns, with no collections from remote areas where there are larger areas of indigenous forest. It therefore seems likely that the level of species diversity in *Hasinamelissa* is much greater than the 17 species described to date.

The extent of deforestation in Madagascar is one of the world's great biodiversity tragedies (Myers et al. 2000.) and it is worth noting that our only sample of *Hasinamelissa* from the Haut Plateaux was from an extremely small remnant forest 85 km from Antananarivo, despite extensive searching in the Antananarivo region. *Hasinamelissa* represents one of the earliest radiations in allodapine bees, a tribe of bees that has the potential to provide major insights into insect social evolution (Schwarz et al. 2007). It is clear that many species are yet to be described, and work based on the few species whose biology has been briefly explored promises a wealth of insights into insect social evolution. Conserving the remaining species warrants a very high priority.

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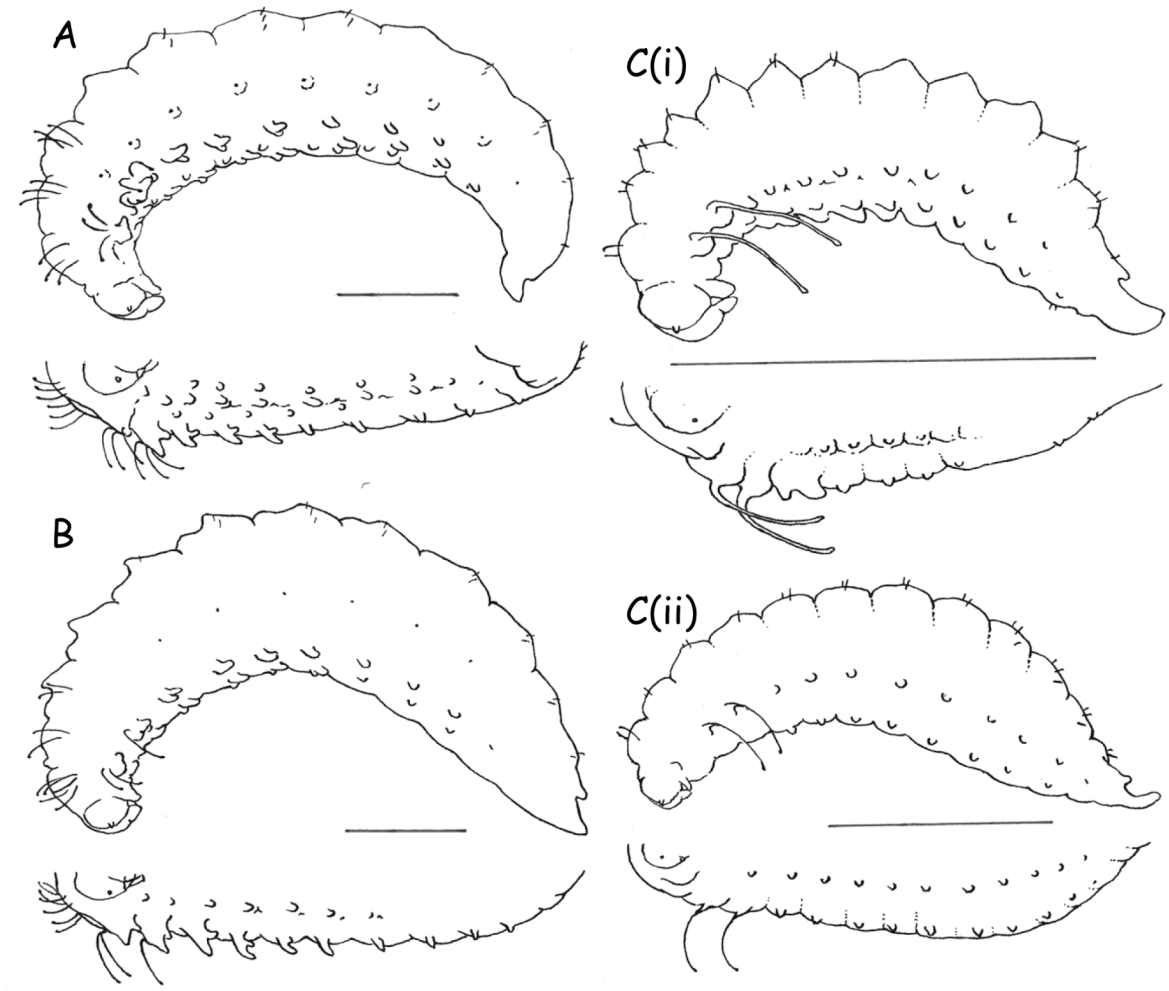

Fig. 1. Lateral and ventral views of mature larvae for *H. seyrigi* (A), *H. isaloensis* (B) and early and late instar larva of *H. minuta* (C(i) and C(ii) respectively). Scale bars represent 2 mm.

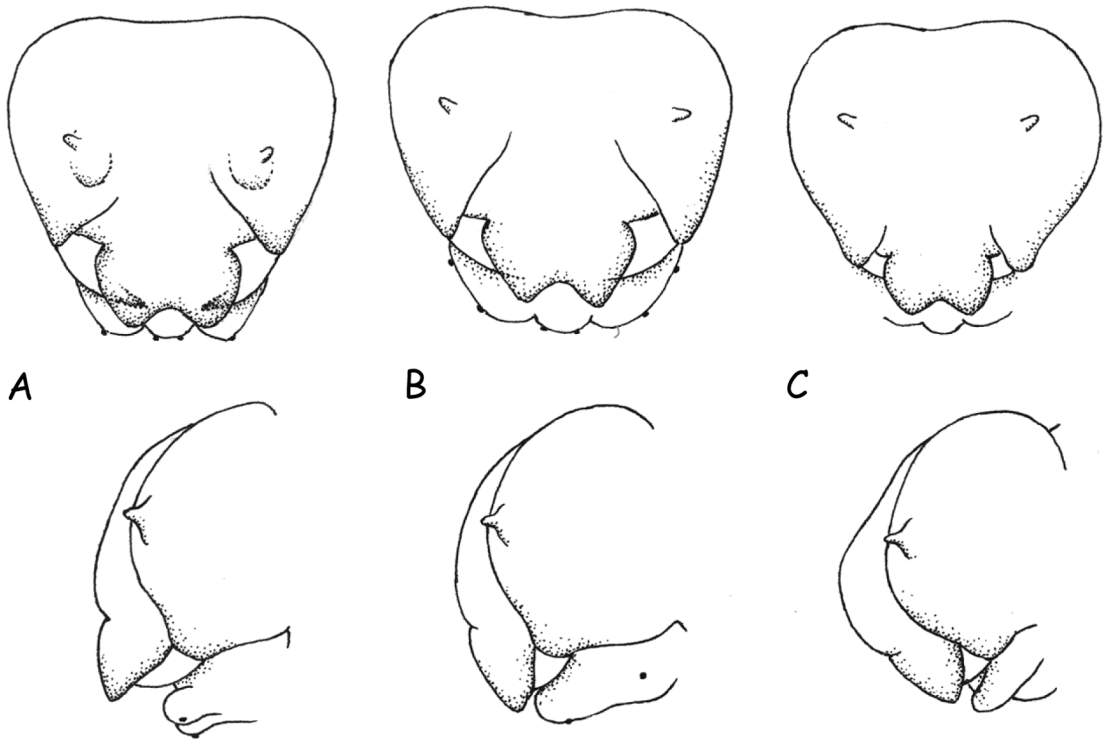

Fig. 2. Anterior and lateral views of head capsules from mature larvae for *H. seyrigi* (A), *H. isaloensis* (B) and *H. minuta* (C).

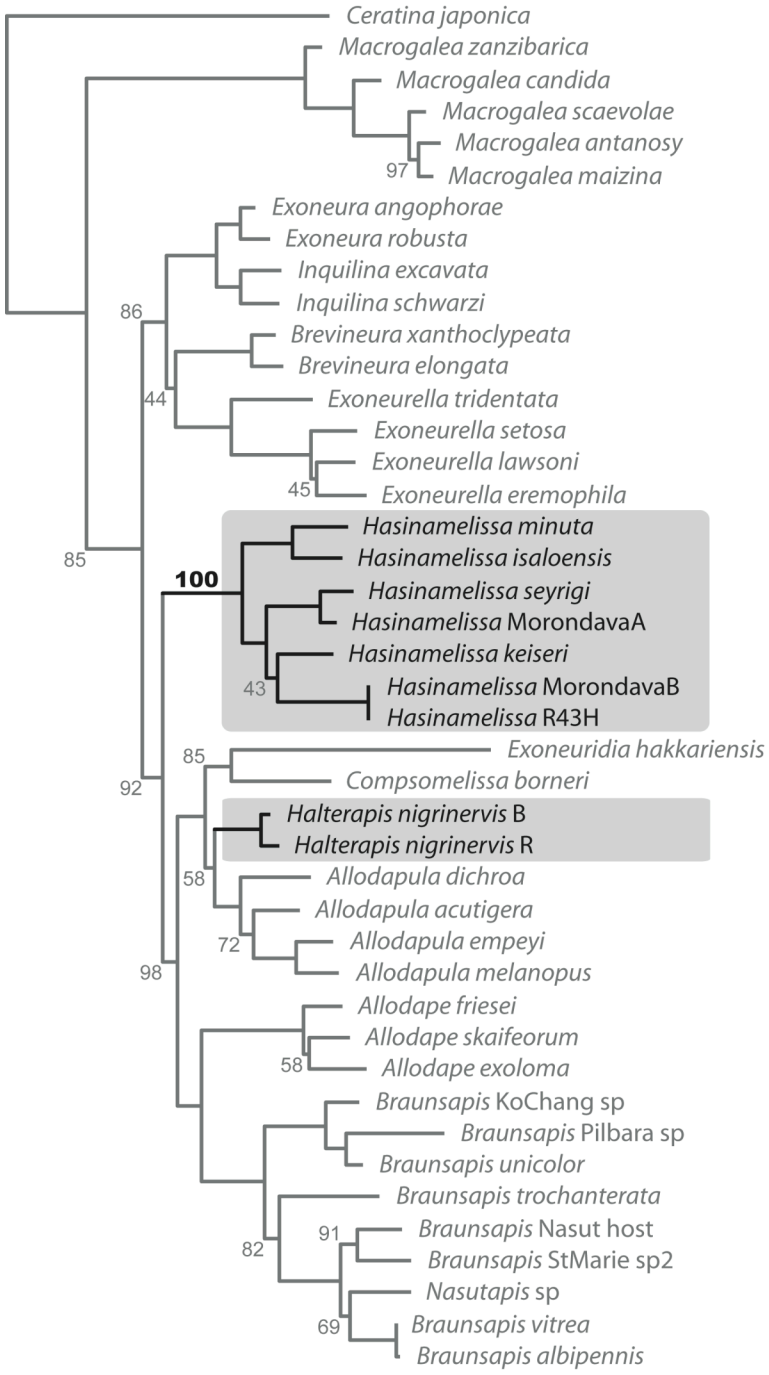

Fig. 3. Consensus phylogram from Bayesian analysis of *Hasinamelissa* species and representatives of all other non-parasitic allodapine genera

Posterior probabilities, based on 3000 sampled post-burnin generations are indicated for each node. Posterior probabilities are shown for all nodes with less than 100% support and for the root node of *Hasinamelissa*. Species belonging to the previous designation of the genus *Halterapis* are highlighted in grey.

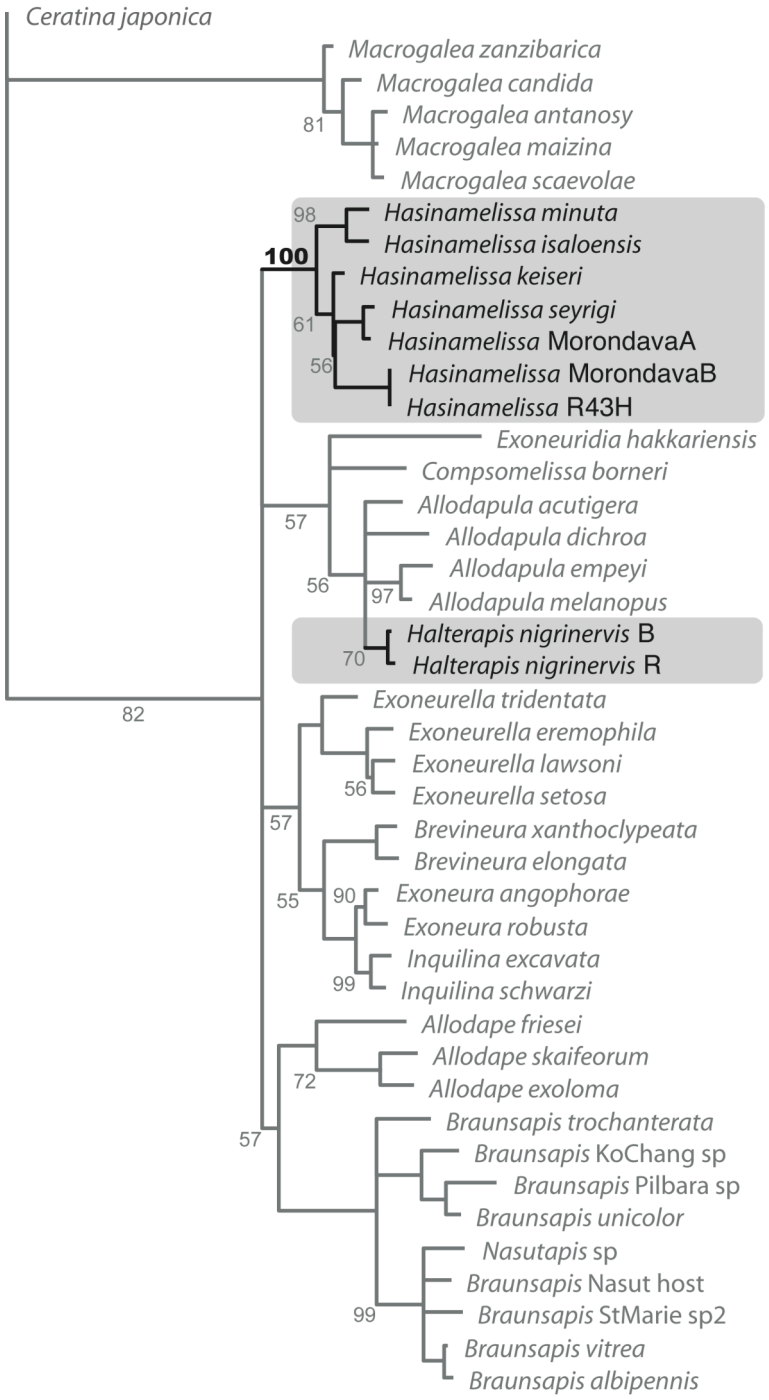

Fig. 4. 50% majority rule phylogram from maximum parsimony analysis.

Bootstrap values based on 1000 pseudo-replicates are indicated for each node. Bootstrap values are shown for all nodes with less than 100% support, and for the root node of *Hasinamelissa*. Species belonging to the previous designation of the genus *Halterapis* are highlighted in grey.

CHAPTER III

Biogeographical Origins and Diversification of the Exoneurine Allodapine Bees of Australia (Hymenoptera, Apidae).

ABSTRACT

Aim Early diversification of allodapine bees occurred in Africa *c.* 50 Ma. They are most abundant in sub-Saharan Africa and Australia, and one of the oldest phylogenetic divergences in the tribe involves a split between an African + Malagasy clade and an Australian clade. The historical biogeographical scenario for this has been highly problematic, entailing an Eocene dispersal from Africa to Australia, followed by an unresolved, and apparently rapid, set of bifurcations leading to the Australian 'exoneurine' genera. Here we use an expanded taxon set of Australian species to explore the timing and historical biogeography of the exoneurine radiation.

Location Australia, Africa, Madagascar.

Methods One nuclear gene (F2 copy of elongation factor 1α) and two mitochondrial genes (cytochrome *c* oxidase subunit I and cytochrome *b*) were sequenced for 33 Australian exoneurine species from all five genera found on the continent as well as for an additional 37 species from all non-parasitic genera in the remainder of the tribe. We used Bayesian inference analyses to study phylogenetic topology and penalized likelihood (PL) analyses to infer key dates of divergence within the tribe. We also used lineage-through-time (LTT) analyses and Bayesian analyses to explore the tempo of radiations and biogeographical history of the exoneurines.

Results Results from the phylogenetic analyses were congruent with previous studies, indicating a single colonization event *c.* 34 Ma, too late for Gondwanan vicariance models, and too early for a Laurasian dispersal route. In contrast to earlier studies, we show that this colonization event did not result in an ancient rapid radiation. However, LTT patterns indicated a rapid radiation of the temperate-adapted genera *Exoneura* and *Brevineura*, but not of the xeric-adapted genus *Exoneurella*, from 10 to 6 Ma.

Main conclusions Our results indicate a trans-oceanic dispersal event from Africa to Australia, most likely via Antarctica, with an accelerated diversification of temperate-adapted lineages during the major Late Miocene event referred to as the 'Hill Gap'. This is the first study to link radiations in Australian bee faunal elements to changing climate, and

differs from many other plant and insect phylogenetic studies by showing increased radiation of temperate clades, rather than xeric clades, with increasing aridification of Australia.

Keywords

Allodapini, aridification, Australia, bees, dispersal, exoneurines, Hill Gap, historical biogeography.

INTRODUCTION

Australia has the most unusual bee fauna in the world (Michener, 1965, 2007), over half of which are from the family Colletidae. Morphology-based studies have traditionally regarded this family as basally situated among the bees and consequently it was thought that the Australian bee fauna was largely dominated by ancient bee clades. More recent studies suggest that the Colletidae are in fact a relatively derived family, and that the most basal of the extant bee fauna are the mellitids *sensu stricto*, meganomiines and dasypodaiines (Danforth *et al.,* 2006), which are entirely absent from Australia. This suggests that the Australian bee fauna may not be nearly as old as first thought.

The most basal bee families found in Australia are Apidae and Megachilidae. Among the Apidae, the subfamily Xylocopinae is basally situated and possibly even the sister group to the rest of the family (Roig-Alsina & Michener, 1993), suggesting a relatively early origin. Three of the four Xylocopinae tribes are found in Australia and all appear to have post-Gondwanan origins outside of the continent. Leys *et al.* (2002) suggested that one Australian lineage, leading to the subgenus *Lestis*, first dispersed into Australia about 30 Ma. This timeframe is broadly coincident with the estimated arrival of another major component of Australia's bee fauna, the Halictinae (*c.* 30 Ma, Danforth *et al.,* 2004) and both groups were hypothesized to have arrived in Australia via dispersals from Laurasia through the Sunda Arc. However, current palaeogeographical models suggest that Australia was still a significant distance from southern Asia at this time (Hall, 2009), potentially raising problems for this dispersal route into Australia.

Within the Xylocopinae, the tribe Allodapini has been the subject of several phylogenetic studies exploring the origins of Australian bees. The allodapines are largely restricted to sub-Saharan Africa, Australia, Madagascar, and southern Asia. Only the derived genus *Exoneuridia*, comprising three rare Middle Eastern species, exists outside of this distribution, and only a single genus, *Braunsapis*, has a distribution that spans Africa, southern Asia and Australia.

Phylogenetic studies indicate that allodapines originated in Africa, with two temporally distinct migrations into Australia. The first of these migrations led to four 'exoneurine' genera endemic to southern Australia (Schwarz *et al.,* 2006), and the second involved the genus *Braunsapis* (Fuller *et al.,* 2005), which is largely absent from southern Australia. *Braunsapis* has an African origin, and Fuller *et al.* (2005) showed that it moved

into Australia via Indian Ocean Rim (IOR) dispersal during the Late Miocene, about 12-7 Ma.

In contrast to *Braunsapis*, the exoneurine genera (comprising *Exoneura*, *Exoneurella*, *Brevineura* and *Inquilina*) apparently derive from a single and much earlier dispersal into Australia, some 50-30 Ma (Schwarz *et al.,* 2006), when Australia's geographical relationship with other continents was very different from that in the Late Miocene. Unlike *Braunsapis*, the exoneurines are almost entirely restricted to the temperate areas of Australia. The primary centre of diversity of the exoneurine genera lies in the south-eastern temperate zone, with relatively few species in the south-western temperate zone and more northerly arid/semi-arid regions. Consequently, the exoneurines and *Braunsapis* differ strongly in both the times that they arrived in Australia and their extant distributions. This suggests that they may have arrived in Australia via different dispersal corridors and were subject to very different climatic histories.

Three broad hypotheses for dispersals between Africa and Australia, post-dating their rifting, have been proposed: (1) Indian Ocean Rim (IOR) dispersal; (2) cross-water dispersal over the Indian Ocean, perhaps aided by now-submerged elements of the Broken Ridge Province, and the Crozet, South Madagascan and Kerguelen Plateaus; and (3) dispersal via a land bridge connecting Indo-Madagascar to Antarctica when Antarctica was still joined to Australia.

IOR dispersals into Australia are well documented for a large number of animal and plant groups and typically post-date the Late Miocene, when no significant trans-ocean expanses remained in either the Tethys Sea or the Sunda Arc. More controversially, Krause *et al.* (1997), Sampson *et al.* (1998) and Cooper *et al.* (2001) argued that the distributions of some Cretaceous mammals, abelosaurids and ratites, respectively, are best explained by a continuous land-bridge model, although whether or not such a continuous land bridge existed at the required times has been strongly contested (Ali & Aitchison, 2009). Trans-oceanic disjunctions between Africa and Australia have been reported for a moderately large number of groups (de Queiroz, 2005), including some Passerida songbirds (Fuchs *et al.,* 2006) parrots (Schweizer *et al.,* 2010), and some Proteaceae (Barker *et al.,* 2007), and generally explained by long-distance dispersal. These dispersals are thought to have taken place since the Late Cretaceous, and for songbirds and parrots at least, the role of now-submerged subaerial Kerguelen and other Southern Indian Ocean elements was conjectured. However, for parrots, a key role in some Southern Hemisphere

disjunctions was thought also to involve Antarctica, via both rifting from Australia, and dispersal into Africa via Kerguelen Plateau elements.

Here we examine the relative likelihood of the ancestral exoneurine allodapines reaching Australia from Africa via each of the three broad scenarios described above. Each of these scenarios posits a different entry point into Australia, and each entails different geographical impediments to dispersal along with different climatic regimes at the points of entry. Resolving the historical biogeography of the exoneurines may also help recognize the origins of some other Australian bee groups where dispersals from Laurasia through the Sunda Arc have been assumed but where alternative origins have not been considered in detail.

Previous attempts to determine the dispersal route of exoneurines into Australia have been hampered by inability to resolve the basal polytomy involving the exoneurine genera, as well as small sample sizes and an absence of species from the western half of that continent. As a result, node ages were unclear and it was not possible to determine the ancestral range distribution for this clade or how it may have subsequently diversified. Here we use an expanded set of exoneurine species, including representatives from all regions where they are known to occur, to re-examine the clades' molecular phylogeny and explore their biogeographical history.

MATERIALS AND METHODS

Taxa, collection localities, and DNA sequencing methods

We used 33 representatives of the Australian exoneurine clade, covering all four genera. We included a further 37 allodapine species in our analysis to ensure that multiple representatives of all major clades were used wherever possible to reduce long-branch attraction due to low taxon sampling. Only one species each of *Compsomelissa* and the rare genus *Exoneuridia* were included due to lack of additional material. The majority of species in our analyses have been used in previous studies (Bull *et al.,* 2003; Schwarz *et al.,* 2003, 2004, 2005; Tierney, 2004; Fuller *et al.,* 2005; Chenoweth *et al.,* 2007, 2008; Smith, 2009) and GenBank accession numbers are provided within these papers. Thirteen additional allodapines, including twelve new exoneurine species, were collected from locations throughout Australia and full details of collection localities of each new species

are provided in Table 1 together with NCBI accession numbers for new gene fragments. In addition to the allodapines, we included three species of Ceratinini, the sister tribe to the Allodapini. The presence of *Ceratina* in our analysis also provides a calibration point based on a Baltic amber fossil of the tribe Boreallodapini, which is the extinct sister group of the Allodapini (Engel, 2000, 2001). To root the node connecting these two tribes we also included four other apids (one species of *Xylocopa*, two ctenoplectrines, and *Apis mellifera*) and we used two halictine bees (*Agapostemon tyleri* and *Lasioglossum lanarium*) to root the apid taxa.

Our sequence data comprised the exon region of one nuclear gene [F2 copy of elongation factor 1α (EF1α-F2); 772 bp] and two mitochondrial genes [cytochrome *b* (cyt *b*); 428 bp, and cytochrome oxidase I (COI); 1279 bp]. DNA extraction, amplification and sequencing methods used for gene fragments are identical to those described in Chenoweth *et al.* (2008).

Phylogenetic methods

Estimation of phylogeny for our taxa used Bayesian inference (BI) implemented in the UNIX version of MRBAYES 3.1.2 (Ronquist & Huelsenbeck, 2003). Sequence data were partitioned into four parts: $1^{st} + 2^{nd}$ and 3^{rd} codon positions for the combined mitochondrial genes, and $1^{st} + 2^{nd}$ and 3^{rd} codon positions for the F2 copy of EF1α. We adopted an 'objective' Bayesian approach (Berger, 2006) and therefore used the MRBAYES default priors because these are mostly uninformative (Huelsenbeck & Ronquist, 2001). We did not fit models to partitions (e.g. using MODELTEST, Posada & Crandall 1998) and then use these as priors because model fitting requires an a priori tree to derive substitution models and we wanted to avoid the possibility that prior belief in a likely topology might influence subsequent models, including topology. We used a 6-parameter ($N_{ST} = 6$) rate transition matrix, with gamma shape for variation in rates and a proportion of invariant sites assumed corresponding to a GTR + I + Γ model. This is the least restrictive model available in MRBAYES and allows more restrictive models, such as HKY and K2P, which are subsets of the GTR + I + Γ model, to arise if they provide a better fit to the data. All parameters were unlinked between partitions. Two sets of four Markov Chain Monte Carlo (MCMC) analyses with Metropolis coupling were run in parallel for each BI analysis. Convergence was assessed by the average standard deviation of split frequencies, and viewing log

likelihood (ln *L*) values using TRACER (Rambaut & Drummond, 2007) version 1.4.1 to assess stationarity. The analysis was run for 10 million generations, sampling every 500th generation to reduce auto-correlation among sampled generations. We repeated the above analyses three times to check for convergent topologies and branch lengths. All runs gave extremely similar results, including very similar branch lengths, and we used the topology and associated branch lengths from the first run in subsequent analyses.

Dating analysis

Penalized likelihood implemented in R8S 1.71 (Sanderson, 2002) was used to transform phylograms obtained from MRBAYES into chronograms. Mean ages for key nodes of interest were obtained using the 'profile' command, and the standard deviations of these means were used to calculate 95% confidence intervals for each node.

We were unable to use the relaxed clock model implemented in BEAST 1.5.2 (Drummond *et al.,* 2009) because initial trees had ln *L* values that were too low to allow the procedure to continue. Although it is possible to provide a 'likely' tree as a topological prior, we did not do so because this could have influenced the final topology in a way that our prior beliefs did not justify. We did not wish to use earlier versions of BEAST that do not allow separate partitioning of both genes and codon position because earlier allodapine studies (e.g. Schwarz *et al.,* 2004) show that combining codon positions across nuclear and mitochondrial genes, or partitioning genes without also partitioning 3rd codon positions, can lead to inappropriate models.

For our R8S analyses, cross validation was first used to estimate an appropriate smoothing value. There are no internal calibration points for allodapine phylogenies. However, the existence of fossil Boreallodapini species from 45 Ma Baltic amber provides a calibration point for divergence between Allodapini and Ceratinini. Boreallodapini is the extinct sister tribe of the Allodapini, with the Ceratinini comprising the next-most basal tribe (Engel, 2001). The divergence between Allodapini and Ceratinini was set at a minimum age of 45 Ma, but it should be noted that this must be a very conservative estimate since it assumes effectively zero time required for divergences of the three tribes. In addition, a fixed age of 90 Ma was set for the node connecting the xylocopine tribes to the corbiculate apines. Fossils of the plant family Clusiaceae, whose floral morphology is closely linked to pollination by corbiculate bees, are dated to 90 Ma (Crepet & Nixon, 1998) and Ramírez *et al.* (2010) have estimated a crown age for the corbiculates at about

90 Ma. However, Danforth *et al.* (2004) have dated the crown age of the Halictidae, a far more derived family than the Apidae, at approximately 120 Ma, suggesting that setting the root node at 90 Ma is likely to be conservative. Additional analyses were therefore performed exploring the effects of setting this node to 100, 110 and 120 Ma.

Biogeographical methods

The MultiState analysis implemented in BAYESTRAITS (Pagel *et al.,* 2004; Pagel & Meade, 2006) was used to infer ancestral states and dispersal events that have resulted in the current distribution of the exoneurines. This method allows for both polymorphisms in character states (i.e. ecological or geographical localities) within species as well as uncertainty in phylogeny. Various priors were explored, with a criterion that acceptance rates had to be bounded by 20 and 40% (Pagel & Meade, 2006). We used a rate deviation of 15 with both an exponential (0, 10) reverse jump hyperprior (rjhp), and also explored an exponential (0, 5) rjhp with a rate deviation of 20. The two sets of priors did not give appreciably different results and results from the first set of priors are presented here. Stationarity was interpreted as a plateau in the harmonic mean. We subsequently used 40 x 10^6 iterations with a burn-in of 10 x 10^6, sampling every 1000^{th} generation.

In order to infer ancestral geographical ranges for the exoneurines, we classified the distributions of our species using two schemes shown in Figure 1. Firstly, species were categorized by their presence in the principal climatic regions of Australia. We used three broad bioclimatic regions characterized by a combination of factors, including growth responses of dominant plants to seasonal temperature variations, climate, and endemism (Crisp *et al.,* 2004, and references therein): (1) a primarily inland 'Eyrean' region which is arid and semi-arid; (2) a northern tropical 'Torresian' region, extending southwards to south-east Queensland; and (3) a temperate 'Bassian' region, primarily in the south-east and south-west, but with some pockets extending north into Queensland. Secondly, species were classed as having either eastern or western distributions, using the Nullarbor Plain and deserts to its north as the divider between the two regions. This Eyrean region effectively divides the south-east and south-west Bassian regions of the continent, forming a significant barrier for dispersal of temperate-adapted species. Only a single exoneurine species, *Exoneurella tridentata,* has an Eyrean distribution crossing the Nullarbor and was thus classified as being east/west polymorphic.

Exploring diversification rates

We used a combination of lineage-through-time (LTT) plots and birth–death models to explore diversification rates within the exoneurines. To check the reliability of the LTT signal in our consensus chronogram, we generated plots for both the consensus chronogram as well as 100 randomly chosen post-burn-in chronograms using the mltt.plot module in APE (Paradis *et al.,* 2004). Caution is needed when interpreting LTT plots (e.g. Ricklefs, 2007), and using LTT plots to infer changes in speciation–extinction ratios for the exoneurines is problematic because our taxon sampling was uneven across genera. We therefore used MEDUSA (Alfaro *et al.,* 2009) to detect changes in diversification rates in a way that does not require all species within a clade to be included in a chronogram. MEDUSA uses a stepwise Aikake information criterion (AIC) to determine whether changes in speciation/extinction ratios lead to increased model fit, based on a flexible rate shift model by Rabosky et al. (2007). We constructed a pruned version of our chronogram and a richness matrix assigning all described exoneurine species, including those not present in our analysis, into tip clades. Our chronogram included all four described *Exoneurella* species and all eight described *Inquilina* species, but only six species of *Brevineura* and 14 species of *Exoneura*, whereas there are 27 and 38 described species for these genera, respectively. Consequently, we allowed each *Exoneurella* and *Inquilina* species to remain as a single species tip, and collapsed all species of *Brevineura* and *Exoneura* into two separate clades representing the known species richness of each genus. Our samples also included one undescribed species from Western Australia where adult morphology suggests assignment to *Brevineura* but phylogenetic analyses strongly indicate membership of the *Exoneurella* clade. In the absence of larvae, whose morphology is critical for delineation between these genera (Michener, 2007), we refer to this species as belonging to *Exoneurella*, but note that future studies of larval morphology may raise questions about generic assignment.

RESULTS

Phylogenetic analyses

The three separate Bayesian analyses, each comprising two parallel runs, all converged on almost identical consensus topologies and very similar branch lengths. We used results from the first run in subsequent analyses. Plots of ln *L* values and examination of the standard deviations of split frequencies showed that parallel runs converged after about two million generations and we chose a conservative burn-in of 5 million. Posterior probability (PP) values from our consensus cladogram (See Figure S1 in Supporting Information) are presented in Figure 2. Four nodes in the tree had support ≤ 50%, all of which involved intra-generic relationships and only two of these involved exoneurine species. However, in both the latter nodes (one involving *Brevineura* and the other *Exoneura*), descendant clades that encompassed a disjunction in ecoregions or east–west distribution were very strongly supported (100% PP support), so that random resolution of these weakly supported nodes would not have consequences for inferring ancestral distributions.

We found high support (100 PP) for *Macrogalea* as sister clade to all other allodapines, concordant with earlier molecular studies. There was also strong support for a sister clade relationship between the Malagasy genus *Hasinamelissa* and the non-*Macrogalea* African clades and their descendants, which include the Middle Eastern genus *Exoneuridia* and the widespread genus *Braunsapis*. Importantly, we found that the position of the exoneurines, between *Macrogalea* and the African + Malagasy clade, was strongly supported (98 PP).

Monophyly of the exoneurine clade was well supported (97 PP) in contrast to previous studies, and high (100 PP) support was found for the monophyly of each of the four genera. There was strong support (93 PP) for monophyly of *Brevineura*, *Exoneura* and *Inquilina*, indicating that *Exoneurella* (including the undescribed Western Australian species D) forms the sister clade to the remaining exoneurines.

Molecular dating

A pruned penalized likelihood (PL) transformation of the Bayesian phylogram is given in Figure 2 (See Figure S2 in Supporting Information for the unpruned phylogram), where geographical distributions of each exoneurine species are also indicated. PL estimates for key node ages are given in Table 2 along with 95% confidence intervals. These indicate a divergence between the Allodapini and the Ceratinini at *c*. 70 Ma, a crown age for the extant allodapine lineages of *c.* 49 Ma, and a crown age for the exoneurines at *c*. 34 Ma, with divergence of the exoneurine clade from the combined African non-*Macrogalea* and Malagasy *Hasinamelissa* lineage about 42 Ma. This provides a window of *c.* 42-34 Ma for the ancestral exoneurine clade to evolve and then disperse into Australia.

Analyses based on a set divergence time of 90 Ma between the corbiculates and the Xylocopinae indicate a crown age for the *Exoneurella* clade of 26.5 Ma, a crown age for *Brevineura* + *Exoneura* + *Inquilina* of *c.* 27.5 Ma, and crown ages for *Exoneura* + *Inquilina* (where *Inquilina* are all obligate social parasites of *Exoneura*) and *Brevineura* of *c.* 16.5 Ma and 12 Ma, respectively. The crown age of *Exoneura* is dated at 8.5 Ma. Varying the 'fixed' age of the root node joining the Xylocopinae and the corbiculates from 90 to 120 Ma resulted in the estimated ages of internal nodes increasing in a proportionately linear manner so that the crown age of the exoneurines increased to 45 Ma after diverging from the African + Malagasy lineages at *c.* 55 Ma, and the crown ages of *Exoneura* + *Inquilina* and *Brevineura* increased to 22 and 16 Ma, respectively. The analyses above suggest that changing the assumed time for the basal divergence between the corbiculate apines and the Xylocopinae could have important consequences for dating early inter-continental dispersal/vicariance events, but is unlikely to have strong effects for dating exoneurine radiations after colonization of Australia.

Biogeographical analyses

Our Bayesian MultiState analysis showed lack of clear support for either an eastern or western origin of the exoneurines (Fig. 2, Node A), but there was support for a temperate origin for the clade (Bayes factor (BF) = 3.21). There was also support for a temperate region as ancestral for both *Brevineura* (Node B, BF = 2.50) and *Exoneura* + *Inquilina* (Node C, BF = 5.67). For both these nodes there was also support for an eastern ancestral range (BF > 2.0 for both tests). Our analyses did not indicate positive support for an

ancestral ecoregion or east–west origin for the *Exoneurella* clade (BF < 2.0 for both characters), although both Bassian and eastern states had greater support as ancestral regions than their alternatives.

Given the eastern temperate origins for *Exoneura* and *Inquilina*, Figure 2 suggests either three range expansions into the western temperate region, or vicariance events leaving three surviving lineages in the west and with their sister clades now having eastern ranges. These possibilities are explored further in the Discussion below. Only one species of *Brevineura*, *B. minutissima* and not represented in our sample, has been recorded from Western Australia (Erickson & Rayment, 1951) and this would therefore represent a fourth dispersal or vicariant origin from an eastern lineage. Of the east–west disjunctions observed in *Exoneura* (Table 2, Fig. 3), the first occurred *c.* 6.2 Ma (Node D, Fig. 3) and was followed by subsequent speciation in Western Australia. The second is more recent, and is represented by a single known species whose lineage diverged from an eastern clade *c.* 1.6 Ma (Node E, Fig. 3). Our analysis suggests that the most recent common ancestor (MRCA) of all three western Australian species originated in the eastern temperate zone (Nodes E and F, Fig. 3). We were unable to determine the ancestral range of the MRCA joining the western species *Exoneura pictifrons*, and *E.* Western Australia C with its sister clade of *E. robusta*, *E.* Cobboboonee sp. 2, and *E.* South Australia A, however this node also had very low PP support (46) in our Bayesian analysis (Node D, Fig. 3).

Diversification rates over time

Our semi-log LTT plot (Fig. 4) for the consensus chronogram of exoneurines was broadly similar to the randomly chosen 100 post-burn-in trees from our Bayesian analysis. Diversification rates do not appear to change markedly up until *c.* 10 Ma, whereupon there is an increased accumulation of lineages, with this increase declining at *c*. 6 Ma. A subsequent decline renders this increase in diversification inconsistent with a 'pull of the present' effect where extinction/speciation events become offset (Nee *et al.,* 1994). There also appears to be a slight increase in the slope of the LTT plots in the most recent 2 Myr. Whilst we can not rule out a 'pull of the present' effect here, this observation may also be indicative of response to wet cool/warm dry cycling present after the initial mid-Miocene aridification event. Our stepwise AIC analysis implemented in MEDUSA indicates a uniform rate of diversification in the *Exoneurella* clade with a low speciation-extinction rate of B-D = 0.050, and extremely low speciation/extinction ratio of 9.83×10^{-7}. In

contrast, we found a slightly higher B-D rate of 0.08 and a much higher B/D ratio of 0.9 for *Brevineura*, *Inquilina* and *Exoneura*, indicating a substantially higher diversification rate in this mostly temperate clade.

DISCUSSION

The allodapine bees form a major component of the bee fauna in sub-Saharan Africa, Madagascar, southern Asia and Australia. Understanding where they evolved and their subsequent radiations may therefore have important implications for understanding angiosperm radiations in these regions.

An African origin for extant allodapines is well established (Bull *et al.,* 2003; Schwarz *et al*., 2003, 2006), and is further supported by an African origin for their extant sister tribe, the Ceratinini (Rehan *et al.,* 2010). However, attempts to understand how the exoneurine allodapines evolved have been beset by problems with unresolved basal nodes, and being able to assess the relative likelihoods of alternative dispersal pathways from Africa to Australia has been problematic. Our analyses suggest divergence of the exoneurine clade from an African clade *c.* 42 Ma, with radiation of extant exoneurine lineages beginning *c.* 34 Ma. This suggests a window of about 8 Myr or greater, given uncertainties for these dates, for exoneurines to disperse from Africa to Australia.

Vicariance via the Gondwanan rifting of Africa from a combined Australia + Antarctica + South America cannot explain the biogeography of the exoneurines because such an early rifting event (Rabinowitz *et al.,* 1983) pre-dated the likely origin of bees *per se* (Engel, 2001; Poinar & Danforth, 2006). This leaves only three plausible scenarios for how allodapines may have expanded their range from Africa to Australia (Fig. 5): (1) Indian Ocean Rim (IOR) dispersal; (2) dispersal across the Indian Ocean; and (3) dispersal via Antarctica. We now address each of these scenarios and then explore factors that may have influenced radiations of the exoneurines once they colonized Australia.

Indian Ocean Rim dispersal. The allodapine genus *Braunsapis* provides a typical example of African–Australian dispersal via the Indian Ocean Rim (IOR) (Fuller *et al.,* 2005). However, this involved only a single dispersal event into Asia in the Early Miocene after the Tethys Sea had largely closed, followed by radiation in southern Asia and then a single dispersal into Australia *c*. 9 Ma when there were no large water barriers in the Sunda

Arc (Fuller *et al.,* 2005). However, a Laurasian dispersal route for the exoneurines in the Late Eocene would require skirting the Tethys Sea and then moving south into southern Asia, followed by cross-ocean dispersal to Australia at a time when the two landmasses were still distantly separated (Fig. 5). This seems extremely unlikely given the poor ability of allodapines to cross even moderate ocean expanses over long time periods (Fuller *et al.,* 2005). IOR dispersal would also imply substantial dispersal through tropical regions followed by extinction of all ancestral lineages except for a single temperate lineage in the south that subsequently radiated to become a major faunal element.

It is possible that global cooling towards the end of the Eocene (McGowran *et al.,* 2004) could have allowed temperate-adapted exoneurines to enter Australia via Laurasia. The subsequent onset of aridification in central Australia, also precipitated by persistent global cooling, may have then contracted the range of the early exoneurine lineages southwards, resulting in their present distribution. However, palaeoclimatic models suggest that equatorial latitudes probably retained stable, tropical climates during Late Eocene cooling (Pearson *et al.,* 2007), so the current distribution of exoneurines would still require complete loss of what must have been tropical-adapted ancestral lineages.

Dispersal across the Indian Ocean. This second possibility was suggested by Schwarz *et al.* (2006) and involves direct dispersal from Africa to Australia across the Indian Ocean via subaerial regions of various large igneous provinces (LIPs) during the Eocene. Direct dispersal relies on the presence of sufficient subaerial LIPs to allow 'island hopping' across an otherwise large ocean expanse. Although there is reason to believe that some LIPs in the Indian Ocean did have subaerial elements during the Eocene (Hay *et al.,* 1999; Coffin *et al.,* 2002; Duncan, 2002; Wallace *et al.,* 2002), we do not know if these elements were all in place at the right times, and with sufficiently small separations to allow cross-ocean dispersal. Current reconstructions of these southern Indian Ocean LIPs in the Eocene (Fig. 5) allow the possibility of island hopping, if subaerial elements all occurred in the 'right places at the right times'.

Dispersal via Antarctica. The third possibility for dispersal from Africa to Australia is via Antarctica. Antarctica was connected to Australia via what is now Tasmania up until *c.* 33 Ma, perhaps via an arc of islands in the latter stages of rifting (Exon *et al.,* 2004). This dispersal route is different from a trans-Indian ocean scenario in that island 'stepping stones' from Africa to Antarctica would probably have involved different expanses of

ocean, in a different climatic region, to be crossed. This route would also lead to a south-eastern entry point to Australia rather than a more northern and western arrival point.

Current understandings of Eocene palaeocoastlines raise strong concerns about the likelihood of island hopping from Africa to either Australia or Antarctica. This is because both scenarios rely heavily on substantial regions of the Kerguelen Plateau remaining subaerial during this period. Palaeogeographical data suggest that only a small fraction of the Kerguelen plateau was above sea level after the Middle Cretaceous, and this igneous province never directly adjoined any Southern Hemisphere continents (Ali & Aitchison, 2009). This means that any dispersal route over the Indian Ocean would have required crossing ocean stretches in excess of 1000 km.

Ancestral distribution and radiation of the exoneurines. An important clue to the route by which the exoneurines reached Australia may come from their inferred ancestral distributions in Australia. Our results differ from previous studies by resolving bifurcation order among the major exoneurine lineages, with *Exoneurella* forming the sister clade to *Brevineura* + (*Exoneura* + *Inquilina*). Resolving this basal node means that we can explore likely ancestral regions and patterns of radiation.

Our BAYESTRAITS analyses indicate a temperate ecoregion for the ancestral exoneurine lineage, as well as for *Brevineura* and *Exoneura* + *Inquilina*. BF support for a temperate origin of the *Exoneurella* clade was neither positive nor negative (but with reconstructed probabilities slightly favouring a temperate origin). Reconstructed probabilities for east or west origins also favoured an eastern origin for all of the above nodes, though BF tests were less than 2.0 for the root of *Exoneurella* and the crown exoneurine node. When combined, the above BAYESTRAITS analyses do not provide support for either western or non-temperate origins for any of the major clades, but provide positive BF support for both an eastern and temperate origin for three of the above four nodes. An eastern temperate origin is consistent with potentially much shorter cross-ocean dispersals from Africa to Antarctica via Indian Ocean LIP stepping stones, compared to a more direct but geologically more problematic Africa to Australia route during the Eocene.

Our LTT analysis suggests that once the exoneurines arrived in Australia, there was a relatively uniform birth–death ratio up until *c.* 10 Ma. This contrasts with earlier studies of the exoneurines that suggested a very rapid radiation after initial colonization of Australia (Bull *et al.,* 2003; Schwarz *et al.,* 2006), which was hypothesized to result from a radical disjunction in biotic environments following cross-ocean dispersal. At the time

when the exoneurines entered Australia, much of Antarctica contained temperate woodland (Francis *et al.,* 2008) and the southern and central regions of Australia were wet and forested (Byrne *et al.,* 2008), so environmental barriers preventing Antarctica–Australia dispersal of temperate adapted taxa should have been weak. The stable rate of early radiation shown by our study is therefore more consistent with entry from Antarctica over a continuous land route or island arc with no significant ecological disjunction.

At *c.* 10 Ma, our LTT analysis shows a sudden, strong change in diversification. This change is due to radiations in *Brevineura*, *Exoneura*, and its obligate parasite genus *Inquilina*. One possibility is that increased radiation in *Exoneura* could have been due to its unique ability to cofound new colonies. Nest cofounding is thought to provide substantial protection against predators at the nest (Schwarz *et al.,* 2006) and, for at least one *Exoneura* species, allows it to persist in habitats where it could otherwise not (Zammit, 2008). However, our MEDUSA analysis suggests the increased diversification of exoneurines also involved *Brevineura*, where nest co-founding has never been reported but which shares the same nesting substrates. As such, the increase in speciation *c*. 10 Ma cannot be explained by this change in nest founding strategy alone. One property that *Brevineura* and *Exoneura*, but not *Exoneurella*, share is that they are both almost entirely wet-temperate genera. This suggests that the marked diversification of exoneurines over the period of 10-6 Ma may have been linked to changes in climate.

Current understanding suggests that the aridification of Australia started in the Middle Miocene *c.* 10 Ma (Clapperton, 1990; Zheng *et al.,* 1999; Crisp *et al.,* 2004; Byrne *et al.,* 2008). This change from the mild Middle Miocene climate of Australia is referred to as the 'Hill Gap' and covers a period of 10-6 Ma (Hill, 1994; Byrne *et al.,* 2008). During this time there was a dramatic shift in the constituent flora of the continent, with forest receding southwards in the wake of growing xeric conditions. Radiation of temperate-adapted plant lineages slowed or even stagnated due to increased extinction (Crisp *et al.,* 2004), and arid-adapted taxa flourished inland (Martin, 2006). This appears to contrast to what was happening with the exoneurines, where temperate-adapted *Brevineura* and *Exoneura* (along with *Exoneura'*s parasitic genus *Inquilina*) were showing accelerated diversification, whilst the more xeric-adapted *Exoneurella* showed very low and unchanging diversification.

In a study on diversification patterns in several plant groups, Crisp & Cook (2009) argued that semi-log LTT plots that might seemingly suggest sudden increases in diversification, followed by attenuating cladogenesis, might instead reflect massive

extinction events. However, our LTT plot did not show the characteristically strong 'elbow' in diversification prior to the apparent increase in diversification associated with an extinction event. Whilst we cannot rule out extinction events as a possible explanation for the apparent diversification *c.* 10 Ma, one alternative scenario seems very plausible. Increasing aridification would have contracted the ranges of wet-temperate adapted species, fragmenting distributions and promoting allopatric speciation. However, for xeric-adapted species aridification would have tended to extend species' ranges, rather than fragmenting them. In this respect, it is worth noting that each of the four extant *Exoneurella* species have more extensive distributions than any species of *Exoneura*, *Brevineura* or *Inquilina* – ranging from just under 2000 km for *Exoneurella lawsoni*, *Exoneurella setosa* and *Exoneurella eremophila*, to nearly 2500 km for *Exoneurella tridentata* (Houston, 1976; Cardale, 1993). Understanding why some temperate bee groups may have shown increased diversification in response to aridification, whilst temperate plants groups showed significant decline, may have important ramifications for understanding the genesis of modern Australian terrestrial ecosystems.

CONCLUSIONS

Our findings implicate trans-oceanic migration between Africa and Australia to explain colonization of Australia by the exoneurines. Current palaeogeographical reconstructions of the Southern Hemisphere during the Eocene do not fit easily with the timing of this migration. However, we find evidence for the route to be via Antarctica. Importantly, Almeida & Danforth (2009) found that the exclusively Australasian colletid bee subfamily Euryglossinae and the exclusively African subfamily Scrapterinae form sister clades, while some other colletid groups show strong relationships between Australian and South American clades. These scenarios also appear to suggest post-Gondwanan, Antarctic connections between the Southern Hemisphere landmasses. Together with other non-bee studies showing connections between African and Australian clades, there is a clear need to study further possible dispersal routes between these two continents, despite the problems suggested by current understandings of Indian Ocean palaeogeology.

Our findings also implicate a role for Miocene climate change in the radiation of temperate allodapine lineages in Australia. Aridification has been associated with increased diversification of many arid-adapted flora (Crisp *et al.,* 2004; Martin, 2006) and fauna

(Chapple & Keogh, 2004; McLeish *et al.,* 2007). Interestingly, our findings suggest no evidence of increased diversification in arid-adapted exoneurine lineages, but do suggest a significant impact on diversification rates of the temperate lineages.

The effects of climate on the diversification of taxa that play an integral role in ecosystem function will have far reaching implications on the subsequent course of an ecosystem's progression. This study highlights how both dispersal mechanisms and the dynamic nature of climate have strongly influenced the biogeographical history of an important group of Australian pollinators. A clearer understanding of how ecosystems evolve over long periods of evolutionary time requires that we consider how both these factors, as well as the interplay between them, can help better inform the history of diversification in key faunal elements of terrestrial ecosystems across the globe.

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Table 1 List of newly sequenced allodapine species for this study along with GenBank accession numbers and their collection location. Species distributions are indicated by ecozones (Te, temperate; E, Eyrean; Tr, tropical) and by geographical distribution (E, eastern; W, western). Localities where new taxa were collected are as follows: Denmark (34°57'36" S 117°21'11" E), Augusta (34°18'43" S 115°09'32" E), and the Margaret River area (33°57'18" S 117°18'04" E) in south-west Western Australia. Bellingen in northern New South Wales (330°448' S 152°901' E). The Noosa region of south-eastern Queensland (26°23′4″ S, 153°6′46″ E). The Dandenong Ranges in Victoria (37°48'37" S 145°23'08" E). Scott Creek in South Australia (35°05' S 138°41' E). Non-exoneurine taxa were not used for biogeographical analyses.

Genus	Species	Collection location/Distribution	Accession numbers		
			EF1α-F2	Cyt *b*	COI
Exoneura	*illustris*	Denmark, Augusta (Te, W)	HQ268575	HQ268574	HQ268588
Exoneura	*pictifrons*	Denmark, Augusta, Margaret River (Te, W)	HQ268563	HQ268562	HQ268582
Exoneura	Western Australia C	Denmark (Te, W)	HQ268565	HQ268564	HQ268583
Exoneura	South Australia A	Scott Creek (Te, E)	HQ268567	HQ268566	HQ268584
Exoneura	South Australia B	Scott Creek (Te, E)	HQ268577	HQ268576	HQ268589
Exoneura	Dandenong A	Dandenong Ranges (Te, E)	HQ268569	HQ268568	HQ268585
Exoneura	Bellingen A	Bellingen (Te, E)	HQ268573	HQ268572	HQ268587
Exoneura	Queensland D	Noosa (Te, E)	HQ268571	HQ268570	HQ268586
Brevineura	Bellingen B	Bellingen (Te, E)	HQ268557	HQ268556	HQ268579
Brevineura	Queensland A	Noosa (Te, E)	HQ268561	HQ268560	HQ268581
Brevineura	Queensland C	Noosa (Te, E)	HQ268559	HQ268558	HQ268580
Exoneurella	Western Australia D	Denmark (Te, W)	HQ268555	HQ268554	HQ268578
Braunsapis	Queensland	Noosa (Te, E)	HQ322268	HQ322267	HQ322266

Table 2 Age estimates (in Ma) and 95% confidence intervals for key allodapine clades using penalized likelihood (R8S) analysis.

	Age (Ma)	
	Mean	95% CI
Divergence between Allodapini & Ceratinini	70.8	61-81
Divergence of extant Allodapini	48.8	42-56
Divergence between exoneurine & African clades	42.1	36-48
Divergence between extant exoneurines	33.9	28-40
Divergence of extant *Exoneurella*	26.5	21-32
Divergence of 'temperate' genera *Brevineura*, *Inquilina*, *Exoneura*	27.7	22-34
Divergence of *Inquilina*/*Exoneura*	16.6	13-20
Divergence of extant *Brevineura*	12.3	9-16
Divergence of extant *Exoneura*	8.5	6-11
First dispersal of *Exoneura* to western Australia	6.2	4-8
Second dispersal of *Exoneura* to Western Australia	1.6	0.7-2.5

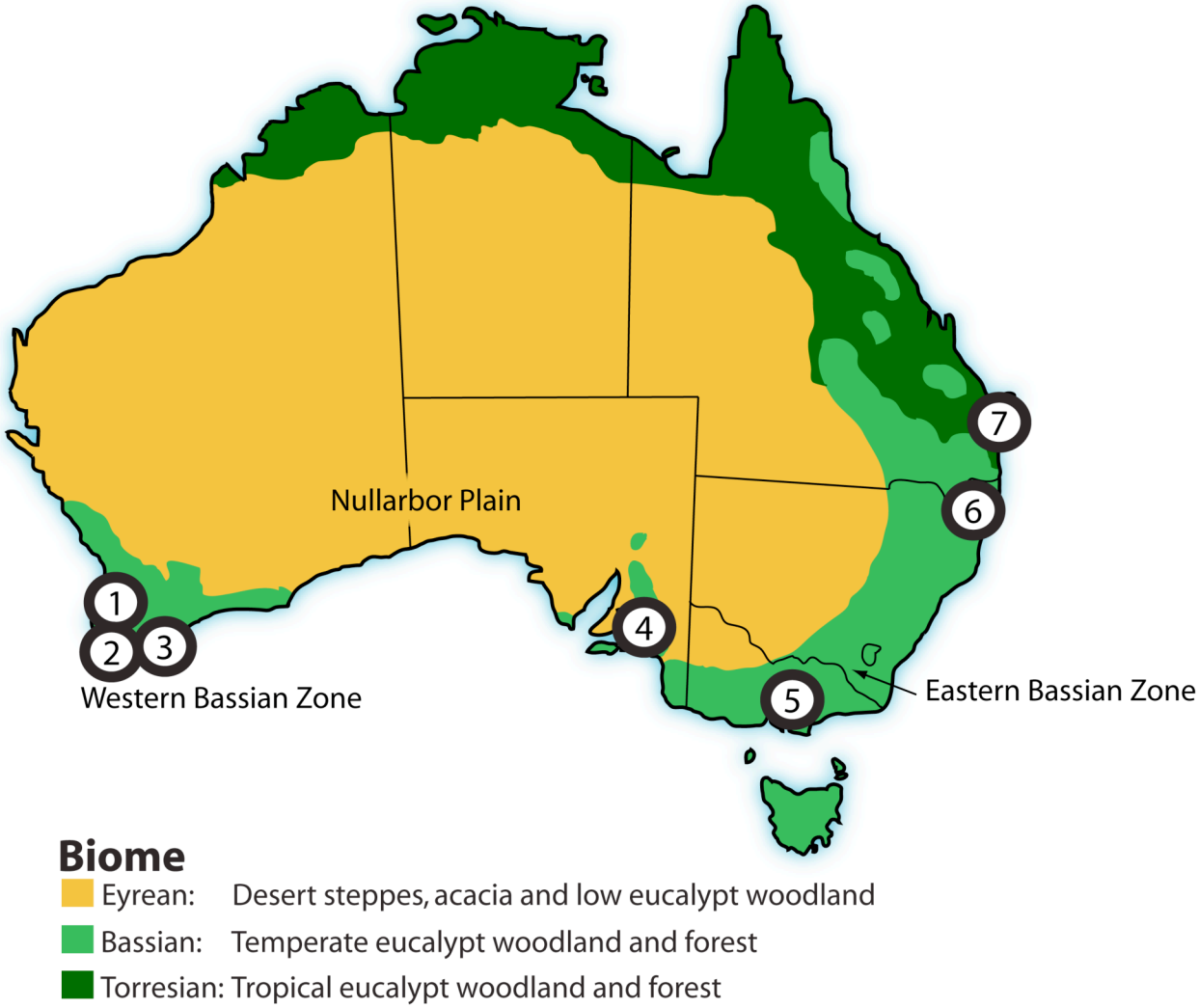


Figure 1 Map and brief descriptions of the biomes in present-day Australia, modified from Burbridge (1960) and Crisp *et al.* (2004). Collection localities of allodapine specimens newly sequenced for this study are numbered as follows: (1) Margaret River; (2) Augusta; (3) Denmark; (4) Scott Creek; (5) Dandenong Ranges; (6) Bellingen; (7) Noosa. Further details about each collection locality are provided in Table 1.

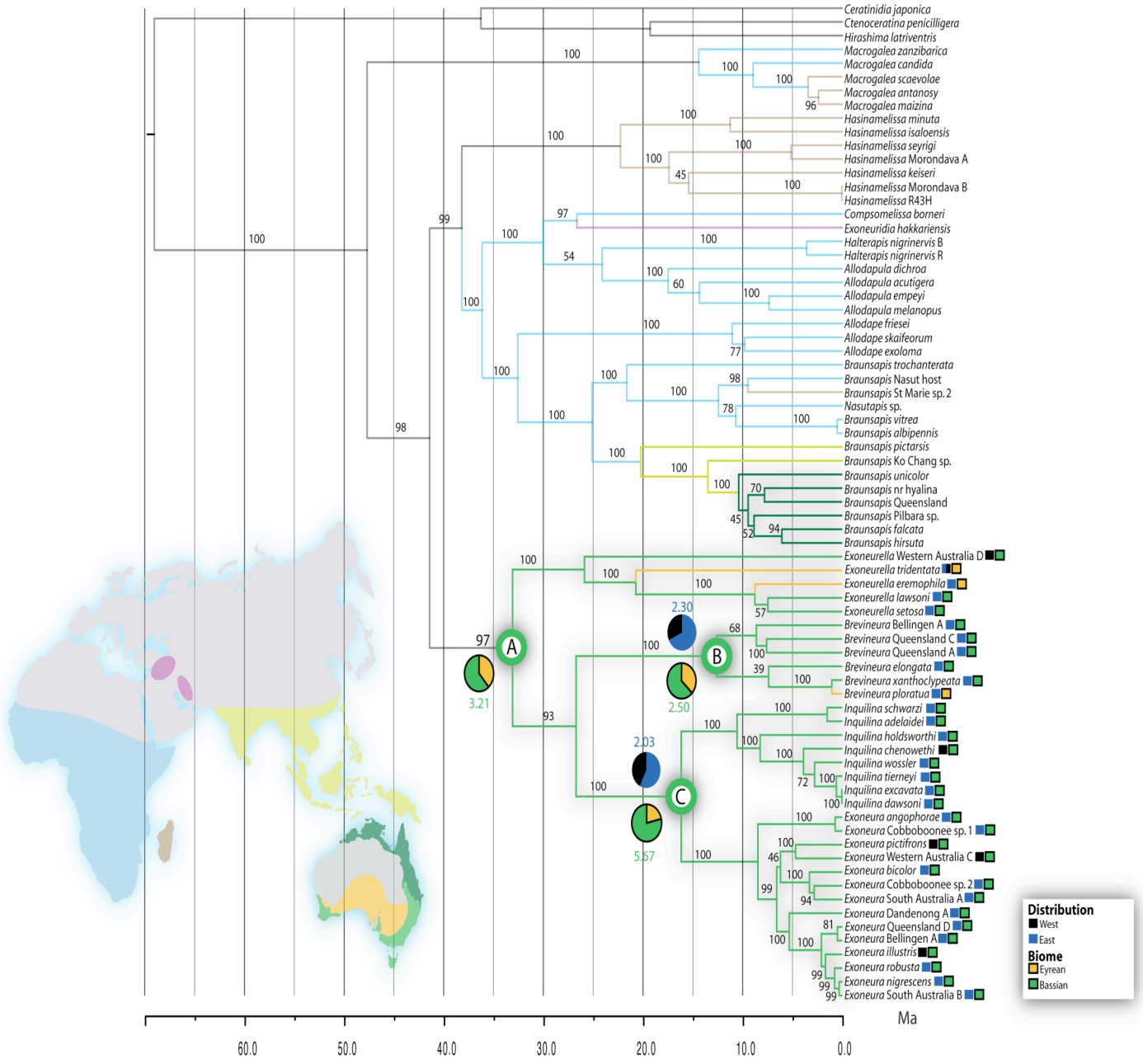


Figure 2 Chronogram of the allodapine bees derived from penalized likelihood transformation of the consensus Bayesian phylogram. Posterior probabilities are indicated for each node. Geographical distributions of each species are colour coded according to the map, and exoneurine taxa are also colour coded according to geographical and environmental distribution. Bayes MultiState analyses of ancestral geographical reconstructions, indicating the relative likelihoods for each ecoregion and east–west distribution, are indicated for nodes of interest as pie charts. Corresponding Bayes factor tests for validity of these analyses are shown where results were significant. These nodes represent the most recent common ancestor (MRCA) of the exoneurine clade as a whole (node A), and the exoneurine genera *Brevineura* and *Inquilina* + *Exoneura* (nodes B and C, respectively).

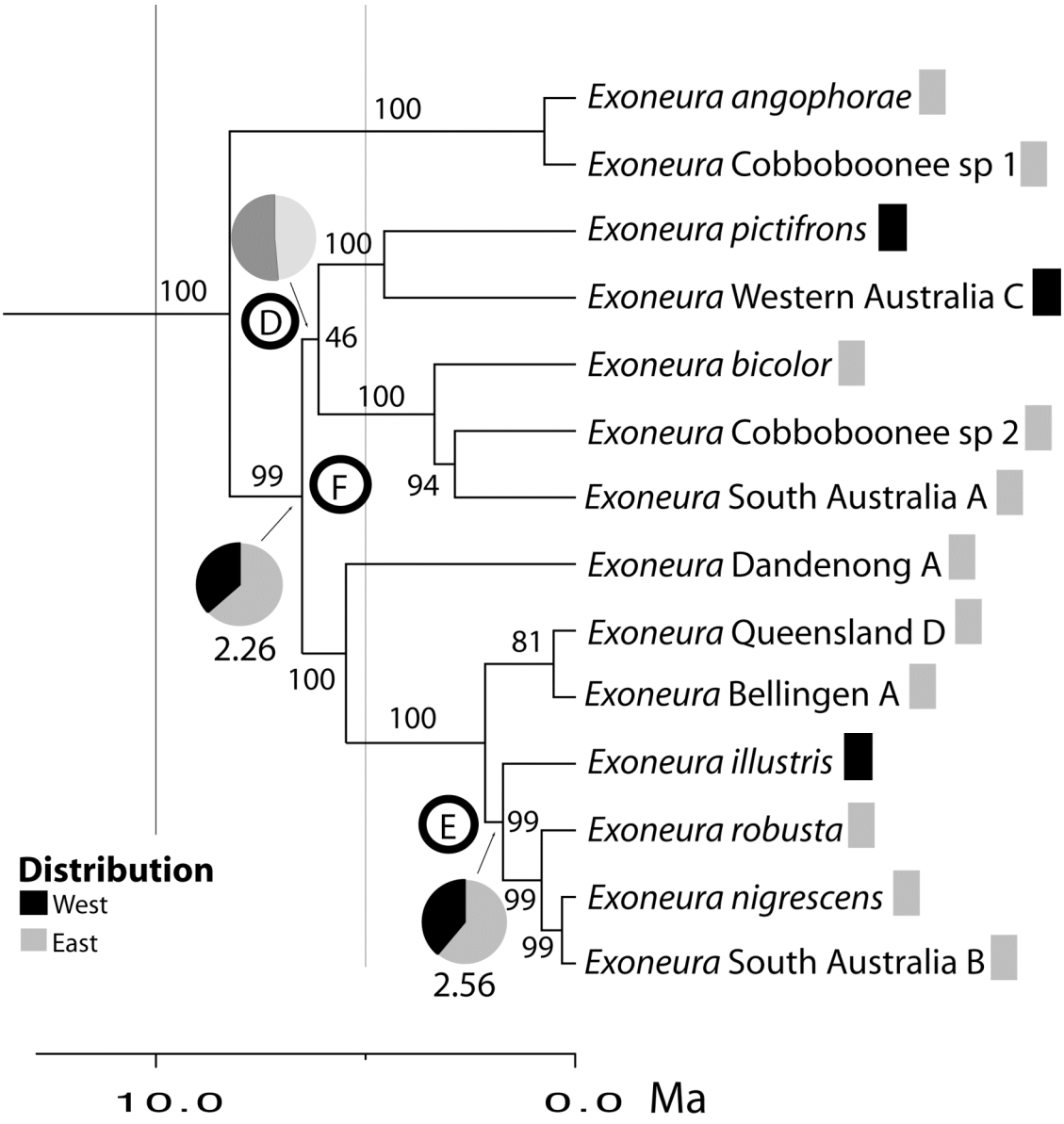


Figure 3 Chronogram of *Exoneura* species derived from penalized likelihood transformation of the consensus Bayesian phylogram with posterior probabilities provided for each node. Geographical distributions of each species are shaded according to the figure legend. Bayes MultiState analysis of ancestral geographical reconstructions for each East-West divergence between taxa (nodes D, E, and F) are indicated as pie charts indicating the relative likelihoods for east/west ancestral distribution, with corresponding Bayes factor test for validity at each node where results were significant.

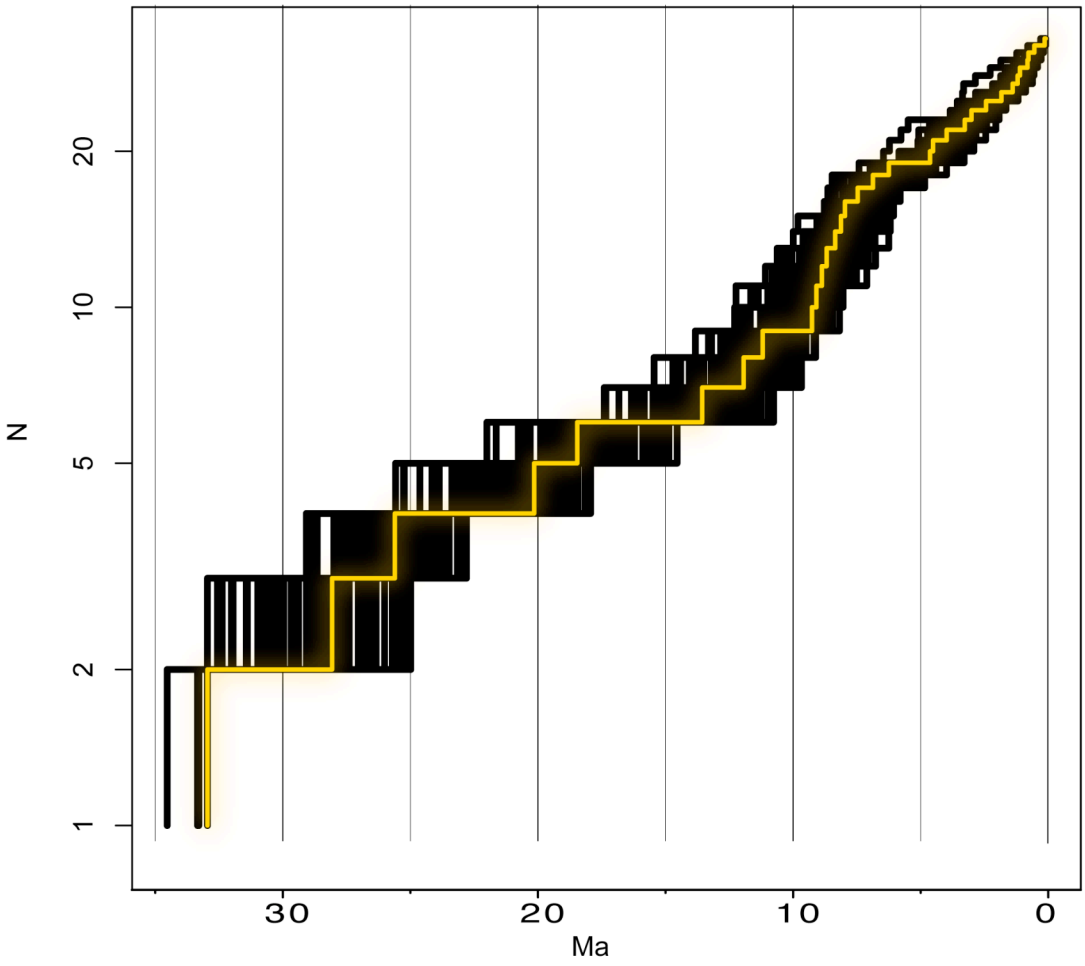


Figure 4 Lineage-through-time (LTT) plot of exoneurine cladogenesis over time, produced using the mltt.plot module in APE. Dark lines represent 100 randomly selected post-burn-in samples, with the LTT plot of our consensus chronogram superimposed as a light line.

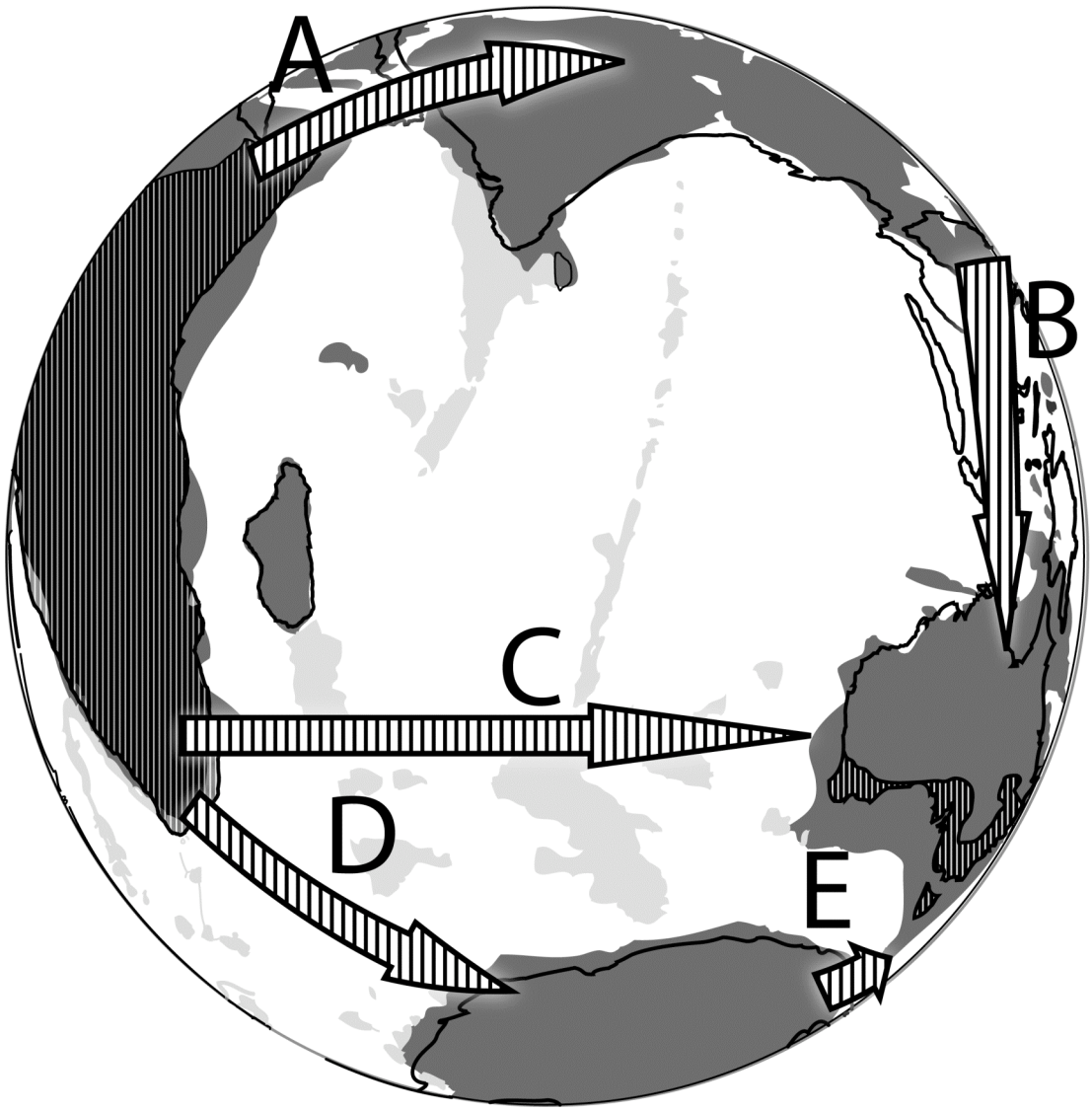


Figure 5 Orthographic azimuthal map centred on the Southern Indian Ocean at 33 Ma. The approximate extents of subaerial landmasses are indicated as dark regions on the map, with terranes, oceanic plateaus, and large igneous provinces (LIPs) indicated in lighter shading. These latter areas were not necessarily subaerial at 33 Ma. Current shorelines are projected onto the map as outlines. Striped areas indicate the present distribution of African and Australian 'exoneurine' allodapine taxa on their respective continents. The three potential dispersal routes of the exoneurine taxa into Australia are indicated as follows: (1) dispersal from African into southern Asia (A) followed by subsequent dispersal into northern Australia (B); (2) Direct dispersal over the Indian Ocean into western Australia (C); and (3) Dispersal into Antarctica (D) followed by subsequent dispersal into south-eastern Australia (E). Map reconstruction modified from Scotese (2002)

SUPPORTING INFORMATION

Biogeographical origins and diversification of the Australian allodapine bees (Hymenoptera, Apidae)

Luke B. Chenoweth and Michael P. Schwarz

Journal of Biogeography

Figure S1 Consensus cladogram of the allodapine bees. Posterior probability values are provided as percentages above nodes. The outgroup taxa pruned from our penalized likelihood analysis (Fig. S2, Supporting Information) are highlighted in red.

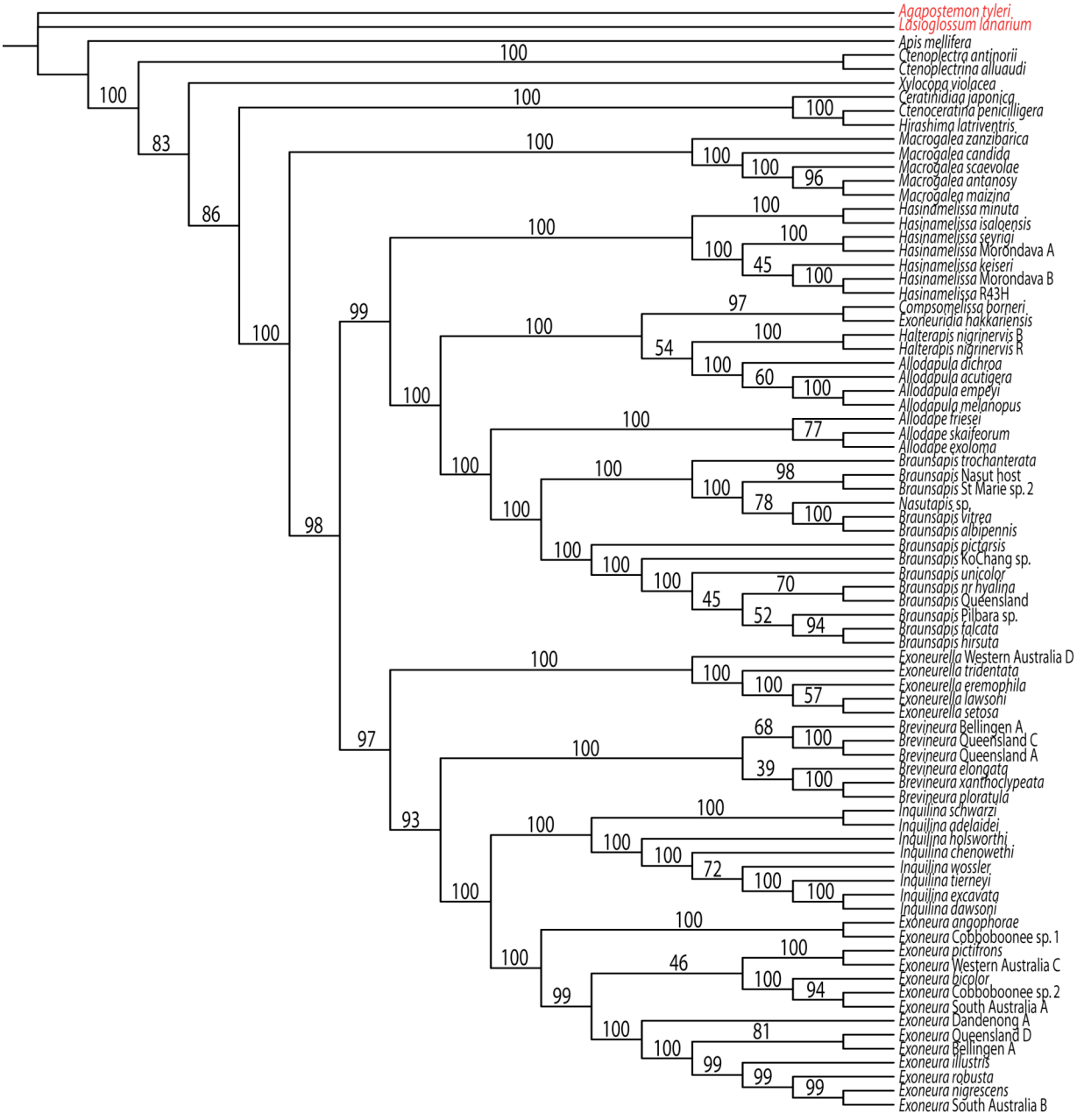
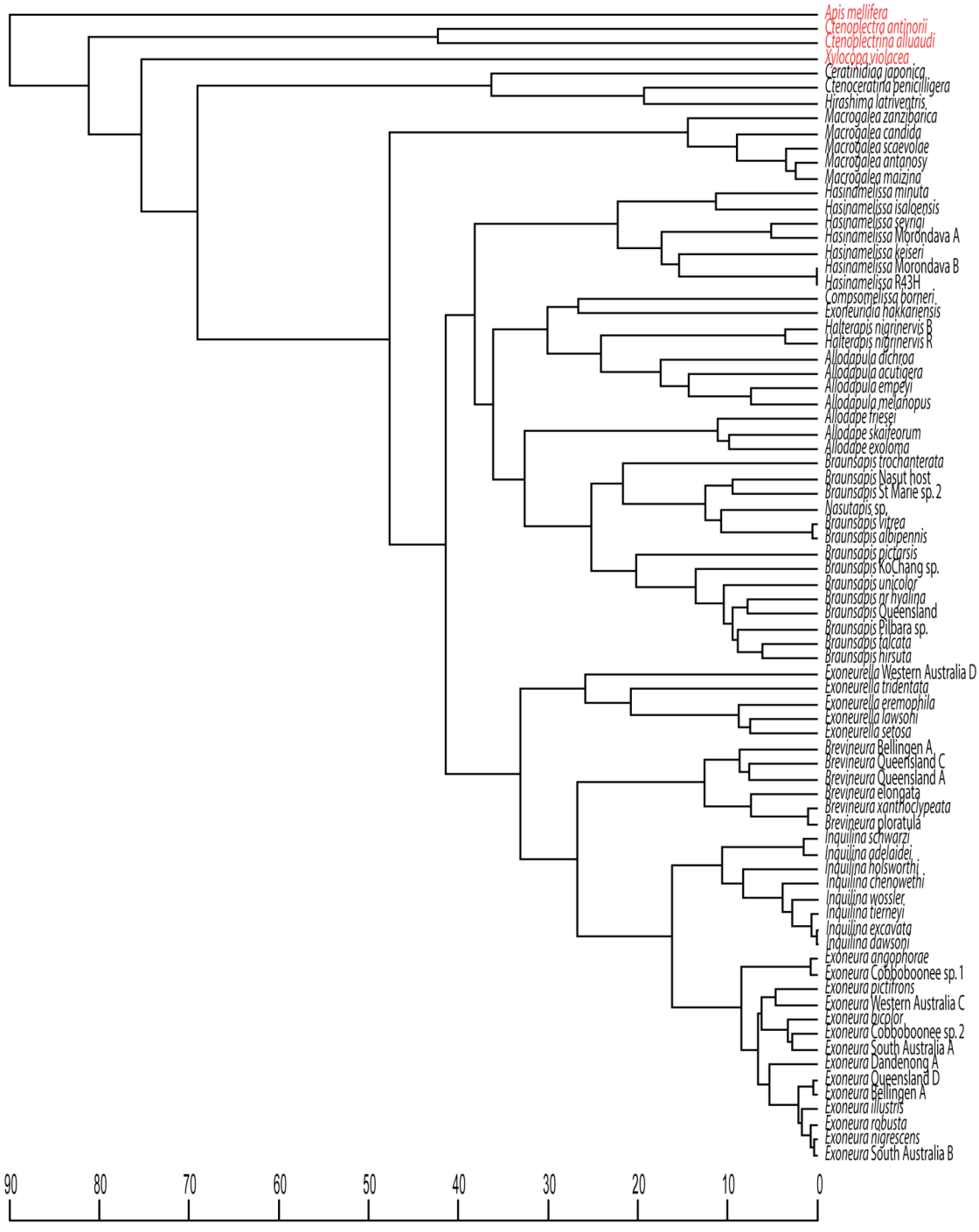


Figure S2 Consensus chronogram of the allodapine bees. Taxa pruned from the chronogram presented in the manuscript body (Fig. 2, Main Article) are highlighted in red.



CHAPTER IV

Biogeographical history of the African allodapine bees (Hymenoptera, Apidae)

ABSTRACT

The African allodapine bees constitute a widespread and diverse group of pollinators throughout Sub-Saharan Africa. Previous phylogenetic studies on this group have been restricted in the range of taxa have been sampled. Here we utilize an expanded taxa set, collected from a significantly broader portion of the African allodapines' range in order to explore the history of this bee tribe. Our results indicate a crown age for the African clade of between 40 and 53 Ma resulting in the divergence of two groups with very different behavioural and environmental traits. The African allodapines do not exhibit any evidence of ancient rapid radiations, but we show that the origin of a large group of temperate taxa amongst the otherwise tropical/subtropical genus *Allodape* corresponds with the aridification of the southern Africa. This aridification event was apart of a global-scale climate shift during the Miocene linked to the formation of the Antarctic Circumpolar Current (ACC). Moreover, this response to aridification mirrors that found in Australian allodapine groups during the ACC-induced aridification of Australia, suggesting that both Australian and African groups of an important bee faunal element responded similarly to a single global climactic event.

INTRODUCTION

For organisms that play pivotal roles in the function of terrestrial environments, understanding biogeographical history has the potential to provide important clues to the course of an entire ecosystem's development. Additionally, understanding how and why certain ecological constraints may restrict the biology or distribution of such organisms can have important ramifications, as a biome's taxonomic composition is linked to the frequency or rarity of species' transitions between neighbouring regions. Due to their key role as the largest group of angiosperm pollinators, bees comprise an integral part of almost every terrestrial environment on Earth. Understanding the history of diversification, speciation, and adaptation of bee taxa is therefore crucial in better understanding the ecological and evolutionary history of many ecosystems across the globe.

A recent study has shown that a key element of the endemic Australian bee fauna, the 'exoneurine' allodapine bees (tribe Allodapini), experienced a significant increase in speciation of temperate-adapted taxa that is temporally linked to the reduction and fragmentation of temperate habitat during the Middle Miocene aridification of Australia (Chenoweth and Schwarz, in press). The allodapine bees are an important group of pollinators throughout sub-Saharan Africa, Australia, Madagascar, and southern Asia. Only the derived genus *Braunsapis* spans the entirety of this range, and only the rare Middle-Eastern genus *Exoneuridia* occurs outside of this distribution. The tribe is presently thought to comprise four major clades. Schwarz et al. (2003) showed that the most basal allodapine clade consists of the African genus *Macrogalea*. The remaining non-*Macrogalea* taxa are divided into the endemic exoneurine and *Hasinamelissa* clades in Australia and Madagascar respectively, with *Exoneuridia*, *Braunsapis*, and the remaining African taxa constituting a fourth 'largely African' clade.

One of the primary factors implicated in the aridification of Australia has been attributed to changes in global ocean dynamics brought about by the separation of South America and Antarctica and the formation of the Antarctic Circumpolar Current (ACC). Another prominent ecological shift coincident with the formation of the ACC was the aridification of Southern Africa and the formation of the highly unique Cape Floristic Region in the area's south-west. Numerous studies have proposed a link between the aridification of this region and a local increase in the endemic diversification of numerous key floral and faunal elements (Richardson et al. 2009). A major aspect of this ecological shift was significant increases in angiosperm diversification and endemicity, and this may

be associated with dramatic shifts in pollinator regimes. (Johnson 1995). Sub-Saharan Africa constitutes the primary centre of diversity for the allodapines, containing 9 of the 15 allodapine genera currently recognized (Michener 1975; Terzo 1999; Eardley and Urban 2010). Whilst *Macrogalea* is a relatively depauperate African genus and is restricted to tropical and subtropical Africa and Madagascar, the range of the remaining African allodapines is far more extensive, encompassing the varied environments of Sub-Saharan Africa, Madagascar, and the Middle East (Figure 1).

Although the allodapines are relatively widespread across the southern Old World, the tribe displays surprising uniformity in many behavioural traits and nesting habits compared to other bee groups, including that found in their sister tribe the Ceratinini (Rehan et al. 2010). Comparing histories of the African and Australian allodapines may therefore provide insight into how two biologically similar yet geographically isolated pollinator groups have responded to a single, major climactic event. Moreover, the key role both groups play as pollinators means that both the African and exoneurine clades' responses to aridification have likely had significant repercussions on their ecosystems' subsequent development.

Despite the importance of exploring these relationships, very little has been inferred about either the nature of the African allodapines' ancestral ranges, or how these ranges may have changed over the course of the clade's history. This is because the phylogenetic and biogeographical relationships amongst the African allodapine taxa are perhaps the least understood amongst all allodapines. A major impediment to our understanding is due to previous studies' inability to provide strong support for some key generic and species-level bifurcations amongst the African taxa, making a clear resolution of topology difficult to obtain. Additionally, previous molecular phylogenies have traditionally relied solely on taxa from the southern regions of the continent, limiting the interpretation of broader biogeographical trends within the clade (Bull et al. 2003; Schwarz et al. 2004, 2006; Tierney 2004).

Here we present a phylogenetic analysis of the African allodapine taxa, using an expanded data set, in order to more accurately infer the phylogenetic associations within the clade and explore the history of this important group of pollinators and their relationship with the ecological history of the African continent.

METHODS

Taxa and collection localities

We used 56 species drawn from all African genera. Multiple representatives of all genera were used wherever possible, however only one species of the rare Middle Eastern genus *Exoneuridia* was included due to lack of additional taxa in our collections. Duplicate specimens, typically from nest mates, were incorporated whenever possible for initial analysis, however they were discarded for our primary analyses. In addition we included a total of 40 allodapine species from all other described genera in our analysis, to ensure that multiple representatives of all major clades were used whenever possible to reduce long-branch attraction when recovering basal nodes. The majority of species in our analyses have been used in previous studies (Bull et al. 2003; Schwarz et al. 2003, 2004, 2005 Tierney 2004; Fuller et al. 2005; Chenoweth et al. 2007, 2008; Smith 2009) and GenBank accession numbers are provided within these publications. Eleven additional allodapine taxa were collected from localities throughout the Kwa Zulu Natal region of South Africa, Lesotho, and Swaziland in southern Africa, and an additional twelve species from Kibale Forest National Park in Uganda, and regions near Tsavo West and Mount Elgon National Parks in Kenya, in eastern Africa. Collection localities of newly sequenced species are in Table 1. In addition to the allodapines, we included three species of Ceratinini, the sister tribe to the Allodapini: *Ceratina* (*Ceratinidia) japonica*, *Ceratina (Ctenoceratina) penicilligera*, and *Ceratina (Hirsashima) latriventris*). The presence of *Ceratina* in our analysis also provides a calibration point based on a Baltic amber fossil of the tribe Boreallodapini, which is the extinct sister group of the Allodapini (Engel 2000, 2001). To root the node connecting these two tribes we also included one species of Xylocopini (*Xylocopa violacea*), two ctenoplectrines (*Ctenoplectra antenorii* and *Ctenoplectrina alluaudi*), two halictines (*Agapostemon tyleri* and *Lasioglossum lanarium*) and one apine (*Apis mellifera*).

DNA sequencing methods

DNA was extracted from either leg or metasomal tissue of ethanol preserved specimens using Gentra Puregene Cell Kit (Quaigen) protocols. DNA pellets were subsequently resuspended in 50µl of TE buffer and stored frozen.

PCR reactions were carried out in 25µl reaction volumes containing 16.8µl of H_2O, 2.5µl taq polymerase buffer, 2.0µl dNTPs, 1µl forward primer, 1µl reverse primer, 0.2µl of

HotMaster taq DNA polymerase (with 25 mM Mg^{2+} included) with 1.5µl of diluted DNA per reaction. Amplification conditions included an initial hot start of 94°C for 9 minutes, typically followed by 35 cycles of denaturation at 94°C for 30 seconds; annealing at 54°C for 45 seconds, extension at 72°C for 1 minute; and then a final extension step of 72°C for 6 minutes. One nuclear and two mitochondrial gene regions were amplified and sequenced bi-directionally. The F2 copy of elongation factor 1α (EF-1α F2; 772 bp) comprised the nuclear region, and the mitochondrial regions were from protein coding genes cytochrome *b* (Cyt *b*; 428 bp sequenced) and cytochrome oxidase I (COI; 1279 bp). EF-1α occurs as two copies, EF-1α F1 and EF-1α F2, in bees, which are expressed at different stages of development (Danforth and 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 Danforth (pers. comm.) and the F2-specific reverse primer designed by Danforth and Ji (2001) for halictid bee species (EF1F2rev1: 5'-AAT CAG CAG CAC CTT TAG GTG G-3'). The primers used for PCR amplification of the Cyt *b* region were designed by Y. C. Crozier (Latrobe University, Melbourne, Australia, Schwarz et al. 2004): cb1: 5'-TAT GTA CTA CCA TGA GGA CAA ATA TC-3' and cb2: 5'-ATT ACA CCT CCT AAT TTA TTA GGA AT-3'. Two sets of primers were used to sequence the COI region. The first set were designed by Lunt et al. (1996) and comprised of 620 bp at the 3' end of COI: UEA7: 5'-TAC AGT TGG AAT AGA CGT TGA TAC-3' and UEA10: 5'-TCC AAT GCA CTA ATC TGC CAT ATT A-3'. The second set of primers were designed by Folmer et al. (1994), M414 (LC01490): 5'-CCT TTT ATA ATT GGA GGA TTT GG-3' and from bee sequences by S. Cooper (Schwarz et al. 2004) M399: 5'-TCA TCT AAA AAC TTT AAT TCC TG-3', providing a 759bp upstream of, and overlapping 91 bp of, the region amplified by the primers of Lunt et al. (1996). PCR products were then purified using Multiscreen PCR-384 Filter Plates (Millipore), and ~50 ng of product was sequenced in 10µl using the Big Dye Sequencing Ready Reaction kit Version 3.1 (Applied Biosystems), with the original PCR primers used as sequencing primers. Sequencing reaction products were then purified by Sequencing Filter plates (Millipore) and sequenced on a capillary DNA sequencer at the Australian Genome Research Institute (AGRF), Adelaide, Australia.

Phylogenetic methods

We used Bayesian Inference (BI) analyses to recover the phylogeny of our taxa. We adopted an 'objective' approach (Berger 2006) and used the MrBayes default priors as these are mostly uninformative (Huelsenbeck and Ronquist 2001), preventing the need for an *a priori* tree to derive substitution models for priors which may subsequently bias tree topology. We used a 6-parameter (Nst = 6) rate transition matrix, with gamma shape for variation in rates and a proportion of invariant sites assumed, corresponding to a GTR + I + Γ model. This is the least restrictive model available in MrBayes and allows more restrictive models, such as HKY and K2P, which are subsets of the GTR + I + Γ model, to arise if they provide a better fit to the data. All parameters were unlinked between partitions. Two sets of four Monte Carlo Markov Chains (MCMC) with Metropolis Coupling were run in parallel for each BI analysis and convergence was assessed by the average standard deviation of split frequencies, and stationarity was assessed by viewing LnL values using Tracer version 1.4.1. (Rambaut and Drummond 2007). The analysis was run for 15 million generations, sampling every 200th generation to reduce auto-correlation among sampled generations.

Dating analysis

We used penalised likelihood implemented in r8s 1.71 (Sanderson 2002) to transform phylograms obtained from MrBayes into chronograms. We first used cross validation to estimate an appropriate smoothing value. There are no internal calibration points for allodapine phylogenies. However, the age of the most recent common ancestor (MRCA) for the allodapines and their extant sister tribe Ceratinini has been estimated in several studies using calibration points for deeper nodes. Ages of this node range from 76 Ma (Schwarz et al. 2006), 73 Ma (Cardinal et al. 2010) and 58 Ma (Fuller et al. 2005). One calibration point is based on the existence of fossil Boreallodapini species from 45 million year old Baltic amber. Boreallodapini is the extinct sister tribe of the Allodapini, with the Ceratinini situated as the next-most basal tribe (Engel 2001) and as such suggests a highly conservative minimum age for the divergence of the Allodapini and Ceratinini. Lastly, we set a fixed age of 90 Ma for the node connecting the xylocopine tribes to the corbiculate apines. Fossils of the plant family Clusiaceae, whose floral morphology is closely linked to pollination by corbiculate bees, are dated to 90 Ma (Crepet and Nixon 1998). This node age is also likely to be conservative, so we followed Chenoweth et al. (2007) by exploring

the effects of setting this node to 100, 110 and 120 Ma. However, both Danforth et al. (2004) and Cardinal et al. (2010) have dated the crown age of the Halictidae at approximately 120 Ma, and this family is much more derived than the Apidae, again suggesting that setting the root node at 90 Ma is conservative.

Exploring diversification rates

We used a combination of LTT plots, gamma values, and diversification models to explore diversification rates within the exoneurine clade. Because the consensus phylogram from our Bayesian analysis had low PP support for some nodes of interest (see Results), we generated a LTT plot for the consensus chronogram as well as for 2000 randomly chosen post-burnin chronograms. We used the mltt.plot module in APE (Paradis 2004) to generate 2000 LTT plots for the post-burnin samples and superimposed the LTT plot for the consensus chronogram onto these. In addition, we used the gamma statistic (γ, Pybus and Harvey 2000) as a numerical means to quantify changing rates of diversification over time.

Using LTT plots to infer changes in speciation/extinction can be problematic when taxon sampling is incomplete and uneven across genera. Incomplete and uneven taxon sampling will also tend to produce gamma values that will suggest higher rates of cladogenesis closer to the root (Pybus and Harvey 2000). Our included taxa represent only 47 of the approximately 108 African allodapines (Eardley and Urban 2010), and the number of recorded species is likely to be less than the actual number (Eardley et al. 2009). Moreover, any single-tree topology may not accurately indicate the branching order of some of the nodes, and if unreliability of nodes varies with time since the root, any single estimate of γ may be biased. In order to address these problems, we randomly selected 2000 trees from our post-burnin iterations and subjected them to r8s transformations using the same procedure as for the consensus phylogram. We then used PAUP* (Swofford 2002) to prune all non-African taxa and used the mccrTest module in Laser 2.2 (Rabosky 2009) to calculate γ values. These γ values were then compared to those obtained from 2000 simulated trees. Simulated trees were generated in Geiger 1.3-1 (Harmon et al. 2008) by using the birthdeath.tree module to produce phylogenetic trees under a uniform birth/death process ending with 108 tip species, which were then randomly pruned down to 47 tip species using the prune.random.taxa module.

As an additional means to explore changes in diversification in a way that can accommodate uneven and incomplete taxon sampling, we employed the stepwise Aikake information criterion (AIC) approach in the MEDUSA procedure in LASER (Alfaro et al. 2009). We constructed a pruned version of our chronogram and a richness matrix assigning all described exoneurine taxa not present in our analysis into tip clades. All African species in our analysis could be firmly assigned to one of the seven African genera, and we used these to estimate stem ages for each genus and then constructed a matrix containing richness data for all described species within each genus.

RESULTS

Phylogenetic analysis

Three separate Bayesian analyses converged on almost identical consensus topologies and highly similar branch lengths. We used results from the first run in subsequent analyses. Comparing the standard deviations of split frequencies with plots of LnL values showed that the parallel runs converged after about two million generations. We chose a conservative burnin of 3.8 million or approximately 25% of the total number of generations.

Posterior probabilities from the BI consensus tree are shown Figure 2. Eight nodes in the tree had support ≤ 50%. Three involved bifurcations amongst species of the Australian exoneurine genera *Exoneura* and *Brevineura*. Another poorly supported node involved bifurcations among some species of the Malagasy genus *Hasinamelissa*. Poor support for these nodes has been reported in previous studies (Chenoweth et al. 2008; Chenoweth and Schwarz in press) and do not affect our analyses of diversification among African taxa. The remaining four poorly supported nodes involve bifurcations within the African clade and these are discussed in detail below.

Consistent with previous studies, the monophyly of *Macrogalea* was highly supported (100 PP), as was its position as sister clade to the remainder of the tribe (Schwarz et al. 2003, 2006; Chenoweth et al. 2007). We also found strong (100 PP) support for monophyly of the Malagasy *Macrogalea* nested within the African lineages. The remaining taxa were strongly recovered as comprising an Australian exoneurine clade (100 PP), the Malagasy genus *Hasinamelissa* (100 PP), and a largely African clade (96 PP) that also contained the Middle Eastern genus *Exoneuridia* and Asian and Australian

members of *Braunsapis*. The Australian exoneurines were strongly supported as sister group to the African + Malagasy clades (99 PP). Within the African clade, there was strong (100 PP) support for the monophyly of four of the five non-parasitic genera within the African clade with the monophyly of *Allodape* receiving only moderate support (80 PP). There was strong (100 PP) support for the formation of two monophyletic groups of genera within the African clade; one consisting of the tropical/temperate genera *Braunsapis* + *Allodape*, and a second consisting of *Exoneuridia* and the genera *Compsomelissa*, *Halterapis*, and *Allodapula*.

Within *Braunsapis*, taxa were basally divided into two geographically distinct clades. The first consists of the African taxa, which also contains two distinct dispersals into Madagascar; one resulting in *B. madecassa*, and a second giving rise to three unidentified species in our analysis (*B.* StMarie sp. 2, *B.* Toliara sp., and *B.* Taolagnaro sp.). The second group of *Braunsapis* consists of Oriental + Australian taxa, with the African species *B. trochanterata* placed basally but with vey low support (48 PP) for this. There was strong support (100 PP) for the monophyly of the Oriental + Australian group and also strong support (99 PP) for monophyly of the Australian *Braunsapis* nested within the Oriental species.

The topology of *Allodape* was well supported in a majority of nodes, however three nodes received poor (<50 PP) support. Despite these nodes, those with strong support point to the monophyly of the southern temperate lineages (99 PP), with moderate (82 PP) indication that this group is nested within the tropical/subtropical *Allodape* lineages of eastern Africa (Figure 3).

There was strong (100 PP) support for the monophyly of the clade comprising the genera *Exoneuridia*, *Allodapula*, *Halterapis* and *Compsomelissa*, however bifurcation order among these genera received relatively weak support in our analyses.

Molecular dating

We used penalized likelihood (PL) transformation of the Bayesian consensus phylogram to produce the chronogram shown in Figure 2. The PL estimates for the ages of various nodes are given in Table 2 along with 95% confidence intervals for each estimate. Varying the 'fixed' age of the root node joining the Xylocopinae and the corbiculates from 90 to 120 Ma resulted in the estimated ages of internal nodes increasing in a proportionately linear manner, so that the crown age of the exoneurines increased to 44 Ma. This has been found in previous studies in the allodapine phylogenetics (Chenoweth et

al. 2007) and is probably because the estimated ages for our minimum-age Baltic calibration point was much older than the set minimums, so that the fixed age of the root node had the strongest effect on scaling the tree. We therefore discuss each node of interest below as a range between a Xylocopinae + corbiculate root node of 90 Ma and 120 Ma with 95% confidence intervals (CIs) provided for each date in parentheses.

Our analysis indicated a divergence between the Allodapini and the Ceratinini at approximately 70 (±8.8) – 94 (±11.2) Ma, and a crown age for the extant allodapine lineages at about 50 (±7.2) – 66 (±8.7) Ma. Our analysis indicates a relatively recent origin of 15 (±5.4) – 20 (±4.9) Ma for the crown age of the basal allodapine genus *Macrogalea*, with subsequent colonization of Madagascar approximately 3 (±2.0) - 4.3 (±2) Ma. The divergence between the primary non-*Macrogalea* clades appears relatively rapid; divergence of the African + Malagasy lineage from the Australian 'exoneurine' lineage is dated at approximately 44 (±5.1) – 58 (±6.8) Ma, with divergence between the African clade and the Malagasy genus of *Hasinamelissa* at approximately 41 (±5) – 54 (±6.7) Ma. The crown age of the exoneurines is dated at 35 (±6.4) – 46 (±7.9) Ma, and the extant *Hasinamelissa* appear to have radiated approximately 23 (±4) – 30 (±4.9) Ma.

The crown age of the African clade is older than that of both the exoneurines and *Hasinamelissa*, with an origin of approximately 40 (±5) – 53 (±6.7) Ma. Within the *Exoneuridia* + *Allodapula* group, the split between the African *Halterapis* + *Compsomelissa* + *Allodapula* clade and the Middle-Eastern *Exoneuridia* appears to have occurred relatively early in the tribe's history, 33 (±7.5) – 43 (±10) Ma). The crown age of *Braunsapis*, involving divergence between the African and Oriental clades, occurred approximately 28 (±4) – 36 (±5.3) Ma, with radiation of Oriental taxa beginning around 22 (±4) – 29 (±5) Ma and radiation of the Australian taxa beginning around 10 (±2.5) – 14 (±3.2) Ma. The dispersal of *Braunsapis* into Madagascar giving rise to a single described species, *B. madecassa*, is derived from a divergence from the African taxa no earlier than 20 (±3.2) – 25 (±4.2) Ma. The second crown group age resulting in *B.* StMarie sp2, *B.* Toliara sp, and *B.* Taolagnaro sp appears more recent at around 4 (±1.3) – 5.1 (±1.7) Ma. The crown age of *Allodape* is approximately 31 (±4.4) – 40 (±5.8) Ma, with the crown age of the temperate clade 15 (±2.6) – 19 (±3.3) Ma.

Diversification rates over time

Our semi-log LTT plot for the consensus chronogram of the African clade was very similar in pattern to the (100) post-burnin trees chosen at random from our Bayesian analysis (Figure 4) Diversification rates appear linear, suggesting that the rate of cladogenesis has been relatively uniform throughout the history of the African taxa. The simulated γ values, when random trees containing 108 tips are randomly pruned to 47, are given in Figure 5 along with the observed gamma values from 200 randomly chosen post-burnin trees. It is not statistically possible to compare these distributions, as our observed post-burnin trees represent statistically generated permutations rather than actual sampling and the arbitrary nature of the number of trees generated in such analyses could potentially lead to erroneous indications of significance. However, it is evident that the observed γ values cannot be explained purely in terms of taxon under-sampling.

Our stepwise AIC analysis provides a statistical means to assess changes in diversity over time. We find largely uniform rates of diversification amongst the African lineages, with a relatively low but positive speciation-extinction rate (B-D = 0.086) and an extremely low birth/death ratio of 4.64×10^{-7}. These three analyses combined indicate that the African taxa have experienced a relatively uniform rate of diversification throughout their history.

DISCUSSION

An African origin for the extant allodapines is well established (Bull et al. 2003; Schwarz et al. 2003, 2006; Chenoweth and Schwarz 2011), however the history of diversification within the African taxa is poorly understood. This has primarily been due to previous studies lacking strong support for many important generic and species-level bifurcations amongst the African taxa. Additionally, these molecular phylogenies have traditionally relied on taxa from the southern regions of the continent, limiting the interpretation of broader biogeographical trends within the clade (Bull et al. 2003; Schwarz et al. 2004, 2006). Whilst the taxa used in our study do not encompass the entire range of the African allodapines, and the West African allodapine fauna are unrepresented, this is the first molecular phylogeny to incorporate a significant proportion of taxa from the tropical/subtropical north-eastern margins of the allodapines' African distribution. The incorporation of these lineages allows us to explore for the first time both the historical

relationships between taxa from two very different environmental regions within Africa, and also the species-level relationships within the genera that span both ranges.

Phylogeny of the African allodapine bees

Our findings support previous phylogenetic studies in grouping the non-*Macrogalea* allodapine taxa into three discrete clades: an Australian 'exoneurine' clade, a Malagasy '*Hasinamelissa*' clade, and an 'African' clade which also contains derivative clades found in Madagascar, Asia and Australia. Our results indicate that this African clade is composed of two well-supported monophyletic groups; an *Allodapula* group, composed of *Exoneuridia*, *Halterapis*, *Allodapula*, *Compsomelissa*, and the parasitic genus *Eucondylops*, and a *Braunsapis* group comprised of *Braunsapis*, *Allodape*, and the parasitic genus *Nasutapis*. Difficulties in resolving bifurcation orders among clades in the *Allodapula* group were reported in prior studies (Schwarz et al. 2006; Chenoweth et al. 2007), and could be due to either rapid radiations within this group or long-branch attraction. Despite the inclusion of a number of additional *Allodapula*-group species in our analyses compared to previous studies, generic-level bifurcations in this group remain unresolved and understanding the reasons for this may require a still-larger sequence data set. At the same time, lack of resolution for this group, when other major allodapine clades have been resolved using the same genetic data, suggests the existence of rapid early radiation in this clade.

We found strong support for the monophyly of both *Braunsapis* and *Allodape*, consistent with previous studies, where a sister relationship between these two genera is indicated by both morphological and molecular data (Michener 1975; Schwarz et al. 2004; Fuller et al. 2005).

Age and diversification of the African taxa

Earlier studies on allodapines have suggested an African origin, but did not explore whether this origin may have had a particular source region within Africa. Whether or not African allodapines are ultimately derived from a more temperate or more tropical habitat is important if we are to understand factors affecting their diversification over time. For example, a tropical origin may lead to very different kinds of diversification in the face of changing Eocene and Miocene climates than a temperate origin.

The African clade encompasses a wide range of diverse habitats (Figure 1), and very similar biological and ecological characteristics exist within, but not between, the two

groups in the African clade. Within the *Allodapula* group, *Exoneuridia* is restricted to montane and highly seasonal regions of Iran, the Middle East, and southern Turkey (Terzo 1999), and *Halterapis* and *Allodapula* are recorded as wholly temperate in distribution (Michener 1975; Eardley and Urban 2010). Although the range of *Compsomelissa* encompasses a significant portion of tropical Africa, they are largely restricted to the dry pockets of savannah in these regions (Michener 1975; Chenoweth and Schwarz pers. obs.). The limited representation of wet tropical taxa in this group suggests that adaptations to temperate habitats likely occurred early, or are possibly relictual, to this group.

Both *Braunsapis* and *Allodape* are far more widespread and speciose than any of the *Allodapula* group, and both genera have ranges that span both the tropical/subtropical north of sub-Saharan Africa as well as the temperate and Mediterranean climates of southern Africa and the Cape Floristic Region. Within *Braunsapis*, the placement of *B. trochanterata* was poorly supported in our analyses and previous studies noted difficulty in resolving this species' phylogenetic position, placing it as either basal to the Oriental + Australian *Braunsapis* (eg. Fuller et al. 2005) or basal to all *Braunsapis* (Schwarz et al. 2004). B. *trochanterata* displays many morphological characteristics in common with *Allodape*, and its basal placement within *Braunsapis* supports a close relationship between the more basal *Braunsapis* and *Allodape* lineages (Michener 1975), as well as evidence for an early monophyletic derivation of the Oriental/Australian *Braunsapis* from African ancestors. The vast majority of the African *Braunsapis* taxa, and all of the Oriental and nearly all of the Australian *Braunsapis*, are tropical or subtropical in distribution. Morphological intergradation with *Allodape* is present in a number of *Braunsapis* taxa, including *B. trochanterata*, *B. calidula, aureoscopa, lyrata,* and *somatotheca*, all of which are tropical northern species (Michener 1975).

Allodape appears to be near-equal in speciosity in both the northern and southern regions of Africa, and a clear delineation exists between the ranges of northern and southern *Allodape* species (Michener 1975; Eardley and Urban 2010). The inclusion of Ugandan and Kenyan *Allodape* species in our study has allowed the exploration of the relationships between these northern and southern groups using molecular techniques. Strong support for the monophyly of *Allodape* was found, with the southern *Allodape* lineages radiating from a single origin nested within older northern lineages. The distribution of *Allodape* species in our analyses indicates existence of a largely temperate clade nested with a tropical/subtropical clade, and given the largely tropical distribution of *Braunsapis*, this suggests that the *Braunsapis* + *Allodape* clade is tropical in origin.

A tropical origin for the *Braunsapis* group and a temperate origin for the *Allodapula* group suggests a period of diversification into tropical and temperate habitats early in the formation of the African clade, however this provides little insight into the ancestral environment of the African clade *per se*, or the tribe as a whole. Knowing whether African allodapines had a tropical or temperate origin is important for understanding what factors have influenced their radiation over the last 50 million years. We argue here that there are strong reasons to believe that the ancestral allodapine lineage was tropical and we now outline these:

1. The genus *Macrogalea* is sister clade to all other extant allodapines and does not contain any temperate species. Species diversity in *Macrogalea* is greatest in East Africa (Malawi to Kenya) with a secondary centre of abundance in northern Madagascar arising from a single colonisation event from Africa (Tierney 2004; Tierney et al. 2008).
2. The sister tribe to the Allodapini is the extinct fossil tribe Boreallodapini from Baltic amber dated at 45.1 Ma. The Baltic amber fossils are from a period when the Baltic region was tropical and the bee fossils contain a large number of meliponine species, and the Meliponini is today strictly tropical and subtropical across both eastern and western hemispheres.
3. Of the Afro-Malagasy allodapine clades, the most basal split is between the Malagasy *Hasinamelissa* clade and another clade comprising the *Allodapula* group and the *Braunsapis* group. *Hasinamelissa* is restricted to Madagascar and is therefore primarily tropical and subtropical in distribution. *Braunsapis* has its greatest diversity in tropical Africa, and our results indicate that the temperate clade of *Allodape* is nested within a tropical/subtropical *Allodape* clade.

A tropical origin for the allodapines has important implications for our understanding of how climate has shaped allodapine diversification across three continents. A tropical origin leaves only two allodapine clades that have largely temperate distributions; the Australian exoneurines and the African *Allodapula* group. The Australian exoneurines diverged from the African taxa very early in the tribes' history, about 44-58 Ma, and their diversification is strongly linked to aridification of Australia since the Miocene, probably due to contractions in the extent of temperate vegetation some 10-6 Ma (Chenoweth et al. in press). Diversification of the African *Braunsapis* genus has involved dispersals into southern Asia and then northern Australia, but in these cases, the

appearance of dispersal corridors into new continental regions seems to have been the driver of speciation rather than movement into new climate zones (Fuller et al. 2005).

Among the African allodapines, our molecular clock analysis suggests that the derivation of the temperate *Allodape* lineage occurred between 19 and 15 Ma. This timeframe corresponds with the formation of the Benguela arm of the Antarctic Circumpolar Current (ACC), an event believed to have played an important role in the aridification of the then-tropical southern regions of Africa into the temperate/Mediterranean environments of today (Richardson et al. 2001). Like the diversification of temperate exoneurines, the origin of the temperate *Allodape* is thus associated with aridification events resulting from the formation of the ACC. The African and Australian clades are of similar age, both groups show a similar history of early diversification into generic clades, but neither shows evidence of early rapid radiations. On the other hand, both temperate *Allodape* and exoneurine lineages show evidence of increased radiations of temperate-adapted taxa that correspond to changing climate in the Miocene.

Climate change and bee diversification

Our findings support an origin and diversification of the earliest allodapine clades during the Early Eocene, as well as increased diversification of temperate African lineages during the Middle Miocene that mirrors the diversification found in temperate Australian lineages at this time (Chenoweth and Schwarz 2011). An Early Eocene origin for the allodapines is coincident with the African origin of the allodapine's extant sister tribe, the Ceratinini (Rehan et al. 2010), as well as a period of increased radiation of Afrotropical meliponine lineages (Rasmussen and Cameron 2010). This points to a temporally distinct period of increased diversification amongst three major African bee lineages along the Paleocene/Eocene boundary around 50-60 Ma. The reasons why a sudden and major diversification in bee lineages would occur at this time have not been explored, but the increased diversification observed in the allodapines during the Middle Miocene may provide a possible explanation. The ACC-induced aridification of Africa and Australia coincides with the increased diversification of allodapine lineages that inhabit seasonal, temperate environments. Similarly, the diversification of allodapine, ceratinine and African meliponine bee lineages in the Early Eocene coincides with the end of Early Eocene Climatic Optimum (EECO) that occurred at around 50-52 Ma and which was the culmination of the most pronounced warming trend since the Cretaceous (Zachos et al.

2001). This event and the subsequent period of global cooling over the remaining Eocene has been linked to the encroachment of more seasonal climates, previously restricted to high latitudes, on formerly aseasonal middle and lower latitudes (Archibald et al. 2010).

A coincidence between bee diversification and significant climatic changes in the Early Eocene and then Middle Miocene may have important implications for understanding how and why modern bee diversity has arisen. The bee tribe Ctenoplectrini, which is also a part of the bee family Apidae like allodapines, ceratinines and meliponines, has an African origin in the Early Eocene, followed by multiple Eocene dispersals into Asia (Schaeffer and Renner 2008). The Ctenoplectrini is a very depauperate tribe compared to the Allodapini, Ceratinini, and Meliponini, containing only 20 species in two genera across Africa, Asia and Australia (Schaeffer and Renner 2008; Sung et al. 2009). Subsequent to their Early Eocene origin, their diversification in the Middle Miocene is far smaller than that of allodapines and ceratinines and involved the division of a single ancestral lineage into West African and East/South African daughter lineages that remained wholly tropical in distribution. The reason for this very low diversification is intriguing. Like the ctenoplectrines, meliponine bees are also restricted to tropical and subtropical habitats, suggesting that an occupation of warm tropical environments is not sufficient to explain patterns of diversification during the Miocene. One important trait distinguishing the ctenoplectrines from the allodapines, ceratinines, and meliponines is their oligolectic foraging behaviour (Michener 2007; Sung et al. 2009). We suggest that changes to flowering phenology associated with a transition from aseasonal to seasonal climates may have favoured the diversification of polylectic lineages over oligolectic lineages.

Polylectic bees are not limited to a small subset of host plant species and, we propose, would be more resilient to the development of seasonal patterns in flowering phenology due to their capacity to switch between different plants as flowering activity changed between seasons. As such, the diversification of allodapines into temperate ecosystems during the Miocene climate shifts may have been enabled by their ability to exploit a wide range of angiosperms and be consequently buffered from the effects of flowering seasonality in any particular host plant. It is interesting to note that another group of oligolectic bees, Melittidae *sensu lato*, is also depauperate in Africa, comprising a mere 66 species across the Dasypodainae, Meganomiinae and Melittinae (Eardley and urban 2010), despite this group comprising one of the oldest extant bee lineages (Danforth et al. 2006). The wealth of melittid specimens recorded from Baltic amber suggests that the

group was diverse by the Early Eocene (Engel 2001) suggesting that little, if any, increase in melittid diversification has occurred over the last 40-45 My. Oligolecty appears to be an ancestral trait for bees (Danforth et al. 2006), and the present-day pervasiveness of more generalist foraging strategies in bees raises the question as to whether or not oligolecty has entailed a lower capacity to adapt to increasing seasonality since the EECO. Climate-induced changes in the diversification of taxa that play key functional roles in ecosystem function could have far-reaching implications on the subsequent course of an ecosystem's development. Our study suggests that large-scale climactic events have had significant, and similar, influences on the diversification of the very disjunct bee faunas of Australia and Africa. Understanding how ecosystems respond to significant periods of climate change therefore requires that we consider the extensive effects that large-scale climatic shifts can have across multiple biomes.

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Table 1 List of species sequenced for this study along with their collection location.

Genus	Species	Collection location
Allodape	*interrupta*	Uganda: 00° 34′ N, 30° 22′ E; 1586m.
Allodape	*chapini*	Kenya: 00° 39′ N, 34° 20′ E; 1248m.
Allodape	*collaris*	Uganda: 00° 35′ N, 30° 22′ E; 1500m.
Allodape	*nula*	Uganda: 00° 34′ N, 30° 22′ E; 1493m.
Allodape	Uganda sp.	Uganda: 00° 34′ N, 30° 22′ E; 1485m.
Allodape	*laeviceps*	Uganda: 00° 34′ N, 30° 22′ E; 1485m.
Allodape	nr derufata	Uganda: 00° 33′ N, 30° 22′ E; 1493m.
Allodape	*mea*	Kenya: --
Allodape	*derufata*	Uganda: 00° 33′ N 30 22′ E; 1533m.
Allodape	*pernix* B	South Africa: 27° 32′ S 32° 41′ E; 58m.
Allodape	nr ceratinoides B	South Africa: 30° 40′ S 30° 29′ E; 74m.
Allodape	*pernix* R	South Africa: 30° 40′ S 30° 29′ E; 74m.
Allodape	nr ceratinoides A	South Africa: 30° 43′ S, 29° 56′ E; 663m.
Allodape	*rufogastra* (Natal variant)	South Africa: 29° 46′ S 29° 30′ E; 1579m.
Allodape	nr exoloma A	Lesotho: 28° 54′ S 28° 26′ E; 1940m.
Allodape	nr exoloma B	Lesotho: 28° 54′ S 28° 26′ E; 1940m.
Allodapula	nr dichroa B	Lesotho: 28° 54′ S, 28° 26′ E; 1940m.
Allodapula	nr variegata	South Africa: 31° 37′ S, 29° 26′ E; 9m
Compsomelissa	*stigmoides*	Kenya: 00° 33′ N, 38° 32′ E; 610m.
Braunsapis	Kenya sp. 2	Kenya: 00° 33′ N, 34° 27′ E; 1205m.

Table 2 Age estimates for key clades using penalized likelihood (r8s) analysis, with the root node joining the xylocopine tribes to the corbiculate apines set at both 90 Ma and 120 Ma.

	Root node set at 90 Ma		Root node set at 120 Ma	
	Mean	95% CI	Mean	95% CI
Divergence between Allodapini & Ceratinini	70	62-79	94	83-105
Crown age of extant Allodapini	50	43-57	66	61-70
Divergence between exoneurine & African clades	44	39-50	58	51-65
Divergence between Malagasy & African clades	41	36-47	54	48-61
Crown age of extant African clade	40	36-44	53	46-59
Crown age of extant exoneurines	35	28-42	46	38-54
Divergence of *Exoneuridia*	33	25-41	43	33-53
Crown age of *Allodape*	31	27-36	40	35-46
Crown age of *Braunsapis*	28	24-32	36	31-42
Crown age Malagasy '*Hasinamelissa*' clade	23	19-26	30	25-35
Crown age of *Allodapula*	22	17-26	29	23-35
Dispersal of *Braunsapis* into the Orient	22	18-26	29	23-34
Crown age of *Compsomelissa*	20	15-24	26	20-31
1st dispersal of *Braunsapis* to Madagascar	20	16-23	25	21-30
Crown age of *Macrogalea*	15	10-22	20	15-25
Crown age of temperate *Allodape* lineages	15	12-17	19	16-22
Dispersal of *Braunsapis* into Australia	10	8-13	14	10-18
Crown age of *Halterapis*	4	2-6	5	3-7
2nd dispersal of *Braunsapis* to Madagascar	4	3-5	5	4-7
Dispersal of *Macrogalea* to Madagascar	3	1-5	4	2-6

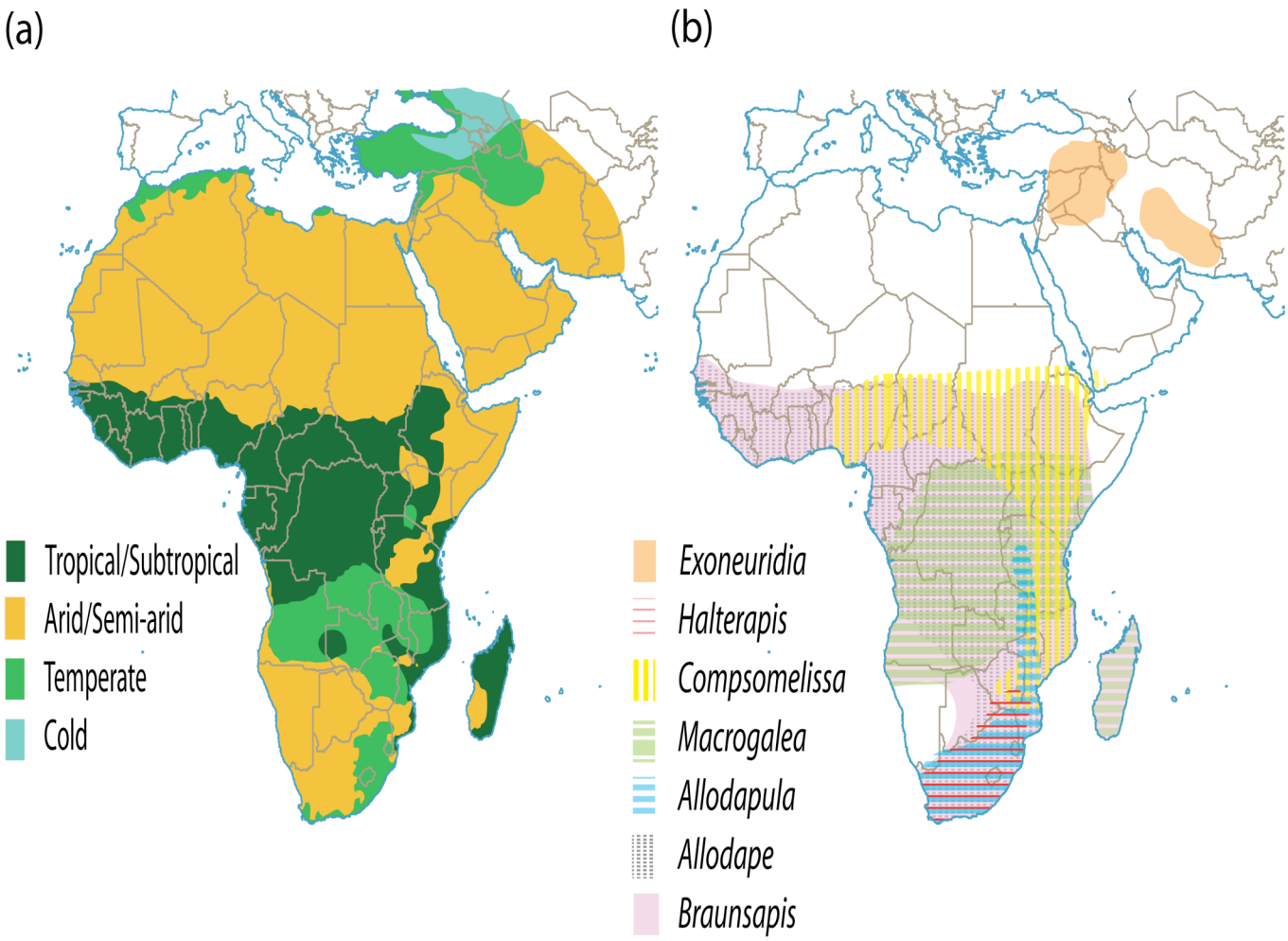

Figure 1 (a) Climate map of Africa, Madagascar, the Arabic Peninsula and Southern Europe simplified from Köppen-Geiger classifications presented in Peel et al. (2007). (b) Distributions of the Allodapine genera of African origin across these regions, compiled from Michener (1975), Terzo (1999), Pauly et al. (2001), and Eardley and Urban (2010).

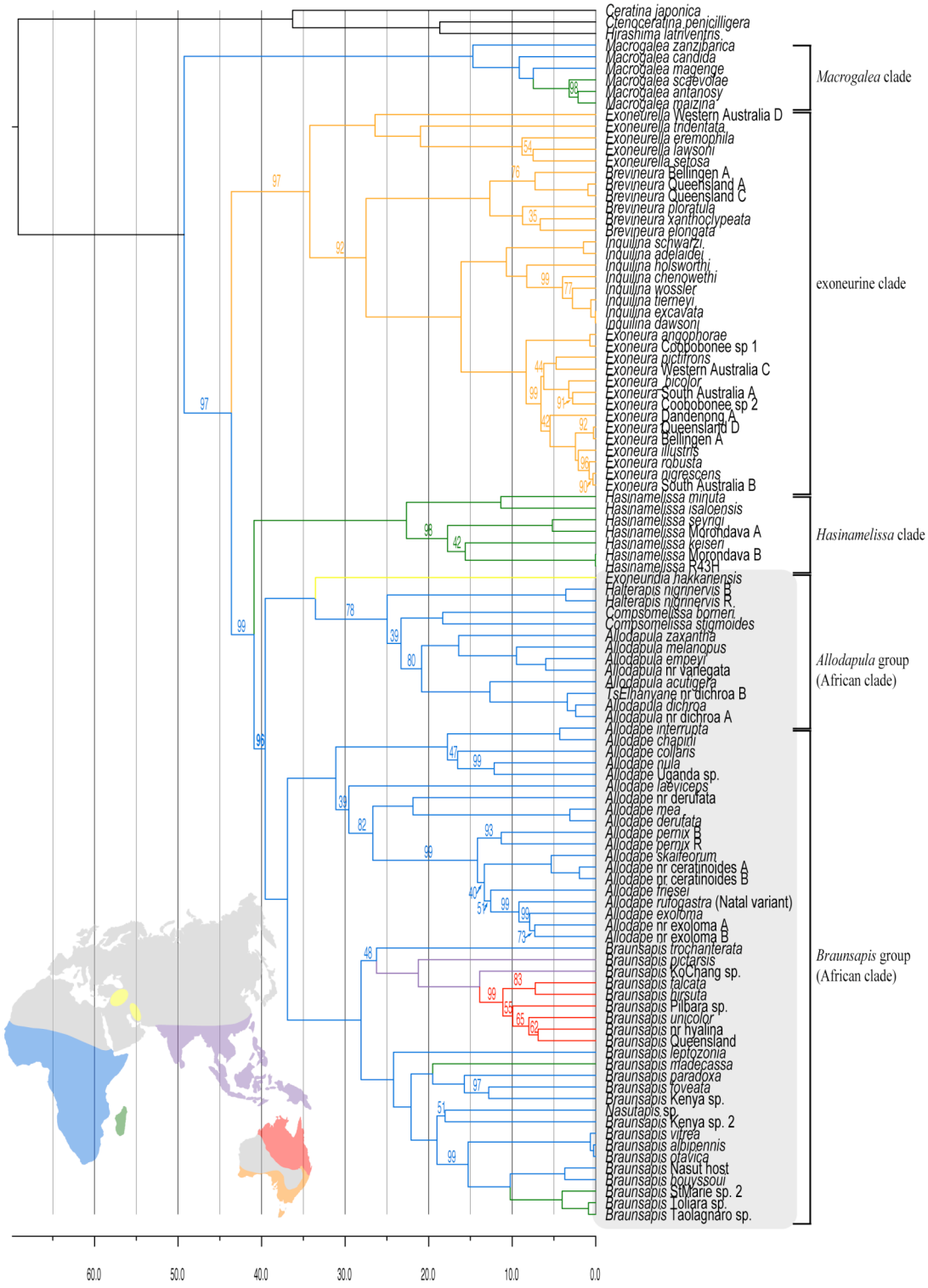

Figure 2 Chronogram of the allodapines derived from penalized likelihood transformation of the consensus Bayesian phylogram. Posterior probabilities are indicated for each node except for nodes with support >99. Geographic distributions of each species are colour coded according to the map.

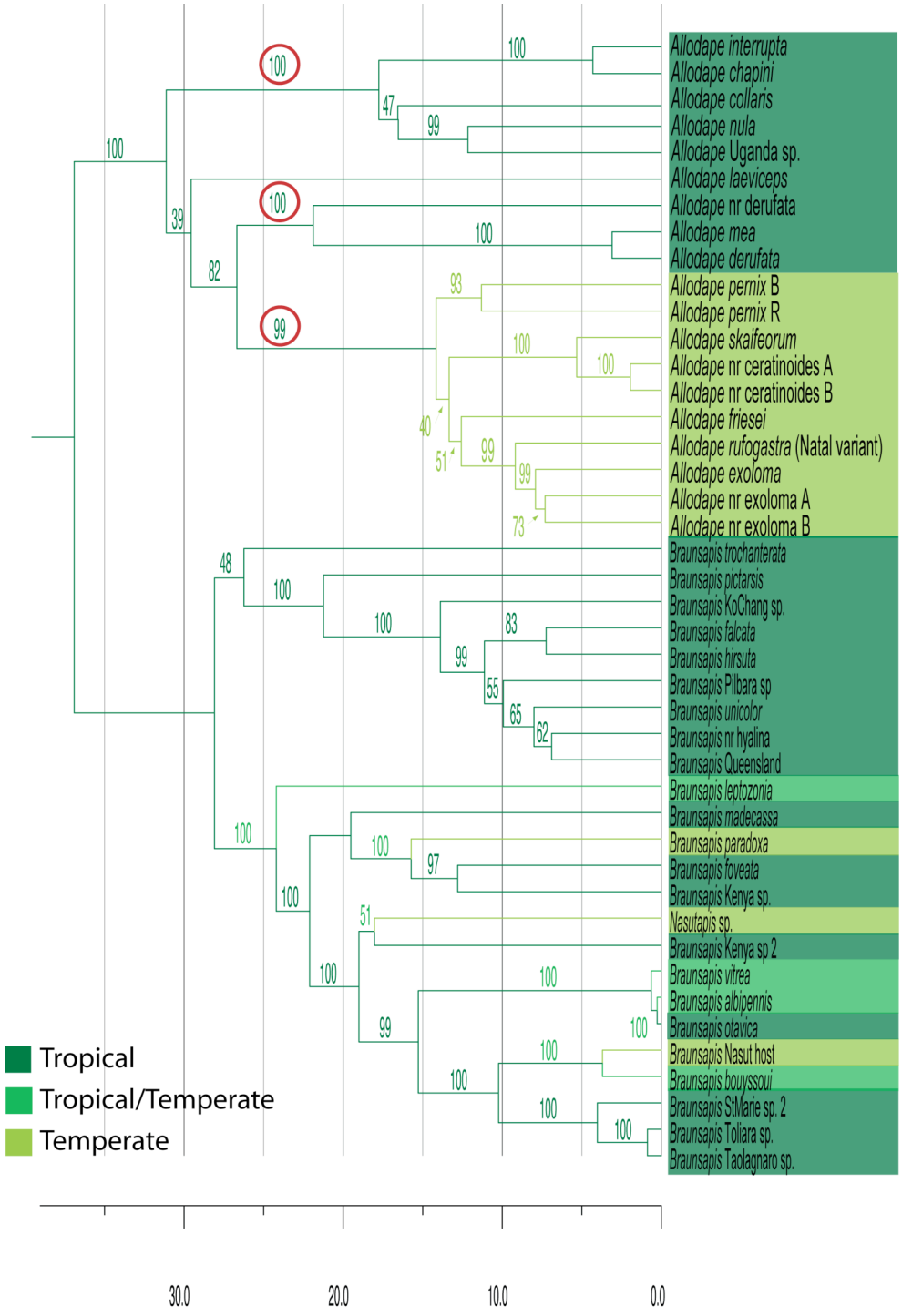

Figure 3 Chronogram of *Allodape* and *Braunsapis* derived from penalized likelihood transformation of the consensus Bayesian phylogram with posterior probabilities provided for each node. Environmental distributions of each species are colour coded according to the map. Highlighted nodes indicate strongly supported nodes regarding the phylogenetic relationship between Tropical and Temperate *Allodape* taxa.

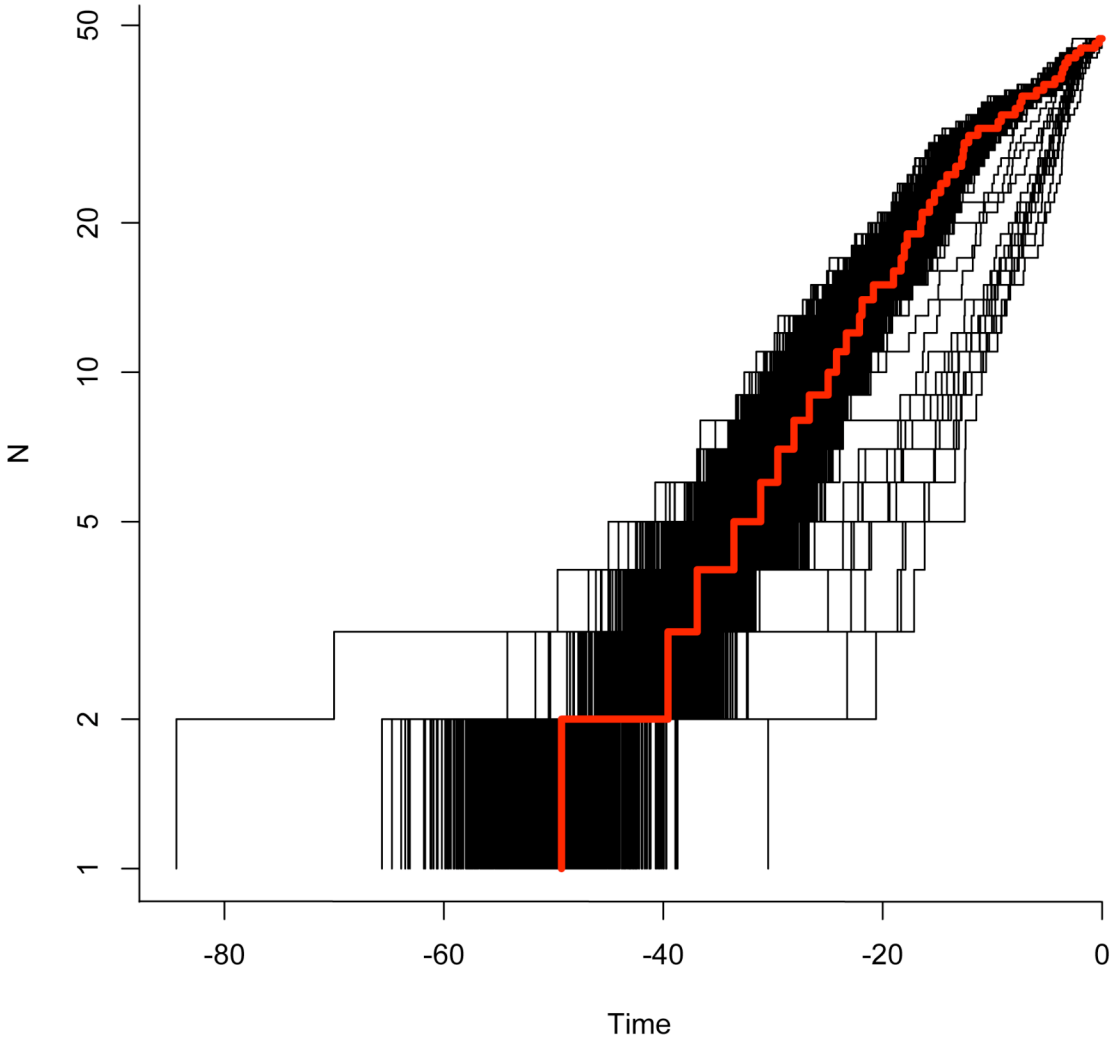

Figure 4 Lineage through time (LTT) plot of exoneurine cladogenesis over time. Dark lines represent 2000 randomly selected post-burnin samples, with the consensus chronogram superimposed in red.

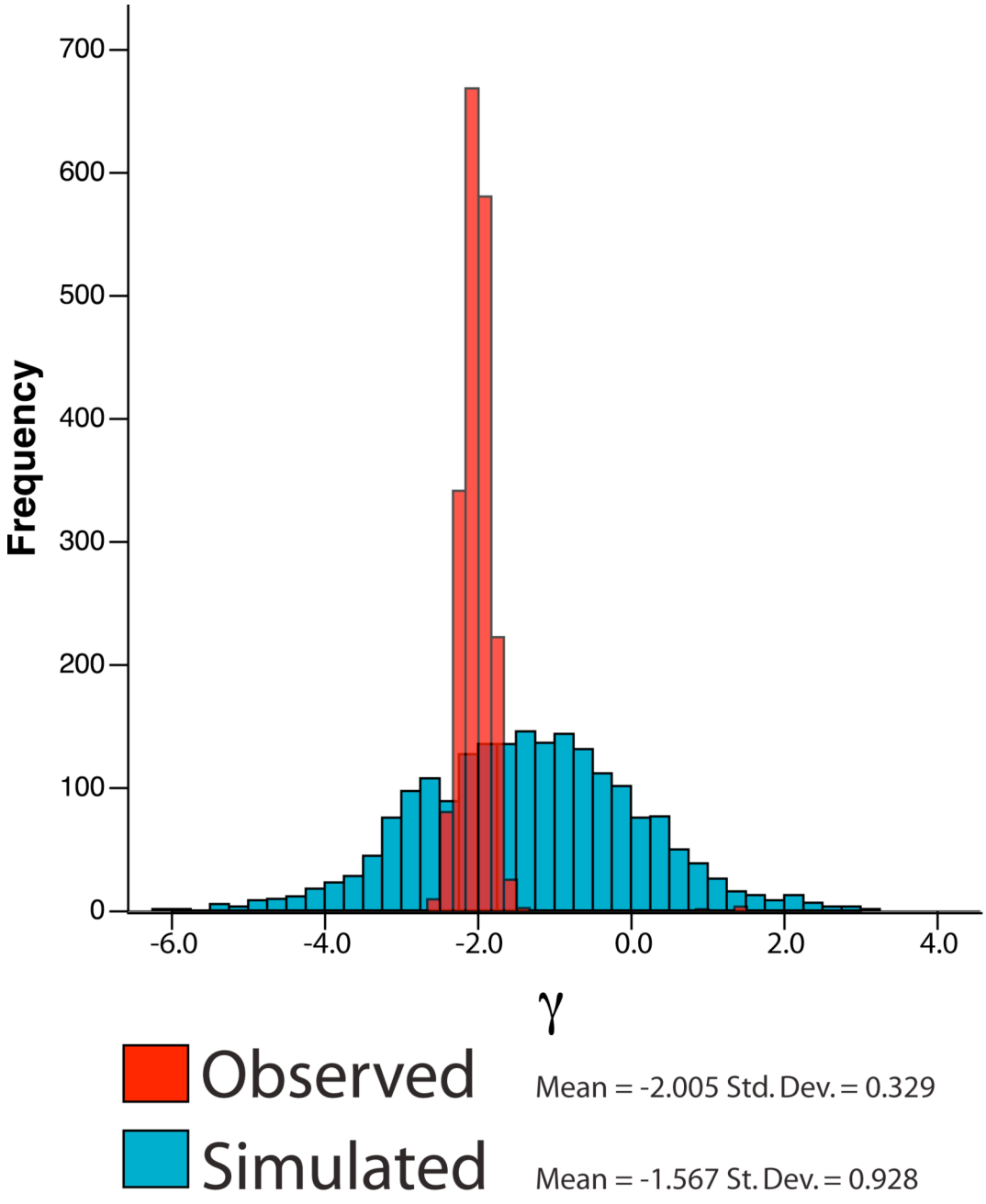

Figure 5 Gamma distributions of sampled versus simulated phylogenies. Blue: Distribution of 2000 randomly sampled post-burnin trees of the 47 allodapine species of the African clade sampled in our study. Red: Gamma distribution of 2000 trees based on described Allodapine diversity (108) with all but 47 terminals randomly deleted.

GENERAL CONCLUSION

The research presented here has provided important insight into both the evolution and the history of diversification of the allodapine bees. The comprehensive study of the nesting biology of *Halterapis nigrinervis* presented in Chapter I indicates the presence of social nesting strategies, meaning that there have been no losses of sociality amongst the extant allodapine clades. Furthermore, this implies that the origin of allodapine sociality is the result of a single origin that preceded the extant members of the tribe, shown in Chapter I to be relatively ancient at no less than 40 Ma. Within the allodapines, strong benefits exist for both alloparental care via sib rearing, and the tolerance of potential alloparents by foundresses. The unusual allodapine trait of progressively rearing brood in an open nest renders protection of brood from predation problematic for solitary-nesting females, as they must episodically leave their nest to forage in order to provision their young. This points to an ecological underpinning in the retention of social nesting in the allodapines, with protection from predation creating large benefits for alloparental care and tolerance of potential alloparents. Chapter I argues that similar vulnerabilities of brood may also help explain the lack of reversions to solitary living in other ubiquitously social taxa such as ants, termites, and vespid wasps, which also rely solely on the presence of adults for the protection of brood.

The taxa sets utilized in this thesis support the previous molecular phylogenies of Schwarz et al. (2003) by placing the African genus *Macrogalea* as a discrete sister clade to the remaining allodapine genera. However the inclusion of additional taxa from all major allodapine centres of diversity have provided much greater insight into the history of speciation within the tribe. Chapter II incorporates no less than six species of Malagasy allodapine previously assigned to *Halterapis* into molecular phylogenetic analyses, and shows that the Malagasy taxa form a monophyletic group unrelated to the African species of *Halterapis*. Moreover, newly collected larval specimens of the Malagasy *Halterapis* species presented in Chapter II display morphological characteristics that are distinct from any other allodapine larvae studied to date. The combined morphological and phylogenetic data presented here suggest that the Malagasy *Halterapis* constitute an endemic Malagasy clade, no less than 20 Myo, that is sister clade to the non-*Macrogalea* African genera. A new genus, *Hasinamelissa*, is erected to contain the Malagasy taxa formerly placed in the genus *Halterapis*. This old and endemic Malagasy bee clade further highlights the age and

distinctiveness of the island's bee fauna, and emphasizes the dire need for the conservation of Madagascar's flora and fauna.

Amongst the allodapine 'exoneurine' genera examined in Chapter III, comprehensive molecular phylogenies supports Schwarz et al.'s (2003) argument of a monophyletic origin of the clade's four genera. These analyses suggest that this colonization event took place *c.* 34 Ma, too late for Gondwanan vicariance models, and too early to be accommodated by a Laurasian dispersal route. As such, this dispersal must have involved direct trans-oceanic dispersal from Africa to Australia over the Indian Ocean. Such dispersals have been proposed in a number of other taxa, but the mechanisms by which these dispersals occurred have remained highly controversial. The implication of Antarctica in the exoneurine's ancestral dispersal suggests a hitherto overlooked avenue for Indian Ocean dispersal to occur, and points to a potential explanation for the paradoxical dispersal patterns observed in many other Australian taxa. Contrary to previous studies, no evidence of ancient rapid radiations upon the formation of the exoneurines is found. Instead, analyses show evidence of accelerated diversification of temperate adapted lineages that coincide with the Late Miocene aridification of the continent, showing for the first time links between the radiation of Australian bee fauna to historical episodes of climate change.

The phylogenetic analyses presented in this thesis all support the placement of all non-*Macrogalea* allodapine genera in Africa, along with the Middle-Eastern genus *Exoneuridia* and the widespread genus *Braunsapis*, as a monophyletic 'African' clade. Further investigation undertaken in Chapter IV shows that the basal divergence of this clade occurs between 40 and 53 Ma, and results in the divergence of an '*Allodapula'* group consisting of the temperate *Halterapis*, *Compsomelissa*, *Allodapula* and the montane *Exoneuridia*, and a '*Braunsapis'* group, consisting of the largely tropical *Allodape* and *Braunsapis*. Amongst the latter, all instances of temperate taxa represent recent derivations from older tropical lineages, pointing to a tropical origin. *Macrogalea* is strictly tropical and subtropical in distribution, as are Baltic amber specimens of the allodapines extinct sister tribe, the Boreallodapini (Engel 2001), which suggests that the tribe's adaptation to tropical habitats occurred early, or is perhaps ancestral, to the tribe *per se*. Similarly to the exoneurines, no evidence of rapid radiations are apparent upon the formation of this African clade. However, Chapter IV shows that the origin of a large group of temperate-adapted taxa amongst the otherwise tropical *Allodape* corresponds with the aridification of Southern Africa. This response to aridification mirrors that found amongst the exoneurines

during the aridification of Australia, suggesting that both Australian and African allodapine taxa have responded in a similar manner to similar climatic shifts in their respective habitats.

A tropical origin for the allodapines would imply that the formation of the Malagasy, exoneurine, and African clades saw significant diversification of life-history traits and environmental preferences upon their divergence. Interestingly, these divergences occurred almost simultaneously, with the divergence of the exoneurines, *Hasinamelissa*, as well as the tropical '*Braunsapis*' and temperate '*Allodapula*' African groups, occurring in a very short period of 2-4 million years. In stark contrast, these groups remain highly constrained in morphological and behavioural characters thereafter (Michener 1975, 1977; Schwarz *et al.* 1998, 2005; Chenoweth & Schwarz 2007) and show few examples of subsequent transitions in habitat or major dispersal events. This raises interesting questions as to the underlying factors that promoted this short-lived yet significant period of diversification. The sudden increase in diversification of temperate lineages amongst the temperate Australian exoneurines and the monophyletic derivation of temperate lineages from the otherwise tropical African genus *Allodape* both appear to coincide with the onset of episodic aridification in their respective environments. This observation potentially implicates climatic change and ensuing climatic instability as a powerful driving force behind speciation in the allodapines. The correlation between the sudden divergence of the African, Malagasy, and exoneurine clades and their radical dissimilarity in climatic and ecological tolerances may point to phenological factors as a major influence in the history of diversification of the allodapines, and provides an interesting avenue for future study.

The investigations provided in this thesis have shown the allodapines to be a valuable group for studying many facets of evolution, biogeography, and cladistics. Studies of allodapine life history traits have provided important insight into the possible ways in which social nesting strategies evolve and are maintained. This work highlights the advantages of cladistic analyses that utilize composite datasets, including often-overlooked characteristics such as larval morphology. Phylogenetic examinations of the allodapines have highlighted the age and pervasiveness of endemic diversity in important southern-hemisphere ecosystems such as the Afrotropic zone, the Cape Floristic Region, the southern temperate regions of Australia, and the island of Madagascar. Finally, the history of allodapine diversification has provided valuable insight into the mechanisms by which periods of significant environmental change have influenced ecological diversity and

speciation within these regions. Closer scrutiny of the role that ecological factors play in the evolution and maintenance of social behaviour is warranted, and investigation of the interplay between diversification patterns and climate in other taxa is an enticing avenue for further exploration of the often capricious nature of bee diversification and evolution.

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