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Molecular phylogeny of the small carpenter bees (Hymenoptera: Apidae: Ceratinini) indicates early and rapid global dispersal

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ABSTRACT

The small carpenter bees (tribe Ceratinini, family Apidae) are recorded from all continents except Antarctica. The Ceratinini have a near-global distribution which contrasts strongly with their sister tribe, the Allodapini which has a largely southern Old World distribution. The Ceratinini therefore provides an excellent group to understand the factors that help determine the biogeography and radiation of the bees. This is the first molecular study of ceratinine bees covering representatives from both northern and southern hemisphere Old and New World regions. We use two mitochondrial and one nuclear marker (totalling 2807 nucleotides) to examine the age, cladogenesis and historical biogeography of this tribe. Tree topology and molecular dating support an African origin at about 47 Mya with subsequent dispersal into Eurasia 44 Mya, and followed by an American invasion 32 Mya. Concentrated African and Malagasy sampling revealed there were two or three dispersals events into Madagascar ranging from 25 to 9 Mya. Lineage through time analyses suggest higher rates of cladogenesis close to the origin of the tribe, and this corresponds to both major dispersal events and divergences of lineages leading to extant subgenera. Ceratinini have potentially great importance for future studies to understand the relative roles of dispersal ability and time of origin in determining bee biogeography.

1. Introduction

Recent molecular phylogenetic studies of various bee groups are beginning to radically change our understanding of early bee evolution, including identification of the most primitive clades (Danforth et al., 2006), early bifurcations in phylogeny, and some likely biogeographical scenarios for the origins and subsequent spread of bees via dispersal and/or vicariance (e.g. Leys et al., 2002; Schwarz et al., 2006; Hines, 2008; Schaefer and Renner, 2008).

The first bees probably evolved in the early to mid Cretaceous, corresponding with the rapid diversification of the angiosperms at this time (Grimaldi, 1999; Engel, 2001; Michener, 2007), and this timeframe corresponds with the oldest known bee fossil dated at about 90 million years ago (Mya) and not belonging to any extant family (Poinar and Danforth, 2006). There are only two confirmed Cretaceous-age bee fossils, the other being a meliponine bee from New Jersey amber, dated to approximately 65 Mya (Engel, 2000). This very limited fossil record means that there are few calibration points when considering the earliest bee divergence dates. However, there are relatively rich fossil bee records from Dominican amber (Miocene) and Baltic amber (Eocene) comprising species from multiple extant tribes, and these have allowed several studies to begin exploring bee phylogeographic and social evolutionary events occurring from the early Eocene until recent times.

A revelation into the origin and evolution of the bees came from the first molecular assessment of the seven extant bee families (Danforth et al., 2006a). Families that were once thought to be relatively derived, including the long-tongued bee families Megachilidae, Apidae and Melittidae, now appear to be much more basal. Molecular analysis of the seven bee families coincides with a morphology-based study suggesting a derived origin of the Colletidae along with other short-tongued bees (Andrenidae, Halicitidae and Strenotritidae) (Alexander and Michener, 1995). The ability to explore evolutionary patterns in bees with independent data sets has strengthened our understanding, especially when morphology and genetics are congruent (Danforth et al., 2006b). Current diver-

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sity and distributions suggest that bees originated in the arid interior of western Gondwana (Michener, 1979). Recent molecular phylogenetic data also suggests an African origin as the earliest branches are predominately African lineages (Danforth et al., 2006b).

Molecular studies of the Halictidae suggest an African origin 70–55 Mya with subsequent dispersals into South America (70–55 Mya) and North America (55–50 Mya) (Danforth et al., 2004). Molecular studies of allodapine bees (Schwarz et al., 2006; Chenoweth et al., 2007) suggest an African origin for this tribe about 47 Mya, with dispersal from Africa to Australia occurring about 25 Mya, and Fuller et al. (2005) inferred a secondary eastward dispersal from Africa into southern Asia about 18 Mya. Schaefer and Renner (2008) inferred a 56 Mya African origin of the ctenoplectrine bees, with dispersal into Asia 40–30 Mya, from which one lineage reached Australia via Indonesia and New Guinea around 13 Mya. Robust phylogenetic analyses of *Bombus* by Cameron et al. (2007) provided a comprehensive data set to examine their historical biogeography, and using these data Hines (2008) inferred an Asian origin 40–25 Mya with subsequent Nearctic and Neotropical dispersal via Bering and Panamanian continental connections around 20 and 7 Mya, respectively. Leys et al. (2002) proposed a Eurasian origin of *Xylocopa* 55–35 Mya with holarctic radiation 34 Mya and subsequent southern dispersal into South America, Africa and Australia <25 Mya.

The molecular studies of halictids, allodapines, ctenoplectrines, *Xylocopa* and *Bombus* provide insights in terms of current distributions of some bee groups and how those came about. Halictids, *Bombus* and *Xylocopa* all have nearly global distributions (excluding Antarctica, and also excluding the Australasian and sub-Saharan regions for *Bombus*), whereas ctenoplectrines and allodapines both have Old World distributions, with minimal extension into the Palaearctic for allodapines and minimal austral expansion for ctenoplectrines.

These contrasting distributions raise very interesting questions: do current distributions reflect dispersal ability, times of origin, ecological constraints, or have they been shaped by all three? For example, more global distributions could have arisen from long-range dispersal ability *per se*, or it could reflect times of origin that allowed ancestral clades to be dispersed by plate tectonic movements or for dispersal to have occurred over barriers that are large now but were much smaller in the past. The bee tribe Ceratinini (tribe Ceratinini, family Apidae) is the extant sister clade to the Allodapini, but unlike that tribe has a near-global distribution. As such it holds enormous promise for helping to identify factors that may explain differences in geographic distributions among closely related taxa.

The Ceratinini is one of four tribes of the apid subfamily Xylocopinae: Allodapini, Ceratinini, Maneuliini and Xylocopini. To date all studies (Sakagami and Michener, 1987; Roig-Alsina and Michener, 1993; Engel, 2001) agree that ceratinines comprise the extant sister group to the allodapine bees, but while the latter are largely restricted to the southern Old World, with only minimal Palearctic representation, the ceratinines are recorded from all continents except Antarctica (Michener, 1979), and the only continent where they are depauperate is Australia (only one recorded species, Michener, 1962).

Michener (2007) recognized only one genus in the tribe Ceratinini, containing 21 subgenera, with 16 subgenera endemic to the eastern hemisphere and five endemic to the western hemisphere. Terzo et al. (2007) recently proposed a new subgenus *Dalyatina*, and Eardley and Daly (2007) described eight new species and provided 30 new synonyms in southern Africa without placing many species into subgenera due to a lack of revision of African *Ceratina* species. Some earlier studies accorded generic status to the subgenera *Megaceratina* (Hirashima, 1971), *Pithitis* (Klug, 1807), and *Ctenoceratina* (Daly, 1988) because of their morphological distinctness. However, in a phylogenetic analysis based on morphological characters, Terzo (2000) found that these three latter groups were nested within other clades of *Ceratina* and generic recognition of these groups would render *Ceratina* polyphyletic. Despite extensive effort, Terzo (2000) was unable to definitively resolve the relationships among subgenera based on morphological characters; and therefore the historical biogeography of the Ceratinini has remained largely speculative.

Here we apply molecular phylogenetic techniques to 71 species from 15 ceratinine subgenera to infer phylogenetic relationships, the approximate times of major divergences and the historical biogeography of this tribe. In particular we explore the most likely centre or origin for this tribe, subsequent patterns of dispersal, and what factors may help explain the near-global distribution of the Ceratinini compared to its sister tribe Allodapini.

2. Methods

2.1. Choice of included taxa

Taxa and sampling localities along with NCBI accession numbers are listed in Table 1. Our ingroup comprised 71 species from 15 of the 21 described subgenera, covering all 6 ecozones of *Ceratina* diversity: Afrotropical (31 species), Madagascar (four species), Indo-Malayan (17 species), Nearctic (four species), Neotropical (five species) and Palearctic (six species). For brevity ingroup species are written using subgeneric names throughout the results as all subgenera and species belong to the genus *Ceratina*. Michener's (2007) subgeneric classification is employed in our study due to a degree of uncertainty of recent subgenera and species groups. Voucher specimens are housed in the collections of M.P. Schwarz at Flinders University of South Australia. In addition to the *Ceratina* species, we included ten species representing all three tribes of the Xylcopinae: Manueliini (two species), Allodapini (seven species) and Xylocopini (one species), as well as two ctenoplectrine, four corbiculate and two halictine bees to provide fossil calibration points and to help root the ingroup. The allodapines were included because this tribe is the extant sister group to Ceratinini (Sakagami and Michener, 1987; Roig-Alsina and Michener, 1993; Engel, 2001) and, therefore, likely to be most appropriate for rooting the ceratinine clade. The split between Ceratinini and Allodapini also provides a minimum-age calibration point because there is support for a sister relationship between allodapines and the Baltic amber fossil tribe Boreallodapini with the Ceratinini being the next-most basal clade (Engel, 2000). *Manuelia* and *Xylocopa* species were also included to sample each of the four tribes and explore the monophyly of the subfamily Xylcopinae. The inclusion of four corbiculate and two ctenoplectrine bees provides another age calibration point between the xylocopines and apines (Schwarz et al., 2006), and two short-tongued halictine bees were included to root this node.

2.2. DNA extraction, amplification and sequencing methods

Tissue samples of approximately 5 mg were taken from up to three legs from each specimen. DNA extractions followed Gentra Puregene Cell Kit (Qiagen) standard protocols. PCR amplification was achieved in 20 μl reactions containing 2 μl 10 mM dNTPs (2.5 mM each), 5 μl each primer (5 mM), 1 U HotMaster *Taq* DNA polymerase, 2.5 μl Hot Master *Taq* Buffer ($MgCl_2$ included) and 50 ng DNA template.

Two mitochondrial gene regions and one nuclear gene region were amplified and sequenced bi-directionally. The nuclear exon region was from the F2 copy of elongation factor 1α (EF-1α F2)

Table 1
List of species sequenced for this study along with Genbank accession numbers and their collection location. Species distributions are indicated in any ecozone as: A = Afrotropical, I = Indo-Malayan, N = Nearctic, M = Madagascar, P = Palearctic, S = Neotropical, and U = Australasian. Outgroups (*Manuelia* spp.) were not used for biogeographic analyses thus distributions are omitted with dashes.

Subgenus	Species	Distribution	Collection location	Accession numbers		
				EF-1α F2	Cyt *b*	COI
Calloceratina	Panama sp.	S	Panama	GU321643	GU321574	GU321508
Calloceratina	blue sp.	S	Argentina	GU321639	N/A	GU321504
Ceratina	*minutula*	I	Turkey	GU321671	GU321601	GU321536
Ceratina	*subquadrata*	A	South Africa	GU321669	GU321599	GU321534
Ceratina	*braunsi*	A	South Africa	N/A	GU321597	GU321532
Ceratina	*rhodura*	A	South Africa	GU321672	GU321602	GU321537
Ceratina	*aloes*	A	South Africa	GU321670	GU321600	GU321535
Ceratina	*perpolita*	A	South Africa	GU321673	N/A	GU321538
Ceratina	*speculifrons*	A	Kenya	GU321668	GU321598	GU321533
Ceratinidia	*papuana*	I U	Malaysia	GU321609	GU321546	GU321474
Ceratinidia	*bowringi*	I	India	GU321611	GU321548	GU321476
Ceratinidia	*hieroglyphica*	I	India	GU321614	GU321551	GU321479
Ceratinidia	*moderata*	I	India	GU321607	GU321544	GU321472
Ceratinidia	*bryanti*	I	Malaysia	GU321612	GU321549	GU321477
Ceratinidia	*japonica*	P	Japan	GU321605	GU321542	GU321470
Ceratinidia	*okinawana*	I P	Japan	GU321613	GU321550	GU321478
Ceratinidia	*nigrolateralis*	I	Malaysia	GU321606	GU321543	GU321471
Ceratinidia	*accusator*	I	Malaysia	GU321610	GU321547	GU321475
Ceratinidia	*cognata*	I	Malaysia	GU321608	GU321545	GU321473
Ceratinula	*breviceps*	S	Bolivia	GU321642	GU321573	GU321507
Ceratinula	Paraguay sp.	S	Paraguay	GU321635	GU321568	GU321500
Ceratinula	*cockerelli*	N	U.S.A.	GU321641	N/A	GU321506
Copoceratina	*minuta*	A	South Africa	GU321667	N/A	GU321531
Ctenoceratina	*pencillata*	A	Kenya	GU321632	GU321565	GU321497
Ctenoceratina	*penicilligera*	A	Kenya	GU321629	N/A	GU321494
Ctenoceratina	*malindae*	A	Kenya	GU321631	GU321564	GU321496
Ctenoceratina	*ericia*	A	Zambia	GU321624	GU321559	GU321489
Ctenoceratina	*lineola*	A	Tanzania	GU321630	GU321563	GU321495
Ctenoceratina	*bilobata*	A	Kenya	GU321626	GU321561	GU321491
Ctenoceratina	Zambia sp.	A	Zambia	GU321625	GU321560	GU321490
Ctenoceratina	*rufigastra*	A	Kenya	GU321628	N/A	GU321493
Ctenoceratina	Kenya sp.	A	Kenya	GU321627	GU321562	GU321492
Euceratina	*chrysomalla*	P	Turkey	GU321620	N/A	GU321485
Euceratina	*mandibularis*	P	Turkey	GU321617	GU321554	GU321482
Euceratina	*tibialis*	P	Turkey	GU321619	GU321556	GU321484
Hirashima	S Africa sp1	A	South Africa	GU321618	GU321555	GU321483
Hirashima	S Africa sp2	A	South Africa	GU321646	GU321576	GU321511
Hirashima	Malagasy sp1	M	Madagascar	GU321644	N/A	GU321509
Hirashima	Malagasy sp2	M	Madagascar	GU321645	GU321575	GU321510
Hirashima	*lativentris*	M	Madagascar	GU321649	GU321579	GU321514
Hirashima	Zambia sp1	A	Zambia	GU321650	GU321580	GU321515
Hirashima	Zambia sp2	A	Zambia	GU321647	GU321577	GU321512
Lioceratina	*flavolateralis*	I	Malaysia	GU321648	GU321578	GU321513
Malgatina	*azurea*	M	Madagascar	GU321615	GU321552	GU321480
Neoceratina	*australensis*	U	Australia	GU321616	GU321553	GU321481
Neoceratina	*dentipes*	I P U	Mauritius	GU321633	GU321566	GU321498
Neoceratina	*dentipes*	I P U	Malaysia	GU321651	GU321581	GU321516
Neoceratina	*propinqua*	I	India	GU321655	GU321585	GU321520
Neoceratina	Solomons_sp.	U	Solomon Islands	GU321652	GU321582	GU321517
Neoceratina	*bispinosa*	P	Israel	GU321657	GU321587	GU321521
Neoceratina	*satoi*	P	Japan	GU321653	GU321583	GU321518
New subgenus	sp.	A	Kenya	GU321656	GU321586	N/A
Pithitis	*unimaculata*	I	Malaysia	GU321654	GU321584	GU321519
Pithitis	*fastigata*	A	Zambia	GU321674	GU321603	GU321539
Pithitis	*waini*	A	Zambia	GU321665	GU321595	GU321529
Pithitis	*citriphila*	A	Zambia	GU321661	GU321591	GU321525
Pithitis	*smaragdula*	I P	Indonesia	GU321659	GU321589	GU321523
Pithitis	*tarsata*	A	Zambia	GU321666	GU321596	GU321530
Pithitis	*nasalis*	A	Swaziland	GU321664	GU321594	GU321528
Pithitis	*binghami*	I	India	GU321662	GU321592	GU321526
Pithitis	Kenya sp.	A	Kenya	GU321663	GU321593	GU321527
Simioceratina	*lunata*	A	Zambia	GU321658	GU321588	GU321522
Simioceratina	*tanganyicensis*	A	Tanzania	GU321660	GU321590	GU321524
Simioceratina	*moerenhouti*	A	Kenya	GU321621	GU321557	GU321486
Zadontomerus	*dupla*	N	U.S.A.	GU321623	N/A	GU321488
Zadontomerus	*floridana*	N	U.S.A.	GU321622	GU321558	GU321487
Zadontomerus	*calcarata*	N	Canada	GU321634	GU321567	GU321499
Zadontomerus	*strenua*	N	Canada	GU321640	GU321572	GU321505
Zadontomerus	*cyaniventris*	S	Cuba	GU321638	GU321571	GU321503
Manuelia	*gayi*	–	Chile	GU321636	GU321569	GU321501
Manuelia	*gayatina*	–	Chile	GU321637	GU321570	GU321502

and the mitochondrial regions were from the protein coding genes cytochrome oxidase I (COI) and cytochrome *b* (Cyt *b*). The primers used for PCR amplification of the EF-1α F2 region included the F2 specific combination HaF2For1/F2-Rev1 (Danforth et al., 1999) to produce an approximately 1100-bp fragment. In the case where the initial primers failed we used a set of primers designed by S.J.B. Cooper: forward (G1553) 5′-ACTATGTTACCATTATTGACGC-3′ and reverse (G1554) 5′-GCTTCTTGCA(G/A)AGC(C/T)TCGTG-3′ to amplify a 1060-bp fragment for 36 of the 71 ingroup taxa. Cycle conditions for nuclear DNA were as follows: 94 °C, 1 min denaturation; 54 °C, 1 min annealing; 72 °C, 1 min 30 s extension for a total of 35 cycles (Danforth et al., 1999). The overlapping primer combinations of UEA7/UEA10 (Lunt et al., 1996) and M414/M399 (Schwarz et al., 2004) were used to amplify a 1279-bp COI region when possible. When this failed the COI primer combination of mtd-8 and 12 (Simon et al., 1994; University of British Columbia Biotechnology Laboratory, Vancouver) produced 900-bp PCR product. The Cyt *b* primer combination of cb1/cb2 designed by Y.C. Crozier (Latrobe University, Melbourne, Australia; Schwarz et al., 2004) produced a consistently amplified 428-bp product. Cycle conditions for mtDNA amplification were as follows: 94 °C, 1 min denaturation; 50 °C, 1 min annealing; 72 °C, 1 min 30 s extension for a total of 34 cycles.

PCR products were purified directly using the Multiscreen PCR_{384} Filter Plate (Millipore), and sequenced using 2 μl product in 10 μl reaction volumes for each original PCR primer using the Big Dye Ready Reaction kit Version 3.1 (Applied Biosystems). Sequencing reaction products were then purified by Millipore Filter plate and sent to the Institute of Medical and Veterinary Science (IMVS), Adelaide, Australia for automated sequencing. Forward and reverse sequences were assembled and edited using SeqEd 1.03 (Applied Biosystems). As with the sister tribe Allodapini, the intron regions of EF-1α F2 were largely unalignable at subgeneric and generic levels and were excluded from analyses.

2.3. Phylogenetic analyses

Maximum parsimony (MP) analyses were conducted using PAUP* b4.10 (Swofford, 1999) and for Bayesian inference (BI) analyses MrBayes version 3.1.2 (Huelsenbeck and Ronquist, 2001) was utilized. We relied on BI rather than MP for recovering phylogenies, however, MP analyses were also used to see whether broad topological features were recovered using a very different approach to BI. One hundred random sequence stepwise additions were used in the MP analysis, holding 10 trees at each step and with tree bisection and reconnection for searching tree space. Node support was estimated using 500 bootstrap pseudoreplicates, using the same methods as for the heuristic search, and retaining compatible groups with less than 50% bootstrap support.

Molecular analyses of allodapine bees, the extant sister clade to Ceratinini, found substantial problems in resolving phylogenetic relationships using maximum parsimony when 3rd codon positions for mitochondrial genes were given equal weight to other gene partitions (Bull et al., 2003; Schwarz et al., 2003, 2004) and explored the effects of down weighting this position between 0 and 0.5. This is likely due to the high level of homoplasic changes for mitochondrial nucleotides where AT bias is extreme for 3rd positions (Schwarz et al., 2004). This problem is likely to be at least as problematic where AT composition for 3rd mitochondrial positions in our sample was 82% and where the more basal bifurcations in ceratinines are likely to be older than for allodapines. At the same time, mitochondrial 3rd codon differences are likely to be useful for recent divergences where overwriting is less likely. We used exploratory analyses to examine what kind of weighting for 3rd codon positions minimized the number of equally most-parsimonious trees, and this involved a trade-off between resolution of basal and distal nodes. We settled on a weighting of 0.2 to generate a first topology, and then re-weighted all sites using the re-scaled consistency index implemented in PAUP*.

In the BI analyses the data were partitioned into six parts: 1st, 2nd and 3rd codon positions for the two mitochondrial genes combined, and 1st, 2nd and 3rd codon positions for EF-1α. All genes were partitioned into three parts due to the varying base composition found between codon positions. We prefer an 'objective' Bayesian approach (Berger, 2006) and therefore used the MrBayes version 3.1.2 default priors because these are mostly uninformative. 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 indicated by plateauing of log likelihood values. The analysis was run for 20 million generations, sampling every 500th generation to reduce auto-correlation among sampled generations and we used a burnin of four million generations, well after stationarity was reached.

2.4. Dating analysis

We used a penalized likelihood method, implemented in r8s version 1.70 (Sanderson, 2002) to estimate the ages of key nodes in our phylogeny. We employed three calibration points: (i) the minimum divergence between the Ceratinini and Allodapini was set at 45 Mya because of the fossil Boreallodapini species found in Baltic amber (Engel, 2001). Boreallodapini is the sister tribe to the Allodapini and the Ceratinini is the next-most basal clade in the Xylocopinae. This minimum-age restriction is likely to be highly conservative since the Allodapini + Boreallodapini clade is likely to have diverged from the Ceratinini much earlier than this. (ii) We also set a minimum-age for the node separating *Apis mellifera* from *Liotrigona* B1 because of the fossil meliponine bee *Cretotrigona prisca* recovered from New Jersey amber (Michener and Grimaldi, 1988) and most recently dated at 65 Mya (Engel, 2000). (iii) Lastly, we set a fixed age of 90 Mya 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 Mya (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 Mya. However, Danforth et al. (2004) have dated the crown age of the Halictidae at approximately 120 Mya, and this family is much more derived than the Apidae, again suggesting that setting the root node at 90 Mya is conservative. The only fossil assigned to the tribe Ceratinini, *Ceratina disrupta* Cockerell (1906), from the Oligocene Florissant shale was not included because the specimen is not confidently placed in this tribe (see Daly, 1973).

Because the consensus phylogram had low PP support for several nodes close to the root node of the Ceratinini, any differences between the consensus phylogeny and the actual phylogeny are likely to generate compounding errors when estimating crown ages for descendent clades, even though many of those may have strong support for monophyly. In order to take phylogenetic uncertainty into account when estimating nodes ages we used the following procedure. Firstly, we used MS Excel to randomly select 1000 out of the 24,000 post-burnin phylograms from the MrBayes analysis and we transformed these into chronograms using r8s,

with the same smoothing value that was used to generate the chronogram from the consensus phylogram. We then identified a number of internal nodes that had strong PP support ($\geqslant$95%) for monophyly from the MrBayes analysis and used the Most Recent Common Ancestor (MRCA) command in r8s to define these nodes and we then estimated their ages. For each of these nodes we estimated the arithmetic mean age and then sorted the individual estimates, based on the 1000 randomly selected post-burnin generations, in ascending order. For these 1000 sorted age estimates, we then removed the lowest and highest 25 values, leaving us with a 95% central distribution of ages based on the r8s transformed post-burnin phylograms.

To explore the robustness of our r8s dating analysis we also carried out a relaxed clock Bayesian analysis implemented in BEAST version 1.5.2 (Drummond et al., 2002, 2009). The combined mtDNA dataset and EF-1α data set were used with unlinked GTR models of nucleotide substitution, gamma rate heterogeneity and a proportion of invariant sites for different codon positions of mtDNA and EF-1α, giving a total of 6 separate partitions. A single relaxed molecular clock using the uncorrelated lognormal model was applied to the entire data set and a constant population coalescent with the Yule Prior was used (Drummond et al., 2002). We used the same calibration points as in the PL analysis, except that instead of setting a minimum-age for the MRCA of allodapines and ceratinines we used uniform prior bounded between 45 and 80 Mya, and a uniform prior bounded between 65 and 80 Mya was used for the MRCA of the corbiculates and root of our tree was assigned a normal distribution with a mean of 90 Mya. The analyses were carried out for 20 million generations, sampling every 1000 generations, after which the program Tracer (version 1.4.1) was used with a burnin of 3.5 million generations to check for convergence of the parameter estimates and determine the mean and 95% confidence intervals of the time to MRCA estimates. Time to MRCA estimates along with high probability densities (HPDs) were only obtained for the highly supported clades identified in the MrBayes analysis.

2.5. Exploring diversification rates using lineage through time (LTT) plots and gamma values

LTT plots are frequently used to graphically explore diversification rates, though caution is needed in their interpretation (e.g. Ricklefs, 2007). Because our consensus phylogram from the MrBayes analysis had low PP support for some critical nodes close to the root node (see Section 3 below) we generated a LTT plot for the consensus chronogram as well as for 49 randomly chosen post-burnin chronograms. We used the mltt.plot module in APE (Paradis et al., 2004) to generate 49 LTT plots for the post-burnin samples and superimposed the LTT plot for the consensus chronogram onto these.

The gamma statistic (γ, Pybus and Harvey, 2000) is frequently used to quantify changing rates of diversification over time, with lower values indicating greater diversification closer to the root node. However, there are two possible confounding factors that may make interpretation of γ problematic. Firstly, any particular tree topology may not indicate the true 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. Low support for many basal nodes in our results (see below) make this a potential problem. Secondly, our included taxa represent only 71 of the 339 described species in *Ceratina*, and incomplete taxon sampling will tend to produce gamma values that will suggest higher rates of cladogenesis closer to the root (Pybus and Harvey, 2000). To explore these possible confounding effects we used the following procedure. We randomly selected 1000 trees from the 24,000 post-burnin trees, subjected these to r8s transformations, and then used TreeEdit (Rambaut and Charleston, 2001) to prune all non-ceratinine taxa from the trees. We then used the mccrTest module in Laser 2.2 (Rabosky, 2006) to calculate gamma values for these trees. We then used Laser to generate 5000 random trees with a total number of 339 tip species and randomly pruned species to end up with only 71 terminals, and then calculated γ values for these trees.

2.6. Biogeographic analysis

We used BayesMultiState implemented in BayesTraits (Pagel et al., 2004; Pagel and Meade, 2006) to infer ancestral states and likely vicariance and dispersal events that shaped the current distribution of ceratinines. This method was used because it allows for both polymorphism in character states (ecozone regions in our analyses) within species as well as uncertainty in phylogeny, which is critical in our analyses where some nodes had low support (see below). Various priors were explored, with a criterion that acceptance rates had to be bounded by 20% and 40% (Pagel and Meade, 2006). We used a rate deviation prior of 15 with both an exponential (0.0, 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 in the Bayesian run was explored by plotting the harmonic mean and looking for a plateau in this. We subsequently used $40 * 10^6$ iterations with a burnin of $10 * 10^6$, sampling every 1000th generation.

We recorded members of each subgenus as being present in any of seven ecozones: Afrotropical (A), Madagascar (M), Nearctic (N), Neotropical (S), Indo-Malayan (I), Palearctic (P), and Australasian (U). Outgroups were not included when inferring ancestral regions for the Ceratinini.

3. Results

3.1. Phylogenetic analyses

The maximum parsimony bootstrap analysis (Fig. 1) showed very low levels of support for nearly all nodes except those that correspond to subgeneric groupings. The monophyly of the Ceratinini was well supported and all subgenera except *Ceratina sensu stricto* were resolved as monophyletic clades. The main features of the bootstrapped topology suggest *Neoceratina* as sister to all other subgenera and *Ceratina s. s.* basal to the remaining subgenera. The apical nodes of the tree suggest the Asian subgenera *Liocerati-na* and *Ceratinidia* as well as American subgenera *Ceratinula, Calloceratina* and *Zadontomerus* are the most recently derived clades.

The BI consensus phylogram is shown in Fig. 2. Posterior probability (PP) support is indicated for each node where support was less than 100%. Monophyly of the ceratinines was strongly supported (100 PP), and there was high support (94 PP) for *Neoceratina* as sister clade to the remaining subgenera in our sample. Conversely, there was weak support (54 PP) for the placement of *Megaceratina* at the base of the African clade and the placement of *Ceratina s. s.* is polyphyletic around *Copoceratina* with weak support (69 PP).

Hirashima, *Ctenoceratina* and *Simioceratina* formed a weakly supported clade (64 PP). The *Hirashima* clade presented two strongly supported (100 PP) African clades with a Malagasy clade contained within one of these. *Ctenoceratina* and *Simioceratina* were recovered as strongly supported sister groups (100 PP). The Malagasy *Malgatina azurea* and four Palaearctic species placed in *Euceratina* were recovered as a strongly supported (100 PP) monophyletic grouping. The position of an undescribed African species

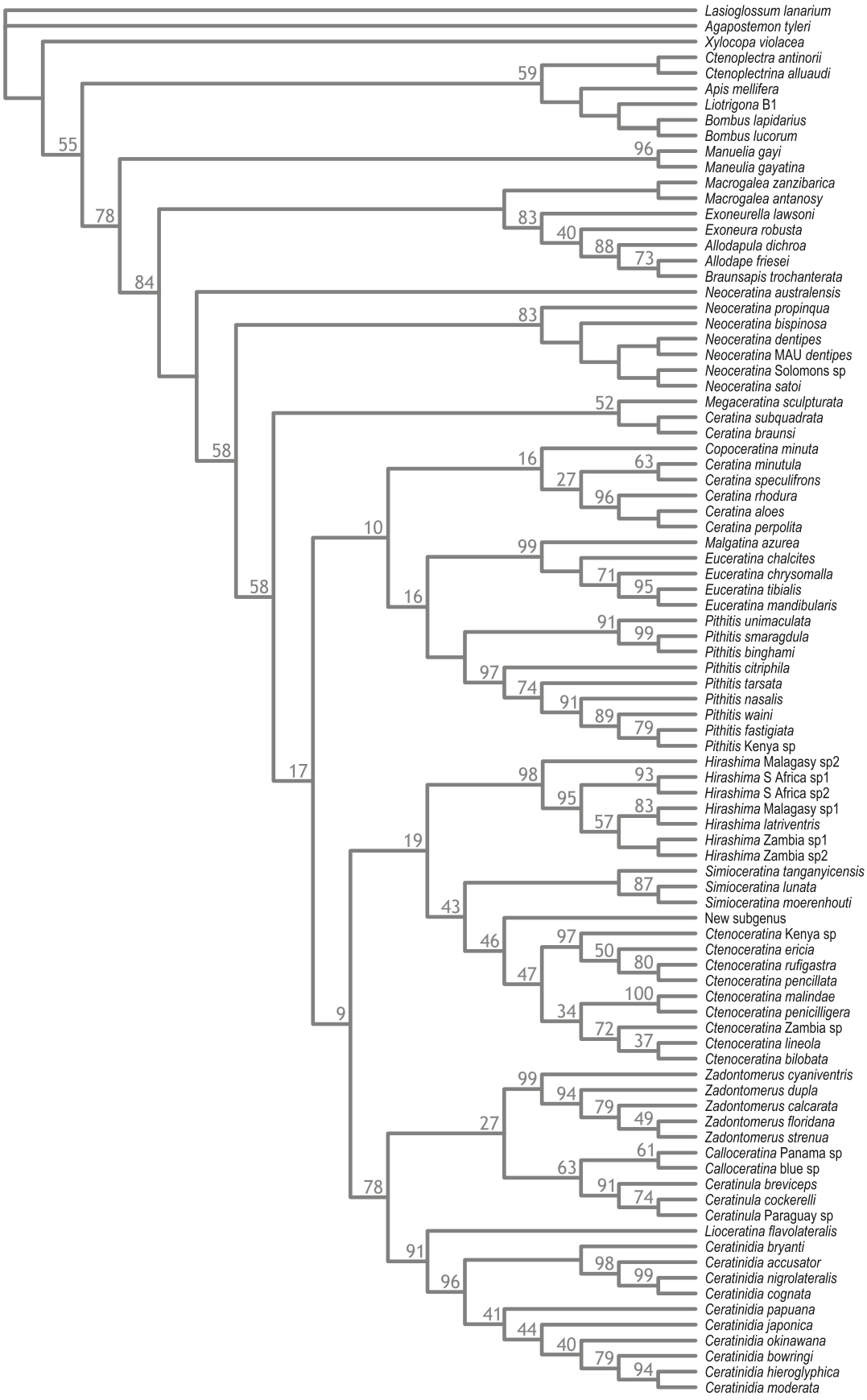

Fig. 1. MP bootstrap tree. Bootstrap support is indicated for each node except nodes with 100% support.

Fig. 2. Consensus phylogram from Bayesian analysis. Posterior probabilities are indicated for each node.

whose morphology justifies subgeneric ranking (and referred to here as 'New subgenus') with respect to *Pithitis* had moderate support (84 PP). Monophyly of the *Pithitis* group was well supported (100 PP), containing a strongly supported Asian (99 PP) and African (100 PP) clade. The node joining the Asian species contained in *Ceratinidia* and *Lioceratina*, and the American species in *Zadontomerus*, *Calloceratina* and *Ceratinula* was highly supported (100 PP). The placement of *Lioceratina* and *Ceratinidia* were highly supported (100 PP), however, the relationship among the three American subgenera was ambiguous (48 PP). Within the *Neoceratina* clade the Mauritian and Malaysian specimens were identical across all three gene regions suggesting these are one species with a recent translocation to Mauritius (see Section 4 below).

Subgeneric groups, with the exception of *Ceratina s. s.*, were all highly supported clades (100 PP). The low PP support values in our BI analysis generally coincided with very short basal branch lengths in the consensus phylogram (Fig. 2). Interestingly, these nodes involve bifurcations among clades with very different global distributions (viz. Madagascar and Palearctic, Africa and Asia, Asia + North America). Understanding these bifurcation events in an historical biogeographical scenario requires that we have some indication of the likely ages of key nodes, and we explore this in the following section.

3.2. Molecular dating

We used penalized likelihood transformation of the Bayesian consensus phylogram to produce a chronogram (Fig. 3), which also indicates the geographic distribution of each species. Results from our BEAST relaxed clock analysis for key node estimated ages and HPDs are given in Table 2 where they are directly compared to results from the r8s analysis. We found broad concordance in estimated ages from the two approaches suggesting that given the fossil calibration points available and the species sampled in this study age estimates are robust to the methods employed. Age estimates were largely identical with the exception of the root node of *Hirashima* and subsequent Malagasy bifurcations (Table 2). This suggests that age estimates are sensitive between methods for recent nodes. For the remainder of this section and the discussion we refer to r8s age estimate as these are most comparable in methodology to phylogenetic literature on other bee groups.

The penalized likelihood point estimate for the crown age of the tribe Ceratinini is 47 ± 8.8 Mya and the relaxed clock analysis gave a very similar result (Table 2). The divergence of the New World *Ceratinula/Zadontomerus* lineage from the lineage leading to the Asian *Lioceratina/Ceratinidia* was estimated at about 32 ± 8.1 Mya and the latter Asian clade had a crown age of 27 ± 7.5 Mya. Relaxed clock dates for these nodes were very similar (Table 2). Dispersal from Africa into Madagascar occurred in at least two lineages. First, the lineage leading to the Malagasy subgenus *Malgatina* split from an African clade some 25 ± 8.4 Mya. Second, the crown group age for the African/Malagasy *Hirashima* was 23 ± 9.3 Mya. Relaxed clock estimates for these two nodes were substantially younger, though confidence intervals were all overlapping (Table 2). It should be remembered that the above estimates are based on two calibration points that are likely to be conservative, so that actual dates may be older, but are unlikely to be younger. When we increased the set age of the root connecting the corbiculates to the Xylocopinae clades from 90 to 120 Mya, we found that the estimated ages of internal nodes increased proportionately and in a linear manner, as Chenoweth et al. (2007) found in their allodapine study. This is probably because the estimated ages for the internal minimum-age calibration points were much older than the set minimums, so that the fixed age of the root node had the strongest effect on scaling the tree.

3.3. Biogeographic analyses

Ancestral geographic ranges were estimated for eight well supported nodes in the Bayesian tree (Fig. 3). BayesMultiState analyses allowed for free rates of biogeographic exchange between the seven ecozones. Analyses suggest an Afrotropical origin at the root of the Ceratinini (node A) where the reconstructed probability for an Afrotropical origin was more than three times greater than for any alternative region. The centre of origin for *Neoceratina* (node B) is less clear, with the Australasian, Indo-Malayan and Palearctic regions having probabilities ranging from 16% to 33% for being ancestral regions. These three regions are geographically contiguous and several species in our analyses occurred in more than one region. Our analyses therefore do not permit us to infer in which ecozone the *Neoceratina* lineage arose, but support for an Afrotropical origin of Ceratinini suggests that *Neoceratina* arose from a north-eastern dispersal from Africa. The next-most distal bifurcations after the split of *Neoceratina* from the other ceratinine clade all have low PP support. This means that we are unable to be confident about related dispersal events among the associated regions. However, strong support for subgeneric nodes and patterns in their regional distributions indicate an African origin with early dispersals extending into all other ecozones prior to 20 Mya.

Distribution ranges suggest three dispersal events subsequent to African diversification. First, the centre of origin of *Hirashima* (node D) suggests an African origin with two dispersal events into Madagascar or a Malagasy origin with two dispersals westward to Africa. Second, the analyses indicated that a Palearctic origin is more likely than a Malagasy origin for the *Malgatina* and *Euceratina* common ancestor (node F) though any dispersals between these regions would have required a presence in Africa with subsequent extinction in that region. Third, *Pithitis* was found as two distinct Afrotropical and Indo-Malayan clades and the root node of these clades had a higher likelihood of comprising an Indo-Malayan lineage than being Afrotropical (node G). Subsequent dispersal out of Africa into the Holarctic was supported by node H, suggesting an Afrotropical to Neotropical, or Indo-Malayan to Neotropical genesis of the New World subgenera and a Palearctic to Indo-Malayan expansion and genesis of *Lioceratina* and *Ceratinidia* (node I).

3.4. Diversification rates over time

The lineage through time (LTT) for the consensus chronogram (Fig. 4) showed a very similar pattern to that of the randomly chosen post-burnin trees with a strong deviation from the linearity that would otherwise be expected if speciation/extinction ratios had remained constant over time. The plots suggest higher rates of cladogenesis up until about 37 Mya, with a levelling off in rates after this time. The graph suggests a further slowing of cladogenesis from about 5 Mya, but this could reflect, at least partially, our taxon sampling regime where we largely avoided inclusion of taxa that were not clearly morphologically distinct. While the LTT plot for the consensus chronogram showed some potentially interesting deviations from linearity between about 30 Mya and the present, variation in the post-burnin LTT plots makes it difficult to discern any clear patterns.

Although LTT plots provide a graphical means for representing diversification rates over time they do not permit any numerical interpretation in themselves. Our estimates of the gamma parameter do, however, allow this but with some strong limitations. The distribution of gamma values for 1000 randomly selected post-burnin trees is contrasted with gamma values based on 5000 randomly generated trees, assuming an actual clade size of 339 terminal taxa (Integrated Taxonomic Information System on-line database, http://www.itis.gov) and reduced to 71 sampled species, in Fig. 5. It is not possible to statistically compare these two distri-

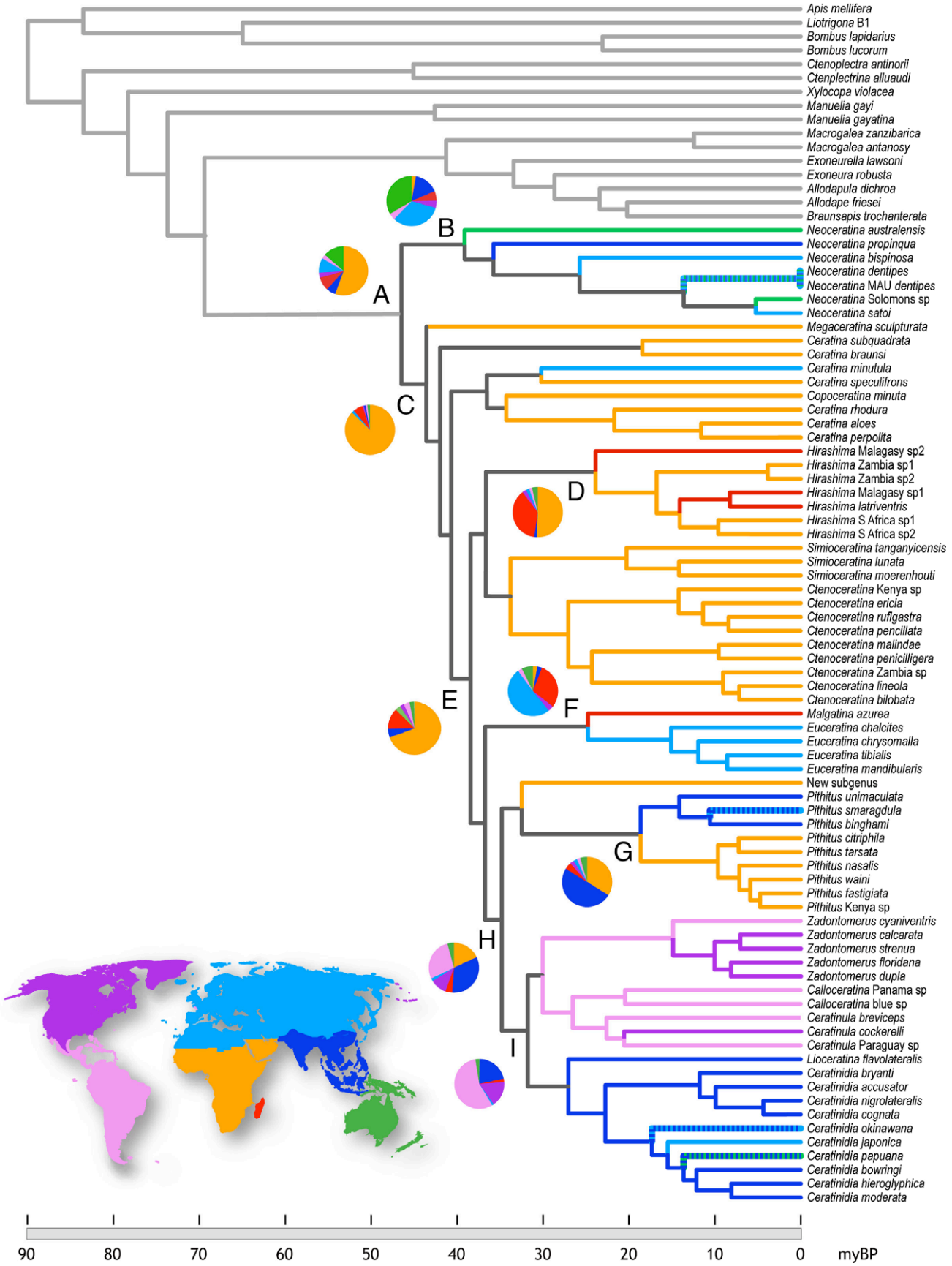

Fig. 3. Chronogram of the Ceratinini derived from penalized likelihood transformation of the consensus Bayesian phylogram. Geographic distributions of each species are colour coded according to the map. BayesMultistate analysis of ancestral geographic reconstructions indicated as pie charts indicating the relative likelihoods of each region at respective nodes (A–I).

Table 2
Comparison of crown age estimates for some key clades, using penalized likelihood (r8s) and relaxed clock (BEAST) methods.

	Penalized likelihood (r8s)		Bayesian relaxed clock (BEAST)	
	Mean	95% CI	Mean	95% CI
Ceratinini	47	39–56	47	32–63
Hirashima	23	14–32	15	6–24
Hirashima lativentris + Malagasy sp1	9	5–13	4	0–9
Simioceratina + *Ctenoceratina*	32	23–40	24	14–36
Malgatina + *Euceratina*	25	17–33	19	4–36
Euceratina	15	8–22	12	2–27
Pithitis	19	12–25	26	8–43
New World subgenera + *Lioceratina* + *Ceratinidia*	32	26–40	32	19–47
Lioceratina + *Ceratinidia*	23	16–30	25	15–38

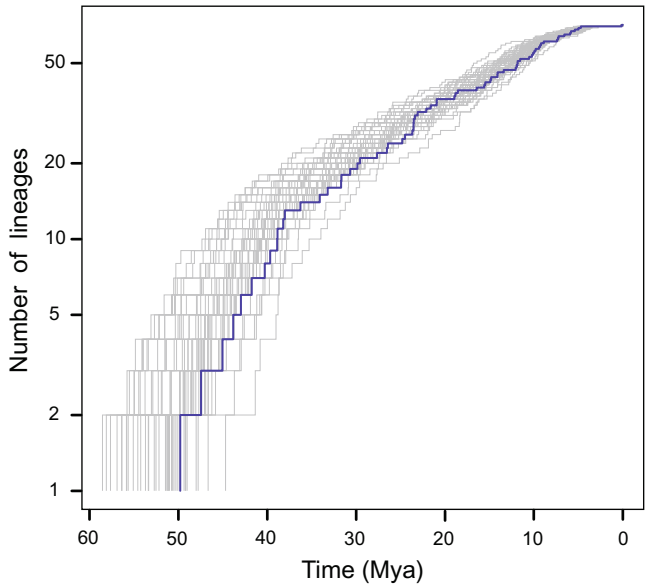

Fig. 4. Lineage through time plot of Ceratinini cladogenesis over time. Grey lines represent 49 randomly selected post-burnin samples and the blue line represents the LTT plot from the consensus chronogram.

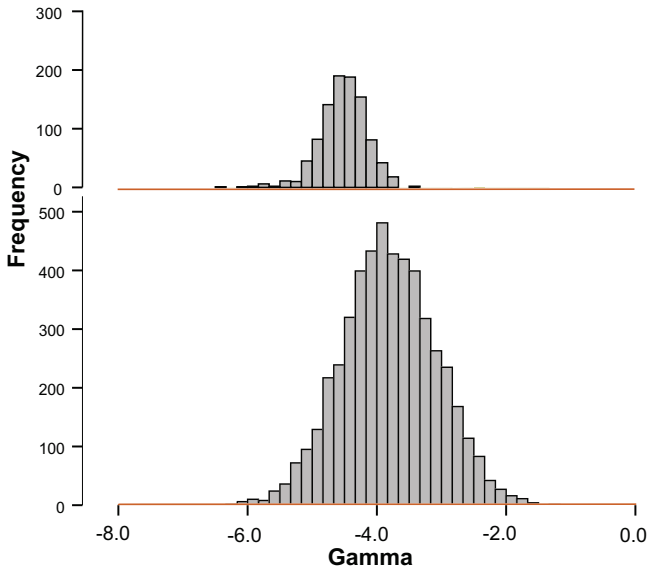

Fig. 5. Gamma distributions of sampled (71 species) versus simulated (339 species) phylogenies. Top: distribution of 1000 randomly-sampled post-burnin trees of the 71 ceratinine species sampled in this study. Bottom: gamma distribution of 5000 trees based on described ceratinine diversity (339 species) with all but 71 terminals randomly deleted. Lower gamma values indicate increasing rates of cladogenesis closer to the root node.

butions since the empirically-derived post-burnin trees do not represent independent samples from a population. Furthermore, the number of post-burnin trees and the number of simulated trees can be arbitrarily large, so that even very small differences in their central tendency could be made significant by simply increasing the post-burnin generations or the number of simulated trees. Given this caveat, the two distributions clearly differ in their central tendencies, with the empirically-derived values tending to lower values, which indicate declining rates of cladogenesis over time. This means that our gamma values suggest that diversification rates were higher in the past than would be expected by undersampling of taxa alone. This concords with our LTT plots and branch lengths separating basal nodes for the consensus chronogram.

4. Discussion

4.1. Phylogeny and evolution of the Ceratinini

The only molecular study of *Ceratina* phylogenetics to date (Cronin, 2004) used a restricted number of species from the Indo-Malayan and Palearctic regions and did not explore divergence times. While Terzo's morphology-based study (2000) examined a large proportion of the described subgenera, the morphological characters used did not permit resolution of many key relationships. Our study takes advantage of an unprecedented DNA sequence database of newly sequenced *Ceratina* species from both the Old and New Worlds. Our resulting phylogenetic hypotheses show some convergences with previous studies, but there are also sharp contrasts. These differences have some important consequences for our understanding of the evolutionary history of this group of bees.

Our analyses recovered all included subgenera as monophyletic groups with the exception of *Ceratina s. s.*, which was paraphyletic. Terzo et al. (2007) have recently described a new subgenus *Dalyatina* with one Mediterranean and six sub-Saharan species from species groups within *Ceratina s. s.*; *C. aloes* and *C. subquadrata* are represented here and *Dalyatina* appears to be polyphyletic (Fig. 2). *Ceratina s. s.* is systematically problematic, found worldwide and contains many species groups (Yasumatsu and Hirashima, 1969; Hirashima, 1971; Pauly et al., 2001; Eardley and Daly, 2007). This subgenus is a potentially important group for understanding the evolutionary patterns in the tribe, but the current taxonomy is clearly in need of revision.

In order to infer a New or Old World origin for this ubiquitous tribe it is important to understand the relationships between the New and Old World subgenera, and it is significant that our results are incongruent with the earlier morphology-based studies by Ter-

zo (2000). We inferred that *Neoceratina* is sister group to all other included ceratinines, including the clade from which the Afrotropical subgenera *Megaceratina* and *Ceratina s. s.* evolved (Figs. 1–3). Conversely, Terzo (2000) recovered the New World subgenus *Zadontomerus* as sister clade to a Holarctic clade in which the widespread Old World subgenus *Neoceratina* and then the New World subgenus *Ceratinula* evolved. On the other hand, our molecular analyses and the previous morphology-based analyses (Terzo, 2000) of the Ceratinini produced broadly similar topologies for the African clades. Both studies strongly support *Hirashima* as sister to the *Ctenoceratina + Simioceratina* clade. Moreover, a close sister-subgenus relationship between *Malgatina* and *Euceratina* is supported by both approaches. Terzo's phylogeny was largely unresolved for older nodes, with a basal polytomy including numerous Old World subgenera, so that inferring origins and subsequent dispersal patterns was difficult. Our results indicated that the phylogenetic signal in our molecular data set was stronger for these deeper nodes, and provides strong support for an Old World origin with a single dispersal into the New World followed by radiation there and no back-dispersal to the Old World. The historical biogeography of the tribe will be discussed in more detail in the following section.

4.2. Age and origin of the Ceratinini

Incomplete sampling of subgenera in our study could create some problems for inferring ancestral regions if missing subgenera are geographically biased. We did not have specimens for seven of the 23 subgenera. These missing subgenera contain about 30 species from a total number of about 200 described species that Michener (2007) ascribes to each subgenus, or about 15% of described ceratinines. In terms of geographic representation our samples do not appear to be biased: we included three of the five New World subgenera, nine of the eleven subgenera with representatives in Africa and Madagascar, three of the five subgenera with representatives in the Indo-Malayan region (although two of the missing Indo-Malayan subgenera are monotypic), and three of the four subgenera with representatives in the Palaearctic.

Our results suggested an African origin for the tribe approximately 47 Mya. An African origin is similar to that proposed for the closely related and similarly aged (~47 Mya) bee tribe Allodapini (Schwarz et al., 2006). However, both the inferred origin times and regions of origin for these two tribes is complicated by a key factor, the fossil tribe Boreallodapini. Three species from this tribe are recorded from Baltic amber dated at 44.1 ± 1.1 Mya (Engel, 2001) and Engel (2001) proposed that the Boreallodapini forms the sister tribe to the Allodapini, with the Ceratinini being the next-most basal tribe in the Xylocopinae. An Oriental origin was proposed for the closely related and similarly aged (~45 Mya), and globally distributed large carpenter bee genus *Xylocopa* (Leys et al., 2002).

Our results preclude a New World origin for the Ceratinini since the Nearctic and Neotropical clades are clearly distal in our phylogeny. A Eurasian origin would be concordant with the existence of the Baltic fossil tribe Boreallodapini and a Palearctic/Indo-Malayan origin for *Neoceratina*. However, an African origin for the tribe seems more likely since a Eurasian origin would require minimal diversification of what would be a relictual Eurasian *Neoceratina* clade, with a single dispersal into Africa, followed by large scale diversification there and subsequent dispersals out of Africa. Moreover, both biodiversity considerations (Michener, 1979) and morphological phylogenetics (Terzo, 2000) of the ceratinines have suggested an African origin with subsequent dispersals into Asia and the New World. Given an African origin of the Ceratinini, our analyses suggest multiple dispersals out of Africa, represented by the *Neoceratina* clade, the clade leading to *Ceratina minutula*, the clade leading to *Euceratina*, and the clade leading to the Asian *Ceratinidia/Lioceratina* and the New World subgenera. Presently, we cannot be certain of the number and direction of these dispersal events due to the low support for basal nodes.

The New World ceratinines present two possible biogeographic scenarios. The sister relationship between the New World subgenera and the Old World Asian *Ceratinidia* and *Lioceratina* support the notion of a Bering Strait dispersal some 32 Mya. This dispersal timing is similar to that of two other cosmopolitan bee genera *Bombus* and *Xylocopa*, both of which are inferred to have had the same dispersal route across the Bering Strait, approximately 20 and 34 Mya, respectively (Hines, 2008; Leys et al., 2002). Conversely, the low support at basal nodes and African antecedents cannot preclude an Afrotropical to Neotropical dispersal as found in some halictid bees (Danforth et al., 2008). Southern hemisphere long-range oceanic dispersals have also been proposed for stem nesting allodapine bees (Schwarz et al., 2006).

The Ceratinini are of cosmopolitan distribution whereas their sister tribe, the Allodapini, are found only across the Old World and with limited representation in the Palearctic. In contrast to the Ceratinini, *Xylocopa* (Leys et al., 2002) and *Bombus* (Hines, 2008), the Allodapini (Schwarz et al., 2006) and Ctenoplectrini (Schaefer and Renner, 2008) are limited to an Old World distribution. This limited distribution could be explained if dispersal in Laurasia was limited by requirements for tropical or subtropical habitats, and indeed Eurasian Allodapini and Ctenoplectrini are found in low latitude landscapes. The only Eurasian allodapines that occur outside tropical and subtropical areas are in the rare Middle Eastern genus *Exoneuridia*. The only *Exoneuridia* species where nests have been found is *E. hakkariensis* and it is unique among allodapines by nesting in rock cavities on cliff faces (Schwarz unpub. data). Conversely the Ceratinini, *Xylocopa* and *Bombus* are found across the Holarctic with species distributions into the boreal forests above 50°N latitude (Bishop and Armbruster, 1999; Janzon and Svensson, 1988; Malyshev, 1931). These species are known for their cold hardiness and resilience (Sakagami et al., 1981; Somanathan and Borges, 2001; Corlett, 2001, 2004) a requisite adaptation to surviving northern climates. In addition, the Ceratinini and *Xylocopa* have truly cosmopolitan ranges with more flexible habitat preferences, also being able to spread in warm habitats (Michener, 1979). Conversely, *Bombus* do not extend into tropical areas and therefore has a less cosmopolitan range than Ceratinini. The remarkable range covering both boreal and tropical habitats and physiological adaptation to a mix of cold and thermotolerance make the Ceratinini and *Xylocopa* of interest for further studies on diversification and dispersal abilities of the bees.

4.3. Malagasy bee fauna

There have been at least two dispersals of *Ceratina* from Africa to Madagascar. The first dispersal of ceratinines across the Mozambique Channel is estimated at 25 Mya giving rise to the endemic *Malgatina*. This was followed by a second and perhaps third dispersal and radiation by *Hirashima* 23 and 9 Mya. Our analyses indicate that a Malagasy origin and subsequent dispersal westward into Africa, or two distinct dispersals from Africa to Madagascar, are equally parsimonious.

The endemism of the Malagasy fauna has been well documented in recent years (Pauly et al., 2001). Phylogenetic studies have shown recent and recurrent dispersal of African fauna into Madagascar across the 450 km wide Mozambique Channel. Madagascar reached its current distance from Africa some 80 Mya, yet some fauna appear to have arrived more recently (Yoder and Nowak, 2006). Rafting and wind dispersion are common hypotheses for this long-range oceanic dispersal.

The bee fauna of Madagascar has recently been surveyed, with Pauly et al. (2001) documenting nine endemic genera, and Chenoweth et al. (2008) describing an additional endemic genus. Molecular dating analyses indicate that all of the inferred African-Malagasy bee dispersal events were less than 30 Mya. Furthermore, there are no bee tribes in Madagascar that are not present in Africa (Pauly et al., 2001), suggesting that the distinctive nature of the Malagasy bee fauna is unlikely to have a very ancient origin (Eardley et al., 2009). The recent and recurrent origins of Malagasy bee genera may instead reflect moderately old to recent events followed by radiation in a new environment. The multiple dispersals of Ceratinini from Africa to Madagascar is similar to *Charaxes* butterflies, where there have been at least three dispersal events over the period of 20–13 Mya (Aduse-Poku et al., 2009).

One major puzzle that arises from our analyses is the monophyly of the Palearctic *Euceratina* and Malagasy *Malgatina* species without any African representation of either subgenus. Comparison of 51 morphological characters across the Ceratinini suggested that *Euceratina* and *Malgatina* are sister subgenera nested within the African taxa (Terzo, 2000). The elaborate male genitalia, metallic colouration and dense punctuation are but a few of the commonalities. It is possible that the *Malgatina* in Madagascar are truly indigenous and evidence of dispersal from Eurasia to Madagascar has been lost through extinction in Africa or that dispersal did not involve an African route. It is difficult to see how the lineage leading to *Malgatina* could have reached Madagascar without an African presence, suggesting that such an African clade must have become extinct. This possibility was also suggested by Terzo (2000) in his analysis of *Euceratina* exemplars and the Malagasy *Malgatina azurea*. Conversely, anthropogenic dispersal seems likely to explain the occurrence of *Neoceratina dentipes* in Mauritius. *Neoceratina dentipes* is abundant and wide-spread across Asia but unknown in Africa. Finding the same species off the coast of Africa, therefore, suggests anthropogenic dispersal from Malaysia to Mauritius, a known trade route over the past century or more (Mountain and Proust, 2000; Rudwick, 2005).

4.4. Rapid radiations

Ancient rapid radiations, defined as rapid speciation over short evolutionary time scales, have been found in numerous plant and animal groups (Whitfield and Lockhart, 2007). The phylogenetic topology is one of compressed cladogenesis compared to that expected by constant diversification (Rokas et al., 2005). Rapid radiations are especially recurrent across insect orders and many of these seem to correspond with angiosperm radiations of the Cretaceous and Tertiary including Lepidoptera and their parasitoids, phytophagous Coleoptera, and corbiculate bees (reviewed in Whitfield and Kjer, 2008). Phylogenies of ancient groups often lack resolution during times of rapid radiation generating patterns of molecular and morphological changes that are difficult to resolve phylogenetically. Here we observed signature short basal branch lengths (Fig. 3) and rapid cladogenesis (Figs. 4 and 5) suggesting high rates of diversification during early evolution of the ceratinines.

Comparing phylogenies among closely related groups can reveal the differences in rates of cladogenesis and signs of relaxed constraint in some taxa. The poor resolution of the basal nodes of the Ceratinini using the same molecules as its relatively well resolved sister tribe Allodapini, suggests that the ceratinines are somewhat unique; radiating rapidly and potentially relaxed from evolutionary constraints seen in the Allodapini. Thus, the aforementioned taxonomic uncertainty among early African ceratinines is not so surprising considering the marked morphological variation among subgenera; species ranging from 2.2 to 12.5 mm in body length, with an array of: dull black to metallic blue green colouration, smooth to punctuate surface sculpturing, hairless to plumose appendages, and elaborate abdominal setae, tegument maculation, and clypeal protrusion unique among subgeneric groups. Conversely, allodapine bees are relatively monomorphic possessing some size and morphological variation, but to a much lesser extent than the ceratinines. Revision of poorly resolved microgastrine wasps found that additional genes did not and, after modelling putative genes, likely will never resolve short internal branches (Banks and Whitfield, 2006). However, these authors do suggest combining molecular and morphological characters to increase support for deep branches in the phylogeny. This approach is certainly worth pursuing for the ceratinines following further taxonomic revision of the group.

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References

Aduse-Poku, K., Vingerhoedt, E., Wahlberg, N., 2009. Out-of-Africa again: a phylogenetic hypothesis of the genus *Charaxes* (Lepidoptera: Nymphalidae) based on 5 gene regions. Mol. Phylogenet. Evol. 53, 463–478.

Alexander, B.A., Michener, C.D., 1995. Phylogenetic studies of the families of short-tongued bees (Hymenoptera: Apoidea). Univ. Kansas Sci. Bull. 55, 377–424.

Banks, J.C., Whitfield, J.B., 2006. Dissecting the ancient rapid radiation of microgastrine wasp genera using additional nuclear genes. Mol. Phylogenet. Evol. 41, 690–703.

Berger, J., 2006. The case for objective Bayesian analysis. Bayesian Anal. 1, 385–402.

Bishop, J.A., Armbruster, W.S., 1999. Thermoregulatory abilities of Alaskan bees: effects of size phylogeny and ecology. Funct. Ecol. 13, 711–724.

Bull, N.J., Schwarz, M.P., Cooper, S.J.B., 2003. Phylogenetic divergence of the Australian allodapine bees (Hymenoptera: Apidae). Mol. Phylogenet. Evol. 27, 212–222.

Cameron, S.A., Hines, H.M., Williams, P.H., 2007. A comprehensive phylogeny of the bumble bees (*Bombus*). Biol. J. Linn. Soc. 91, 161–188.

Chenoweth, L.B., Fuller, S., Tierney, S.M., Park, Y.C., Schwarz, M.P., 2008. *Hasinamelissa*: a new genus of allodapine bee from Madagascar revealed by larval morphology and DNA sequence data. Syst. Entomol. 33, 700–710.

Chenoweth, L.B., Tierney, S.M., Smith, J.A., Cooper, S.J.B., Schwarz, M.P., 2007. Social complexity in bees is not sufficient to explain lack of reversions to solitary living over long time scales. BMC Evol. Biol. 7, 246.

Cockerell, T.D.A., 1906. Fossil Hymenoptera from Florissant Colorado. Bull. Mus. Comp. Zool., Harvard 50, 33–58.

Corlett, R.T., 2001. Pollination in a degraded tropical landscape: a Hong Kong case study. J. Trop. Ecol. 17, 155–161.

Corlett, R.T., 2004. Flower visitors and pollination in the Oriental (Indomalayan) region. Biol. Rev. 79, 497–532.

Crepet, W.L., Nixon, K.C., 1998. Fossil Clusiaceae from the Late Cretaceous (Turonian) of New Jersey and implications regarding the history of bee pollination. Am. J. Bot. 85, 1122–1133.

Cronin, A.L., 2004. A molecular phylogeny and social behaviour of Japanese *Ceratina* (Hymenoptera, Apidae, Xylocopinae). Insect Syst. Evol. 35, 137–146.

Daly, H.V., 1973. Bees of the Genus *Ceratina* in America North of Mexico. University of California Press, Berkley, CA. 113 pp.

Daly, H.V., 1988. Bees of the New Genus *Ctenoceratina* in Africa, South of the Sahara (Hymenoptera: Apoidea). University of California Press, Berkley, CA. 69 pp.

Danforth, B.N., Brady, S.G., Sipes, S.D., Pearson, A., 2004. Single-copy nuclear genes recover Cretaceous-age divergences in bees. Syst. Biol. 53, 309–326.

Danforth, B.N., Eardley, C., Packer, L., Walker, K., Pauly, A., Rankdrianambinintsoa, F.J., 2008. Phylogeny of Halictidae with emphasis on endemic African Halicitinae. Apidologie 39, 86–101.

Danforth, B.N., Sauquet, H., Packer, L., 1999. Phylogeny of the bee genus *Halictus* (Hymenoptera: Halictidae) based on parsimony and likelihood analyses of nuclear EF-1a sequence data. Mol. Phylogenet. Evol. 13, 605–618.

Danforth, B.N., Fang, J., Sipes, S., 2006a. Analysis of family-level relationships in bees (Hymenoptera: Apiformes) using 28S and two previously unexplored nuclear genes: CAD and RNA polymerase II. Mol. Phylogenet. Evol. 39, 358–372.

Danforth, B.N., Sipes, S., Fang, J., Brady, S.G., 2006b. This history of early bee diversification based on five genes plus morphology. Proc. Natl. Acad. Sci. (USA) 103, 15118–15123.
Drummond, A.J., Nicholls, G.K., Rodrigo, A.G., Solomon, W., 2002. Estimating mutation parameters, population history and genealogy simultaneously from temporally spaced sequence data. Genetics 161, 1307–1320.
Drummond, A.J., Rambaut, A. Suchard M., 2009. BEAST version 1.5.2. Available from: <http://evolve.zoo.ox.ac.uk/beast/>.
Eardley, C., Daly, H.V., 2007. Bees of the genus *Ceratina* Latreille in Southern Africa (Hymenoptera, Apoidea). Entomofauna Suppl. 13, 1–93.
Eardley, C., Gikungu, M., Schwarz, M.P., 2009. Bee conservation in Sub-Saharan Africa and Madagascar: diversity, status and threats. Apidologie 40, 355–366.
Engel, M.S., 2000. A new interpretation of the oldest fossil bee (Hymenoptera: Apidae). Am. Mus. Novit. 3296, 1–11.
Engel, M.S., 2001. A monograph of the Baltic amber bees and the evolution of the Apoidea. Bull. Am. Mus. Nat. Hist. 259, 1–192.
Fuller, S., Schwarz, M.P., Tierney, S.M., 2005. Phylogenetics of the allodapine bee genus *Braunsapis*: historical biogeography and long-range dispersal over water. J. Biogeogr. 32, 2135–2144.
Grimaldi, D., 1999. The co-radiations of pollinating insects and angiosperms in the Cretaceous. Ann. Mo. Bot. Gard. 86, 373–406.
Hines, H.M., 2008. Historical biogeography, divergence times, and diversification patterns of bumble bees (Hymenoptera: Apidae: *Bombus*). Syst. Biol. 57, 58–75.
Hirashima, Y., 1971. *Megaceratina*, a new genus of bees from Africa (Hymenoptera, Anthophoridae). J. Nat. Hist. 5, 251–256.
Huelsenbeck, J.P., Ronquist, F., 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17, 754–755.
Janzon, L.A., Svensson, B.G., 1988. 110 years with the carpenter bee *Ceratina cyanea* (Myn., Anthophoridae) in Sweden. Entomol. Tidskr. 109, 19–23.
Klug, J.C.F., 1807. Vergleichung der Gattungen der Hautfliiger Piezata Fabr. Hymenoptera Linn. Jur. Magazin für Insektenkunde 6, 189–199.
Leys, R., Cooper, S.J.B., Schwarz, M.P., 2002. Molecular phylogeny and historical biogeography of the large carpenter bees, genus *Xylocopa* (Hymenoptera: Apidae). Biol. J. Linn. Soc. 77, 249–266.
Lunt, D.H., Zang, D.X., Szymura, J.M., Hewitt, G.M., 1996. The insect cytochrome oxidase I gene: evolutionary patterns and conserved primers for phylogenetic studies. Insect Mol. Biol. 5, 153–165.
Malyshev, S.J., 1931. Lebensgeschichte der holzbienen *Xylocopa* Latr. (Apoidea). Z. Morphol. Oekol. Tiere. 23, 754–809.
Michener, C.D., 1962. The genus *Ceratina* in Australia with notes on its nests (Hymenoptera: Apoidea). J. Kansas Entomol. Soc. 35, 414–421.
Michener, C.D., 1979. Biogeography of the bees. Ann. Mo. Bot. Gard. 66, 277–347.
Michener, C.D., 2007. The Bees of the World, second ed. John Hopkins University Press, Baltimore. 953 pp.
Michener, C.D., Grimaldi, D.A., 1988a. A *Trigonia* from Late Cretaceous amber of New Jersey (Hymenoptera: Apidae: Meliponinae). Am. Mus. Novaitates 2917, 10.
Michener, C.D., Grimaldi, D.A., 1988b. The oldest fossil bee: apiod history, evolutionary stasis, and antiquity of social behaviour. Proc. Natl. Acad. Sci. (USA) 85, 6424–6426.
Mountain, A., Proust, A., 2000. This is Mauritius. Struik, Cape Town. 160 pp.
Pagel, M., Meade, A., Barker, D., 2004. Bayesian estimation of ancestral character states on phylogenies. Syst. Biol. 53, 673–684.
Pagel, M., Meade, A., 2006. Bayesian analysis of correlated evolution of discrete characters by reversible-jump Markov chain Monte Carlo. Am. Nat. 167, 808–825.
Paradis, E., Claude, J., Strimmer, K., 2004. APE: An R package for analyses of phylogenetics and evolution. Bioinformatics 20, 289–290.
Pauly, A.R., Brooks, W., Nilsson, L.A., Persenko, Y.A., Eardley, C.D., Terzo, M., Griswold, T., Schwarz, M., Patiny, S., Munzinger, J., Barbier, Y., 2001. Hymenoptera Apoidea de Madagascar. Annalen Zoologische Wetenschappen 128, 1–390.
Poinar Jr., G.O., Danforth, B.N., 2006. A fossil bee from Early Cretaceous Burmese amber. Science 314, 614.
Pybus, O.G., Harvey, P.H., 2000. Testing macro-evolutionary models using incomplete molecular phylogenies. Proc. Roy. Soc. Lond. B 267, 2267–2272.
Rabosky, D.L., 2006. LASER: a maximum likelihood toolkit for detecting temporal shifts in diversification rates from molecular phylogenies. Evol. Bioinform. Online 2, 247–250.
Rambaut, A., Charleston, M., 2001. TreeEdit: phylogenetic tree editor. Version 1.0. Department of Zoology, University of Oxford. URL <http://evolve.zoo.ox.ac.uk/software/TreeEdit/main.html>.
Ricklefs, R.E., 2007. Estimating diversification rates from phylogenetic information. Trends Ecol. Evol. 22, 601–610.
Roig-Alsina, A., Michener, C.D., 1993. Studies of the phylogeny and classification of long-tongued bees (Hymenoptera: Apoidea). Univ. Kansas Sci. Bull. 55, 123–173.
Rokas, A., Kruger, D., Carroll, S.B., 2005. Animal evolution and the molecular signature of rapidations compressed in time. Science 310, 1933–1938.
Rudwick, M.J.S., 2005. Bursting the Limits of Time: The Reconstruction of Geohistory in the Age of Revolution. University of Chicago Press, Chicago. 780 pp.
Sanderson, M.J., 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. Mol. Biol. Evol. 19, 101–109.
Sakagami, S.F., Tanno, K., Enomoto, O., 1981. Cold resistance of the small carpenter bee *Ceratina flavipes* restudied. Low Temp. Sci. Ser. B 39, 1–7.
Sakagami, S.F., Michener, C.D., 1987. Tribes of the Xylocopinae and origin of the Apidae. Ann. Entomol. Soc. Am. 80, 439–450.
Schaefer, H., Renner, S.S., 2008. A phylogeny of the oil bee tribe Ctenoplectrini (Hymenoptera: Anthophila) based on mitochondrial and nuclear data: evidence for early Eocene divergence and repeated out-of-Africa dispersal. Mol. Phylogenet. Evol. 47, 799–811.
Schwarz, M.P., Bull, N.J., Cooper, S.J.B., 2003. Molecular phylogenetics of allodapine bees, with implications for the evolution of sociality and progressive rearing. Syst. Biol. 52, 1–14.
Schwarz, M.P., Fuller, S., Tierney, S.M., Cooper, S.J.B., 2006. Molecular phylogenetics of the exoneurine allodapine bees reveal an ancient and puzzling dispersal from Africa to Australia. Syst. Biol. 55, 31–45.
Schwarz, M.P., Tierney, S.M., Cooper, S.J.B., Bull, N.J., 2004. Molecular phylogenetics of the allodapine bee genus *Braunsapis*: A–T bias and heterogeneous substitution parameters. Mol. Phylogenet. Evol. 32, 110–122.
Simon, C., Frati, F., Bechenbach, A., Crespi, B., Liu, H., Flook, P., 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. 69, 168–176.
Somanathan and Borges, 2001. Nocturnal pollination by the carpenter bee *Xylocopa tenuiscapa* (Apidae) and the effect of floral display on fruit set of *Heterophargma quadriloculare* (Bignoniacceae) in India. Biotropica 33, 78–89.
Swofford, D.L., 1999. PAUP. Phylogenetic analysis using parsimony (*and other methods). Version 4.0b. Sinauer Associates, Sunderland, MA.
Terzo, M., 2000. Classification phylogénétique des Cératines du monde et monographie des espèces de la région ouest-paléarctique et de l'Asie Centrale (Hymenoptera, Apoidea, Xylocopinae: *Ceratina* Latreille) Ph.D. dissertation, Universite de Mons-Hainaut.
Terzo, M., Iserbyt, S., Rasmont, P., 2007. Révision des Xylocopinae (Hymenoptera: Apidae) de France et de Belgique. Ann. Soc. Entomol. Fr. 43, 445–491.
Whitfield, J.B., Kjer, K.M., 2008. Ancient rapid radiations of insects: challenges for phylogenetic analysis. Ann. Rev. Entomol. 53, 449–472.
Whitfield, J.B., Lockhart, P.J., 2007. Deciphering ancient rapid radiations. Trends Ecol. Evol. 22, 258–265.
Yasumatsu, K., Hirashima, Y., 1969. Synopsis of the small carpenter bee genus *Ceratina* of Japan (Hymenoptera, Anthophoridae). Kontyu 37, 61–70.
Yoder, A.D., Nowak, M.D., 2006. Has vicariance or dispersal been the predominant biogeographic force in Madagascar? Only time will tell. Ann. Rev. Ecol. Evol. Syst. 37, 405–431.