



Global stingless bee phylogeny supports ancient divergence, vicariance, and long distance dispersal

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Stingless bees (Meliponini) are one of only two highly eusocial bees, the other being the well studied honey bee (Apini). Unlike Apini, with only 11 species in the single genus *Apis*, stingless bees are a large and diverse taxon comprising some 60 genera, many of which are poorly known. This is the first attempt to infer a phylogeny of the group that includes the world fauna and extensive molecular data. Understanding the evolutionary relationships of these bees would provide a basis for behavioural studies within an evolutionary framework, illuminating the origins of complex social behaviour, such as the employment of dance and sound to communicate the location of food or shelter. In addition to a global phylogeny, we also provide estimates of divergence times and ancestral biogeographic distributions of the major groups. Bayesian and maximum likelihood analyses strongly support a principal division of Meliponini into Old and New World groups, with the Afrotropical+Indo-Malay/Australian clades comprising the sister group to the large Neotropical clade. The meliponine crown clade is inferred to be of late Gondwanan origin (approximately 80 Mya), undergoing radiations in the Afrotropical and Indo-Malayan/Australasian regions, approximately 50–60 Mya. In the New World, major diversifications occurred approximately 30–40 Mya. © 2010 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2010, 99, 206–232.

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INTRODUCTION

Stingless bees (Meliponini) are a large monophyletic group of highly eusocial bees (Michener, 1974) found in abundance in warm humid forests around the globe. They are indispensable pollinators within tropical ecosystems (Roubik, 1989), and vary widely in both individual and colony size. They share the presence of a corbicula, a pollen-carrying structure on the hind legs, with the other corbiculate bees, which include the highly eusocial honey bees (Apini), primitively eusocial bumble bees (Bombini), and the mostly solitary orchid bees (Euglossini) (Michener, 2007). Although stingless bees and honey bees both exhibit highly eusocial behaviour (Michener, 1974), including perennial colonies of workers and a single queen, the two tribes have likely evolved their particular kind of sociality independently (Cameron & Mardulyn, 2001; Kawakita *et al.*, 2008; Whitfield *et al.*, 2008). Stingless bees are therefore a vital key to understanding

independent evolution of complex social behaviour, such as the employment of dance and sound to communicate the location of food or shelter.

As well as advancing our understanding of social evolution, stingless bees are the only group of social bees to have left an imprint in the fossil record spanning most of the Cenozoic. Hence, they offer an unusual opportunity to examine the early biogeographic history and colonization pattern of a highly eusocial bee. A Gondwanan origin appears possible because they are an old group with a worldwide distribution restricted to tropical regions. This scenario is testable only with a robust phylogeny from which ancestral distribution patterns and the direction of evolution of the different biogeographically distinct clades (Rasmussen & Cameron, 2007) can be inferred within an estimated time frame. These comprise the aims of the present study.

FOSSIL ORIGINS

A good fossil record is important in the estimation of divergence times, both for placing a minimum age on

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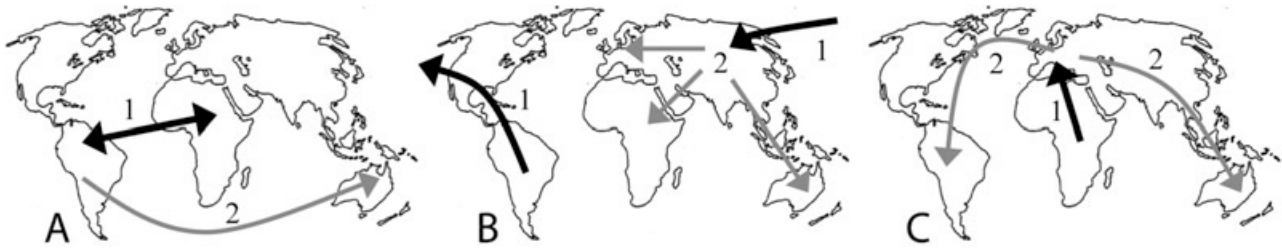


Figure 1. Three proposed biogeographical hypotheses of the stingless bees. A, based on the distribution of a putative *Plebeia* lineage, with extant taxa in the Neotropical, Afrotropical, and Australasian regions, Camargo & Wittmann (1989) proposed a Gondwanan origin in which South American taxa became separated from Afrotropical taxa during the opening of the Atlantic Ocean. Subsequently, the Neotropical *Plebeia* dispersed to Australasia through Antarctica. The number on the arrow refers to the order in which the event happened, the double arrowhead refers to a vicariant event, a single arrowhead refers to dispersal. B, Kerr & Maule (1964) suggested an origin in South America, later northerly movement with dispersal via the Bering land bridge to the Palearctic region, followed by dispersal to Africa and Asia. C, Wille (1979) argued that stingless bees originated in Africa, then dispersed to Europe and later moved into the Indo-Malay/Australasian and Neotropical regions.

the crown group of interest and for calibrating the ages of multiple clades across the group. Several fossils can be brought to bear on estimating the divergence times of Meliponini. That they are an ancient lineage is evident from *Cretotrigona prisca*, a Nearctic meliponine fossil that dates at least to the Late Cretaceous (approximately 65 Mya) and is the oldest known Apidae (Michener & Grimaldi, 1988b; Engel, 2000). The age of the stingless bees must therefore be older than 65 Myr and probably younger than the oldest known bee-like fossil, *Melittosphex burmensis* (100 Mya; Poinar & Danforth, 2006), or at least younger than the radiation of the angiosperms (125 Mya; Morley, 2003; Soltis *et al.*, 2005), which may have prompted the early radiation of bees (Grimaldi, 1999; Grimaldi & Engel, 2005). More recent stingless bee fossils include *Proplebeia* from Early Miocene Dominican and Mexican amber (15–20 Mya; Wille, 1959; Wille & Chandler, 1964; Camargo, Grimaldi & Pedro, 2000) and *Liotrigonopsis* and *Kelneriapis* from Middle Eocene Baltic amber (44.1 Mya; Kelner-Pillault, 1969, 1970; Engel, 2001a). Additional Middle Eocene Baltic taxa belonging to the corbiculate bees are known, but their systematic position remains to be explored rigorously (Engel, 2001a). Of these, only the extinct tribe Melikertini resemble stingless bees and may be an extinct sister clade (Engel, 2001a, b; Michener, 2007).

BIOGEOGRAPHIC HYPOTHESES

Stingless bees have been recorded from the Nearctic (from amber of the Late Cretaceous) and Palearctic (amber of the Middle Eocene and Late Oligocene), but have subsequently gone extinct in those regions (Tosi, 1896; Engel, 2000, 2001a). Currently, they are a pantropical group found in South and Central America,

including Mexico, sub-Saharan Africa, India, and southern China to Australia. They are not native on most volcanic islands (Michener, 1979, 2007). Stingless bees have limited dispersal ability because of their gradual and progressive colony establishment (Nogueira-Neto, 1954) and a short flight range (Araújo *et al.*, 2004), making successful transport across water highly unlikely by individual reproductives or swarms (Michener, 2007). This leads to the questions of when and how the stingless bees achieved their disjunct global distribution.

Four hypotheses have been proposed to explain the phases and modes of stingless bee distribution. Camargo & Wittmann (1989) hypothesized an Early-Cretaceous Gondwanan origin of the stingless bees, with subsequent interchange through a land connection between southern South America, Antarctica, and Australia (Fig. 1A). This hypothesis developed out of their interest in explaining the current distribution of *Plebeia*, a putative monophyletic clade thought to be widely distributed in the Neotropical (*Plebeia*, *Friesella*, *Mourella*, *Schwarziana*), Afrotropical (*Meliplebeia*, *Plebeiella*, *Plebeina*), and Australasian (*Austroplebeia*) Regions, and nowhere else. We assume, however, from the recent phylogeny of Rasmussen & Cameron (2007) that New World *Plebeia*-like taxa are not related to any of the Old World clades, and therefore the framework for their argument is not supported.

By contrast, other dispersal models have hypothesized a post-Gondwanan Laurasian/Australasian interchange. For example, Kerr & Maule (1964) suggested that stingless bees originated and diversified in South America, expanded their range during the Eocene, reached the Nearctic and Palearctic via the Bering Strait, finally arriving in Indo-Malay/Australasia and Africa during the Late Oligocene

(Fig. 1B). Their argument rested on the relatively large number of South American stingless bee species, a number of characters thought to be 'primitive', and the Late Oligocene European fossils known at that time. The hypothesis assumed a land bridge between South and Central America during the Palaeocene–Eocene, but it is now known that South America was isolated for most of the past 100 million years, joining North America via the Isthmus of Panama only within the last 3–4 Myr (Burnham & Graham, 1999; Burnham & Johnson, 2004; Corlett & Primack, 2006). Their South American dispersal scenario is therefore unlikely during the inferred time period.

Michener (1990) proposed that stingless bees arose in tropical America at a time when a tropical climate extended into North America. He based this on his inferred phylogeny (Michener, 1990: 92), in which the genus *Melipona*, an exclusively New World taxon, was the sister group to the rest of the stingless bees. He argued that a number of the basal groups supported a New World origin of the stingless bees. Without discussing the time frame of events, Michener (1990) suggested that the stingless bees would have dispersed or migrated from the New World to achieve their current pantropical range. In addition, he noted that no stingless bee taxa are shared between Africa and South America, suggesting the two faunas originated after the opening of the South Atlantic Ocean with the breakup of Gondwana. He did not explain the distribution of *Trigona s.l.* (Rasmussen & Cameron, 2007) in both the Neotropical and Indo-Malayan/Australasian regions.

By contrast to these New World hypotheses, Wille (1979: 255) proposed that stingless bees originated in Africa during the Late Cretaceous or Early Tertiary and then dispersed to southern Europe during the Eocene, when land bridges formed between the two continents; later, he argued, they dispersed to their current range (Fig. 1C). His arguments rested on his views of the taxonomic distribution of 'primitive' characters (although he had no quantitative phylogeny) and the known Palearctic fossils.

Testing the validity of these or any newly-informed biogeographic hypotheses will depend largely on the availability of a robust phylogeny. Only Kerr & Maule's (1964) scenario can be rejected at this time, based on a lack of congruence with known geological history.

PHYLOGENETIC BACKGROUND

Encompassing more than 600 described (and many undescribed) species in approximately 61 genera (for a discussion of generic ranking, see Rasmussen & Cameron, 2007), the taxonomic diversity of the stingless bees is higher than that of all of the other

corbiculate bees combined (Moore, 1961; Pauly *et al.*, 2001; Eardley, 2004; Camargo & Pedro, 2007; Michener, 2007; Rasmussen & Cameron, 2007; Rasmussen, 2008). Several studies over the last two decades using morphological characters (Michener, 1990; Camargo & Pedro, 1992) and a single mitochondrial DNA gene fragment with limited (34 species) taxon sampling (Costa *et al.*, 2003) resulted in phylogenies, although the patterns were inconsistent. Recently, Rasmussen & Cameron (2007) published a four-gene phylogeny of 64 meliponine taxa belonging to 22 of 25 Old World genera and 15 taxa belonging to 13 of 36 New World genera. Their analysis included 16S mitochondrial DNA and three nuclear fragments: long-wavelength rhodopsin copy 1 (opsin), elongation factor-1 α F2 (EF-1 α) and arginine kinase (ArgK), each of which indicated strong support for three major splits corresponding to a Neotropical clade, an Afrotropical clade, and an Indo-Malay/Australasian clade. This analysis, however, was unable to resolve the relationship among these three basal-most splits with strong support, which may have been the result of limited taxon sampling of the New World groups or an insufficient number of slowly-evolving nuclear markers, or both.

In the present study, with significantly increased taxon sampling of New World taxa and new sequence data from an additional five nuclear genes, we largely resolve the meliponine phylogeny, elucidate the temporal patterns of clade diversification, and hypothesize a different historical biogeography of the stingless bees with new insights, providing a test of the opposing hypotheses.

MATERIAL AND METHODS

The phylogenetic analyses are presented in two parts. The first incorporates a majority of New World genera (Table 1) and adds sequences from nuclear 28S rDNA to the original four-gene data matrix of Rasmussen & Cameron (2007). The 28S gene has been useful for resolving deeper phylogenies in several groups of Hymenoptera (Dowton & Austin, 2001; Pilgrim, Dohlen & Pitts, 2008), including bees (Danforth, Fang & Sipes, 2006a). The second part of our analysis is a more intensive examination of the relationships among the three basal splits, wherein we selected 17 exemplar taxa to represent these well supported clades and included sequences from another four nuclear markers (see genes below) in a nine-gene analysis.

TAXA

We sequenced 202 stingless bee terminals, representing 186 different taxa from 55 of the 61 world genera

Table 1. Stingless bee and outgroup taxa included in the present study, their voucher numbers, collection localities, and GenBank (<http://www.ncbi.nlm.nih.gov>) accession numbers for each of the genes (16S, ArgK, EF-1 α , opsin, and 28S)

	Voucher number	Collection localities	16S	ArgK	EF-1 α	opsin	28S
<i>*Aparatrigona impunctata</i> (Ducke)	28	Peru, San Martín	FJ041911	FJ042187	FJ042288	FJ042390	FJ042011
<i>Apotrigona infuscata</i> (Cockerell)	410	Uganda, Bwindi	DQ790447	DQ813029	DQ813104	DQ813182	FJ042172
<i>Austrolebeia symei</i> (Rayment)	223	Australia, QLD	DQ790445	–	DQ813105	DQ813183	FJ042170
<i>Austrolebeia symei</i> (Rayment)	224	Australia, QLD	DQ790446	–	DQ813106	DQ813184	FJ042171
<i>Axestotrigona ferruginea</i> (Lepeletier)	415	Uganda, Bwindi	DQ790448	DQ813030	DQ813107	DQ813185	FJ042173
<i>Axestotrigona ferruginea</i> (Lepeletier)	428	Cameroon, Ngaoundere	DQ790449	DQ813031	DQ813108	DQ813186	FJ042174
<i>Axestotrigona ferruginea</i> (Lepeletier)	429	Cameroon, Ngaoundere	DQ790450	DQ813032	DQ813109	DQ813187	FJ042175
<i>*Celetrigona aff. longicornis</i> (Friese)	62	Peru, San Martín	FJ041912	FJ042188	FJ042289	FJ042391	FJ042012
<i>*Cephalotrigona aff. capitata</i> (Smith)	66	Peru, San Martín	FJ041913	FJ042189	FJ042290	FJ042392	FJ042013
<i>*Cephalotrigona capitata</i> (Smith)	463	Brazil, São Paulo	FJ041915	FJ042191	FJ042292	FJ042394	FJ042015
Cephalotrigona sp. nov. A	74	Peru, San Martín	DQ790465	DQ813033	DQ813110	DQ813188	EU049743
*Cephalotrigona sp. nov. B	374	Peru, Loreto	FJ041914	FJ042190	FJ042291	FJ042393	FJ042014
<i>Dactylurina staudingeri</i> (Gribodo)	424	Cameroon, Yaounde	DQ790451	DQ813034	DQ813111	DQ813189	FJ042176
<i>Dolichotrigona browni</i> Camargo & Pedro	360	Peru, San Martín	DQ790457	DQ813035	DQ813112	DQ813190	FJ042017
<i>*Dolichotrigona chachapoya</i> Camargo & Pedro	51	Peru, San Martín	FJ041916	FJ042192	FJ042293	FJ042395	FJ042016
<i>*Duceola ghiliani</i> (Spinola)	331	Brazil, Amazonas	FJ041917	FJ042193	FJ042294	FJ042396	FJ042018
<i>*Melipona (Eomelipona) bicolor</i> Lepeletier	456	Brazil, Minas Gerais	FJ041919	FJ042195	FJ042296	FJ042398	FJ042020
<i>*Melipona (Eomelipona) bradleyi</i> Schwarz	600	Colombia, Vaupés	FJ041921	FJ042197	FJ042298	FJ042400	FJ042022
<i>*Melipona (Eomelipona) marginata</i> Lepeletier	461	Brazil, São Paulo	FJ041920	FJ042196	FJ042297	FJ042399	FJ042021
<i>*Melipona (Eomelipona) tumupusae</i> Schwarz	131	Peru, Madre de Dios	FJ041918	FJ042194	FJ042295	FJ042397	FJ042019
<i>*Friesella schrottkyi</i> (Friese)	445	Brazil, São Paulo	FJ041922	FJ042198	FJ042299	FJ042401	FJ042023
<i>*Friesiellina silvestrii</i> (Friese)	462	Brazil, São Paulo	FJ041925	FJ042201	FJ042302	FJ042404	FJ042026
<i>*Friesiellina trichocera</i> Moure	375	Peru, Loreto	FJ041923	FJ042199	FJ042300	FJ042402	FJ042024
<i>*Friesiellina varia</i> (Lepeletier)	457	Brazil, São Paulo	FJ041924	FJ042200	FJ042301	FJ042403	FJ042025
<i>Geniotrigona incisa</i> (Sakagami & Inoue)	192	Indonesia, Sulawesi	DQ790392	DQ813036	DQ813113	DQ813191	FJ042118
<i>Geniotrigona thoracica</i> (Smith)	312	Malaysia, Kedah	DQ790393	DQ813037	DQ813114	DQ813192	FJ042119
<i>Geniotrigona thoracica</i> (Smith)	436	Malaysia, Sabah, Taman	DQ790394	DQ813038	DQ813115	DQ813193	FJ042120
<i>*Geotrigona fulvata</i> Camargo & Moure	377	Peru, San Martín	FJ041927	FJ042203	FJ042304	FJ042406	FJ042028
<i>Geotrigona fulvohirta</i> (Friese)	7	Peru, San Martín	DQ790466	DQ813039	DQ813116	DQ813194	EU049744
<i>*Geotrigona mombuca</i> (Smith)	464	Brazil, São Paulo	FJ041928	FJ042204	FJ042305	FJ042407	FJ042029
<i>*Geotrigona subgrisea</i> (Cockerell)	149	Colombia, Amazonas	FJ041926	FJ042202	FJ042303	FJ042405	FJ042027
<i>Heterotrigona bakeri</i> (Cockerell)	388	Malaysia, Sabah, Mahua	DQ790397	DQ813042	DQ813119	DQ813197	FJ042123
<i>Heterotrigona erythrogaster</i> (Cameron)	198	Malaysia, Pasoh	DQ790395	DQ813040	DQ813117	DQ813195	FJ042121
<i>Heterotrigona imata</i> (Cockerell)	311	Malaysia, Kedah	DQ790396	DQ813041	DQ813118	DQ813196	FJ042122
<i>Homotrigona fimbriata</i> (Smith)	558	Thailand, Tak	DQ790403	–	DQ813120	DQ813198	FJ042129
<i>Hypotrigona gribodoi</i> (Magretti)	405	Uganda, Bwindi	DQ790440	DQ813043	DQ813121	DQ813199	FJ042165
<i>Hypotrigona ruspollii</i> (Magretti)	425	Cameroon, Yaounde	DQ790441	DQ813044	DQ813122	DQ813200	FJ042166
<i>Lepidotrigona terminata</i> (Smith)	266	Indonesia, Sulawesi	DQ790398	DQ813045	DQ813123	DQ813201	FJ042124
<i>Lepidotrigona terminata</i> (Smith)	270	Malaysia, Sabah	DQ790399	DQ813046	DQ813124	DQ813202	FJ042125
<i>Lepidotrigona ventralis</i> (Smith)	478	Malaysia, Sabah, Labang	DQ790400	DQ813047	DQ813125	DQ813203	FJ042126
<i>*Lestrimelitta aff. limao</i> (Smith)	119	Peru, Madre de Dios	FJ041930	FJ042206	FJ042307	FJ042409	FJ042031
<i>*Lestrimelitta limao</i> (Smith)	449	Brazil, São Paulo	FJ041931	FJ042207	FJ042308	FJ042410	FJ042032
<i>*Lestrimelitta maracaia</i> Marchi & Melo	40	Peru, San Martín	FJ041929	FJ042205	FJ042306	FJ042408	FJ042030
<i>*Leurotrigona muelleri</i> Moure	466	Brazil, São Paulo	FJ041933	FJ042209	FJ042310	FJ042412	FJ042034
<i>*Leurotrigona pusilla</i> Moure & Camargo	35	Peru, San Martín	FJ041932	FJ042208	FJ042309	FJ042411	FJ042033
<i>Liotrigona madecassa</i> (Saussure)	404	Madagascar, Mahajanga	DQ790443	DQ813049	DQ813127	DQ813205	FJ042168
<i>Liotrigona mahafalya</i> Moure & Camargo	403	Madagascar, Mahajanga	DQ790442	DQ813048	DQ813126	DQ813204	FJ042167
<i>Lisotrigona furva</i> Engel	420	Thailand, Pathumthani	DQ790444	–	DQ813128	DQ813206	FJ042169
<i>Lophotrigona canifrons</i> (Smith)	196	Malaysia, Pasoh	DQ790404	DQ813050	DQ813129	DQ813207	FJ042130
<i>*Melipona (Melikerria) becheii</i> (Bennet)	128	Costa Rica, Guanacaste	FJ041947	FJ042224	FJ042325	FJ042427	FJ042049
<i>*Melipona (Melikerria) becheii</i> (Bennet)	294	Mexico, Chiapas	FJ041948	FJ042225	FJ042326	FJ042428	FJ042050
<i>*Melipona (Melikerria) fasciculata</i> Smith	120	Brazil, São Paulo	FJ041946	FJ042223	FJ042324	FJ042426	FJ042048
<i>Melipona (Melikerria) grandis</i> Guérin	17	Peru, San Martín	DQ790458	DQ813051	DQ813130	DQ813208	EU049745
<i>Meliplebeia ogouensis</i> (Vachal)	414	Uganda, Bwindi	DQ790453	DQ813053	DQ813132	DQ813210	FJ042178
<i>*Melipona (M.) variegatipes</i> Gribodo	563	Lesser Antilles, Montserrat	FJ041949	FJ042226	FJ042327	FJ042429	FJ042051
<i>Meliponula bocandei</i> (Spinola)	406	Uganda, Bwindi	DQ790452	DQ813052	DQ813131	DQ813209	FJ042177
<i>*Melipona (Michmelia) aff. brachychaeta</i> Moure	129	Peru, Madre de Dios	FJ041941	FJ042218	FJ042319	FJ042421	FJ042043
<i>*Melipona (Michmelia) aff. mimetica</i> Cockerell	18	Ecuador, Loja	FJ041934	FJ042210	FJ042311	FJ042413	FJ042035
<i>*Melipona (Michmelia) crinita</i> Moure & Kerr	48	Peru, San Martín	FJ041936	FJ042212	FJ042313	FJ042415	FJ042037
<i>*Melipona (Michmelia) crinita</i> Moure & Kerr	586	Peru, Madre de Dios	FJ041942	FJ042219	FJ042320	FJ042422	FJ042044
<i>*Melipona (Michmelia) eburnea</i> Friese	19	Peru, San Martín	FJ041935	FJ042211	FJ042312	FJ042414	FJ042036

Table 1. Continued

	Voucher number	Collection localities	16S	ArgK	EF-1 α	opsin	28S
* <i>Melipona (Michmelia) fasciata</i> Latreille	596	Mexico, Guerrero	FJ041945	FJ042222	FJ042323	FJ042425	FJ042047
* <i>Melipona (Michmelia) illota</i> Cockerell	82	Peru, San Martín	–	FJ042215	FJ042316	FJ042418	FJ042040
* <i>Melipona (Michmelia) illota</i> Cockerell	587	Colombia, Amazonas	FJ041943	FJ042220	FJ042321	FJ042423	FJ042045
* <i>Melipona (Michmelia) irisae</i> Camargo & Melo	73	Peru, San Martín	FJ041938	FJ042214	FJ042315	FJ042417	FJ042039
* <i>Melipona (Michmelia) scutellaris</i> Latreille	121	Brazil, Minas Gerais	FJ041939	FJ042216	FJ042317	FJ042419	FJ042041
* <i>Melipona (Michmelia) seminigra</i> merrillae Cockerell	122	Brazil, Amazonas	FJ041940	FJ042217	FJ042318	FJ042420	FJ042042
* <i>Melipona (Michmelia) sp. nov. B</i>	588	Colombia, Amazonas	FJ041944	FJ042221	FJ042322	FJ042424	FJ042046
* <i>Melipona (Michmelia) titania</i> Gribodo	65	Peru, San Martín	FJ041937	FJ042213	FJ042314	FJ042416	FJ042038
* <i>Mourella caerulea</i> (Friese)	423	Brazil, Rio Grande do Sul	FJ041950	FJ042227	FJ042328	FJ042430	FJ042052
<i>Nannotrigona melanocera</i> (Schwarz)	111	Peru, Junín	DQ790460	DQ813054	DQ813133	DQ813211	FJ042053
* <i>Nannotrigona perilampoides</i> (Cresson)	296	Mexico, Chiapas	FJ041952	FJ042229	FJ042330	FJ042432	FJ042055
* <i>Nannotrigona schultzei</i> (Friese)	113	Peru, Madre de Dios	FJ041951	FJ042228	FJ042329	FJ042431	FJ042054
* <i>Nannotrigona testaceicornis</i> (Lepelletier)	450	Brazil, São Paulo	FJ041953	FJ042230	FJ042331	FJ042433	FJ042056
* <i>Nogueirapis butteli</i> (Friese)	364	Peru, Loreto	FJ041954	FJ042231	FJ042332	FJ042434	FJ042057
* <i>Nogueirapis mirandula</i> (Cockerell)	s/n	Costa Rica	FJ041955	FJ042232	FJ042333	FJ042435	–
<i>Odontotrigona haematoptera</i> (Cockerell)	475	Malaysia, Sabah, Labang	DQ790405	DQ813055	DQ813134	DQ813212	FJ042131
* <i>Oxytrigona mediorufa</i> (Cockerell)	301	Mexico, Chiapas	FJ041959	FJ042236	FJ042337	FJ042439	FJ042061
* <i>Oxytrigona mellicolor</i> (Packard)	114	Costa Rica, Guanacaste	FJ041958	FJ042235	FJ042336	FJ042438	FJ042060
* <i>Oxytrigona mulfordi</i> (Schwarz)	12	Peru, San Martín	FJ041957	FJ042234	FJ042335	FJ042437	FJ042059
* <i>Oxytrigona obscura</i> (Friese)	6	Peru, San Martín	FJ041956	FJ042233	FJ042334	FJ042436	FJ042058
* <i>Oxytrigona tataira</i> (Smith)	448	Brazil, São Paulo	FJ041960	FJ042237	FJ042338	FJ042440	FJ042062
* <i>Parapartamona fumata</i> Moure	567	Ecuador, Cañar	FJ041977	FJ042255	FJ042356	FJ042457	FJ042082
<i>Parapartamona tungurahua</i> (Schwarz)	14	Ecuador, Loja	DQ790464	DQ813062	DQ813141	DQ813219	FJ042081
* <i>Parapartamona tungurahua</i> (Schwarz)	583	Ecuador, Cañar	FJ041978	FJ042256	FJ042357	FJ042458	FJ042083
* <i>Paratrigona guigliae</i> Moure	115	Peru, Pasco	FJ041968	FJ042246	FJ042347	FJ042449	FJ042071
* <i>Paratrigona lineata</i> (Lepelletier)	465	Brazil, São Paulo	FJ041969	FJ042247	FJ042348	FJ042450	FJ042072
* <i>Paratrigona lineatifrons</i> (Schwarz)	37	Peru, San Martín	FJ041966	FJ042244	FJ042345	FJ042447	FJ042069
<i>Paratrigona onorei</i> Camargo & Moure	23	Peru, Pasco	DQ790462	DQ813057	DQ813136	DQ813214	FJ042068
* <i>Paratrigona pacifica</i> (Schwarz)	107	Peru, San Martín	FJ041967	FJ042245	FJ042346	FJ042448	FJ042070
<i>Partamona auripennis</i> Pedro & Camargo	13	Peru, San Martín	DQ790463	DQ813056	DQ813135	DQ813213	EU049746
* <i>Partamona epiphytophila</i> Pedro & Camargo	20	Peru, Pasco	FJ041962	FJ042239	FJ042340	FJ042442	FJ042064
* <i>Partamona epiphytophila</i> Pedro & Camargo	422	Peru, Madre de Dios	FJ041964	FJ042242	FJ042343	FJ042445	–
* <i>Partamona musarum</i> (Cockerell)	593	Costa Rica, Guanacaste	FJ041965	FJ042243	FJ042344	FJ042446	FJ042067
* <i>Partamona orizabaensis</i> (Strand)	117	Costa Rica, Guanacaste	–	FJ042241	FJ042342	FJ042444	FJ042066
* <i>Partamona testaceae</i> (Klug)	5	Peru, San Martín	FJ041961	FJ042238	FJ042339	FJ042441	FJ042063
* <i>Partamona vicina</i> Camargo	55	Peru, San Martín	FJ041963	FJ042240	FJ042341	FJ042443	FJ042065
<i>Platytrigona hobbyi</i> (Schwarz)	305	Malaysia, Sabah, Sugud	DQ790401	DQ813058	DQ813137	DQ813215	FJ042127
* <i>Plebeia droryana</i> (Friese)	444	Brazil, São Paulo	FJ041974	FJ042252	FJ042353	FJ042455	FJ042078
<i>Plebeia frontalis</i> (Friese)	339	Mexico, Puebla	DQ790459	DQ813059	DQ813138	DQ813216	FJ042077
* <i>Plebeia minima</i> (Gribodo)	86	Peru, San Martín	FJ041971	FJ042249	FJ042350	FJ042452	FJ042074
* <i>Plebeia moureana</i> Ayala	295	Mexico, Chiapas	FJ041973	FJ042251	FJ042352	FJ042454	FJ042076
* <i>Plebeia sp. nov. A</i>	577	Ecuador, Cañar	FJ041975	FJ042253	FJ042354	FJ042456	FJ042079
* <i>Plebeia sp. nov. B</i>	43	Peru, San Martín	FJ041970	FJ042248	FJ042349	FJ042451	FJ042073
* <i>Plebeia sp. nov. C</i>	104	Peru, San Martín	FJ041972	FJ042250	FJ042351	FJ042453	FJ042075
* <i>Plebeia tobagoensis</i> Melo	s/n	Trinidad	FJ041976	FJ042254	FJ042355	–	FJ042080
<i>Plebeiella lendliana</i> (Friese)	418	Uganda, Bwindi	DQ790454	DQ813060	DQ813139	DQ813217	FJ042179
<i>Plebeina hildebrandti</i> (Friese)	435	South Africa, L. Trichardt	DQ790455	DQ813061	DQ813140	DQ813218	FJ042180
* <i>Ptilotrigona lurida</i> (Smith)	378	Peru, San Martín	FJ041980	FJ042258	FJ042359	FJ042460	FJ042085
* <i>Ptilotrigona pereneae</i> (Schwarz)	3	Peru, San Martín	FJ041979	FJ042257	FJ042358	FJ042459	FJ042084
* <i>Scaptotrigona bipunctata</i> (Lepelletier)	453	Brazil, São Paulo	FJ041987	FJ042265	FJ042366	FJ042467	FJ042092
* <i>Scaptotrigona depilis</i> (Moure)	454	Brazil, São Paulo	FJ041988	FJ042266	FJ042367	FJ042468	FJ042093
* <i>Scaptotrigona mexicana</i> (Guérin)	336	Mexico, Puebla	FJ041986	FJ042264	FJ042365	FJ042466	FJ042091
* <i>Scaptotrigona pectoralis</i> (Dalla Torre)	297	Mexico, Chiapas	FJ041985	FJ042263	FJ042364	FJ042465	FJ042090
<i>Scaptotrigona polysticta</i> Moure	10	Peru, San Martín	DQ790461	DQ813063	DQ813142	DQ813220	EU049747
* <i>Scaptotrigona sp. nov. A</i>	25	Peru, Pasco	FJ041981	FJ042259	FJ042360	FJ042461	FJ042086
* <i>Scaptotrigona sp. nov. B</i>	69	Peru, San Martín	FJ041983	FJ042261	FJ042362	FJ042463	FJ042088
* <i>Scaptotrigona sp. nov. C</i>	31	Peru, San Martín	FJ041982	FJ042260	FJ042361	FJ042462	FJ042087
* <i>Scaptotrigona tricolorata</i> Camargo	96	Peru, San Martín	FJ041984	FJ042262	FJ042363	FJ042464	FJ042089
* <i>Scaura latitarsis</i> (Friese)	9	Peru, San Martín	FJ041991	FJ042269	FJ042370	FJ042471	FJ042096
* <i>Scaura longula</i> (Lepelletier)	2	Peru, San Martín	FJ041989	FJ042267	FJ042368	FJ042469	FJ042094
* <i>Scaura tenuis</i> (Ducke)	8	Peru, San Martín	FJ041990	FJ042268	FJ042369	FJ042470	FJ042095

Table 1. Continued

	Voucher number	Collection localities	16S	ArgK	EF-1 α	opsin	28S
* <i>Schwarziana quadripunctata</i> (Lepeletier)	472	Brazil, São Paulo	FJ041992	FJ042270	FJ042371	FJ042472	FJ042097
* <i>Schwarzula coccidophila</i> Camargo & Pedro	368	Peru, San Martín	FJ041994	FJ042272	FJ042373	FJ042474	FJ042099
* <i>Schwarzula timida</i> (Silvestri)	362	Peru, San Martín	FJ041993	FJ042271	FJ042372	FJ042473	FJ042098
<i>Sundatrigona moorei</i> (Schwarz)	389	Malaysia, Sabah, Mahua	DQ790402	DQ813064	DQ813143	DQ813221	FJ042128
* <i>Tetragona clavipes</i> (Fabricius)	1	Peru, San Martín	FJ042004	FJ042281	FJ042383	FJ042484	FJ042111
* <i>Tetragona clavipes</i> (Fabricius)	452	Brazil, São Paulo	FJ042010	FJ042287	FJ042389	FJ042490	FJ042117
* <i>Tetragona dissecta</i> (Moure)	110	Peru, San Martín	FJ042007	FJ042284	FJ042386	FJ042487	FJ042114
* <i>Tetragona goettei</i> (Friese)	27	Peru, San Martín	FJ042006	FJ042283	FJ042385	FJ042486	FJ042113
* <i>Tetragona perangulata</i> (Cockerell)	138	Costa Rica, Puntarenas	FJ042009	FJ042286	FJ042388	FJ042489	FJ042116
*<i>Tetragona</i> sp. nov.	21	Peru, Pasco	FJ042005	FJ042282	FJ042384	FJ042485	FJ042112
* <i>Tetragona zieglerei</i> (Friese)	137	Costa Rica, Guanacaste	FJ042008	FJ042285	FJ042387	FJ042488	FJ042115
<i>Tetragonilla atripes</i> (Smith)	191	Malaysia, Pasoh	DQ790409	DQ813072	DQ813151	DQ813229	FJ042135
<i>Tetragonilla collina</i> (Smith)	543	Malaysia, Sabah, Tawan	DQ790412	DQ813075	DQ813154	DQ813232	FJ042138
<i>Tetragonilla fuscibasis</i> (Cockerell)	313	Malaysia, Kedah	DQ790411	DQ813073	DQ813152	DQ813230	FJ042137
<i>Tetragonilla rufibasalis</i> (Cockerell)	502	Malaysia, Sabah, Labang	DQ790410	DQ813074	DQ813153	DQ813231	FJ042136
<i>Tetragonisca angustula</i> (Latreille)	64	Peru, San Martín	DQ790470	DQ813076	DQ813155	DQ813233	FJ042107
* <i>Tetragonisca angustula</i> (Latreille)	451	Brazil, São Paulo	FJ042002	–	FJ042381	FJ042482	FJ042109
* <i>Tetragonisca weyrauchi</i> (Schwarz)	136	Peru, Madre de Dios	FJ042001	FJ042279	FJ042380	FJ042481	FJ042108
<i>Tetragonula aff. iridipennis</i> (Smith)	552	India, Tamil Nadu	DQ790423	DQ813102	DQ813180	DQ813259	FJ042148
<i>Tetragonula aff. laeviceps</i> (Smith)	230	Thailand, Ban Dong	DQ790420	DQ813085	DQ813164	DQ813242	FJ042146
<i>Tetragonula aff. pagdeni</i> (Schwarz)	536	Malaysia, Sabah	DQ790437	DQ813098	DQ813177	DQ813255	FJ042162
<i>Tetragonula carbonaria</i> (Smith)	228	Australia, QLD	DQ790429	DQ813083	DQ813162	DQ813240	FJ042154
<i>Tetragonula clypearis</i> (Friese)	225	Australia, QLD	DQ790414	DQ813080	DQ813159	DQ813237	FJ042140
<i>Tetragonula clypearis</i> (Friese)	234	Papua New Guinea	DQ790415	DQ813086	DQ813165	DQ813243	FJ042141
<i>Tetragonula fuscobalteata</i> (Cameron)	194	Indonesia, Sulawesi	DQ790416	DQ813078	DQ813157	DQ813235	FJ042142
<i>Tetragonula fuscobalteata</i> (Cameron)	514	Malaysia, Sabah, Labang	DQ790417	DQ813095	DQ813174	DQ813252	FJ042143
<i>Tetragonula fuscobalteata</i> (Cameron)	529	Malaysia, Sabah, Taman	DQ790418	DQ813096	DQ813175	DQ813253	FJ042144
<i>Tetragonula geissleri</i> (Cockerell)	197	Malaysia, Pasoh	DQ790426	DQ813079	DQ813158	DQ813236	FJ042151
<i>Tetragonula geissleri</i> (Cockerell)	303	Malaysia, Sabah	DQ790433	DQ813091	DQ813170	DQ813248	FJ042158
<i>Tetragonula hirashimai</i> (Sakagami)	291	Thailand, Chiang Mai	DQ790432	DQ813090	DQ813169	DQ813247	FJ042157
<i>Tetragonula hockingsi</i> (Cockerell)	229	Australia, QLD	DQ790430	DQ813084	DQ813163	DQ813241	FJ042155
<i>Tetragonula laeviceps</i> (Smith)	308	Malaysia, Sabah, Sukau	DQ790438	DQ813092	DQ813171	DQ813249	FJ042163
<i>Tetragonula melanocephala</i> (Gribodo)	401	Malaysia, Sabah, G. Alab	DQ790434	DQ813093	DQ813172	DQ813250	FJ042159
<i>Tetragonula melina</i> (Gribodo)	532	Malaysia, Sabah, Labang	DQ790436	DQ813097	DQ813176	DQ813254	FJ042161
<i>Tetragonula mellipes</i> (Friese)	226	Australia, WA	DQ790428	DQ813081	DQ813160	DQ813238	FJ042153
<i>Tetragonula minor</i> (Sakagami)	290	Thailand, Phanga	DQ790419	DQ813089	DQ813168	DQ813246	FJ042145
<i>Tetragonula pagdeni</i> (Schwarz)	193	Indonesia, Sulawesi	DQ790413	DQ813077	DQ813156	DQ813234	FJ042139
<i>Tetragonula sapiens</i> (Cockerell)	235	Papua New Guinea	DQ790425	DQ813087	DQ813166	DQ813244	FJ042150
<i>Tetragonula sapiens</i> (Cockerell)	227	Australia, QLD	DQ790427	DQ813082	DQ813161	DQ813239	FJ042152
<i>Tetragonula sarawakensis</i> (Schwarz)	441	Malaysia, Sabah	DQ790435	DQ813094	DQ813173	DQ813251	FJ042160
<i>Tetragonula sirindhornae</i> (Michener & Boengird)	289	Thailand, Ranong	DQ790431	DQ813088	DQ813167	DQ813245	FJ042156
<i>Tetragonula</i> sp. nov. A	547	India, Haryana	DQ790421	DQ813100	DQ813179	DQ813257	FJ042147
<i>Tetragonula</i> sp. nov. B	550	India, Orissa	DQ790422	DQ813101	–	DQ813258	–
<i>Tetragonula</i> sp. nov. C	553	India, Tamil Nadu	DQ790424	–	DQ813181	DQ813260	FJ042149
<i>Tetragonula zucchini</i> (Sakagami)	541	Malaysia, Sabah, Sukau	DQ790439	DQ813099	DQ813178	DQ813256	FJ042164
<i>Tetrigona apicalis</i> (Smith)	195	Malaysia, Pasoh	DQ790406	DQ813065	DQ813144	DQ813222	FJ042132
<i>Tetrigona binghami</i> (Schwarz)	498	Malaysia, Sabah, Labang	DQ790408	DQ813067	DQ813146	DQ813224	FJ042134
<i>Tetrigona melanoleuca</i> (Cockerell)	439	Malaysia, Sabah	DQ790407	DQ813066	DQ813145	DQ813223	FJ042133
* <i>Trichotrigona extranea</i> Camargo & Moure	330	Brazil, Amazonas	FJ042003	FJ042280	FJ042382	FJ042483	FJ042110
<i>Trigona aff. fuscipennis</i> Friese A	458	Brazil, São Paulo	EU049717	EU049767	EU049791	EU049812	EU049742
<i>Trigona aff. fuscipennis</i> Friese B	169	Peru, Madre de Dios	EU049706	EU049756	EU049779	EU049801	EU049731
<i>Trigona aff. recursa</i> Smith	167	Peru, Madre de Dios	EU049704	EU049754	EU049777	EU049799	EU049729
<i>Trigona albipennis</i> Almeida	168	Peru, Junín	EU049705	EU049755	EU049778	EU049800	EU049730
<i>Trigona amalthea</i> (Olivier)	52	Peru, San Martín	DQ790468	DQ813070	EU049772	DQ813227	EU049722
<i>Trigona amazonensis</i> (Ducke)	200	Peru, Madre de Dios	DQ790469	DQ813071	EU049781	DQ813228	EU049731
<i>Trigona chanchamayoensis</i> Schwarz	16	Peru, San Martín	EU049698	EU049748	EU049769	EU049793	EU049723
<i>Trigona cilipes</i> (Fabricius)	45	Peru, San Martín	EU049699	EU049749	EU049771	EU049794	EU049724
<i>Trigona corvina</i> Cockerell	219	Costa Rica, Puntarenas	EU049710	EU049760	EU049784	EU049805	EU049735
<i>Trigona crassipes</i> (Fabricius)	60	Peru, San Martín	EU049701	EU049751	EU049774	EU049796	EU049726
<i>Trigona dallatorreana</i> Friese	76	Peru, San Martín	EU049703	EU049753	EU049776	EU049798	EU049728
<i>Trigona fulviventris</i> Guérin	299	Mexico, Chiapas	EU049712	EU049762	EU049786	EU049807	EU049737

Table 1. Continued

	Voucher number	Collection localities	16S	ArgK	EF-1 α	opsin	28S
<i>Trigona fuscipennis</i> Friese	218	Costa Rica, Limón	EU049709	EU049759	EU049783	EU049804	EU049734
<i>Trigona guianae</i> Cockerell	24	Peru, Pasco	DQ790467	DQ813069	EU049770	DQ813226	EU049720
<i>Trigona hyalinata</i> (Lepeleter)	459	Brazil, São Paulo	EU049718	EU049768	EU049792	EU049813	EU049742
<i>Trigona hypogaea</i> Silvestri	380	Peru, San Martín	EU049714	EU049764	EU049788	EU049809	EU049739
<i>Trigona lacteipennis</i> Friese	58	Peru, San Martín	EU049700	EU049750	EU049773	EU049795	EU049725
<i>Trigona nigerrima</i> Cresson	220	Costa Rica, Guanacaste	EU049711	EU049761	EU049785	EU049806	EU049736
<i>Trigona pallens</i> (Fabricius)	61	Peru, San Martín	EU049702	EU049752	EU049775	EU049797	EU049727
<i>Trigona recursa</i> Smith	446	Brazil, São Paulo	EU049715	EU049765	EU049789	EU049810	EU049740
<i>Trigona silvestriana</i> Vachal	187	Costa Rica, Guanacaste	EU049707	EU049757	EU049780	EU049802	EU049732
<i>Trigona spinipes</i> (Fabricius)	447	Brazil, São Paulo	EU049716	EU049766	EU049790	EU049811	EU049741
<i>Trigona truculenta</i> Almeida	212	Peru, Madre de Dios	EU049708	EU049758	EU049782	EU049803	EU049733
<i>Trigona williana</i> Friese	371	Peru, Loreto	EU049713	EU049763	EU049787	EU049808	EU049738
* <i>Trigonisca bidentata</i> Albuquerque & Camargo	151	Peru, Madre de Dios	FJ041999	FJ042277	FJ042378	FJ042479	FJ042104
* <i>Trigonisca buyssoni</i> (Friese)	150	Costa Rica, Guanacaste	FJ041998	FJ042276	FJ042377	FJ042478	FJ042103
* <i>Trigonisca nataliae</i> (Moure)	471	Brazil, Matto Grosso	FJ042000	FJ042278	FJ042379	FJ042480	FJ042106
* <i>Trigonisca</i> sp. nov. A	44	Peru, San Martín	FJ041995	FJ042273	FJ042374	FJ042475	FJ042100
* <i>Trigonisca</i> sp. nov. B	71	Peru, San Martín	FJ041996	FJ042274	FJ042375	FJ042476	FJ042101
* <i>Trigonisca</i> sp. nov. C	72	Peru, San Martín	FJ041997	FJ042275	FJ042376	FJ042477	FJ042102
<i>Trigonisca townsendi</i> (Cockerell)	383	Peru, Tumbes	DQ790456	DQ813068	DQ813147	DQ813225	FJ042105
<i>Apis dorsata</i> Fabricius		(India, Bangalore)	L22893	AY267178	AY267146	AF091733	FJ042186
<i>Bombus terrestris</i> (Linnaeus)		Italy, San Quirico	DQ788118	AF492888	DQ788288	AF493022	FJ042181
<i>Bombus willmatiae</i> Cockerell		Mexico, Chiapas	DQ788136	DQ788521	DQ788304	AY739496	FJ042182
<i>Euglossa imperialis</i> Cockerell		Brazil, São Paulo	AJ581085	AY267176	AY267144	AY267160	FJ042183
<i>Eulaema boliviensis</i> (Friese)		Bolivia, La Paz	DQ788139	DQ788523	DQ788307	DQ788387	FJ042184
<i>Exaerete smaragdina</i> (Guérin)			AJ581101	AJ581738	AJ582379	DQ813103	FJ042185

Asterisks indicate the 102 new taxa included in the present study that were not examined by Rasmussen & Cameron (2007).

(Camargo & Pedro, 2007; Rasmussen, 2008), and recorded their identity, locality, and voucher numbers (Table 1). This included 102 newly-sequenced taxa plus 76 Old World taxa, 24 *Trigona* s.s. taxa, and six outgroup taxa from Rasmussen & Cameron (2007) and Rasmussen & Camargo (2008). J. Camargo (Universidade de São Paulo, Ribeirão Preto, Brazil) verified identifications of all New World taxa. Voucher specimens for all sampled taxa are deposited at the University of Illinois Natural History Survey (Urbana, IL, USA).

GENES, POLYMERASE CHAIN REACTION (PCR), AND DNA SEQUENCING

Gene fragments (16S rRNA, ArgK, EF-1 α and opsin) that provided useful signal in Rasmussen & Cameron's (2007) study of Old World taxa were sequenced in this study for the additional 102 New World taxa. To strengthen support for intergeneric relationships, we also sequenced the relatively conserved nuclear 28S rRNA (D2–D3 expansion regions and related core elements) for all 202 taxa.

For a more intensive examination of the three basal ingroup splits, we analysed a subset of 22 taxa (17 ingroup) for a total of nine gene fragments. These

genes were the five used in the comprehensive analysis plus four others that have been useful in analyses of tribal and intratribal relationships of corbiculate bees (Kawakita *et al.*, 2008; Whitfield *et al.*, 2008): RNA polymerase II (Pol II), mitotic checkpoint control protein (Bub3), deoxyribonucleoside kinase (Dnk), and glycerol kinase (Gyk).

DNA extraction, PCR, and sequencing protocols are reported in Rasmussen & Cameron (2007). PCR amplification of 28S was performed using the published primers of Hines *et al.* (2007) (For28SVsp, Rev28SVsp; annealing temperature of 50 °C and extension at 72 °C). For the smaller subset of 22 taxa, we used the primers reported by Danforth *et al.* (2006a) for Pol II (polfor2a, polrev2a; annealing of 50 °C) and by Kawakita *et al.* (2008) for Bub3 (Bub3f2, Bub3r2; annealing of 50 °C), Dnk (dNKf2, dNKr2; annealing of 50 °C), and Gyk (GlyK-F, GlyK-R; annealing of 55 °C). Sequence products for both strands were run on an ABI 3730XL automated sequencer (Applied Biosystems) at the W. M. Keck Center for Comparative and Functional Genomics, University of Illinois (Urbana, IL, USA). Sequences are available in GenBank under the accession numbers provided in Tables 1, 2.

Table 2. GenBank accession numbers for a subset of taxa for which additional genes were sequenced (Pol II, Bub3, Dnk, Gyk)

		Pol II	Bub3	Dnk	Gyk
<i>Cephalotrigona capitata</i>	463	EU184728	EU184819	EU184782	EU184761
<i>Dactylurina staudingeri</i>	424	EU980075	EU980039	–	–
<i>Dolichotrigona chachapoya</i>	51	–	EU980040	–	EU980063
<i>Hypotrigona ruspolii</i>	425	EU980076	EU980041	EU980053	EU980064
<i>Lepidotrigona ventralis</i>	478	EU980077	EU980042	EU980054	EU980065
<i>Liotrigona madecassa</i>	404	EU980078	EU980043	–	EU980066
<i>Lisotrigona furva</i>	420	EU980079	EU980044	EU980055	EU980067
<i>Melikerria grandis</i>	17	EU980080	EU980045	EU980056	EU980068
<i>Meliponula bocandei</i>	406	EU184729	EU184820	EU184783	EU184762
<i>Odontotrigona haematoptera</i>	475	EU980081	EU980046	EU980057	EU980069
<i>Plebeia</i> sp. nov. A	577	EU980082	EU980047	EU980058	EU980070
<i>Scaptotrigona tricolorata</i>	96	–	EU980048	–	EU980071
<i>Tetragonula fuscobalteata</i>	529	EU980086	EU980052	EU980062	EU980074
<i>Tetrigona binghami</i>	498	EU980083	EU980049	EU980059	EU980072
<i>Trigona amazonensis</i>	200	EU980085	EU980051	EU980061	–
<i>Trigona fuscipennis</i>	218	EU184727	EU184818	EU184781	EU184760
<i>Trigonisca</i> sp. nov. A	44	EU980084	EU980050	EU980060	EU980073
<i>Apis dorsata</i>		EU184732	EU184822	–	EU184764
<i>Bombus diversus</i>		EU184725	EU184817	EU184780	EU184759
<i>Bombus ardens</i>		EU184724	EU184816	EU184779	EU184758
<i>Euglossa imperialis</i>		EU184721	EU184813	EU184776	EU184755
<i>Exaerete frontalis</i>		EU184722	EU184814	EU184777	EU184756

Outgroup taxa (*Bombus terrestris*, *Bombus willmattae*, and *Exaerete smaragdina*) were not sequenced directly for these genes and are represented by sequences obtained from GenBank for closely-related taxa (*Bombus diversus*, *Bombus ardens*, and *Exaerete frontalis*, respectively).

PHYLOGENETIC ANALYSIS

Sequences were edited and aligned using default parameters in BIOEDIT, version 7.0.0 (Hall, 1999), with minor adjustment by eye if similar regions within introns and variable regions were aligned differently across taxa. The aligned data matrix is available in TREEBASE (<http://www.treebase.org>). Stingless bee relationships were largely inferred from Bayesian analyses implemented in MRBAYES, version 3.1.2 (Ronquist & Huelsenbeck, 2003). Gene fragments were analysed both individually and combined, and partitioned into exon and intron regions when appropriate to account for variable evolutionary rates between gene regions (Huelsenbeck & Crandall, 1997; Schwarz *et al.*, 2004). Appropriate substitution models for each gene partition were based on Akaike information criterion in MODELTEST, version 3.7 (Posada & Crandall, 1998). MRBAYES does not support all of the suggested models and we therefore used these more general models for the analysis: GTR+I+G (for 16S, 28S, ArgK exon, EF-1 α exon, EF-1 α intron, opsin exon, opsin intron, Dnk exon, Pol II), GTR+I (for Bub3 exon, Bub3 intron, Dnk intron), HKY+I+G (for ArgK intron, Gyk exon), and HKY+I

(for Gyk intron). For maximum likelihood (ML) criteria, single models were estimated for the five-gene (TVM+I+G) and nine-gene (GTR+I+G) datasets, respectively.

From three to six independent analyses were carried out for each gene fragment and for each combined dataset (eight million generations for individual genes, 12 million for combined analyses, four chains, mixed-models, flat priors, saving trees every 1000 generations). Majority rule consensus trees were estimated from a compilation of at least three independent analyses, after log-likelihood plots were examined in TRACER, version 1.3 (Rambaut & Drummond, 2006). All trees estimated prior to stationarity (burn-in) were discarded, and trees remaining after convergence were combined to create a single majority-rule consensus tree. Posterior probability (PP) values represent the proportion of all Markov chain samples, excluding burnin, that contain a particular node, and are interpreted as the probability of a clade conditioned on the observations.

To compare the Bayesian PP support values, we performed ML nonparametric bootstrapping (Felsenstein, 1985). ML bootstrap values (ML BV) were esti-

mated with PHYML, version 3.0 (Guindon & Gascuel, 2003) (200 replicates for the five-gene analyses, 500 replicates for the nine-gene analyses, GTR model, $p\text{-invar} = 0.449$, gamma shape parameter = 0.474) with a ML starting tree from PAUP* (Swofford, 2002) [100 replicates, tree bisection-reconnection (TBR) branch swapping, retaining 500 trees per replicate].

Maximum parsimony (MP) analyses were run for comparison with results from Bayesian and likelihood analyses to determine whether results were robust, independent of model assumptions. Both MP (heuristic search, 1000 random additions, TBR branch swapping, retaining 500 trees per replicate) and MP bootstrap (heuristic search, 500 replicates, ten random additions per replicate, retaining 200 trees per replicate) were implemented in PAUP*. For the nine-gene dataset, which included the subset of 22 taxa, we ran both Bayesian (three independent runs, six million generations, four chains, mixed-models, flat priors, saving trees every 1000 generations) and MP analyses (heuristic search, 10 000 random additions, TBR branch swapping; bootstrap: heuristic search, 500 replicates, ten random additions per replicate).

Potential conflicts among individual gene histories were examined for the five-gene and nine-gene phylogenies in SPLITSTREE, version 4.10 (Huson & Bryant, 2006) by creating a consensus supernetwork from the individual gene trees using Z-closure methods (Huson *et al.*, 2004). Because a consensus network includes all relationships (splits) appearing in the multiple input trees, such a network can represent more information than a single tree with support values. To visualize the number of relationships in the supernetwork that are represented only sporadically as phylogenetic 'noise' among the source (gene) trees, we calculated filtered supernetworks (Huson & Bryant, 2006; Whitfield *et al.*, 2008), displaying only relationships found in three or more of the five source trees ($\text{min-trees} = 3$). We did this for the nine source trees with a higher filter, displaying relationships found in five or more trees ($\text{min-trees} = 5$). The resulting networks best summarize the relationships found repeatedly among the individual trees.

HISTORICAL BIOGEOGRAPHIC ANALYSIS

The disjunct pantropical distribution of the extant stingless bees can be explained by historical dispersal across pre-existing barriers, range expansions, and fragmentation of a once widespread ancestor by past vicariant (isolating) events. To infer the ancestral distribution of stingless bees, we performed a dispersal-vicariance analysis implemented in DIVA, version 1.1 (Ronquist, 1996). By broadly categorizing the presence/absence distribution of all included extant taxa into four main regions (Neotropical, Afro-

tropical, Indo-Malay/Australasia, and Palearctic), the most parsimonious ancestral distribution from the resolved Bayesian phylogeny (including the outgroups) can be estimated by minimizing the number of dispersal and extinction events, thus favouring vicariance (Ronquist, 1997). The advantage of this approach is that it does not require an a priori general hypothesis of area relationships; this is instead a product of the analysis. DIVA does not accommodate large datasets, so taxa were pruned to represent only the generic clades and their distributions under default settings. The maximum number of areas occupied by the ancestral lineage was allowed to vary from one to four in multiple analyses.

To account for phylogenetic uncertainty and branch length in the analyses, we explored BAYESTRAITS, version 1.0 (Pagel, Meade & Barker, 2004; Pagel & Meade, 2006), which has been used in other studies to infer ancestral geographic range (Praz *et al.*, 2008; Xiang & Thomas, 2008). We used a sample of 1000 trees with branch lengths from two of the final Bayesian runs. To avoid autocorrelation ('TAC' in TRACER, version 1.3), trees selected after burn-in were sampled from the t-files every 20 000 generations. The outgroup was deleted before analysis as the branch linking the outgroup to the ingroup cannot be estimated in BAYESTRAITS and may influence the outcome of the analysis (Huelsenbeck, Bollback & Levine, 2002; A. Meade, pers. comm.). We employed the following techniques for the analysis: 'multistate' model, Markov chain Monte Carlo (MCMC) method, an exponential prior seeded from a uniform on the interval 0–30 ('rjhp exp 0 30'), sampling every 300 iterations, a burn-in of 100 000. Using a rate deviation parameter of 3 ('ratedev 3') provided an average acceptance rate of between 20 and 40%, as recommended. Five nodes, including all taxa from the Old World (0) and New World (1) clades, and, in a separate analysis, Neotropical (0), Afrotropical (1), and Indo-Malayan/Australasian (2) clades, were specified using the command 'addMRCA'. A total of five to ten million generations were run three times for each of the nodes. To assess the robustness of each of these five ancestral node reconstructions, we constrained the ancestral states at each node to each of the states (0, 1 or 0, 1, 2) using the 'fossil' command in BAYESTRAITS. A Bayes factor above 2 (i.e. twice the difference in average harmonic means between states) is considered significant when comparing reconstructions (Pagel, 1999).

DIVERGENCE ESTIMATION

Stingless bee divergence times were estimated from the Bayesian phylogeny using penalized likelihood implemented in r8s 1.71 (Sanderson, 2003), with the

smoothing parameter determined by cross-validation. Time estimation is dissociated from branch length estimation in this analysis via calibration of node divergences based on known stingless bee amber fossils. Fossils were placed tentatively on the phylogeny according to estimates of relationship from the literature. The oldest fossil (*Cretotrigona prisca*, 65 Mya; Engel, 2000) was used to place a minimum age on the stingless bee crown clade. The exact systematic position of *Cretotrigona* is controversial (Michener & Grimaldi, 1988b; Engel, 2000) and we have not assigned it to any extant genus. Because of its possession of several apomorphies (Michener, 1990: 91; Engel, 2000: 3) we do, however, consider *Cretotrigona* to belong to the crown clade comprising all extant stingless bees, rather than an older extinct sister lineage. A maximum age of 125 Mya for the stingless bee clade was based on the age of the radiation of the Angiosperm plants (125 Mya; Grimaldi, 1999; Soltis *et al.*, 2005).

A constraint of r8s is that at least one node age constraint must be fixed. We therefore fixed the root age at intervals spanning 65–125 Mya (i.e. 65, 70, 80, 90, 100, 110, 125 Mya). Three additional stingless bee fossil genera were used to assign minimum age constraints to other nodes of the phylogeny. *Proplebeia* from Dominican Republic amber (15–20 Mya; Camargo *et al.*, 2000) was placed at the node of *Plebeia s.l.* (*Friesella*, *Mourella*, and *Plebeia*), which corresponds to Michener's (1990, 2007) subgenus *Plebeia* (*Plebeia*), although the hypothesized relationship with *Plebeia* is based on limited investigation. The Baltic amber fossils *Liotrigonopsis* and *Kelneriapis* (44.1 Mya; Kelner-Pillault, 1969; Kelner-Pillault, 1970; Engel, 2001a) were placed, respectively, at the nodes uniting *Liotrigona* and *Hypotrigona*, based on the study of Engel (2001a: 134, 136). To test the effect on the age of the root node, we also fixed the age of *Liotrigonopsis* (the younger of the two fossils) at 44.1 Mya and left *Kelneriapis* as a minimum age constraint.

To contrast the divergence dates of the ingroup estimated from penalized likelihood in r8s, we used the Bayesian relaxed clock uncorrelated lognormal method (with a Yule process for the tree prior) implemented in BEAST, version 1.4.7b (Drummond *et al.*, 2006; Drummond & Rambaut, 2007). For the age parameter of the root node, we assumed a normal prior distribution (mean \pm SD; 95 ± 30), and lognormal prior distributions for the age parameters of the other three fossil-calibrated nodes: *Proplebeia* (2.0 ± 0.5 , zero offset lower bound of 15 Mya), *Liotrigonopsis* (2.5 ± 0.5 , zero offset lower bound of 44 Mya) and *Kelneriapis* (2.5 ± 0.5 , zero offset lower bound of 44 Mya). MCMC searches were run for ten million generations with the first two million discarded as burn-in.

RESULTS

The combined dataset of five gene fragments comprised 3596 aligned nucleotides: 16S = 579 bp; 28S = 859 bp; ArgK = 724 bp (including a 174 bp intron); EF-1 α = 839 bp (267 bp intron); and opsin = 595 bp (147 bp intron). The 22-taxon dataset of nine gene fragments comprised 5995 aligned nucleotides, including the above five fragments plus Bub3 = 463 bp (117 bp intron); Dnk = 492 bp (117 bp intron); Gyk = 601 bp (192 bp intron); and Pol II = 843 bp.

RESOLUTION AND SUPPORT FOR THE PHYLOGENY

Bayesian analysis of each of the five individual datasets (see Supporting information, Fig. S1) provided weak support for deeper relationships, with generic clades frequently collapsed into polytomies, but most previously designated genera or subgenera (Camargo & Pedro, 2007; Rasmussen & Cameron, 2007; Rasmussen, 2008) were supported. Each dataset largely supported the monophyly of the broadly defined genera, including *Melipona s.l.* (*Eomelipona*, *Melikerria*, *Melipona*, and *Michmelia*; Michener, 2007) and *Trigonisca s.l.* (*Leurotrigona*, *Celetrigona*, *Dolichotrigona*, and *Trigonisca*; Michener, 2007). Bayesian analyses of the concatenated gene sequences resulted in a highly resolved tree with good branch support across most of the tree (Figs 2, 3). Three major clades were well defined: an Afrotropical (PP = 1.00/ML BV = 98%), an Indo-Malay/Australasian (PP = 1.00/ML BV = 100%), and a Neotropical clade (PP = 1.00/ML BV = 100%). Falling within the Afrotropical clade with good support (PP = 1.00/ML BV = 98%), however, were *Austroplebeia* from Australasia and *Lisotrigona* from the Indo-Malayan region. The two Old World clades (Afrotropical and Indo-Malay/Australasian) were weakly supported as sister clades (PP = 0.88/ML BV = 65%).

Three previously recognized genera were paraphyletic: *Leurotrigona* with respect to the remaining *Trigonisca s.l.* (PP = 0.99/ML BV = 54%), *Trigonisca* with respect to *Dolichotrigona* (PP = 0.90/ML BV = 75%), and *Scaura* with respect to *Schwarzula* (PP = 1.00/ML BV = 85%). Three genera were polyphyletic: *Eomelipona* (PP = 0.99/ML BV = 82%), *Plebeia* (PP = 1.00/ML BV = 99%), and *Geniotrigona* (PP = 1.00/ML BV = 0.86%).

ML bootstrap results were largely congruent with Bayesian results at the generic level, with most of the discrepancies between the two analytical methods occurring at the tips of the tree (Figs 2, 3). By contrast, MP (Fig. 4) did not recover the three major splits of the Bayesian tree (the MP basal relationships

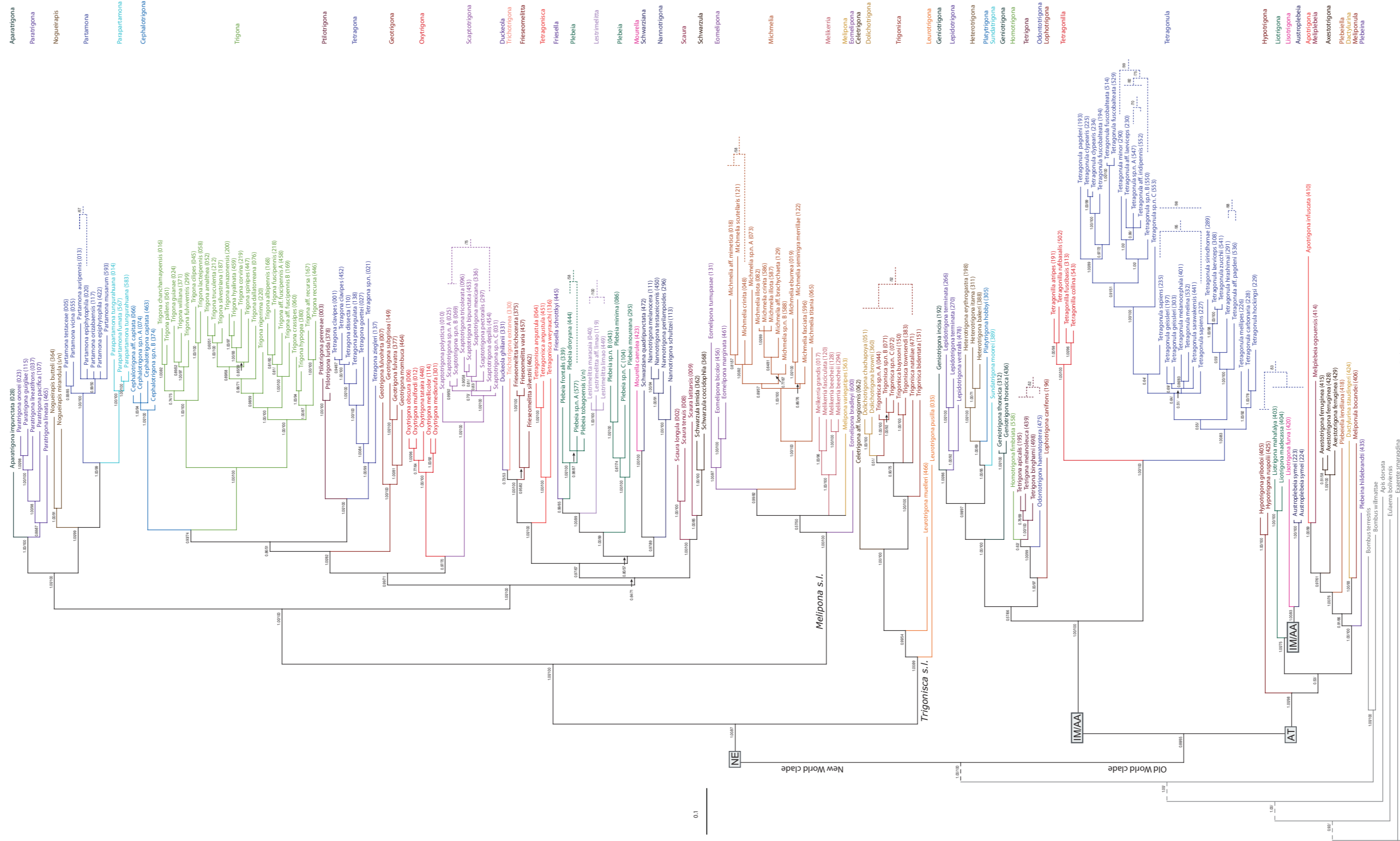


Figure 2. Bayesian phylogeny of stingless bees estimated from concatenated sequences of five gene fragments (16S, opsin, EF-1 α , ArgK, and 28S). Species within the two major splits (New World and Old World) are colour-coded by genus and labelled in the column to the right. Voucher numbers are provided at the end of the taxon names. Values above branches are Bayesian posterior probability values followed by maximum likelihood bootstrap values ($\geq 50\%$). Alternative resolution of maximum likelihood analysis is shown as dotted lines. Outgroup branches (grey dashed lines) have been shortened for clarity. NE, Neotropical; IM/AA, Indo-Malay/Australasia; AT, Afrotropical.

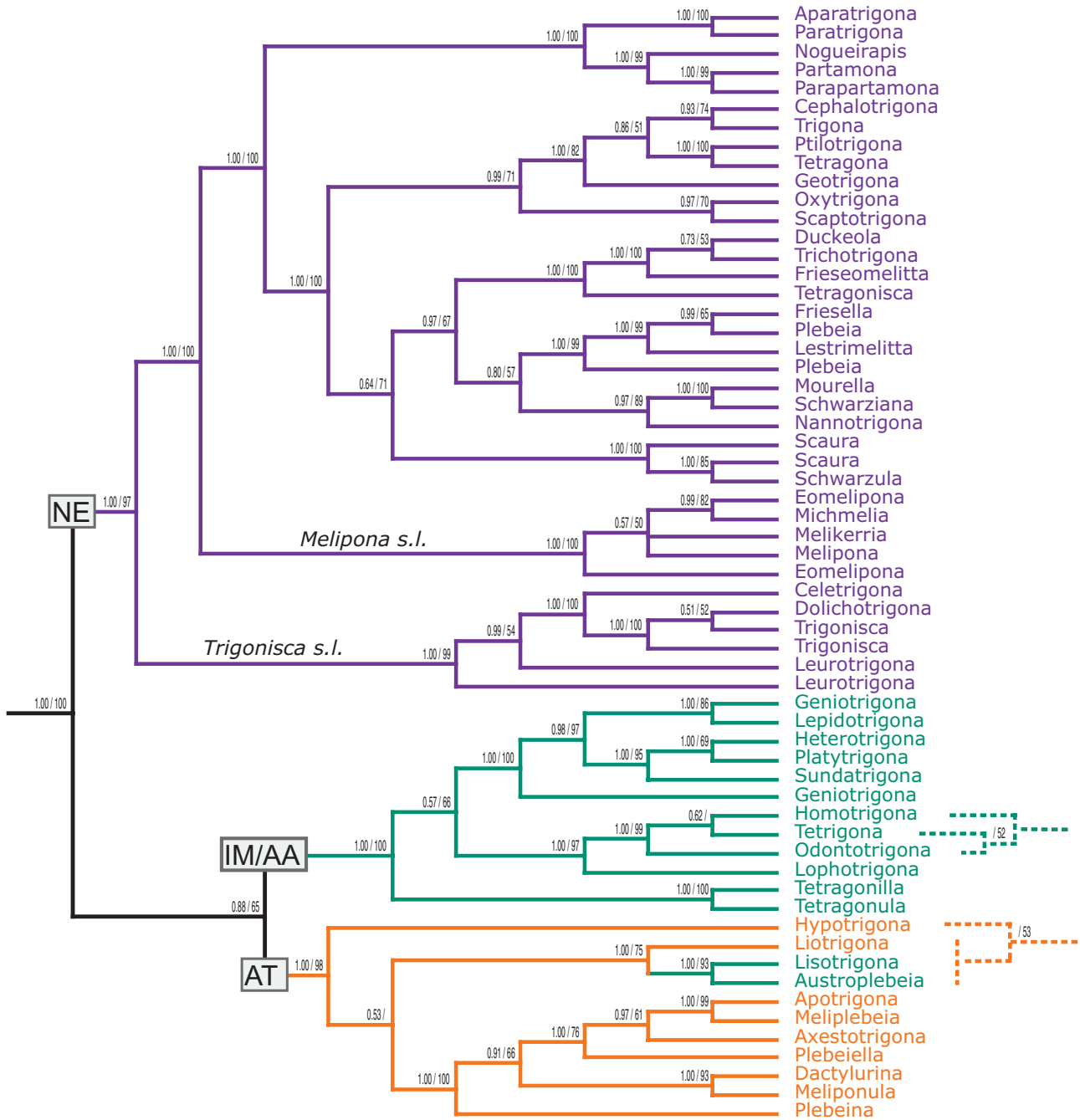


Figure 3. Summary Bayesian tree for Fig. 2, indicating the generic level relationships. Alternative resolutions from maximum likelihood analysis are shown as dotted lines. Values above branches are as given in Fig. 2. NE, Neotropical; IM/AA, Indo-Malay/Australasia; AT, Afrotropical.

in general were poorly supported; BV < 60%) but the tip relationships were largely congruent with those of the Bayesian tree. The MP tree was rooted with poor support (BV < 50%) at *Hypotrigona*, an Afrotropical genus. The remaining ingroup taxa were split (BV < 50%) into an essentially Afrotropical clade (MP BV = 65%), including a small Afrotropical–

Australian clade (*Liotrigona–Austroplebeia*) and an Indo-Malayan/Australasian plus Neotropical clade (MP BV = 60%). The tip relationships were largely congruent with Bayesian results.

Results from the nine-gene/22-taxon analyses (Fig. 5) reinforced the strong support for three major monophyletic lineages (Afrotropical PP = 1.00/ML

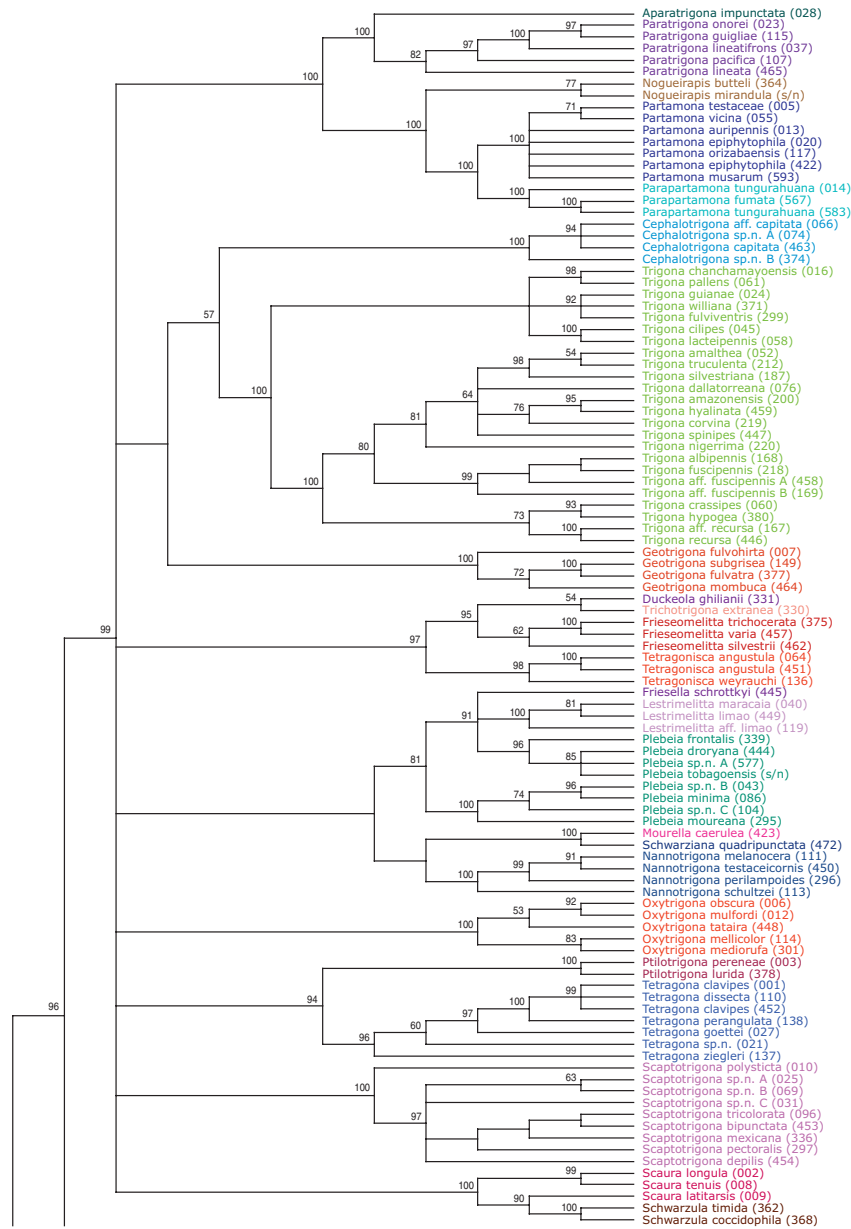


Figure 4. Maximum parsimony (MP) phylogeny (strict consensus of 49 000 trees) of stingless bees estimated from the same five concatenated gene fragments as in Fig. 2. Tree length, Tree length = 7286, consistency index (CI) = 0.34, retention index (RI) = 0.78. Values above branches are MP bootstrap values ($\geq 50\%$). Outgroup branches shortened (grey dashed lines) for visual purposes. NE, Neotropical; IM/AA, Indo-Malay/Australasia; AT, Afrotropical.

BV = 89%; Indo-Malay/Australasian PP = 1.00/ML BV = 100%; Neotropical PP = 1.00/ML BV = 92%), and resolved the uncertainty regarding the relationships among these basal splits. There was strong support for an Old World grouping (Afrotropical plus Indo-Malay/Australasian, PP = 0.96/ML BV = 77%) as sister to the New World clade. MP for the nine-gene subset (Fig. 6) recovered the two Old World clades as sister taxa (MP BV = 62%), but the New World clade

was paraphyletic with *Trigonisca*+*Dolichotrigona* falling out as sister group to the rest of the stingless bees, although with poor support (MP BV = 58%).

The full Z-closure supernetwork of five gene trees (Fig. 7) resembles the Bayesian phylogeny (Fig. 2) in general outline, but indicates regions of topological difference (conflict) among the trees with a web of reticulations (supernetwork) within and between clades. Applying a filter to the supernetwork, allowing

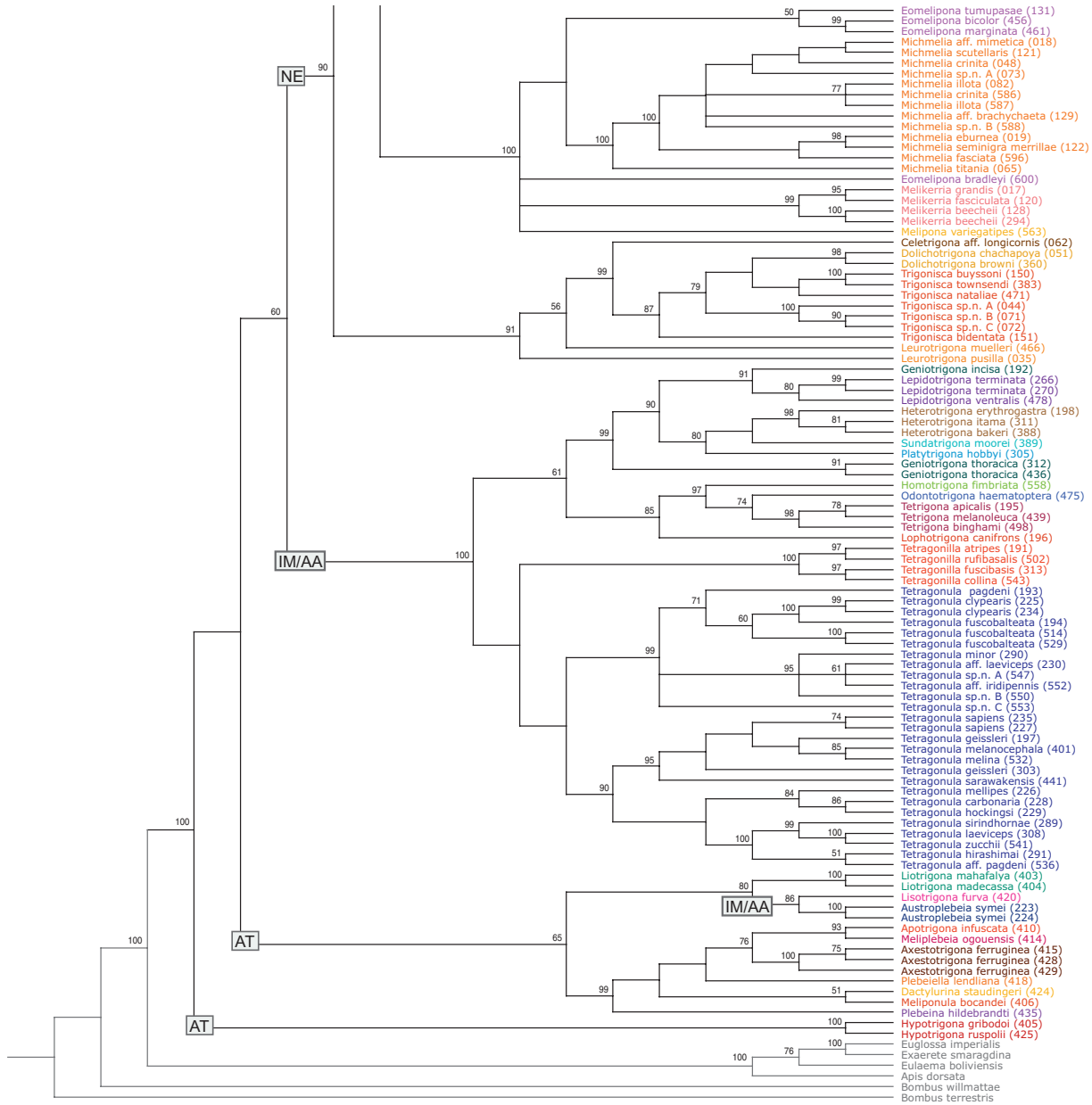


Figure 4. Continued

only the recurrent splits that occur in three or more (mintrees = 3) of the five gene trees, yields a largely resolved set of the same three major biogeographic clades as the Bayesian tree. In particular, *Melipona s.l.* and *Trigonisca s.l.*, which were the source of most of the conflicts, were largely resolved as monophyletic branches in the New World clade. The full supernetwork of nine gene trees (Fig. 8) similarly shows conflicts among the basal group relationships, including a lack of monophyly for the Afrotropical clade and uncertain attachment of both *Melipona s.l.* and

Trigonisca s.l. This is largely resolved with application of a stringent filter, reducing the conflict and further supporting the same three major biogeographic clades as the Bayesian tree.

BIOGEOGRAPHICAL RECONSTRUCTIONS

The DIVA analyses indicated an inclusive New and Old World ancestral region for stingless bees, suggesting that a vicariant event, rather than dispersal, in their early history could explain the current dis-

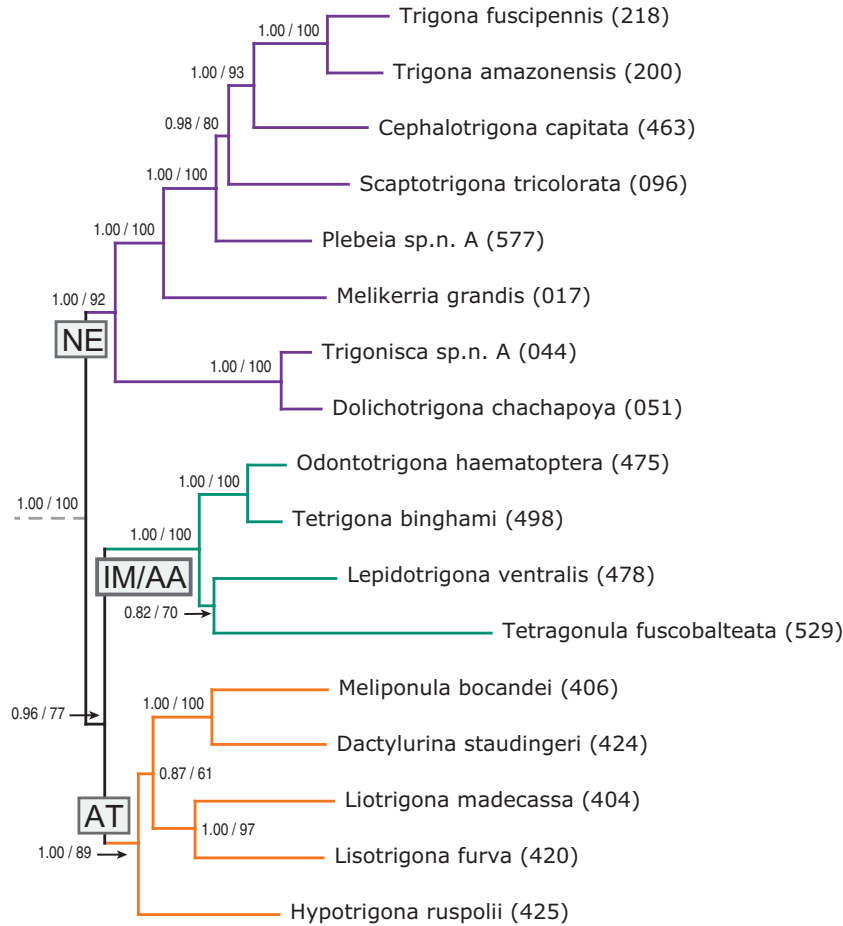


Figure 5. Bayesian phylogeny of stingless bees estimated from concatenated nucleotide sequences of nine gene fragments (16S, opsin, EF-1 α , ArgK, 28S, Pol II, Bub3, Dnk, and Gyk). Values on branches are as given in Fig. 2. Outgroups pruned; NE, Neotropical; IM/AA, Indo-Malay/Australasia; AT, Afrotropical.

tribution. From this analysis, one would infer a widespread ancestral distribution of the stingless bees as a whole, in which they would have inhabited the Neotropical, Afrotropical, and Indo-Malayan/Australasian regions. DIVA also suggests that the Afrotropical clade, with its two Indo-Malayan/Australasian genera (*Lisotrigona* and *Austroplebeia*) is the result of a single dispersal from the Afrotropical region into the Indo-Malay region, followed by dispersal into Australia.

BAYESTRAITS, in contrast, supports an exclusively Old World ancestral geographic origin of the stingless bees (PP = 80% for an Old World clade; Bayes Factor 4). In particular, the Afrotropical region is supported as the ancestral geographic range for all extant stingless bees (PP = 92% for an Afrotropical clade; Bayes Factor 5). The ancestral geographic range for each of the three main clades (Neotropical, Afrotropical, Indo-Malayan/Australasian) is recovered as from within each of the three distinct regions,

and not secondary dispersal to each region (PP = 99–100%).

DIVERGENCE DATES

Estimates for the divergence times of the three major splits and the root-node are listed in Table 3 for each of the different dating methods and fossil constraints. Penalized likelihood with a fixed node for *Liotrigonopsis* places the root-node within the middle (81 Mya) of the range of ages given in Table 3. This age was used for the chronogram (Fig. 9), which also indicates the placement of fossil node age constraints in the phylogeny. Based on the *Liotrigonopsis* fixed age, the Old World clade diverged 73 Mya, the Afrotropical clade diverged 61 Mya, and the Indo-Malayan/Australasian clade diverged 49 Mya. The New World clade began to diverge 71 Mya. Beast provided older estimates for all of the regions compared to r8s (Table 3), including for the root-node (96 Mya).

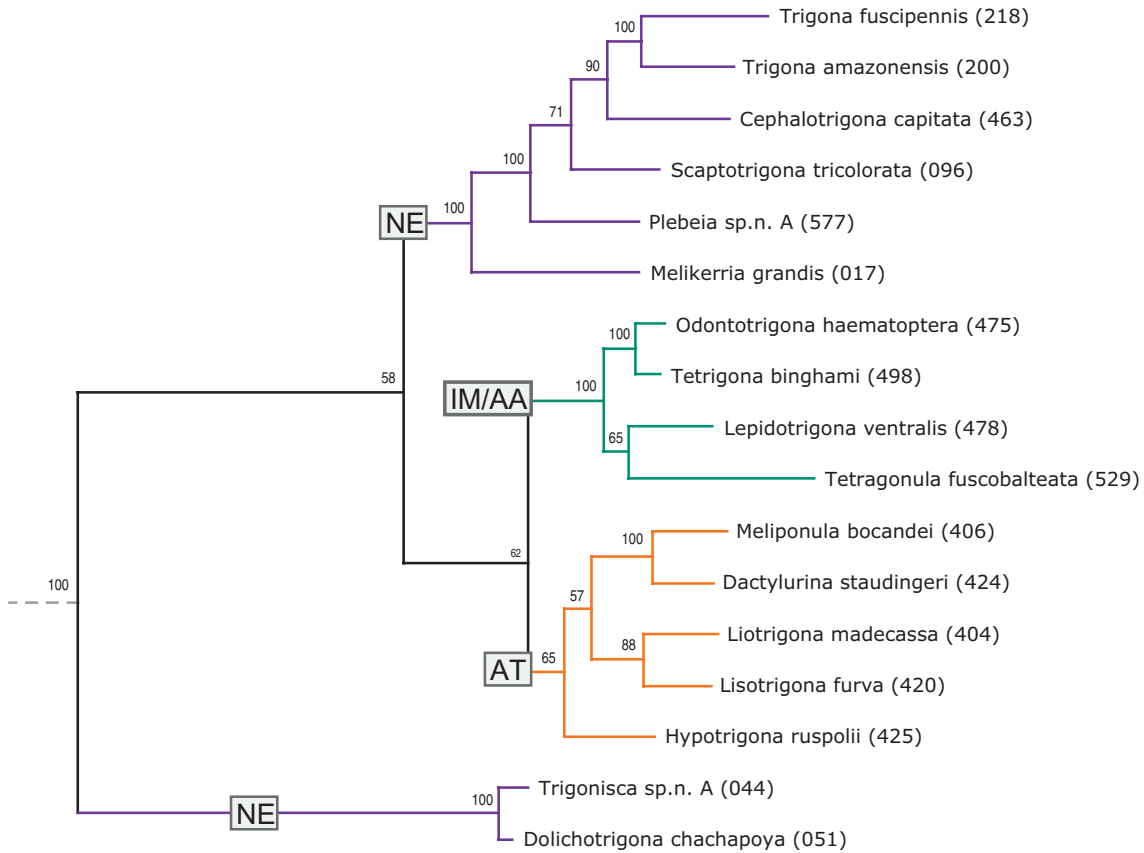


Figure 6. Maximum parsimony (MP) phylogeny (single best tree) of stingless bees estimated from concatenated sequences of the same nine gene fragments as in Fig. 5. Tree length, Tree length = 3611, consistency index (CI) = 0.66, retention index (RI) = 0.65. Outgroups pruned; NE, Neotropical; IM/AA, Indo-Malay/Australasia; AT, Afrotropical.

DISCUSSION

BIOGEOGRAPHICAL CONCLUSION

Comprehensive sampling of almost all genera of stingless bees and the resulting robust molecular phylogeny allows credible estimation of divergence times of the major clades and a solid framework for testing the variously proposed biogeographic hypotheses. It appears that the biogeographic history of the stingless bees is complex. They are at least Late Cretaceous in age (81 or 96 Mya, depending on the estimation method) and are the only group of bees with a global distribution restricted to tropical and subtropical areas (Michener, 2007). Their distribution suggests either a widespread Gondwanan origin or an origin of interplate movement via plate connections or land bridges during periods of tropical climate. A late Gondwanan origin, as proposed by Camargo & Wittmann (1989) cannot be rejected, even though they argued this from the incorrect view that *Plebeia*-like taxa from the Neotropical, Afrotropical, and Australasian regions are closely related. Although the phylogeny indicates clearly that these groups do not form a

monophyletic clade, their argument that stingless bees are Gondwanan could still stand. Dispersal-vicariance analysis based on the current phylogeny (DIVA) suggests that early stingless bees occurred throughout the range they currently occupy (Neotropical, Afrotropical, Indo-Malay/Australasia) or maybe (BAYESTRAITS) the bees were initially restricted to the Afrotropical region followed by range-expansion to the Neotropical and Indo-Malay/Australasia regions. It is possible that as the continental plates moved and topographic barriers appeared, the ancestral fauna split into subgroups. The first major split occurred between the Old and New Worlds, followed by the isolation and diversification of the Afrotropical and the Indo-Malay/Australasian faunas. There is no support for the hypothesis of an original Eocene dispersal out of South America (Kerr & Maule, 1964; Michener, 1990) leading to the three basal clades. However, more recent dispersals of taxa near the tips of the phylogeny have occurred, such as those from Africa to Australasia (i.e. *Lisotrigona*, *Austroplebeia*). Wille (1979) might have been correct in suggesting an origin in Africa, but the dispersal route to South

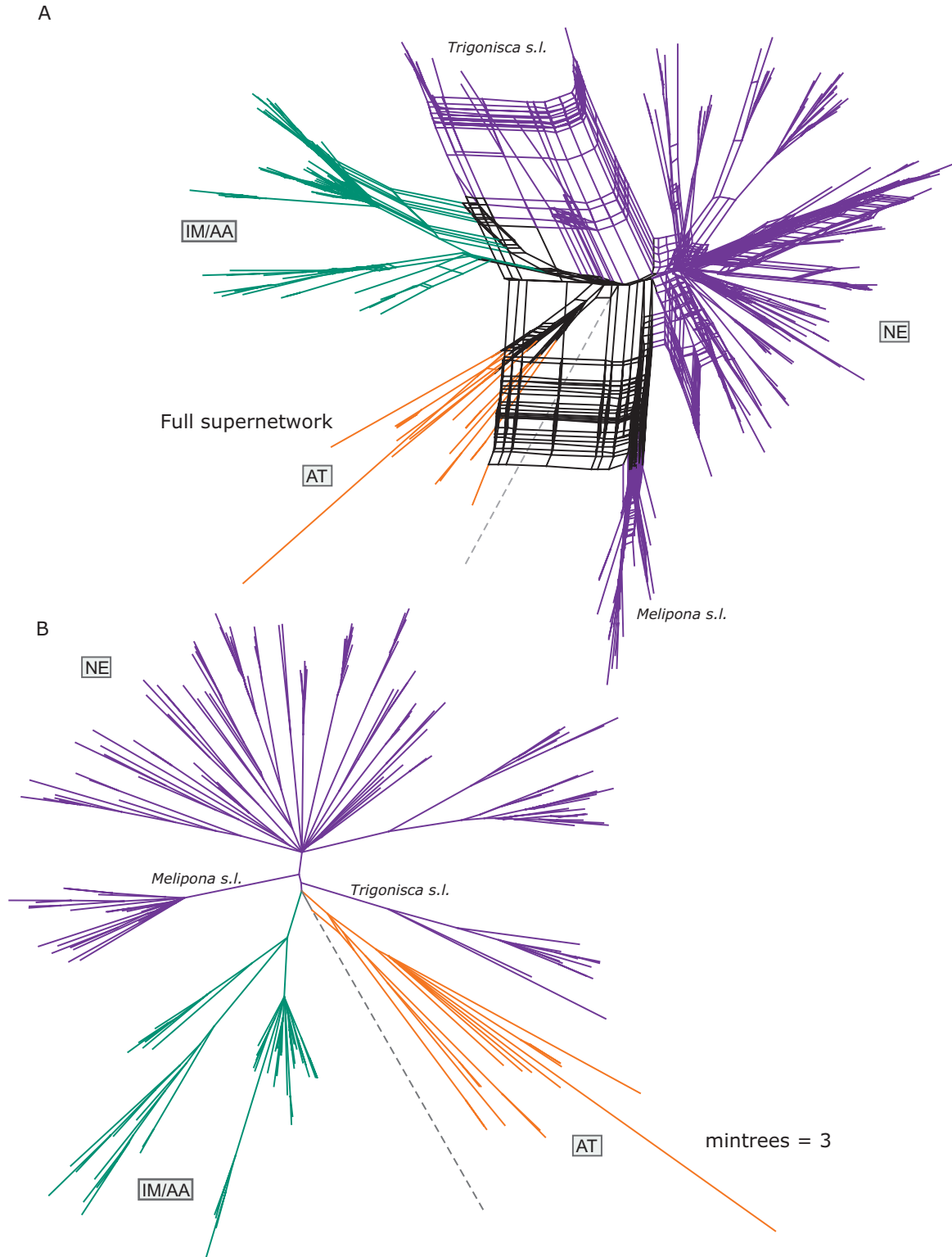


Figure 7. A, Z-closure supernetwork of five meliponine gene trees (see Supporting information, Fig. S1) prior to filtering. The supernetwork resembles the Bayesian phylogeny (Fig. 2) with conflicts among gene trees indicated by black boxes (between clade conflict) and coloured boxes (within clade conflict). B, Z-closure supernetwork after filtering (mintrees = 3), displaying all splits found in (or fully compatible with) three or more of the five gene trees. Taxon names are omitted and outgroup branches are pruned (grey dashed line) and shortened. Stingless bee branches are colour-coded by region: Neotropical (purple, NE), Indo-Malay/Australasia (green, IM/AA), and Afrotropical (orange, AT).

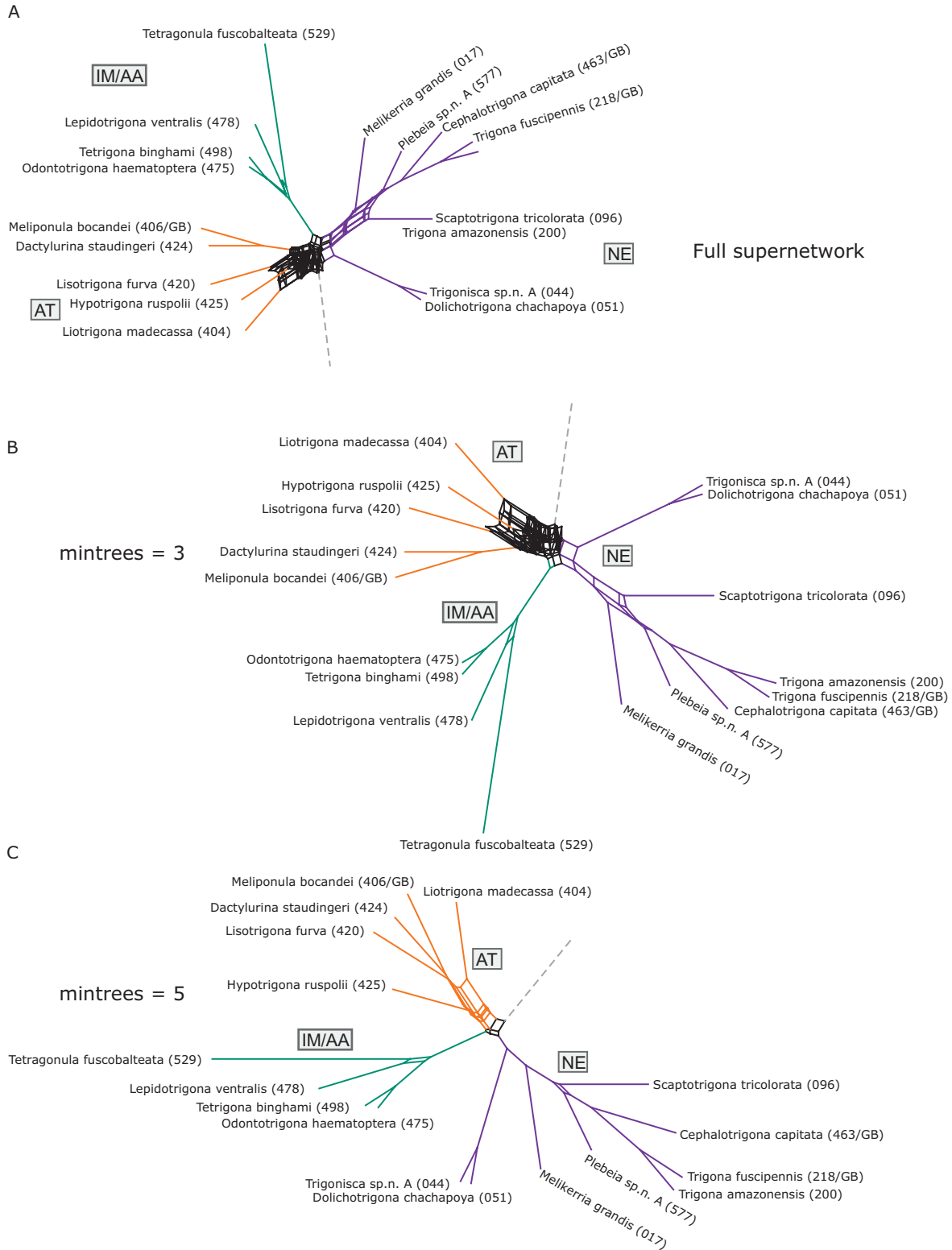


Figure 8. A, Z-closure supernetwork of nine meliponine gene trees (22-taxon dataset) prior to filtering (= full supernetwork). B, Z-closure supernetwork of the nine gene trees, indicating filtering with mintrees = 3 and (C) mintrees = 5, respectively, showing only the splits found in (or compatible with) at least three or five of the nine original source trees. Taxon names are omitted and outgroup branches are pruned (grey dashed line) and shortened. Stingless bee branches are colour-coded by region: Neotropical (purple, NE), Indo-Malay/Australasia (green, IMM/AA), and Afrotropical (orange, AT).

Table 3. Inferred divergence time estimates of key-nodes in the phylogeny, inferred using the computer program r8s under different constraints and BEAST

Analyses	Meliponini root age	Old World	Afrotropical	Indo-Malay/ Australasia	Neotropical	<i>Kelneriapis</i>	<i>Liotrigonopsis</i>
r8s	81.06	73.44	61.39	48.63	70.73	54.90	44.1*
	65*	60.65	54.10	40.77	56.83	50.40	44.1
	70*	64.64	56.41	43.20	61.16	51.85	44.1
	80*	72.60	60.93	48.10	69.81	54.62	44.1
	90*	81.55	68.17	53.99	78.53	60.96	48.97
	100*	90.61	75.74	59.99	87.26	67.74	54.41
	110*	99.67	83.31	65.99	95.98	74.51	59.85
	125*	113.26	94.68	74.99	109.07	84.67	68.01
BEAST	95.51	93.67	65.74	69.40	81.46	61.99	56.31

An asterisk '*' indicates a fixed age. The inferred ages for the crown clade of the two main fossils (*Kelneriapis* and *Liotrigonopsis*) are included for comparison.

America across Europe is probably incorrect and this is more likely to have been a vicariant event.

EVIDENCE FOR A VICARIANT SPLIT BETWEEN THE NEW AND OLD WORLD

The Gondwanan continental separation of South America from Africa occurred near the end of the Albian, 100 Mya (Parrish, 1993). Although this geological split is older than the age inferred for the crown clade of the stingless bees (81 Mya), trans-Atlantic floral dispersal between the continents (Morley, 2003; Morley & Dick, 2003) occurred via island chains along the Rio Grande Rise–Walvis Ridge and the Ceará Rise–Sierra Leone Rise (Parrish, 1993; Culver & Rawson, 2000) until long after the supercontinent split. Thus, plants frequently appeared simultaneously on both sides of the Atlantic Ocean until the Maastrichtian (70.6–65.5 Mya) and later (Morley, 2000). It is possible that Old World and New World stingless bees maintained contact via these island chains after the geological separation at 100 Mya, until the inferred divergence of the Neotropical clade of stingless bees at approximately 71 Mya.

Few scenarios other than a Gondwanan origin could place stingless bees in South America by approximately 70 Mya. The South American continent has been separated for most of the time subsequent to the split from Africa and became connected to North America only recently via the Isthmus between Panama and South America (3–4 Mya; Donnelley, 1992). Filter bridges to North America existed earlier, however, during the Late Miocene, Middle Palaeocene, and Late Cretaceous (Stehli & Webb, 1985; Morley, 2003). Evidence that stingless bees could have existed along these filter bridges is sug-

gested by the presence of *Proplebeia* fossils found in 15–20 million-year-old amber from Central America (Mexico, Chiapas) and the Caribbean (Dominican Republic) (Camargo *et al.*, 2000; Camargo & Pedro, 2007).

EVIDENCE FOR THE OLD WORLD SPLITS AND DIVERGENCE TIMES

Today, the Old World stingless bees are restricted to tropical and subtropical regions of the world (Michener, 2007). Extinct Old World taxa are known, however, from Baltic and Sicilian amber (Tosi, 1896; Engel, 2001a), documenting a past presence in the Palearctic region. Climate data (Morley, 2000; Scotese, 2008), the current and fossil ranges (Michener, 2007), and past extension of land area (Smith, Smith & Funnell, 2004), makes it plausible that stingless bees would have had a wide distribution during the global warming from the first half of the Cretaceous through the Tertiary (Scotese, 2008). The distribution of the Old World clade would have included all of the present-day Old World tropical and subtropical regions, extending into the paratropical (mid-latitude tropical) areas (Scotese, 2008) of the Palearctic, and possibly into the tropical parts of the Nearctic region, as documented by the presence of *Cretotrigona* from New Jersey (Michener & Grimaldi, 1988a). The honey bee (*Apis*), for example, had both a Palearctic and Nearctic distribution but later went extinct in the Americas (Engel, Hinojosa-Díaz & Rasnitsyn, 2009).

Widespread Old World stingless bees could have maintained contact (gene flow) through available corridor connections until being interrupted by cooling, and isolated by the position of, in particular, the Obik

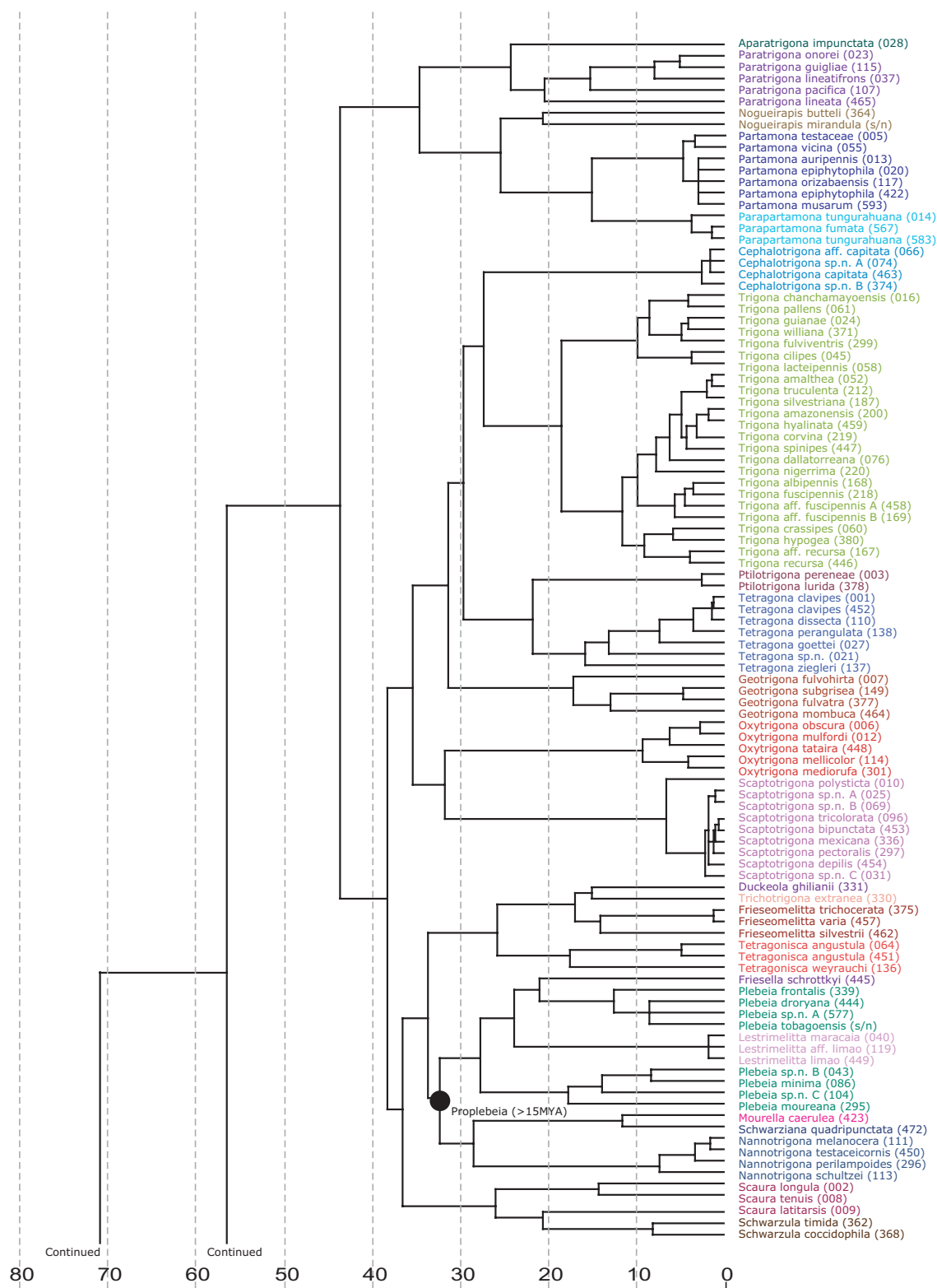


Figure 9. A time-calibrated Bayesian phylogeny (chronogram) of stingless bees, with age estimates for the diversification times of all clades. Dating analysis is based on r8s, with *Liotrigonopsis* age fixed at 44.1 Mya. Shaded circles represent node age constraints of fossils: *Proplebeia* (Mexico, Dominican Republic), *Cretotrigona* (New Jersey, USA), *Kelneriapis* and *Liotrigonopsis* (Baltic amber, northern Europe). NE, Neotropical; IM/AA, Indo-Malay/Australasia; AT, Afrotropical.

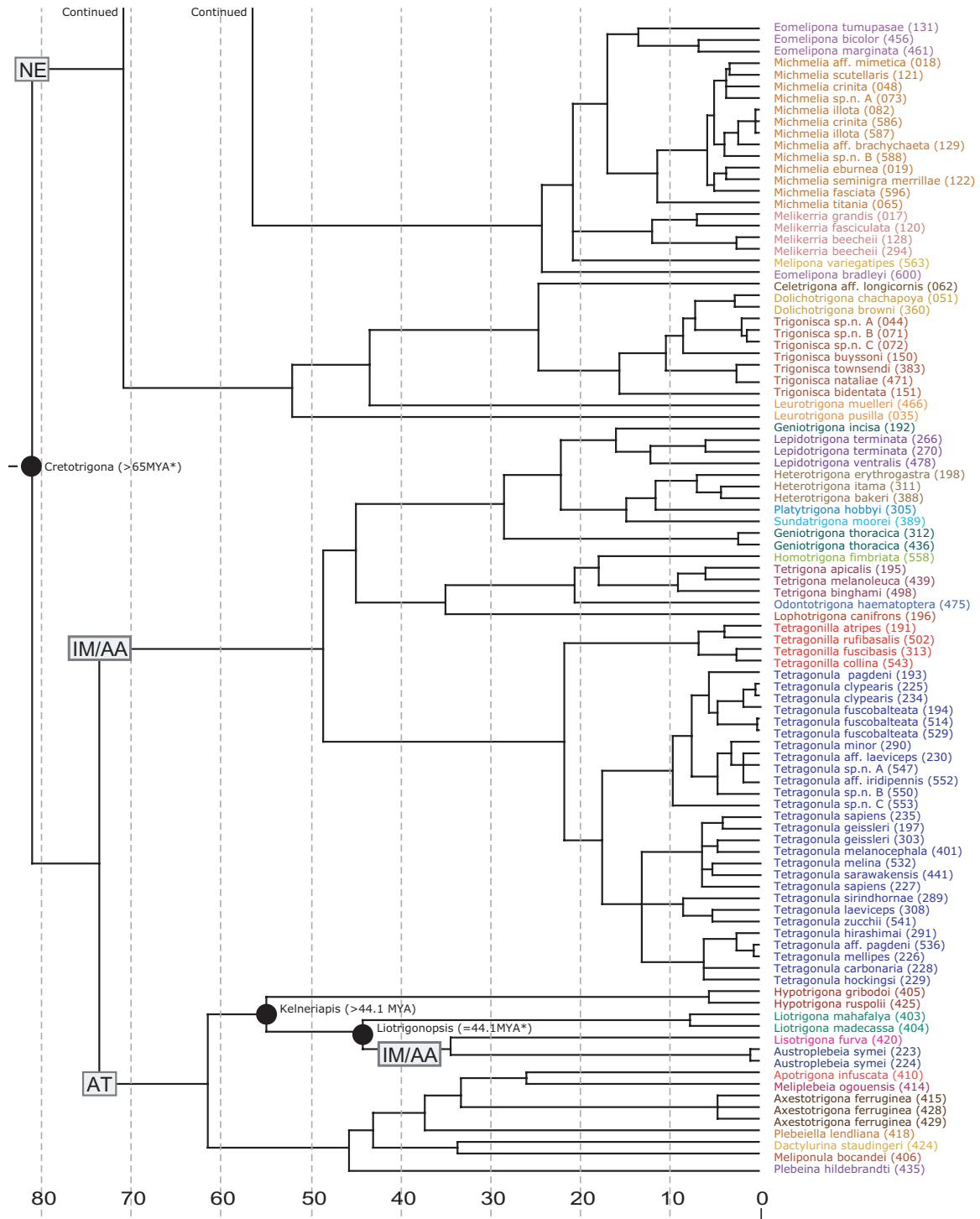


Figure 9. Continued

Sea and the Tethys Sea (Culver & Rawson, 2000). The Obik Sea separating Europe from Asia was dry during the warm Palaeocene, permitting sustained contact between Europe and Asia until the climatic change from the Eocene to Oligocene (50–35 Mya). Tempera-

tures in the Palearctic region are estimated to have decreased by 20 °C during this period and caused the extinctions known as ‘Grand Coupure’ (‘big cut’) (Culver & Rawson, 2000). The climatic change from principally warm humid forest types during the

Eocene, to more zonal climates during the Oligocene, with arid, colder, and more open savannah-like habitats (Prothero & Berggren, 1992; Prothero, 1994), would eventually have driven the Palearctic stingless bee fauna to extinction, presumably part as a result of constraints in the thermoregulatory abilities of their nests (Engels, Rosenkranz & Engels, 1995).

The phylogeny indicates an Afrotropical and Indo-Malayan/Australasian sister group relationship, with the Palearctic fossils from Baltic amber nested within the Afrotropical clade (Engel, 2001a). Contact zones between Africa and Europe existed during the Late Cretaceous (Morley, 2003). Subsequently, Africa was separated from Europe by a shallow seaway until the Late Eocene, when contact between Europe and Africa is recorded again (Raven & Axelrod, 1974; Cavagnetto & Anadón, 1996; Morley, 2003; Smith *et al.*, 2004) and, eventually, Africa united with Eurasia in the Early Miocene (Potts & Behrensmeyer, 1992). These connections could have allowed scattered contact between the Afrotropical and Palearctic region, as documented for ctenoplectrine bees (Schaefer & Renner, 2008).

The presence of stingless bees throughout the Indo-Malay region, including isolated islands, is the result of an intricate geological history, including dramatic changes in climate, sea level, and land form (Holloway & Hall, 1998), where Borneo and the Malay Peninsula were connected by land bridges until the Miocene (Hall, 1998; Holloway & Hall, 1998; Smith *et al.*, 2004). This period was followed by cyclical glaciations during the Pleistocene, with the sea level occasionally being low enough to expose land bridges that would have reconnected Borneo, Sumatra, and Java to the Malay Peninsula as a single landmass (Morley, 2000; Voris, 2000).

ARE THERE ANY ALTERNATIVE BIOGEOGRAPHICAL SCENARIOS?

With all of the known stingless bee fossils located in the former Laurasia (i.e. *Proplebeia* from Mexico and Dominican Republic, *Cretotrigona* from New Jersey, *Kelneriapis* and *Liotrigonopsis* from Baltic amber of northern Europe), it is tempting to hypothesize a Laurasian origin for the stingless bees, with subsequent range-expansion southward into South America, Africa, and Asia after plate contacts. The earlier derived groups within a clade are expected to be found in areas where the clade initially diversified, and the absence of such groups from a region may indicate that the region was occupied after the diversification occurred. The higher generic endemism of the South American fauna (Camargo & Pedro, 2007) and the lack of earlier derived groups in Central America does not suggest a movement from Laurasia into South America through Central America, and evidence of the more

recent movements within genera rather suggests a northward dispersal from South America into Central America (Camargo, Moure & Roubik, 1988; Camargo & Wittmann, 1989; Camargo & Moure, 1996; Camargo & Pedro, 2003, 2007). A better understanding of the systematic position of the fossil *Cretotrigona* from New Jersey could help to test an ancient Laurasian origin of the stingless bees. For example, if *Cretotrigona* were the sister group to the extant Neotropical clade, it would document an ancient relationship between South and North America as hypothesized from a Laurasian origin (stingless bees secondarily present in South America). If instead *Cretotrigona* belongs to an Old World clade, presumably dispersing through connections between North America and Eurasia (Culver & Rawson, 2000: 328; Morley, 2003), the Gondwanan scenario proposed here would be the preferred explanation, with only recent movements between South and Central America.

The documented South America–Antarctica–Australia connection from 52–35 Mya was used by temperate South American flora and fauna, as exemplified by affinities between taxa currently distributed in southern South America and the Australian region (Sanmartín & Ronquist, 2004; Almeida & Danforth, 2009). By contrast, taxa inhabiting tropical portions of South America have more relatives in tropical portions of Africa and the Nearctic Region. Such trans-Antarctic connection among tropical stingless bees from the Neotropical and Indo-Malay/Australian Regions does not appear to be a plausible scenario. The Australian taxa are deeply nested within otherwise exclusively Indo-Malayan and Afrotropical clades (Rasmussen & Cameron, 2007), and the general paucity of stingless bee genera and species in Australia (Dollin, Dollin & Sakagami, 1997; Rasmussen, 2008) supports a later dispersal from within the Old World Region to Australia, rather than an interchange with South America (Rasmussen & Cameron, 2007), as proposed by Camargo & Wittmann (1989) for *Austroplebeia*. Terrestrial dispersal routes connecting Madagascar to Africa, India or Antarctica (Upchurch, 2008) also do not provide an explanation for most of the current Old World meliponine distribution. Only a single widespread Afrotropical genus (*Liotrigona*) occurs on Madagascar. Only *Lisotrigona* (Indo-Malay) and *Austroplebeia* (Australasia), the sister-groups to *Liotrigona*, represent a possible connection among Madagascar–India–Asia–Australia, as has been reported for other bees (Schwarz *et al.*, 2006).

ADDITIONAL INTERPRETATIONS OF THE AGE OF STINGLESS BEES

The divergence time estimates of 81 Mya (r8s) to 96 Mya (Beast) for the stingless bee crown clade is

older than the age suggested by Hines (2008), who reported the date for the Meliponini-Bombini split as between 80 and 100 Mya, with radiation of the stingless bees supposedly occurring later. When Hines (2008) used stingless bee fossil calibration alone, in the absence of upper age constraints on the bees, this resulted in much older dates (> 160 Mya) for the Bombini–Meliponini split as a result the long branch subtending the stingless bees. If stingless bees are younger (80–65 Mya) than the age inferred from our analysis, as implied by Hines (2008), the diversity of South American stingless bees remains to be explained in light of the biogeographical history of the continent. If, on the other hand, stingless bees are as old as inferred here, the remaining 32 extant tribes of Apidae must have had an ancient and possibly rapid radiation, following the radiation of the angiosperm plants (Grimaldi, 1999; Grimaldi & Engel, 2005; Soltis *et al.*, 2005).

The corbiculate bees, including Meliponini, which underwent extensive extinction of entire tribes during the Eocene (Engel, 2001b), could provide additional biogeographical details for a broader discussion of the age of all the Apidae. Examination of all four of the corbiculate tribes, including fossil clades, has resulted in inference of a date approximately 70 Mya for the stingless bee crown clade (Engel, 2004; Grimaldi & Engel, 2005), intermediate in range between what is proposed in the present study and that proposed by Hines (2008). Anthophorini is the only other documented Apidae tribe of considerable age (*Paleohabropoda oudardi*, 60 Mya; Michez *et al.*, 2009), adding support to an ancient age of the Apidae bees. Large-scale molecular studies of all bee families (Danforth *et al.*, 2006a, b) have not yet presented divergence time estimates, but such a large-scale approach will be necessary for a rigorous assessment of the age of Meliponini in a larger context.

CLASSIFICATORY IMPLICATIONS

Our comprehensive Neotropical taxon sampling suggests that several classificatory modifications are in order. We make no nomenclatural decisions in the present study, however, because specific recommendations concerning the recognition of groups will be made elsewhere. Some of the relevant details can be highlighted. *Tetragonisca*, *Frieseomelitta*, and *Duckeola* are not part of the *Trigona s.l.* clade, contrary to the classification of Michener (1990, 2007). *Cephalotrigona* was excluded from *Trigona s.l.* by Michener (1990), but is here the sister group to *Trigona s.s.* The most surprising discrepancy between the phylogeny and the current classification (Camargo & Pedro, 2007) is the nonmonophyly of *Plebeia s.s.*, because the robber bees, *Lestrimelitta*,

fall out as sister group to one of the two *Plebeia* clades. There is no morphological support for *Plebeia* being two distinct clades, with *Lestrimelitta* nested within, even based on examination of the male terminalia (J. M. F. Camargo, unpubl. data). Because of their unique morphology (Michener, 1990) and biology (Sakagami, Roubik & Zucchi, 1993), we do not recommend synonymizing *Plebeia* under *Lestrimelitta* at this early stage in their analysis. That decision should await a more rigorous study of the speciose *Plebeia*-like group. To enforce monophyletic taxa, the genus *Schwarzula* should be synonymized under *Scaura*, *sensu* Michener (1990), and *Dolichotrigona* should be synonymized under *Trigonisca*, *sensu* Camargo & Pedro (2005). The revised morphological diagnosis for *Scaura* follows Moure (1951: 51) and that for *Trigonisca* follows Camargo & Pedro (2005: 70). Variable branch support among the *Melipona s.l.* makes it difficult to assess whether the polyphyletic relationship of *Eomelipona* should be corrected. Further data are needed to better resolve the internal relationships among the *Melipona s.l.* genera. Of the Old World genera, *Geniotrigona* is the only lineage not recovered as monophyletic, as previously reported in Rasmussen & Cameron (2007).

FUTURE DIRECTIONS

Diverse biological data are available for a large number of stingless bee taxa (Eardley, 2004; Camargo & Pedro, 2007; Rasmussen, 2008) and this phylogeny provides the first significant global and comprehensive molecular approach for reconstructing the generic relationships. As such, we have a unique opportunity to synthesize information on relationships with new and previously gathered biological data in an evolutionary context. With the overall phylogeny in place, species-level phylogenies should be pursued for testing hypotheses of behavioural evolution (Rasmussen & Camargo, 2008) and to provide an indirect record of the speciation events that have led to extant species. This can provide information on the tempo of diversification within clades (Barraclough & Nee, 2001) and could be used to correlate local speciation with environmental data. Comprehensive species-level sampling would also greatly enhance the biogeographic hypotheses. Dated dispersal events between South and Central America could provide support for directionality of such movements, corroborate divergence time results, and explain the fossil distributions in North and Central America.

Branch support was generally good throughout the tree, although the internodes of certain clades are short or have low support values (e.g. *Melipona s.l.*). These more specific parts of the tree should be

addressed with additional sets of molecular markers and extended taxon sampling. Several genera were also poorly sampled compared to the number of known extant species, such as *Plebeia* (eight of approximately 80 taxa sampled, J. M. F. Camargo, pers. comm.), and further assessment of the evolutionary history awaits a rigorous sampling of those groups. Finally, the five missing genera should be sampled so that morphology-based relationships can be tested [i.e. *Camargoia* as putative sister group of *Ptilotrigona* (Camargo & Pedro, 2004); *Cleptotrigona* as sister group of *Liotrigona* (Michener, 1990); *Meliwillea* as sister group to *Scaptotrigona* (Roubik, Segura & Camargo, 1997); *Papuatrigona* unplaced within *Trigona s.l.* (Michener, 1990); *Paratrigonoides* as sister group to *Paratrigona*–*Aparatrigona* (Camargo & Roubik, 2005); and *Pariotrigona* as sister group of *Hypotrigona* (Michener, 1990)].

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Phylogeny of the stingless bees estimated from Bayesian analysis of individual sequence data from five gene fragments (16S, opsin, EF-1 α , ArgK, and 28S). Colour coding, NE, IM/AA, and AT, as in Fig. 2.

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