MOLECULAR PHYLOGENETIC ANALYSIS OF THE BEES (HYMENOPTERA), WITH AN EMPHASIS ON APIDAE AND THE EVOLUTIONARY HISTORY OF SOCIAL AND CLEPTOPARASITIC BEHAVIOR

A Dissertation
Presented to the Faculty of the Graduate School
of Cornell University
in Partial Fulfillment of the Requirements for the Degree of
Doctor of Philosophy

by
Sophie Carole Cardinal
February 2010

MOLECULAR PHYLOGENETIC ANALYSIS OF THE BEES (HYMENOPTERA), WITH AN EMPHASIS ON APIDAE AND THE EVOLUTIONARY HISTORY OF SOCIAL AND CLEPTOPARASITIC BEHAVIOR

Sophie Carole Cardinal, Ph.D.

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Apidae (Hymenoptera) is the most speciose family of bees with over 5600 species. The family is notable for having some of the most important pollinators of managed crops, yet also comprises a rich diversity of social and parasitic lifestyles, host plant affinities, and ecosystem services. Despite its importance, relationships among the tribes within Apidae remain unclear. To date, rigorous phylogenetic analysis has been challenged by long-standing assumptions about the relatedness of cleptoparasitic groups in relation to their hosts. I performed the first large-scale phylogenetic study of the family Apidae based on DNA sequence data, including representative taxa from all 33 apid tribes. I then used this phylogeny to investigate the origins and antiquity of cleptoparasitism and sociality. Results indicate that most cleptoparasitic apid bees form a monophyletic group, and therefore stem from a single origin of cleptoparasitism (with two more origins in the Euglossini orchid bees and one in the tribe Ctenoplectrini). Divergence time analysis using a relaxed fossil-calibrated molecular clock model reveals that cleptoparasitism is an ancient behavior in apid bees that first evolved ~100 Ma. Results also indicate that primitive eusociality is the ancestral state for corbiculate Apidae, and that orchid bees represent a reversal from eusociality to solitary, communal, and weakly social behavior. According to my divergence time analysis, eusociality first evolved ~87 Ma in the corbiculates, much earlier than in other groups of bees. To date the origin of bees and their major clades, I performed a phylogenetic analysis of bees including representatives from every subfamily, and almost all tribes, using sequence data from seven genes. I then conducted a fossil-calibrated relaxed clock divergence time analysis. I estimate that bees originated at the start of the Aptian, concurrently with the origin of the eudicot angiosperms. All of the major bee clades are estimated to have originated during the middle to late Cretaceous, which is when angiosperms diversified to becom the dominant group of land plants. This study firmly establishes temporal overlap in the diversification of bees and angiosperms, a necessary precondition for the role of bees in the angiosperms' rise to dominance during the late Cretaceous.

BIOGRAPHICAL SKETCH

Sophie grew up in New Liskeard, a small town in northern Ontario. An arch south of town inscribed with "Gateway to the North" declares a certain pride of latitude. Meanwhile, local agricultural pride is expressed by Miss Claybelt, an 18-foot high fiberglass Holstein cow. More important to Sophie however, is the large francophone community found in this area – a community she grew up in, with French as her first language, and that she was proud to be a part of. Nevertheless, like many rebellious teenagers she was inspired to leave the land of Miss Claybelt to study marine biology, attending Dalhousie University in Halifax, Nova Scotia, for the first two years of her B.Sc. (Biology). She then transferred to the University of Toronto to complete her B.Sc. where her interests shifted to entomology. There, she was fortunate to work for Prof. Chris Darling (Royal Ontario Museum/U of T). Long hours spent in the museum pinning and labeling insects from Vietnam is what got her seriously interested in insect systematics. She then attended York University (Toronto) for her M.Sc. (Biology) with Prof. Laurence Packer. Here her interests shifted from parasitoid wasps to bees. At the time she believed that she would one day return to working on parasitoid wasps, but then she discovered cleptoparasitic bees, and she knew that my PhD. would have to focus on them. In 2004, she began her Ph.D. at Cornell University (Ithaca, New York) with Prof. Bryan Danforth. During her dissertation, she has travelled to Arizona, French Guiana, Paraguay and South Africa to collect and study bees. In January 2010, she will continue to work in Dr. Danforth's lab as a postdoctoral associate.

ACKNOWLEDGMENTS

I am most indebted to my advisor, Bryan N. Danforth, for both accepting me into his lab and, more importantly, for providing me with invaluable support and encouragement throughout my Ph.D. I am also thankful to my committee members, James L. Liebherr and Anurag Agrawal, for their advice, constructive criticism and editing of my dissertation. I also benefitted from participating in classes and discussion groups led by Jim and Anurag during my time at Cornell.

Members of the Danforth lab throughout the years have contributed greatly to my professional and personal development. I am thankful to Eduardo Almeida, Jennifer Fang, Neha Bodapati, Kojun Kanda, and Andrew Debevec for assistance in the lab. Over the years, Eduardo, Jesse Litman, and Margarita Lopez-Uribe have not only shared with me their enthusiasm and knowledge about bees and phylogenetics, but more importantly their friendship. I am especially grateful to Jesse and Margarita for their support over the last few years.

My family has always supported me unconditionally throughout my life and the time during my dissertation was no exception. They have always made sure that I know that they are proud of me and wish only the best for me. I could also always count on my parents to take care of Chebucto when I had to leave on longer bee-collecting trips. Chebucto is in part to credit for the maintenance of my sanity at times through his constant affection, general excitement and enthusiasm for the simpler things in life (especially cardboard boxes).

I am indebted to Chris Darling and Laurence Packer for my time spent in their labs before coming to Cornell. I never would have made it to the point of starting a Ph.D. without the help, encouragement and training that both of them provided for me.

Some of my most insightful conversations at Cornell have been with Stuart Campbell. His questions and comments have pushed me to better understand the logic behind the work that I do. This dissertation was also greatly improved by comments and careful editing provided by Stuart. His support, encouragement and love during the last few stages of my thesis have convinced me not just to settle for that which is acceptable but to expect more from myself and life. For that, I will always be grateful.

The Hortorium Systematics Discussion Group led by Kevin Nixon helped me develop some critical thinking skills and prevented me from becoming overly zealous about molecular phylogenetics. I have also greatly benefited from discussions with people here at Cornell who are too numerous to name. I am grateful to John Ascher for his help with some of the bee identifications and to Jerome G. Rozen, Jr., and Charles D. Michener for discussing my thesis with me and sharing their amazing wealth of knowledge on all that is bees.

Most of the funding for the research came from a National Science Foundation Research Grant to B.N. Danforth (DEB- 0412176) and a Doctoral Dissertation Improvement Grant. Funding for the field trips was provided by the Grace Griswold Fund (Cornell University), the A.C. Rawlins Endowment (Cornell University), the Theodore Roosevelt Memorial Fund (AMNH), and The Einaudi Center International Travel Grant (Cornell University). My graduate studies were funded through teaching assistantships in the introductory biology course, research assistantships, the Palmer Fellowship, and the Bradley Fellowship.

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CHAPTER ONE

Comprehensive phylogeny of apid bees (Apidae: Hymenoptera) reveals the evolutionary origins and antiquity of cleptoparasitism

Abstract

Apidae is the most speciose family of bees with over 5600 species. The family is notable for having some of the most important pollinators of managed crops, yet it also comprises a rich diversity of social and parasitic lifestyles, host plant affinities, and ecosystem services. Despite its importance, relationships among the tribes within Apidae remain unclear. To date, rigorous phylogenetic analysis has been challenged by long-standing assumptions about the relatedness of cleptoparasitic groups (bees that lay eggs in nests of a different host bee) in relation to their hosts. We performed the first large-scale phylogenetic study of the family Apidae based on DNA sequence data, including representatives from all 33 apid tribes. We then used this phylogeny to investigate the origins and antiquity of cleptoparasitism. Results indicate that most cleptoparasitic apid bees form a monophyletic group, and therefore stem from a single origin of cleptoparasitism (with two more origins in the orchid bees and one in the tribe Ctenoplectrini). This large cleptoparasitic clade, which consists of Nomadinae and most cleptoparasitic Apinae, renders the Apinae paraphyletic, and indicates that drastic changes are needed to the higher level classification of Apidae. Divergence time estimates using a relaxed fossil-calibrated molecular clock model reveal that cleptoparasitism is an ancient behavior in apid bees that first evolved ~100 Ma, much earlier then the appearance of the first cleptoparasitic bee in the fossil record.

Introduction

Apid bees have a worldwide distribution and are important pollinators in most natural and agro-ecosystems. Of the seven currently recognised extant families of bees, Apidae includes the greatest number of species (~5687), genera (209) and tribes (33). The behavioral diversity found in Apidae is unsurpassed by any other bee family with solitary, communal, primitively eusocial, advanced eusocial, cleptoparasitic and socially parasitic taxa. Most species (~52%) are solitary, ground or stem-nesting, with variable host-plant preferences. Solitary apid bees include oil-collecting bees (Centredini, *Ctenoplectra*, *Tetrapedia,* Tapinotaspidini), small and large carpenter bees (Ceratinini and Xylocopini), long-horned bees (Eucerini), and many other less well-known groups. The most thoroughly studied apid bee is the honey bee, which has long been a model organism for understanding many aspects of insect communication (Frisch 1967), decision making (Seeley 1995), genetics (Ben-Shahar 2002; Weinstock et al. 2006), and social behavior (Evans and Wheeler 1999).

Previous attempts at resolving the phylogenetic relationships of apid bees have either utilized only morphological data (Roig-Alsina and Michener 1993; Straka and Bogusch 2007) or, if molecular data were used, have investigated relationships within only a single clade of Apidae (e.g. (Cameron and Mardulyn 2001; Bull et al. 2003; Cameron et al. 2007; Rasmussen and Cameron 2007)). No previous molecular study has attempted to resolve relationships among all of the subfamilies and tribes.

Cleptoparasitism in the Apidae

The cleptoparasitic Apidae, which comprise almost one third of all apid species, have posed particular problems for previous analyses of apid relationships (Roig-Alsina & Michener 1993). *Cleptoparasitic* bees do not build or provision their own nests. Instead, they enter the nests of other bees and lay their eggs in either closed cells or in open, partially provisioned, cells. In a few cases, the female adult bee destroys the host egg (e.g. (Garófalo and Rozen 2001)), but more commonly, a specialized larval instar kills the host larvae (e.g. (Alves-Dos-Santos et al. 2002)). The parasitic larva then consumes the provisions of pollen and nectar that had been left for the host larva and completes its development before emerging from the host nest.

The cleptoparasitic Apidae as a whole attack a wide range of host taxa including bees from other bee families, including Colletidae, Andrenidae, Halictidae, and Melittidae (Figure 1.1). However, most species specialize on only a few host species, as expected in most host-parasite relationships based on the need to specialise on a particular host species in order to overcome its defence strategies (Poulin et al. 2000). Some species with a wide host spectrum seem to contradict this prediction, but it has been shown that individual females in some of these species show some degree of host specialization (Bogusch et al. 2006).

Cleptoparasitic bees are highly adapted for their parasitic lifestyle and are morphologically divergent from their pollen collecting relatives (Michener 2007). Because they no longer forage for pollen, they lack a scopa or corbicula (pollen carrying structures) and associated structures for manipulating pollen (combs, brushes, etc). They also tend to have a thick integument, often bearing spines, which probably serves to protect them against the stings of hosts. They usually have reduced basitibial and pygidial plates, which when present are used in nest construction and movement within the nest. Cleptoparasites also have a larger number of mature oocytes in their ovaries compared to solitary nest-making bees (Rozen 2003), allowing them to take full advantage of any host nest found. Most of these characters are associated with no longer needing to forage for pollen or for increased defence against the host.

Previous authors have assumed that the morphological similarity among the many cleptoparasitic species, genera and tribes arose, in many cases, through convergence associated with repeated origins of cleptoparasitism (Roig-Alsina and

Michener 1993). Whereas the overall similarity of the cleptoparasites could be interpreted as evidence of close relationship, bee systematists have often assumed that this was due to convergence. Rozen (2000), for example, estimates that cleptoparasitism arose 11 times within Apidae, and Straka (2007) estimated 6 independent origins. Roig-Alsina & Michener (1993) excluded characters that they considered to have arisen convergently in cleptoparasites, effectively forcing multiple origins of cleptoparasitism on their own data.

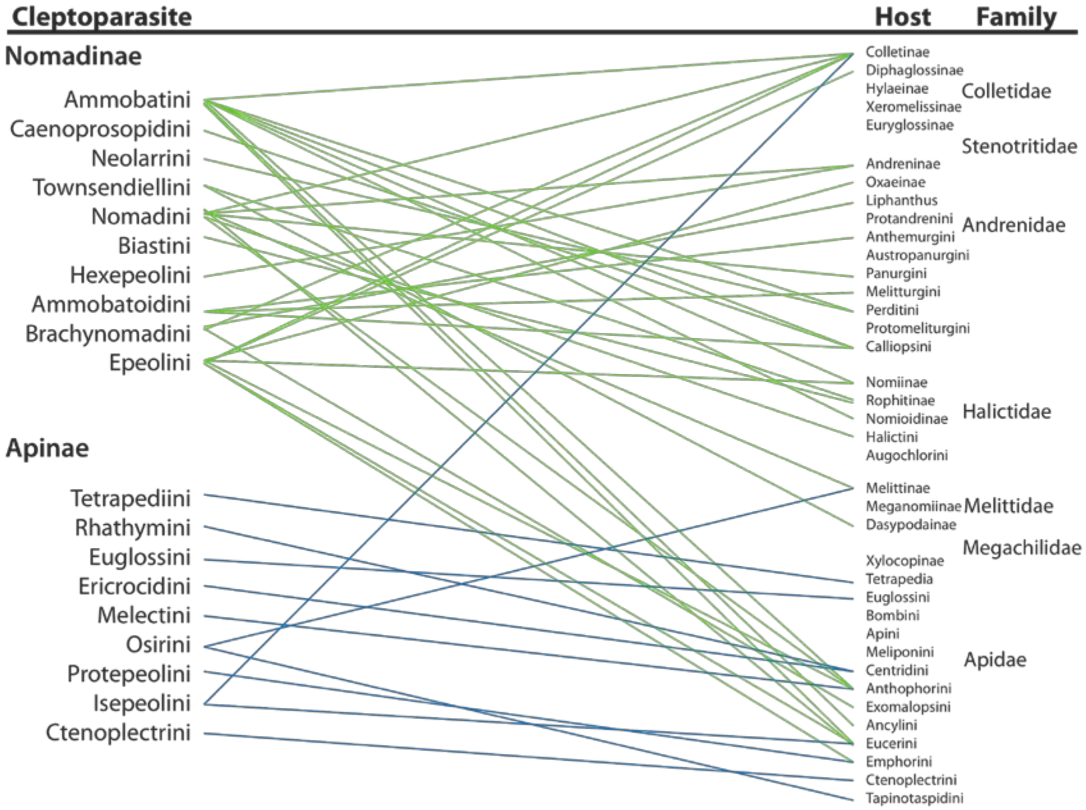


Figure 1.1. Known host-parasite relationships of apid cleptoparasites.

To some extent, this is because of the *a priori* assumption that parasites always arise from their host (Emery's rule [Emery 1909]). There are two forms of Emery's rule, each with different implications for how parasites evolve. The strict form of Emery's rule states that parasites form the sister group to their hosts, implying that they either evolved sympatrically through intraspecific parasitism with subsequent reproductive isolation (Bourke and Franks 1991; Savolainen and Vepsäläinen 2003), or allopatrically with secondary sympatry (Lowe et al. 2002). The loose form of Emery's rule states that parasites are close relatives of their host, but not necessarily sister to them. This would imply that the parasite evolved from a lineage other than their host species (Bourke and Franks 1991), or if they evolved from their host lineage, either a host switching event occurred or they underwent speciation following a speciation event in their host taxon (Buschinger 1990). Emery's rule almost certainly applies for some cleptoparasitic bee lineages, especially in the family Halictidae, where there are repeated origins of cleptoparasites from within host genera: *Lasioglossum* (*Paralictus*) [parasite] and *Lasioglossum* (*Dialictus*) [host], *Lasioglossum* (*Echthralictus*) and *Lasioglossum* (*Homalictus*), *Megalopta* (*Noctoraptor*) and *Megalopta* (*Megalopta*), *Megommation* (*Cleptommation*) and other pollen-collecting subgenera of *Megommation*, *Parathincostom*a and *Thrinchostoma* (Danforth et al. 2008). However, it is not clear whether Emery's rule applies to apid cleptoparasites.

Emery's rule has been tested for *social parasites* in Hymenoptera using molecular techniques in ants (Sumner et al. 2004; Buschinger 2009), wasps (Carpenter et al. 1993), and bees (Danforth et al. 1999; Smith et al. 2007). In social parasites, a female enters the nest of a closely related eusocial species and either displaces the queen or usurps her role as primary egg layer (Fisher 1988; Batra et al. 1993; Küpper and Schwammberger 1995). In contrast to cleptoparasites, social parasites remain in the nest for an extended period of time.

Previous phylogenetic analyses of the family Apidae

Roig-Alsina and Michener (1993), analyzed the relationships among the subfamilies and tribes of Apidae based on larval and adult morphology. They included 31 of the 33 apid tribes and used 131 adult and 77 larval morphological characters. The initial analyses (including all characters) failed to recover monophyly of all 3 subfamilies. In several analyses, the Apinae were rendered paraphyletic by the Xylocopinae. There was significant uncertainty about the placement of several cleptoparasitic tribes, including Osirini, Isepeolini, Protepeolini, Rathymini, Melectini, and Ericrocidini. In fact, this initial analysis found that cleptoparasitism would appear to be ancestral for the Apinae; a hypothesis rejected by the authors as implausible. Subsequent analyses excluded characters related to cleptoparasitism, or went so far as to exclude cleptoparasitic taxa. Addition of larval data resulted in additional tree topologies. Despite the large number of morphological characters used, no analysis robustly supported the subfamily or tribal relationships within Apidae. Figure 1.2 summarizes the "preferred" tree obtained by Roig-Alsina and Michener (1993) (analysis C including adult characters only: 5 characters associated with parasitism excluded). Based on this tree, the Apidae are now classified into 3 subfamilies: Nomadinae (~1214 spp.), Xylocopinae (~999spp.), and Apinae (~3474 spp.) (Michener 2007).

The Nomadinae is a diverse group of exclusively cleptoparasitic bees, including 10 tribes and 33 genera. Relationships among the tribes of Nomadinae have been examined based on both larval (Rozen 1966; Rozen 1977; Rozen et al. 1978; Rozen 1996; Straka and Bogusch 2007) and adult characters (Alexander 1990; Roig-Alsina and Michener 1993), but in neither case where relationships clearly and robustly resolved.

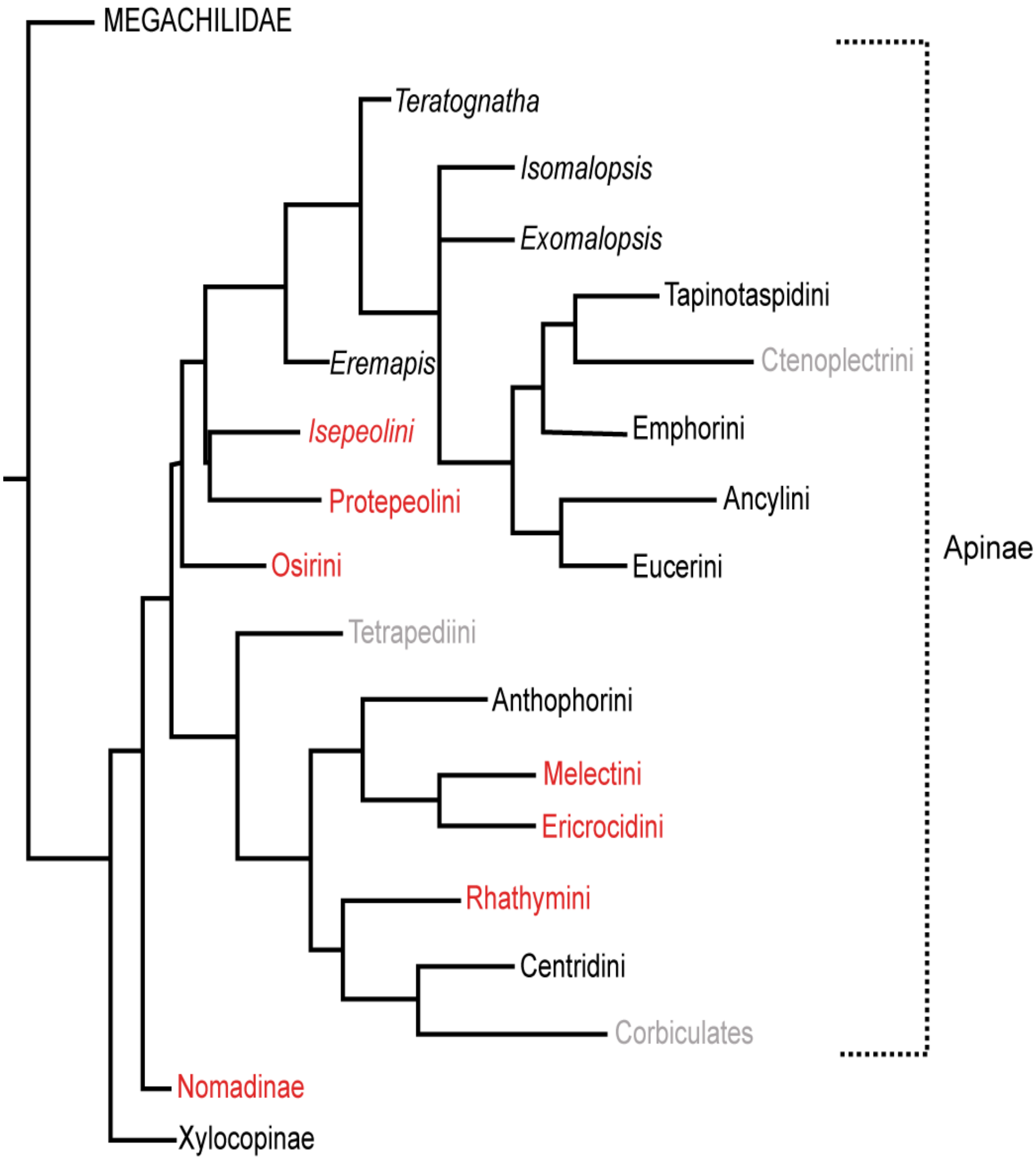


Figure 1.2. Previously hypothesized apid relationships derived from analysis C of Roig-Alsina and Michener (1993). Clades whose members are all cleptoparasitic are indicated in red, and clades that contain cleptoparasitic taxa are indicated in grey.

The Xylocopinae includes 4 tribes and 16 genera, whose members mostly nest in dead plant material. There is a tendency toward social behavior in the xylocopines, especially in the tribe Allodapini, which includes eusocial and socially parasitic species. Relationships among the four extant tribes remain somewhat unclear. Engel (Engel 2001) placed the extinct tribe Boreallodapini as sister to the extant tribe Allodapini, with the extant Ceratinini sister to Boreallodapini+Allodapini (see also (Sakagami and Michener 1987; Roig-Alsina and Michener 1993)). The positions of Manuelini and Xylocopini, however, differ between analyses with Manuelini being sister to all other xylocopines (Sakagami and Michener 1987; Roig-Alsina and Michener 1993) or alternatively Xylocopini being sister to all other xylocopines (Roig-Alsina and Michener 1993; Engel 2001). Several characters unite the xylocopines, but their affinity to other apids remains unclear. In some morphological analyses, they appear as the sister group to the Apinae (Roig-Alsina and Michener 1993), or to Apinae+Nomadinae (Roig-Alsina and Michener 1993). In other analyses, they fall within the Apinae (Roig-Alsina and Michener 1993; Straka and Bogusch 2007) suggesting that Apinae is not monophyletic.

Apinae is the largest of the three subfamilies with ~3474 species classified into 160 genera and 19 tribes. It includes the highly eusocial honey bees and stingless bees, the primitively eusocial bumble bees, numerous solitary and communal species, as well as socially parasitic and cleptoparasitic bees. There are no distinctive characters that unite all members of the Apinae (Roig-Alsina and Michener 1993; Michener 2007). There are exceptions to every subfamilial character described, once again casting doubt on the validity of the Apinae as a natural group. Relationships within the Apinae are also unclear, but there appears to be support for dividing the Apinae into two large clades (Roig-Alsina and Michener 1993; Silveira 1993) referred to by Silveira as the “eucerine line” and “apine line”.

While relationships within the Apidae remain unclear, the monophyly of the Apidae has been well established by both morphological (Roig-Alsina and Michener 1993; Alexander and Michener 1995) and molecular (Danforth et al. 2006; Danforth et al. 2006) phylogenetic analyses. A unique character to all Apidae is the presence of 4 or more ovarioles per ovary in females (Michener 2007). The sister group relationship between the two long-tongued bee families, Apidae and Megachilidae, is also recovered in both morphological (Roig-Alsina and Michener 1993; Alexander and Michener 1995) and molecular analyses (Danforth et al. 2006).

In this paper we provide the first comprehensive phylogeny of the family Apidae based on molecular data. Our goals are two-fold. First, we conducted phylogenetic analyses based on molecular data which provide a hypothesis on the relationships of the Apidae independent of possible morphological convergence in the cleptoparasites. This hypothesis that can now be used in evolutionary and comparative studies on the ecological and behavioral diversity of apid bees. Because of the phylogenetic importance of the cleptoparasites, we extensively sampled all the presumed origins of cleptoparasitism (in addition to all the extant tribes within the family). This sampling allowed us to use our phylogeny to address a second goal, namely to investigate the evolutionary origins of cleptoparasitism in relation to other common apid life-histories. We estimate the number of independent origins of cleptoparasitism using both parsimony and model based methods. We then estimate the antiquity cleptoparasitism and of Apidae and its major clades using a relaxed fossil-calibrated molecular clock model.

Methods

Taxon sampling

We sampled representatives from all 33 currently recognized tribes (Michener 2007) of the family Apidae, including representatives from every presumed independent lineage of cleptoparasitic apid bees (Rozen 2000). We also included all apid genera for which we had access to high quality molecular material (106/209 genera). An effort was made to choose taxa representing the morphological diversity found within tribes for a total of 160 ingroup exemplars. As outgroups, representatives from all currently recognized tribes of the family Megachilidae -- which has been well supported as the sister clade to the Apidae (Roig-Alsina and Michener 1993; Alexander and Michener 1995; Danforth et al. 2006; Danforth et al. 2006) -- were included in addition to taxa from all 3 Melittidae subfamilies hypothesized to be the sister clade(s) to the long-tongued bees (Danforth et al. 2006; Danforth et al. 2006). This totaled 30 outgroup taxa. The entire dataset comprises 190 taxa.

Most of the material was collected by the authors on various field trips within the USA, French Guiana, Paraguay, South Africa, and Australia. We are grateful to the following collaborators for important specimens: Jerome G. Rozen, Jr., Laurence Packer, Eduardo Almeida, Robert L. Minckley, Jack Neff, Christophe Praz, John Ascher, David L. Wagner, Hanno Schaefer, and Santiago Ramírez. Most specimens used for sequencing were killed using cyanide and preserved in either 95% EtOH or in an airtight vile with Drierite, but pinned specimens up to 5 years old were also used. Table 1.1 lists all of the species included in the study along with taxonomic, voucher, and locality information.

DNA Extraction

DNA extractions followed standard phenol-chloroform protocols (Danforth et al. 1999), with the elimination of the use of liquid nitrogen and RNase which were both found to be unnecessary. Tissue samples were taken from either 1-3 leg(s), the thoracic muscles or the entire thorax depending on the size, and the state of preservation of the bee.

Table 1.1. Taxonomic, voucher, and locality information for species included in the study.

Voucher #	Family	Subfamily	Tribe	Species	Collecting data
sc250	Apidae	Apinae	Ancylini	*Ancyla anatolica* Warncke, 1979	Turkey: Adana Prov.
sc202	Apidae	Apinae	Ancylini	*Ancyla asiatica* Friese, 1922	Greece: Rhodes, Afantou. 10.v.2005
793	Apidae	Apinae	Anthophorini	*Amegilla asserta* (Cockerell, 1926)	Australia: SA, 59km N. Cowell. 6.i.1999
504	Apidae	Apinae	Anthophorini	*Anthophora urbana* Cresson, 1878	USA: California, Santa Clara Co., Del Puerto Canyon. 27.v.1999
sc190	Apidae	Apinae	Anthophorini	*Deltoptila aurulentocaudata* (Dours, 1869)	Mexico: Estado de Jalisco, Reserva Biosfera Sierra de Manantlan. 11.ix.2004
1254	Apidae	Apinae	Anthophorini	*Habropoda laboriosa* (Fabricius, 1804)	USA: Florida, Alachua Co., Gainesville. 16.iiv.2002
985	Apidae	Apinae	Anthophorini	*Pachymelus peringueyi* (Friese, 1911)	South Africa: NCP, 14km E. Kamiesksroon, 16.ix.2001
apis_cer	Apidae	Apinae	Apini	*Apis cerana* Fabricius, 1793	Japan: Kyoto
apis_dor	Apidae	Apinae	Apini	*Apis dorsata* Fabricius, 1793	Laos: Laksao
apis_flo	Apidae	Apinae	Apini	*Apis florea* Fabricius, 1787	Loas: Mahaxai
Bom_ard	Apidae	Apinae	Bombini	*Bombus ardens* Smith, 1879	Japan: Kyoto
Bom_div	Apidae	Apinae	Bombini	*Bombus diversus* Smith, 1869	Japan: Kyoto
Bom_men	Apidae	Apinae	Bombini	Bombus mendax Gerstäcker, 1869	Italy: Monte Rosa
sc9	Apidae	Apinae	Centridini	*Centris decolorata* Lepeletier, 1841	French Guiana: Cayenne, Cayenne. 7.vii.2006
sc12	Apidae	Apinae	Centridini	*Centris analis* (Fabricius, 1804)	French Guiana: Roura, Cacao. 9.vii.2006
sc2	Apidae	Apinae	Centridini	*Centris dimidiata* (Olivier, 1789)	French Guiana: Cayenne, Cayenne. 6.vii.2006
228	Apidae	Apinae	Centridini	*Centris atripes* Mocsáry, 1899	USA: Arizona, Cochise Co., W. Turkey Creek
sc4	Apidae	Apinae	Centridini	*Centris longimana* Fabricius, 1804	French Guiana: Maripasoula, Saül. vil. 2.vii.2006
503	Apidae	Apinae	Centridini	*Centris hoffmanseggiae* Cockerell, 1897	USA: California, Kern Co.,5mi S. Mojave. 13.vi.1999
sc13	Apidae	Apinae	Centridini	*Epicharis* sp.	French Guiana: Kourou, Kourou. 11.vii.2006
sc120	Apidae	Apinae	Centridini	*Epicharis analis* Lepeletier, 1841	Paraguay: Paraguarí, Salto Cristal. 10.ii.2007
983	Apidae	Apinae	Ctenoplectrini	*Ctenoplectra albolimbata* Magretti, 1895	South Africa: KZN, 20km N. Hluhluwe. 9-12.iiv.2002
sc227	Apidae	Apinae	Ctenoplectrini	*Ctenoplectra bequaerti* Cockerell, 1930	Nigeria: Cross River State, Afi Mountain. 12.iii.2006
sc204	Apidae	Apinae	Ctenoplectrini	C*tenoplectrina* sp.	Nigeria: 2006
sc220	Apidae	Apinae	Emphorini	*Alepidosceles* sp.	Argentina: Salta Prov., Cafayate env. 10.II.2006
sc169	Apidae	Apinae	Emphorini	*Ancyloscelis* sp.	Paraguay: Cordillera, 6km SW Pirebebuy. 16.i.2007
sc252	Apidae	Apinae	Emphorini	Ancyloscelis sp.	Argentina: Misiones Prov.
490	Apidae	Apinae	Emphorini	*Diadasia bituberculata* (Cresson, 1878)	USA: California, Contra Costa Co., Mitchell canyon. 5.vi.1999
sc148	Apidae	Apinae	Emphorini	*Diadasina distincta* (Holmberg, 1903)	Paraguay: Boquerón, 2km NE. Filadélfia. 6.ii.2007
sc221	Apidae	Apinae	Emphorini	*Meliphilopsis* sp.	Argentina: Salta Prov., Cafayate env. 10.II.2006
sc77	Apidae	Apinae	Emphorini	*Melitoma* sp.	Paraguay: Cordillera, 2km S. Atyra. 18.i.2007
648	Apidae	Apinae	Emphorini	*Ptilothrix* sp.	USA: New Mexico, Hidalgo Co. Rodeo. 22.ix.1999

Table 1.1. (Continued)

sc193	Apidae	Apinae	Ericrocidini	*Ctenioschelus goryi* (Romand, 1840)	Mexico: Estado de Jalisco, Reserva Biosfera Chamela-Cuixmala. 03.ix.2004
1362	Apidae	Apinae	Ericrocidini	*Epiclopus gayi* Spinola, 1851	Chile: Curico prov., Laguna de Teno
616	Apidae	Apinae	Ericrocidini	*Ericrocis lata* (Cresson, 1878)	USA: Arizona, Cochise Co., 2mi N. Rodeo. 8.xi.1999
sc11	Apidae	Apinae	Ericrocidini	*Hopliphora velutina* (Lepeletier & Audinet-Serville, 1825)	French Guiana: Kourou, Kourou. 15.xii.2006
sc118	Apidae	Apinae	Ericrocidini	*Mesocheira bicolor* (Fabricius, 1804)	Paraguay: Concepción, Vallemi. 30.i.2007
sc100	Apidae	Apinae	Ericrocidini	*Mesonychium asteria* (Smith, 1854)	Paraguay: Boquerón, Fn. Toledo. 4.ii.2007
sc106	Apidae	Apinae	Ericrocidini	*Mesoplia rufipes* (Perty, 1833)	Paraguay: Paraguarí, P.N. Ybycui. 12.ii.2007
491	Apidae	Apinae	Eucerini	*Eucera frater* (Cresson, 1878)	USA: California, Contra Costa Co., Lafayette. 26.v.1999
1101	Apidae	Apinae	Eucerini	*Martinapis luteicornis* (Cockerell, 1896)	USA: Arizona, Cochise Co., 4mi E. Willcox. 25.iix.2001
485	Apidae	Apinae	Eucerini	*Melissodes desponsa* Smith, 1854	USA: New York, Tompkins Co., Ithaca. 29.vii.1997
sc197	Apidae	Apinae	Eucerini	*Melissoptila* sp.	Paraguay: Presidente Hayes, Campo León. 5.ii.2007
1103	Apidae	Apinae	Eucerini	*Peponapis pruinosa* (Say, 1837)	USA: Arizona, Santa Cruz Co., Patagonia. 18.iix.2000
631	Apidae	Apinae	Eucerini	*Svastra obliqua* (Say, 1837)	USA: New Mexico, Luna Co., 34mi NE Deming. 13.ix.1999
1256	Apidae	Apinae	Eucerini	*Svastrides melanura* (Spinola, 1851)	Chile: Valparaíso Reg., Petorca. 1.xii.2004
sc225	Apidae	Apinae	Eucerini	*Svastrina subapicalis* (Brèthes, 1910)	Argentina: Corrientes Prov., Mburucuyá env. 26-27.I.2006
1045	Apidae	Apinae	Eucerini	*Tetralonia cinctula* Cockerell, 1936	South Africa: Limpopo Prov., Alldays. 10.i.2004
sc254	Apidae	Apinae	Eucerini	*Tetraloniella glauca* (Fabricius, 1775)	Turkey: Adana Prov.
sc200	Apidae	Apinae	Eucerini	*Thygate*r sp.	French Guiana: Roura, Cacao vil. 9.vii.2006
623	Apidae	Apinae	Eucerini	*Xenoglossa angustior* Cockerell, 1899	USA: Arizona, Cochise Co., 2mi N. Portal. 11.ix.1999
sc229	Apidae	Apinae	Euglossini	*Aglae caerulea* Lepeletier & Audinet-Serville, 1825	Peru: Lagunas, Loreto
Euf_pul	Apidae	Apinae	Euglossini	*Eufriesea pulchra* (Smith, 1854)	Panama
sc23	Apidae	Apinae	Euglossini	*Eufriesea surinamensis* (Linnaeus, 1758)	French Guiana: Kaw, Rt D6, 30.xi.2006
sc201	Apidae	Apinae	Euglossini	*Euglossa piliventris* Guérin-Méneville, 1845	French Guiana: Kaw, Rt D6, 30.xi.2006
Eugl_imp	Apidae	Apinae	Euglossini	*Euglossa imperialis* Cockerell, 1922	Panama
sc14	Apidae	Apinae	Euglossini	*Eulaema meriana* (Olivier, 1789)	French Guiana: Maripalousa, Saül, 4.vii.2006
Exae_fro	Apidae	Apinae	Euglossini	*Exaerete frontalis* (Guérin-Méneville, 1845)	Panama
sc104	Apidae	Apinae	euglossini	*Exaerete* sp.	Paraguay:San Pedro, 30km S. Cororo, 9.ii.2007
sc222	Apidae	Apinae	Exomalopsini	*Anthophorula (Isomalopsis)* sp.	Argentina: La Rioja prov., Pagancillo env. 16-17.ii.2004
sc223	Apidae	Apinae	Exomalopsini	*Anthophorula (Isomalopsis)* sp.	Argentina: Salta prov., Cafayate env. 14.II.2004

Table 1.1. (Continued)

627	Apidae	Apinae	Exomalopsini	*Anthophorula completa* (Cockerell, 1935)	USA: Arizona, Cochise Co., Comm. Rd. 12.ix.1999
sc253	Apidae	Apinae	Exomalopsini	*Exomalopsis* sp.	Argentina: Salta Prov.
1359	Apidae	Apinae	Isepeolini	*Isepeolus atripilis* Roig-Alsina, 1991	Chile: Curico prov., Laguna de Teno
1258	Apidae	Apinae	Isepeolini	*Isepeolus cortesi* Toro & Rojas, 1968	Argentina: Santa Cruz Prov., 25km E. Los Antigos
1360	Apidae	Apinae	Isepeolini	*Isepeolus luctuosus* (Spinola, 1851)	Chile: Chacabuco Province, La Dormida
1361	Apidae	Apinae	Isepeolini	*Isepeolus wagenknechti* Toro & Rojas, 1968	Chile: Huasco province, Carrizal Bajo
sc246	Apidae	Apinae	Isepeolini	*Melectoides bellus* (Jörgensen, 1912)	Argentina: Salta Prov.
sc247	Apidae	Apinae	Melectini	*Melecta albifrons* (Forster, 1771)	Czeck Republic
sc218	Apidae	Apinae	Melectini	*Tetralonioidella* sp.	Thailand: Chiang Mai, Doi Inthonun NP checkpoint 2. 22.vii-2.viii.2006
987	Apidae	Apinae	Melectini	*Thyreus delumbatus* (Vachal, 1903)	South Africa: NP, 14km E. Vivo. 17.iiv.2002
499	Apidae	Apinae	Melectini	*Xeromelecta californica* (Cresson, 1878)	USA: California, Santa Clara Co., Del Puerto Canyon. 27.v.1999
650	Apidae	Apinae	Melectini	*Zacosmia maculata* (Cresson, 1879)	USA: New Mexico, Grant Co., Hachita. 24.ix.1999
ceph_cap	Apidae	Apinae	Meliponini	*Cephalotrigona capitata* (Smith, 1854)	Costa Rica
1040	Apidae	Apinae	Meliponini	*Hypotrigona gribodoi* (Magretti, 1884)	South Africa: Limpopo Prov., 27km E. Waterpoort. 7.i.2004
sc228	Apidae	Apinae	Meliponini	*Lestrimelitta* sp.	Argentina: Jujuy prov., Libertador General San Martín env., 2-3.ii.2006
522	Apidae	Apinae	Meliponini	*Melipona* sp.	Bolivia: Dept. Santa Cruz, San José. 26.vi.1999
989	Apidae	Apinae	Meliponini	*Meliponula ferruginea*	South Africa: NP, 40km W. Sibasa. 20.iiv.2002
meli_boc	Apidae	Apinae	Meliponini	*Meliponula bocandei* (Spinola, 1853)	Gabon
1042	Apidae	Apinae	Meliponini	*Meliponula ferruginea*	South Africa: Limpopo Prov. Soutpansberg Mts. 9.i.2004
991	Apidae	Apinae	Meliponini	*Plebeina hildebrandti* (Friese, 1900)	South Africa: NP, 53 km S. Louis Trichardt, 16.iii.2002
sc199	Apidae	Apinae	Meliponini	*Scaptotrigona hellwegeri* (Friese, 1900)	Mexico: Estado de Jalisco, Carretera. 2.ix.2004
scau_lat	Apidae	Apinae	Meliponini	*Scaura latitarsis* (Friese, 1900)	not available
685	Apidae	Apinae	Meliponini	*Tetragonula carbonaria* (Smith, 1854)	Australia: NSW Windsor, 70 km N. 2xii.1999
trig_fus	Apidae	Apinae	Meliponini	*Trigona fuscipennis* Friese, 1900	not available
sc245	Apidae	Apinae	Osirini	*Epeoloides coecutiens* (Fabricius, 1775)	Czeck Republic
1364	Apidae	Apinae	Osirini	*Epeoloides pilosula* (Cresson, 1878)	USA: Connecticut, New London Co., Bozrah Rt 163. 22.vi.2006
sc248	Apidae	Apinae	Osirini	*Osiris* sp. 1	Peru
sc20	Apidae	Apinae	Osirini	*Parepeolus aterrimus* (Friese, 1906)	French Guiana: Kourou
sc219	Apidae	Apinae	Protepeolini	*Leiopodus abnormis* (Jörgensen, 1912)	Argentina: Salta Prov., Cachi env. 4-8.ii.2006

Table 1.1. (Continued)

651	Apidae	Apinae	Protepeolini	*Leiopodus singularis* (Linsley & Michener, 1937)	USA: New Mexico, Grant Co., Hachita. 24.ix.1999
sc249	Apidae	Apinae	Protepeolini	*Leiopodus trochantericus* Ducke, 1907	Argentina: Chaco Prov.
sc107	Apidae	Apinae	Protepiolini	*Leiopodus* sp.	Paraguay: Presidente Hayes, Campo Maria. 5.ii.2007
1330	Apidae	Apinae	Rhathymini	*Nanorhathymus* sp.	West Indies: Trinidad, Cauara Valley. 17.vii.2003
1329	Apidae	Apinae	Rhathymini	*Rhathymus* sp.	West Indies: Trinidad, Cauara Valley. 6-7.ii.2005
sc111	Apidae	Apinae	rhathymini	*Rhathymus unicolor* (Smith, 1854)	Paraguay:San Pedro, 30km S. Cororo, 27.i.2007
sc226	Apidae	Apinae	tapinotaspidini	*Arhysoceble* sp.	Argentina: Jujuy prov., Libertador General San Martín env. 2-3.ii.2006
sc127	Apidae	Apinae	tapinotaspidini	C*aenonomada* sp.	Paraguay: Concepción, Vallemi. 1.ii.2007
sc181	Apidae	Apinae	tapinotaspidini	*Paratetrapedia* sp.	Paraguay: Paraguarí, 6 km SE La Colmena. 21.i.2007
sc196	Apidae	Apinae	tapinotaspidini	*Arhysoceble picta* (Friese, 1899)	Paraguay: Paraguarí, Caballero. 21.i.2007
sc198	Apidae	Apinae	tapinotaspidini	*Tapinotaspoides* sp.	Paraguay: Cordillera, 2km NE. Atyra. 18.i.2007
sc130	Apidae	Apinae	tetrapediini	*Coeleoxoides* sp.	Paraguay: Paraguarí, P.N. Ybycui. 9.ii.2007
sc235	Apidae	Apinae	tetrapediini	*Coelioxoides waltheriae* Ducke, 1908	Argentina: Jujuy Prov.
sc233	Apidae	Apinae	tetrapediini	*Tetrapedia (Lagobata)* sp.	Argentina: Misiones Prov.
sc234	Apidae	Apinae	tetrapediini	*Tetrapedia (Tetrapedia)* cf. *diversipes*	Argentina: Jujuy Prov.
1340	Apidae	Apinae	Tetrapediini	*Tetrapedia maura* Cresson, 1878	Mexico: Estado de Jalisco, Reserva Biosfera Chamela-Cuixmala. 1.ix.2004
sc238	Apidae	Nomadinae	Ammobatini	*Ammobates punctatus* (Fabricius, 1804)	Turkey: Adana Prov.
sc205	Apidae	Nomadinae	Ammobatini	*Ammobates* sp.	Jordan, 10km N Jerash 23.4.2007
637	Apidae	Nomadinae	Ammobatini	*Oreopasites barbarae* Rozen, 1992	USA: Arizona, Cochise Co., Apache 14 mi SW. 10.ix.1999
sc239	Apidae	Nomadinae	Ammobatini	*Pasites maculatus* Jurine, 1807	Romania
896	Apidae	Nomadinae	Ammobatini	*Sphecodopsis capensis* (Friese, 1915)	South Africa: NCP, Kamieskroon. 16.ix.2001
sc240	Apidae	Nomadinae	Ammobatoidini	*Ammobatoides luctuosus* (Friese, 1911)	Mongolia
sc16	Apidae	Nomadinae	Ammobatoidini	*Holcopasites arizonicus* (Linsley, 1942)	USA: Arizona, Cochise Co., 1 mi E. Douglas. 23.iix.2006
519	Apidae	Nomadinae	Ammobatoidini	*Holcopasites calliopsidis* (Linsley, 1943)	USA: New York, Schuyler Co. Valois. 18.vi.1999
sc74	Apidae	Nomadinae	Ammobatoidini	*Holcopasites minimus* (Linsley, 1943)	USA: Arizona, Cochise Co., 14 mi W. Apache. 28.iix.2006
sc17	Apidae	Nomadinae	Ammobatoidini	*Holcopasites insoletus* (Linsley, 1942)	USA: New Mexico, Hidalgo Co. 20 mi S. Animas. 7.ix.2006
511	Apidae	Nomadinae	Ammobatoidini	*Holcopasites ruthae* Cooper, 1993	USA: California, Riverside Co. 18.v.1993
sc51	Apidae	Nomadinae	Ammobatoidini	*Holcopasites stevensi* Crawford, 1915	USA: Arizona, Cochise Co., 14 mi W. Apache. 27.iix.2006
sc241	Apidae	Nomadinae	Biastini	*Biastes truncatus* (Nylander, 1848)	Czeck Republic

Table 1.1. (Continued)

sc195	Apidae	Nomadinae	Biastini	*Neopasites cressoni* Crawford, 1916	Mexico: Sonora, 30 km E Aqua Prieta. 4.iv.2005
sc242	Apidae	Nomadinae	Brachynomadini	*Brachynomada* sp.	Argentina: Salta Prov.
1380	Apidae	Nomadinae	Brachynomadini	*Brachynomada margaretae* (Rozen, 1994)	AZ: Cochise Co., 14 mi. SE Apache 25.iix.2006
652	Apidae	Nomadinae	Brachynomadini	*Paranomada velutina* Linsley, 1939	USA: Arizona, Cochise Co., 2 mi E. Apache. 10.ix.1999
653	Apidae	Nomadinae	Brachynomadini	*Triopasites penniger* (Cockerell, 1894)	USA: New Mexico, Grant Co., Hachita. 24.ix.1999
sc213	Apidae	Nomadinae	Caenoprosopidini	*Caenoprosopina holmbergi* Roig-Alsina, 1987	Argentina: Salta Prov., Cachi env. 4-8.ii.2006
sc214	Apidae	Nomadinae	Caenoprosopidini	*Caenoprosopis crabronina* Holmberg, 1887	Argentina: Salta Prov., Cachi env. 4-8.ii.2006
sc215	Apidae	Nomadinae	Epeolini	*Doeringiella* sp.	Argentina: Chaco Prov., Capitan Solari env. 3-7.ii.2004
635	Apidae	Nomadinae	Epeolini	*Triepeolus robustus* (Cresson, 1878)	USA: Arizona, Cochise Co, Chiricahua Monument. 14.ix.1999
sc243	Apidae	Nomadinae	Epeolini	*Epeolus variegatus* (Linnaeus, 1758)	Czeck Republic
sc216	Apidae	Nomadinae	Epeolini	*Epeolus* sp.	Argentina: Corrientes Prov., Mburucuyá env. 26-27.I.2006
489	Apidae	Nomadinae	Epeolini	*Epeolus scutellaris* Say, 1824	NY: Tompkins Co. Ithaca. 22.iix.1997
1251	Apidae	Nomadinae	epeolini	*Odyneropsis* sp.	Costa Rica: Puntarenas Prov., Las Cruces Biol. Sta., 30.v.2004
sc217	Apidae	Nomadinae	Epeolini	*Rhinepeolus rufiventris* (Friese, 1908)	Argentina: Corrientes Prov., Mburucuyá env. 26-27.I.2006
sc114	Apidae	nomadinae	epeolini	*Thalestria spinosa* (Fabricius, 1804)	Paraguay:San Pedro, 30km S. Cororo, 27.i.2007
1363	Apidae	Nomadinae	Hexepeolini	*Hexepeolus rhodogyne* Linsley & Michener, 1937	USA: Arizona, Pima Co.
1379	Apidae	Nomadinae	Neolarrini	*Neolarra orbiculata* Shanks, 1978	USA: Arizona: Cochise Co., 2 mi E Willcox 1.ix.2004
501	Apidae	Nomadinae	Nomadini	*Nomada maculata* Cresson, 1863	USA: New York, Tompkins Co., Ithaca. 3.v.1999
sc244	Apidae	Nomadinae	Nomadini	*Nomada signata* Jurine, 1807	Czeck Republic
as72	Apidae	Nomadinae	Townsendiellini	*Townsendiella* sp.	USA: California
1166	Apidae	Xylocopinae	Allodapini	*Braunsapis madecassella* Michener, 1977	Madagascar: 4km N. Tulear. 17.x.2004
1169	Apidae	Xylocopinae	Allodapini	*Compsomelissa keiseri* (Benoist, 1962)	Madagascar: Parc National Ranomafana, 8 km N. Ranomafana. 8.x.2004
679	Apidae	Xylocopinae	Allodapini	*Exoneura bicolor* Smith, 1854	Australia: VIC Flowerdale Forest. 20.xi.1999
1171	Apidae	Xylocopinae	Allodapini	*Macrogalea ellioti* (Saussure, 1890)	Madagascar: Ifaty, N. Tulear. 17.x.2004
sc236	Apidae	Xylocopinae	Ceratinini	*Ceratina cyanea* (Kirby, 1802)	Czeck Republic
sc208	Apidae	Xylocopinae	Ceratinini	*Ceratina (Ceratinula)* sp.	Argentina: Chaco prov., Capitan Solari env. 29-31.i.2006
sc168	Apidae	xylocopinae	ceratinini	*ceratina (Crewella)* sp.	Paraguay: Cordillera, 6km SW Pirebebuy. 16.i.2007
sc194	Apidae	Xylocopinae	ceratinini	*Ceratina (Simioceratina)* sp.	South Africa: NP, 29km NW. Waterpoort. 17.iiv.2002
sc209	Apidae	Xylocopinae	Ceratinini	Ceratina (Xanthoceratina) sp.	Malaysia: Borneo isl., Gudung Gad env. 2003
656	Apidae	Xylocopinae	Ceratinini	*Ceratina calcarata* Robertson, 1900	USA: New York, Tompkins Co., Ithaca. 4.iix.1999

Table 1.1. (Continued)

1255	Apidae	Xylocopinae	Manueliini	*Manuelia gayatina* (Spinola, 1851)	Chile: Reg VIII, Entrepiernas, W. of Recinto, 8.xii.2004
sc212	Apidae	Xylocopinae	Xylocopini	*Xylocopa pubescens* Spinola, 1838	TUNISIA: Blidette vill. 25-27.iii.2006
sc3	Apidae	Xylocopinae	Xylocopini	*Xylocopa fimbriata* Fabricius, 1804	French Guiana: Cayenne, Cayenne. 6.vii.2006
500	Apidae	Xylocopinae	Xylocopini	*Xylocopa tabaniformis* Smith, 1854	USA: California, Santa Clara Co., Mt. Hamilton. 27.v.1999
sc211	Apidae	Xylocopinae	Xylocopini	*Xylocopa (Proxylocopa)* sp.	Turkey: Gaziantep prov., Birecik env. 13.iix.2006
sc19	Apidae	Xylocopinae	Xylocopini	*Xylocopa muscaria* (Fabricius, 1775)	French Guiana: Kourou, Kourou. 11.vii.2006
sc237	Apidae	Xylocopinae	Xylocopini	*Xylocopa violacea* (Linnaeus, 1758)	Turkey: Adana Prov.
sc57	Apidae	Xylocopinae	Xylocopini	*Xylocopa californica arizonensis* Cresson, 1879	USA: Arizona, Cochise Co., 14 mi W. Apache. 6.ix.2006
1153	Apidae	Xylocopinae	Xylocopini	*Xylocopa virginica*	USA: New York, Tompkins Co., Ithaca. 8.v.2001
sc210	Apidae	Xylocopinae	Xylocopini	*Xylocopa iris* (Christ, 1791)	Turkey: Izmir Prov., Selcuk env. 24.iix.2006
948	Megachilidae	Fideliinae	Fideliini	*Fideliopsis major* (Friese, 1911)	South Africa: WCP, 5km N. Clanwilliam. 20.ix.2001
802	Megachilidae	Fideliinae	Fideliini	*Neofidelia* sp.	Chile: Atacama Prov., Inca-havas 5 km N. 3.x.1997
sc231	Megachilidae	Fideliinae	Pararhophitini	*Pararhophites quadratus* (Friese, 1898)	Tunisia
630	Megachilidae	Megachilinae	Anthidiini	*Anthidiellum notatum* (Latreille, 1809)	USA: Arizona, Cochise Co., Comm. Rd. 12.ix.1999
645	Megachilidae	Megachilinae	Anthidiini	*Anthidium porterae* Cockerell, 1900	USA: New Mexico, Hidalgo Co. 20 mi S. Animas. 17.ix.1999
1267	Megachilidae	Megachilinae	Anthidiini	*Dianthidium subparvum* Swenk, 1914	USA: Utah, Cache Co. BSFC. 5.iix.2003
495	Megachilidae	Megachilinae	Anthidiini	*Paranthidium jugatorium* (Say, 1824)	NY: Tompkins Co. Ithaca. 31.vii.1997
1271	Megachilidae	Megachilinae	anthidiini	*Stelis linsleyi* Timberlake, 1941	USA: California, Madera Co. Yosemite N.P. 11.vii.2005
1142	Megachilidae	Megachilinae	Anthidiini	*Trachusa larreae* (Cockerell, 1897)	USA: Nevada, Clark Co. Las Vegas Dunes. 1.iv.2004
1152	Megachilidae	Megachilinae	Dioxyini	*Dioxys pomonae* Cockerell, 1910	USA: Nevada, Clark Co. McCullough Mts. 12.v.2004
863	Megachilidae	Megachilinae	Lithurgini	*Lithurgus echinocacti* Cockerell, 1898	USA: Arizona, Pima Co., Tucson. 4.iix.2000
sc207	Megachilidae	Megachilinae	Lithurgini	*Microthurge* sp.	Argentina: Jujuy Prov., Libertador General San Martín env. 2-3.ii.2006
1275	Megachilidae	Megachilinae	Lithurgini	*Trichothurgus herbsti* (Friese, 1905)	Chile: Region VIII, Las Trancas, 78 km E. Chillan. 12.xii.2003
487	Megachilidae	Megachilinae	Megachilini	*Coelioxys alternata* Say, 1837	USA: New York, Tompkins Co., Ithaca. 29.vii.1997
sc232	Megachilidae	Megachilinae	Megachilini	*Megachile ericetorum* Lepeletier, 1841	Czeck Republic
1283	Megachilidae	Megachilinae	Megachilini	*Megachile angelarum* Cockerell, 1902	USA: Nevada, Clark Co., 2.5 mi S. Wheeler Well. 30.vi.2004
1281	Megachilidae	Megachilinae	Osmiini	*Afroheriades hyalinus*	South Africa: WCP, 7 km W. Nieuwoudtville.. 9.iix.2002
1270	Megachilidae	Megachilinae	Osmiini	*Ashmeadiella aridula* Cockerell, 1910	USA: Utah, Garfield Co., Long Canyon, 1.ix.2003
1269	Megachilidae	Megachilinae	Osmiini	*Chelostoma californica*	USA: California, Mariposa Co., El Portal. 12.iv.2004

Table 1.1. (Continued)

1149	Megachilidae	Megachilinae	Osmiini	*Heriades crucifer* Cockerell, 1897	USA: Arizona, Cochise Co, Chiricahua Mts. 25.iix.2003
507	Megachilidae	Megachilinae	Osmiini	*Hoplitis albifrons* (Kirby, 1837)	USA: California, Contra Costa Co., Donner canyon. 30.v.1999
1280	Megachilidae	Megachilinae	Osmiini	*Hoplosmia scutellari*s (Morawitz, 1868)	GREECE: Lesvos, 1 km N Mytilene. 6.v.2004
1265	Megachilidae	Megachilinae	Osmiini	*Osmia lignaria* Say, 1837	not available
497	Megachilidae	Megachilinae	Osmiini	*Protosmia rubifloris* (Cockerell, 1898)	USA: California, Santa Clara Co., San Antonio summit. 28.v.1999
973	Melitidae	Dasypodainae	Dasypodaini	*Dasypoda argentata* Panzer, 1809	France: Gard, Générac, 22.vi.2002
488	Melitidae	Dasypodainae	Dasypodaini	*Hesperapis larreae* Cockerell, 1907	USA: California, Los Angeles Co., Palmada. 13.vi.1999
sc230	Melitidae	Dasypodainae	Promelittini	*Promelitta alboclypeata* (Friese, 1900)	United Arab Emirates
1021	Melitidae	Meganomiinae		*Meganomia binghami* (Cockerell, 1909)	South Africa: Limpopo Prov., 8.5 km N. Vivo. 7.i.2004
17ja	Melitidae	Melittinae	Melittini	*Macropis nuda* (Provancher, 1882)	USA: NY, Rensselaer Co., Rensselaerville, Huyck Nature Preserve
942	Melitidae	Melittinae	Melittini	*Melitta arrogans* (Smith, 1879)	South Africa: NCP, 76 km S. Port Nolloth. 11.ix.2001

Data

The dataset consists of sequences of two nuclear ribosomal genes (18S, 28S), and 5 nuclear protein-coding genes (wingless, pol II, opsin, Nak, Ef1α). Genes were selected based on their previously demonstrated ability to resolve phylogenetic relationships in other insect groups of various ages. The nuclear ribosomal gene 18S has been used in numerous studies of ordinal relationships in insects (e.g. (Engel 1999; Caterino et al. 2000; Wheeler et al. 2001; Whiting 2002)). It has also more recently been used in bee family-level phylogenies to provide resolution at the base of the trees (Danforth et al. 2006). In this study we amplified an ~900bp fragment which spans 18S helices H367-H960 of *Apis mellifera* (Gillespie et al. 2006).

The nuclear ribosomal gene 28S has also been extensively used for higher-level insect phylogenetic studies, and due to its higher rate of substitution as compared to 18S (Hillis and Dixon 1991) has been included in studies of phylogenetic relationships within families of bees (eg. (Cameron and Mardulyn 2001; Almeida and Danforth 2009)). For this study, we sequenced an ~1500bp fragment from the large subunit 28S rRNA locus spanning regions D1-D5 and helices H234-H1011 of *Apis mellifera* (Gillespie et al. 2006).

Wnt-1 is one of several subfamilies of the large family of protein-coding genes Wingless (wnt), which is involved in early embryogenesis in insects and vertebrates (e.g. (Rijsewijk et al. 1987; Uzvölgyi et al. 1988)). Wnt-1 has been found to be phylogenetically informative at a variety of taxonomic levels, including among metazoan phyla (Schubert et al. 2000), within the stalk-eyed fly family Diopsidae (Baker et al. 2001), and has been used to recover subfamilies, tribes and genera of the bee family Halictidae (Danforth et al. 2004). In this study we sequenced an ~500bp intronless fragment of the wnt-1 paralogue.

RNA Polymerase II (Pol II) is a protein-coding gene which codes for the two largest subunits of the RNA polymerase II enzyme. It evolves at a slower rate than many other commonly used protein-coding genes and has been found useful in resolving higher-level arthropod phylogenies (Shultz and Regier 2000). It has also been used in studies on the relationships of bee families and subfamilies (Danforth et al. 2006) For this study we sequenced a ~900bp fragment of Pol II which lacks introns.

LW rhodopsin is a G-protein-coupled receptor protein that performs the first steps in visual transduction in many organisms (Chang et al. 1996; Towson et al. 1998). LW opsin has been used in numerous phylogenetic analyses of various groups of insects (Hsu et al. 2001; Danforth et al. 2006), including relationships among the corbiculate bee tribes (Cameron and Mardulyn 2003). For this study, we amplified a ~750pb fragment of LW opsin which spanned two introns. Because the intron regions could not be aligned unambiguously, they were excluded from the phylogenetic analysis.

Sodium-Potassium ATPase (NaK) is a protein-coding gene not widely used yet in phylogenetic studies (but see (Tsang et al. 2008)). The primers used in this study were developed by blasting the ouabain-binding site consensus sequence of various insects (Labeyrie and Dobler 2004) against the honey bee genome. Our primers amplify an intronless region ~1500bp long encompassing the extracellular ouabain-binding site near the N-terminus of Segment 1 of the Nak alpha-subunit (Fagan and Saier 1993).

Elongation factor-1 alpha (EF1-α) is a widely-used nuclear protein-coding gene in phylogenetics (e.g., (Cho et al. 1995; Moulton 2000)). It encodes a protein involved in the GTP-dependent binding of charged tRNAs to the acceptor site of the ribosome during translation (Maroni 1993). It occurs as two copies in bees (Danforth and Ji 1998). For this study, we used primers specific to the F2 copy, which amplify a ~1050bp long fragment spanning 1 intron (intron 2 in Danforth et al 2004). The intron was removed from the dataset prior to phylogenetic analyses due to difficulties of alignment.

Sequencing

Most sequences used in the study are previously unpublished sequences. Previously published sequences used were downloaded from Genbank (see Table 1.2. for accession numbers). All new sequences were obtained following standard PCR and sequencing protocols (Danforth et al. 1999). PCR products were gel-purified overnight on low-melting-point agarose gels, the gel slices were extracted with the Promega Wizard PCR purification system (Promega, Madison, WI), and all PCR products were sequenced in both directions with an Applied Biosystems Automated 3730 DNA Analyzer using Big Dye Terminator chemistry and AmpliTaq-FS DNA polymerase at Cornell University Life Sciences Core Laboratories Center. Primer pairs and PCR conditions for all genes are listed in Table 1.3.

Table 1.2. Genbank accession numbers for sequences used in the study.

Taxa	**18S**	**28S**	**Wingless**	**Pol II**	**Opsin**	**Nak**	**ef1a**
Ancyla asiatica	GU244590	GU244752	GU245509	GU245351	GU245234	GU245056	GU244912
Ancyla anatolica	GU244591	GU244753	GU245510	GU245352	GU245235	GU245057	GU244913
Pachymelus peringueyi	AY995685	AY654544	GU245514	AY945151	DQ116678	GU245061	AY585114
Anthophora urbana	GU244593	GU244755	GU245512	GU245354	AF344585	GU245059	GU244915
Amegilla asserta	GU244594	GU244756	GU245513	GU245355	GU245237	GU245060	GU244916
Habropoda laboriosa	GU244592	GU244754	GU245511	GU245353	GU245236	GU245058	GU244914
Deltoptila aurulentocaudata	GU244595	GU244757	GU245515	GU245356	GU245238	GU245062	GU244917
Apis cerana	no # avail.	no # avail.	EU184716	EU184733	EU184839	EU184750	EU184774
Apis dorsata	no # avail.	no # avail.	EU184715	EU184732	AY267162	EU184749	AY267146
Apis florea	no # avail.	no # avail.	EU184714	EU184731	EU184838	EU184748	EU184773
Bombus ardens	no # avail.	no # avail.	EU184707	EU184724	AF493031	EU184741	AF492964
Bombus diversus	no # avail.	no # avail.	EU184708	EU184725	AF493028	EU184742	AF492961
Bombus mendax	no # avail.	no # avail.	EU184709	EU184726	AF493024	EU184743	AF492957
Centris atripes	GU244596	GU244764	GU245522	GU245363	GU245239	GU245069	AY362993
Centris hoffmanseggiae	GU244597	GU244765	GU245523	GU245364	AF344590	GU245070	GU244918
Centris analis	GU244598	GU244758	GU245516	GU245357	GU245240	GU245063	GU244919
Epicharis analis	GU244599	GU244759	GU245517	GU245358	GU245241	GU245064	GU244920
Epicharis sp.	GU244600	GU244760	GU245518	GU245359	GU245242	GU245065	GU244921
Centris dimidiata	GU244601	GU244761	GU245519	GU245360	GU245243	GU245066	GU244922
Centris longimana	GU244602	GU244762	GU245520	GU245361	GU245244	GU245067	GU244923
Centris decolorata	GU244603	GU244763	GU245521	GU245362	GU245245	GU245068	GU244924
Ctenoplectra albolimbata	AY995681	AY654538	GU245526	AY945111	DQ116677	GU245073	AY585118
Ctenoplectrina sp.	GU244604	GU244766	GU245524	GU245365	GU245246	GU245071	GU244925
Ctenoplectra bequaerti	GU244605	GU244767	GU245525	GU245366	GU245247	GU245072	GU244926
Diadasia bituberculata	GU244606	GU244768	GU245527	GU245367	AF344594	GU245074	GU244927
Ptilothrix sp.	GU244607	GU244769	GU245528	GU245368	AF344630	GU245075	GU244928
Diadasina distincta	GU244608	GU244770	GU245529	GU245369	GU245248	GU245076	GU244929
Ancyloscelis sp.	GU244609	GU244771	GU245530	GU245370	GU245249	GU245077	GU244930
Alepidosceles sp.	GU244610	GU244772	GU245531	GU245371	GU245250	GU245078	GU244931
Meliphilopsis sp.	GU244611	GU244773	GU245532	GU245372	GU245251	GU245079	GU244932
Ancyloscelis sp.	GU244612	GU244774	GU245533	GU245373	GU245252	GU245080	GU244933
Melitoma sp.	GU244613	GU244775	GU245534	GU245374	GU245253	GU245081	GU244934
Ericrocis lata	GU244615	GU244777	GU245536	GU245376	GU245255	GU245083	GU244936
Epiclopus gayi	GU244614	GU244776	GU245535	GU245375	GU245254	GU245082	GU244935
Mesonychium asteria	GU244616	GU244778	GU245537	GU245377	GU245256	GU245084	GU244937
Mesoplia rufipes	GU244617	GU244779	GU245538	GU245378	GU245257	GU245085	GU244938
Hopliphora velutina	GU244618	GU244780	GU245539	GU245379	GU245258	GU245086	GU244939
Mesocheira bicolor	GU244619	GU244781	GU245540	GU245380	GU245259	GU245087	GU244940
Ctenioschelus goryi	GU244620	GU244782	GU245541	GU245381	GU245260	GU245088	GU244941
Melissodes desponsa	GU244625	GU244785	GU245546	GU245384	AF344603	GU245093	GU244942
Eucera frater	GU244626	GU244786	GU245547	GU245385	GU245232	GU245094	
Xenoglossa angustior	GU244627	GU244787	GU245548	GU245386	GU245233	GU245095	
Svastra obliqua	GU244628	GU244788	GU245549	GU245387	AF344632	GU245096	
Tetralonia cinctula	GU244621	DQ072157	GU245542	AY945205	GU245229	GU245089	
Martinapis luteicornis	GU244622	DQ072147	GU245543	DQ069333	GU245230	GU245090	
Peponapis pruinosa	GU244623	GU244783	GU245544	GU245382	GU245261	GU245091	

Table 1.2. (Continued)

Svastrides melanura	GU244624	GU244784	GU245545	GU245383	GU245262	GU245092	
Melissoptila sp.	GU244629	GU244789	GU245550	GU245388	GU245263	GU245097	
Thygater sp.	GU244630	GU244790	GU245551	GU245389	GU245264	GU245098	
Florilegus sp.	GU244631	GU244791	GU245552	GU245390	GU245265	GU245099	GU244943
Svastrina subapicalis	GU244632	GU244792	GU245553	GU245391	GU245266	GU245100	
Tetraloniella glauca	GU244633	GU244793	GU245554	GU245392	GU245267	GU245101	
Exaerete sp.	GU244634	GU244794	GU245555	GU245393	GU245268	GU245102	GU244944
Eulaema meriana	GU244635	GU244795	GU245556	GU245394	GU245269	GU245103	GU244945
Euglossa piliventris	GU244636	GU244796	GU245557	GU245395	GU245270	GU245104	GU244946
Aglae caerulea	GU244637	GU244797	GU245558	GU245396	GU245271	GU245105	
Eufriesea surinamensis	GU244638	GU244798	GU245559	GU245397	GU245272	GU245106	GU244947
Eufriesea pulchra	no # avail.	no # avail.	EU184706	EU184723	EU184834	EU184740	EU184769
Euglossa imperialis	no # avail.	no # avail.	EU184704	EU184721	AY267160	EU184738	AY267144
Exaerete frontalis	no # avail.	no # avail.	EU184705	EU184722	AY267159	EU184739	AY267143
Anthophorula completa	GU244639	GU244799	GU245560	GU245398	AF344622	GU245107	GU244948
Anthophorula (Isomalopsis) sp.	GU244640	GU244800	GU245561	GU245399	GU245273	GU245108	GU244949
Anthophorula (Isomalopsis) sp.	GU244641	GU244801	GU245562	GU245400	GU245274	GU245109	GU244950
Exomalopsis sp.	GU244642	GU244802	GU245563	GU245401	no # avail.	GU245110	GU244989
Isepeolus cortesi	GU244643	GU244803	GU245564	GU245402	GU245275	GU245111	GU244951
Isepeolus atripilis	GU244644	GU244804	GU245565	GU245403	GU245276	GU245112	GU244952
Isepeolus luctuosus	GU244645	GU244805	GU245566	GU245404	GU245277	GU245113	GU244953
Isepeolus wagenknechti	GU244646	GU244806	GU245567	GU245405	GU245278	GU245114	GU244954
Melectoides bellus	GU244647	GU244807	GU245568	GU245406	no # avail.	GU245115	GU244999
Thyreus delumbatus	AY995687	AY654546	GU245571	AY945169	DQ116679	GU245118	AY585119
Zacosmia maculata	AY995689	AY654548	GU245570	AY945176	AF344637	GU245117	AY585117
Xeromelecta californica	GU244648	GU244808	GU245569	GU245407	AF344613	GU245116	GU244955
Tetralonioidella sp.	GU244649	GU244809	GU245572	GU245408	GU245279	GU245119	GU244956
Melecta albifrons	GU244650	GU244810	GU245573	GU245409	no # avail.	GU245120	GU244998
Melipona sp.	GU244653	GU244813	GU245576	GU245410	AF344607	GU245123	GU244959
Tetragonula carbonaria	GU244654	GU244814	GU245577	GU245411	GU245282	GU245124	GU244960
Meliponula ferruginea	GU244652	GU244812	GU245575	AY945192	GU245281	GU245122	GU244958
Plebeina hildebrandti	GU244656	GU244816	GU245579	GU245413	GU245284	GU245126	GU244962
Hypotrigona gribodoi	GU244651	GU244811	GU245574	AY945189	GU245280	GU245121	GU244957
Meliponula ferruginea	GU244655	GU244815	GU245578	GU245412	GU245283	GU245125	GU244961
Scaptotrigona hellwegeri	GU244657	GU244817	GU245580	GU245414	GU245285	GU245127	GU244963
Lestrimelitta sp.	GU244658	GU244818	GU245581	GU245415	GU245286	GU245128	GU244964
Scaura latitarsis	no # avail.	no # avail.	EU184713	EU184730	EU184837	EU184747	EU184772
Trigona fuscipennis	no # avail.	no # avail.	EU184710	EU184727	EU184835	EU184744	EU184770
Cephalotrigona capitata	no # avail.	no # avail.	EU184711	EU184728	EU184836	EU184745	EU184771
Meliponula bocandei	no # avail.	no # avail.	EU184712	EU184729	AY267161	EU184746	AY267145
Epeoloides pilosula	GU244659	GU244819	GU245582	GU245416	GU245287	GU245129	GU244966
Parepeolus aterrimus	GU244660	GU244820	GU245583	GU245417	GU245288	GU245130	GU244967
Epeoloides coecutiens	GU244661	GU244821	GU245584	GU245418	no # avail.	GU245131	GU244987
Osiris sp. 1	GU244662	GU244822	GU245585	GU245419	no # avail.	GU245132	GU245033

Table 1.2. (Continued)

Leiopodus singularis	AY995684	AY654542	GU245586	AY945137	AF344624	GU245133	AY585113
Leiopodus sp.	GU244663	GU244823	GU245587	GU245420	GU245289	GU245134	GU244968
Leiopodus abnormis	GU244664	GU244824	GU245588	GU245421	GU245290	GU245135	GU244969
Leiopodus trochantericus	GU244665	GU244825	GU245589	GU245422	GU245291	GU245136	GU244970
Rhathymus sp.	GU244666	GU244826	GU245590	GU245423	GU245292	GU245137	GU244971
Nanorhathymus sp.	GU244667	GU244827	GU245591	GU245424	GU245293	GU245138	GU244972
Rhathymus unicolor	GU244668	GU244828	GU245592	GU245425	GU245294	GU245139	GU244973
Caenonomada sp.	GU244669	GU244829	GU245593	GU245426	GU245295	GU245140	GU244974
Paratetrapedia sp.	GU244670	GU244830	GU245594	GU245427	GU245296	GU245141	GU244975
Tapinotaspoides sp.	GU244672	GU244832	GU245596	GU245429	GU245298	GU245143	GU244977
Arhysoceble picta	GU244671	GU244831	GU245595	GU245428	GU245297	GU245142	GU244976
Arhysoceble sp.	GU244673	GU244833	GU245597	GU245430	GU245299	GU245144	GU244978
Tetrapedia maura	GU244674	GU244834	GU245598	GU245431	GU245300	GU245145	GU244979
Coeleoxoides sp.	GU244675	GU244835	GU245599	GU245432	GU245301	GU245146	GU244980
Tetrapedia (Lagobata) sp.	GU244676	GU244836	GU245600	GU245433	GU245302	GU245147	GU244991
Tetrapedia (Tetrapedia) sp.	GU244677	GU244837	GU245601	GU245434	no # avail.	GU245148	GU24503
Coelioxoides waltheriae	GU244678	GU244838	GU245602	GU245435	GU245303	GU245149	GU244986
Oreopasites barbarae	GU244703	GU244863	GU245632	GU245460	AF344626	GU245176	GU245008
Sphecodopsis capensis	GU244704	GU244864	GU245633	GU245461	GU245317	GU245177	GU245009
Ammobates sp.	GU244705	GU244865	GU245634	GU245462	GU245318	GU245178	GU245010
Ammobates punctatus	GU244706	GU244866	GU245635	GU245463	no # avail.	GU245179	GU245011
Pasites maculatus	GU244707	GU244867	GU245636	GU245464	no # avail.	GU245180	GU245035
Holcopasites ruthae	AY995683	AY654540	GU245637	AY945154	AF344602	GU245181	AY585112
Holcopasites calliopsidis	GU244708	GU244868	GU245638	GU245465	AF344600	GU245182	GU245012
Holcopasites arizonicus	GU244709	GU244869	GU245639	GU245466	GU245319	GU245183	GU245013
Holcopasites insoletus	GU244710	GU244870	GU245640	GU245467	GU245320	GU245184	GU245014
Ammobatoides luctuosus	GU244711	GU244871	GU245641	GU245468	no # avail.	GU245185	GU244965
Holcopasites stevensi	GU244712	GU244872	GU245642	GU245469	GU245321	GU245186	GU245015
Holcopasites minimus	GU244713	GU244873	GU245643	GU245470	GU245322	GU245187	GU245016
Neopasites cressoni	GU244714	GU244874	GU245644	GU245471	GU245323	GU245188	GU245017
Biastes truncatus	GU244715	GU244875	GU245645	GU245472	no # avail.	GU245189	GU244981
Paranomada velutina	AY995686	AY654545	GU245646	AY945154	AF344627	GU245190	AY585115
Triopasites penniger	GU244716	GU244876	GU245647	GU245473	AF344633	GU245191	GU245018
Brachynomada margaretae	GU244718	GU244878	GU245649	GU245475	GU245324	GU245193	GU245019
Brachynomada sp.	GU244717	GU244877	GU245648	GU245474	no # avail.	GU245192	GU244982
Caenoprosopina holmbergi	GU244719	GU244879	GU245650	GU245476	GU245325	GU245194	GU244983
Caenoprosopis crabronina	GU244720	GU244880	GU245651	GU245477	GU245326	GU245195	GU245020
Triepeolus robustus	AY995688	AY654547	GU245654	AY945170	AF344634	GU245198	GU245023
Epeolus scutellaris	GU244722	GU244882	GU245653	GU245479	AF344596	GU245197	GU245022
Odyneropsis sp.	GU244721	GU244881	GU245652	GU245478	GU245327	GU245196	GU245021
Thalestria spinosa	GU244723	GU244883	GU245655	GU245480	GU245328	GU245199	GU245024
Doeringiella sp.	GU244724	GU244884	GU245656	GU245481	GU245329	GU245200	GU245025
Epeolus sp.	GU244725	GU244885	GU245657	GU245482	GU245330	GU245201	GU245026
Rhinepeolus rufiventris	GU244726	GU244886	GU245658	GU245483	GU245331	GU245202	GU245027

Table 1.2. (Continued)

Epeolus variegatus	GU244727	GU244887	GU245659	GU245484	no # avail.	GU245203	GU244988
Hexepeolus rhodogyne	GU244728	GU244888	GU245660	GU245485	GU245332	GU245204	GU245028
Neolarra orbiculata	GU244729	GU244889	GU245661	GU245486	GU245333	GU245205	GU245029
Nomada maculata	GU244730	GU244890	GU245662	GU245487	AF344609	GU245206	GU245030
Nomada signata	GU244731	GU244891	GU245663	GU245488	no # avail.	GU245207	GU245031
Townsendiella sp.	GU244732	GU244892	GU245664	GU245489	GU245334	GU245208	GU245032
Exoneura bicolor	GU244736	GU244896	GU245668	GU245493	GU245337	GU245212	GU245041
Braunsapis madecassella	GU244733	GU244893	GU245665	GU245490	GU245231	GU245209	GU245038
Compsomelissa keiseri	GU244734	GU244894	GU245666	GU245491	GU245335	GU245210	GU245039
Macrogalea ellioti	GU244735	GU244895	GU245667	GU245492	GU245336	GU245211	GU245040
Ceratina calcarata	AY995679	AY654535	GU245669	AY945099	AF344620	GU245213	AY585108
Ceratina (Crewella) sp.	GU244737	GU244897	GU245670	GU245494	GU245338	GU245214	GU245042
Ceratina (Simioceratina) sp.	GU244738	GU244898	GU245671	GU245495	GU245339	GU245215	GU245043
Ceratina (Ceratinula) sp.	GU244739	GU244899	GU245672	GU245496	GU245340	GU245216	GU245044
Ceratina (Xanthoceratina) sp.	GU244740	GU244900	GU245673	GU245497	GU245341	GU245217	GU245045
Ceratina cyanea	GU244741	GU244901	GU245674	GU245498		GU245218	GU244984
Manuelia gayatina	GU244742	GU244902	GU245675	GU245499	GU245342	GU245219	GU245046
Xylocopa tabaniformis	GU244744	GU244904	GU245677	GU245501	AF344614	GU245221	GU245048
Xylocopa virginica	GU244743	GU244903	GU245676	GU245500	GU245343	GU245220	GU245047
Xylocopa muscaria	GU244745	GU244905	GU245678	GU245502	GU245344	GU245222	GU245049
Xylocopa iris	GU244746	GU244906	GU245679	GU245503	GU245345	GU245223	GU245050
Xylocopa (Proxylocopa) sp.	GU244747	GU244907	GU245680	GU245504	GU245346	GU245224	GU245051
Xylocopa pubescens	GU244748	GU244908	GU245681	GU245505	GU245347	GU245225	GU245052
Xylocopa violacea	GU244749	GU244909	GU245682	GU245506	GU245348	GU245226	GU245055
Xylocopa fimbriata	GU244750	GU244910	GU245683	GU245507	GU245349	GU245227	GU245053
Xylocopa californica arizonensis	GU244751	GU244911	GU245684	GU245508	GU245350	GU245228	GU245054
Fideliopsis major	AY995692	AY654539	GU245606	AY945119	EU851628	GU245152	AY363024
Neofidelia sp.	GU244680	GU244840	GU245605	GU245437	GU245305	GU245151	GU244990
Pararhophites quadratus	GU244681	GU244841	GU245607	GU245438	GU245306	GU245153	GU245034
Paranthidium jugatorium	GU244684	GU244844	GU245610	GU245441	AF344611	GU245156	GU244994
Anthidiellum notatum	GU244685	GU244845	GU245611	GU245442	AF344617	GU245157	GU244995
Anthidium porterae	GU244686	GU244846	GU245612	GU245443	AF344619	GU245158	GU244996
Trachusa larreae	GU244682	GU244842	GU245608	GU245439	GU245307	GU245154	GU244992
Dianthidium subparvum	GU244683	GU244843	GU245609	GU245440	GU245308	GU245155	GU244993
Stelis linsleyi	GU244690	GU244850	GU245617	GU245447	GU245312	GU245162	GU245002
Dioxys pomonae	GU244687	GU244847	GU245613	GU245444	GU245309	GU245159	GU244997
Lithurgus echinocacti	AY995694	AY654541	GU245615	AY945136	DQ116702	EF646390	AY363026
Trichothurgus herbsti	GU244688	GU244848	GU245614	GU245445	GU245310	GU245160	GU245000
Microthurge sp.	GU244689	GU244849	GU245616	GU245446	GU245311	GU245161	GU245001
Coelioxys alternata	GU244692	GU244852	GU245619	GU245449	AF344591	GU245164	GU245004
Megachile angelarum	GU244691	GU244851	GU245618	GU245448	GU245313	GU245163	GU245003
Megachile ericetorum	GU244693	GU244853	GU245620	GU245450		GU245165	GU244985
Protosmia rubifloris	GU244701	GU244861	GU245630	GU245458	EU851724	GU245174	EU851619
Hoplitis albifrons	GU244702	GU244862	GU245631	GU245459	AF344598	GU245175	EU851577

Table 1.2. (Continued)

Heriades crucifer	GU244695	GU244855	GU245624	GU245452	EU851661	GU245168	EU851555
Osmia lignaria	GU244696	GU244856	GU245625	GU245453	EU851715	GU245169	EU851610
Chelostoma californica	GU244697	GU244857	GU245626	GU245454	GU245315	GU245170	GU245006
Ashmeadiella aridula	GU244698	GU244858	GU245627	GU245455	EU851641	GU245171	EU851535
Hoplosmia scutellaris	GU244699	GU244859	GU245628	GU245456	EU851693	GU245172	EU851587
Afroheriades hyalinus	GU244700	GU244860	GU245629	GU245457	GU245316	GU245173	GU245007
Dasypoda argentata	AY995660	AY654518	GU245603	AY945112	DQ116680	EF646418	AY585148
Hesperapis larreae	AY995664	AY654521	AY222552	AY945121	EF416862	EF646410	AY230131
Promelitta alboclypeata	GU244679	GU244839	GU245604	GU245436	GU245304	GU245150	GU245036
Meganomia binghami	AY995674	AY654528	GU245623	AY945144	DQ116689	EF646406	DQ141114
Macropis nuda	AY995670	AY654454	GU245621	AY945139	DQ116686	GU245166	AY585155
Melitta arrogans	GU244694	GU244854	GU245622	GU245451	GU245314	GU245167	GU245005

Aligning

All genes were separately aligned in the Lasergene DNAStar (DNASTAR 1999) software package using ClustalW. Alignments for 28S and 18S were subsequently adjusted by referring to the secondary structure of these genes proposed for *Apis mellifera* (Gillespie et al. 2006). Regions that could not be aligned with confidence were excluded from the analysis. Introns of LW rhodopsin were quite variable in length, and could not be aligned with confidence, and so were excluded from all analyses. The intron of EF1-α was also difficult to align across the entire dataset. Short portions of the intron that were aligneable were used in preliminary analyses, but were then eliminated from subsequent analyses because they represented too little data to inform parameters in their own partition. Reading frames and intron/exon boundaries for the protein-coding genes were determined by comparison with published sequences from the honey bee, *Apis mellifera.*

Partitioning of data

The dataset was analyzed under various partitioning schemes. The model-based analyses were first done with the data partitioned by genes (7 partitions). However, examination of the parameter trace files after 50 million generations revealed that some parameters of the 18S partition had not converged on a stationary phase. Therefore, it appeared that the 18S partition did not contain enough data to adequately estimate all parameters in the model. To reduce the number of partitions, subsequent analyses combined the two ribosomal genes into one partition and kept the protein-coding genes separately partitioned or combined together into one partition. The dataset was also analyzed with the two ribosomal genes in one partition, and the protein-coding genes combined together and partitioned by codon position. We did not partition each protein-coding gene separately by codon position because this would have produced a 17-partition analyses. Such an analysis would have been

Table 1.3. Primer pairs and PCR conditions for all genes.

Locus	Primer	Sequence
18S[1]	H17F (Heraty et al. 2004)	5'-AAA TTA CCC ACT CCC GGC A-3'
	H35R (Heraty et al. 2004)	5'-TGG TGA GGT TTC CCG TGT T-3'
28S[2]	A-28S-For (Ward and Brady 2003)	5'-CCC CCT GAA TTT AAG CAT AT-3'
	Bel28S-For (Belshaw and Quicke 1997)	5'-AGA GAG AGT TCA AGA GTA CGT G-3'
	Mar28S-Rev (Mardulyn and Whitfield 1999)	5'-TAG TTC ACC ATC TTT CGG GTC CC-3'
	28SD4-Rev (Danforth et al. 2006)	5'-GTT ACA CAC TCC TTA GCG GA-3'
Wingless[3]	Wg-Collet-For (Almeida and Danforth 2009)	5'-CAC GTG TCB TCB GRG ATG MGR SAG GA-3'
	Lep-Wg1a-For (Brower and DeSalle 1998)	5'-GAR TGY AAR TGY CAY GGY ATG TCT GG-3'
	Bee-wg-For (Danforth et al. 2004)	5'-TGC ACN GTS AAG ACC TGY TGG ATG AG-3'
	Lep-Wg2a-Rev (Brower and DeSalle 1998)	5'-ACT ICG CAR CAC CAR TGG AAT GTR CA-3'
Pol II[4]	polfor2a (Danforth et al. 2006)	5'-AAY AAR CCV GTY ATG GGT ATT GTR CA-3'
	polrev2a (Danforth et al. 2006)	5'-AGR TAN GAR TTC TCR ACG AAT CCT CT-3'
Opsin[5]	Opsin-For (Mardulyn and Whitfield 1999)	5'-AAT TGC TAT TAY GAR ACN TGG GT-3'
	Opsin-For3(mod) (Almeida and Danforth 2009)	5'-TTC GAY AGA TAC AAC GTR ATC GTN AAR GG-3'
	Opsin-Rev (Mardulyn and Whitfield 1999)	5'-ATA TGG AGT CCA NGC CAT RAA CCA-3'
	Opsin-Rev(mod) (Almeida and Danforth 2009)	5'-ATA NGG NGT CCA NGC CAT GAA CCA-3'
NaK[6]	NaKfor1	5'-GGY GGT TTC GCS WTG YTG YTG TGG ATC GG-3'
	NaKfor2	5'-GCS TTC TTC TCB ACS AAC GCC GTY GAR GG-3'
	NaKrev1a	5'-CCG ATN ARR AAG ATR TGM GCG TCN AGC CAA TG-3'
	NaKrev2	5'-ACC TTG ATR CCG GCY GAW CGG CAC TTG GC-3'
EF-1a[7]	HaF2For1 (Danforth et al. 1999)	5'-GGG YAA AGG WTC CTT CAA RTA TGC-3'
	EmphF2For (Sipes and Wolf 2001)	5'-GCC TGG GTA TTG GAT AAG CTG AA-3'
	F2-rev1 (Danforth et al. 1999)	5'-A ATC AGC AGC ACC TTT AGG TGG-3'
	F2-rev2	5'-AAR TCA GCA GCR CCY TTH GGT GG-3'
	EmphF2Rev (Sipes and Wolf 2001)	5'-TGG ATT GTT YTT RGA GTC ACC AG-3'

[1] PCR conditions 18S-H17F / 18S-H35R: 94°C for 1 min, 58°C for 1 min, 72°C for 1 min (35 cycles).

[2] PCR conditions A-28S-For / Mar28S-Rev: 94°C for 1 min, 54-58°C for 1 min, 72°C for 1.5 min (35 cycles); Bel28S-For / 28SD4-Rev: 94°C for 1 min, 58°C for 1 min, 72°C for 1.5 min (35 cycles).

[3] PCR conditions Wg-Collet-For / Lep-Wg2a-Rev: 94°C for 1 min, 55°C for 1 min, 72°C for 1 min (35 cycles); Lep-Wg1a-For / Lep-Wg2a-Rev: 94°C for 45 sec, 54°C for 45 sec, 72°C for 45 sec (35 cycles); Bee-wg-For / Lep-Wg2a-Rev: 94°C for 45 sec, 58°C for 45 sec, 72°C for 45 sec (35 cycles).

[4] PCR conditions polfor2a / polrev2a: 94°C for 1 min, 46-58°C for 1 min, 72°C for 1 min (35 cycles).

[5] PCR conditions Opsin-For / Opsin-Rev: 94°C for 1 min, 52°C for 1 min, 72°C for 1 min (35 cycles); Opsin-For3(mod) / Opsin-Rev(mod): 94°C for 1 min, 54°C for 1 min, 72°C for 1 min (35 cycles).

[6] PCR conditions NaKfor1 / NaKrev1a: 94°C for 1 min, 46-54°C for 1 min, 72°C for 1 min (35 cycles); NaKfor2 / NaKrev2: 94°C for 1 min, 48-58°C for 1 min, 72°C for 1.5 min (35 cycles).

[7] PCR conditions HaF2For1 / F2-rev1: 94°C for 1 min, 54°C for 1 min, 72°C for 1.5 min (35 cycles); HaF2For1 / F2-rev2: 94°C for 1 min, 46-54°C for 1 min, 72°C for 1.5 min (35 cycles); EmphF2For / EmphF2Rev: 94°C for 1 min, 52°C for 1 min, 72°C for 1.5 min (35 cycles).

computationally prohibitive and would have created some partitions with very little information for parameter estimation.

Model selection

Selection of best-fit models of nucleotide substitution for each data partition used in a Bayesian or Maximum Likelihood (ML) analysis was based on the Akaike (AIC) and Bayesian Information Criteria (BIC) as implemented in JModelTest v.0.1.1 (Posada 2008). Likelihood calculations were carried out for 88 models which included 11 substitution schemes (of which the general time reversible model (GTR) was the most complex), equal or unequal base frequencies, a proportion of invariable sites (I), and rate variation among sites with 4 rate categories (G) on an ML optimized tree. Model-selection uncertainty was quantified using the AIC and BIC delta values (the difference in AIC or BIC score between the model with the smallest score and the model in question); values within 2 delta units of the best model were considered to have substantial support and were therefore considered. The AIC and BIC weights were used to approximate a 95% confidence set of models by summing the weights of the ranked models until the sum was 0.95 (as suggested in the manual). Any model that was part of that set of models was considered to have substantial support and was also considered. If a model selected under the above criteria could not be implemented in MrBayes, the least complex model that included all of the parameters of the selected model, and could be implemented in MrBayes, was used instead.

Parsimony analyses

We used Winclada v.10.00.08 (Nixon 1999) to remove parsimony uninformative sites, and analyzed the concatenated dataset in TNT (Goloboff et al. 2008). A traditional heuristic search was conducted first with tree bisection-reconnection (TBR), 1000 random-taxon-addition replicates holding 10 trees per replicate, and treating sequence indels as missing data. Several new technology searches employing Ratchet and Drift options were also done. The minimum number of steps found in these previous searches was used as the target for a new technology driven search. This analysis was run until trees with this number of minimum steps were hit 1550 times. The initial sequence additions were set to 10. Support for the branches in the strict consensus of all the equally parsimonious trees found was assessed with 1000 bootstrap pseudo-replicates (Felsenstein 1985). Each re-sampled matrix was searched 10 times using a combination of drifting, tree fusing, and random sectorial searches, and the consensus tree of each iteration was saved. The 1000 consensus trees from the resampled matrices were opened in Winclada for calculation of nodal support of the strict consensus of the most parsimonious trees.

Maximum likelihood analysis

Maximum likelihood analyses were done in the program RaxML-VI-HPC (Stamatakis 2006) with the concatenated dataset partitioned into 4 partitions: (1) ribosomal genes, (2) 1st codon positions, (3) 2nd codon positions, and (4) 3rd codon positions. We used RaxML's rapid bootstrap algorithm which uses the GTR+CAT approximation to conduct 1000 BS replicates. Every 5th BS tree was then used as a starting point to search for the best-scoring ML tree under GTR+I+G for a total of 200 ML searches. The bootstrap support values were shown on the maximum likelihood tree.

Bayesian analysis

The Bayesian analyses were done in MrBayes v.3.1.2 (Huelsenbeck and Ronquist 2001). Each gene was individually analyzed under 1 or 2 different models according to the results of the model tests described above (Table 1.4). The individual gene datasets were analyzed with two independent runs with 4 chains each for approximately 20 million generations. The parameter trace files of each run were observed to verify that the runs had converged on the stationary distribution, and to

Table 1.4. Results of model tests and models used in the Bayesian analyses of each partition and combined dataset.

Partitions	**AIC**	**BIC**	**Model(s) used**
28S	GTR+I+G TIM1+I+G TVM+I+G	TIM1+I+G TVM+I+G	GTR+I+G
18S	TrN+I+G TIM1+I+G TIM3+I+G TIM2+I+G GTR+I+G	TrNef+I+G TIM1ef+I+G TrN+I+G	GTR+I+G SYM+I+G
Opsin	TPM3uf+I+G GTR+I+G TrN+I+G TVM+I+G	TPM3uf+I+G TrN+I+G HKY+I+G	GTR+I+G HKY+I+G
Wingless	GTR+I+G TIM1+I+G TVM+I+G	TIM1+I+G TVM+I+G	GTR+I+G
Pol II	TVM+I+G GTR+I+G TIM3+I+G	TPM1uf+I+G TPM2uf+I+G TVM+I+G TIM3+I+G TPM3uf+I+G	GTR+I+G
Nak	TVM+I+G GTR+I+G	TVM+I+G TIM3+I+G HKY+I+G TPM1uf+I+G	GTR+I+G HKY+I+G
Ef1α	TIM1+I+G GTR+I+G TIM2+I+G TVM+I+G TPM1uf+I+G	TIM1+I+G HKY+I+G TPM1uf+I+G TrN+I+G TIM2+I+G TPM2uf+I+G	GTR+I+G HKY+I+G
pos1	GTR+I+G	GTR+I+G	GTR+I+G
pos2	TVM+I+G GTR+I+G	TVM+I+G	GTR+I+G
pos3	TVM+I+G TPM1uf+I+G	TPM1uf+I+G	GTR+I+G
28S+18S	GTR+I+G	GTR+I+G	GTR+I+G

Combined analyses	**Model used for each partition**
|28S|18S|Ops|Win|Pol|Nak|Ef1|	GTR+I+G; GTR+SSR
|28S,18S|Ops|Win|Pol|Nak|Ef1|	GTR+I+G
|28S|18S|pos1|pos2|pos3|	GTR+I+G
|28S,18S|pos1|pos2|pos3|	GTR+I+G

decide on the appropriate number of generations to discard as burn-in. Convergence was also assessed by observing the posterior probabilities of clades over generations in the program Awty (Nylander et al. 2008).

The concatenated dataset was analyzed under the different partitioning schemes described above. For the concatenated analyses, all partitions used the GTR+I+G model with each parameter unlinked across partitions. Two individual runs of 20 to 50 million generations with 4 chains each were conducted for the variously partitioned concatenated dataset (|28S|18S|Ops|Win|Pol|Nak|Ef1|, |28S,18S|Ops|Win|Pol|Nak|Ef1|, and |28S|18S|pos1|pos2|pos3|). Our preferred partitioning scheme, where the two ribosomal genes are combined into 1 partition and the protein-coding genes are combined together and partitioned by codon position (|28S,18S|pos1|pos2|pos3|), was much more thoroughly analyzed. A total of 46 independent runs, 44 with 4 chains and 2 with 8 chains, were conducted. The number of generations for each run varied from 4 096 000 generations to 23 353 000 generations. The tree files and parameter files with the burn-in removed from each run that had reached convergence were combined. Runs that had not converged were discarded. A maximum clade credibility tree was constructed from these 268 560 000 post-burn-in generations in TreeAnnotator v1.4.8 (Rambaut and Drummond 2008). We chose to do numerous shorter runs instead of a few longer runs because of run time restrictions on the computer clusters at Cornell University's Computational Biology Service Unit. Also, doing a large number of independent runs from different starting points allowed us to more fully explore tree space.

Properties of the molecular data sets were explored with further analyses in MrBayes. To estimate the base frequencies and the shape of the gamma distribution (alpha) of each gene, a combined analysis partitioned by locus with a GTR+I+G model applied to each partition with all parameters unlinked across partitions was conducted. To estimate the rates of substitution, the dataset was analyzed with a site-specific rates model (GTR+SSR) where the rate categories corresponded to each codon position within each protein-coding gene, to 28S and to 18S rRNA. For each analysis done, a maximum clade credibility tree was constructed from the post burn-in trees and examined for topological congruence with all other analyses.

Phylogenetic signal of individual gene trees

The phylogenetic signal of each gene partition was assessed by comparing the tree topologies obtained from the Bayesian analysis of each gene to the topology of the combined analysis (|28S,18S|pos1|pos2|pos3|). The ratio between the number of nodes congruent between each gene tree and the combined tree, and the maximum number of nodes which can be resolved for a given number of taxa, gave a measure of the 'Congruent topological information' (CTI) for each gene partition 104 Almeida, E.A.B. 2009. Only nodes within the family Apidae were considered for the calculations of the CTIs.

Ancestral state reconstruction of cleptoparasitic behavior

All terminals in the tree were coded for a behavioral character, based on information in the literature, consisting of 2 states; cleptoparasitic and nest-making (Table 1.5). Parsimony methods were used to reconstruct the evolution of cleptoparasitism in apid bees on the Bayesian maximum clade credibility tree using MacClade v.4.0 (Maddison and Maddison 2000).

To take phylogenetic uncertainty, branch lengths, and relative rates of gains and losses into account, Bayesian ancestral state reconstruction methods were implemented in the program BayesTraits v.1.0 (Pagel et al. 2004b). We used a random sample of 10 000 trees from the Bayesian analysis of the phylogeny. We initially ran a likelihood analysis in order to get reasonable starting values (priors), on the transition rate from one character state to another, for the Bayesian MCMC

Table 1.5. Behavioral character state assigned to each taxa used in the study.

Character state distribution		
Nest making		Cleptoparasitic
Afroheriades hyalinus	*Megachile ericetorum*	*Aglae caerulea*
Alepidosceles sp.	*Meganomia binghami*	*Ammobates punctatus*
Amegilla asserta	*Meliphilopsis sp.*	*Ammobates sp.*
Ancyla anatolica	*Melipona sp.*	*Ammobatoides luctuosus*
Ancyla asiatica	*Meliponula bocandei*	*Biastes truncatus*
Ancyloscelis sp.	*Meliponula bocandei*	*Brachynomada margaretae*
Ancyloscelis sp.	*Meliponula ferruginea*	*Brachynomada sp.*
Anthidiellum notatum	*Meliponula ferruginea*	*Caenoprosopina holmbergi*
Anthidium porterae	*Melissodes desponsa*	*Caenoprosopis crabronina*
Anthophora urbana	*Melissoptila sp.*	*Coeleoxoides sp.*
Anthophorula (Isomalopsis) sp.	*Melitoma sp.*	*Coelioxoides waltheriae*
Anthophorula (Isomalopsis) sp.	*Melitta arrogans*	*Coelioxys alternata*
Anthophorula completa	*Microthurge sp.*	*Ctenioschelus goryi*
Apis cerana	*Neofidelia sp.*	*Ctenoplectrina sp.*
Apis dorsata	*Osmia lignaria*	*Dioxys pomonae*
Apis florea	*Pachymelus peringueyi*	*Doeringiella sp.*
Arhysoceble sp.	*Paranthidium jugatorium*	*Epeoloides coecutiens*
Ashmeadiella aridula	*Pararhophites quadratus*	*Epeoloides pilosula*
Bombus ardens	*Paratetrapedia sp.*	*Epeolus scutellaris*
Bombus diversus	*Peponapis pruinosa*	*Epeolus sp.*
Bombus mendax	*Plebeina hildebrandti*	*Epeolus variegatus*
Braunsapis madecassella	*Promelitta alboclypeata*	*Epiclopus gayi*
Caenonomada sp.	*Protosmia rubifloris*	*Ericrocis lata*
Centris analis	*Ptilothrix sp.*	*Exaerete frontalis*
Centris atripes	*Scaptotrigona hellwegeri*	*Exaerete sp.*
Centris decolorata	*Scaura latitarsis*	*Hexepeolus rhodogyne*
Centris dimidiata	*Svastra obliqua*	*Holcopasites arizonicus*
Centris hoffmanseggiae	*Svastrides melanura*	*Holcopasites calliopsidis*
Centris longimana	*Svastrina subapicalis*	*Holcopasites insoletus*
Cephalotrigona capitata	*Tapinotaspidini sp.*	*Holcopasites minimus*
Ceratina (Ceratinula) sp.	*Tapinotaspoides sp.*	*Holcopasites ruthae*
ceratina (Crewella) sp.	*Tetragonula carbonaria*	*Holcopasites stevensi*
Ceratina (Simioceratina) sp.	*Tetralonia cinctula*	*Hopliphora velutina*
Ceratina (Xanthoceratina) sp.	*Tetraloniella glauca*	*Isepeolus atripilis*
Ceratina calcarata	*Tetrapedia (Lagobata) sp.*	*Isepeolus cortesi*
Ceratina cyanea	*Tetrapedia (Tetrapedia) sp.*	*Isepeolus luctuosus*
Chelostoma californica	*Tetrapedia maura*	*Isepeolus wagenknechti*
Compsomelissa keiseri	*Thygater sp.*	*Leiopodus abnormis*
Ctenoplectra albolimbata	*Trachusa larreae*	*Leiopodus singularis*
Ctenoplectra bequaerti	*Trichothurgus herbsti*	*Leiopodus sp.*
Dasypoda argentata	*Trigona fuscipennis*	*Leiopodus trochantericus*
Deltoptila aurulentocaudata	*Xenoglossa angustior*	*Melecta albifrons*
Diadasia bituberculata	*Xylocopa (Proxylocopa) sp.*	*Melectoides bellus*
Diadasina distincta	*Xylocopa californica arizonensis*	*Mesocheira bicolor*
Dianthidium subparvum	*Xylocopa fimbriata*	*Mesonychium asteria*
Epicharis analis	*Xylocopa iris*	*Mesoplia rufipes*
Epicharis sp.	*Xylocopa muscaria*	*Nanorhathymus sp.*

Table 1.5. (Continued)

Eucera frater	*Xylocopa pubescens*	*Neolarra orbiculata*
Eufriesea pulchra	*Xylocopa tabaniformis*	*Neopasites cressoni*
Eufriesea surinamensis	*Xylocopa violacea*	*Nomada maculata*
Euglossa imperialis	*Xylocopa virginica*	*Nomada signata*
Euglossa piliventris		*Odyneropsis sp.*
Eulaema meriana		*Oreopasites barbarae*
Exomalopsis sp.		*Osiris sp. 1*
Exoneura bicolor		*Paranomada velutina*
Fideliopsis major		*Parepeolus aterrimus*
Florilegus sp.		*Pasites maculatus*
Habropoda laboriosa		*Rhathymus sp.*
Heriades crucifer		*Rhathymus unicolor*
Hesperapis larreae		*Rhinepeolus rufiventris*
Hoplitis albifrons		*Sphecodopsis capensis*
Hoplosmia scutellaris		*Stelis linsleyi*
Hypotrigona gribodoi		*Tetralonioidella sp.*
Lestrimelitta sp.		*Thalestria spinosa*
Lithurgus echinocacti		*Thyreus delumbatus*
Macrogalea ellioti		*Townsendiella sp.*
Macropis nuda		*Triepeolus robustus*
Manuelia gayatina		*Triopasites penniger*
Martinapis luteicornis		*Xeromelecta californica*
Megachile angelarum		*Zacosmia maculata*

analysis, as recommended in the manual (Pagel et al. 2004b). Both transition rates were below 1.3. We therefore used a reversible jump model with priors obtained from an exponential prior seeded from a uniform on the interval 0 to 5 (hyperprior). A reversible-jump model automatically finds the posterior distribution of models of evolution for the data (Pagel and Meade 2006). In this case, it would determine if a model where the rate of transition from nest-making to cleptoparasitic was the same as the rate of transition from cleptoparasitic to nest-making, or if a model with these two transition rates being different, better fit the data. Using a hyperprior allowed us to choose an exponential distribution for the prior, and let the program estimate this prior from the data using a uniform hyperprior to seed the prior (Pagel et al. 2004a). We ran the analysis several times for 2 million generations and discarded the first 1 million as burn-in. Examination of the output files indicated that our burn-in was sufficient because parameter estimates were stable over the post burn-in period.

In order to test whether there was significant support for 4 independent origins of cleptoparasitism instead of just 3, we ran the MCMC analysis 100 times with the common ancestor of *Euglossa, Exaerete and Aglae* fixed alternatively as cleptoparasitic and nest-making. We compared the log of the harmonic means obtained for each of the 100 replicates. Twice the difference in the harmonic mean of the log likelihood scores represents the Bayes Factor. Values above 2 are considered positive support for the hypothesis, values above 5 are considered strong support for the hypothesis, and values above 10 are considered very strong support (Kass & Raftery 1995, Nylander et al. 2004).

Estimating divergence times

We used a Bayesian phylogenetic relaxed molecular clock model (Drummond et al. 2006) with multiple calibration points to estimate divergence times in the program BEAST v1.4.8 (Drummond and Rambaut 2007). We applied a GTR+I+G model, as in the phylogenetic analysis described above. Branch rates were estimated with an uncorrelated relaxed clock model in which the rate at each branch was drawn from an underlying log-normal distribution. This allowed for the rate of evolution to vary among the branches of the tree with no *a priori* correlation between a lineage's rate and that of its ancestor. Parameters were unlinked across partitions. The Yule tree prior was used, which assumes a constant per lineage selection rate as recommended in the manual for species-level phylogenies. We randomly selected a starting tree from the posterior distribution of trees from the MrBayes analysis.

The tree was time calibrated by setting priors on the ages of 10 internal nodes (Figure 1.3) and the root of the tree. Age estimates were based on paleontological evidence as described below. Uncertainty in the age of the calibration points was incorporated into the analysis by assuming that the probability of the node being a certain age follows a lognormal distribution with a rigid minimum bound. This required us to specify a mean, standard deviation and rigid lower bound for the age of each calibration point. Selection of the values for these parameters is somewhat subjective, but below we outline the reasoning behind each of our choices for every calibration point. Applying a lognormal distribution to our age estimates allows us to assume that the actual divergence event took place some time prior to the earliest appearance of fossil evidence, but that the age of the node is more likely to be close to the age of the oldest known fossil, and less likely to be significantly older. Fossils can only provide minimum age estimates and their appearance must postdate the origin of the clade to which they belong. By how much the appearance of a clade predates the age of the first fossil is unclear. We therefore made sure that the 95% probability included the oldest reasonable age for the clade. For our more basally positioned calibration points, that meant including the start of the Cretaceous (145 Ma). The fossil record for angiosperms extends back to the early Cretaceous (Crane et al. 2004).

Figure 1.3. Bayesian maximum clade credibility tree showing placement of 10 calibration points.

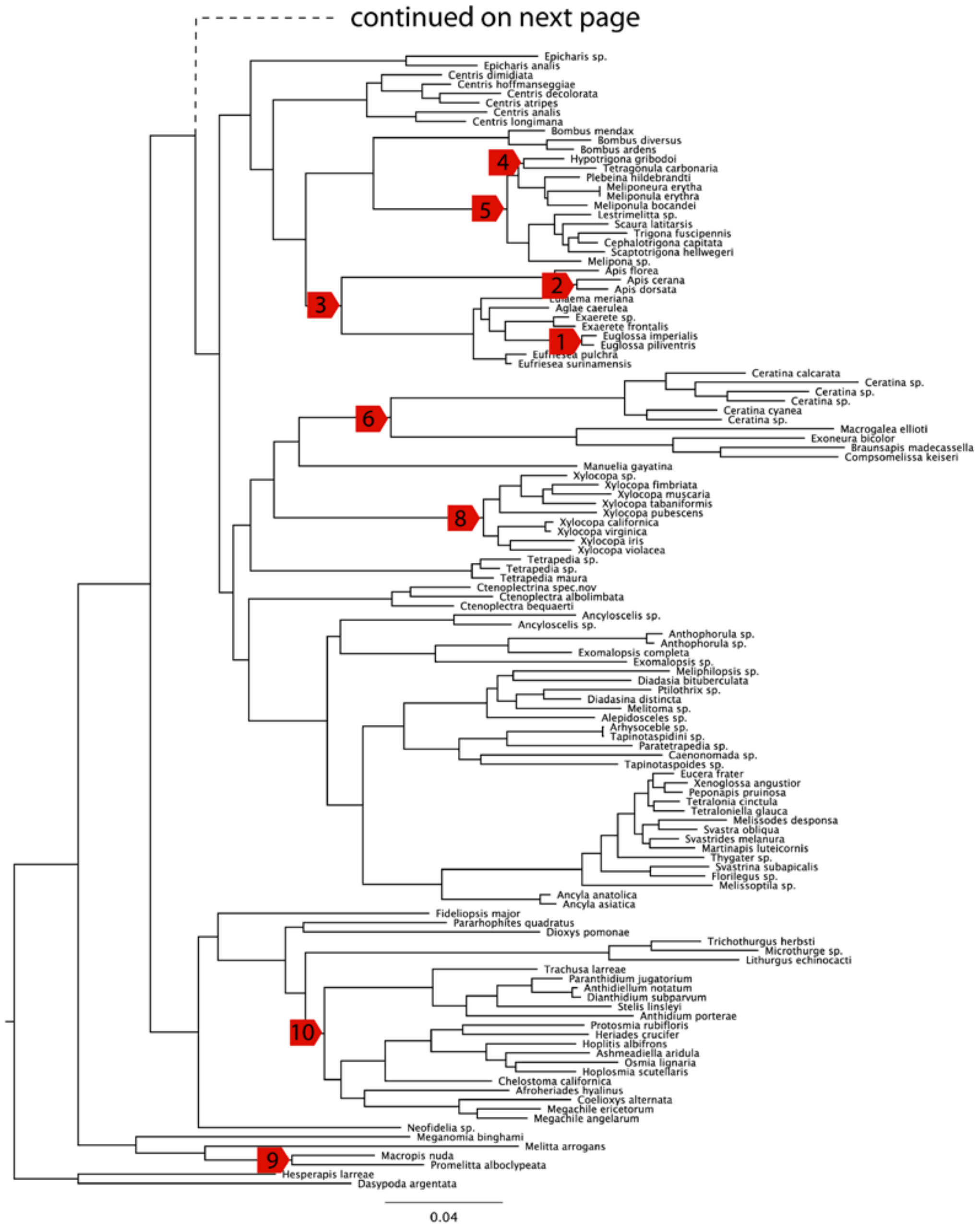
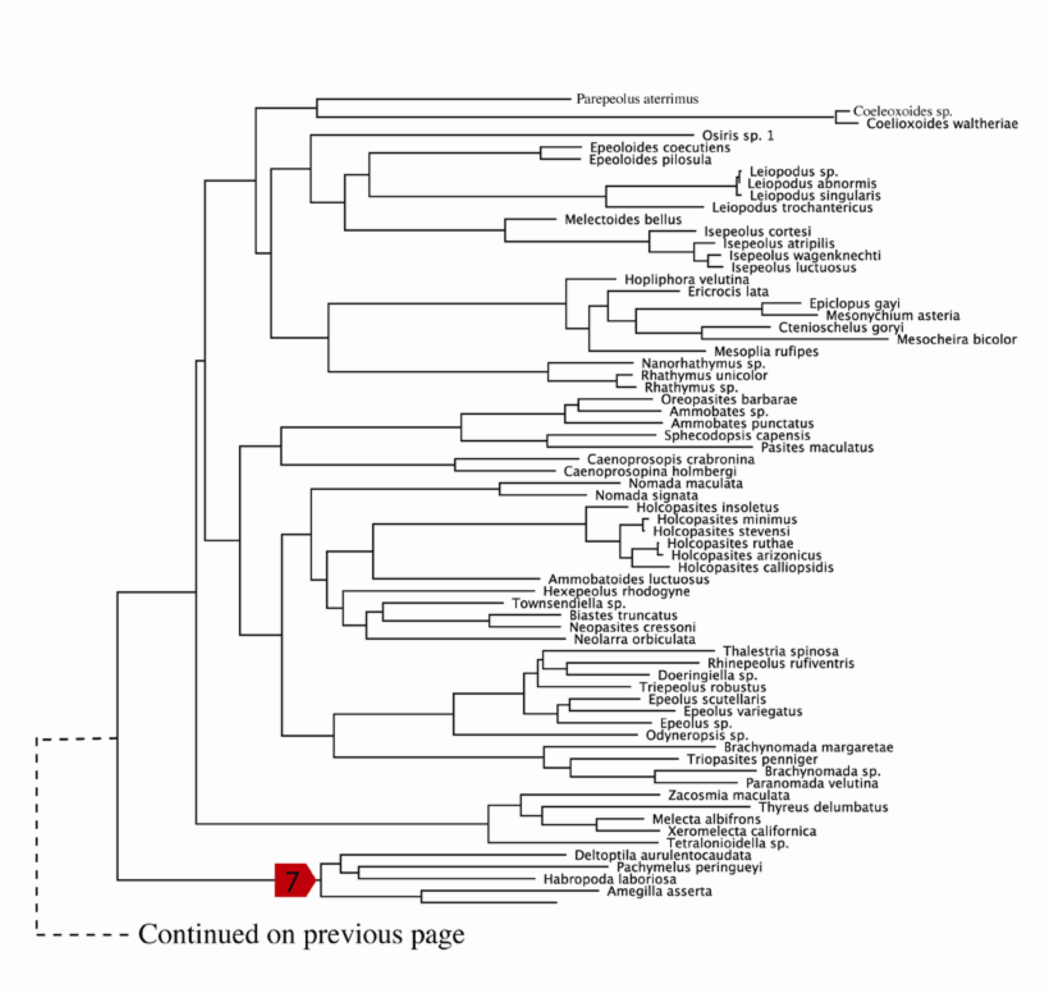

continued on next page
Epicharis sp.
Epicharis analis
Centris dimidiata
Centris hoffmanseggiae
Centris decolorata
Centris atripes
Centris analis
Centris longimana
Bombus mendax
Bombus diversus
Bombus ardens
4
Hypotrigona gribodoi
Tetragonula carbonaria
Plebeina hildebrandti
Meliponeura erytha
Meliponula erythra
Meliponula bocandei
5
Lestrimelitta sp.
Scaura latitarsis
Trigona fuscipennis
Cephalotrigona capitata
Scaptotrigona hellwegeri
Melipona sp.
Apis florea
2
Apis cerana
Apis dorsata
3
Eulaema meriana
Aglae caerulea
Exaerete sp.
Exaerete frontalis
1
Euglossa imperialis
Euglossa piliventris
Eufriesea pulchra
Eufriesea surinamensis
Ceratina calcarata
Ceratina sp.
Ceratina sp.
Ceratina sp.
Ceratina cyanea
Ceratina sp.
6
Macrogalea ellioti
Exoneura bicolor
Braunsapis madecassella
Compsomelissa keiseri
Manuelia gayatina
Xylocopa sp.
Xylocopa fimbriata
Xylocopa muscaria
Xylocopa tabaniformis
Xylocopa pubescens
8
Xylocopa californica
Xylocopa virginica
Xylocopa iris
Xylocopa violacea
Tetrapedia sp.
Tetrapedia sp.
Tetrapedia maura
Ctenoplectrina spec.nov
Ctenoplectra albolimbata
Ctenoplectra bequaerti
Ancyloscelis sp.
Ancyloscelis sp.
Anthophorula sp.
Anthophorula sp.
Exomalopsis completa
Exomalopsis sp.
Meliphilopsis sp.
Diadasia bituberculata
Ptilothrix sp.
Diadasina distincta
Melitoma sp.
Alepidosceles sp.
Arhysoceble sp.
Tapinotaspidini sp.
Paratetrapedia sp.
Caenonomada sp.
Tapinotaspoides sp.
Eucera frater
Xenoglossa angustior
Peponapis pruinosa
Tetralonia cinctula
Tetraloniella glauca
Melissodes desponsa
Svastra obliqua
Svastrides melanura
Martinapis luteicornis
Thygater sp.
Svastrina subapicalis
Florilegus sp.
Melissoptila sp.
Ancyla anatolica
Ancyla asiatica
Fideliopsis major
Pararhophites quadratus
Dioxys pomonae
Trichothurgus herbsti
Microthurge sp.
Lithurgus echinocacti
Trachusa larreae
Paranthidium jugatorium
Anthidiellum notatum
Dianthidium subparvum
Stelis linsleyi
Anthidium porterae
10
Protosmia rubifloris
Heriades crucifer
Hoplitis albifrons
Ashmeadiella aridula
Osmia lignaria
Hoplosmia scutellaris
Chelostoma californica
Afroheriades hyalinus
Coelioxys alternata
Megachile ericetorum
Megachile angelarum
Neofidelia sp.
Meganomia binghami
Melitta arrogans
9
Macropis nuda
Promelitta alboclypeata
Hesperapis larreae
Dasypoda argentata
0.04

Figure 1.3. (Continued)



Angiosperms are thought to have originated about 130 Ma, shortly before the origin of the eudicots about 125 Ma (Davies et al. 2004; Soltis and Soltis 2004; Magallon and Castillo 2009). Bees are mostly dependent on floral resources from eudicots, therefore it would be very unlikely for bees to predate the origin of eudicots or angiosperms. Furthermore, it is estimated that the crabronid-bee divergence took place ~120 Ma (Grimaldi and Engel 2005). In most cases, we chose a mean that was slightly older then the oldest fossil belonging to the group because the divergence event of the group necessarily predates any fossil belonging to it. We set the rigid lower bounds (zero offset) for each calibration point to the boundary between two geological time scales younger then the age of the fossil to allow for the chance that the fossil is younger than the age assigned to it and to allow for possible mistakes in the phylogenetic placement of the fossil. We describe below the paleontological evidence upon which our 10 calibration points are based. A lognormal Stdev of 1 was used in all cases. Figure 1.3 shows the placement of the calibration points on our phylogenetic tree.

Calibration 1 –The fossil bee *Euglossa morenei*, which is from Miocene Dominican amber, was described and placed within *Euglossa* based on morphological characters (Engel 1999). Several characters however differentiated this species from any of the described subgenera, suggesting that it represents a lineage of euglossine bees that is no longer extant (Engel 1999). We therefore placed this fossil as part of the stem group of the genus *Euglossa*. Based on biostratigraphic and paleogeographic data, Dominican amber has been dated to be of late Early Miocene through early Middle Miocene (15-20 Ma) age (Iturralde-Vinent and MacPhee 1996). A lognormal distribution with a lognormal mean of 3 (=20 Ma), Stdev of 1 and zero offset of 11.6 Ma was applied as a prior for calibration point 1.

Note- *Eufriesea melissiflora* was first described under the name *Paleoeuglossa melissiflora* (Poinar 1998), but was later transferred to *Eufriesea* (Engel 1999). This fossil cannot be placed within the crown group of *Eufriesea* with confidence, only it's stem group. It is also from Dominican amber of the same single sedimentary basin as *E. morenei.* Therefore, this fossil was not used to inform a calibration point because *E. morenei* already informs a calibration node nearer the tips of the tree .

Calibration 2– *Apis lithohermaea* (Engel 2006) is the oldest fossil record for crown-group *Apis*. The fossil is from the Chôjabaru Formation of Iki Island, Japan which has been dated to be from the middle Miocene (Engel 2006). Based on some key morphological characters, it was assigned to the *dorsata* species group and was described as being quite similar to modern *Apis dorsata* (Engel 2006). This fossil therefore provides a minimum age for the diversification of *Apis dorsata* from *Apis cerana*, a member of the mellifera species group. A lognormal distribution with a lognormal mean of 2.8 (=17 Ma), Stdev of 1 and zero offset of 11.6 Ma was applied as a prior for calibration point 2.

Calibration 3 – There are several fossil honey bee species that are thought to belong to the stem group of *Apis* (Engel 1998). The oldest of these fossils is a compression fossil of *A. henshawi* from the Oligocene, Rott, Germany. Age of this famous European shale deposit is somewhat ambiguous, being either late Oligocene (Chattian) or early Miocene (Aquitanian) age (Grimaldi and Engel 2005). A lognormal distribution with a lognormal mean of 3.5 (=33 Ma), Stdev of 1 and zero offset of 20.4 Ma was applied as a prior for calibration point 3.

Note – The two fossil bee species of the armbrusteri species group, *Apis armbrusteri* and *Apis nearctica,* are thought to share the most recent common ancestor with extant *Apis* species (Engel 2006; Engel et al. 2009). The type specimen of *A. armbrusteri* was described from worker bees preserved on a slab of thermal limestone found near Böttingen in the Swabian Alb, Württemburg, Germany (Engel 2006). The Randecker Maar is thought to be from the Early Miocene(Grimaldi and Engel 2005). *Apis* *nearctica* was recently described from a worker preserved in paper shale from the Middle Miocene (Late Barstovian 14-14.5 Ma) of the Stewart Valley Basin in west-central Nevada (Engel et al. 2009). K/Ar dating indicates an age of 16-10.5 Ma (Grimaldi and Engel 2005).

Note – There have been a few reliable *Bombus* compression fossils described from the Miocene. These are *Bombus vetustus, Bombus luianus,* and *Bombus proavus.* However, these fossils do not reveal sufficient morphological synapomorphies of *Bombus* to be placed within the crown group of *Bombus* instead of the stem group (Hines 2008). Grimaldi and Engel (2005) mention the existence of various European Oligocene *Bombus* spp., but give no citation for these. We could not find any described *Bombus* fossils from the Oligocene that are still believed to belong to *Bombus*, therefore we assume that these fossils have not yet been described, and do not know if they would belong to the stem or crown group of *Bombus*. Therefore, the *Bombus* fossils could only be used as a minimum age on the node uniting Bombini with Meliponini. But there have been much older fossils described that could be placed as a minimum age for this node, so we do not use any of the *Bombus* fossils in this analysis.

Calibration 4 – *Kelneriapis eocenica* is a fossil bee from the Baltic amber thought to be most similar to, and perhaps sister to *Hypotrigona* (Engel 2001). Therefore, this fossil can be used to set a minimum age on the node of the most recent common ancestor of *Hypotrigona* and its sister lineage in the phylogeny. *Liotrigonopsis rozeni* is also a fossil bee from the Baltic amber and is thought to be morphologically similar to the extant genus *Liotrigona* (Engel 2001), which is hypothesized to be the sister genus to *Hypotrigona* (Rasmussen and Cameron 2007). *Liotrigona* was not included in this analysis however, but this fossil also serves to inform a minimum age on the split of *Hypotrigona* from other extant stingless bees. K-Ar radiometric studies of the

Baltic amber which occurs in the *blau Erde* ("blue Earth") throughout northern Europe indicate it to be 44.1 ±1.1 Ma (middle of the Lutetian stage of the Eocene) (Ritzkowski 1997). A lognormal distribution with a lognormal mean of 3.85 (=47 Ma), Stdev of 1 and zero offset of 33.9 Ma was applied as a prior for calibration point 4.

Note – *Proplebeia dominicana, Proplebeia tantilla,* and *Proplebeia vetusta* are fossil bees from Dominican amber. A study of the extinct fauna of stingless bees in Dominican Amber, corroborated the hypothesis that *Proplebeia* is a distinct group within the lineage of Neotropical *Plebeia* (s.s) (Camargo et al. 2000). *Plebeia* was not included in this phylogeny, but has been placed within a large Neotropical clade of stingless bees (Rasmussen and Cameron 2007) which is represented in this analysis by the most recent common ancestor of *Trigona* and *Melipona*. Therefore a minimum age of 15-20 Ma (see calibration 1 for discussion on age of Dominican amber) could be placed on this node, but calibration point 4 already provides a much older age estimate for this node.

Calibration 5 - *Cretotrigona prisca* is the oldest crown group bee fossil recorded. It is from the Late Cretaceous amber of New Jersey (Michener and Grimaldi 1988). It was first placed within *Trigona* (Michener and Grimaldi 1988), but was later placed sister to the African genus *Dactylurina* (Engel 2000). These two genera superficially resemble each other but are not phylogenetically close to one another (Rasmussen and Cameron 2007). Therefore, this fossil can only be used as a minimum age on the node uniting all of the extant stingless bees.

The age of this fossil has been debated. It was first thought to be Campanian (ca. 80 Ma) in origin based on chemical comparison with other New Jersey ambers (Michener and Grimaldi 1988). However, based on the other insects found in this amber, it was suggested that it was of Tertiary age -- probably from the Paleocene (Rasnitsyn and

Michener 1991). It was once again argued that it was of Cretaceous age based on infrared spectroscopy and pyrolysis gas chromatography and the presence of other insects in the amber fragment dated to the Turonian (Grimaldi 1999). This fossil is usually now treated as being of Late Maastrichtian (ca. 65-70Ma) age (Engel 2000). A lognormal distribution with a lognormal mean of 4.25 (=70 Ma), Stdev of 1 and zero offset of 55 Ma was applied as a prior for calibration point 5.

Note - There are numerous fossils from the Baltic amber that have been placed within the corbiculate bees (Engel 2001). Many of these do not fall within extant tribes but have been instead assigned to their own fossil tribes. Electrobombini, Electrapini, and Milikertini are corbiculate tribes containing only fossil bees. In a cladistic analysis with representatives of extant corbiculates and a few outgroups, the phylogenetic relationships among and within these tribes were analyzed (Engel 2001). But, because of the difference in topology of the corbiculate tribes between most morphological and molecular studies described in the paper, these fossils can at best be used as a minimum age for the common ancestor of corbiculates. Pending a clearer resolution of extant and fossil corbiculate relationships, these fossil corbiculates provide limited information on the age of the extant groups. However, *Cretotrigona prisca* is also placed within the corbiculates and is of much older age.

Calibration 6 – There have been three fossil bee species described from the Baltic amber that have been placed in the fossil bee tribe Boreallodapini. Boreallodapini most resembles the extant Ceratinini and Allodapini (Xylocopinae). A phylogenetic analysis placed it as sister to the Allodapini with Ceratinini being sister to Boreallodapini+Allodapini (Engel 2001). These tribal relationships are in agreement with those found in this present phylogeny of extant taxa only, and so these fossils were used to inform a minimum age for the node uniting Allodapini and Ceratinini. See above for discussion on the age of the Baltic amber. A lognormal distribution with a lognormal mean of 3.85 (=47 Ma), Stdev of 1 and zero offset of 33.9 Ma was applied as a prior for calibration point 6.

Calibration 7 – *Paleohabropoda oudardi* from the lacustrine shales of Menat in France has been placed within the tribe Anthophorini based on a morphological cladistic analysis and wing morphometrics (Michez et al. 2008). Evidence from pollen and the mammals also found in Menat suggest it is of Paleocene age. K/Ar analysis propose a date of ~56 Ma for the Menat fossils (Russel 1982). This fossil is used to inform the calibration of the node uniting the Anthophorini. A lognormal distribution with a lognormal mean of 4.09 (=60 Ma), Stdev of 1 and zero offset of 23 Ma was applied as a prior for calibration point 7.

Calibration 8 – *Xylocopa gabrielae* was described from a female bee preserved as a compression fossil from the Eocene-Oligocene boundary (34 Ma) of Florissant, Colorado (Engel 2001). The combination of characters present in the fossil indicate that it is a species of *Xylocopa*, however because of a general lack of preserved features it is not possible to assign it to any recognizable subgenera (Engel 2001). Therefore, there is the possibility that this fossil is part of the stem, rather than crown, group of *Xylocopa*. However this age seems reasonable for the node of extant xylocopines because there is a Miocene fossil from China closely resembling extant *Xylocopa (Nyctomelitta)* (Zhang 1990), and fossils from Switzerland of Middle Oligocene age (Zeuner and Manning 1976) most likely related to *Xylocopa s.s.* and *Copoxyla* (Leys et al. 2000). A lognormal distribution with a lognormal mean of 3.53 (=34 Ma), Stdev of 1 and zero offset of 23 Ma was applied as a prior for calibration point 8.

Calibration 9 - The oldest fossil of a melittid bee is *Palaeomacropis eocenicus* from the early Eocene amber of Oise (France) (Michez et al. 2007). In a cladistic analysis, it formed the sister group to *Macropis* with another fossil bee *Eomacropis glaesaria*, falling as the sister to *Macropis + Palaeomacropis* (Michez et al. 2007). *Eomacropis glaesaria* is from Baltic amber of the late Eocene (Engel 2001). The Paris Basin amber of Oise, France is thought to be of Ypresian age (Grimaldi and Engel 2005). This fossil is used to calibrate the node representing the common ancestor of *Macropis* and its sister group. A lognormal distribution with a lognormal mean of 4.01 (=55 Ma), Stdev of 1 and zero offset of 40.4 Ma was applied as a prior for calibration point 9.

Calibration 10 – *Probombus hirsutus* was first described as a bumblebee. However, after doubts were placed on this (Rasnitsyn and Michener 1991), it was re-examined and transferred to the Megachilidae (Nel and Petrulevicius 2003). The fossil is thought to be part of the Megachilinae, probably related to Osmiini or Megachilini (Nel and Petrulevicius 2003), which together form a monophyletic group. We therefore use this fossil to calibrate the node uniting the Osmiini, Megachilini and Anthidiini. This fossil is from a spongodiatomitic volcanic paleolake (maar) deposit, in Menat, Puy-de-Dôme, France. K/Ar analysis propose a date of ~56 Ma for the Menat fossils (Russel 1982). A lognormal distribution with a lognormal mean of 4.09 (=60 Ma), Stdev of 1 and zero offset of 48.6 Ma was applied as a prior for calibration point 10.

Note - *Protolithurgus ditomeus* was described from Baltic amber (Engel 2001). In a cladistic analasis, it was placed sister to all other lithurgines due to the plesiomorphic retention of some characters (Engel 2001). This fossil can therefore only be used to calibrate the node uniting Lithurgini with the other Megachilinae.

Note - Four fossil species described from the Baltic amber have been assigned to the fossil genus *Glyptapis* (Engel 2001). The phylogenetic position of *Glyptapis* is uncertain and may possibly be more closely allied to Anthidiini than other Osmiini. Therefore, these fossils can tentatively be used as a minimum age on the node representing the most recent common ancestor of Anthidiini and extant Osmiini which is the same node used for calibration point 10.

Note – Four fossil species described from the Baltic amber, and one from Rovno Amber (of roughly contemporaneous age) have been assigned to the fossil genus *Ctenoplectrella* of the subtribe Ctenoplectriellina (Engel and Perkovsky 2006; Engel 2008). *Glaesosmia genalis* has also been described from a fossil of the Baltic amber (Engel 2001). A third genus was recently added to the tribe Ctenoplectrellini or subtribe Ctenoplectrellina. The type species, *Friccomelissa schopowi* (Wedmann et al. 2009), is described from a fossil recovered from a Messel pit near Darmstadt, Hesse, Germany. The Messel Formation is from the lower mid-Eocene, Geiseltalian, ca. 47 Ma (Wedmann et al. 2009). *Friccomelissa* might represent a stem group to Ctenoplectrellini (Wedmann et al 2009). Ctenoplectrellina has been placed in the tribe Osmiini which might be paraphyletic with respect to the Megachilini (Engel 2001). Ctenoplectrellina is now it's own tribe (Ctenoplectrellini) (Wedmann et al. 2009), and may be sister to extant Osmiini+Megachilini, and therefore cannot provide a minimum age to the most recent common ancestor of Osmiini +Megachilini but to one node back from there which is where calibration point 10 is placed.

Bees are thought to have originated in the mid-Cretaceous about 120-125 Ma (Grimaldi and Engel 2005). We therefore ran separate analyses with the age of the root node sampled from a normal distribution with a mean of 145.5, 130, 120, 110, 100 and 90 Ma ± 1 Ma to see the effect of different root node ages on the age estimates of internal nodes. We also ran analyses for all of these different root node ages with the internal calibration point age priors changed to be sampled from a uniform distribution with a specified minimum and maximum age. In all cases, the start of the Cretaceous was used as an absolute maximum (145.5 Ma), and the youngest age assigned to a fossil was used as the minimum age for the group. We did five independent runs of 10 million generations for each of these exploratory analyses. Results of the 5 independent runs were combined together, giving us a total of 50 million generations. Two longer analyses of 100 million generations were run with the root node set to 120 Ma and the calibration points set with a lognormal distribution.

Results

Molecular data sets

The final dataset consisted of 190 taxa and 6047 aligned nucleotide sites. Of those, 1959 are parsimony-informative characters (Table 1.6). The information content was calculated as the difference between the maximum number of steps for all characters in a partition minus the minimum number of steps (calculated in Winclada). The Nak partition contained the most characters (1464) and the highest information content (24 984 steps) among the gene partitions, but the Opsin partition, which contained the least number of characters (454), had higher information per character (21.21steps/character). The partition containing the 3rd position codons for all 5 protein-coding genes had the highest total information and information/character of all partitions, whereas the partition of 1st position codons contained comparatively little information.

The best-fit models selected by the AIC and BIC differed in some cases. Both model choice criteria always selected models with a proportion of invariable sites (I) and rate variation among sites (Gamma), but at times differed on the nucleotide substitution scheme (HKY vs. GTR, for example) and on whether base frequencies were equal or unequal. Modeltest often selected models whose number of nucleotide substitution rates could not be implemented in MrBayes which only allows for 1, 2, or 6 different substitution rates. When the models selected had more then 2 substitution rates but less then 6, we analyzed that partition using 6 substitution rates which is the

GTR model (Table 1.4). Under-parameterization has been shown to produce inflated estimates of the posterior probability (PP) while few repercussions are seen to slight over parameterization of the model (Cunningham et al. 1998; Huelsenbeck and Rannala 2004).

Table 1.6. Summary of the number of characters, parsimony informative characters, and information content for each partition and the concatenated dataset.

Partition	Number of Characters	Parsimony-Informative characters	Total information	Information/ number of characters
28S	1331	279	3790	2.85
18S	754	78	1048	1.39
Opsin	454	237	9630	21.21
Wingless	457	196	6530	14.29
Pol II	841	296	15919	18.93
Nak	1464	589	24984	17.07
Ef1-α	746	284	12133	16.26
pos 1	1320	249	6250	4.73
pos 2	1321	111	1659	1.26
pos 3	1321	1242	59588	45.11
Combined	6047	1959	72335	11.96

Models which fell within the approximate 95% confidence set of models based on the summed weights or were within 2 delta values of the best fit model are listed in ranked order for both the AIC and BIC. The models under which each partition were analyzed are also shown along with the different partitioning schemes used for the combined analyses.

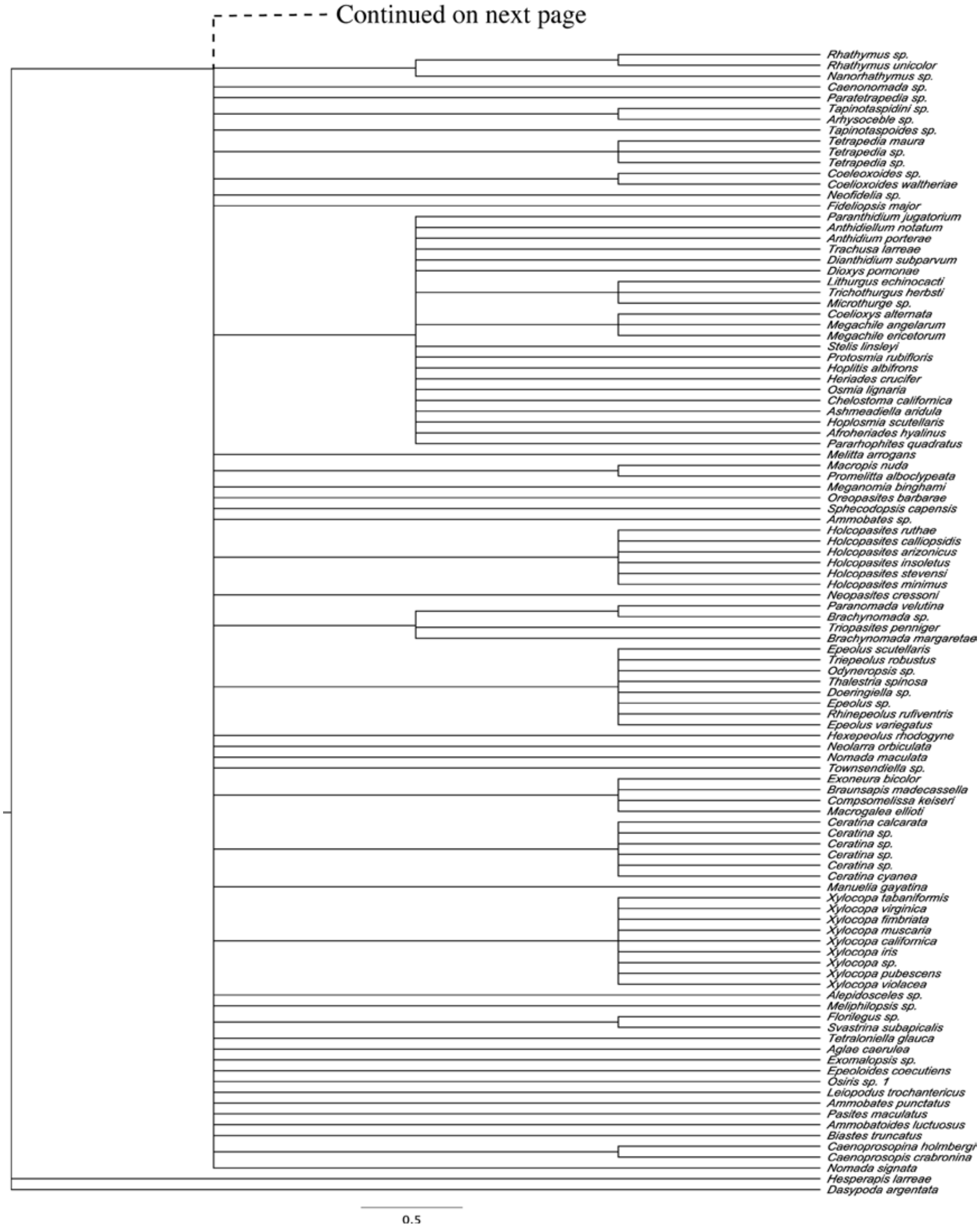
Individually analyzing the gene partitions with the different models selected by the AIC and BIC had very little effect on the tree topology (Figures 1.4-1.10). There also did not seem to be a substantial difference in the efficiency of the runs in terms of time to reach convergence. We therefore used the GTR+I+G model for all partitions in the combined analyses.

Properties of the data sets

The frequencies of the nucleotide bases for each gene locus (Figure 1.11) show wingless deviating the most from equal base composition, with a strong G-C bias. Ef1-α and Pol II show an A-T bias. Nak has a slight G-C bias while the ribosomal and Opsin gene partitions have fairly even base composition, although 18S was the only partition whose model test supported a model with equal base frequencies (SYM+I+Γ).

The relative rates of substitution for the two ribosomal genes and for each codon position of the protein-coding genes are shown in Figure 1.12. The 3rd codon position of each gene has the highest substitution rate, the 1st codon position has an intermediate substitution rate, and the 2nd codon position has the lowest substitution rate. 28S has a substitution rate comparable to the protein-coding genes 2nd codon position, whereas 18S has a lower substitution rate comparable to 1st codon position nucleotides.

Figure 1.4 Bayesian majority rule consensus tree based on sequence data from 28S analysed with a GTR+I+G model.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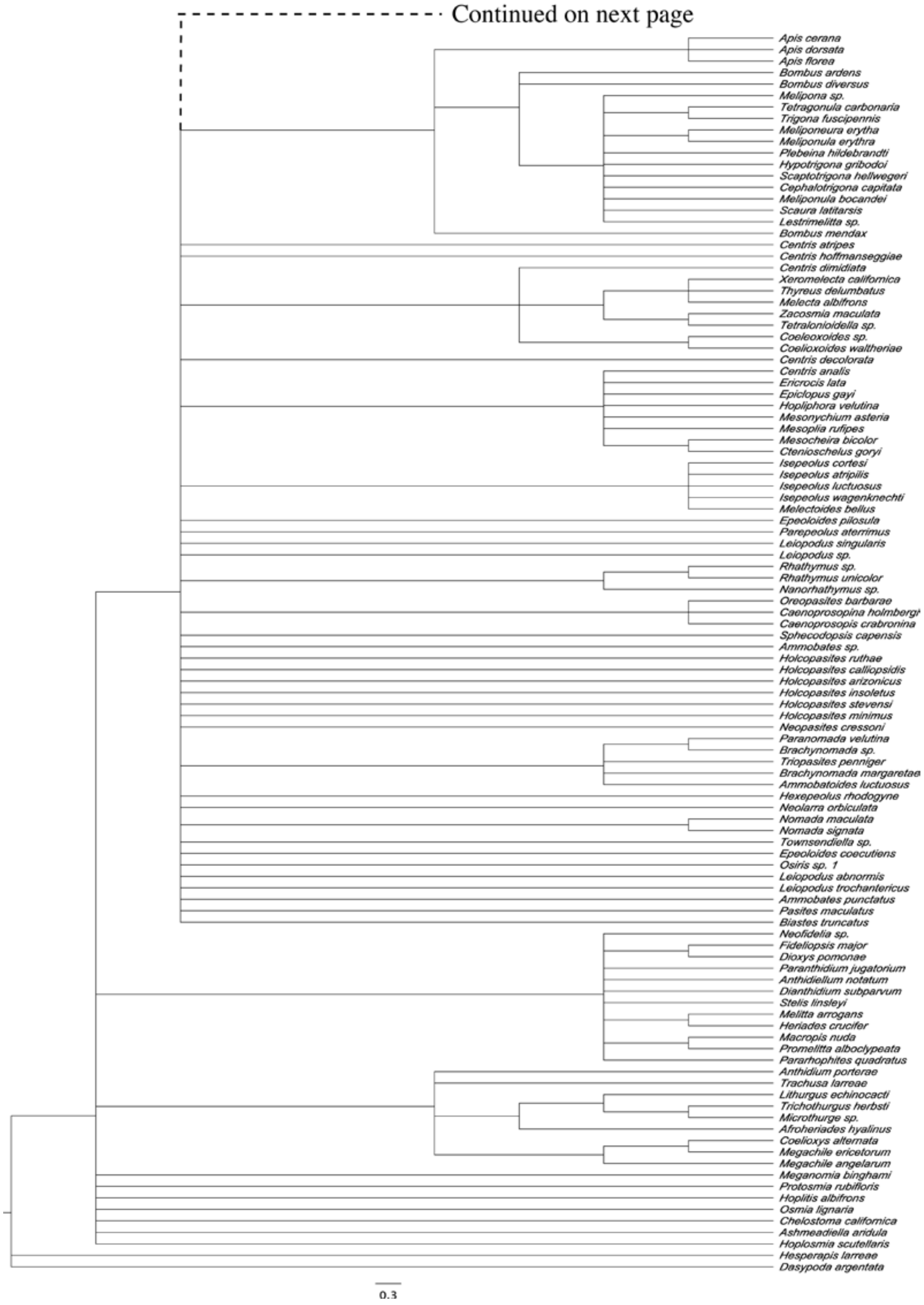
Rhathymus sp.
Rhathymus unicolor
Nanorhathymus sp.
Caenonomada sp.
Paratetrapedia sp.
Tapinotaspidini sp.
Arhysoceble sp.
Tapinotaspoides sp.
Tetrapedia maura
Tetrapedia sp.
Tetrapedia sp.
Coeleoxoides sp.
Coelioxoides waltheriae
Neofidelia sp.
Fideliopsis major
Paranthidium jugatorium
Anthidiellum notatum
Anthidium porterae
Trachusa larreae
Dianthidium subparvum
Dioxys pomonae
Lithurgus echinocacti
Trichothurgus herbsti
Microthurge sp.
Coelioxys alternata
Megachile angelarum
Megachile ericetorum
Stelis linsleyi
Protosmia rubifloris
Hoplitis albifrons
Heriades crucifer
Osmia lignaria
Chelostoma californica
Ashmeadiella aridula
Hoplosmia scutellaris
Afroheriades hyalinus
Pararhophites quadratus
Melitta arrogans
Macropis nuda
Promelitta alboclypeata
Meganomia binghami
Oreopasites barbarae
Sphecodopsis capensis
Ammobates sp.
Holcopasites ruthae
Holcopasites calliopsidis
Holcopasites arizonicus
Holcopasites insoletus
Holcopasites stevensi
Holcopasites minimus
Neopasites cressoni
Paranomada velutina
Brachynomada sp.
Triopasites penniger
Brachynomada margaretae
Epeolus scutellaris
Triepeolus robustus
Odyneropsis sp.
Thalestria spinosa
Doeringiella sp.
Epeolus sp.
Rhinepeolus rufiventris
Epeolus variegatus
Hexepeolus rhodogyne
Neolarra orbiculata
Nomada maculata
Townsendiella sp.
Exoneura bicolor
Braunsapis madecassella
Compsomelissa keiseri
Macrogalea ellioti
Ceratina calcarata
Ceratina sp.
Ceratina sp.
Ceratina sp.
Ceratina sp.
Ceratina cyanea
Manuelia gayatina
Xylocopa tabaniformis
Xylocopa virginica
Xylocopa fimbriata
Xylocopa muscaria
Xylocopa californica
Xylocopa iris
Xylocopa sp.
Xylocopa pubescens
Xylocopa violacea
Alepidosceles sp.
Meliphilopsis sp.
Florilegus sp.
Svastrina subapicalis
Tetraloniella glauca
Aglae caerulea
Exomalopsis sp.
Epeoloides coecutiens
Osiris sp. 1
Leiopodus trochantericus
Ammobates punctatus
Pasites maculatus
Ammobatoides luctuosus
Biastes truncatus
Caenoprosopina holmbergi
Caenoprosopis crabronina
Nomada signata
Hesperapis larreae
Dasypoda argentata
0.5

Figure 1.4. (Continued)

Figure 1.5 Bayesian majority rule consensus tree based on sequence data from 18S analysed with a GTR+I+G model.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Apis cerana
Apis dorsata
Apis florea
Bombus ardens
Bombus diversus
Melipona sp.
Tetragonula carbonaria
Trigona fuscipennis
Meliponeura erytha
Meliponula erythra
Plebeina hildebrandti
Hypotrigona gribodoi
Scaptotrigona hellwegeri
Cephalotrigona capitata
Meliponula bocandei
Scaura latitarsis
Lestrimelitta sp.
Bombus mendax
Centris atripes
Centris hoffmanseggiae
Centris dimidiata
Xeromelecta californica
Thyreus delumbatus
Melecta albifrons
Zacosmia maculata
Tetralonioidella sp.
Coeleoxoides sp.
Coelioxoides waltheriae
Centris decolorata
Centris analis
Ericrocis lata
Epiclopus gayi
Hopliphora velutina
Mesonychium asteria
Mesoplia rufipes
Mesocheira bicolor
Ctenioschelus goryi
Isepeolus cortesi
Isepeolus atripilis
Isepeolus luctuosus
Isepeolus wagenknechti
Melectoides bellus
Epeoloides pilosula
Parepeolus aterrimus
Leiopodus singularis
Leiopodus sp.
Rhathymus sp.
Rhathymus unicolor
Nanorhathymus sp.
Oreopasites barbarae
Caenoprosopina holmbergi
Caenoprosopis crabronina
Sphecodopsis capensis
Ammobates sp.
Holcopasites ruthae
Holcopasites calliopsidis
Holcopasites arizonicus
Holcopasites insoletus
Holcopasites stevensi
Holcopasites minimus
Neopasites cressoni
Paranomada velutina
Brachynomada sp.
Triopasites penniger
Brachynomada margaretae
Ammobatoides luctuosus
Hexepeolus rhodogyne
Neolarra orbiculata
Nomada maculata
Nomada signata
Townsendiella sp.
Epeoloides coecutiens
Osiris sp. 1
Leiopodus abnormis
Leiopodus trochantericus
Ammobates punctatus
Pasites maculatus
Biastes truncatus
Neofidelia sp.
Fideliopsis major
Dioxys pomonae
Paranthidium jugatorium
Anthidiellum notatum
Dianthidium subparvum
Stelis linsleyi
Melitta arrogans
Heriades crucifer
Macropis nuda
Promelitta alboclypeata
Pararhophites quadratus
Anthidium porterae
Trachusa larreae
Lithurgus echinocacti
Trichothurgus herbsti
Microthurge sp.
Afroheriades hyalinus
Coelioxys alternata
Megachile ericetorum
Megachile angelarum
Meganomia binghami
Protosmia rubifloris
Hoplitis albifrons
Osmia lignaria
Chelostoma californica
Ashmeadiella aridula
Hoplosmia scutellaris
Hesperapis larreae
Dasypoda argentata
0.3

Figure 1.5. (Continued)

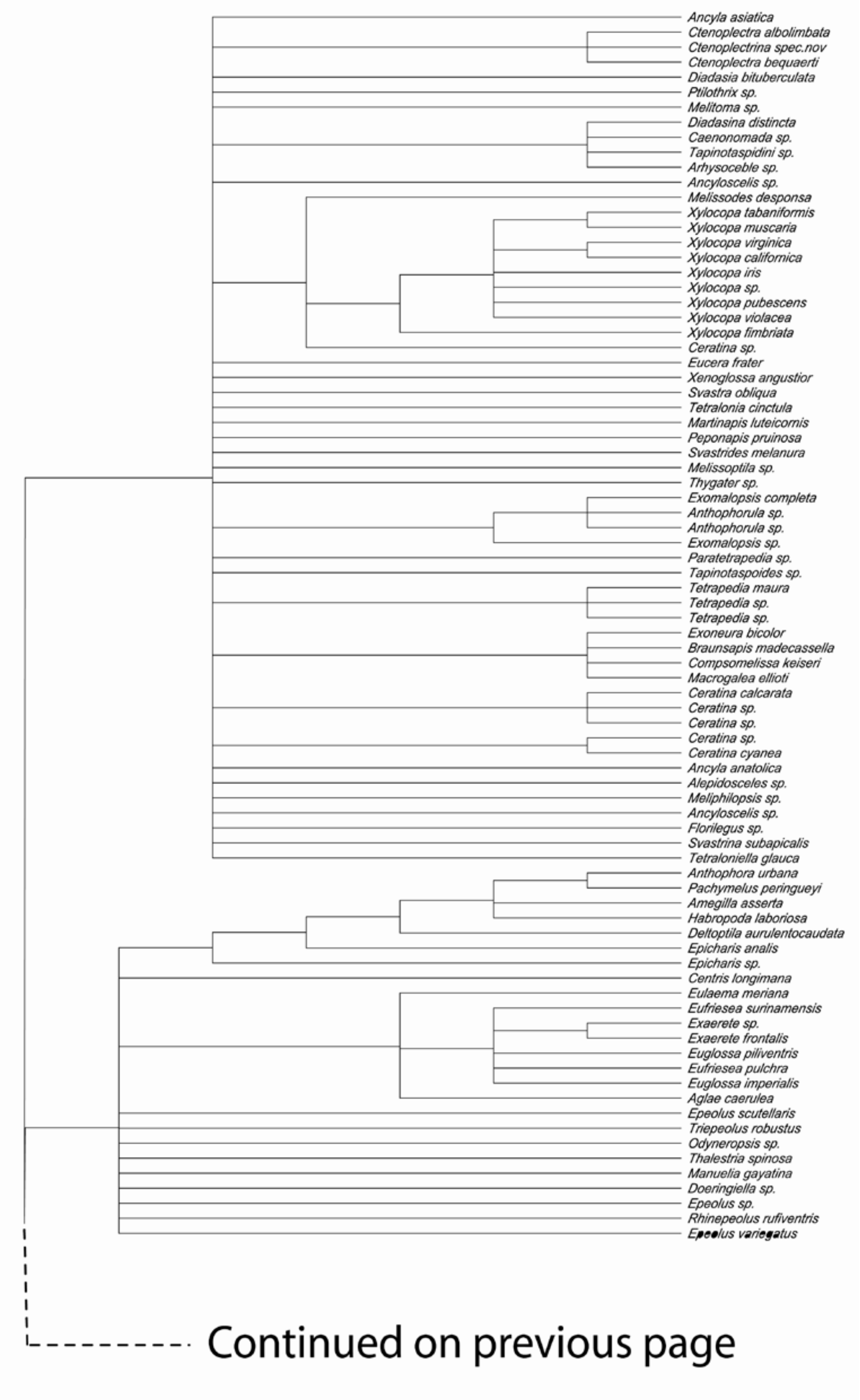

Figure 1.6 Bayesian majority rule consensus tree based on sequence data from Opsin analysed with a GTR+I+G model.

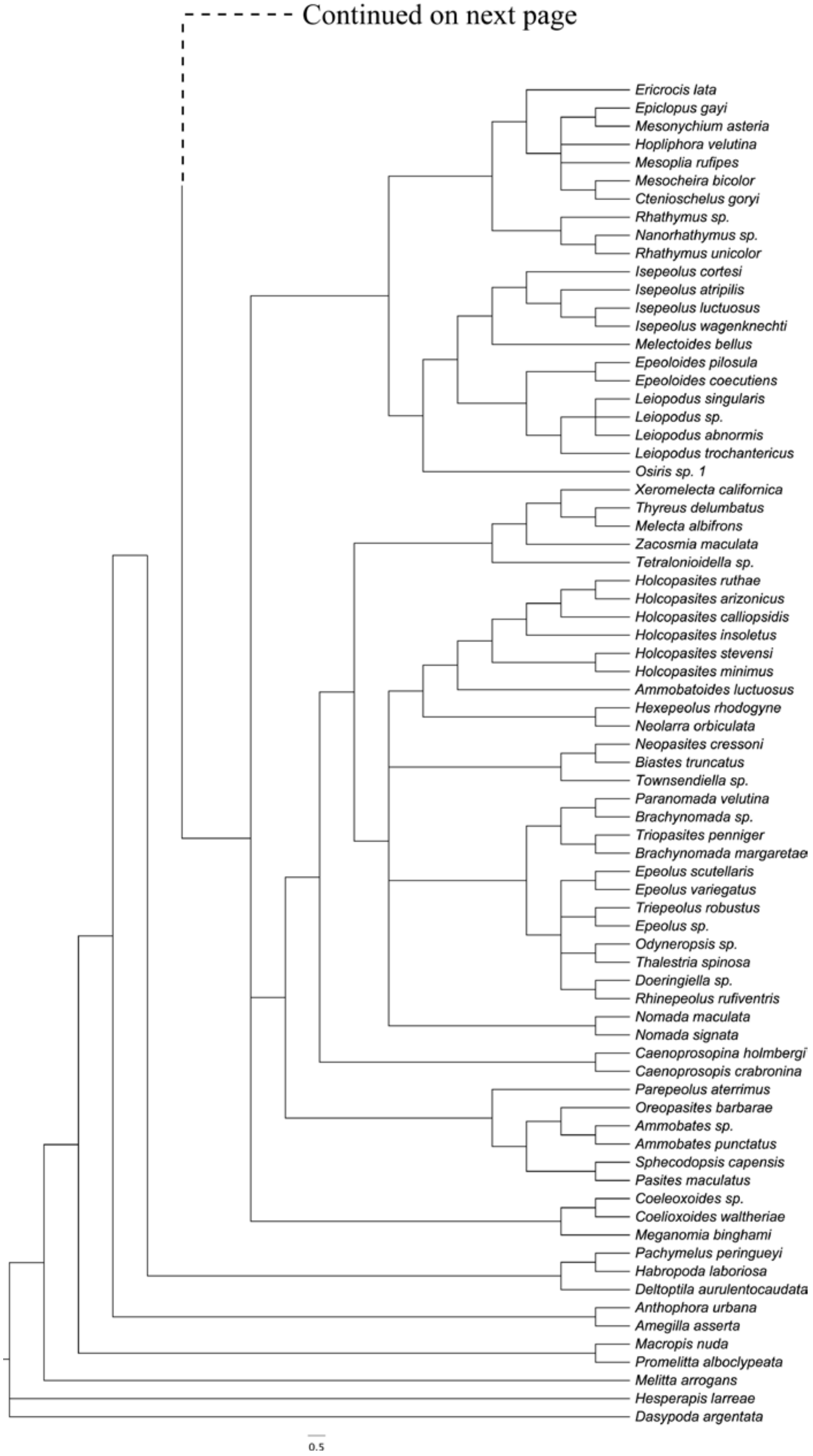
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Figure 1.6. (Continued)

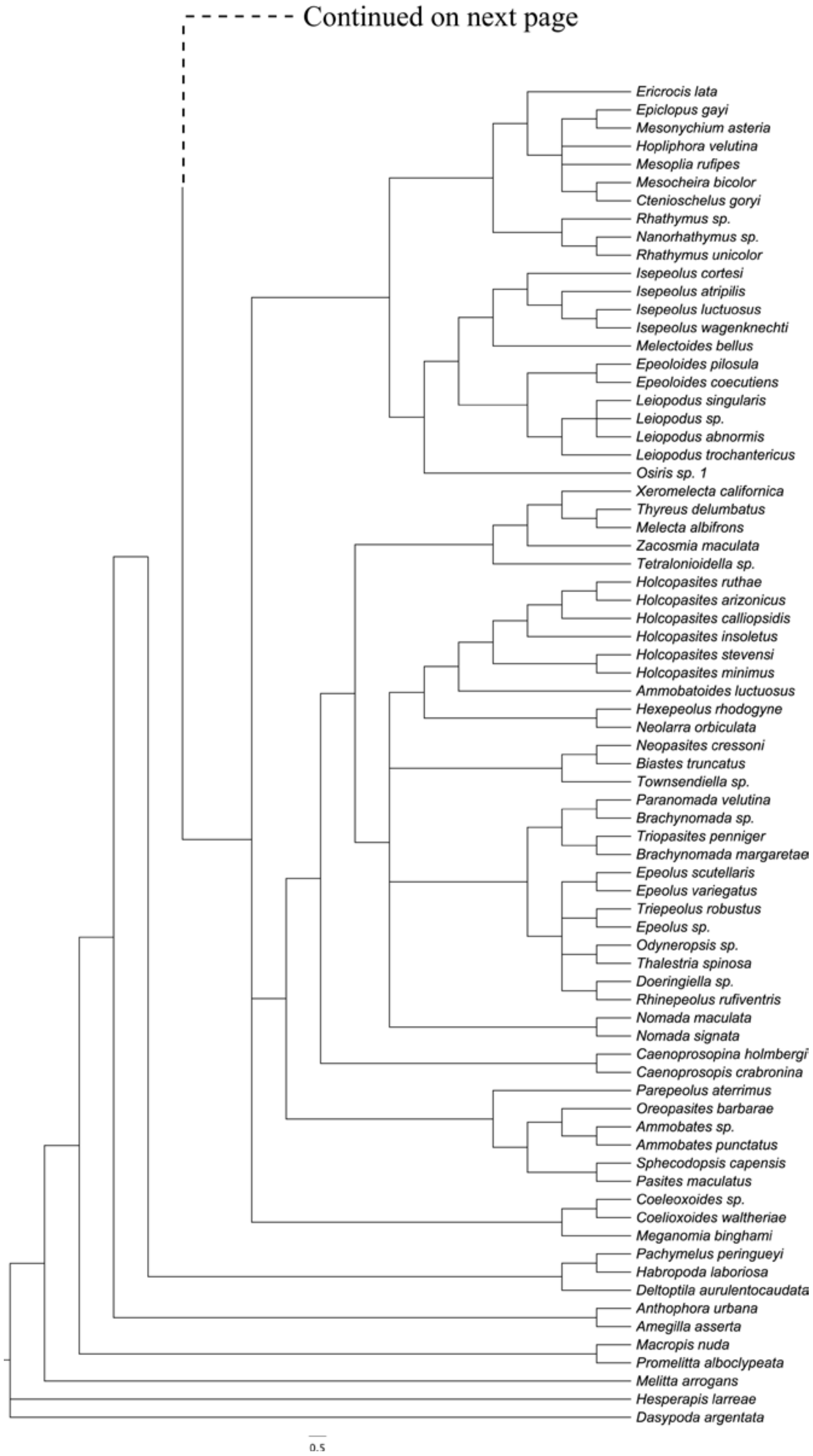
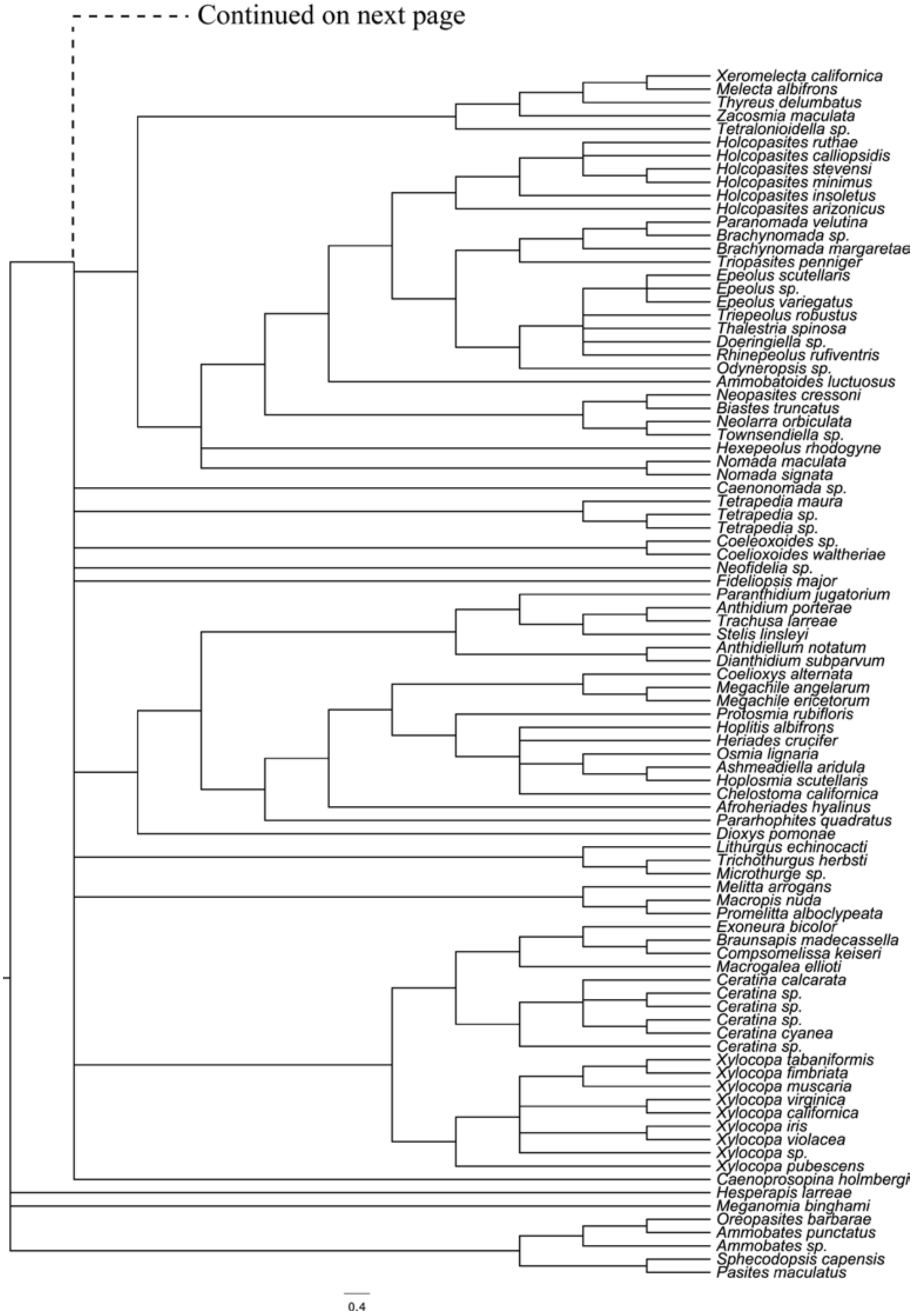


Figure 1.7. Bayesian majority rule consensus tree based on sequence data from Wingless analysed with a GTR+I+G model.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Xeromelecta californica
Melecta albifrons
Thyreus delumbatus
Zacosmia maculata
Tetralonioidella sp.
Holcopasites ruthae
Holcopasites calliopsidis
Holcopasites stevensi
Holcopasites minimus
Holcopasites insoletus
Holcopasites arizonicus
Paranomada velutina
Brachynomada sp.
Brachynomada margaretae
Triopasites penniger
Epeolus scutellaris
Epeolus sp.
Epeolus variegatus
Triepeolus robustus
Thalestria spinosa
Doeringiella sp.
Rhinepeolus rufiventris
Odyneropsis sp.
Ammobatoides luctuosus
Neopasites cressoni
Biastes truncatus
Neolarra orbiculata
Townsendiella sp.
Hexepeolus rhodogyne
Nomada maculata
Nomada signata
Caenonomada sp.
Tetrapedia maura
Tetrapedia sp.
Tetrapedia sp.
Coeleoxoides sp.
Coelioxoides waltheriae
Neofidelia sp.
Fideliopsis major
Paranthidium jugatorium
Anthidium porterae
Trachusa larreae
Stelis linsleyi
Anthidiellum notatum
Dianthidium subparvum
Coelioxys alternata
Megachile angelarum
Megachile ericetorum
Protosmia rubifloris
Hoplitis albifrons
Heriades crucifer
Osmia lignaria
Ashmeadiella aridula
Hoplosmia scutellaris
Chelostoma californica
Afroheriades hyalinus
Pararhophites quadratus
Dioxys pomonae
Lithurgus echinocacti
Trichothurgus herbsti
Microthurge sp.
Melitta arrogans
Macropis nuda
Promelitta alboclypeata
Exoneura bicolor
Braunsapis madecassella
Compsomelissa keiseri
Macrogalea ellioti
Ceratina calcarata
Ceratina sp.
Ceratina sp.
Ceratina sp.
Ceratina cyanea
Ceratina sp.
Xylocopa tabaniformis
Xylocopa fimbriata
Xylocopa muscaria
Xylocopa virginica
Xylocopa californica
Xylocopa iris
Xylocopa violacea
Xylocopa sp.
Xylocopa pubescens
Caenoprosopina holmbergi
Hesperapis larreae
Meganomia binghami
Oreopasites barbarae
Ammobates punctatus
Ammobates sp.
Sphecodopsis capensis
Pasites maculatus
0.4

Figure 1.7. (Continued)

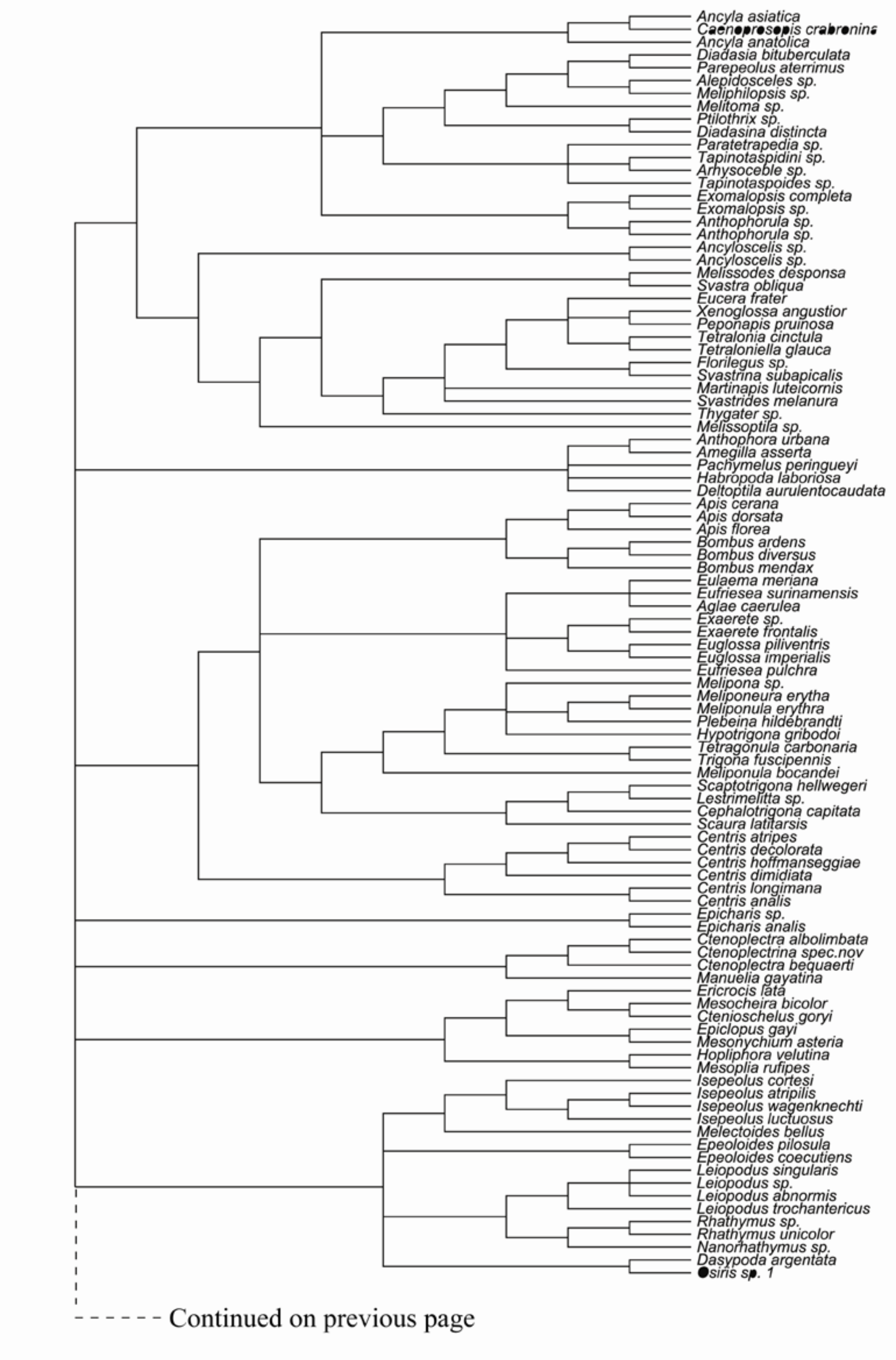
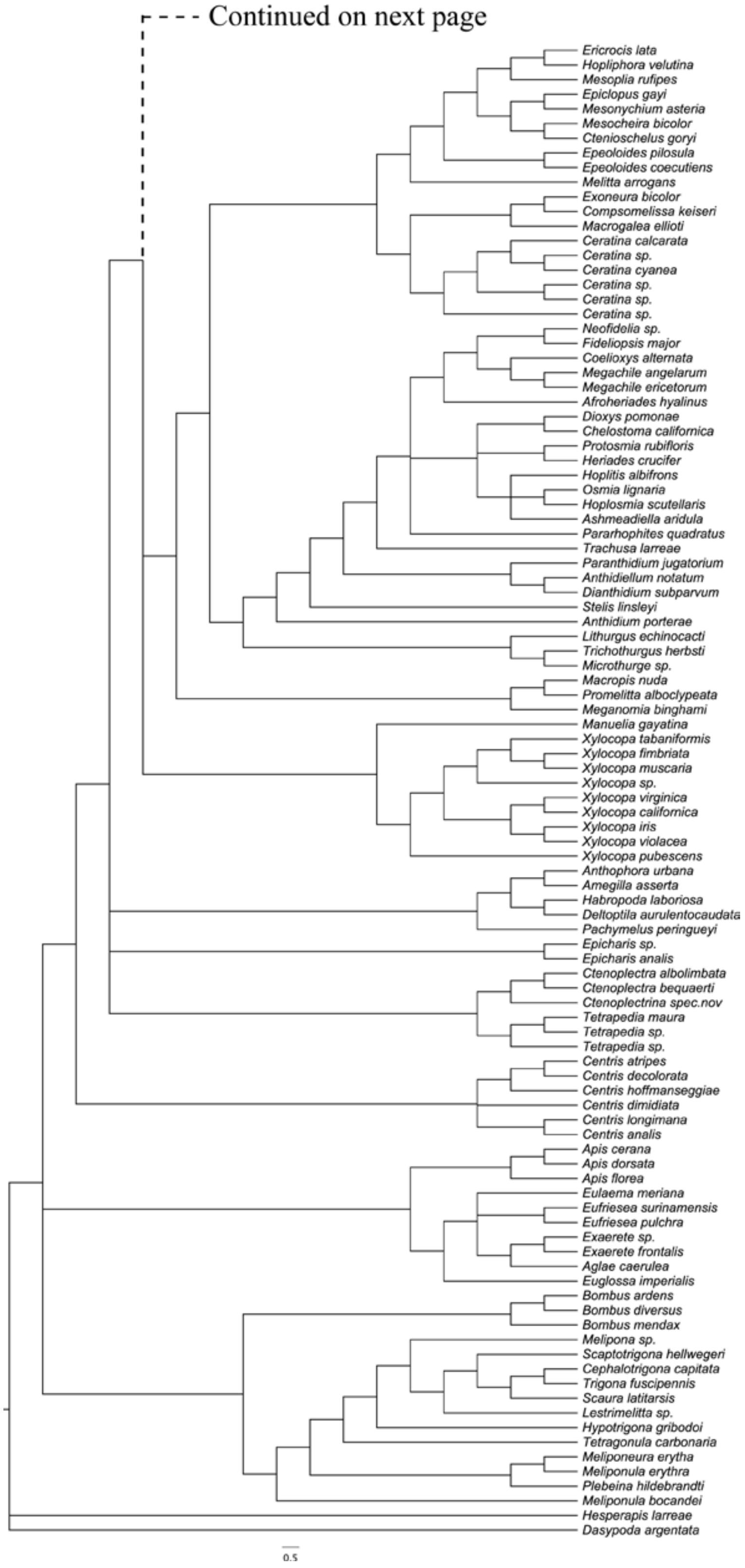


Figure 1.8. Bayesian majority rule consensus tree based on sequence data from Pol II analysed with a GTR+I+G model.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Ericrocis lata
Hopliphora velutina
Mesoplia rufipes
Epiclopus gayi
Mesonychium asteria
Mesocheira bicolor
Ctenioschelus goryi
Epeoloides pilosula
Epeoloides coecutiens
Melitta arrogans
Exoneura bicolor
Compsomelissa keiseri
Macrogalea ellioti
Ceratina calcarata
Ceratina sp.
Ceratina cyanea
Ceratina sp.
Ceratina sp.
Ceratina sp.
Neofidelia sp.
Fideliopsis major
Coelioxys alternata
Megachile angelarum
Megachile ericetorum
Afroheriades hyalinus
Dioxys pomonae
Chelostoma californica
Protosmia rubifloris
Heriades crucifer
Hoplitis albifrons
Osmia lignaria
Hoplosmia scutellaris
Ashmeadiella aridula
Pararhophites quadratus
Trachusa larreae
Paranthidium jugatorium
Anthidiellum notatum
Dianthidium subparvum
Stelis linsleyi
Anthidium porterae
Lithurgus echinocacti
Trichothurgus herbsti
Microthurge sp.
Macropis nuda
Promelitta alboclypeata
Meganomia binghami
Manuelia gayatina
Xylocopa tabaniformis
Xylocopa fimbriata
Xylocopa muscaria
Xylocopa sp.
Xylocopa virginica
Xylocopa californica
Xylocopa iris
Xylocopa violacea
Xylocopa pubescens
Anthophora urbana
Amegilla asserta
Habropoda laboriosa
Deltoptila aurulentocaudata
Pachymelus peringueyi
Epicharis sp.
Epicharis analis
Ctenoplectra albolimbata
Ctenoplectra bequaerti
Ctenoplectrina spec.nov
Tetrapedia maura
Tetrapedia sp.
Tetrapedia sp.
Centris atripes
Centris decolorata
Centris hoffmanseggiae
Centris dimidiata
Centris longimana
Centris analis
Apis cerana
Apis dorsata
Apis florea
Eulaema meriana
Eufriesea surinamensis
Eufriesea pulchra
Exaerete sp.
Exaerete frontalis
Aglae caerulea
Euglossa imperialis
Bombus ardens
Bombus diversus
Bombus mendax
Melipona sp.
Scaptotrigona hellwegeri
Cephalotrigona capitata
Trigona fuscipennis
Scaura latitarsis
Lestrimelitta sp.
Hypotrigona gribodoi
Tetragonula carbonaria
Meliponeura erytha
Meliponula erythra
Plebeina hildebrandti
Meliponula bocandei
Hesperapis larreae
Dasypoda argentata
0.5

Figure 1.8. (Continued)

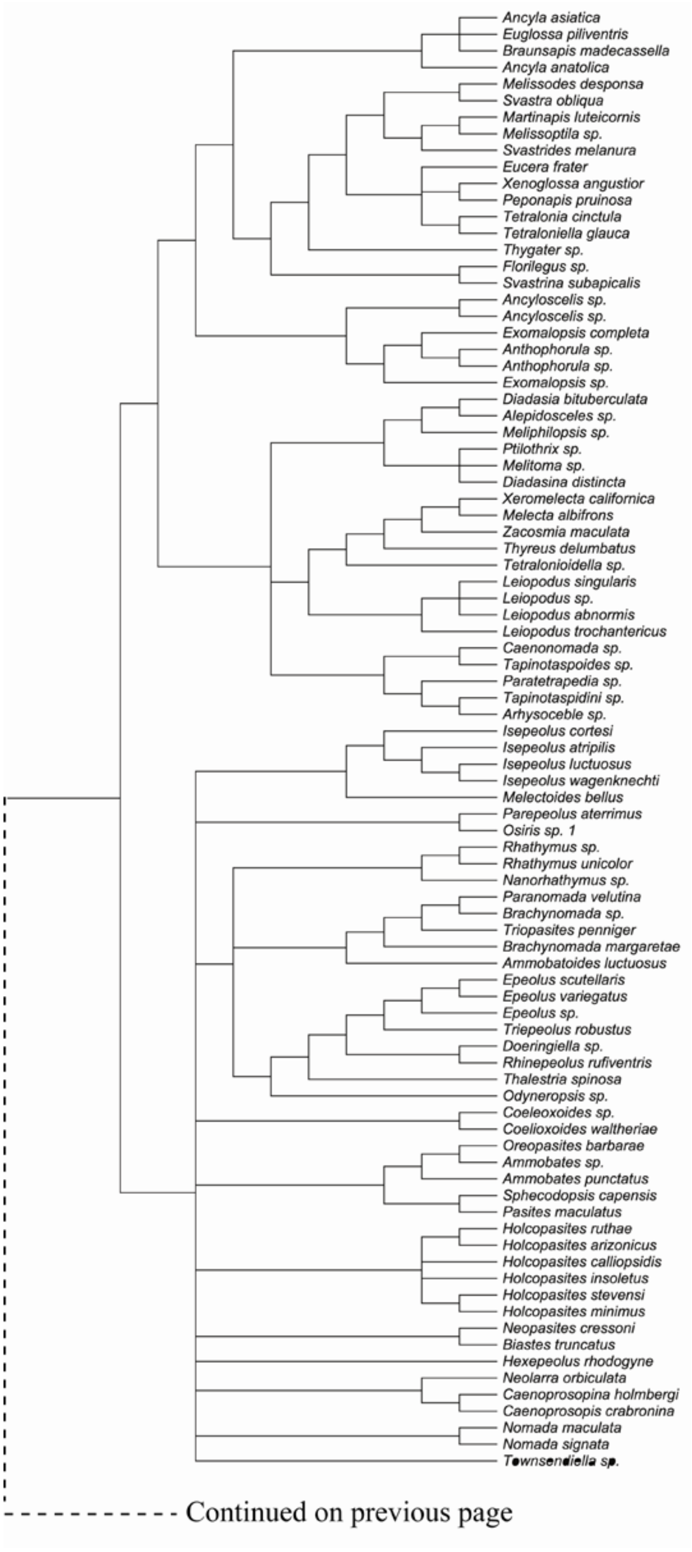
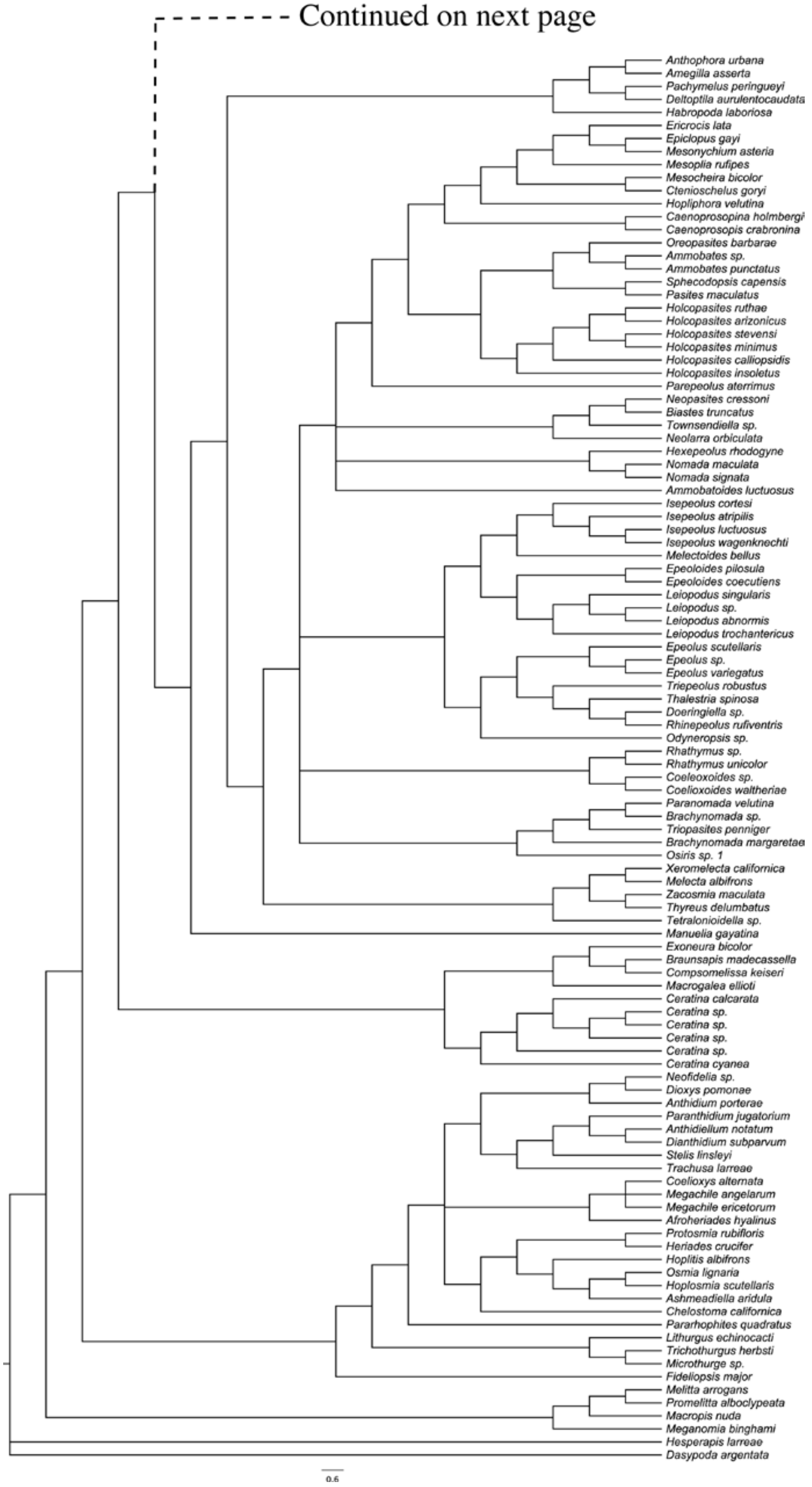


Figure 1.9. Bayesian majority rule consensus tree based on sequence data from Nak analysed with a GTR+I+G model.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Anthophora urbana
Amegilla asserta
Pachymelus peringueyi
Deltoptila aurulentocaudata
Habropoda laboriosa
Ericrocis lata
Epiclopus gayi
Mesonychium asteria
Mesoplia rufipes
Mesocheira bicolor
Ctenioschelus goryi
Hopliphora velutina
Caenoprosopina holmbergi
Caenoprosopis crabronina
Oreopasites barbarae
Ammobates sp.
Ammobates punctatus
Sphecodopsis capensis
Pasites maculatus
Holcopasites ruthae
Holcopasites arizonicus
Holcopasites stevensi
Holcopasites minimus
Holcopasites calliopsidis
Holcopasites insoletus
Parepeolus aterrimus
Neopasites cressoni
Biastes truncatus
Townsendiella sp.
Neolarra orbiculata
Hexepeolus rhodogyne
Nomada maculata
Nomada signata
Ammobatoides luctuosus
Isepeolus cortesi
Isepeolus atripilis
Isepeolus luctuosus
Isepeolus wagenknechti
Melectoides bellus
Epeoloides pilosula
Epeoloides coecutiens
Leiopodus singularis
Leiopodus sp.
Leiopodus abnormis
Leiopodus trochantericus
Epeolus scutellaris
Epeolus sp.
Epeolus variegatus
Triepeolus robustus
Thalestria spinosa
Doeringiella sp.
Rhinepeolus rufiventris
Odyneropsis sp.
Rhathymus sp.
Rhathymus unicolor
Coeleoxoides sp.
Coelioxoides waltheriae
Paranomada velutina
Brachynomada sp.
Triopasites penniger
Brachynomada margaretae
Osiris sp. 1
Xeromelecta californica
Melecta albifrons
Zacosmia maculata
Thyreus delumbatus
Tetralonioidella sp.
Manuelia gayatina
Exoneura bicolor
Braunsapis madecassella
Compsomelissa keiseri
Macrogalea ellioti
Ceratina calcarata
Ceratina sp.
Ceratina sp.
Ceratina sp.
Ceratina sp.
Ceratina cyanea
Neofidelia sp.
Dioxys pomonae
Anthidium porterae
Paranthidium jugatorium
Anthidiellum notatum
Dianthidium subparvum
Stelis linsleyi
Trachusa larreae
Coelioxys alternata
Megachile angelarum
Megachile ericetorum
Afroheriades hyalinus
Protosmia rubifloris
Heriades crucifer
Hoplitis albifrons
Osmia lignaria
Hoplosmia scutellaris
Ashmeadiella aridula
Chelostoma californica
Pararhophites quadratus
Lithurgus echinocacti
Trichothurgus herbsti
Microthurge sp.
Fideliopsis major
Melitta arrogans
Promelitta alboclypeata
Macropis nuda
Meganomia binghami
Hesperapis larreae
Dasypoda argentata
0.6

Figure 1.9. (Continued)

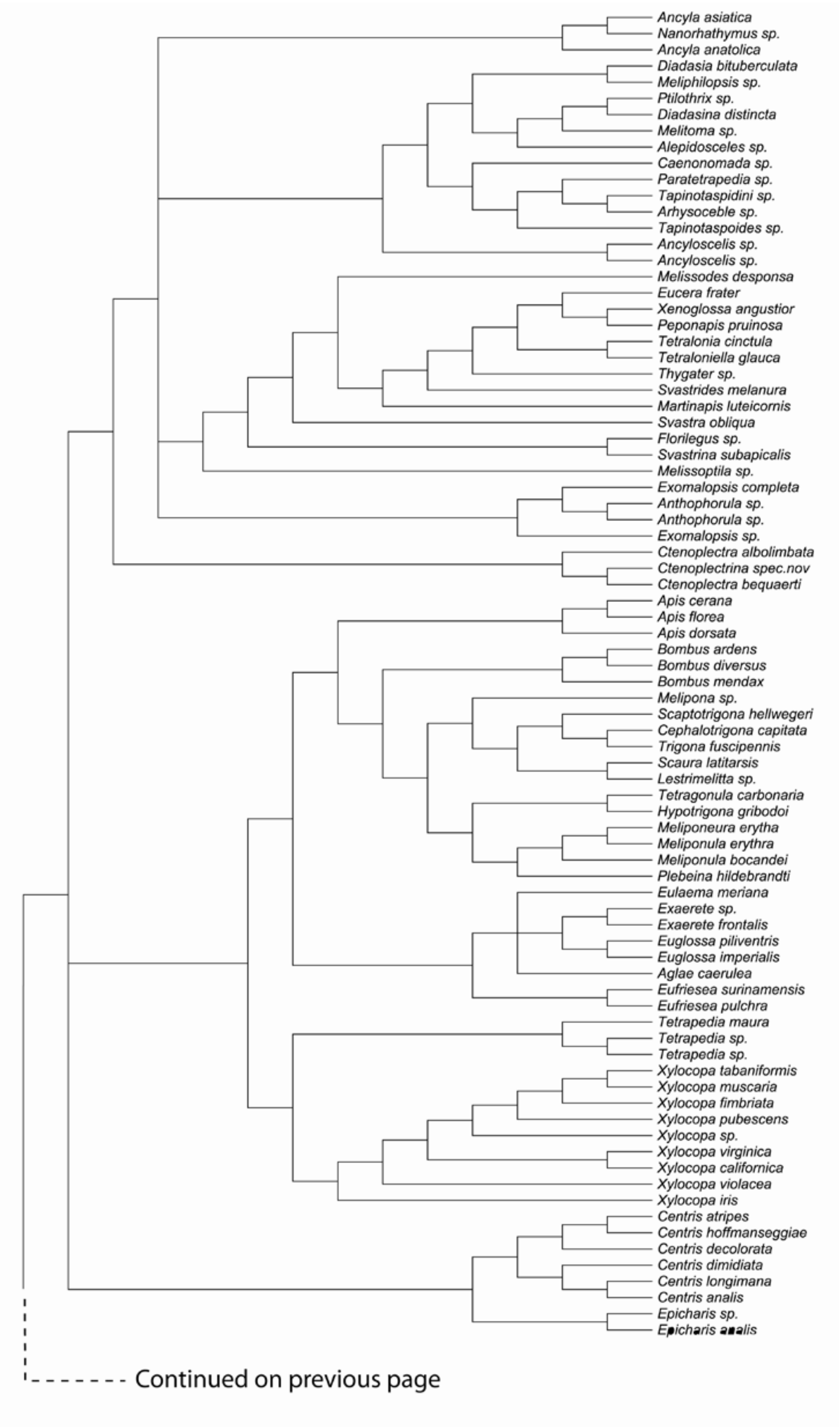
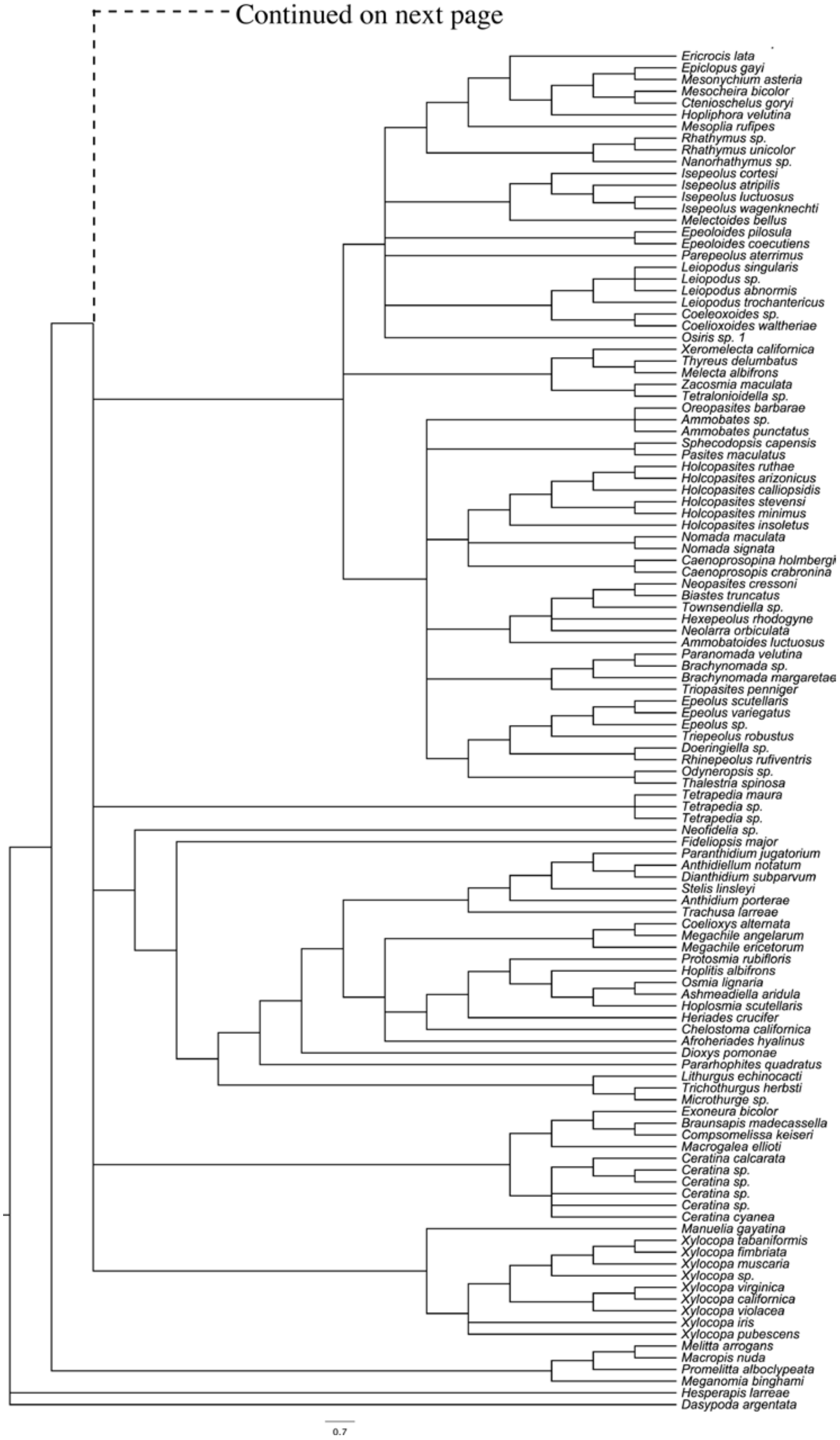


Figure 1.10. Bayesian majority rule consensus tree based on sequence data from EF1α analysed with a GTR+I+G model.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Ericrocis lata
Epiclopus gayi
Mesonychium asteria
Mesocheira bicolor
Ctenioschelus goryi
Hopliphora velutina
Mesoplia rufipes
Rhathymus sp.
Rhathymus unicolor
Nanorhathymus sp.
Isepeolus cortesi
Isepeolus atripilis
Isepeolus luctuosus
Isepeolus wagenknechti
Melectoides bellus
Epeoloides pilosula
Epeoloides coecutiens
Parepeolus aterrimus
Leiopodus singularis
Leiopodus sp.
Leiopodus abnormis
Leiopodus trochantericus
Coeleoxoides sp.
Coelioxoides waltheriae
Osiris sp. 1
Xeromelecta californica
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Tetralonioidella sp.
Oreopasites barbarae
Ammobates sp.
Ammobates punctatus
Sphecodopsis capensis
Pasites maculatus
Holcopasites ruthae
Holcopasites arizonicus
Holcopasites calliopsidis
Holcopasites stevensi
Holcopasites minimus
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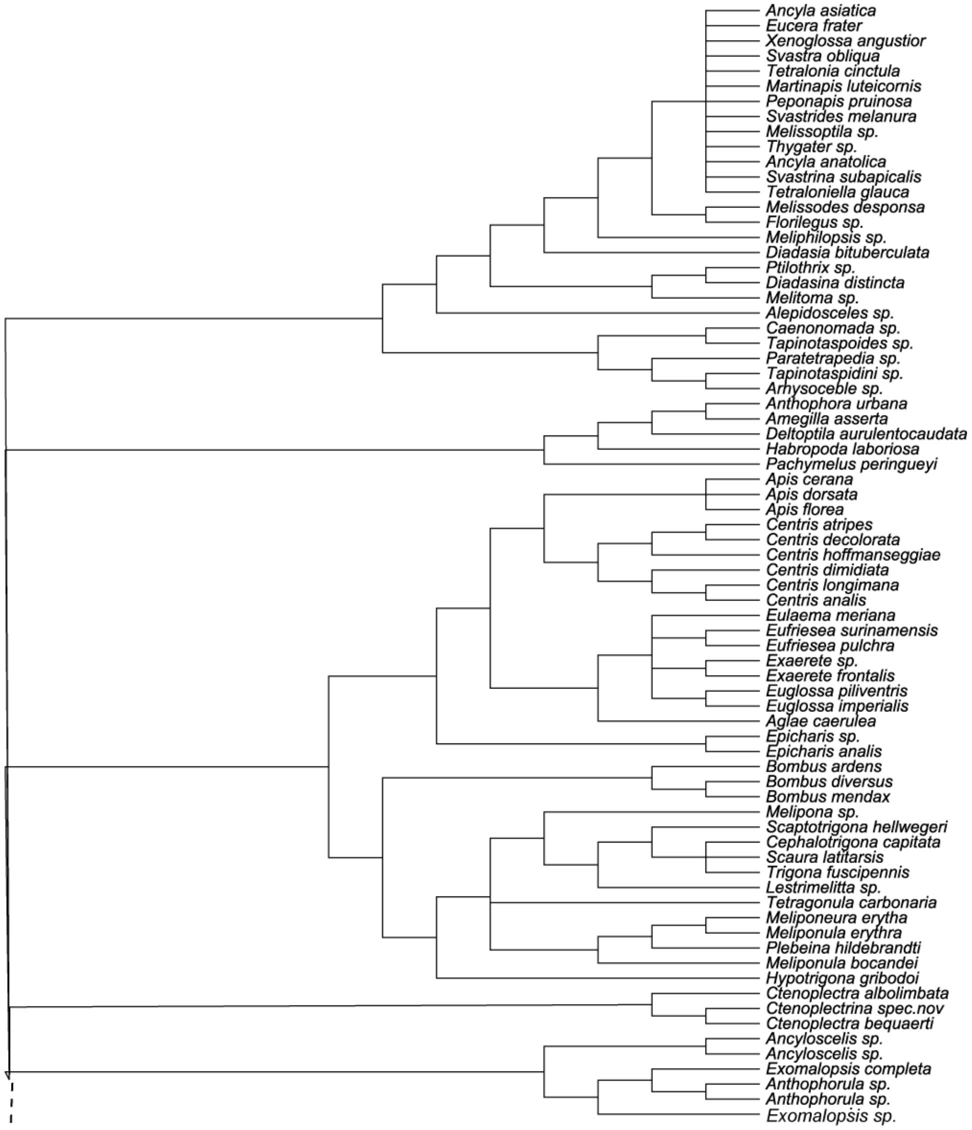
Nomada maculata
Nomada signata
Caenoprosopina holmbergi
Caenoprosopis crabronina
Neopasites cressoni
Biastes truncatus
Townsendiella sp.
Hexepeolus rhodogyne
Neolarra orbiculata
Ammobatoides luctuosus
Paranomada velutina
Brachynomada sp.
Brachynomada margaretae
Triopasites penniger
Epeolus scutellaris
Epeolus variegatus
Epeolus sp.
Triepeolus robustus
Doeringiella sp.
Rhinepeolus rufiventris
Odyneropsis sp.
Thalestria spinosa
Tetrapedia maura
Tetrapedia sp.
Tetrapedia sp.
Neofidelia sp.
Fideliopsis major
Paranthidium jugatorium
Anthidiellum notatum
Dianthidium subparvum
Stelis linsleyi
Anthidium porterae
Trachusa larreae
Coelioxys alternata
Megachile angelarum
Megachile ericetorum
Protosmia rubifloris
Hoplitis albifrons
Osmia lignaria
Ashmeadiella aridula
Hoplosmia scutellaris
Heriades crucifer
Chelostoma californica
Afroheriades hyalinus
Dioxys pomonae
Pararhophites quadratus
Lithurgus echinocacti
Trichothurgus herbsti
Microthurge sp.
Exoneura bicolor
Braunsapis madecassella
Compsomelissa keiseri
Macrogalea ellioti
Ceratina calcarata
Ceratina sp.
Ceratina sp.
Ceratina sp.
Ceratina sp.
Ceratina cyanea
Manuelia gayatina
Xylocopa tabaniformis
Xylocopa fimbriata
Xylocopa muscaria
Xylocopa sp.
Xylocopa virginica
Xylocopa californica
Xylocopa violacea
Xylocopa iris
Xylocopa pubescens
Melitta arrogans
Macropis nuda
Promelitta alboclypeata
Meganomia binghami
Hesperapis larreae
Dasypoda argentata
0.7

Figure 1.10. (Continued)



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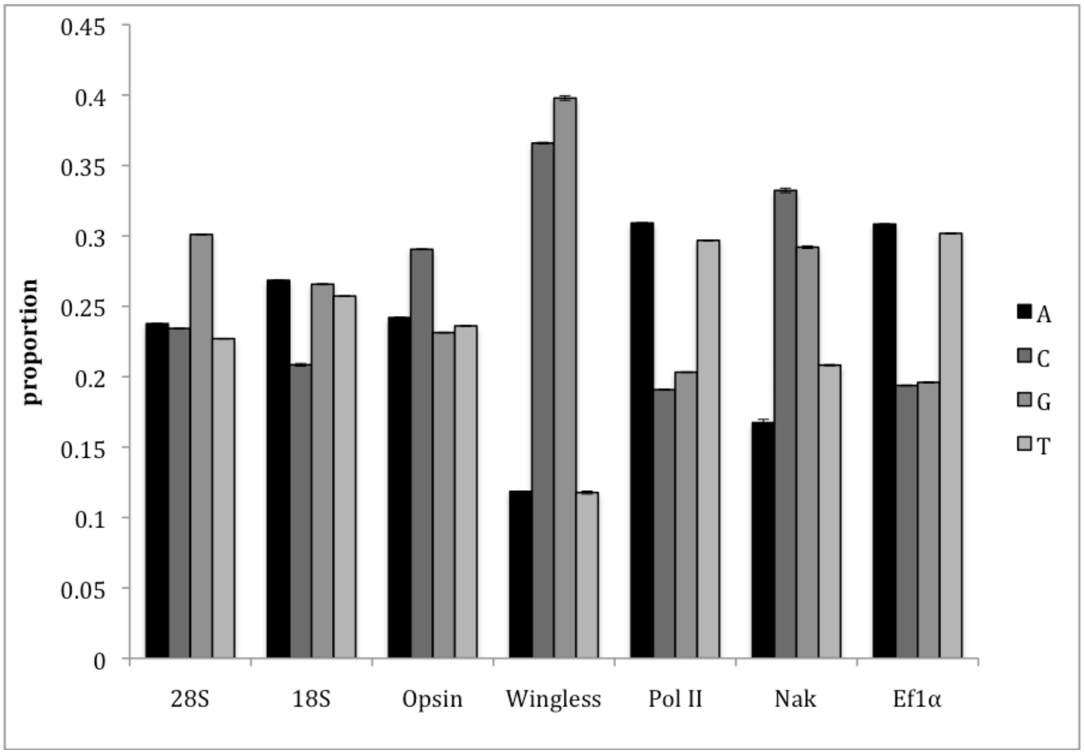


Figure 1.11. Nucleotide frequencies for each gene partition.

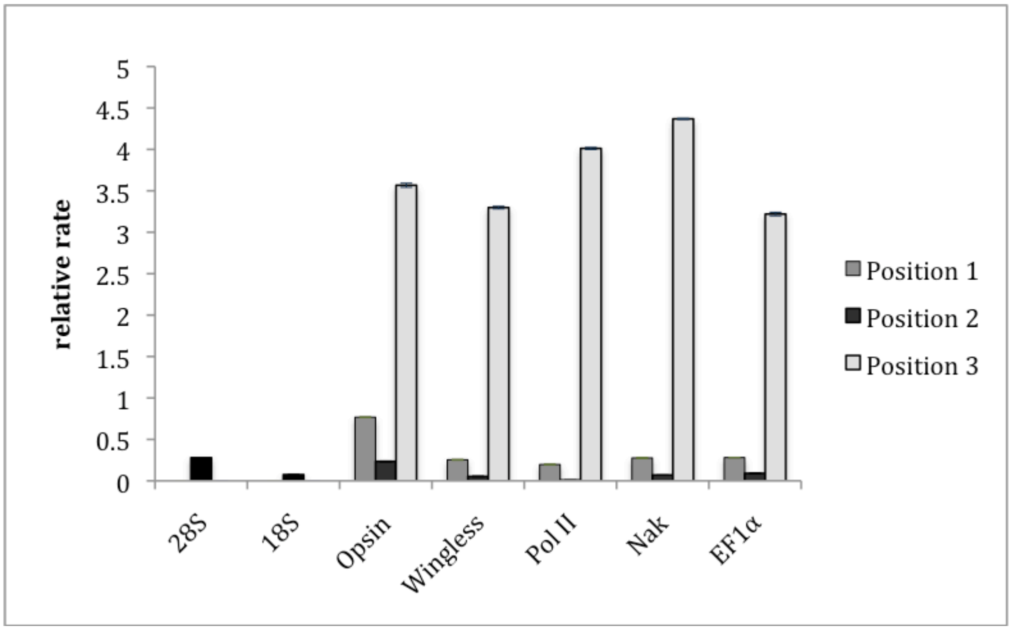


Figure 1.12. Relative rates of substitution for the ribosomal genes and for each codon position of the protein-coding genes.

The variation in substitution rates across sites within each gene locus was estimated by the alpha parameter of the gamma distribution and is shown in Figure 1.13. High alpha values correspond to homogeneous rates across sites and low alpha values indicate heterogeneity in among-site variation. The 18S locus has the lowest alpha value (i.e., the most heterogeneous pattern of among site rate variation) because most nucleotide sites have a very low substitution rate but a few sites have a high substitution rate.

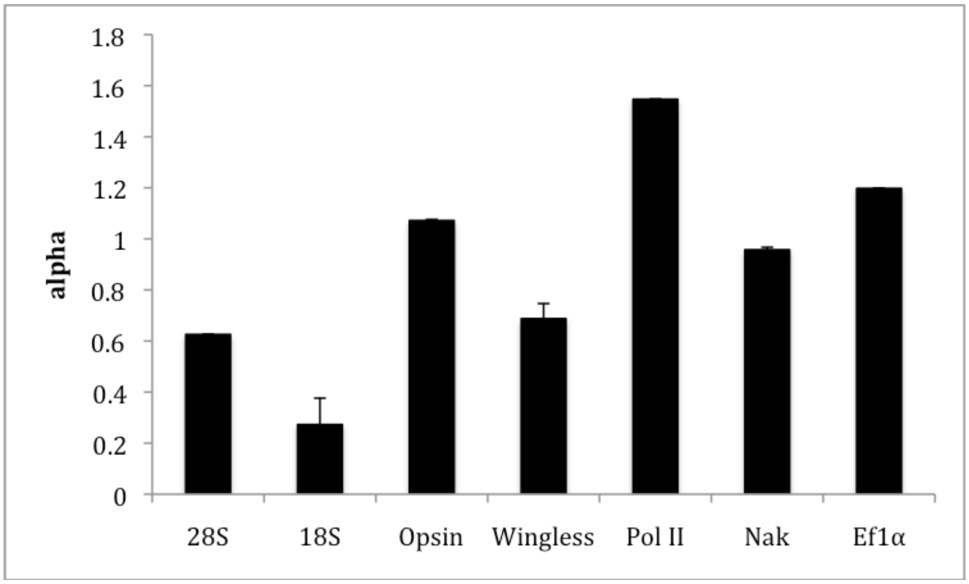


Figure 1.13. The variation in substitution rates across sites within each gene locus as estimated by the alpha parameter of the gamma distribution.

Phylogenetic analyses

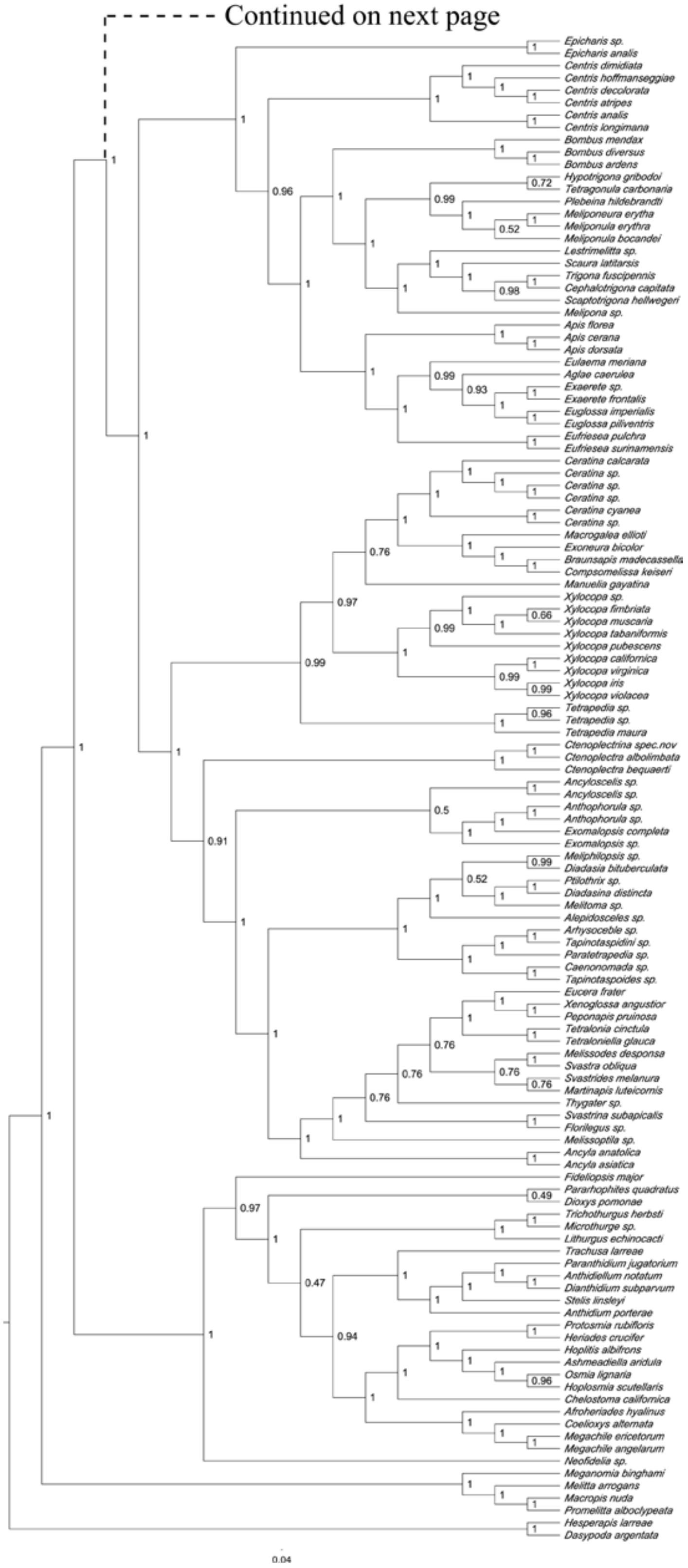
The maximum clade credibility tree constructed from the 268 560 000 post burn-in generations of the 46 independent Bayesian runs was well resolved (Figure 1.14). Figure 1.15 is a simplified version of this tree summarizing the subfamily and tribal relationships. Table 1.7 summarizes the results from each analysis. Eleven of the 46 runs did not converge on the stationary distribution. In one case, stationarity was not even reached after 23 million generation. Most analyses had a burn-in between 1 and 3 million generations. Doubling the number of chains did not decrease the number of generations needed to converge on the stationary phase.

The tree obtained with maximum likelihood (Figure 1.16) was also well resolved and nearly identical to the Bayesian tree (Figure 1.14); the only major difference being the position of Melectini. In the Bayesian tree, Melectini is sister to the large cleptoparasitic clade, whereas in the ML tree Melectini is sister to the Nomadinae.

The strict consensus tree of the 32 equally parsimonious trees of 31 744 steps (Figure 1.17) was slightly less resolved, but the topology was mostly congruent with that obtained using model-based methods. The MP tree differed by the monophyletic Anthophorini+Manuelini+Ctenoplectrini comprising the sister group to the eucerine line (minus Tetrapediini).

Bayesian posterior probabilities were higher than the ML bootstrap proportions (Figure 1.18) which in turn were higher than the bootstrap proportions found with parsimony (Figure 1.19).

Figure 1.14. Bayesian maximum clade credibility tree of the concatenated dataset (|28S,18S|pos1|pos2|pos3|) using a GTR+I+G model. Posterior probabilities for each node are shown.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Epicharis sp.
Epicharis analis
Centris dimidiata
Centris hoffmanseggiae
Centris decolorata
Centris atripes
Centris analis
Centris longimana
Bombus mendax
Bombus diversus
Bombus ardens
Hypotrigona gribodoi
Tetragonula carbonaria
Plebeina hildebrandti
Meliponeura erytha
Meliponula erythra
Meliponula bocandei
Lestrimelitta sp.
Scaura latitarsis
Trigona fuscipennis
Cephalotrigona capitata
Scaptotrigona hellwegeri
Melipona sp.
Apis florea
Apis cerana
Apis dorsata
Eulaema meriana
Aglae caerulea
Exaerete sp.
Exaerete frontalis
Euglossa imperialis
Euglossa piliventris
Eufriesea pulchra
Eufriesea surinamensis
Ceratina calcarata
Ceratina sp.
Ceratina sp.
Ceratina sp.
Ceratina cyanea
Ceratina sp.
Macrogalea ellioti
Exoneura bicolor
Braunsapis madecassella
Compsomelissa keiseri
Manuelia gayatina
Xylocopa sp.
Xylocopa fimbriata
Xylocopa muscaria
Xylocopa tabaniformis
Xylocopa pubescens
Xylocopa californica
Xylocopa virginica
Xylocopa iris
Xylocopa violacea
Tetrapedia sp.
Tetrapedia sp.
Tetrapedia maura
Ctenoplectrina spec.nov
Ctenoplectra albolimbata
Ctenoplectra bequaerti
Ancyloscelis sp.
Ancyloscelis sp.
Anthophorula sp.
Anthophorula sp.
Exomalopsis completa
Exomalopsis sp.
Meliphilopsis sp.
Diadasia bituberculata
Ptilothrix sp.
Diadasina distincta
Melitoma sp.
Alepidosceles sp.
Arhysoceble sp.
Tapinotaspidini sp.
Paratetrapedia sp.
Caenonomada sp.
Tapinotaspoides sp.
Eucera frater
Xenoglossa angustior
Peponapis pruinosa
Tetralonia cinctula
Tetraloniella glauca
Melissodes desponsa
Svastra obliqua
Svastrides melanura
Martinapis luteicornis
Thygater sp.
Svastrina subapicalis
Florilegus sp.
Melissoptila sp.
Ancyla anatolica
Ancyla asiatica
Fideliopsis major
Pararhophites quadratus
Dioxys pomonae
Trichothurgus herbsti
Microthurge sp.
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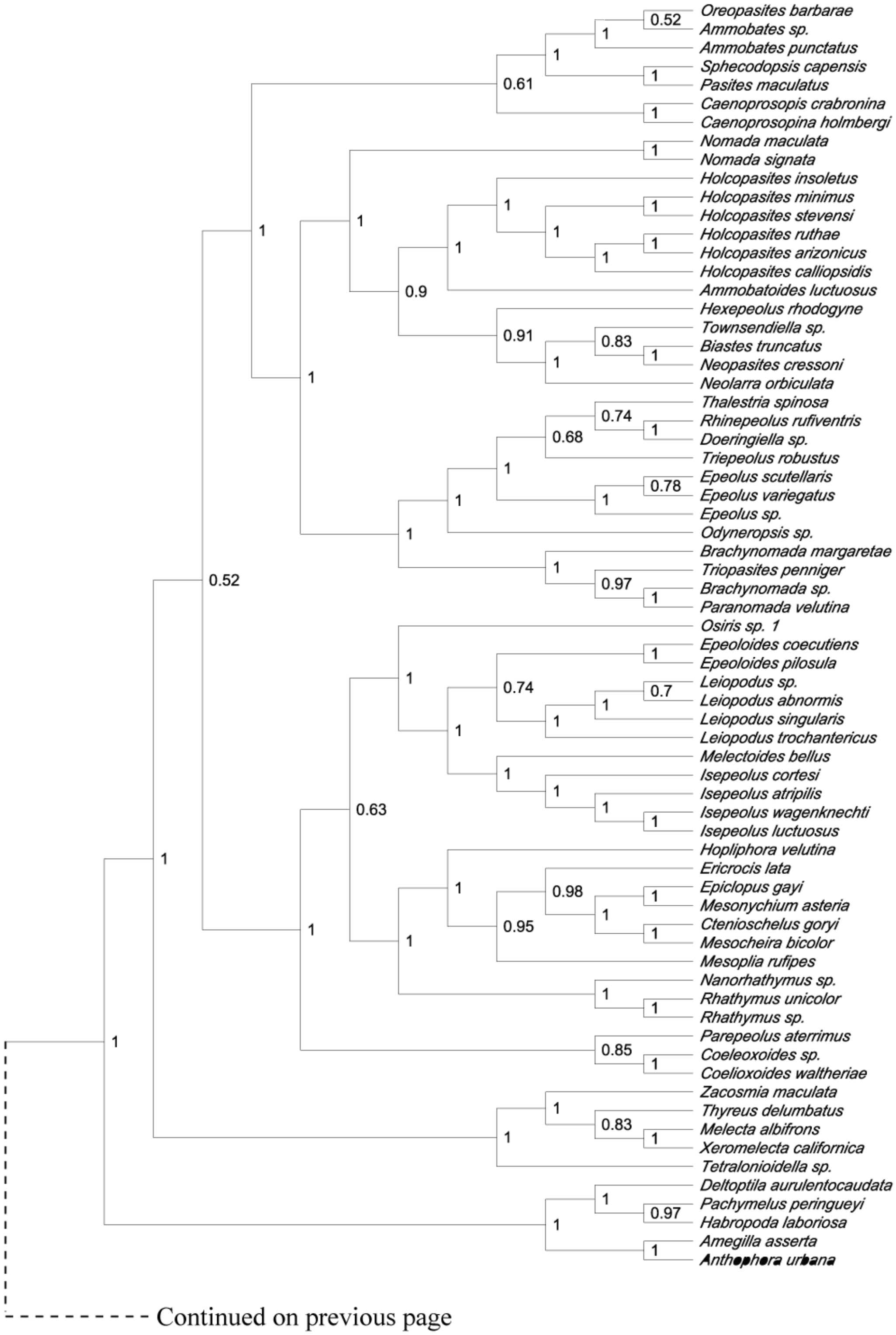
Anthidium porterae
Protosmia rubifloris
Heriades crucifer
Hoplitis albifrons
Ashmeadiella aridula
Osmia lignaria
Hoplosmia scutellaris
Chelostoma californica
Afroheriades hyalinus
Coelioxys alternata
Megachile ericetorum
Megachile angelarum
Neofidelia sp.
Meganomia binghami
Melitta arrogans
Macropis nuda
Promelitta alboclypeata
Hesperapis larreae
Dasypoda argentata
0.04

Figure 1.14. (Continued)



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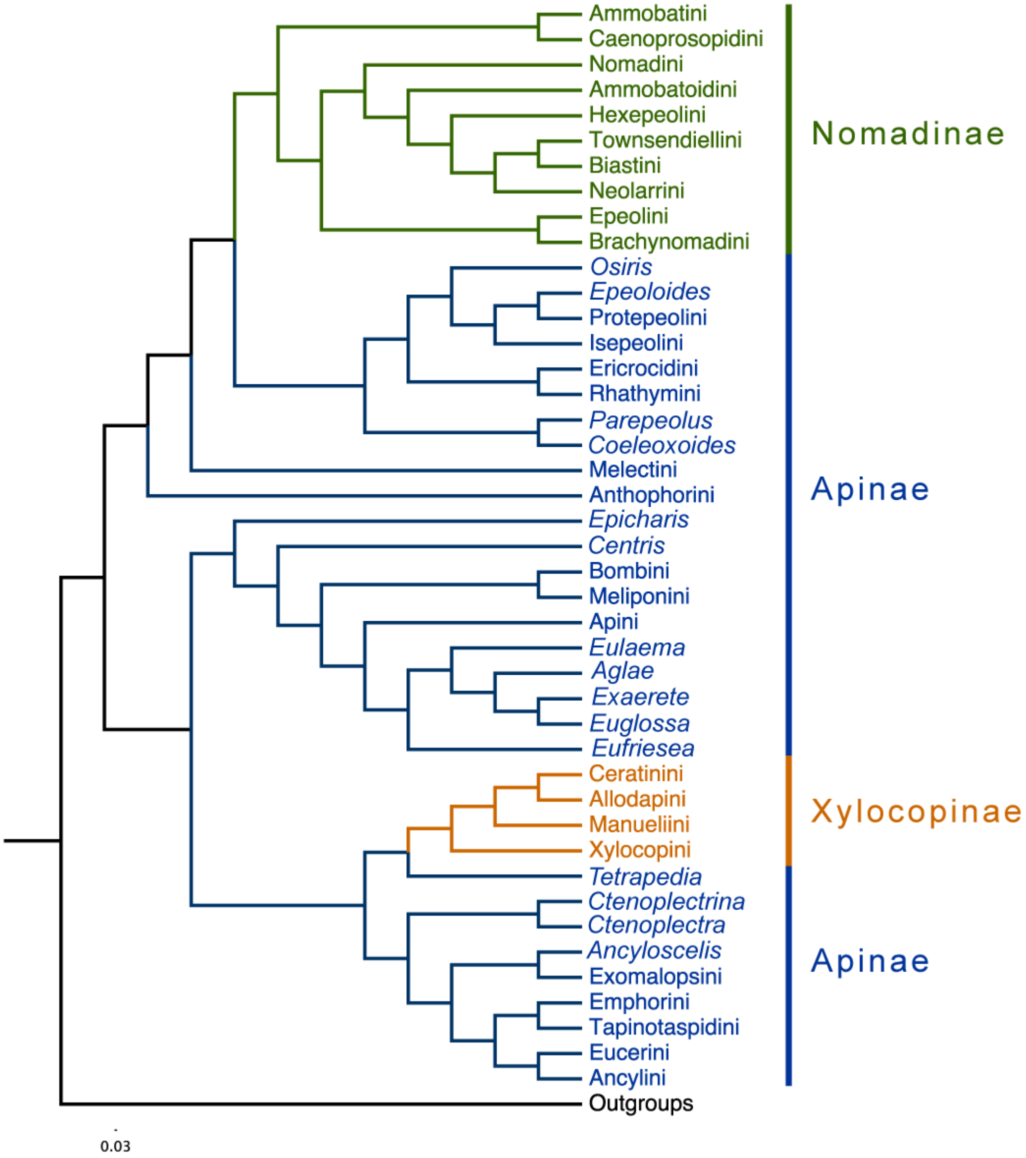
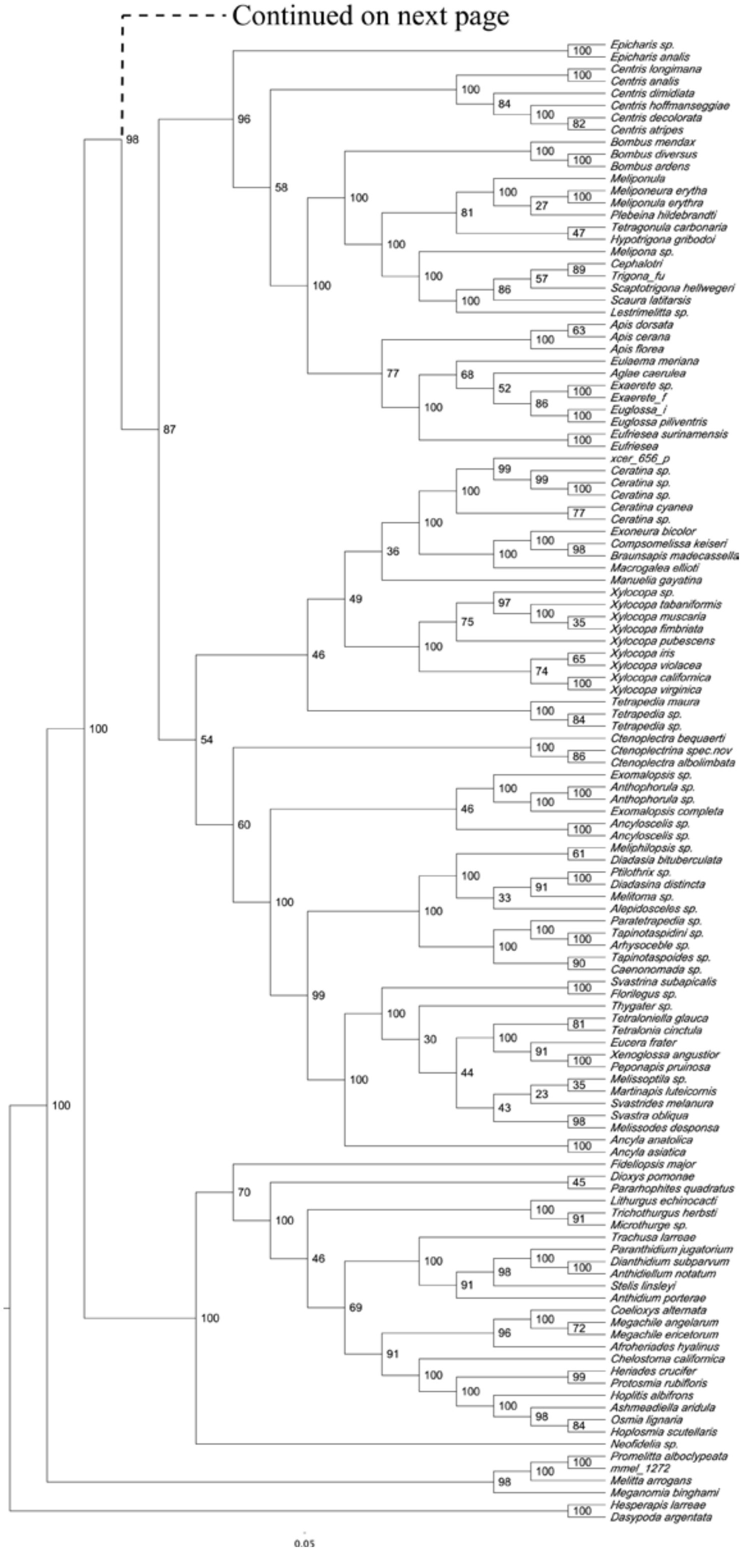


Figure 1.15. Pruned version of the Bayesian maximum clade credibility tree of the concatenated dataset (|28S,18S|pos1|pos2|pos3|) using a GTR+I+G model.

Table 1.7. Number of chains, likelihood, burn-in, number of generations, and split frequencies of the 46 independent Bayesian runs of the concatenated dataset (|28S,18S|pos1|pos2|pos3|). Runs shaded in grey did not converge on the stationary phase.

Analysis	run	#chains	likelihood	Burn-in	# generation	split freq
1	1	4	-146640.6044	na	5260000	
	2	4	-146342.8208	1700000	5260000	0.021561
2	1	4	-146344.9347	2000000	5466000	
	2	4	-146343.1271	1200000	5466000	0.010962
3	1	4	-146641.4253	na	5261000	
	2	4	-146342.1068	1700000	5261000	0.021257
4	1	4	-146341.5785	2000000	5432000	
	2	4	-146342.713	1700000	5432000	0.013767
5	1	4	-146653.961	na	5416000	
	2	4	-146640.0465	na	5416000	0.052178
6	1	4	-146345.6715	700000	4112000	
	2	4	-146346.3264	1600000	4112000	0.024822
7	1	4	-146344.6883	1500000	4096000	
	2	4	-146650.9018	na	4096000	0.030326
8	1	4	-146340.3586	1800000	5212000	
	2	4	-146640.5796	na	5212000	0.025241
9	1	4	-146344.7762	1000000	5359000	
	2	4	-146607.675	na	5359000	0.024577
10	1	4	-146344.6466	2000000	5195000	
	2	4	-146341.1059	2000000	5195000	0.015922
11	1	4	-146343.5124	2000000	5450000	
	2	4	-146344.706	2000000	5450000	0.016117
12	1	4	-146342.5527	3000000	23353000	
	2	4	-146497.9922	na	23353000	0.059348
13	1	8	-146613.8364	na	4848000	na
14	1	8	-146349.024	2100000	4999000	na
15	1	4	-146342.5218	1500000	7295000	
	2	4	-146344.0981	2000000	7295000	0.009682
16	1	4	-146346.2445	2200000	4128000	
	2	4	-146345.4288	1800000	4128000	0.011741
17	1	4	-146345.481	1500000	21704000	
	2	4	-146346.7403	1700000	21704000	0.017492
18	1	4	-146342.6722	2500000	21998000	
	2	4	-146343.6936	2000000	21998000	0.00592
19	1	4	-146344.0291	2000000	22242000	
	2	4	-146344.9236	2000000	22242000	0.007474
20	1	4	-146343.0947	2000000	22126000	
	2	4	-146342.6195	2000000	22126000	0.003379
21	1	4	-146653.5502	na	5197000	
	2	4	-146347.4811	2000000	5197000	0.029127
22	1	4	-146637.3141	na	5179000	
	2	4	-146348.9153	2000000	5179000	0.044708
23	1	4	-146348.1456	1700000	4099000	
	2	4	-146342.645	2500000	4099000	0.012565
24	1	4	-146339.7711	1400000	5225000	
	2	4	-146342.3162	1500000	5225000	0.010817

Figure 1.16. ML tree of the concatenated (|28S,18S|pos1|pos2|pos3|) using a GTR+I+G model with bootstrap support values over 50% drawn in front of the nodes.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Epicharis sp.
Epicharis analis
Centris longimana
Centris analis
Centris dimidiata
Centris hoffmanseggiae
Centris decolorata
Centris atripes
Bombus mendax
Bombus diversus
Bombus ardens
Meliponula
Meliponeura erytha
Meliponula erythra
Plebeina hildebrandti
Tetragonula carbonaria
Hypotrigona gribodoi
Melipona sp.
Cephalotri
Trigona_fu
Scaptotrigona hellwegeri
Scaura latitarsis
Lestrimelitta sp.
Apis dorsata
Apis cerana
Apis florea
Eulaema meriana
Aglae caerulea
Exaerete sp.
Exaerete_f
Euglossa_i
Euglossa piliventris
Eufriesea surinamensis
Eufriesea
xcer_656_p
Ceratina sp.
Ceratina sp.
Ceratina sp.
Ceratina cyanea
Ceratina sp.
Exoneura bicolor
Compsomelissa keiseri
Braunsapis madecassella
Macrogalea ellioti
Manuelia gayatina
Xylocopa sp.
Xylocopa tabaniformis
Xylocopa muscaria
Xylocopa fimbriata
Xylocopa pubescens
Xylocopa iris
Xylocopa violacea
Xylocopa californica
Xylocopa virginica
Tetrapedia maura
Tetrapedia sp.
Tetrapedia sp.
Ctenoplectra bequaerti
Ctenoplectrina spec.nov
Ctenoplectra albolimbata
Exomalopsis sp.
Anthophorula sp.
Anthophorula sp.
Exomalopsis completa
Ancyloscelis sp.
Ancyloscelis sp.
Meliphilopsis sp.
Diadasia bituberculata
Ptilothrix sp.
Diadasina distincta
Melitoma sp.
Alepidosceles sp.
Paratetrapedia sp.
Tapinotaspidini sp.
Arhysoceble sp.
Tapinotaspoides sp.
Caenonomada sp.
Svastrina subapicalis
Florilegus sp.
Thygater sp.
Tetraloniella glauca
Tetralonia cinctula
Eucera frater
Xenoglossa angustior
Peponapis pruinosa
Melissoptila sp.
Martinapis luteicornis
Svastrides melanura
Svastra obliqua
Melissodes desponsa
Ancyla anatolica
Ancyla asiatica
Fideliopsis major
Dioxys pomonae
Pararhophites quadratus
Lithurgus echinocacti
Trichothurgus herbsti
Microthurge sp.
Trachusa larreae
Paranthidium jugatorium
Dianthidium subparvum
Anthidiellum notatum
Stelis linsleyi
Anthidium porterae
Coelioxys alternata
Megachile angelarum
Megachile ericetorum
Afroheriades hyalinus
Chelostoma californica
Heriades crucifer
Protosmia rubifloris
Hoplitis albifrons
Ashmeadiella aridula
Osmia lignaria
Hoplosmia scutellaris
Neofidelia sp.
Promelitta alboclypeata
mmel_1272
Melitta arrogans
Meganomia binghami
Hesperapis larreae
Dasypoda argentata
0.05

Figure 1.16. (Continued)

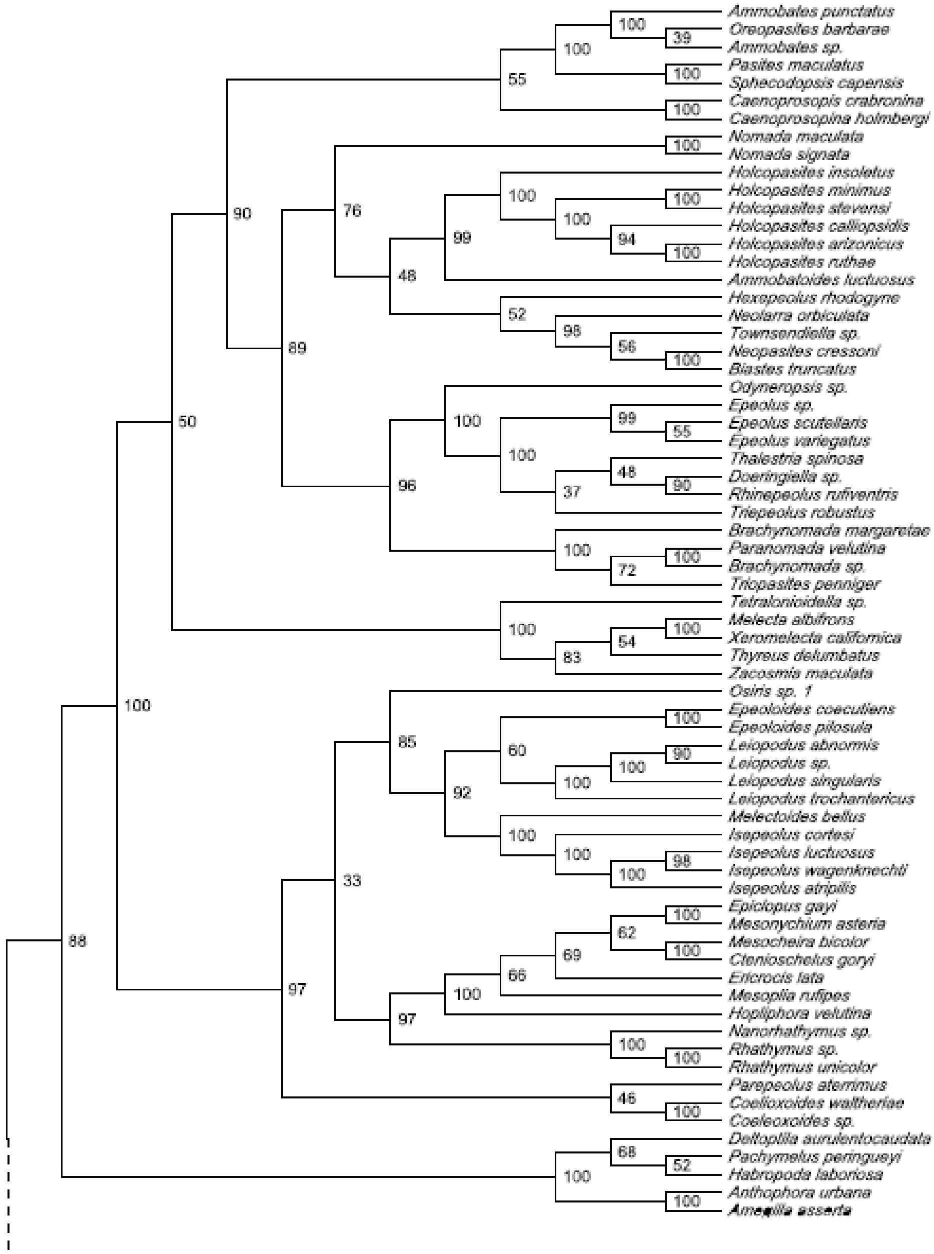

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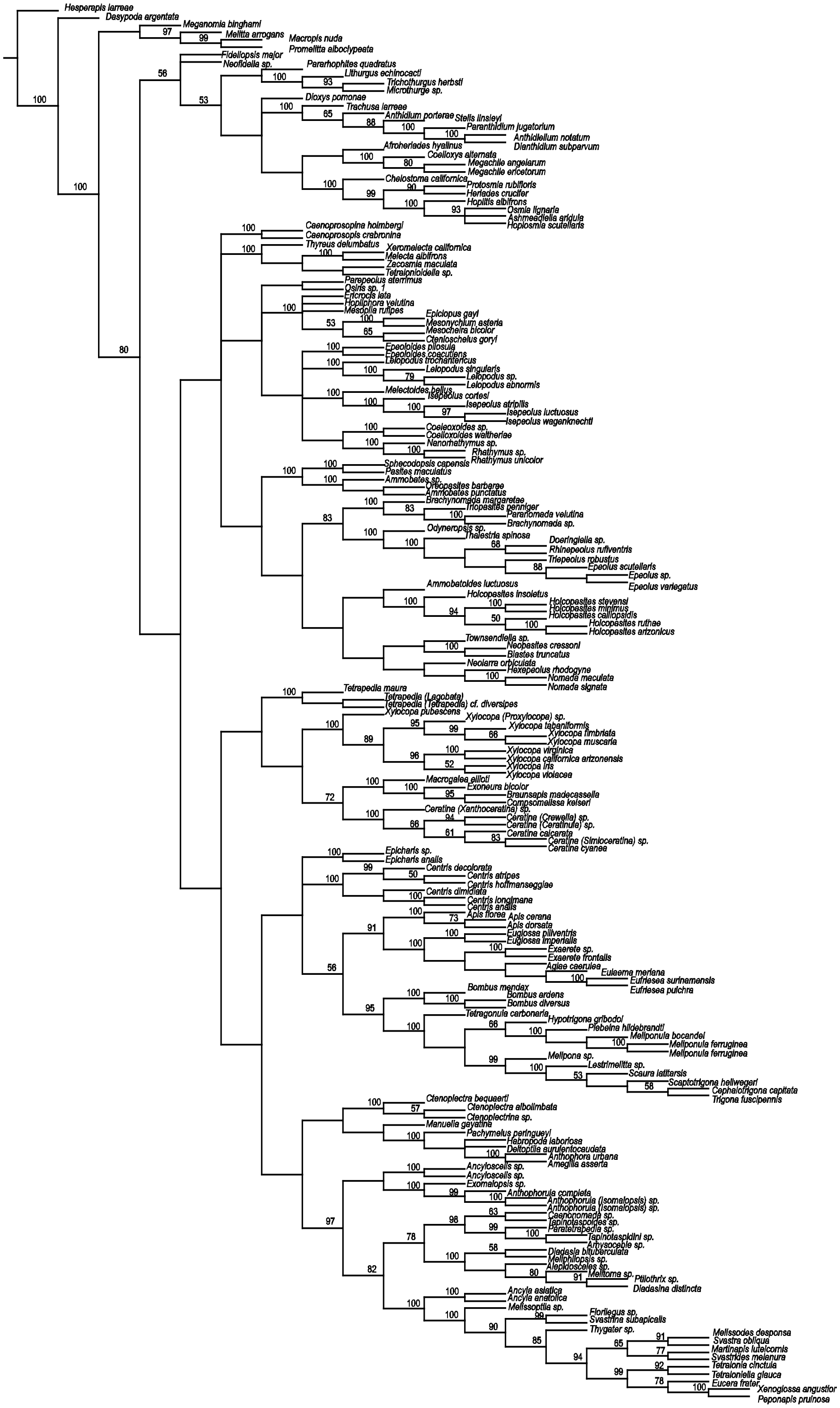
Figure 1.17. Stict concensus of 32 equally parsimonious trees of the concatenated dataset. Bootstrap support values above 50% are drawn above branches.


Hesperapis larreae
Desypoda argentata
Meganomia binghami
Melitta arrogans
Macropis nuda
Promelitta alboclypeata
Fidelliopsis major
Neofidelia sp.
Pararhophites quadratus
Lithurgus echinocacti
Trichothurgus herbsti
Microthurge sp.
Dioxys pomonae
Trachusa larreae
Anthidium porterae
Stelis linsleyi
Paranthidium jugatorium
Anthidiellum notatum
Dianthidium subparvum
Afroheriades hyalinus
Coelioxys alternata
Megachile angelarum
Megachile ericetorum
Chelostoma californica
Protosmia rubifloris
Heriades crucifer
Hoplitis albifrons
Osmia lignaria
Ashmeadiella aridula
Hoplosmia scutellaris
Caenoprosopina holmbergi
Caenoprosopis crabronina
Thyreus delumbatus
Xeromelecta californica
Melecta albifrons
Zacosmia maculata
Tetralonioidella sp.
Parepeolus aterrimus
Osiris sp. 1
Ericrocis lata
Hopliphora velutina
Mesoplia rufipes
Epiclopus gayi
Mesonychium asteria
Mesocheira bicolor
Ctenioschelus goryi
Epeoloides pilosula
Epeoloides coecutiens
Leiopodus trochantericus
Leiopodus singularis
Leiopodus sp.
Leiopodus abnormis
Melectoides bellus
Isepeolus cortesi
Isepeolus atripilis
Isepeolus luctuosus
Isepeolus wagenknechti
Coeleoxoides sp.
Coelioxoides waltheriae
Nanorhathymus sp.
Rhathymus sp.
Rhathymus unicolor
Sphecodopsis capensis
Pasites maculatus
Ammobates sp.
Oreopasites barbarae
Ammobates punctatus
Brachynomada margaretae
Triopasites penniger
Paranomada velutina
Brachynomada sp.
Odyneropsis sp.
Thalestria spinosa
Doeringiella sp.
Rhinepeolus rufiventris
Triepeolus robustus
Epeolus scutellaris
Epeolus sp.
Epeolus variegatus
Ammobatoides luctuosus
Holcopasites insoletus
Holcopasites stevensi
Holcopasites minimus
Holcopasites calliopsidis
Holcopasites ruthae
Holcopasites arizonicus
Townsendiella sp.
Neopasites cressoni
Biastes truncatus
Neolarra orbiculata
Hexepeolus rhodogyne
Nomada maculata
Nomada signata
Tetrapedia maura
Tetrapedia (Lagobata)
Tetrapedia (Tetrapedia) cf. diversipes
Xylocopa pubescens
Xylocopa (Proxylocopa) sp.
Xylocopa tabaniformis
Xylocopa fimbriata
Xylocopa muscaria
Xylocopa virginica
Xylocopa californica arizonensis
Xylocopa iris
Xylocopa violacea
Macrogalea ellioti
Exoneura bicolor
Braunsapis madecassella
Compsomelissa keiseri
Ceratina (Xanthoceratina) sp.
Ceratina (Crewella) sp.
Ceratina (Ceratinula) sp.
Ceratina calcarata
Ceratina (Simioceratina) sp.
Ceratina cyanea
Epicharis sp.
Epicharis analis
Centris decolorata
Centris atripes
Centris hoffmanseggiae
Centris dimidiata
Centris longimana
Centris analis
Apis florea
Apis cerana
Apis dorsata
Euglossa piliventris
Euglossa imperialis
Exaerete sp.
Exaerete frontalis
Aglae caerulea
Eulaema meriana
Eufriesea surinamensis
Eufriesea pulchra
Bombus mendax
Bombus ardens
Bombus diversus
Tetragonula carbonaria
Hypotrigona gribodoi
Plebeina hildebrandti
Meliponula bocandei
Meliponula ferruginea
Meliponula ferruginea
Melipona sp.
Lestrimelitta sp.
Scaura latitarsis
Scaptotrigona hellwegeri
Cephalotrigona capitata
Trigona fuscipennis
Ctenoplectra bequaerti
Ctenoplectra albolimbata
Ctenoplectrina sp.
Manuelia gayatina
Pachymelus peringueyi
Habropoda laboriosa
Deltoptila aurulentocaudata
Anthophora urbana
Amegilla asserta
Ancyloscelis sp.
Ancyloscelis sp.
Exomalopsis sp.
Anthophorula completa
Anthophorula (Isomalopsis) sp.
Anthophorula (Isomalopsis) sp.
Caenonomada sp.
Tapinotaspoides sp.
Paratetrapedia sp.
Tapinotaspidini sp.
Arhysoceble sp.
Diadasia bituberculata
Meliphilopsis sp.
Alepidosceles sp.
Melitoma sp.
Ptilothrix sp.
Diadasina distincta
Ancyla asiatica
Ancyla anatolica
Melissoptila sp.
Florilegus sp.
Svastrina subapicalis
Thygater sp.
Melissodes desponsa
Svastra obliqua
Martinapis luteicornis
Svastrides melanura
Tetralonia cinctula
Tetraloniella glauca
Eucera frater
Xenoglossa angustior
Peponapis pruinosa

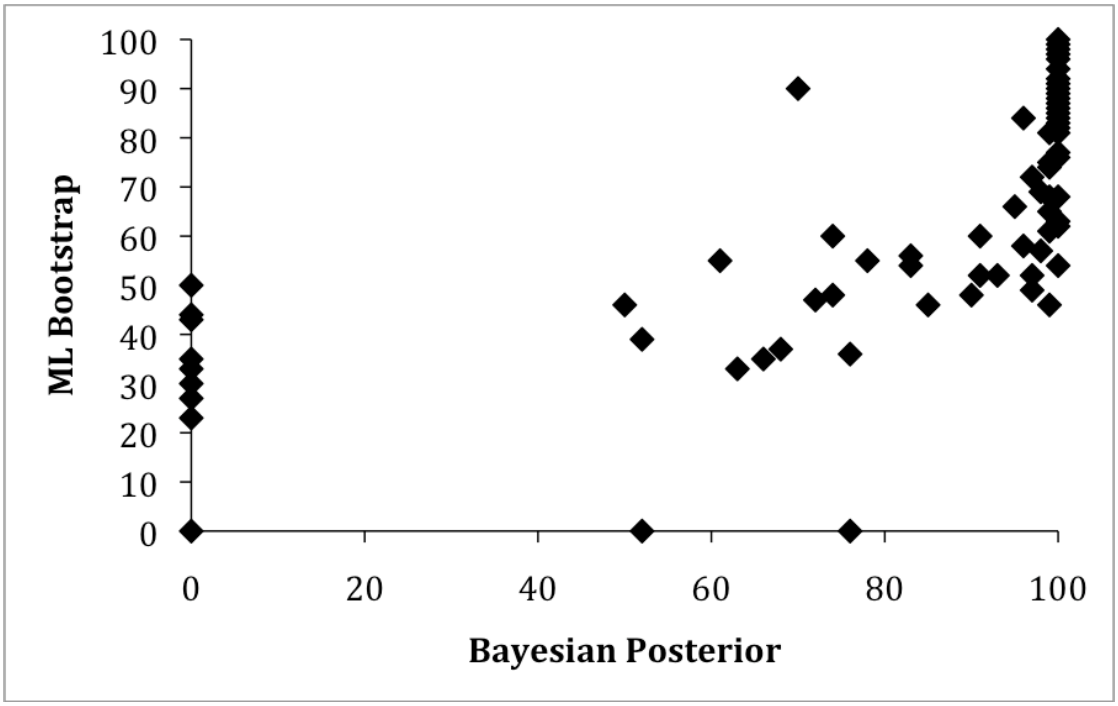


Figure 1.18. Relationship between the ML bootstrap values and the Bayesian posterior probabilities for every node.

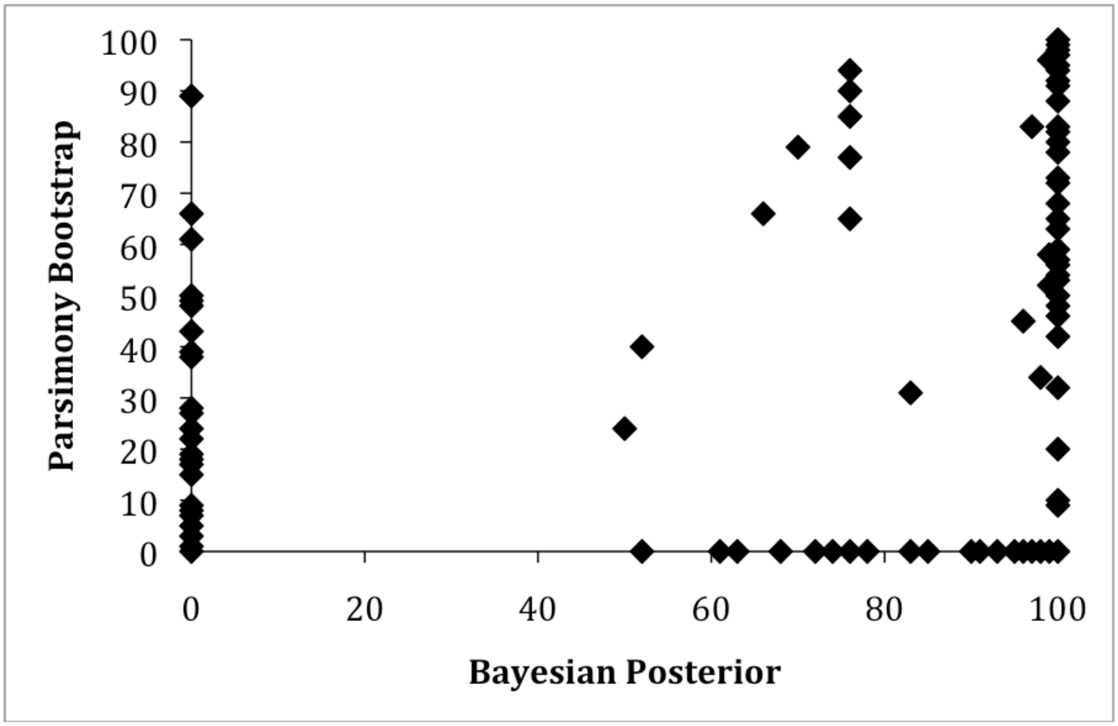


Figure 1.19. Relationship between the MP bootstrap values and the Bayesian posterior probabilities for every node.

None of the phylogenetic analyses based on individual genes were able to recover all of the groups found in the Bayesian tree based on all 7 genes combined. Nak recovered the most nodes in common with the combined tree, and is therefore considered to have the highest phylogenetic signal based on the CTI. The other protein-coding genes were similar to one another and only slightly worse than Nak. 28S recovered less “correct” nodes then the protein-coding genes, but still performed relatively well. 18S however recovered significantly less nodes then all other gene partitions. The phylogenetic signal of each gene as determined by the CTI is shown in Figure 1.20.

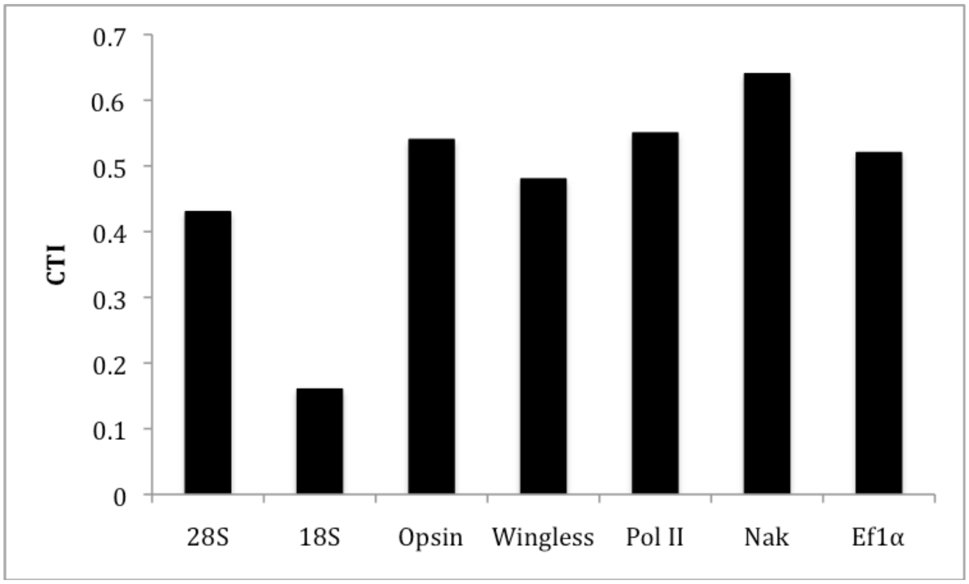


Figure 1.20. Congruent topological information (CTI) for each gene partition.

Phylogenetic relationships

Monophyly of Apidae was recovered by all three analytical methods although with very low nodal support in the parsimony analysis. The subfamily Nomadinae was recovered with high support in the Bayesian and ML analyses, but did not include the tribe Caenoprosopidini in the MP tree. In MP, Caenoprosopidini formed an unresolved polytomy with Nomadinae and most of the cleptoparasitic Apinae. The subfamily Xylocopinae was recovered with high support in the Bayesian tree and low bootstrap support in the ML tree, but the tribe Manueliini was not placed there in the MP tree. The Nomadinae and the Xylocopinae rendered the subfamily Apinae paraphyletic (see below).

Most tribes were found to be monophyletic with variable levels of support except for Emphorini, Tetrapediini, Centridini, and Osirini. The genus *Ancyloscelis* (Emphorinri) was found to be sister to the Exomalopsini. The cleptoparasitic genus *Coelioxoides* (Tetrapediini) did not group with its tribal members of the genus *Tetrapedia*, but instead was part of the large cleptoparasitic clade. The two genera of Centridini did not form a monophyletic group, *Centris* was sister to the corbiculate bees and *Epicharis* was sister to *Centris* + corbiculates in the Bayesian and ML trees. In the parsimony tree, the position of *Epicharis* was unresolved, therefore allowing for the possibility of a monophyletic Centridini sister to the corbiculates. The three represented genera of Osirini did not form a monophyletic group, but all three were part of the cleptoparasitic clade.

The most unexpected result of the phylogeny was the formation of the large cleptoparasitic clade comprised of the subfamily Nomadinae and most of the cleptoparasitic Apinae, with the exception of the cleptoparasitic orchid bees and *Ctenoplectrina*. We will refer to this clade as the “clepto clade”. The clepto clade has a posterior probability of 100, ML bootstrap proportion of 98 and parsimony bootstrap proportion of 42. Within the clepto clade, the position of Melectini is unclear. The Bayesian topology places it as sister to the remaining members of the clepto clade whereas the ML topology places it as sister to the Nomadinae. In the parsimony topology, it is part of an unresolved polytomy at the base of the clepto clade.

Evolution of cleptoparasitism

It is equally parsimonious to hypothesize four independent origins of cleptoparasitism in the Apidae or three independent origins with one reversal to nest-making (Figure 1.21). Using parsimony to reconstruct the evolutionary history of cleptoparasitism in Apidae, it is clear that cleptoparasitism evolved once in the common ancestor of the clepto clade, and once in the common ancestor of *Ctenoplectrina.* It is unclear, however, if cleptoparasitsm evolved once in the common ancestor of *Exaerete, Algae,* and *Euglossa,* with a reversal to nest-making in the *Euglossa*, or if it evolved twice independently in *Exaerete* and *Aglae.* Incorporating branch length information, transition rates, and uncertainty in tree topology into the ancestral state reconstruction of cleptoparasitism using ML, supports 4 independent origins with no reversals. The rate of transition from cleptoparasitism to nest-making was found to be 0 in the ML analysis. Results from the ancestral state reconstruction using Bayesian methods also supports the hypothesis of 4 independent origins with no reversals (Figure 1.22). In this analysis, the rate of transition from nest-making to cleptoparasitic was 1.31 ± 0.22, and from cleptoparasitic to nest-making was 0.05 ± 0.05. The common ancestor of *Exaerete, Algae,* and *Euglossa* is reconstructed as being nest-making with a probability of 0.95. The common ancestor of the clepto clade, and that of *Ctenoplectrina* are reconstructed as being cleptoparasitic with a probability of 1.0.

Figure 1.21. Pruned version of the Bayesian maximum clade credibility tree of the concatenated dataset (|28S,18S|pos1|pos2|pos3|) using a GTR+I+G model. Alternative equally parsimonious reconstructions of cleptoparasitism are mapped onto the tree. Cleptoparasitic taxa are shown in red and nest-making taxa in black. The main tree shows the hypothesis of 4 independent origins of cleptoparasitism, whereas the inset shows the reconstruction within orchid bees when 3 independent origins with one reversal back to nest-making are hypothesized.

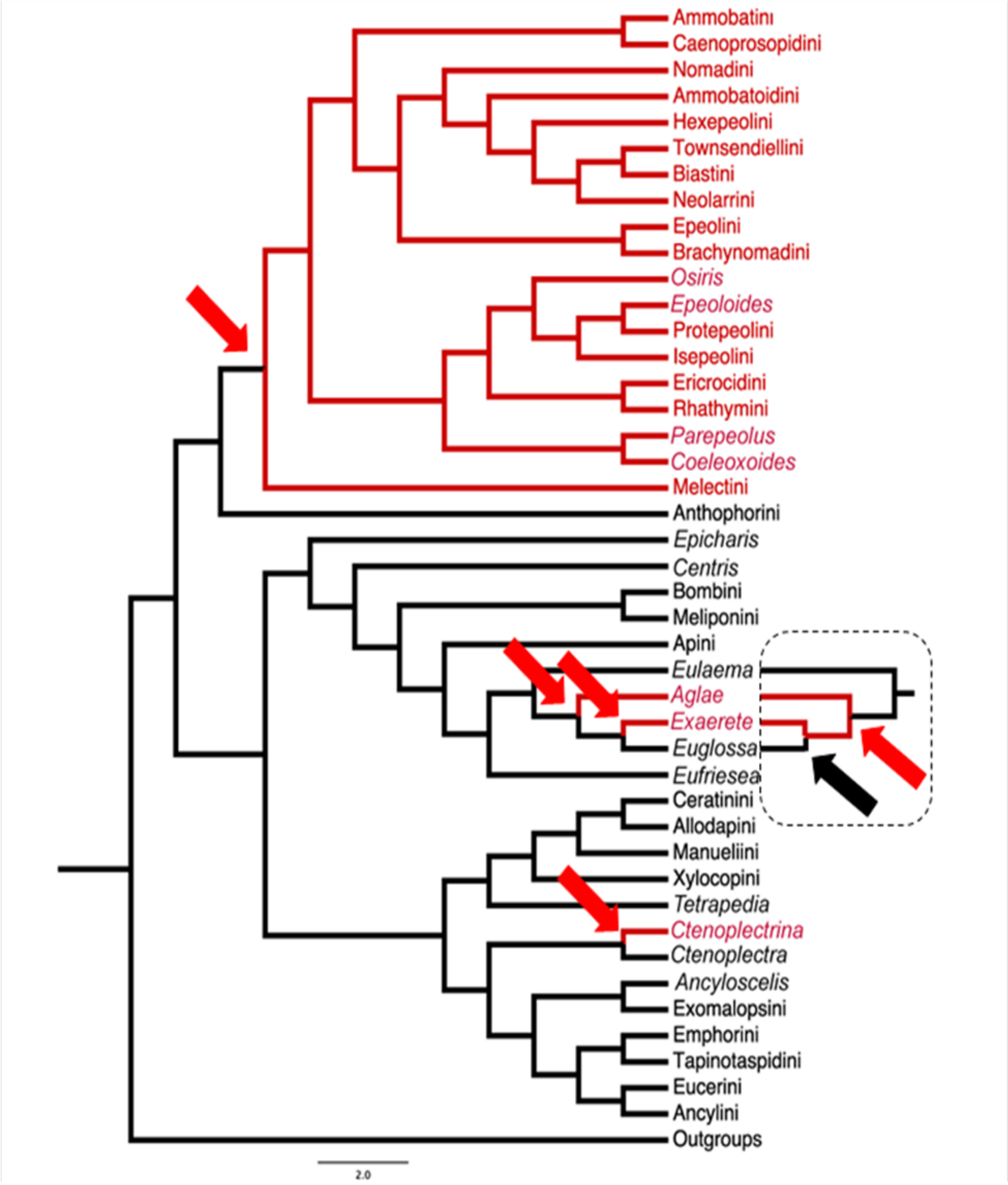
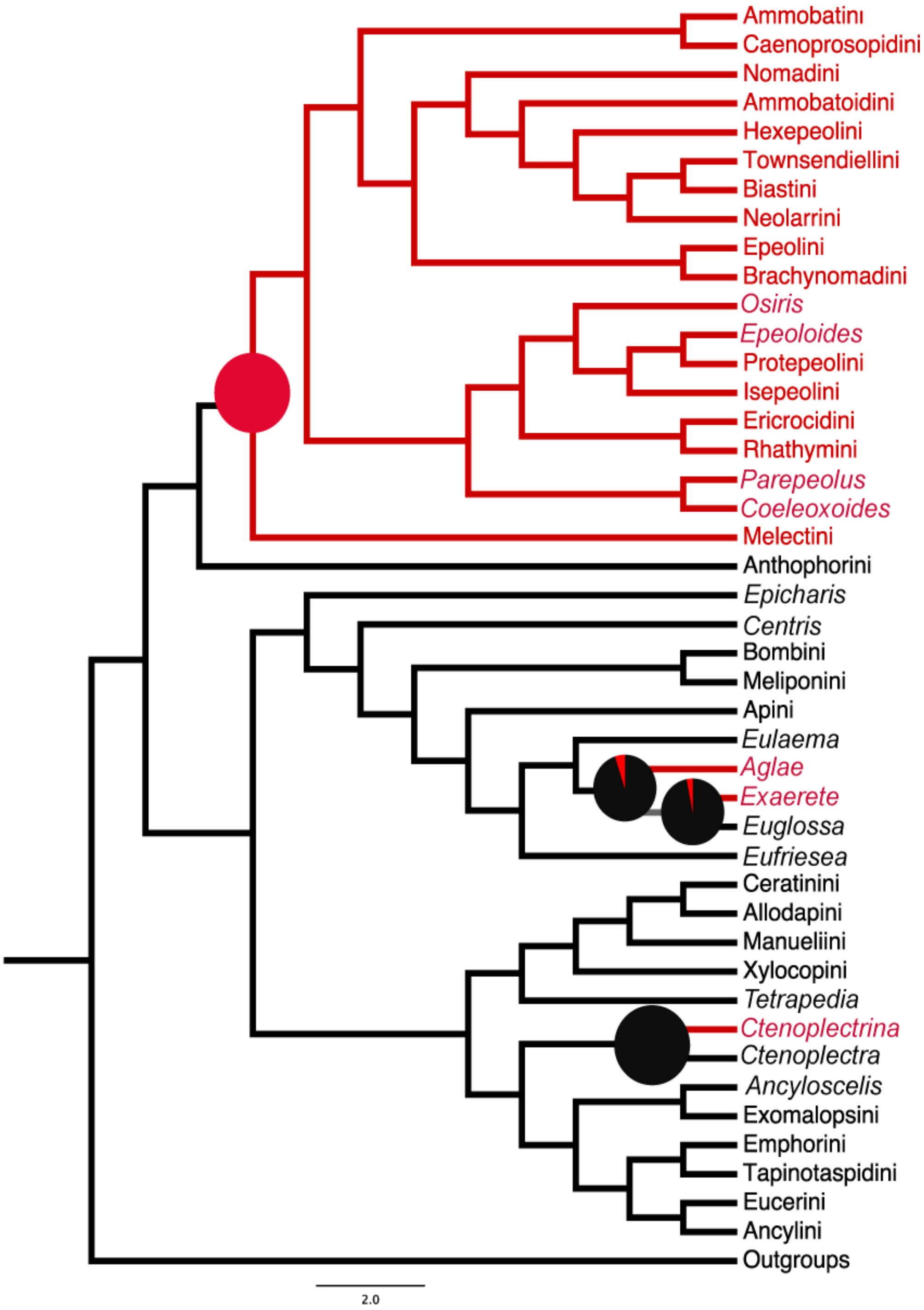

Ammobatini
Caenoprosopidini
Nomadini
Ammobatoidini
Hexepeolini
Townsendiellini
Biastini
Neolarrini
Epeolini
Brachynomadini
Osiris
Epeoloides
Protepeolini
Isepeolini
Ericrocidini
Rhathymini
Parepeolus
Coeleoxoides
Melectini
Anthophorini
Epicharis
Centris
Bombini
Meliponini
Apini
Eulaema
Aglae
Exaerete
Euglossa
Eufriesea
Ceratinini
Allodapini
Manueliini
Xylocopini
Tetrapedia
Ctenoplectrina
Ctenoplectra
Ancyloscelis
Exomalopsini
Emphorini
Tapinotaspidini
Eucerini
Ancylini
Outgroups
2.0

Figure 1.22. Pruned version of the Bayesian maximum clade credibility tree of the concatenated dataset (|28S,18S|pos1|pos2|pos3|) using a GTR+I+G model. Cleptoparasitic taxa are in red and nest-making taxa in black. Cleptoparasitism is mapped onto the tree according to the results of our model based ancestral state reconstruction. Pie charts show the probability of the nodes ancestral state being cleptoparasitic or nest-making.


Ammobatini
Caenoprosopidini
Nomadini
Ammobatoidini
Hexepeolini
Townsendiellini
Biastini
Neolarrini
Epeolini
Brachynomadini
Osiris
Epeoloides
Protepeolini
Isepeolini
Ericrocidini
Rhathymini
Parepeolus
Coeleoxoides
Melectini
Anthophorini
Epicharis
Centris
Bombini
Meliponini
Apini
Eulaema
Aglae
Exaerete
Euglossa
Eufriesea
Ceratinini
Allodapini
Manueliini
Xylocopini
Tetrapedia
Ctenoplectrina
Ctenoplectra
Ancyloscelis
Exomalopsini
Emphorini
Tapinotaspidini
Eucerini
Ancylini
Outgroups
2.0

We then used the Bayes Factor test to see if the dataset significantly supports 4 independent origins over 3 origins with one reversal. The Bayes Factor was 8.04 ± 1.2 (Figure 1.23), which indicates very strong support for the hypothesis of 4 independent origins of cleptoparasitism.

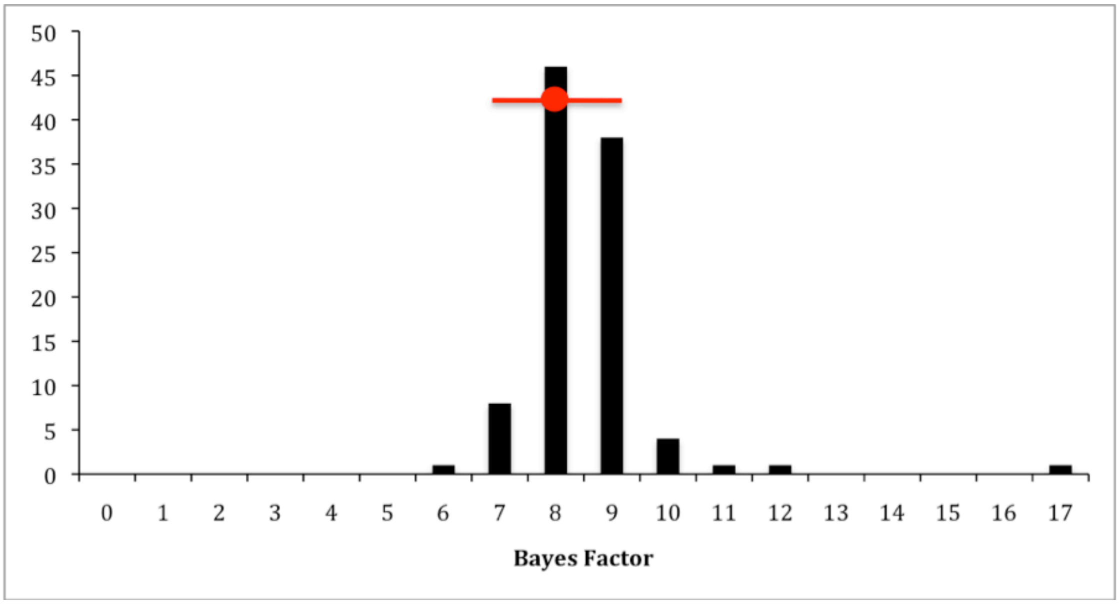


Figure 1.23. Distribution, mean and variance of the 100 Bayes Factor tests when the node uniting *Euglossa*, *Exaerete,* and *Aglae* was constrained to be nest-making vs. cleptoparasitic. Values above 0 indicate support for a nest-making ancestor, and those above 6 are considered to be strong support for this hypothesis.

Divergence time estimates

Varying the prior age set on the root node affected the estimated divergence times of nodes close to the root of the tree (e.g. Figure 1.24) but did not have an effect on nodes near the tips of the tree (e.g. Figure 1.25). Because bees are thought to have originated in the mid-Cretaceous about 125-120 Ma (Grimaldi and Engel 2005), we present results based on the analysis where the root node was set to 120 Ma (Figure 1.26).

We estimate that Apidae evolved ~112 Ma (107-117Ma) in the Early Cretaceous. The Xylocopinae are estimated to have evolved ~92 Ma (83-101Ma), and the Nomadinae ~85 Ma (76-94), in the Late Cretaceous. Because the Xylocopinae and Nomadinae appear to have evolved from the Apinae, our age estimate for Apinae would be the same as for Apidae. We also have age estimates for the 4 major clades found in this phylogeny: cleptoparasites+Anthophorini ~107 Ma (100-113), Xylocopinae + *Tetrapedia* ~98 Ma (90-106), Centridini + corbiculates ~ 98 Ma (90-106), and the eucerine line ~97 Ma (89-106 Ma).

Cleptoparasitism appears to be an ancient behavior in apid bees. It first evolved ~95 Ma (87-103 Ma) in the common ancestor of the Nomadinae and cleptoparasitic Apinae (i.e., the clepto clade). The other three origins of cleptoparasitism are much more recent: ~21 Ma (11-32 Ma) in the Ctenoplectrini, ~23 Ma (17-30 Ma) in *Aglae*, and some time after *Exaerete* diverged from *Euglossa* ~19 Ma (13-24 Ma).

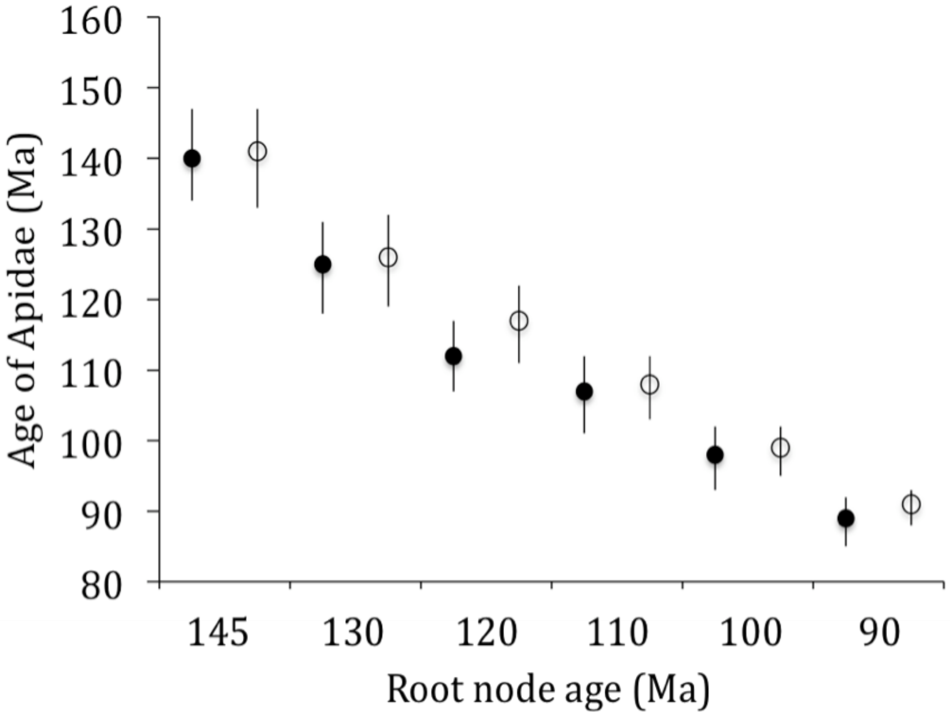


Figure 1.24. Mean and variance of the estimated age of Apidae for each root node age prior tested, using lognormal (closed circles) and uniform (open circles) priors for internal calibration points.

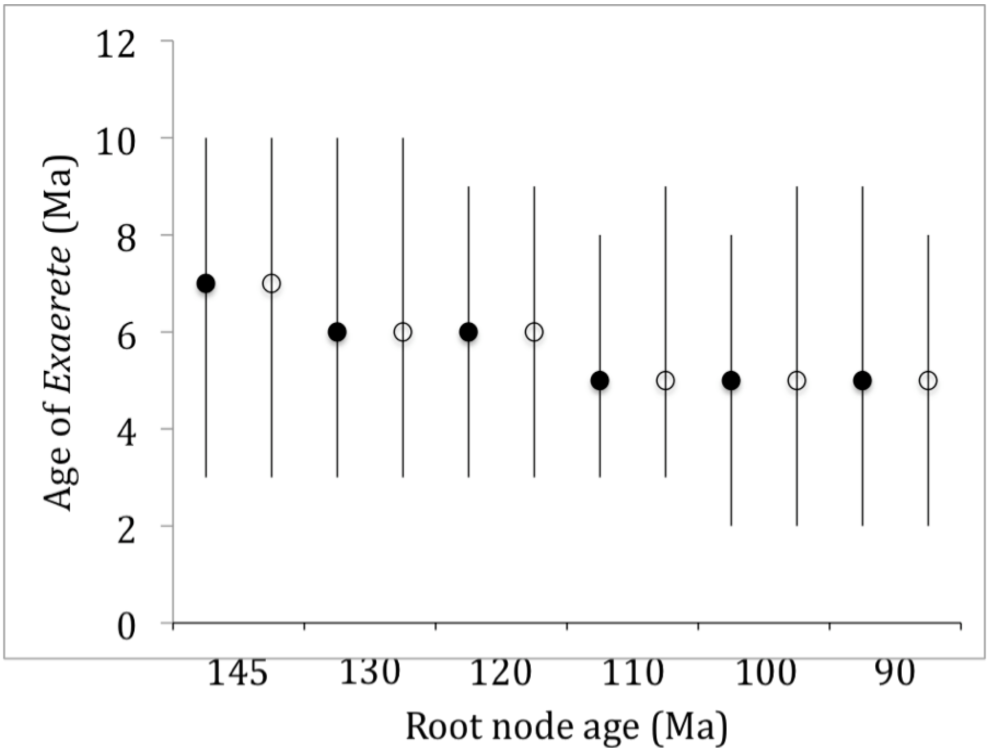


Figure 1.25. Mean and variance of the estimated age of *Exaerete* for each root node age prior tested, using lognormal (closed circles) and uniform (open circles) priors for internal calibration points.

Figure 1.26. Chronogram of the Apidae with outgroups removed. Red error bars indicate the 95% HPD on our estimate of the age of each cleptoparasitic origin. The black error bar represents the 95% HPD on our estimate of the age of Apidae.

Allodapini
Ceratinini
Manuelilni
Xylocopini
Xylocopinae
Tetrapedia
Exomalopsini
Emphorini
Tapinotaspidini
Ancylini
Eucerini
Ctenoplectrini
Centridini
Apini
Euglossini
Bombini
Meliponini
Anthophorini
Ericrocidini
Rhathymini
Isepeolini
Protepeolini
Osirini
Coelioxoides
Melectini
Apinae
Ammobatoidini
Neolarrini
Biastini
Townsendiellini
Hexepeolini
Nomadini
Brachynomadini
Epeolini
Ammobatini
Caenoprosopidini
Nomadinae
120
100
80
60
40
20
0
EARLY
LATE
PALEOCENE
EOCENE
OLIGOCENE
MIOCENE
CRETACEOUS
PALEOGENE
NEOGENE
■ NEST-MAKING
🟥 CLEPTOPARASITIC

Discussion

Phylogenetic relationships within the Apidae

Our phylogenetic hypothesis indicates that drastic changes are needed to the higher level classification of Apidae. This is mostly due to the grouping of most cleptoparasitic Apinae with the cleptoparasitic Nomadinae, and the Xylocopinae arising from within Apinae. The Nomadinae and Xylocopinae are recovered as monophyletic, but render the Apinae paraphyletic (Figure 1.15). Therefore, the current subfamilial classification of Apidae is not tenable.

Recovery of the large parasitic clade is not a completely unexpected result. When Roig-Alsina and Michener (1993) included all of the adult characters in their phylogenetic analysis, the cleptoparasitic Apinae did not form a large monophyletic clade (as found here), but were placed at the base of the Apinae and Xylocopinae. This topology was rejected by the authors since it suggested that nest-making Apidae are derived from cleptoparasitic ancestors. However, the basal placement of all of the cleptoparasitic Apinae, with the Nomadinae as sister to all other apids, suggested the possibility of a large cleptoparasitic clade near the base of Apidae.

Both Roig-Alsina and Michener (1993), and Straka and Bugusch (2007), recovered a monophyletic Tetrapediini, with the cleptoparasitic *Coeleoxoides* sister to its host *Tetrapedia*. However, in our phylogenetic hypothesis, *Coeleoxoides* is part of the clepto clade and therefore not closely related to its host. In their study of the immature stages of Tetrapediini, Alves-Dos-Santos et. al. (2002), concluded that *Coelioxoides* and *Tetrapedia* were probably sister genera based on similarities previously identified by Roig-Alsina and Michener (1993), but they also found that features of the eggs, first instars, and pupae were quite different from one another. Furthermore, in Roig-Alsina and Michener's morphological matrix of adult characters, several characters are coded differently for these two genera, with *Coeleoxoides* often assigned the same state as several other cleptoparasitic Apinae.

We recover a monophyletic Nomadinae, and find that Osirini, Protepeolini, and Isepeolini should not be placed within Nomadinae. Morphological studies have strongly supported monophyly of Nomadinae (Alexander 1990; Roig-Alsina and Michener 1993). However, it has been unclear whether Osirini, Protepeolini, and Isepeolini (three cleptoparasitic Apinae tribes) form a monophyletic group with Nomadinae, or whether they belong to Apinae (Alexander 1990). In our analysis, they are part of the large cleptoparasitic clade.

We recovered the monophyly of every tribe within the Nomadinae, but relationships among the tribes are not fully congruent with previous analyses based on adult (Alexander 1990; Roig-Alsina and Michener 1993), and/or larval (Alexander 1990; Roig-Alsina and Michener 1993; Rozen 1996) characters which are not fully congruent themselves. We recover the sister group relationship between Ammobatini and Caenoprosopidini as do phylogenetic analyses of the mature larvae (Rozen 1996), and adults (Alexander 1990; Roig-Alsina and Michener 1993; Rozen 1996). However, our placement of Ammobatini and Caenoprosopidini as sister to the remaining Nomadinae has not been supported previously by morphology. We also recover (Townsendiellini+Biastini)+Neolarrini, as do analyses based on mature larvae (Rozen 1996), and Brachynomadini+Epeolini, as do some analyses based on adult morphology (Alexander 1990; Rozen 1996).

We hypothesize that Xylocopinae is monophyletic and arose from within the Apinae with *Tetrapedia* as its sister clade. There has previously been evidence for Xylocopinae arising from within Apinae. When all adult characters were included in the analysis of Roig-Alsina and Michener (1993), Xylocopinae was found to be monophyletic, but evolving from within the Apinae. *Tetrapedia* was not sister to the

Xylocopinae as is found in our phylogenetic hypothesis, but Tetrapediini was found to have arisen within the Xylocopinae in the phylogenetic hypothesis of Straka and Bogusch (2007) based on larval characters. Relationships among the four xylocopine tribes are congruent with some previous analyses based on morphology (Roig-Alsina and Michener 1993; Engel 2001).

We do not recover a monophyletic Apinae, for which no un-reversed morphological synapomorphy appears to exist (Roig-Alsina and Michener 1993; Michener 2007). Indeed, there are exceptions to every subfamilial character described, and Roig-Alsina and Michener (1993) only recovered the monophyly of the Apinae after removal of five characters associated with parasitism or by exclusion of the cleptoparasitic taxa. While this raised the possibility of a paraphyletic Apinae, our analysis is the first to clearly and rigorously support this alternative view of the subfamily. We find support for Silveira's (1993) eucerine line (minus *Tetrapedia)*, but our data do not support the apine line as defined by Silveira (1993).

We recovered relationships among the corbiculate tribes congruent with previous molecular studies (Cameron 1993; Koulianos and S. 1999; Cameron and Mardulyn 2001; Kawakita et al. 2008) ((Euglossini+Apini)+(Meliponini+Bombini)). In contrast, most morphological analyses (Prentice 1991; Roig-Alsina and Michener 1993; Engel 2001; Schultz et al. 2001; Cardinal and Packer 2007) and those based on combined morphological and molecular datasets (Chavarria and Carpenter 1994; Schultz et al. 1999) support the phylogeny proposed by Michener (Michener 1944) (Euglossini+(Bombini+(Meliponini+Apini))). *Centris* is found to be the sister group to the corbiculates, but contrary to earlier studies (e.g. (Roig-Alsina and Michener 1993)), corbiculates appear to be a fairly basal branch within Apidae.

The evolution of cleptoparasitism in the Apidae

Our phylogenetic hypothesis for relationships within Apidae has implications for our understanding of the evolution of a wide variety of ecological and behavioural features of this group. Elsewhere, we use our phylogeny to investigate the evolutionary origins and antiquity of sociality in bees (see Chapter 2). Here, we have exploited our extensive sampling of cleptoparasitic taxa in order to test the evolution of this important, but little studied mode of parasitism.

Our analysis reduces the presumed number of independent origins of cleptoparasitism from 6 (Straka and Bogusch 2007) or 11 (Rozen 2000), to 4. Our model-based approach to ancestral state reconstruction resolves ambiguity in the ancestral behavior of the parasitic Euglossini. It was equally parsimonious to hypothesize four independent origins of cleptoparasitism, or three independent origins with one reversal to nest-making in *Euglossa*. Our model-based reconstruction of four independent origins and no reversals from cleptoparasitic back to nest-making is consistent with Dollo's Law (Dollo 1893), which proposes that once a complex character (e.g., nest-making) is lost, it is unlikely to be regained. In this case, females of *Euglossa* would have had to re-evolve the structures associated with foraging for pollen and nest-making (such as the corbicula, or pollen basket). Indeed, Dollo's Law appears to hold across the family: despite the antiquity of cleptoparasitism in apid bees (~95 Ma for the Nomadinae + clepto Apinae), we do not see any reversals back to nest-making. The only clade in which a reversal might have occurred is associated with what we estimate to be the most recent origin of cleptoparasitism (~19 Ma), in the *Exaerete*. However, we strongly believe this does not represent a reversal, as discussed above.

Only one of the most recent cleptoparasitic lineages, *Ctenoplectrina,* can potentially supports Emery's rule in its strict form. Females in the species of

Ctenoplectrina attack the nests of species in the genus *Ctenoplectra*. Our phylogenetic results indicate that *Ctenoplectrina* evolved from within its host lineage *Ctenoplectra,* rendering *Ctenoplectra* paraphyletic. A recent phylogenetic analysis based on molecular data that examined the relationships within Ctenoplectrini found the parasitic *Ctenoplectrina* sister to its host *Ctenoplectra* (Schaefer and Renner 2008), with Eucerini as the closest outgroup. Conversely, based on a much broader taxon sampling of apid bees, we hypothesize Ctenoplectrini to be sister to all other members of the eucerine line.

The other two recently derived cleptoparasitic lineages, *Aglae* and *Exaerete*, support the loose form of Emery's rule by being closely related to, though not sister taxa of their respective hosts, *Eufriesea* and *Eulaema.* The most ancient cleptoparasitic lineage, estimated to be ~95 Ma (87-103 Ma), has a much wider host range then the three more recent lineages; a finding not consistent with Emery's rule. However, the basal clade of this large cleptoparasitic lineage, Melectini, is a parasite of the sister clade to the cleptoparasitic clade, Anthophorini. This suggests that when this cleptoparasitic lineage first diverged from its nest-making ancestor, it may have parasitized closely related hosts, thereby supporting Emery's rule.

The large host range seen in the ancient cleptoparasitic lineage may be due to its age, and the cumulative opportunities to switch hosts during diversification of both host and parasite lineages. However, within this clade, there are two large monophyletic groups, Nomadinae and most cleptoparasitic Apinae. Despite being the same age, the Nomadinae have a much wider host range (Figure 1.1). This could simply be due to the difference in the number of species in both groups, with 1214 species in the Nomadinae and only 135 in its sister clade. This raises the question of why the Nomadinae is so much more speciose and diverse in its hosts compared to its sister clade. One possible answer lies in the mode of parasitism of Nomadinae. In all cases, the female enters a nest in which a cell is still open and being provisioned by the host female. The parasite inserts its egg into the wall or lining of the cell while the host is foraging away from the nest (Rozen 1991). The eggs of the Nomadinae tend to be small for the size of the bee, and the chorion often has complex microsculpturing which may help camouflage the egg in the cell wall (Rozen 2003). Only a few of the cleptoparasitic Apinae parasitize open cells, most insert their eggs in cells that have already been provisioned for and closed off by the host. Also, larvae of Nomadinae do not spin cocoons, in contrast to most Apinae (Michener 2007).

Conclusions

Our phylogenetic hypothesis indicates that most cleptoparasitic apid bees form a monophyletic group, and therefore stem from a single origin of cleptoparasitism. We find two more origins of cleptoparasitism in the orchid bees and one in the tribe Ctenoplectrini. The large cleptoparasitic clade formed by the Nomadinae and most cleptoparasitic Apinae renders the subfamily Apinae paraphyletic, and indicates that radical changes are needed to the higher level classification of Apidae. Divergence time estimates using a relaxed fossil-calibrated molecular clock model reveal that cleptoparasitism is an ancient behavior in apid bees that first evolved ~95 Ma, much earlier then the appearance of the first cleptoparasitic bee in the fossil record. We also find that both the strict and loose form of Emery's rule is applicable to cleptoparasitic apid bees.

Our molecular phylogeny is broadly supported, though some nodes in the tree would benefit from additional data and analyses. We also suggest that morphology should be investigated in detail once again to see if synapomorphies can be found supporting the clades suggested in this tree. Once the morphology has been re-examined, we predict that changes to the higher level classification of Apidae will need to be made. Furthermore, our divergence time estimates provide a framework with which the biogeography of Apidae can now be more thoroughly investigated.

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CHAPTER TWO

Phylogeny of apid bees reveals the evolutionary history and antiquity of eusociality

Abstract

The bee family Apidae includes the most well studied of all social bees, the honey bee, as well as other eusocial species (bumble, stingless, and allodapine bees). In spite of their importance for studies of social organization and the genetic basis of eusociality, apid bee phylogeny remains poorly understood. We provide the first comprehensive analysis of apid phylogeny and use this phylogeny to reconstruct the evolutionary history of eusociality using model-based methods. Our results indicate that primitive eusociality is the ancestral state for corbiculate Apidae, and that orchid bees represent a reversal from eusociality to solitary, communal, and weak social behavior. In addition, we present the first fossil-calibrated phylogeny for Apidae, which reveals that eusociality first evolved ~87 Ma in the corbiculates, much earlier than in other groups of bees. Our phylogeny represents a major improvement over the current systematic understanding of this family and provides new insights into both the timing and evolution of eusociality in bees.

Introduction

Apid bees have a worldwide distribution and comprise a major component of the bee fauna in most terrestrial ecosystems. Apidae is the largest of the seven currently recognized bee families, with over 5600 described species in 33 tribes and

177 genera (Michener 2000). Apidae includes all of the highly eusocial bees as well as numerous solitary, primitively eusocial, and parasitic taxa. The most well-known and thoroughly studied of all apid bees is *Apis mellifera* (tribe Apini). The honey bee is a model organism for understanding many aspects of social behavior, including the genetic basis of sociality (Ben-Shahar 2002; Robinson and Ben-Shahar 2002; Whitfield et al. 2003; Weinstock et al. 2006; Weinstock et al. 2006), caste determination (Evans and Wheeler 1999), division of labor (Seeley 1995), and complex forms of communication such as the dance language (Frisch 1967). The diversity in social behavior found within Apidae, and the inclusion of a model organism in Apidae, makes Apidae a key study group for understanding the organization and genetic basis of social behavior. It is therefore essential to have a clear understanding of the evolutionary history of eusocial behavior in this family to put studies into an appropriate evolutionary context.

Of particular importance for resolving the evolutionary history of eusociality in bees is the clade commonly referred to as the Corbiculates (defined by the corbicula or pollen basket on the hind tibia of females). Corbiculates include approximately 1000 of the 6000 species of Apidae and are undoubtedly the most thoroughly studied bee group. This lineage includes the only advanced eusocial bees, the only bees to store harvestable honey, and the most important managed pollinators in agricultural settings (i.e., the honey bee). There are four monophyletic tribes recognized within the corbiculates: the highly eusocial Apini (honey bees) and Meliponini (stingless bees), the primitively eusocial Bombini (bumble bees), and the mostly solitary, communal, and weakly social Euglossini (orchid bees). The advanced eusocial Apini and Meliponini have morphologically distinct queens and workers with nests founded by swarms (Michener 1974), whereas the primitively eusocial Bombini have queens and workers that differ only in size, with new nests established by a single foundress.

Orchid bees are usually referred to as being solitary or communal (Cameron 2004), but intriguing hints of more advanced forms of social behavior, including overlap of generations and cooperative brood care have been recently reported in some taxa (Augusto and Garofalo 2009).

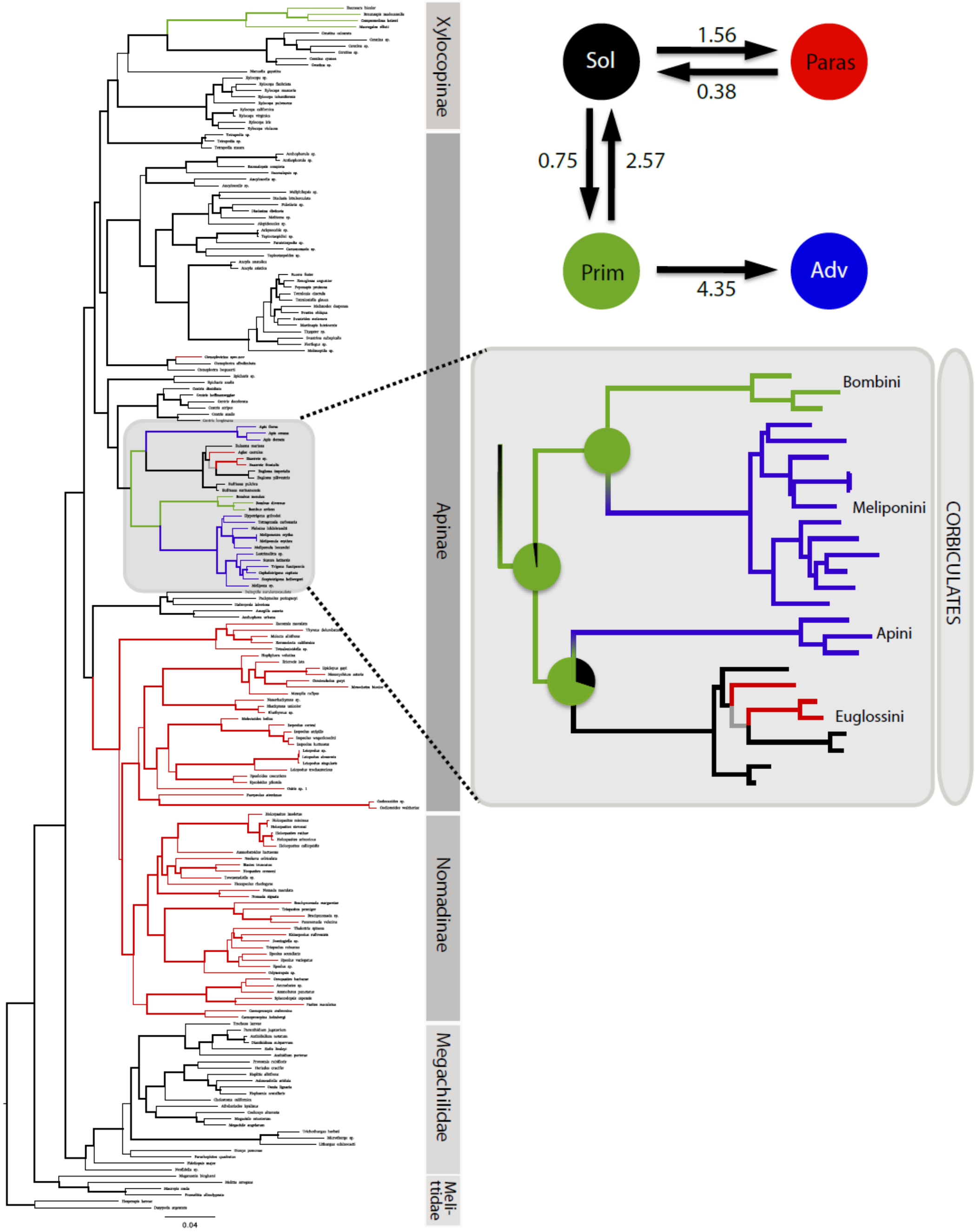
While monophyly of the corbiculate bees as a whole is well supported, relationships among the four tribes remain controversial. Most morphological analyses (Prentice 1991; Roig-Alsina and Michener 1993; Engel 2001; Schultz et al. 2001; Cardinal and Packer 2007) and those based on combined morphological and molecular data sets (Chavarria and Carpenter 1994; Schultz et al. 1999) support the phylogeny proposed by Michener (1944) which is consistent with single origins of primitive eusociality and of advanced eusociality within corbiculates (Euglossini+(Bombini+(Meliponini+Apini))). In contrast, most molecular phylogenies (Cameron 1993; Koulianos et al. 1999; Cameron and Mardulyn 2001; Kawakita et al. 2008) propose a sister group relationship between Bombini and Meliponini, with variable placement of Apini and Euglossini. The latter topology suggests dual origins of eusociality. Thus resolving the relationships among the four tribes of corbiculates has important implications for our understanding of social evolution. Previous molecular analyses have been criticized for poor choices of genes and limited outgroup sampling (Schultz et al. 2001).

In this paper we provide the first comprehensive phylogeny of the family Apidae based on molecular data and use this phylogeny to assess whether eusociality is likely to have evolved once or twice in the corbiculates. By including all 33 tribes of Apidae in our analysis, we not only resolve relationships among the corbiculates (and 29 other apid tribes), but also determine the corbiculates phylogenetic placement within Apidae. This broad taxon sampling, including the non-corbiculate eusocial apids (in the tribe Allodapini), allowed for more accurate estimates of the transition rates from one behavioral state to another, informing our reconstruction of social evolution within corbiculates and across the Apidae as a whole. Our inclusion of representatives from all tribes of Megachilidae, the sister clade to Apidae (Roig-Alsina and Michener 1993; Danforth et al. 2006), and all subfamilies of Melittidae as outgroup taxa in our analysis, permitted us to incorporate information from all known apid, megachilid and melittid fossils into our estimates of the antiquity of social behavior using a relaxed-clock, fossil-calibrated divergence time analysis. Our study provides the first comprehensive analysis of the temporal appearance of eusociality across Apidae and other eusocial bee lineages.

Results and Discussion

To resolve the higher level relationships of apid bees, we analyzed over 6 kb of sequence data from two nuclear ribosomal genes (18S and 28S) and five nuclear protein coding genes (EF-1alpha, long-wavelength opsin, wingless, Na/K ATPase, PolII) for 190 taxa. Analyses with several methods of phylogeny reconstruction (Parsimony, ML, Bayesian) resulted in a well-resolved phylogeny that was robust across numerous alternative analytical methodologies. We recovered the monophyly of the subfamilies Xylocopinae and Nomadinae, however each of these render the Apinae (the largest subfamily) paraphyletic in our analysis (Figure 2.1). This is largely due to the grouping of the cleptoparasitic apine taxa (“cuckoo bees”) with the cleptoparasitic Nomadinae, and the placement of Xylocopinae as a clade derived from the Apinae. Our phylogenetic hypothesis indicates the need for radical changes to the higher level classification of Apidae (Michener 2007).

Figure 2.1. Bayesian maximum clade credibility tree with outgroups removed and thickness of branches representing posterior probabilities. Transition rates between four different behavioral states are also indicated. Pie charts on inset of corbiculate phylogeny represent the probability of the ancestral state of the node.


Xylocopinae
Apinae
Nomadinae
Megachilidae
Meli-ttidae
0.04
Sol
Paras
1.56
0.38
0.75
2.57
Prim
Adv
4.35
Bombini
Meliponini
Apini
Euglossini
CORBICULATES

We recovered relationships among the corbiculate tribes congruent with previous molecular studies based on a much smaller sample of outgroups, suggesting that limited outgoup sampling was not a problem in those studies. *Centris* was found to be the sister group to the corbiculates, but contrary to earlier studies (e.g. Roig-Alsina and Michener 1993), corbiculates appear to be a fairly basal branch within Apidae. Our phylogenetic analysis based on molecular data provides a hypothesis of apid relationships independent of possible morphological convergence (a cited problem in previous analyses (Roig-Alsina and Michener 1993)), and provides a significant improvement in our understanding of apid evolution that will provide a basis for future comparative behavioral, ecological and genomic studies.

We used our phylogenetic hypothesis to investigate the origins of social behavior. Parsimony reconstruction of social behavior on our phylogeny was unable to distinguish between dual or single evolutionary origins of eusociality within corbiculate bees. This ambiguity arises because two equally parsimonious alternative reconstructions are possible: (1) primitive eusociality evolved once in the common ancestor of corbiculates and then reversed to solitary nesting in Euglossini, or (2) primitive eusociality evolved twice, implying a solitary corbiculate common ancestor. Although previous authors have dismissed the hypothesis of a reversal in Euglossini (e.g. Cameron and Mardulyn 2001), these alternatives cannot be distinguished using parsimony.

Bayesian methods have only recently been used to reconstruct ancestral states in social insects (Huelsenbeck et al. 2000; Lin et al. 2004; McLeish and Chapman 2007) but they have not been applied previously to the evolution of bees. Unlike parsimony, Bayesian approaches allow for uncertainty in tree topology, branch lengths, and relative rates of gains/losses to be incorporated into the reconstruction of ancestral states. Using BayesTraits (Pagel et al. 2004), we randomly sampled 1000 trees from a Bayesian phylogenetic analysis of the dataset consisting of 260 million post-burnin generations. We coded all terminal taxa for a behavioral character consisting of one of four states: solitary/communally nesting, parasitic, primitively eusocial, and advanced eusocial. We coded all nonparasitic orchid bee taxa as solitary/communal to remove any bias towards finding a primitively eusocial common ancestor for corbiculates. In our model, we set the prior probability on transitions from solitary nesting to advanced eusociality to zero because we considered it highly unlikely that a bee could evolve from being solitary to having morphologically distinct castes and swarm founding without an intermediate step. Relaxation of this prior (allowing a low but non-zero rate of transition from solitary to advanced eusociality) did not substantially alter our results (see supplementary material for details). We also did not allow transitions from advanced eusocial behavior to any other state because queens in advanced eusocial species cannot forage or found new nests independent of workers (Michener 1974; Winston 1977; Koulianos et al. 1999; Wilson and Hoelldobler 2005).

In our model-based ancestral state reconstructions, the common ancestor of the corbiculates is estimated with high probability (98.3%) to be primitively eusocial. The common ancestor of Bombini + Meliponini is also reconstructed as primitively eusocial with high probability (99.84%), as is the common ancestor of Euglossini + Apini (76.56% primitive eusociality) (Figure 2.1). This implies a single origin of eusocial behavior in corbiculate bees with two independent origins of advanced eusocial behavior (in stingless bees and honey bees), and one reversal from primitively eusocial to solitary/communal nesting in orchid bees.

In order to statistically test whether there was significant support for a primitively eusocial common ancestor, we ran the Bayesian ancestral state reconstruction analysis 100 times with the common ancestor alternatively fixed as solitary, parasitic, primitively eusocial and advanced eusocial. We then conducted pairwise comparisons of the mean of the likelihood scores for each of the 100 replicates under each of these models using a Bayes Factor test. The results strongly support the hypothesis that the common ancestor of corbiculates was primitively eusocial, and not parasitic or advanced eusocial, with significantly high Bayes Factor scores for these comparisons (5.05 ± 1.60 and 153.64 ± 1.92 respectively), whereas the Bayes Factor comparing a primitively eusocial ancestor and a solitary ancestor showed weaker, but positive support for a primitively eusocial common ancestor (1.56 ± 0.25). Collectively, the Bayes factor tests corroborate the Bayesian reconstructions of a primitively eusocial corbiculate ancestor.

This result has important implications for understanding the evolution of eusociality in corbiculates, because Euglossini is commonly taken to represent the primitive solitary condition. If Euglossini are indeed derived from an ancestor that was eusocial, then Euglossini do not represent a primitively solitary phenotype, but a secondarily solitary phenotype (Wcislo and Danforth 1997). Using euglossines as representative of a solitary corbiculate in comparative studies is likely to lead to incorrect assessments of the genetic and behavioral traits underlying the transition from the solitary mode of life typical of the vast majority of bees to the primitive and advanced modes of social organization evident in honey bees and their relatives. A more appropriate choice of a solitary bee for comparison to the eusocial behavior of the corbiculates would be bees in the tribe Centridini (genera *Centris* and *Epicharis*), which are truly solitary and strongly supported as the sister clades to the corbiculates in our phylogenetic analysis (Figure 2.1).

These phylogenetic results are congruent with recent behavioral studies of Euglossini. Whereas many authors have described Euglossini as “solitary/communal” (Zucchi et al. 1969; Dressler 1982; Kimsey 1987; Michener 2000; Cameron 2004), others have noted that some species have multiple-female associations in which some females forage and others guard the nest, suggestive of weak division of labor (Dodson 1966; Roberts and Dodson 1967; Sakagami and Laroca 1967; Zucchi et al. 1969; Eberhard 1988; Olesen 1988), possibly arising from nest re-use by succeeding generations, but also within generations by unrelated individuals. Given our ancestral state analysis, we interpret the weak reproductive division of labor in *Euglossa* as evidence of retention of the primitive eusocial state inferred to exist in the common ancestor of all corbiculates, and suggest that further investigation should reveal additional evidence of sociality in this tribe. Our finding of two independent transitions to advanced eusociality is also congruent with early studies on the divergent morphology, caste determination, nest founding and social behavior of the Meliponini and Apini (e.g. (Winston 1977)), and these differences can now be understood as the product of the independent evolution of advanced eusociality in these two tribes.

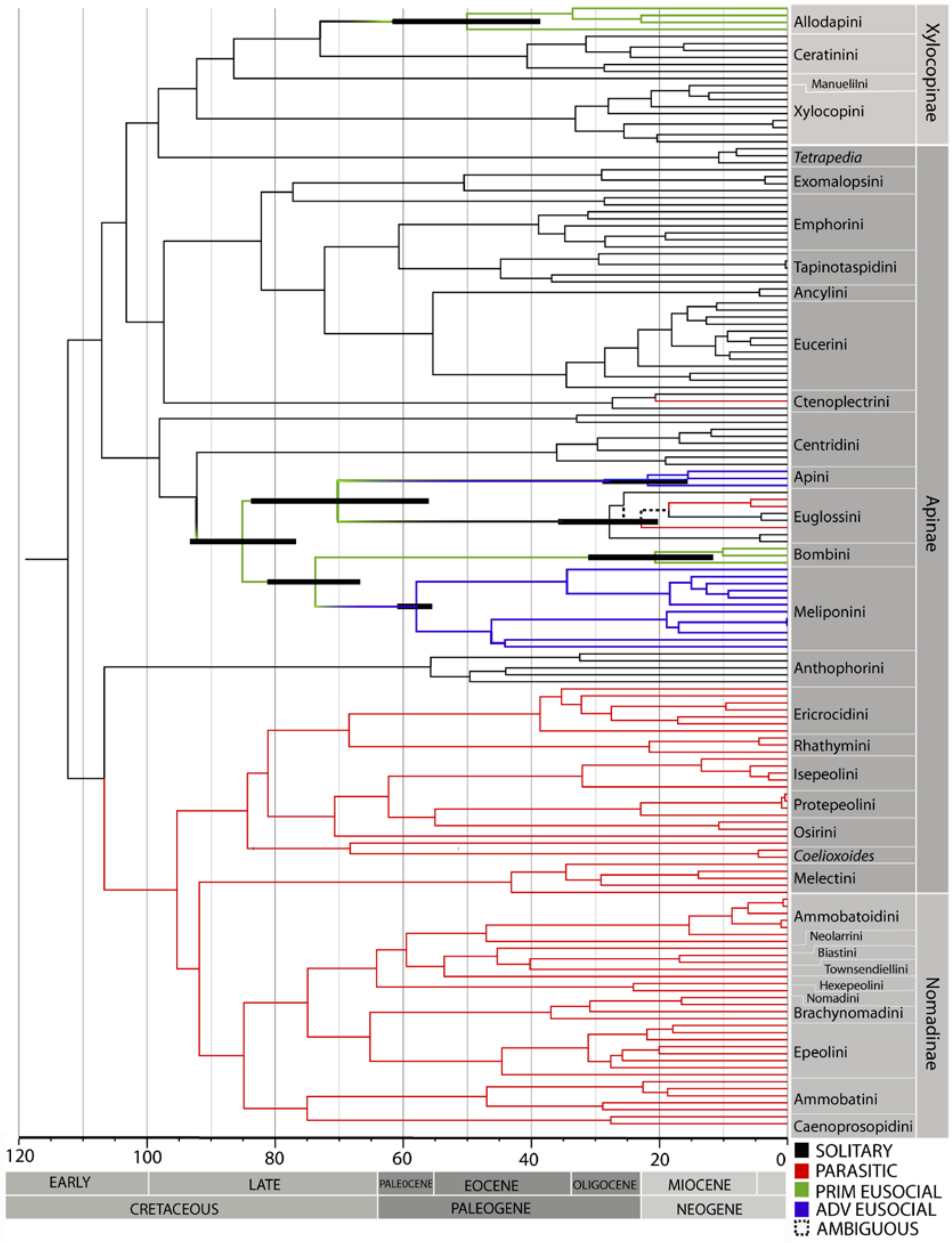
Despite a reluctance to consider the hypothesis of reversals in social behavior when considering bees corbiculate bees (e.g., (Cameron and Mardulyn 2001)), reversals from primitive eusociality to solitary or facultative eusociality have been shown in other insect groups. For example, previous studies indicate multiple reversals to solitary nesting within primitively eusocial lineages of halictid bees (Danforth et al. 2003), although no such reversals have been reported for the allodapine bees (Chenoweth et al. 2007). Similarly, a worker caste may have been lost repeatedly in the termites (Thompson et al. 2000), again suggesting that some aspects of social behavior in insects may be reversible. It is possible that a bias exists towards viewing social evolution in insects (and other organisms) as a steady progression from simple (solitary) to more complex (eusocial), and modern molecular phylogenies may force us to alter this assumption.

In order to date the antiquity of social behavior in apid bees, we applied an uncorrelated lognormal relaxed clock (Drummond et al. 2006) to our phylogenetic dataset, with 10 calibration points whose prior age estimates were based on information from the fossil record. We thoroughly reviewed the literature on fossil bees to inform our calibration points, and confirmed that our results were robust to variation in model priors. See Chapter 1 for details on the methods.

The dating analysis provides estimated divergence times for every node in the tree (Figure 2.2). We estimate that primitive eusociality evolved once in the allodapines, whose extant lineages have an origin ~53 Ma (41 to 65 Ma), and once in the corbiculate bees, whose extant lineages originated ~87 Ma (78 to 95 Ma). The estimated age of origin for extant members of the highly eusocial stingless bees is ~58 Ma (56 to 61 Ma) and for extant members of the highly eusocial honey bees ~23 Ma (15 to 20 Ma).

Therefore, highly eusocial behavior evolved once sometime after the divergence of bumble bees from stingless bees ~75 Ma, and before the diversification of extant stingless bees ~58 Ma. The second origin of advanced eusocial behavior occurred after the divergence of orchid bees from honey bees, ~72 Ma, and sometime before the common ancestor of extant honey bees which we date at ~23 Ma. The oldest *Apis* fossil is a compression fossil of *A. henshawi* from the European shale deposit in Rott, Germany. Age of this deposit is somewhat ambiguous as either late Oligocene or early Miocene age (~25 Ma). Therefore, we conclude highly eusocial behavior evolved a second time sometime between ~72 Ma and the appearance of this oldest fossil honey bee.

Figure 2.2. Chronogram of the Apidae with outgroups removed and error bars for the age of nodes of interest.


Allodapini
Ceratinini
Manuelilni
Xylocopini
Xylocopinae
Tetrapedia
Exomalopsini
Emphorini
Tapinotaspidini
Ancylini
Eucerini
Ctenoplectrini
Centridini
Apini
Euglossini
Bombini
Meliponini
Anthophorini
Ericrocidini
Rhathymini
Isepeolini
Protepeolini
Osirini
Coelioxoides
Melectini
Apinae
Ammobatoidini
Neolarrini
Biastini
Townsendiellini
Hexepeolini
Nomadini
Brachynomadini
Epeolini
Ammobatini
Caenoprosopidini
Nomadinae
120
100
80
60
40
20
0
EARLY
LATE
PALEOCENE
EOCENE
OLIGOCENE
MIOCENE
CRETACEOUS
PALEOGENE
NEOGENE
SOLITARY
PARASITIC
PRIM EUSOCIAL
ADV EUSOCIAL
AMBIGUOUS

Fossil calibrated phylogenies have now been used to date the antiquity of eusociality in all five clades of eusocial bees. Whereas wasps, ants, and termites show origins well within the Cretaceous (65-140 Ma), bees show independent origins over a broad timescale from late Cretaceous (80 Ma) to the Miocene (20 Ma) (Table 2.1).

Table 2.1. Estimated ages of eusocial insect clades

Lineage	**Estimated age (Ma)**
Augochlorine bees	20 ± 1.80(Brady et al. 2006a)
Halictus bees	21 ± 1.05(Brady et al. 2006a)
Lasioglossum bees	22 ± 1.22(Brady et al. 2006a)
Allodapine bees	52 ± 0.23
Corbiculate bees	87 ± 0.17
Extant *Apis*	22 ± 0.11
Extant *Meliponini*	58 ± 0.02
Extant *Bombus*	21 ± 0.15
Vespidae (wasps)	≥65(Wenzel 1990)
Formicidae (ants)	115-135(Brady et al. 2006b)
Isoptera (termites)	≥130(Thorne et al. 2000)

Our results indicate that the complexity of eusocial behavior within bees is roughly correlated with age; more ancient lineages (corbiculates) show more complex social organization. We estimate that complex social behavior characteristic of honey bees and stingless bees, such as caste polymorphism, complex forms of communication, elaborate nest architecture, and age polyethism, have evolved over an 80 million year timespan.

APPENDIX

Materials and Methods

Dataset

See Chapter 1 for a list of all species included in the study along with taxonomic, voucher, and locality information, sequencing protocols, and Genbank accession numbers.

Model selection

Selection of best-fit models of nucleotide substitution for each data partition used in a Bayesian or Maximum Likelihood analysis was based on the Akaike (AIC) and Bayesian Information Criteria (BIC) as implemented in JModelTest v.0.1.1 (Posada 2008).

Partitioning of data

The two ribosomal genes were combined into 1 partition and the protein coding genes were combined together and then partitioned by codon position.

Bayesian analysis

The Bayesian phylogenetic analysis was done in MrBayes v.3.1.2 (Huelsenbeck and Ronquist 2001). We applied a General Time Reversible model with gamma distributed rates among sites in four categories and a proportion of invariant sites (GTR+I+G) to all partitions. We unlinked each parameter across partitions. A total of 46 independent runs, 44 with 4 chains and 2 with 8 chains, were conducted. The number of generations of each run varied from 4 096 000 generations to 23 353 000 generations. The parameter trace files of each individual run was observed to verify that the run had converged on the stationary distribution and to decide on the appropriate number of generations to discard as burn-in. The tree files and parameter files with the burn-in removed from each run that had reached convergence were combined. Runs that had not converged were discarded. A maximum clade credibility tree was constructed from these 268 560 000 post-burnin generations in TreeAnnotator v1.4.8 (Rambaut and Drummond 2008). Nodal support was assessed with posterior probabilities. We chose to do numerous shorter runs instead of a few longer runs because of run time restrictions on the computer clusters at Cornell University's Computanional Biology Service Unit. Also, doing a large number of independent runs from different starting points allowed us to more fully explore the tree space.

Ancestral state reconstruction of eusocial behaviour

All terminals in the tree were coded for a behavioral character, based on information in the literature, consisting of 4 states; parasitic, solitary, primitively eusocial or highly eusocial (Table A2.1). Parsimony methods were used to reconstruct the evolution of eusocial behavior in apid bees on the Bayesian maximum clade credibility tree using MacClade v.4.0 (Maddison and Maddison 2000). Regardless of whether the character was treated as being ordered or unordered, the ancestral state of corbiculate bees remained ambiguous (Figure A2.1).

Table A2.1. Behavioral character state assigned to each taxa used in the study.

Taxa	**Character State**
Ancyla asiatica	Solitary/Communal
Ancyla anatolica	Solitary/Communal
Pachymelus peringueyi	Solitary/Communal
Anthophora urbana	Solitary/Communal
Amegilla asserta	Solitary/Communal
Habropoda laboriosa	Solitary/Communal
Deltoptila aurulentocaudata	Solitary/Communal
Apis cerana	Advanced eusocial
Apis dorsata	Advanced eusocial
Apis florea	Advanced eusocial
Bombus ardens	Primitively eusocial
Bombus diversus	Primitively eusocial
Bombus mendax	Primitively eusocial
Centris atripes	Solitary/Communal
Centris hoffmanseggiae	Solitary/Communal
Centris analis	Solitary/Communal
Epicharis analis	Solitary/Communal
Epicharis sp.	Solitary/Communal
Centris dimidiata	Solitary/Communal
Centris longimana	Solitary/Communal
Centris decolorata	Solitary/Communal
Ctenoplectra albolimbata	Solitary/Communal
Ctenoplectrina sp.	Parasitic
Ctenoplectra bequaerti	Solitary/Communal
Diadasia bituberculata	Solitary/Communal
Ptilothrix sp.	Solitary/Communal
Diadasina distincta	Solitary/Communal
Ancyloscelis sp.	Solitary/Communal
Alepidosceles sp.	Solitary/Communal
Meliphilopsis sp.	Solitary/Communal
Ancyloscelis sp.	Solitary/Communal
Melitoma sp.	Solitary/Communal
Ericrocis lata	Parasitic
Epiclopus gayi	Parasitic
Mesonychium asteria	Parasitic
Mesoplia rufipes	Parasitic
Hopliphora velutina	Parasitic
Mesocheira bicolor	Parasitic

Table A2.1. (Continued)

Ctenioschelus goryi	Parasitic
Melissodes desponsa	Solitary/Communal
Eucera frater	Solitary/Communal
Xenoglossa angustior	Solitary/Communal
Svastra obliqua	Solitary/Communal
Tetralonia cinctula	Solitary/Communal
Martinapis luteicornis	Solitary/Communal
Peponapis pruinosa	Solitary/Communal
Svastrides melanura	Solitary/Communal
Melissoptila sp.	Solitary/Communal
Thygater sp.	Solitary/Communal
Florilegus sp.	Solitary/Communal
Svastrina subapicalis	Solitary/Communal
Tetraloniella glauca	Solitary/Communal
Exaerete sp.	Parasitic
Eulaema meriana	Solitary/Communal
Euglossa piliventris	Solitary/Communal
Aglae caerulea	Parasitic
Eufriesea surinamensis	Solitary/Communal
Eufriesea pulchra	Solitary/Communal
Euglossa imperialis	Solitary/Communal
Exaerete frontalis	Parasitic
Anthophorula completa	Solitary/Communal
Anthophorula (Isomalopsis) sp.	Solitary/Communal
Anthophorula (Isomalopsis) sp.	Solitary/Communal
Exomalopsis sp.	Solitary/Communal
Isepeolus cortesi	Parasitic
Isepeolus atripilis	Parasitic
Isepeolus luctuosus	Parasitic
Isepeolus wagenknechti	Parasitic
Melectoides bellus	Parasitic
Thyreus delumbatus	Parasitic
Zacosmia maculata	Parasitic
Xeromelecta californica	Parasitic
Tetralonioidella sp.	Parasitic
Melecta albifrons	Parasitic
Melipona sp.	Advanced eusocial
Tetragonula carbonaria	Advanced eusocial
Meliponula ferruginea	Advanced eusocial

Table A2.1. (Continued)

Plebeina hildebrandti	Advanced eusocial
Hypotrigona gribodoi	Advanced eusocial
Meliponula ferruginea	Advanced eusocial
Scaptotrigona hellwegeri	Advanced eusocial
Lestrimelitta sp.	Advanced eusocial
Scaura latitarsis	Advanced eusocial
Trigona fuscipennis	Advanced eusocial
Cephalotrigona capitata	Advanced eusocial
Meliponula bocandei	Advanced eusocial
Meliponula bocandei	Advanced eusocial
Epeoloides pilosula	Parasitic
Parepeolus aterrimus	Parasitic
Epeoloides coecutiens	Parasitic
Osiris sp. 1	Parasitic
Leiopodus singularis	Parasitic
Leiopodus sp.	Parasitic
Leiopodus abnormis	Parasitic
Leiopodus trochantericus	Parasitic
Rhathymus sp.	Parasitic
Nanorhathymus sp.	Parasitic
Rhathymus unicolor	Parasitic
Caenonomada sp.	Solitary/Communal
Paratetrapedia sp.	Solitary/Communal
Tapinotaspidini sp.	Solitary/Communal
Tapinotaspoides sp.	Solitary/Communal
Arhysoceble sp.	Solitary/Communal
Tetrapedia maura	Solitary/Communal
Coeleoxoides sp.	Parasitic
Tetrapedia (Lagobata) sp.	Solitary/Communal
Tetrapedia (Tetrapedia) sp.	Solitary/Communal
Coelioxoides waltheriae	Parasitic
Oreopasites barbarae	Parasitic
Sphecodopsis capensis	Parasitic
Ammobates sp.	Parasitic
Ammobates punctatus	Parasitic
Pasites maculatus	Parasitic
Holcopasites ruthae	Parasitic
Holcopasites calliopsidis	Parasitic
Holcopasites arizonicus	Parasitic

Table A2.1. (Continued)

Holcopasites insoletus	Parasitic
Ammobatoides luctuosus	Parasitic
Holcopasites stevensi	Parasitic
Holcopasites minimus	Parasitic
Neopasites cressoni	Parasitic
Biastes truncatus	Parasitic
Paranomada velutina	Parasitic
Triopasites penniger	Parasitic
Brachynomada margaretae	Parasitic
Brachynomada sp.	Parasitic
Caenoprosopina holmbergi	Parasitic
Caenoprosopis crabronina	Parasitic
Triepeolus robustus	Parasitic
Epeolus scutellaris	Parasitic
Odyneropsis sp.	Parasitic
Thalestria spinosa	Parasitic
Doeringiella sp.	Parasitic
Epeolus sp.	Parasitic
Rhinepeolus rufiventris	Parasitic
Epeolus variegatus	Parasitic
Hexepeolus rhodogyne	Parasitic
Neolarra orbiculata	Parasitic
Nomada maculata	Parasitic
Nomada signata	Parasitic
Townsendiella sp.	Parasitic
Exoneura bicolor	Primitively eusocial
Braunsapis madecassella	Primitively eusocial
Compsomelissa keiseri	Primitively eusocial
Macrogalea ellioti	Primitively eusocial
Ceratina calcarata	Solitary/Communal
ceratina (Crewella) sp.	Solitary/Communal
Ceratina (Simioceratina) sp.	Solitary/Communal
Ceratina (Ceratinula) sp.	Solitary/Communal
Ceratina (Xanthoceratina) sp.	Solitary/Communal
Ceratina cyanea	Solitary/Communal
Manuelia gayatina	Solitary/Communal
Xylocopa tabaniformis	Solitary/Communal
Xylocopa virginica	Solitary/Communal
Xylocopa muscaria	Solitary/Communal

Table A2.1. (Continued)

Xylocopa iris	Solitary/Communal
Xylocopa (Proxylocopa) sp.	Solitary/Communal
Xylocopa pubescens	Solitary/Communal
Xylocopa violacea	Solitary/Communal
Xylocopa fimbriata	Solitary/Communal
Xylocopa californica arizonensis	Solitary/Communal
Fideliopsis major	Solitary/Communal
Neofidelia sp.	Solitary/Communal
Pararhophites quadratus	Solitary/Communal
Paranthidium jugatorium	Solitary/Communal
Anthidiellum notatum	Solitary/Communal
Anthidium porterae	Solitary/Communal
Trachusa larreae	Solitary/Communal
Dianthidium subparvum	Solitary/Communal
Stelis linsleyi	Parasitic
Dioxys pomonae	Parasitic
Lithurgus echinocacti	Solitary/Communal
Trichothurgus herbsti	Solitary/Communal
Microthurge sp.	Solitary/Communal
Coelioxys alternata	Parasitic
Megachile angelarum	Solitary/Communal
Megachile ericetorum	Solitary/Communal
Protosmia rubifloris	Solitary/Communal
Hoplitis albifrons	Solitary/Communal
Heriades crucifer	Solitary/Communal
Osmia lignaria	Solitary/Communal
Chelostoma californica	Solitary/Communal
Ashmeadiella aridula	Solitary/Communal
Hoplosmia scutellaris	Solitary/Communal
Afroheriades hyalinus	Solitary/Communal
Dasypoda argentata	Solitary/Communal
Hesperapis larreae	Solitary/Communal
Promelitta alboclypeata	Solitary/Communal
Meganomia binghami	Solitary/Communal
Macropis nuda	Solitary/Communal
Melitta arrogans	Solitary/Communal

Bayesian ancestral state reconstruction methods were implemented in BayesTraits v.1.0 (Pagel et al. 2004). We used a random sample of 10 000 trees from the Bayesian analysis of the phylogeny. We initially ran a likelihhod analysis with no constraints on the rate of transitions among-states in order to get reasonable starting values (priors) for the Bayesian MCMC analysis, as recommended in the manual. All of the transition rates were below 2.8, with most being below 1. We therefore ran the MCMC analysis with all transition rate priors having an exponential distribution. We used a uniform distribution whose values ranged from 0 to 10 to seed the mean of the exponential distribution, giving us exponentially distributed hyperpriors. Some transition rates were fixed to 0 as discussed in the paper. We ran the analysis several times for 2 million generations and discarded the first 1 million as burnin. Examination of the output files indicated that our burnin was sufficient because parameter estimates were stable over the post-burnin period. Allowing a non-zero rate of transition from solitary to advanced eusociality reconstructed the ancestral state of the corbiculates as being primitively eusocial with a probability of 86%.

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CHAPTER THREE

Simultaneous origins of bees and eudicots: implications for Darwin's abominable mystery

Abstract

Bees are important pollinators of flowering plants in both natural and agro-ecosystems. Because of the close association between bees and angiosperms, it has been proposed that bees played a role in the angiosperms' rise to dominance during the lower late Cretaceous. To determine if bees played a role in angiosperm diversification, we first need to establish the extent of temporal overlap in their diversifications. Much work has been done on dating the major divergence events in angiosperms, but little is known on the dates of origin of the major bee lineages. To date the origin of bees and their major clades, we first perform a phylogenetic analysis of bees including representatives from every family, subfamily, and almost all tribes, using sequence data from seven genes. We then use this phylogeny to place 14 time calibration points based on information from the fossil record for a relaxed clock divergence time analysis. We estimate that bees originate at the start of the Aptian, concurrently with the origin of the eudicots. All of the major bee clades are estimated to have originated during the middle to late Cretaceous, which is when angiosperms became the dominant group of land plants.

Introduction

Angiosperms dominate terrestrial ecosystems with over 250 000 species. Perplexed by what appeared to be the sudden appearance of modern flowers in the fossil record, Darwin referred to this great diversity as an "abominable mystery"

(Friedman 2009). Angiosperms became dominant in species numbers during the lower late Cretaceous (especially during the Turonian age) (Crepet 2008). The matter of angiosperm success has received much attention and continues to be a highly debated and controversial area of research (summarized in Crepet (2008)). The presence of characters associated with advanced modes of insect pollination in early fossil angiosperms, and the finding that many basal eudicot families have specialized pollination modes, suggests that specialized pollination modes may have been present during the mid-Cretaceous angiosperm diversification (Hu et al. 2008). More specifically, some Turonian angiosperm flowers have morphologies and characters now associated with bee pollinators (Crepet 2008) suggesting that bees were involved in the diversification of angiosperms. It has been proposed that bees could have caused rapid and extensive speciation in angiosperms through ethological and floral reproductive isolation (Grant 1949; Grant 1994).

A first step in determining whether bees contributed to the diversity of flowering plants seen today is to establish that they were present and diversifying at the same time as the rapid increase in angiosperm diversity. A divergence time analysis of ants indicates that ants diversified during the early to mid-Cretaceous, coincident with angiosperm diversification (Moreau et al. 2006). Angiosperms have also been hypothesized to have played an important role in beetle diversification because angiosperm-feeding beetle lineages appear to have undergone more rapid diversification than their gymnosperm feeding sister taxa (Farrell 1998), but see Hunt et al. (2008).

Determining whether there is co-occurrence in the diversification of bees and angiosperms requires reliable estimates on the dates of divergence events in both groups. Much research has been focused on dating the origins of the major angiosperm clades. Various estimates have been obtained for the same divergence events using different dating methods including minimum age node mapping (Crepet et al. 2004), a supertree calibrated with sequence data and fossils (Davies et al. 2004), and relaxed molecular clock analyses (Wikstrom et al. 2001; Magallon and Castillo 2009). Despite these discrepancies, most authors now seem to accept an age for crown group angiosperms of approximately 130 Ma (Davies et al. 2004; Soltis and Soltis 2004; Magallon and Castillo 2009). The fossil record for angiosperms extends back to the early Cretaceous (Crane et al. 2004). The oldest angiosperm macrofossil, *Archaefructus liaoningensis*, is ~125 Ma (Sun et al. 2002). However, pollen believed to be of angiosperm origin has been dated to ~132 Ma (Brenner and Bickoff 1992; Brenner 1996). Phylogenetic placement of *Archaefructus* has been debated, with some authors placing it in a new basal angiosperm family Archaefructaceae (Sun et al. 2002), and others placing it nearer to the base of the eudicots (Friis et al. 2003).

Eudicots represent ~ 75% of the diversity of flowering plants (Soltis et al. 2005), and a large proportion of families are highly dependent on bees for pollination services. Similarly, bees rely heavily on eudicots for pollen, nectar, and oil resources. We therefore predict that the origin and diversification of bees should coincide more specifically with that of the eudicots. The age of the eudicot crown group is estimated to be ~125 Ma based on the appearance of tricolpate pollen grains in late Barremian/early Aptian sediments (Friis et al. 2006; Magallon and Castillo 2009).

In contract to angiosperms, efforts in dating the major clades of bees using information from both sequence and fossil data have been lacking. To date, there have only been age estimates using such methods for a few groups of bees (Danforth et al. 2004; Brady et al. 2006; Hines 2008; Schaefer and Renner 2008). Estimation of divergence events in bees has been hampered by a previous lack of well supported phylogenies and a scarcity of bee fossils. During the past two decades, great advances in both of these areas have been made, making this an opportune time to incorporate information from phylogenetic hypotheses, the fossil record, and molecular rates of evolution, into estimates of the ages of major bee clades. Phylogenetic analyses combining morphological and molecular data have resolved the relationships among bee families with most nodes in the tree being well supported (Danforth et al. 2006). Also, numerous bee fossils have now been recovered and described (e.g. (Engel 1995; Engel 1996; Engel 1999a; Engel 1999b; Engel 2001; Poinar and Danforth 2006; Michez et al. 2007; Michez et al. 2008; Engel et al. 2009).

Despite the recent publication of numerous new bee fossil discoveries, the bee fossil record remains sparse and highly biased towards resin collecting bees. The oldest described fossil bee, *Melittosphex burmensis*, is from Burmese amber estimated to be 100 to 110 Ma (Poinar and Danforth 2006). This bee appears to be morphologically intermediate between bees and crabronid wasps (the putative sister group to bees), and does not seem to belong to any extant family of bees. Therefore, this fossil cannot be used as a minimum age estimate for the crown group of bees, as it may represent a stem lineage. The oldest fossil that can be attributed to the crown group of bees with confidence is *Cretotrigona prisca* from the Late Cretaceous amber of New Jersey (Michener and Grimaldi 1988). The exact age and phylogenetic affinity of this fossil have been controversial (see calibration node 5 in Methods section for a detailed account). But this bee is most certainly a stingless bee belonging to the crown group or stem lineage of Meliponini (Michener and Grimaldi 1988; Engel 2000a), a tribe within Apidae and not near the base of the bee phylogeny. This fossil is now treated as being of Maastrichtian age (~65 Ma) (Engel 2000a). The lack of crown group bee fossils from the Cretaceous favors the hypothesis of a Tertiary radiation of bees. However, the existence of a ca. 65 Ma stingless bee suggests the possibility that many of the major bee clades were already present by the end of the Cretaceous and that bees radiated during the early Cretaceous, coincident with the diversification of the eudicots.

An incomplete fossil record inherently underestimates the ages of clades. Using molecular substitution rate models calibrated with fossil data is a way to more accurately estimate divergence events. A number of methods have been developed to estimate divergence dates under models that relax the strict molecular clock constraint (Sanderson 1997; Thorne et al. 1998). More recently, a Bayesian Markov chain Monte Carlo (MCMC) method has been introduced for performing relaxed phylogenetics in which the phylogeny and the divergence dates are co-estimated under a relaxed molecular clock using probabilistic calibration priors (Drummond et al. 2006). Simulations have found this approach to outperform previous methods of dating (Drummond et al. 2006).

We expand the molecular dataset of Danforth et. al (2006), by adding 2 new protein coding genes and adding 75 more taxa (for a total of 7 genes and 168 taxa) to see if the family-level relationships found in this previous study are robust to the addition of new data (taxa and genes). The addition of these new taxa allowed us to use information from the fossil record to time calibrate 14 nodes in the phylogeny and estimate divergence times of the major bee clades using the relaxed phylogenetics approach implemented in BEAST (Drummond and Rambaut 2007). We then compare our age estimates for bees with those of angiosperms to see if there is a correspondence in timing of major divergence events in bees and angiosperms.

Methods

<u>*Taxon sampling*</u>

We sampled representatives from all subfamilies of bees and 91% of tribes (Michener 2007). Our dataset therefore represents all major lineages of bees with 152 species included. As outgroups, representatives from all 3 subfamilies of Sphecidae and 4 of the 5 subfamilies of Crabronidae were included, because bees appear to have arisen from within the “spheciform” wasps (Michener 2007). The aculeate wasp family Crabronidae forms the sister group to bees (Lomholdt 1982; Melo 1999).

Most specimens used for sequencing were killed using cyanide and preserved in either 95% EtOH or in an airtight vile with Drierite, but pinned specimens up to 5 years old were also used. Table 3.1 lists all of the species included in the study along with taxonomic, voucher, and locality information. *DNA Extraction*

DNA extractions followed standard phenol-chloroform protocols (Danforth et al. 1999), with the elimination of the use of liquid nitrogen and RNase which were both found to be unnecessary. Tissue samples were taken from either 1-3 leg(s), the thoracic muscles or the entire thorax depending on the size, and the state of preservation of the bee.

Data

The dataset consists of sequences of two nuclear ribosomal genes (18S, 28S), and 5 nuclear protein-coding genes (wingless, pol II, opsin, Nak, Ef1α). Genes were selected based on their previously demonstrated ability to resolve phylogenetic relationships in other insect groups of various ages (see Chapter 1). We amplified an ~900bp fragment which spans 18S helices H367-H960 of *Apis mellifera* (Gillespie et al. 2006). We sequenced an ~1500bp fragment from the large subunit 28S rRNA locus spanning regions D1-D5 and helices H234-H1011 of *Apis mellifera* (Gillespie et al. 2006). We sequenced an ~500bp intronless fragment of the Wingless-1 (wnt-1) paralogue. We sequenced a ~900bp fragment of RNA Polymerase II (Pol II) which lacks introns. We amplified a ~750pb fragment of LW opsin which spanned two introns. Because the intron regions could not be aligned unambiguously, they were excluded from the phylogenetic analysis. We sequenced an intronless region of

Table 3.1. Taxonomic, voucher, and locality information for species included in the study.

Voucher #	Family	Subfamily	Tribe	Species	Collecting data
sc250	Apidae	Apinae	Ancylini	*Ancyla anatolica* Warncke, 1979	Turkey: Adana Prov.
504	Apidae	Apinae	Anthophorini	*Anthophora urbana* Cresson, 1878	USA: California, Santa Clara Co., Del Puerto Canyon. 27.v.1999
985	Apidae	Apinae	Anthophorini	*Pachymelus peringueyi* (Friese, 1911)	South Africa: NCP, 14km E. Kamiesksroon, 16.ix.2001
apis_cer	Apidae	Apinae	Apini	*Apis cerana* Fabricius, 1793	Japan: Kyoto
apis_dor	Apidae	Apinae	Apini	*Apis dorsata* Fabricius, 1793	Laos: Laksao
apis_flo	Apidae	Apinae	Apini	*Apis florea* Fabricius, 1787	Loas: Mahaxai
Bom_ard	Apidae	Apinae	Bombini	*Bombus ardens* Smith, 1879	Japan: Kyoto
Bom_div	Apidae	Apinae	Bombini	*Bombus diversus* Smith, 1869	Japan: Kyoto
Bom_men	Apidae	Apinae	Bombini	*Bombus mendax* Gerstäcker, 1869	Italy: Monte Rosa
503	Apidae	Apinae	Centridini	*Centris hoffmanseggiae* Cockerell, 1897	USA: California, Kern Co.,5mi S. Mojave. 13.vi.1999
sc120	Apidae	Apinae	Centridini	*Epicharis analis* Lepeletier, 1841	Paraguay: Paraguarí, Salto Cristal. 10.ii.2007
983	Apidae	Apinae	Ctenoplectrini	*Ctenoplectra albolimbata* Magretti, 1895	South Africa: KZN, 20km N. Hluhluwe. 9-12.iiv.2002
490	Apidae	Apinae	Emphorini	*Diadasia bituberculata* (Cresson, 1878)	USA: California, Contra Costa Co., Mitchell canyon. 5.vi.1999
sc148	Apidae	Apinae	Emphorini	*Diadasina distincta* (Holmberg, 1903)	Paraguay: Boquerón, 2km NE. Filadélfia. 6.ii.2007
616	Apidae	Apinae	Ericrocidini	*Ericrocis lata* (Cresson, 1878)	USA: Arizona, Cochise Co., 2mi N. Rodeo. 8.xi.1999
491	Apidae	Apinae	Eucerini	*Eucera frater* (Cresson, 1878)	USA: California, Contra Costa Co., Lafayette. 26.v.1999
485	Apidae	Apinae	Eucerini	*Melissodes desponsa* Smith, 1854	USA: New York, Tompkins Co., Ithaca. 29.vii.1997
sc225	Apidae	Apinae	Eucerini	*Svastrina subapicalis* (Brèthes, 1910)	Argentina: Corrientes Prov., Mburucuyá env. 26-27.I.2006
623	Apidae	Apinae	Eucerini	*Xenoglossa angustior* Cockerell, 1899	USA: Arizona, Cochise Co., 2mi N. Portal. 11.ix.1999
sc229	Apidae	Apinae	Euglossini	*Aglae caerulea* Lepeletier & Audinet-Serville, 1825	Peru: Lagunas, Loreto

Table 3.1. (Continued)

sc23	Apidae	Apinae	Euglossini	*Eufriesea surinamensis* (Linnaeus, 1758)	French Guiana: Kaw, Rt D6, 30.xi.2006
Eugl_imp	Apidae	Apinae	Euglossini	*Euglossa imperialis* Cockerell, 1922	Panama
sc14	Apidae	Apinae	Euglossini	*Eulaema meriana* (Olivier, 1789)	French Guiana: Maripalousa, Saül, 4.vii.2006
627	Apidae	Apinae	Exomalopsini	*Anthophorula completa* (Cockerell, 1935)	USA: Arizona, Cochise Co., Comm. Rd. 12.ix.1999
sc253	Apidae	Apinae	Exomalopsini	*Exomalopsis* sp.	Argentina: Salta Prov.
1359	Apidae	Apinae	Isepeolini	*Isepeolus atripilis* Roig-Alsina, 1991	Chile: Curico prov., Laguna de Teno
sc218	Apidae	Apinae	Melectini	*Tetralonioidella* sp.	Thailand: Chiang Mai, Doi Inthonun NP checkpoint 2. 22.vii-2.viii.2006
499	Apidae	Apinae	Melectini	*Xeromelecta californica* (Cresson, 1878)	USA: California, Santa Clara Co., Del Puerto Canyon. 27.v.1999
ceph_cap	Apidae	Apinae	Meliponini	*Cephalotrigona capitata* (Smith, 1854)	Costa Rica
1040	Apidae	Apinae	Meliponini	*Hypotrigona gribodoi* (Magretti, 1884)	South Africa: Limpopo Prov., 27km E. Waterpoort. 7.i.2004
1042	Apidae	Apinae	Meliponini	*Meliponula ferruginea*	South Africa: Limpopo Prov. Soutpansberg Mts. 9.i.2004
scau_lat	Apidae	Apinae	Meliponini	*Scaura latitarsis* (Friese, 1900)	not available
1364	Apidae	Apinae	Osirini	*Epeoloides pilosula* (Cresson, 1878)	USA: Connecticut, New London Co., Bozrah Rt 163. 22.vi.2006
sc248	Apidae	Apinae	Osirini	*Osiris* sp. 1	Peru
sc20	Apidae	Apinae	Osirini	*Parepeolus aterrimus* (Friese, 1906)	French Guiana: Kourou
sc107	Apidae	Apinae	Protepiolini	*Leiopodus* sp.	Paraguay: Presidente Hayes, Campo Maria. 5.ii.2007
1330	Apidae	Apinae	Rhathymini	*Nanorhathymus* sp.	West Indies: Trinidad, Cauara Valley. 17.vii.2003
sc127	Apidae	Apinae	tapinotaspidini	*Caenonomada* sp.	Paraguay: Concepción, Vallemi. 1.ii.2007
sc196	Apidae	Apinae	tapinotaspidini	*Arhysoceble picta* (Friese, 1899)	Paraguay: Paraguarí, Caballero. 21.i.2007
sc235	Apidae	Apinae	tetrapediini	*Coelioxoides waltheriae* Ducke, 1908	Argentina: Jujuy Prov.

Table 3.1. (Continued)

1340	Apidae	Apinae	Tetrapediini	*Tetrapedia maura* Cresson, 1878	Mexico: Estado de Jalisco, Reserva Biosfera Chamela-Cuixmala. 1.ix.2004
637	Apidae	Nomadinae	Ammobatini	*Oreopasites barbarae* Rozen, 1992	USA: Arizona, Cochise Co., Apache 14 mi SW. 10.ix.1999
896	Apidae	Nomadinae	Ammobatini	*Sphecodopsis capensis* (Friese, 1915)	South Africa: NCP, Kamieskroon. 16.ix.2001
519	Apidae	Nomadinae	Ammobatoidini	*Holcopasites calliopsidis* (Linsley, 1943)	USA: New York, Schuyler Co. Valois. 18.vi.1999
sc195	Apidae	Nomadinae	Biastini	*Neopasites cressoni* Crawford, 1916	Mexico: Sonora, 30 km E Aqua Prieta. 4.iv.2005
1380	Apidae	Nomadinae	Brachynomadini	*Brachynomada margaretae* (Rozen 1994)	AZ: Cochise Co., 14 mi. SE Apache 25.iix.2006
sc214	Apidae	Nomadinae	Caenoprosopidini	*Caenoprosopis crabronina* Holmberg, 1887	Argentina: Salta Prov., Cachi env. 4-8.ii.2006
489	Apidae	Nomadinae	Epeolini	*Epeolus scutellaris* Say, 1824	NY: Tompkins Co. Ithaca. 22.iix.1997
sc114	Apidae	nomadinae	epeolini	*Thalestria spinosa* (Fabricius, 1804)	Paraguay:San Pedro, 30km S. Cororo, 27.i.2007
1363	Apidae	Nomadinae	Hexepeolini	*Hexepeolus rhodogyne* Linsley & Michener, 1937	USA: Arizona, Pima Co.
1379	Apidae	Nomadinae	Neolarrini	*Neolarra orbiculata* Shanks, 1978	USA: Arizona: Cochise Co., 2 mi E Willcox 1.ix.2004
501	Apidae	Nomadinae	Nomadini	*Nomada maculata* Cresson, 1863	USA: New York, Tompkins Co., Ithaca. 3.v.1999
as72	Apidae	Nomadinae	Townsendiellini	*Townsendiella* sp.	USA: California
1166	Apidae	Xylocopinae	Allodapini	*Braunsapis madecassella* Michener, 1977	Madagascar: 4km N. Tulear. 17.x.2004
679	Apidae	Xylocopinae	Allodapini	*Exoneura bicolor* Smith, 1854	Australia: VIC Flowerdale Forest. 20.xi.1999
1171	Apidae	Xylocopinae	Allodapini	*Macrogalea ellioti* (Saussure, 1890)	Madagascar: Ifaty, N. Tulear. 17.x.2004
sc168	Apidae	xylocopinae	ceratinini	*Ceratina* (Crewella) sp.	Paraguay: Cordillera, 6km SW Pirebebuy. 16.i.2007
sc194	Apidae	Xylocopinae	ceratinini	*Ceratina* (Simioceratina) sp.	South Africa: NP, 29km NW. Waterpoort. 17.iiv.2002

Table 3.1. (Continued)

1255	Apidae	Xylocopinae	Manueliini	*Manuelia gayatina* (Spinola, 1851)	Chile: Reg VIII, Entrepiernas, W. of Recinto, 8.xii.2004
sc212	Apidae	Xylocopinae	Xylocopini	*Xylocopa pubescens* Spinola, 1838	TUNISIA: Blidette vill. 25-27.iii.2006
500	Apidae	Xylocopinae	Xylocopini	*Xylocopa tabaniformis* Smith, 1854	USA: California, Santa Clara Co., Mt. Hamilton. 27.v.1999
sc211	Apidae	Xylocopinae	Xylocopini	*Xylocopa* (*Proxylocopa*) sp.	Turkey: Gaziantep prov., Birecik env. 13.iix.2006
sc57	Apidae	Xylocopinae	Xylocopini	*Xylocopa californica arizonensis* Cresson, 1879	USA: Arizona, Cochise Co., 14 mi W. Apache. 6.ix.2006
948	Megachilidae	Fideliinae	Fideliini	*Fidelia major*	SOUTH AFRICA: WCP, 5 km N. Clanwilliam 20.ix.2001
802	Megachilidae	Fideliinae	Fideliini	*Neofidelia* sp.	Chile: Atacama Prov., Inca-havas 5 km N. 3.x.1997
sc231	Megachilidae	Fideliinae	Pararhophitini	*Pararhophites quadratus* (Friese, 1898)	Tunisia
630	Megachilidae	Megachilinae	Anthidiini	*Anthidiellum notatum* (Latreille, 1809)	USA: Arizona, Cochise Co., Comm. Rd. 12.ix.1999
645	Megachilidae	Megachilinae	Anthidiini	*Anthidium porterae* Cockerell, 1900	USA: New Mexico, Hidalgo Co. 20 mi S. Animas. 17.ix.1999
1267	Megachilidae	Megachilinae	Anthidiini	*Dianthidium subparvum* Swenk, 1914	USA: Utah, Cache Co. BSFC. 5.iix.2003
495	Megachilidae	Megachilinae	Anthidiini	*Paranthidium jugatorium* (Say, 1824)	NY: Tompkins Co. Ithaca. 31.vii.1997
1271	Megachilidae	Megachilinae	anthidiini	*Stelis linsleyi* Timberlake, 1941	USA: California, Madera Co. Yosemite N.P. 11.vii.2005
1142	Megachilidae	Megachilinae	Anthidiini	*Trachusa larreae* (Cockerell, 1897)	USA: Nevada, Clark Co. Las Vegas Dunes. 1.iv.2004
1152	Megachilidae	Megachilinae	Dioxyini	*Dioxys pomonae* Cockerell, 1910	USA: Nevada, Clark Co. McCullough Mts. 12.v.2004
863	Megachilidae	Megachilinae	Lithurgini	*Lithurgus echinocacti* Cockerell, 1898	USA: Arizona, Pima Co., Tucson. 4.iix.2000
sc207	Megachilidae	Megachilinae	Lithurgini	*Microthurge* sp.	Argentina: Jujuy Prov., Libertador General San Martín env. 2-3.ii.2006
1275	Megachilidae	Megachilinae	Lithurgini	*Trichothurgus herbsti* (Friese, 1905)	Chile: Region VIII, Las Trancas, 78 km E. Chillan. 12.xii.2003

Table 3.1. (Continued)

487	Megachilidae	Megachilinae	Megachilini	*Coelioxys alternata* Say, 1837	USA: New York, Tompkins Co., Ithaca. 29.vii.1997
1283	Megachilidae	Megachilinae	Megachilini	*Megachile angelarum* Cockerell, 1902	USA: Nevada, Clark Co., 2.5 mi S. Wheeler Well. 30.vi.2004
1281	Megachilidae	Megachilinae	Osmiini	*Afroheriades hyalinus*	South Africa: WCP, 7 km W. Nieuwoudtville.. 9.iix.2002
1270	Megachilidae	Megachilinae	Osmiini	*Ashmeadiella aridula* Cockerell, 1910	USA: Utah, Garfield Co., Long Canyon, 1.ix.2003
1269	Megachilidae	Megachilinae	Osmiini	*Chelostoma californica*	USA: California, Mariposa Co., El Portal. 12.iv.2004
1149	Megachilidae	Megachilinae	Osmiini	*Heriades crucifer* Cockerell, 1897	USA: Arizona, Cochise Co, Chiricahua Mts. 25.iix.2003
507	Megachilidae	Megachilinae	Osmiini	*Hoplitis albifrons* (Kirby, 1837)	USA: California, Contra Costa Co., Donner canyon. 30.v.1999
1280	Megachilidae	Megachilinae	Osmiini	*Hoplosmia scutellaris* (Morawitz, 1868)	GREECE: Lesvos, 1 km N Mytilene. 6.v.2004
1265	Megachilidae	Megachilinae	Osmiini	*Osmia lignaria* Say, 1837	not available
497	Megachilidae	Megachilinae	Osmiini	*Protosmia rubifloris* (Cockerell, 1898)	USA: California, Santa Clara Co., San Antonio summit. 28.v.1999
973	Melitidae	Dasypodainae	Dasypodaini	*Dasypoda argentata* Panzer, 1809	France: Gard, Générac, 22.vi.2002
977	Melitidae	Dasypodainae	Dasypodaini	*Dasypoda visnaga*	FRANCE: Herault, Valras Plage. 27.vi.2002
975	Melitidae	Dasypodainae	Dasypodaini	*Dasypoda hirtipes*	FRANCE: Gard, Generac. 22.vi.2002
488	Melitidae	Dasypodainae	Dasypodaini	*Hesperapis larreae* Cockerell, 1907	USA: California, Los Angeles Co., Palmada. 13.vi.1999
940	Melitidae	Dasypodainae	Dasypodaini	*Hesperapis richtersveldensis*	SOUTH AFRICA: NCP, Richtersveld National Park. 12.ix.2001
sc230	Melitidae	Dasypodainae	Promelittini	*Promelitta alboclypeata* (Friese, 1900)	United Arab Emirates
939	Melitidae	Dasypodainae	Sambini	*Haplomelitta griseonigra*	SOUTH AFRICA: WCP, 5 km S. Clanwilliam 7.ix.2001
1021	Melitidae	Meganomiinae		*Meganomia binghami* (Cockerell, 1909)	South Africa: Limpopo Prov., 8.5 km N. Vivo. 7.i.2004
17ja	Melitidae	Melittinae	Melittini	*Macropis nuda* (Provancher, 1882)	USA: NY, Rensselaer Co., Rensselaerville, Huyck Nature Preserve

Table 3.1. (Continued)

980	Melitidae	Melittinae	Melittini	*Macropis europaea*	FRANCE: Herault, Portiragnes. 27.vi.2002
942	Melitidae	Melittinae	Melittini	*Melitta arrogans* (Smith, 1879)	South Africa: NCP, 76 km S. Port Nolloth. 11.ix.2001
508	Melitidae	Melittinae	Melittini	*Melitta eickworti*	USA: New York, Tompkins Co., Ithaca 25.vi.1997
981	Melitidae	Melittinae	Melittini	*Melitta leporina*	FRANCE: Aude, Port la Nouvelle. 17.vi.2002
946	Melitidae	Melittinae	Melittini	*Redivivoides simulans*	SOUTH AFRICA: WCP, 5 km N. Clanwilliam. 20.ix.2001
945	Melitidae	Melittinae	Melittini	*Rediviva mcgregori*	SOUTH AFRICA: NCP, Dassiefontein, Kamieskroon 16.ix.2001
643	Andrenidae	Andreninae		*Andrena brooksi*	USA: Arizona, Cochise Co., Animas, 20 mi S, 17.ix.1999
31ja	Andrenidae	Andreninae		*Megandrena enceliae*	not available
49ja	Andrenidae	Alocandreninae		*Alocandrena porterae*	not available
226	Andrenidae	Oxaeinae		*Protoxaea gloriosa*	USA: Arizona, Portal.
509	Andrenidae	Panurginae	Calliopsini	*Calliopsis pugionis*	USA: California, Riverside Co., San Jacinto WA 18.v.93
597	Andrenidae	Panurginae	Calliopsini	*Calliopsis anthidia*	USA: California, Santa Clara Co., Mt. Hamilton 28.v.1999
959	Andrenidae	Panurginae	Melitturgini	*Melitturga clavicornis*	FRANCE: Herault, Causse de la Selle. 17.vi.2002
514	Andrenidae	Panurginae	Panurgini	*Panurgus calcarata*	Italy: Rome 7.vi.1998
450	Colletidae	Colletinae	Colletini	*Colletes inaequalis*	USA, New York, Tompkins Co., Ithaca
687	Colletidae	Colletinae	Paracolletini	*Callomelitta antipodes*	Australia: NSW, Guyra, 74km E. 5.xii.1999
702	Colletidae	Colletinae	Paracolletini	*Leioproctus fimbriatinus*	Australia: VIC, Hattah, 12 km E. 6.i.1999
708	Colletidae	Colletinae	Paracolletini	*Trichocolletes* sp.	Australia: NSW, Oberon, 53 km S. 30.xi.1999
706	Colletidae	Colletinae	Paracolletini	*Leioproctus plumosus*	Australia: VIC, Torquay 19.xi.1999
568	Colletidae	Colletinae	Paracolletini	*Leioproctus delahozii*	CHILE: Elqui Prov., Llano de la Hignera
848	Colletidae	Diphaglossinae	Caupolicanini	*Caupolicana vestita*	Chile: Region I, Arica Playas las Machas

Table 3.1. (Continued)

654	Colletidae	Diphaglossinae	Caupolicanini	*Caupolicana yarrowi*	USA: New Mexico, Hidalgo Co., 20 mi S Animas, 24.ix.1999
850	Colletidae	Diphaglossinae	Diphaglossini	*Diphaglossa gayi*	Chile: Region X, Aguas Calientes
688	Colletidae	Euryglossinae		*Euryglossa calliopsella*	Australia: VIC, Yan Yaen 20.xi.1999
692	Colletidae	Euryglossinae		*Euryglossina globuliceps*	Australia: VIC, Colquhuon State Forest. 26.xi.1999
709	Colletidae	Euryglossinae		*Xanthesma furcifera*	Australia: VIC, Patchewollock. 10.xii.1999
697	Colletidae	Hylaeinae		*Hylaeus elegans*	Australia: SA, Kimba, 10 km E. 5.i.1999
698	Colletidae	Hylaeinae		*Hylaeus amiculus*	Australia: SA, Kimba, 10 km E. 5.i.1999
699	Colletidae	Hylaeinae		*Hylaeus proximus*	Australia: SA, Kimba, 10 km E. 5.i.1999
903	Colletidae	Scrapterinae		*Scrapter heterodoxus*	SOUTH AFRICA: WCP, 31 km S. Clanwilliam. 7.ix.2001
937	Colletidae	Scrapterinae		*Scrapter ruficornis*	SOUTH AFRICA: WCP, Kunje Farm, Citrusdal 23.ix.2001
905	Colletidae	Scrapterinae		*Scrapter niger*	SOUTH AFRICA: WCP, 21 km N. Hermanus. 28.ix.2001
901	Colletidae	Scrapterinae		*Scrapter erubescens*	SOUTH AFRICA: WCP, Pakhuis Pass. 8.ix.2001
800	Colletidae	Xeromelissinae	Chilicolini	*Chilicola styliventris*	Peru: Junin Dept., Tarma 22.x.1999
857	Colletidae	Xeromelissinae	Xeromelissini	*Chilimelissa rozeni*	Chile: Region III, Panamerican Hwy. Km 1005 NE Chanaral
851/852	Colletidae	Colletinae	Paracolletini	*Leioproctus bathycyaneus*	Chile: Region III, Santa Juana, E. of Vallenar
1015	Stenotritidae			*Stenotritus* sp.	AUSTRALIA: WA, 23 km SW Coorow, 17.xi.1997
122	Stenotritidae			*Ctenocolletes smaragdinus*	not available
592	Halictidae	Halictinae	Augochlorini	*Augochlorella pomoniella*	USA: California, Inyo Co., Big Pine 15.vi.1999
334	Halictidae	Halictinae	Augochlorini	*Augochloropsis metallica*	USA: New York, Tompkins Co., Ithaca
574	Halictidae	Halictinae	Caenohalictini	*Ruizantheda mutabilis*	CHILE: Region VIII, Angol (nr. Temuco)
230	Halictidae	Halictinae	Caenohalictini	*Agapostemon tyleri*	USA: Arizona, Cochise Co., Portal

Table 3.1. (Continued)

32/447	Halictidae	Halictinae	Halictini	*Halictus rubicundus*	USA: Montana, Missoula Co., Missoula 15.iv.1994
98	Halictidae	Halictinae	Halictini	*Mexalictus arizonensis*	USA: Arizona, Cochise Co., Miller Cyn.
299	Halictidae	Halictinae	Halictini	*Lasiglossum hybodinum*	Australia: SA, 6km E. SA/WA border
950	Halictidae	Halictinae	Halictini	*Zonalictus abessinicus*	SOUTH AFRICA: Free State, Harrismith 6.iii.2002
337	Halictidae	Halictinae	Sphecodini	*Sphecodes ranunculi*	USA: New York, Tompkins Co., Ithaca
207	Halictidae	Nomiinae		*Dieunomia nevadensis*	USA: Arizona, Cochise Co., Portal
241	Halictidae	Nomiinae		*Pseudapis albocincta*	Spain: Almeria Prov.
243	Halictidae	Nomioidinae		*Nomioides facilis*	Spain: Almeria Prov.
572	Halictidae	Rophitinae	Penapini	*Penapis penai*	CHILE: Huasco Prov., N. Vallenar
968	Halictidae	Rophitinae	Rophitini	*Rophites algirus*	FRANCE: Var, Entrecasteaux, 14.vi.2002
233	Halictidae	Rophitinae	Rophitini	*Dufourea mulleri*	USA: Michigan, Michigan
350	Halictidae	Rophitinae	Rophitini	*Systropha curvicornis*	Austria
351	Halictidae	Rophitinae	Rophitini	*Conanthalictus wilmattae*	USA: California
566	Halictidae	Rophitinae	Rophitini	*Xeralictus bicuspidariae*	USA: California, San Diego Co., Anza Borrego Desert 28.ii.1998
813	Crabronidae	Bembicinae	Bembicini	*Bicyrtes ventralis*	USA: New York, Oswego Co., Sellkirk SP 5.iix.2000
814	Crabronidae	Bembicinae	Bembicini	*Xerostictia* sp.	USA: Arizona, Cochise Co., Willcox 28.iix.2000
811	Crabronidae	Bembicinae	Bembicini	*Bembix spinolae*	USA: New York, Oswego Co., Sellkirk. 5.iix.2000
815	Crabronidae	Bembicinae	Gorytini	*Ochleroptera bipunctata*	USA: New York, Tompkins Co., Ithaca 22.vii.2000
817	Crabronidae	Bembicinae	Stizini	*Stizoides* sp.	USA: Arizona, Cochise Co., Rucker Cyn. 17.iix.2000
803	Crabronidae	Crabroninae	Crabronini	*Anacrabro ocellatus*	USA: New York, Tompkins Co., Ithaca 22.vii.2000
807	Crabronidae	Crabroninae	Larrini	*Tachysphex* sp.	USA: Arizona, Cochise Co., Willcox 24.iix.2000

Table 3.1. (Continued)

810	Crabronidae	Crabroninae	Miscophini	*Plenoculus* sp.	USA: Arizona, Cochise Co., Willcox 28.iix.2000
805	Crabronidae	Crabroninae	Oxybelini	*Oxybelus* sp.	USA: Arizona, Cochise Co., Willcox 24.iix.2000
818	Crabronidae	Pemphredoninae	Pemphredonini	*Stigmus* sp.	USA: Arizona, Cochise Co., Chiricahua Mts. 20.iix.2000
819	Crabronidae	Philanthinae	Aphilanthopini	*Clypeadon* sp.	USA: New Mexico, Hidalgo Co., 16 mi S. Animas 19.iix.2000
825	Crabronidae	Philanthinae	Cercerini	*Cerceris* sp.	USA: Arizona, Cochise Co., Portal 14.iix.2000
828	Crabronidae	Philanthinae	Philanthini	*Philanthus gibbosus*	USA: New York, Cortland Co., McLean Bog 16.vii.2000
833	Sphecidae	Sphecinae	Ammophilini	*Podalonia* sp.	USA: New Mexico, Hidalgo Co., E. Animas 15.iix.2000
836	Sphecidae	Sphecinae	Sceliphronini	*Sceliphron caementarium*	USA: New York, Tompkins Co. 23.vii.2000
838	Sphecidae	Sphecinae	Sphecini	*Isodontia mexicana*	USA: New York, Tompkins Co. 26.vii.2000

Sodium-Potassium ATPase (NaK) ~1500bp long encompassing the extracellular ouabain-binding site near the N-terminus of segment 1 of the Nak alpha-subunit (Fagan and Saier 1993). For Elongation factor-1 alpha (EF1-α), we used primers specific to the F2 copy, which amplify a ~1050bp long fragment spanning 1 intron (intron 2 in Danforth et al. (2004)). The intron was removed from the dataset prior to phylogenetic analyses due to difficulties of alignment.

Sequencing

Previously published sequences were downloaded from Genbank (Table 3.2). All new sequences were obtained following standard PCR and sequencing protocols (Danforth et al. 1999). PCR products were gel-purified overnight on low-melting-point agarose gels, the gel slices were extracted with the Promega Wizard PCR purification system (Promega, Madison, WI), and all PCR products were sequenced in both directions with an Applied Biosystems Automated 3730 DNA Analyzer using Big Dye Terminator chemistry and AmpliTaq-FS DNA polymerase at Cornell University Life Sciences Core Laboratories Center. Primer pairs and PCR conditions for all genes are listed in Chapter 1.

Table 3.2. Genbank accession numbers for sequences used in the study.

Species	18S	28S	EF1	NaK	Pol II	Wingless	Opsin
Alocandrena porteri Michener, 1986	AY995594	AY654473	AY585099	EF646429	EF646429	GU320165	DQ113659
Andrena brooksi Larkin, 2004	AY995598	AY654474	AY230129	EF646389	AY945092	AY222551	AF344618
Megandrena enceliae (Cockerell, 1927)	AY995605	AY654455	AY585103	GU320119		GU320176	DQ113657
Protoxaea gloriosa (Fox, 1893)	AY995617	AY654480	AY585106	EF646428	AY945157	GU320183	DQ113658
Calliopsis pugionis Cockerell, 1925	AY995601	AY654477	AY585102	EF646434	AY945098	GU320180	AF344588
Calliopsis anthidia Fowler, 1899	AY995599	AY654475	AY585100	EF646436	AY945096	GU320184	DQ113656
Melitturga clavicornis (Latreille, 1808)	AY995606	AY654478	AY585104	EF646442	AY945145		DQ116703
Panurgus calcaratus (Scopoli, 1763)	AY995612	AY654479	AY585105	EF646459	AY945152	GU320166	AF344612
Ancyla anatolica Warncke, 1979	GU244591	GU244753	GU244913	GU245057	GU245352	GU245510	GU245235
Anthophora urbana Cresson, 1878	GU244593	GU244755	GU244915	GU245059	GU245354	GU245512	AF344585
Pachymelus peringueyi (Friese, 1911)	AY995685	AY654544	AY585114	GU245061	AY945151	GU245514	DQ116678
Apis cerana Fabricius, 1793	no # avail.	no # avail.	EU184774	EU184750	EU184733	EU184716	EU184839
Apis dorsata Fabricius, 1793	no # avail.	no # avail.	AY267146	EU184749	EU184732	EU184715	AY267162
Apis florea Fabricius, 1787	no # avail.	no # avail.	EU184773	EU184748	EU184731	EU184714	EU184838
Bombus ardens Smith, 1879	no # avail.	no # avail.	AF492964	EU184741	EU184724	EU184707	AF493031
Bombus diversus Smith, 1869	no # avail.	no # avail.	AF492961	EU184742	EU184725	EU184708	AF493028
Bombus mendax Gerstäcker, 1869	no # avail.	no # avail.	AF492957	EU184743	EU184726	EU184709	AF493024
Epicharis analis Lepeletier, 1841	GU244599	GU244759	GU244920	GU245064	GU245358	GU245517	GU245241
Centris hoffmanseggiae Cockerell, 1897	GU244597	GU244765	GU244918	GU245070	GU245364	GU245523	AF344590
Ctenoplectra albolimbata Magretti, 1895	AY995681	AY654538	AY585118	GU245073	AY945111	GU245526	DQ116677
Diadasia bituberculata (Cresson, 1878)	GU244606	GU244768	GU244927	GU245074	GU245367	GU245527	AF344594
Diadasina distincta (Holmberg, 1903)	GU244608	GU244770	GU244929	GU245076	GU245369	GU245529	GU245248
Ericrocis lata (Cresson, 1878)	GU244615	GU244777	GU244936	GU245083	GU245376	GU245536	GU245255
Melissodes desponsa Smith, 1854	GU244625	GU244785	GU244942	GU245093	GU245384	GU245546	AF344603
Eucera frater (Cresson, 1878)	GU244626	GU244786		GU245094	GU245385	GU245547	GU245232
Xenoglossa angustior Cockerell, 1899	GU244627	GU244787		GU245095	GU245386	GU245548	GU245233
Svastrina subapicalis (Brèthes, 1910)	GU244632	GU244792		GU245100	GU245391	GU245553	GU245266
Eulaema meriana (Olivier, 1789)	GU244635	GU244795	GU244945	GU245103	GU245394	GU245556	GU245269
Aglae caerulea Lepeletier & Audinet-Serville, 1825	GU244637	GU244797	AJ582383	GU245105	GU245396	GU245558	GU245271
Eufriesea surinamensis (Linnaeus, 1758)	GU244638	GU244798	GU244947	GU245106	GU245397	GU245559	GU245272

Table 3.2. (Continued)

Euglossa imperialis Cockerell, 1922	no # avail.	no # avail.	AY267144	EU184738	EU184721	EU184704	AY267160
Anthophorula completa (Cockerell, 1935)	GU244639	GU244799	GU244948	GU245107	GU245398	GU245560	AF344622
Exomalopsis sp.	GU244642	GU244802	GU244989	GU245110	GU245401	GU245563	
Isepeolus atripilis Roig-Alsina, 1991	GU244644	GU244804	GU244952	GU245112	GU245403	GU245565	GU245276
Xeromelecta californica (Cresson 1878)	GU244648	GU244808	GU244955	GU245116	GU245407	GU245569	AF344613
Tetralonioidella sp.	GU244649	GU244809	GU244956	GU245119	GU245408	GU245572	GU245279
Hypotrigona gribodoi (Magretti, 1884)	GU244651	GU244811	GU244957	GU245121	AY945189	GU245574	GU245280
Meliponula ferruginea	GU244652	GU244812	GU244958	GU245122	AY945192	GU245575	GU245281
Cephalotrigona capitata (Smith, 1854)	no # avail.	no # avail.	EU184771	EU184745	EU184728	EU184711	EU184836
Scaura latitarsis (Friese, 1900)	no # avail.	no # avail.	EU184772	EU184747	EU184730	EU184713	EU184837
Epeoloides pilosula (Cresson, 1878)	GU244659	GU244819	GU244966	GU245129	GU245416	GU245582	GU245287
Parepeolus aterrimus (Friese, 1906)	GU244660	GU244820	GU244967	GU245130	GU245417	GU245583	GU245288
Osiris sp. 1	GU244662	GU244822	GU245033	GU245132	GU245419	GU245585	
Leiopodus sp.	GU244663	GU244823	GU244968	GU245134	GU245420	GU245587	GU245289
Nanorhathymus sp.	GU244667	GU244827	GU244972	GU245138	GU245424	GU245591	GU245293
Caenonomada sp.	GU244669	GU244829	GU244974	GU245140	GU245426	GU245593	GU245295
Arhysoceble picta (Friese, 1899)	GU244671	GU244831	GU244976	GU245142	GU245428	GU245595	GU245297
Tetrapedia maura Cresson, 1878	GU244674	GU244834	GU244979	GU245145	GU245431	GU245598	GU245300
Coelioxoides waltheriae Ducke, 1908	GU244678	GU244838	GU244986	GU245149	GU245435	GU245602	GU245303
Oreopasites barbarae Rozen, 1992	GU244703	GU244863	GU245008	GU245176	GU245460	GU245632	AF344626
Sphecodopsis capensis (Friese, 1915)	GU244704	GU244864	GU245009	GU245177	GU245461	GU245633	GU245317
Holcopasites calliopsidis (Linsley, 1943)	GU244708	GU244868	GU245012	GU245182	GU245465	GU245638	AF344600
Neopasites cressoni Crawford, 1916	GU244714	GU244874	GU245017	GU245188	GU245471	GU245644	GU245323
Brachynomada margaretae (Rozen, 1994)	GU244718	GU244878	GU245019	GU245193	GU245475	GU245649	GU245324
Caenoprosopis crabronina Holmberg, 1887	GU244719	GU244879	GU244983	GU245194	GU245476	GU245650	GU245325
Epeolus scutellaris Say, 1824	GU244722	GU244882	GU245022	GU245197	GU245479	GU245653	AF344596
Thalestria spinosa (Fabricius, 1804)	GU244723	GU244883	GU245024	GU245199	GU245480	GU245655	GU245328
Hexepeolus rhodogyne Linsley & Michener, 1937	GU244728	GU244888	GU245028	GU245204	GU245485	GU245660	GU245332

Table 3.2. (Continued)

Neolarra orbiculata Shanks, 1978	GU244729	GU244889	GU245029	GU245205	GU245486	GU245661	GU245333
Nomada maculata Cresson, 1863	GU244730	GU244890	GU245030	GU245206	GU245487	GU245662	AF344609
Townsendiella sp.	GU244732	GU244892	GU245032	GU245208	GU245489	GU245664	GU245334
Braunsapis madecassella Michener, 1977	GU244733	GU244893	GU245038	GU245209	GU245490	GU245665	GU245231
Macrogalea ellioti (Saussure, 1890)	GU244735	GU244895	GU245040	GU245211	GU245492	GU245667	GU245336
Exoneura bicolor Smith, 1854	GU244736	GU244896	GU245041	GU245212	GU245493	GU245668	GU245337
Ceratina sp.	GU244737	GU244897	GU245042	GU245214	GU245494	GU245670	GU245338
Ceratina sp.	GU244738	GU244898	GU245043	GU245215	GU245495	GU245671	GU245339
Manuelia gayatina (Spinola, 1851)	GU244742	GU244902	GU245046	GU245219	GU245499	GU245675	GU245342
Xylocopa tabaniformis Smith, 1854	GU244744	GU244904	GU245048	GU245221	GU245501	GU245677	AF344614
Xylocopa sp.	GU244747	GU244907	GU245051	GU245224	GU245504	GU245680	GU245346
Xylocopa pubescens Spinola, 1838	GU244748	GU244908	GU245052	GU245225	GU245505	GU245681	GU245347
Xylocopa californica arizonensis Cresson, 1879	GU244751	GU244911	GU245054	GU245228	GU245508	GU245684	GU245350
Colletes inaequalis Say, 1837	AY995621	AY654484	AY585123	EF646387	AY945107	DQ884804	DQ115542
Leioproctus delahozii Toro, 1973	AY995636	DQ872762	AF435392	GU320114	AY945134	AY222549	AY227914
Trichocolletes sp.	AY995642	AY654504	AY585139	GU320140	AY945171	DQ884733	DQ115562
Leioproctus fimbriatinus (Cockerell, 1910)	AY995632	AY654494	AY585131	GU320139	AY945130	DQ884786	DQ115554
Leioproctus plumosus (Smith, 1853)	AY995634	AY654496	AY585133	GU320111	AY945132	DQ884789	DQ115556
Callomelitta antipodes (Smith, 1853)	AY995620	AY654483	AY585122	GU320133	AY945105	EF032907	DQ115563
Caupolicana vestita (Smith, 1879)	AY995623	AY654486	AY585124	GU320129	AY945109	DQ884726	DQ115543
Caupolicana yarrowi (Cresson, 1875)	AY995624	AY654487		GU320127	AY945110	DQ884727	DQ115544
Diphaglossa gayi Spinola, 1851	AY995625	AY654488	AY585125	GU320143	AY945115	DQ884728	DQ115545
Euryglossa calliopsella Cockerell, 1910	AY995626	AY654489	AY585126	GU320142	AY945117	DQ884809	DQ115550
Euryglossina globuliceps (Cockerell, 1918)	AY995627	AY654490	AY585127	GU320124	AY945118	DQ884810	DQ115551
Xanthesma furcifera (Cockerell, 1913)	AY995643	AY654505	AY585140	GU320100	AY945173	DQ884811	DQ115552
Hylaeus amiculus (Smith, 1879)	AY995628	AY654491	AY585128		AY945126	DQ884838	DQ115546
Hylaeus elegans (Smith, 1853)	AY995630	AY654492	AY585129	GU320136	AY945127	DQ884839	DQ115547
Hylaeus proximus (Smith, 1879)	AY995631	AY654493	AY585130	GU320117	AY945128	EF032906	DQ115548

Table 3.2. (Continued)

Scrapter erubescens (Friese, 1925)	AY995638	AY654499	AY585135	GU320106	AY945161	DQ884813	DQ115558
Scrapter heterodoxus (Cockerell, 1921)	AY995639	AY654500	AY585136	GU320131	AY945162	DQ884814	DQ115559
Scrapter niger Lepeletier and Audinet-Serville, 1825	AY995640	AY654501	AY585137	GU320112	AY945163	DQ884815	DQ115560
Scrapter ruficornis (Cockerell, 1916)	AY995641	AY654502	AY585138	GU320134	AY945164	DQ884816	DQ115561
Chilicola styliventris (Friese, 1908)	AY995619	AY654482	AY585121	GU320115	AY945103	DQ884832	
Xeromelissa rozeni (Toro & Moldenke, 1979)	AY995618	AY654481	AY585120	GU320141	AY945102	DQ884826	DQ115549
Leioproctus bathycyaneus Toro, 1973	AY995644	AY654498	AY585141	GU320122	AY945135	DQ884741	DQ115553
Bembix americana spinolae Lepeletier 1845	AY995580	AY654459	AY585168	GU320099	AY945095	GU320175	
Bicyrtes ventralis (Say, 1824)	AY995579	AY654458	AY585161	GU320153	AY945094	GU320188	DQ116701
Xerostictia sp.	AY995590	AY654471	AY585165	GU320137	AY945175	GU320187	
Ochleroptera bipunctatus (Say 1824)	AY995583	AY654463		GU320113	AY945147	GU320179	DQ116699
Stizoides sp.	AY995588	AY654469	AY585164	GU320120		GU320178	
Anacrabro ocellatus Packard 1866	AY995578	DQ072142	AY585160	GU320118	AY945091		DQ116700
Tachysphex sp.	AY995589	AY654470	AY585171	GU320138	AY945172	GU320167	DQ116707
Plenoculus sp.	AY995586	AY654465	AY585170	GU320103	AY945150	GU320168	DQ116705
Oxybelus sp.	AY995584	DQ072153	AY585169	GU320145	AY945148		DQ116694
Stigmus sp.	AY995587	AY654467	AY363038	GU320101	AY945165	GU320182	DQ116706
Clypeadon sp.	AY995582	AY654461	AY585163	GU320121	AY945104		DQ116693
Cerceris sp.	AY995581	AY654460	AY585162	GU320130	AY945100	GU320185	DQ116696
Philanthus gibbosus (Fabricius 1775)	AY995585	AY654464		GU320154	AY945149	GU320173	DQ116704
Augochloropsis metallica (Fabricius, 1793)	GU320087	GU320093	AF140315	GU320149	GU320161	AY222571	AY227934
Augochlorella pomoniella (Cockerell, 1915)	AY995647	AY654507	AF435373	GU320105	GU320158	AY222572	AY227935
Agapostemon tyleri Cockerell, 1917	AY995646	AY654506	AF140320	GU320102	AY945089	AY222577	AY227940
Ruizantheda mutabilis (Spinola, 1851)	GU320088	GU320094	AF435406	GU320151	GU320163	AY222586	AY227949
Halictus rubicundus (Christ, 1791)	AY995651	AY654510	AF140335	EF646388	AY945120	AY222592	DQ116674
Mexalictus arizonensis Eickwort, 1978	GU320089	GU320095	AF140322	GU320146	GU320155	AY222595	AY227959
Lasioglossum hybodinum (Cockerell, 1912)	GU320090	GU320096	AF264857	GU320148	GU320160	AY222599	AY227963

Table 3.2. (Continued)

Patellapis abessinica (Friese, 1916)	GU320091	GU320097	EU203267	GU320152	GU320164	EU203236	EU203295
Sphecodes ranunculi Robertson, 1897	GU320092	GU320098	AF140325	GU320150	GU320162	AY222597	AY227961
Dieunomia nevadensis (Cresson, 1874)	AY995653	AY654512	AF435397	GU320123	AY945146	AY222568	AY227931
Pseudapis unidentata albocincta (LUCAS 1846)	AY995655	AY654514	AF435404	GU320110	AY945156	AY222570	AY227933
Nomioides facilis (Smith, 1853)	AY995652	AY654511	AF435394	GU320125	GU320156	AY222566	AY227929
Penapis penai Michener, 1965	AY995654	AY654513	AF435401	GU320104	AY945153	AY222558	AY227921
Conanthalictus wilmattae Cockerell, 1936	AY995648	AY654508	AF435378	GU320109	GU320157	AY222553	AY227934
Dufourea mulleri (Cockerell, 1898)	AY995650	AY654509	AF435383	GU320108	AY945116	AY222555	AY227918
Systropha curvicornis (Scopoli, 1770)	AY995657	AY654516	AF435411	GU320107	AY945168	AY222562	AY227925
Xeralictus bicuspidariae Snelling and Stage, 1995	AY995658	AY654517	AF435413	GU320116	AY945174	AY222564	AY227927
Rophites algirus Pérez, 1895	AY995656	AY654515	AY585144	GU320132	AY945158	EF411184	DQ116675
Neofidelia sp.	GU244680	GU244840	GU244990	GU245151	GU245437	GU245605	GU245305
Fidelia major Friese, 1911	AY995692	AY654539	DQ141113	GU245152	AY945119	GU245606	DQ116708
Pararhophites quadratus (Friese, 1898)	GU244681	GU244841	GU245034	GU245153	GU245438	GU245607	GU245306
Trachusa larreae (Cockerell, 1897)	GU244682	GU244842	GU244992	GU245154	GU245439	GU245608	GU245307
Dianthidium subparvum Swenk, 1914	GU244683	GU244843	GU244993	GU245155	GU245440	GU245609	GU245308
Paranthidium jugatorium (Say, 1824)	GU244684	GU244844	GU244994	GU245156	GU245441	GU245610	AF344611
Anthidiellum notatum (Latreille, 1809)	GU244685	GU244845	GU244995	GU245157	GU245442	GU245611	AF344617
Anthidium porterae Cockerell, 1900	GU244686	GU244846	GU244996	GU245158	GU245443	GU245612	AF344619
Stelis linsleyi Timberlake, 1941	GU244690	GU244850	GU245002	GU245162	GU245447	GU245617	GU245312
Dioxys pomonae Cockerell, 1910	GU244687	GU244847	GU244997	GU245159	GU245444	GU245613	GU245309
Trichothurgus herbsti (Friese, 1905)	GU244688	GU244848	GU245000	GU245160	GU245445	GU245614	GU245310
Lithurgus echinocacti Cockerell, 1898	AY995694	AY654541	DQ141116	EF646390	AY945136	GU245615	DQ116702
Microthurge sp.	GU244689	GU244849	GU245001	GU245161	GU245446	GU245616	GU245311
Megachile angelarum Cockerell, 1902	GU244691	GU244851	GU245003	GU245163	GU245448	GU245618	GU245313
Coelioxys alternata Say, 1837	GU244692	GU244852	GU245004	GU245164	GU245449	GU245619	GU245164
Heriades crucifer Cockerell, 1897	GU244695	GU244855	EU851555	GU245168	GU245452	GU245624	EU851661
Osmia lignaria Say, 1837	GU244696	GU244856	EU851610	GU245169	GU245453	GU245625	EU851715
Chelostoma californicum Cresson, 1878	GU244697	GU244857	GU245006	GU245170	GU245454	GU245626	GU245315

Table 3.2. (Continued)

Ashmeadiella aridula Cockerell, 1910	GU244698	GU244858	EU851535	GU245171	GU245455	GU245627	EU851641
Hoplosmia scutellaris (Morawitz, 1868)	GU244699	GU244859	EU851587	GU245172	GU245456	GU245628	EU851693
Afroheriades hyalinus	GU244700	GU244860	GU245007	GU245173	GU245457	GU245629	GU245316
Protosmia rubifloris (Cockerell, 1898)	GU244701	GU244861	EU851619	GU245174	GU245458	GU245630	EU851724
Hoplitis albifrons (Kirby, 1837)	GU244702	GU244862	EU851577	GU245175	GU245459	GU245631	AF344598
Hesperapis larreae Cockerell, 1907	AY995664	AY654521	AY230131	EF646410	AY945121	AY222552	AF344597
Dasypoda argentata Panzer, 1809	AY995660	AY654518	AY585148	EF646418	AY945112	GU245603	DQ116680
Hesperapis richtersveldensis (Patiny and Michez, 2007)	AY995667	AY654523	AY585152	EF646414	AY945123	GU320169	DQ116683
Dasypoda altercator (Harris, 1780)	AY995661	AY654519	AY585149	EF646416	AY945113	GU320177	DQ116681
Dasypoda visnaga (Rossi, 1790)	AY995662	AY654520	AY585150	EF646420	AY945114	GU320181	DQ116682
Promelitta alboclypeata (Friese, 1900)	GU244679	GU244839	GU245036	GU245150	GU245436	GU245604	GU245304
Haplomelitta griseonigra Michener, 1981	AY995668	AY654524	AY585153	EF646426	AY945125	GU320170	DQ116684
Meganomia binghami (Cockerell, 1909)	AY995674	AY654528	DQ141114	EF646406	AY945144	GU245623	DQ116689
Macropis nuda (Provancher, 1882)	AY995670	AY654454	AY585155	EF646404	AY945139	GU245621	DQ116686
Melitta arrogans (Smith, 1879)	AY995671	AY654526	AY585156	GU245167	AY945140	GU245622	DQ116687
Melitta eickworti Snelling and Stage, 1995	AY995672	AY654527	AY585157	EF646393	AY945141	GU320174	AF344604
Rediviva macgregori Whitehead and Steiner, 2001	AY995675	AY654531	AY585159	EF646400	AY945159	GU320189	DQ116690
Redivivoides simulans Michener, 1981	AY995676	AY654532	AY585142	EF646401	AY945160		DQ116691
Macropis europaea Warncke, 1973	AY995669	AY654525	AY585154	EF646403	AY945138		DQ116685
Melitta leporina (Panzer, 1799)	AY995673	AY654529	AY585158	EF646394	AY945142		DQ116688
Podalonia sp.	AY995592	AY654466		GU320144	AY945155	GU320186	DQ116698
Sceliphron caementarium (Drury 1773)	AY995593	AY654468	AY585167	GU320126	AY945166	GU320171	DQ116695
Isodontia mexicana	AY995591	AY654462	AY585166	GU320128	AY945129	GU320172	DQ116697
Ctenocolletes smaragdinus (Smith, 1868)	GU320086	DQ768461	DQ884572	GU320147	GU320159	DQ884713	DQ884480
Stenotritus sp.	AY995677	AY654503	DQ141115	GU320135	AY945167	DQ884714	DQ115564

Aligning

All genes were separately aligned in the Lasergene DNAStar (DNASTAR 1999) software package using ClustalW. Alignments for 28S and 18S were subsequently adjusted by referring to the secondary structure of these genes proposed for *Apis mellifera* (Gillespie et al. 2006). Regions that could not be aligned with confidence were excluded from the analysis. Introns of LW rhodopsin were quite variable in length, and could not be aligned with confidence, and so were excluded from all analyses. The intron of EF1-α was also difficult to align across the entire dataset. Short portions of the intron that were aligneable were used in preliminary analyses, but were then eliminated from subsequent analyses because they represented too little data to inform parameter estimation in their own partition. Reading frames and intron/exon boundaries for the protein-coding genes were determined by comparison with published sequences from the honey bee, *Apis mellifera.*

Partitioning of data

The dataset was divided into 4 partitions. The two ribosomal genes were placed together into one partition, and the protein-coding genes were combined together and partitioned by codon position (|28S,18S|pos1|pos2|pos3|).

Phylogenetic analysis

The dataset was analyzed in MrBayes v.3.1.2 (Huelsenbeck and Ronquist 2001). All partitions used the General Time Reversible model (GTR)(Tavaré 1986), with a proportion of invariable sites (I), and rate variation among sites with 4 rate categories (G). Parameters were unlinked across partitions. The dataset was analyzed with eight independent runs with 4 chains each. The number of generations for each run varied from 9 741 000 generations to 17 824 000 generations The parameter trace files of each run were observed to verify that the runs had converged on the stationary distribution, and to decide on the appropriate number of generations to discard as burn-in. Convergence was also assessed by observing the posterior probabilities of clades over generations in the program Awty (Nylander et al. 2008).

The tree files and parameter files with the burn-in removed from each run were combined. A maximum clade credibility tree was constructed from these 81 396 000 post-burn-in generations in TreeAnnotator v1.4.8 (Rambaut and Drummond 2008). We chose to do numerous shorter runs instead of a few longer runs because of run time restrictions on the computer clusters at Cornell University's Computational Biology Service Unit. Also, doing a large number of independent runs from different starting points allowed us to more fully explore tree space.

Estimating divergence times

We used a Bayesian phylogenetic relaxed molecular clock model (Drummond et al. 2006) with multiple calibration points to estimate divergence times in the program BEAST v1.4.8 (Drummond and Rambaut 2007). We applied a GTR+I+G model, as in the phylogenetic analysis described above. Branch rates were estimated with an uncorrelated relaxed clock model in which the rate at each branch was drawn from an underlying log-normal distribution. This allowed for the rate of evolution to vary among the branches of the tree with no *a priori* correlation between a lineage's rate and that of its ancestor. Parameters were unlinked across partitions. The Yule tree prior was used, which assumes a constant per lineage selection rate as recommended in the manual for species-level phylogenies. We randomly selected a starting tree from the posterior distribution of trees from the MrBayes analysis.

The tree was time calibrated by setting priors on the ages of 14 internal nodes (Figure 3.1) and the root of the tree. Age estimates were based on paleontological evidence as described below. Uncertainty in the age of the calibration points was incorporated into the analysis by assuming that the probability of the node being a certain age follows a lognormal distribution with a rigid minimum bound. This required us to specify a mean, standard deviation and rigid lower bound for the age of each calibration point. Selection of the values for these parameters is somewhat subjective, but below we outline the reasoning behind each of our choices for calibration points 11 to 14 (see Chapter 1 for calibration points 1 to 10). Applying a lognormal distribution to our age estimates allows us to assume that the actual divergence event took place some time prior to the earliest appearance of fossil evidence, but that the age of the node is more likely to be close to the age of the oldest known fossil, and less likely to be significantly older. Fossils can only provide minimum age estimates and their appearance must postdate the origin of the clade to which they belong. By how much the appearance of a clade predates the age of the first fossil is always unclear. We therefore made sure that the 95% probability included the oldest reasonable age for the clade. For our deepest calibration points, that meant including the start of the Cretaceous (145 Ma). The fossil record for angiosperms extends back to the early Cretaceous (Crane et al. 2004). Angiosperms are thought to have originated about 130 Ma, shortly before the origin of the eudicots about 125 Ma (Davies et al. 2004; Soltis and Soltis 2004; Magallon and Castillo 2009). Bees are mostly dependent on floral resources from eudicots, therefore it would be very unlikely for bees to predate the origin of eudicots or angiosperms. Furthermore, it is estimated that the crabronid-bee divergence took place ~120 Ma (Grimaldi and Engel 2005). In most cases, we chose a mean that was slightly older then the oldest fossil belonging to the group because the divergence event of the group necessarily predates any fossil belonging to it. We set the rigid lower bounds (zero offset) for each calibration point to the boundary between two geological time scales younger then the age of the fossil to allow for the chance that the fossil is younger than the age assigned to it and to allow for possible mistakes in the phylogenetic placement of the fossil. We describe below the paleontological evidence upon which the last four calibration points are based (see Chapter 1 for a description of the first 10). Table 3.3 summarizes the information presented below on the fossils used, phylogenetic position, and priors set for each calibration point.

Table 3.3. Values given for the mean, standard deviation, and zero offset for the time priors of each calibration point. Information on the main fossil used to calibrate each node, and the phylogenetic positions of the calibrations are also shown.

Calibration	Fossil	Age of fossil (Ma)	Fossilization	Node	mean	stdev	zero offset
1	*Euglossa morenei*	15-20	amber	Euglossa	20	1	11.6
2	*Apis lithohermaea*	15	compression	A. cerana + A. dorsata	16	1	11.6
3	*Apis henshawi*	23-30	compression	Apini+Eugossini	33	0.75	20.4
4	*Kelneriapis eocenica*	43-45	amber	Hypotrigona + sister	47	0.58	33.9
5	*Cretotrigona prisca*	65-70	amber	Meliponini	70	0.37	55
6	*Boreallodape* (3 spp.)	43-45	amber	Allodapini+Ceratinini	47	0.57	33.9
7	*Paleohabropoda oudardi*	56	compression	Anthophorini	60	0.45	23
8	*Xylocopa gabrielae*	34	compression	Xylocopini	34	0.74	23
9	*Palaeomacropis eocenicus*	49-56	amber	Macropis + sister	55	0.5	40.4
10	*Probombus hirsutus*	56	compression	Anth.+Megachi.+Osm.	60	0.45	48.6
11	*Electrolictus*	43-45	amber	Halictini+Sphecodini	47	0.57	33.9
12	*Augochlora leptoloba*	15-20	amber	Augochlorini	20	1	11.6
13	*Chilicola*	15-20	amber	Chilicolini+Xeromelissini	20	1	11.6
14	*Heterosarus eickworti*	15-20	amber	Melitturgini+Panurgini	20	1	11.6

Calibration 11 – *Electrolictus aniquus* was described from Baltic amber and placed in the tribe Halictini (Engel 2001). *Electrolictus* might represent a stem group to Halictini. We therefore used this fossil to calibrate the node uniting Halictini and its sister clade Sphecodini. A lognormal distribution with a mean of 3.85 (= 47 Ma), stdev of 1 and zero offset of 33.9 Ma was applied as a prior for calibration point 11.

Calibration 12 – Several fossils from Dominican amber have been assigned to Augochlorini (Engel 1995; Engel 1996; Engel and Rightmyer 2000). One of these, *Augochlora leptoloba* was placed in the extant genus *Augochlora* when it was described (Engel 2000b). Therefore, this fossil can be used to calibrate the node representing the crown group of Augochlorini. A lognormal distribution with a lognormal mean of 3 (= 20 Ma), stdev of 1 and zero offset of 11.6 Ma was applied as a prior for calibration point 12.

Calibration 13 – *Chilicola electrodominica* (Engel 1999b), and *Chilicola gracilis* (Michener and Poinar Jr. 1996) are both from Dominican amber. These fossils can be used to calibrate the node uniting the extant species of *Chilicola* that we have in our dataset with its sister *Chilimelissa*. A lognormal distribution with a lognormal mean of 3 (= 20 Ma), stdev of 1 and zero offset of 11.6 Ma was applied as a prior for calibration point 13.

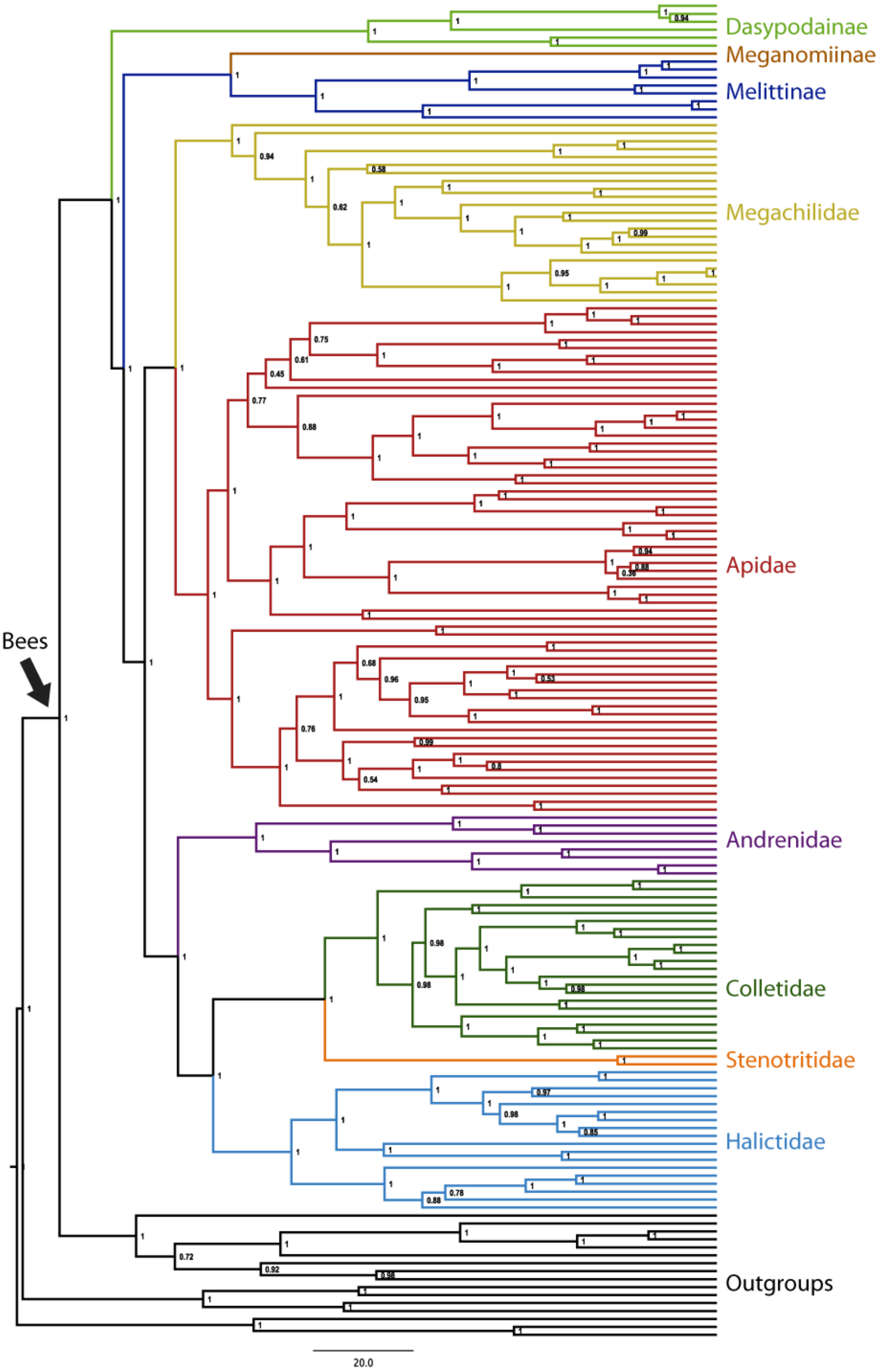
Calibration 14 – *Heterosarus eickworti* is from Dominican amber and belongs to the tribe Protandrenini (Rozen 1996). Our dataset does not contain any Protandrenini, but Protandrenini belongs to the subfamily Panurginae, of which we have representatives. We therefore use this fossil to calibrate the node uniting Panurgini and Melitturgini (both tribes of Panurginae). A lognormal distribution with a mean of 3 (= 20 Ma), stdev of 1 and zero offset of 11.6 Ma was applied as a prior for calibration point 13.

We also had to apply a prior on the age of the root node of the tree which represents the divergence between Sphecidae and Crabronidae+bees. The oldest fossils that can be assigned with certainty to the Sphecidae or Crabronidae come from Burmese amber (Bennett and Engel 2006). Palynomorphs obtained from this amber have been assigned to the Upper Albian (~100-110 Ma) of the Early Cretaceous (Cruickshank and Ko 2003). Based on extrapolation from the fossil record, Grimaldi and Engel (2005) estimate that Sphecidae diverged from other Apoidea during the early ages of the Cretaceous ~140 Ma. We therefore ran 10 independent analyses with the age of the root node sampled from a normal distribution with a mean of 140 ± 1 Ma. Each analysis was run for over 11 million generations, giving us a total of 110 260 000 generations. Results from these 10 runs were combined together once their burn-in periods were removed, and maximum clade credibility tree was constructed. To see the effect of different root node age priors on the age estimates of internal nodes, we ran analyses with the prior on the root node age set to 150 ± 1 Ma, and 130 ± 1 Ma.

Results

Our Bayesian analysis of our concatenated 7 gene dataset results in a well resolved phylogeny with most nodes having high support (Figure 3.1). The monophyly of bees is supported with posterior probability (PP) of 1.0. The melittid subfamily Dasypodainae, is sister to all other bees (PP = 0.86). Meganomiinae and Melitiinae form a monophyletic group (PP = 1.0) sister to all non-melittid bees (PP = 0.97). The long-tongued bees are monophyletic (PP = 1.0) as are the two long-tongued bee families Apidae (PP = 1.0), and Megachilidae (PP = 1.0). The short-tongued bee families Andrenidae, Halictidae, Colletidae, and Stenotritidae form a monophyletic clade (PP = 0.99) sister to the long-tongued bees. The monophyly of these 4 families are each recovered with PP = 1.0. Andrenidae is found to be sister to the remaining members of this group (PP = 1.0), and Colletidae and Stenotritidae form a monophyletic group (PP = 1.0).

Figure 3.1. Bayesian maximum clade credibility tree of the concatenated dataset using a GTR+I+G model. Posterior probabilities for each node are shown.


Dasypodainae
Meganomiinae
Melittinae
Megachilidae
Apidae
Bees
Andrenidae
Colletidae
Stenotritidae
Halictidae
Outgroups
20.0

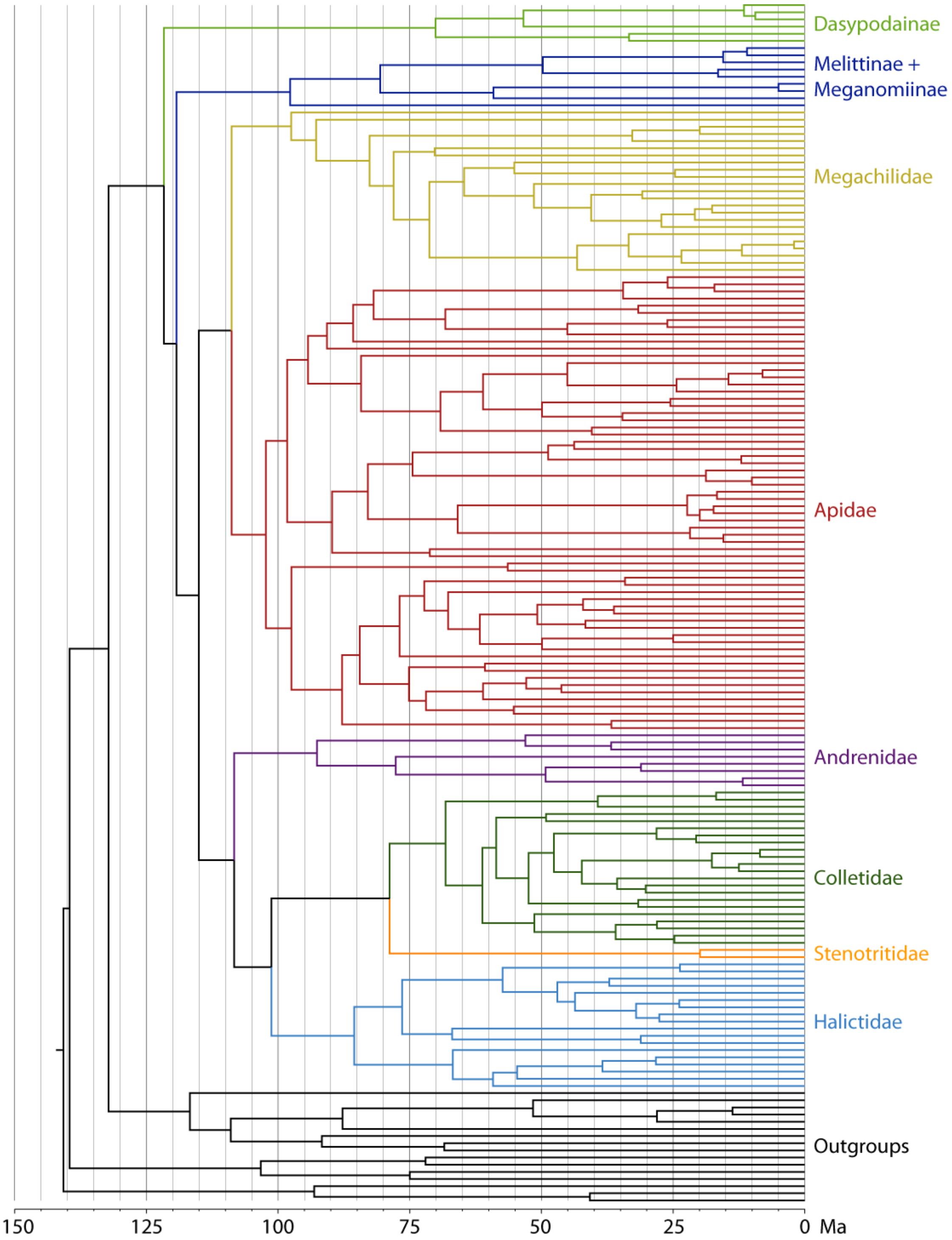
Our study recovers the same phylogenetic relationships among bee families and subfamilies as Danforth et. al. (2006). The addition of more taxa and genes did not alter relationships, but did change the support values for some nodes. The posterior probability for the node uniting all bees excluding Dasypodainae increased to 0.86 from 0.81. The node uniting all non-melittid bees decreased to 0.97 from 1.0. The node uniting Andrenidae, Halictidae, Stenotritidae and Colletidae was lowered to 0.99 from 1.0. In 2 out of 3 cases, the addition of more data slightly decreased the support values for clades instead of increasing them.

We estimate the antiquity of bees to be ~122 Ma (116-127Ma). This estimate is based on the dating analysis where our root node was set to 140 Ma, which we believe to be the best estimate for the age of our root node. The chronogram presented in Figure 3.2 is also based on this root node age. Figure 3.3 shows our age estimates for the major bee clades with the root node set to 150, 140, and 130 Ma. The 95% confidence intervals for the age estimates of the major clades based on the different root node ages overlap with one another in all cases.

Discussion

Researchers have long speculated that bees played a crucial role in angiosperm diversification, and yet the most basic test of this hypothesis (whether bees and angiosperms diversified concurrently) has been lacking. We estimate that the crown group of bees originated ~122 Ma (116-127 Ma). This date coincides closely with the estimated age of ~125 Ma for the eudicot crown group (Friis et al. 2006; Magallon and Castillo 2009) (Figure 3.4). Eudicots represent ~ 75% of the diversity of flowering plants (Soltis et al. 2005). They encompass most of the bee pollinated angiosperm families and include most of the angiosperm orders that have undergone rapid

Figure 3.2. Chronogram of the bees with the root node age set to 140 Ma


Dasypodainae
Melittinae +
Meganomiinae
Megachilidae
Apidae
Andrenidae
Colletidae
Stenotritidae
Halictidae
Outgroups
150
125
100
75
50
25
0
Ma

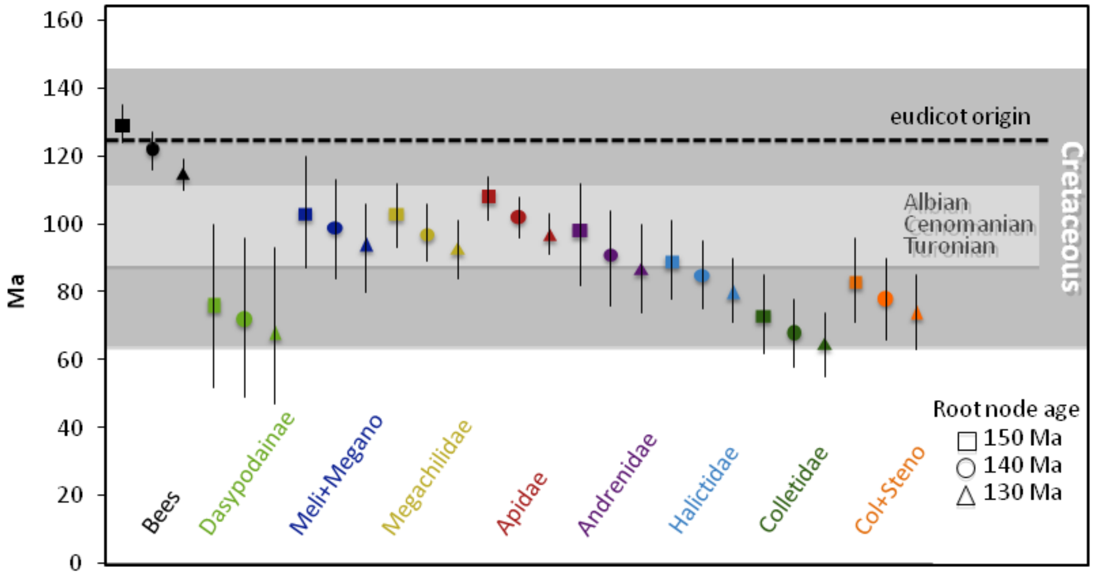


Figure 3.3. Mean and 95% confidence intervals on the estimated divergence times of the major bee clades with the root node set to 150, 140 and 130 Ma.

diversification (Magallon and Sanderson 2001; Magallon and Castillo 2009). All but two of the rapidly evolving clades found in these studies (Poales and Cyperales) belong to the eudicot clade. Furthermore, a large proportion of these rapidly evolving clades are pollinated by bees. Analyses of pollination mode in basal angiosperm lineages suggest that beetles, flies, micropterygid Lepidoptera, thrips and possibly Hymenoptera were important pollinators (Grimaldi 1999; Thien et al. 2000; Hu et al. 2008). Bee pollination does not become important until the origin of the eudicots, and we have shown a very close temporal association between origins of bees and eudicots. Our results provide new insight into how bee origins may have related to the temporal diversification of angiosperms, especially eudicots.

Despite a lack of fossils from the Cretaceous, we hypothesize that the major bee clades diversified during the mid and late Cretaceous. We estimate that five of the major bee clades (Melittinae + Meganomiinae, Megachilidae, Apidae, Andrenidae, and Halictidae), were present by 90 Ma. Likewise, plant fossils representing at least four of the eleven extant angiosperm subclasses (Magnoliidae, Hamamelidae, Rosidae and at least one subclass of Liliatae) appear in the fossil record for the first time before 90 Ma (Lidgard and Crane 1988).

The other major bee clades appear during the late Creatceous (Turonian to Maastrichtian age). The origin of all major bee clades between the Aptian and Maastrichtian, supports the hypothesis that bees diversified during the same time as angiosperms. Analyses of species richness based on both palynofloras and leaf macrofloras indicate a major mid-Cretaceous increase in the within-flora diversity of angiosperms starting in the Aptian (120 Ma) and continuing on through the Maastrichtian (65 Ma) (Lidgard and Crane 1990). While the number of angiosperm species increased through the Cretaceous, a corresponding decline was seen in the diversity of other groups of land plants (Niklas et al. 1985; Lidgard and Crane 1988;

Lidgard and Crane 1990). Conifers however show little change in diversity through the Cretaceous (Lidgard and Crane 1988; Lidgard and Crane 1990), and polypod ferns have been hypothesized to have diversified in the Cretaceous, after angiosperms (Schneider et al. 2004).

Our age estimates for bees are further supported by the angiosperm fossil record. Cenomanian angiosperm pollen from the Dakota Formation suggests that 87% of the fossil pollen species were insect-pollinated, and that 29% had specialized modes of pollination. The percent of species having specialized modes of pollination increases to 65% when considering only the putative eudicot species (Hu et al. 2008). There is evidence that some of these extinct species were more specifically pollinated by bees. For example, fossils of Ericanae which first appear in Turonian deposits, have a suite of characters (elongate sepals, nectary disc, carpels with separate stigmas, a sympetalous corolla) that is now associated with bee pollinators (Crepet 2008). The Turonian also saw the first appearance of taxa that are now specifically associated with bees (Crepet 2008). For example, fossil flowers of Clusiaceae from the Turonian of New Jersey share several characters with extant Clusiaceae that have a close relationship with stingless bees (Crepet and Nixon 1998).

Our estimates on the antiquity of the different bee families are also consistent with the chronological appearance of representatives from these families in the fossil record. The oldest fossil bee that can be placed with confidence into one of the extant families, *Cretotrigona prisca*, is an apid bee found in Jersey Amber (~65 Ma)(Michener and Grimaldi 1988). Likewise, we estimate Apidae to be the oldest family (~102 Ma). A few fossils have been described from the late Paleocene and early Eocene (Engel 2001; Nel and Petrulevicius 2003; Michez et al. 2007; Michez et al. 2008) that belong to Apidae, Melittinae and Megachilidae. We hypothesize that these clades diversified slightly after the appearance of Apidae during the Cenomanian

(93-100 Ma). We estimate that Halictidae diversified later (~85 Ma). The first appearance of Halictidae in the fossil record, *Electrolictus aniquus*, is from middle Eocene Baltic amber (Engel 2001). Colletidae and Andrenidae do not appear in the fossil record until the early Miocene (Michener and Poinar Jr. 1996; Rozen 1996; Engel 1999b). We estimate Colletidae to be one of the youngest families (~78 Ma), but Andrenidae is estimated to be only slightly younger than the long-tongued bees (~91 Ma).

The finding of a correspondence in timing of major divergence events in bees and angiosperms represents an important step in determining if bees played a role in Darwin's "abominable mystery." This correspondence however, does not indicate that bees played a causal role per se in the diversification of angiosperms during the Cretaceous. The next step is to determine temporal correspondence between diversification rates of angiosperms and bees. The diversification rates of angiosperms during their early history have been estimated (Magallon and Castillo 2009). Our divergence time estimates for bees can now be used to document temporal trends in bee diversification rates and compare these to the rates found in angiosperms. Our divergence time estimates can also be used to help interpret the historical biogeography of bees, which could provide further insights into the role of bees in angiosperm diversification.

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