Blackwell Publishing

Cladistics 23 (2007) 99–118

Cladistics

10.1111/j.1096-0031.2006.00137.x

Phylogenetic analysis of the corbiculate Apinae based on morphology of the sting apparatus (Hymenoptera: Apidae)

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Accepted 22 August 2006

Abstract

This study aimed to test the various competing hypotheses regarding the relationships among the four tribes of corbiculate apine bees (Euglossini "orchid bees", Bombini "bumble bees", Meliponini "stingless bees", and Apini "honey bees") with a completely new set of previously unstudied morphological characters derived from the sting apparatus. The result was one most parsimonious tree of 49 steps, CI = 89, RI = 93 that is perfectly congruent with most studies based on morphological and combined morphological/molecular data, i.e., Euglossini + (Bombini + (Meliponini + Apini)), supporting a well accepted scenario of social evolution for these bees. This data matrix was then combined with other published matrices for this group in order to perform simultaneous analyses. The problem of how to best combine the multiple matrices that did not use the same exemplars was investigated.

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Relationships among the four tribes of corbiculate bees (Euglossini "orchid bees", Bombini "bumble bees", Meliponini "stingless bees", and Apini "honey bees") are controversial because phylogenetic hypotheses based on morphological characters often support different patterns from those based on molecular data (for, e.g., Michener 1990, and Cameron and Mardulyn 2001). Of even more concern, different morphological or molecular data sets are frequently incongruent with one another (e.g.: Kimsey 1984, and Michener 1990—for morphology, and Cameron 1991, 1993—for molecular data). Thus, a clear resolution has yet to be achieved concerning relationships among the corbiculate bees.

This problem is of more than academic interest, because different topologies in the literature suggest very different patterns of social evolution in this group (Kimsey, 1984; Kerr, 1987; Plant and Paulus, 1987; Michener, 1990; Pereira-Martins and Kerr, 1991; Mardulyn and Cameron, 1999; Schultz et al., 1999; Cameron and Mardulyn, 2001). The purpose of this paper is to test these various competing hypotheses against a completely new set of previously unstudied morphological characters. A combined analysis of molecular, morphological and behavioral data was also performed. Various methods of how to combine these data sets with few overlapping exemplars were explored.

Taxonomic background

The corbiculate bees are a derived clade of the subfamily Apinae (Hymenoptera: Apidae) (Roig-Alsina and Michener, 1993). Females of the non-parasitic taxa, excluding highly eusocial queens, are characterized by their unique pollen-carrying and pollen-manipulating structures including the tibial corbicula (after which the bees are named), rastellum, and auricle (Roig-Alsina and Michener, 1993). There are four tribes of corbiculate bees as follows.

Apini

The Apini is comprised of one extant genus, *Apis*, in which all species are highly eusocial, meaning they have reproductive division of labor, overlapping generations and morphologically distinct castes. The number of species within the Apini has been thought to range from four (Ruttner, 1988) to 24 species by Maa (1953), but is currently believed to include six to 11 species

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(Michener, 2000). The Apini are found in the Palearctic region as well as throughout the African and Oriental regions (Michener, 2000). *Apis mellifera* L. is now found practically worldwide due to human activity.

Meliponini

Like the Apini, species of Meliponini are highly eusocial. As can be assumed from their common name of "stingless bees", they have a greatly reduced sting. The several hundred species are classified into 22 extant genera (Michener, 2000; Camargo and Roubik, 2005). The oldest fossil bee, *Cretotrigona prisca* Michener and Grimaldi, is a member of this tribe, and was found in amber estimated to be of late Cretaceous age ≈ 65 Ma (Michener and Grimaldi, 1988; Engel, 2000a). The Meliponini are found in tropical and southern subtropical areas throughout the world (Michener, 2000).

Bombini

Unlike the Apini and Meliponini, the Bombini do not have morphologically distinct castes, although queens and workers differ in size (Michener, 1974). Therefore, the non-parasitic Bombini are considered to be "primitively" eusocial (*sensu* Michener, 1974). The 239 species of Bombini belong to one genus, *Bombus* (Williams, 1998). Traditionally, most of the workerless social parasites that inhabit nests of other species have been placed in a separate genus *Psithyrus*. Social parasites do not raise their own offspring. Rather, they induce host workers to rear their offspring by taking the place of the host colony's queen. However, following cladistic analyses based on morphological characters from all parts of the body and both sexes, Williams (1991, 1994) recommended that *Psithyrus* be considered a subgenus of *Bombus*. This avoids rendering the genus *Bombus* paraphyletic in relation to *Psithyrus*.

Euglossini

The Euglossini are non-eusocial bees restricted to the Neotropics. The 190 currently recognized species are classified into five genera (Appendix 1), two of which are cleptoparasitic. Cleptoparasitic bees lay their eggs in the cells of a host bee. The cleptoparasite larvae then feed on the food that had been provided for the host larvae. Cladistic analyses of the genera have been performed by Kimsey (1987), Michener (1990), Engel (1999), and Michel-Salzat et al. (2004).

Previous phylogenetic analyses

The monophyly of the corbiculate bees is well established (Sakagami and Michener, 1987; Roig-Alsina and Michener, 1993), as is the monophyly of each tribe (Michener, 1990). Even authors whose molecular data show non-corbiculate "outgroups" arising within the corbiculate tribes cast doubt on their own findings in this regard (Cameron, 1993; Mardulyn and Cameron, 1999; Ascher et al., 2001; Cameron and Mardulyn, 2001). However, numerous alternate hypotheses regarding the relationships among the four tribes have been proposed (Table 1). In fact, of the 15 theoretically possible rooted tree topologies, nine have been supported by one or more data sets.

Resolving conflict

In order to help resolve this conflict, more characters should be added to existing data (Engel, 2001a). In all of the morphological analyses to date, the posterior abdominal segments of the female, including the sting apparatus, have been largely ignored. After finding considerable variation in sting morphology for all subfamilies of the bees, Packer (2003) suggested that all future studies of bee systematics above the species level should include assessment of variation of the sting apparatus. In this paper it is demonstrated that data from this new suite of morphological characters strongly support one of the more traditional patterns of relationships among the corbiculate tribes.

Materials and methods

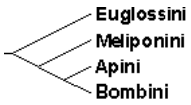
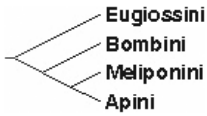
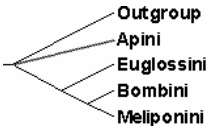
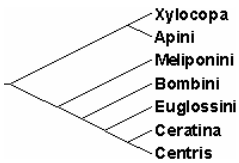
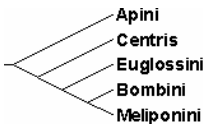
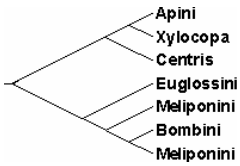
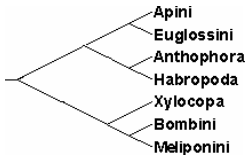
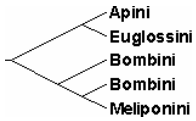
Taxon sampling for sting matrix

Representatives from each of the four tribes were selected following Prendini (2001), who suggested choosing a basal and one or more derived members of each group as exemplars. For the Euglossini, at least one member of each genus was selected, including the two cleptoparasitic genera *Exaerete* and *Aglae*. Selection of representatives of the Bombini was based on Williams' (1994) and Kawakita et al.'s (2004) phylogenies in which the subgenus *Mendacibombus* is found to be basal. Therefore, *Mendacibombus*, in addition to a representative from each of three other more derived subgenera, as well as the parasitic genus *Psithyrus*, were included. For the Meliponini, selection of exemplars was based on Michener (1990), and Costa et al. (2003). These studies produced conflicting phylogenies. Michener (1990) placed *Melipona* as the basal genus based upon morphology, whereas Costa et al.'s (2003) molecular study found *Hypotrigona* to be most basal. Therefore, one *Hypotrigona* and two *Melipona* species were included in the analysis in addition to other derived taxa in Michener (1990). These exemplars also represent the three major lineages of meliponines found by Camargo and Pedro (1992), based on morphological data. For the Apini, five species were included representing all three species groups described in Michener (2000) and supported in Arias and Sheppard (2005). A complete listing

Table 1
Previously proposed topologies for the corbiculate bee tribes. Studies in *italics* presented multiple topologies based on different data sets or methods of analysis.

Topology	Study	Data	Analysis
	Michener (1944)	morphological characters	ET
	Maa (1953)	morphological and behavioral characters	ET
	Michener (1990)	20 morphological characters from Winston and Michener (1977), Kimsey (1984), Plant and Paulus (1987), and Prentice (1991)	P
	Prentice (1991)	26 morphological characters	ET
	**Roig-Alsina and Michener (1993)*	adult morphological characters	P
		adult + larval morphological characters	P
	Chavarría and Carpenter (1994)	54 morphological characters derived from Michener (1990), Prentice (1991), and Alexander (1991a,b) + small and subunit rRNA from Sheppard and McPheron (1991) (seven sites), and mitochondrial 16S rDNA from Cameron (1993) (164 sites)	P
	**Schultz et al. (1999)*	25 morphological characters from Prentice (1991), and Michener (1990)	P
	**Ascher et al. (2001)*	22 morphological/behavioral characters derived from Schultz et al. (1999) + nuclear LW *Rh* (228 sites)	P
	Engel (2001ab) (extinct taxa omitted in tree to left)	50/51 morphological characters including extinct taxa	P
	Noll (2002)	42 behavioral characters	P
	Michener (1974)	morphological characters	
	**Michener (1990)*	20 morphological characters from Winston and Michener (1977), Kimsey (1984), Plant and Paulus (1987) and Prentice (1991)	P
	Schultz et al. (1999)	25 morphological characters from Prentice (1991) and Michener (1990) + 16S rDNA from Cameron (1991), (1993) (171 sites)	P
	Serrão (2001)	four characters from the proventriculus	P
	Sheppard and McPheron (1991)	small and large subunit rRNA (seven sites) for five taxa	P
	Koulianos et al. (1999)	cytochrome *b* amino acid sequences	P
	**Mardulyn and Cameron (1999)*	nuclear LW *Rh* (132 sites)	P
	Cameron (1991)	16S mitochondrial rDNA (129 sites) (position of outgroups not shown)	P ML
	Koulianos et al. (1999)	mitochondrial cytochrome *b* (167 sites)	P ML
	Schultz et al. (1999)	16S rDNA from Cameron (1991), (1993) (171 sites)	P
	**Cameron and Mardulyn (2001)*	LW *Rh* + cytochrome *b* + 28S + 16S (736 sites)	P
	Kerr (1987)	Cytological data	
	Winston and Michener (1977)	17 morphological characters	P
	Kimsey (1984)	23 morphological characters	P
	Sakagami and Maeta (1984)	morphological characters	
	**Cameron and Mardulyn (2001)*	95 morphological characters from Roig-Alsina and Michener (1993) + 16S, 28S, cytochrome *b*, LW *Rh* (736 sites)	P

Table 1
continued

Topology	Study	Data	Analysis
	Plant and Paulus (1987)	five morphological characters from the postmentum	P
	Peixoto & Serrão (2001)	four morphological character from the cardia and cardiac valves	P
	Pereira-Martins and Kerr (1991)	nest architecture	ET
	*Roig-Alsina and Michener (1993)*	larval characters	P
	Cameron (1993) (Outgroup = *Xylocopa*)	16S mitochondrial rDNA (171 sites)	MP ML
	Mardulyn and Cameron (1999) (Outgroup = *Melissodes*)	nuclear LW *Rh* 132 sites	P ML
	Ascher et al. (2001) (Outgroup = *Centris*)	nuclear LW *Rh* 228 sites	P
	*Ascher et al. (2001)*	nuclear LW *Rh* (228 sites)	ML
	*Cameron and Mardulyn (2001)*	nuclear LW *Rh* (144 sites)	P
	*Cameron and Mardulyn (2001)*	cytochrome *b* (239 sites)	P
	*Cameron and Mardulyn (2001)*	nuclear 28S rDNA (168 sites)	P
	*Cameron and Mardulyn (2001)*	mitochondrial 16S rDNA (185 sites)	P

ET = evolutionary taxonomy, P = parsimony, ML = maximum likelihood, * = preferred topology of original authors if multiple ones presented. Number of characters are quoted only for studies with distinct characters that did not include large numbers of taxa that are not relevant to the present study.

of all taxa examined for the cladistic analysis can be found in Appendix 1.

Outgroup taxa were selected based on Roig-Alsina and Michener's (1993) Analysis C. This analysis was of all major groups of long-tongued bees. In this cladogram, the Centridini was placed as the sister tribe of the corbiculate Apinae. Centridini contains two genera, *Centris* and *Epicharis* (Michener, 2000), and an exemplar from each was selected for analysis.

Character selection and coding for sting matrix

The 31 characters chosen for the analysis (Appendix 2) were based on observations of the sting apparatus of the 32 taxa shown in Appendix 1. Multiple specimens of a few species were observed, and additional taxa, mostly identified to genus only, were also observed for all characters to verify that they were consistent within genera. The states of morphological features used for phylogenetic reconstruction are given in Appendix 1. Characters were taken from all skeletal parts of the sting apparatus. The sclerites were measured using an optical micrometer. Angles were measured with a protractor from images taken of the structures. Potentially continuous characters were only used if distinct gaps in measured variables were found. These gaps delimited the upper value of one state and lower value of the next. For example, in character 18, the least number of barbs found in taxa coded as state (0) was two, and the largest number of barbs was four. Taxa coded as state (1) all had 10 barbs. No taxa examined had five to nine barbs; therefore, a distinct gap in the number of barbs permitted unambiguous coding.

A cladistic analysis based on the morphology of the sting apparatus including the Meliponini ("stingless" bees), made for a data matrix with several missing entries. Treatment of missing data is a difficult issue (Platnick et al., 1991; Wilkinson, 1995). Missing entries stem from three different sources: unknown data, inapplicable characters, and polymorphic taxa. All of the missing entries in this data set came from inapplicable characters. If the taxa with the inapplicable character are all members of one, otherwise well supported clade, then the undesirable effects of including missing entries in the data matrix do not occur (Maddison, 1993). Only character 4—incision of lateral lamella—was coded as (?) in more then one clade, the Apini and Meliponini. Exclusion of this character from the analysis had no effect on the resultant topology, therefore it was left in the matrix.

Cladistic analysis of sting matrix

Taxa that had identical character state codings for all of the characters were not included in the data matrix analyzed. Such redundant taxa are indicated by a * in Appendix 1. Parsimony analysis was performed using the exhaustive search command in TNT (Goloboff et al., 2003b). All multistate characters were treated as unordered to allow all possible hypotheses of evolutionary relationship among the character states.

Nodal support was measured in a variety of ways, none of which by themselves are considered entirely without problems. Bremer support (Bremer, 1988, 1994) was estimated using Nona version 2.0 (Goloboff, 1993), by a search keeping trees of 59 steps or less (seven steps more then the most parsimonious tree). Random seed was set to time (i.e., "0"), the number of trees held at 10 000 and the number of iterations of parsimony searches at 20. Symmetric resampling, a method that permits characters to be up- or downweighted with equal probability (Goloboff et al., 2003a) was performed using TNT, with resampling change probability 5% and 10 000 replications. Both GC and frequency slopes measures of support were obtained.

Simultaneous analyses

A simultaneous analysis was performed combining the sting matrix with previously published molecular, morphological and behavioral data sets. The simultaneous analysis was executed, despite little overlap in exemplars between the different data sets, to allow any secondary signal that may be shared by the data sets to emerge, and in agreement with other arguments for simultaneous analyses reviewed in Nixon and Carpenter (1996). Included in the analysis was the aligned molecular data matrix of Cameron and Mardulyn (2001) which included four genes (LW Rhodopsin, cytochrome *b*, 28S, and16S). The morphological matrix of Engel (2001a), which contains extant and fossil corbiculate bee taxa and the behavioral data matrix of Noll (2002) were also added along with the matrix of characters derived from the sting apparatus presented in this paper.

The combined matrix was initially analyzed with only exemplars of the same species from the different matrices fused together. However, this resulted in numerous missing entries, as there is little overlap in taxa between the different data matrices combined. Numerous authors have shown that missing data can influence the results of analysis (Nixon and Davis, 1991; Simmons, 2001). Therefore, a subsequent analysis was performed in which the taxa were fused to genera, except for the Bombini, which were fused to subgenera. This reduced the amount of missing data; however, there was still a great number of taxa with data from only one data set (T1D). Therefore, the data were also analyzed with the taxa fused into tribes. The fossil taxa were only coded for a fraction of the characters of one data set, and therefore may cause an increase in the number of parsimonious trees by being "wildcard" taxa as described in Nixon and Wheeler (1992). Subsequent analyses of these three matrices, in which all T1D were eliminated, were then performed (with Bombini fused to genus in this case).

For all analyses, gaps in the molecular data were treated as missing data. All multistate characters were treated as unordered. Uninformative characters were inactivated, which left 767 informative characters in the combined matrix with terminals fused to species. For all three methods of fusion with and without taxa coded for only one data set, a heuristic search in Nona was performed (see Table 2 for specific commands). Three different Ratchet searches (Nixon, 1999a) were also done to try and find shorter trees by spawning Nona from Winclada version 0.9.9 (Nixon, 1999b). The first Ratchet sampled 10% of the characters, the second sampled 15% and the third 20% of the characters (see Appendix 4 for specific commands).

Nodal support was estimated as noted above.

Results

Sting matrix

The exhaustive search command in TNT yielded one most parsimonious tree of 49 steps with a consistency index of 0.89 and a retention index of 0.93 (Fig. 1). This tree was stable to successive approximations character weighting (Farris, 1969; Carpenter, 1988).

The monophyly of the corbiculate bees was supported by one synapomorphy (character 19(1): gonostylus undivided). The Euglossini came out as the basal tribe of the group and its monophyly was indicated by five unique synapomorphies. One unique synapomorphy plus one parallel character state change supported the cladistic grouping of the Bombini with the Apini and Meliponini. Monophyly of the Bombini was supported by four unique synapomorphies plus one homoplasious character. The monophyletic grouping of the Apini and Meliponini was strongly supported by five synapomorphies and monophyly of each tribe was supported by three synapomorphies. Symmetric resampling indicates that the data set strongly supports all nodes. Each group had a GC value of 85 or above and many had a small negative slope. A high Bremer support value of 6 was found for the Apini + Meliponini clade. All nodal support values are listed in Table 2.

To verify that the Meliponini + Apini grouping is not due to an artifact of missing data in the Meliponini, a matrix was analyzed in which the Bombini were coded as missing for all characters in which the Meliponini also have missing values. Despite this modification, the Meliponini still grouped with the Apini.

Simultaneous analyses

Depending on how the taxa were fused and whether taxa coded for only one data set (T1D) were included or not, different topologies were obtained (Fig. 2). When terminals were fused to species level, Bombini was rendered paraphyletic by Apini and Meliponini (Fig. 2a). Symmetric resampling did not indicate strong

Table 2
Nodal support values for Figs 1 and 2 trees. The difference in frequency between a group and the most frequent contradictory group (GC), and the slope of the frequency of a group (Slope) are listed for nodes indicated on Figs 1 and 2. Bremer support values are listed for nodes found on Fig. 1.

Tree	Node	GC	Slope	Bremer
Fig. 1	1	85	+0.00	1
	2	99	−0.11	2
	3	100	+0.00	6
	4	99	+0.00	3
	5	99	+0.00	4
	6	85	−2.45	1
	7	99	−0.41	2
	8	100	+0.00	5
	9	99	−0.01	3
Fig. 2A	1	100	+0.00	
	2	19	−41.55	
	3	26	−38.37	
	4	1	−49.74	
	5	2	−49.42	
	6	21	−40.50	
	7	7	−46.65	
	8	71	−14.59	
Fig. 2B	1	100	+0.00	
	2	95	−3.74	
	3	96	−2.58	
	4	99	−0.23	
	5	99	−0.67	
	6	52	−37.3	
	7	52	−37.3	
	8	53	−36.72	
Fig. 2C	1	100	+0.00	
	2	100	+0.00	
	3	92	−1.50	
	4	31	−0.06	
	5	94	−0.09	
Fig. 2D	1	100	+0.00	
	2	100	−1.00	
	3	99	−7.28	
	4	57	−45.94	
	5	29	−47.99	
	6	100	−28.21	
	7	99	−6.79	
	8	57	−44.17	
	9	100	+0.00	
	10	57	−47.04	
Fig. 2E	1	100	+0.00	
	2	99	−0.22	
	3	99	−0.14	
	4	100	+0.00	
	5	99	−0.31	
	6	91	−3.83	
	7	100	+0.00	
	8	63	−6.43	
	9	62	−6.83	
	10	73	−6.22	
Fig. 2F	1	[100]	+0.00	
	2	100	+0.00	
	3	94	−1.19	
	4	30	−1.10	

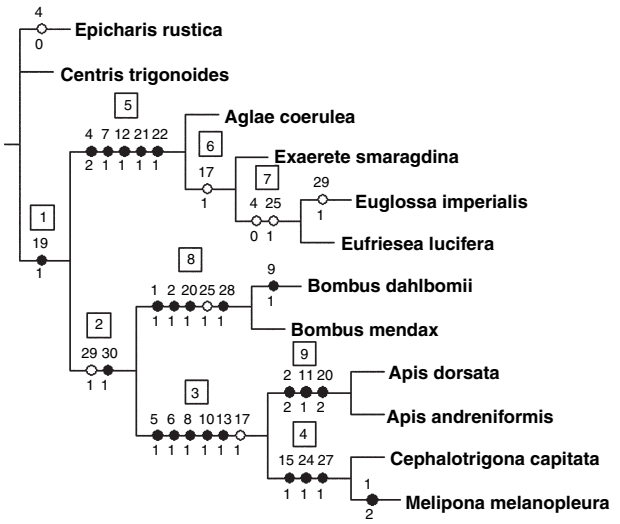


Fig. 1. Most parsimonious tree of 49 steps with a consistency index of 0.89 and a retention index of 0.93. Unambiguous optimizations are shown by circles, filled circles indicate non-homoplasious changes and open ones indicate homoplasies. Character numbers are shown above and derived states below the circles. Node numbers are indicated in boxes. Support values for each numbered node are given in Table 2.

support for any of the ingroup nodes (Table 2). Removing T1D did not resolve the paraphyly of Bombini (Fig. 2d). The Meliponini + Bombini (in part) clade was only weakly supported (Table 2). Fusing terminals at the level of genus resulted in Apini and Meliponini forming an unresolved bush with a monophyletic Bombini (Fig. 2b). All nodes were strongly supported with the exception of those within Euglossini (Table 2). Removing T1D resulted in a monophyletic Meliponini but an unresolved node between this tribe, *Apis* and *Bombus* (Fig. 2e). Once again, the Apini + Meliponini + Bombini node was well supported (Table 2). With the terminals fused at tribal level, relationships between Apini, Bombini and Meliponini were unresolved (Fig. 2c), but monophyly of the three tribes combined was again well supported (Table 2). Removal of T1D resulted in Apini and Meliponini forming a monophyletic group with Bombini sister to them and the Euglossini sister to all the other corbiculate tribes (Fig. 2F). Apini + Meliponini is only moderately supported (Table 2).

Discussion

Cladistic analysis of sting characters and of the combined matrix with terminals fused to genera or tribes (excluding terminals coded for only one data set) produced a cladogram congruent with others based on morphological (Michener, 1944, 1990; Maa, 1953; Prentice, 1991; Roig-Alsina and Michener, 1993; Schultz et al., 1999; Engel, 2001a,b), behavioral (Noll, 2002), and a combination of morphological and molecular characters (Chavarría and Carpenter, 1994; Ascher et al., 2001). However, this topology disagrees with phylogenies based on molecular (Cameron, 1991, 1993; Sheppard and McPheron, 1991; Koulianos et al., 1999; Mardulyn and Cameron, 1999; Schultz et al., 1999; Cameron and Mardulyn, 2001), cytological (Kerr, 1987), and nest architectural data (Pereira-Martin and Kerr, 1991); some phylogenies based on morphological data (Michener, 1974, 1990; Winston and Michener, 1977; Kimsey, 1984; Sakagami and Maeta, 1984; Plant and Paulus, 1987), and some derived from a combination of morphological and molecular characters (Schultz et al., 1999; Cameron and Mardulyn, 2001). However, many of these studies did not use rigorous phylogenetic methods (see Table 1 for details).

Resolving the phylogeny of the corbiculate apine tribes is an integral component to understanding the evolution of social behavior within this clade. Solitary and communal behavior are found in the Euglossini, while the Bombini exhibit primitive eusociality and the Meliponini and Apini are highly eusocial. Therefore, a wide range of behaviors is found in the corbiculate bee clade whose myriad proposed phylogenies bear implications for behavioral evolution. At the center of this debate, is the question of whether advanced eusociality stems from a single or dual origin. Based on the morphology of the sting apparatus as well as numerous previous studies (Michener, 1944, 1990; Maa, 1953; Prentice, 1991; Roig-Alsina and Michener, 1993; Chavarría and Carpenter, 1994; Schultz et al., 1999; Ascher et al., 2001; Engel, 2001a,b; Noll, 2002), advanced eusocial behavior evolved once in the common ancestor of the Apini and Meliponini. Primitive eusociality, as found in the Bombini, perhaps forms an intermediate step between the predominantly solitary Euglossini and the highly eusocial taxa.

The combined analyses showed that how data matrices are combined can have an impact on the resultant topology and consequently on the pattern of evolution of the attributes of interest (Fig. 2). Interestingly, the only analysis that fully resolved tribal relationships was also congruent with the single origin hypothesis (Fig. 2f).

An integral step in answering the question of single versus dual origin would be to investigate why the molecular and morphological data disagree. Earlier studies based on molecular data have been thought to suffer from poor taxon sampling and choice of outgroups (Engel, 2001a; Schultz et al., 2001). For example, Sheppard and McPheron's (1991) analysis using rRNA sequences, was based on only seven informative characters (sites) for five taxa. Cameron (1991, 1993) only used *Xylocopa virginica* L. as an outgroup. This species is not thought to be a close relative of the corbiculate

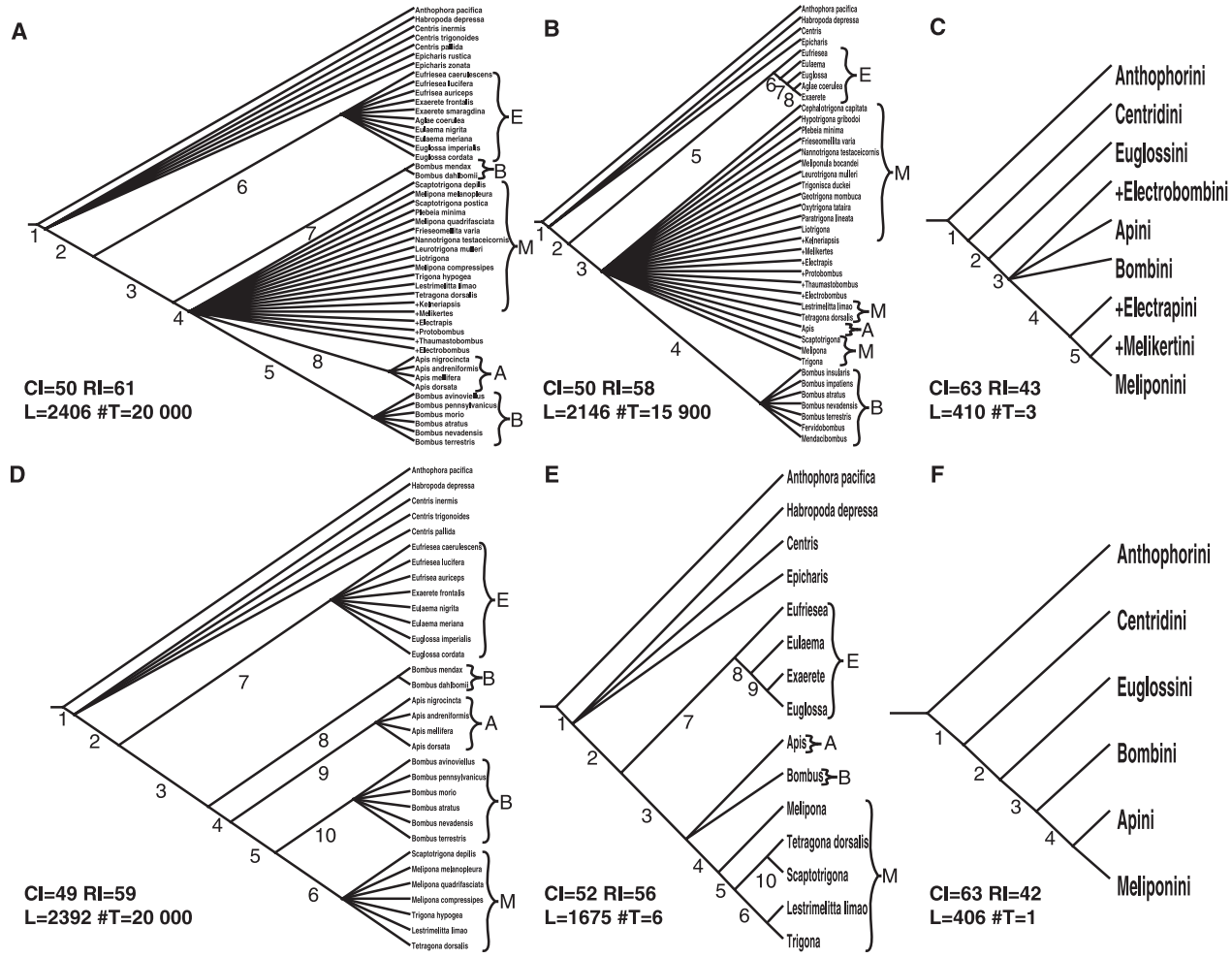


Fig. 2. Consensus trees of combined analyses. A = Apini, B = Bombini, E = Euglossini, M = Meliponini, and fossil taxa are indicated by +0. CI, RI, Length and number of trees given for most equally parsimonious trees the consensus is derived from. (A) Terminals fused to species. (B) Terminals fused to genus. (C) Terminals fused to tribe. (D–E) Same as (A–C) but with terminals coded for only one data set removed. Node numbers are indicated in boxes. Support values for each numbered node are given in Table 2.

apine bees (Roig-Alsina and Michener, 1993). Recent molecular analyses have employed a much greater number of informative sites and a wider array of ingroup and outgroup taxa. The resulting molecular trees however, still unite the Bombini with the Meliponini. The problem then may be the selection of genes sequenced. Cameron (1991, 1993), and Koulianos et al. (1999) employed mitochondrial DNA regions that are commonly used in species-level phylogenies because of their higher rate of substitutions, which can be disadvantageous when trying to resolve divergences of more than 5–10 million years (Lin and Danforth, 2004). Engel (2000a) estimated the divergence among the corbiculate tribes to have occurred at least 75 Ma during the Mesozoic. Therefore, more slowly evolving nuclear genes may be more appropriate for reconstructing the phylogeny of the corbiculates. However, studies using nuclear genes (Mardulyn and Cameron, 1999; Cameron and Mardulyn, 2001) still report topologies incongruent with the theory of a single origin for highly eusocial behavior. The nuclear gene used in Mardulyn and Cameron (1999), and one of the two nuclear genes used in Cameron and Mardulyn (2001) was the major opsin (LW *Rh*) gene, which has been found to provide weak support at the tribal level in bees (Ascher et al., 2001).

Another possible problem plaguing phylogenetic analysis of corbiculate apine bees is ''long-branch attraction'' (Felsenstein, 1978). Ascher et al. (2001) suggested that extant *Apis* species tend to cluster at the end of a long branch due to massive extinction of earlier diverging species (Engel, 2000b) and biased base composition at third positions in the molecular data. Engel (2001a,b) attempted to overcome that difficulty by including numerous extinct corbiculate bees found in Baltic amber in an analysis with extant taxa and found that extinct taxa group cladistically with the honey bees

and stingless bees (Engel, 2001a,b). Therefore, topologies that unite the Bombini with Meliponini cannot account for the character combinations exhibited by fossils.

Lockhart and Cameron (2001) suggest the following possible sources of error in the morphological data to account for the disagreement between morphology and molecules; continuously varying characters coded discretely, non-independent characters coded separately, and characters coded for supraspecific taxa rather than for species. Schultz et al. (2001), reviewed these criticisms. Lockhart and Cameron (2001) also suggested that sociality was driving (Meliponini + Apini) morphology. If this is the case, then support of the morphological phylogeny of (Meliponini + Apini) using sting apparatus data is even more surprising given the completely different directions of evolution of the sting in the two groups: its loss of function as a sting in Meliponini and its increase in stinging function through autotomy in *Apis*.

The discordance between the morphological and molecular phylogenies may not be substantial. Thompson and Oldroyd (2004) found that only one of the four molecular data sets used in Cameron and Mardulyn (2001) could statistically reject any of the 15 possible outgroup-rooted phylogenetic hypotheses. Therefore, support for the dual origins hypothesis is dependent on one gene (cytochrome *b*).

Acknowledgments

This research was funded by the Natural Sciences and Engineering Research Council of Canada through a research grant awarded to L.P. Additional support was obtained from the Ontario Graduate Scholarships in Sciences and Technology through a graduate scholarship to S.C. We thank E. Almeida, J.M. Carpenter, and two anonymous reviewers for comments on the manuscript, and P.A. Goloboff for help with TNT.

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Appendix 1

Data matrix of character states for the parsimony analysis of the corbiculate apine bees. Taxa with an * are coded identically to the taxa listed before them and were removed from the matrix prior to analysis.

		Characters																														
Tribe	Species	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Euglossini	*Aglae coerulea* Lepeletier and Serville	0	0	0	0	2	0	0	1	0	0	0	0	1	0	1	0	0	0	0	1	0	1	1	0	0	0	0	0	1	0	0
	Exaerete smaragdina (Guérin)	0	0	0	0	2	0	0	1	0	0	0	0	1	0	0	0	0	1	0	1	0	1	1	0	0	0	0	0	1	0	0
	Euglossa imperialis Cockerell	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	1	0	1	1	0	0	1	0	0	1	1	0
	**Euglossa decorata* Smith	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	1	0	1	1	0	0	1	0	0	1	1	0
	**Euglossa piliventris* Guérin	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	1	0	1	1	0	0	1	0	0	1	1	0
	Eufriesea lucifera (Kimsey)	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	1	0	1	1	0	0	1	0	0	1	0	0
	**Eufriesea mariana* (Mocsary)	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	1	0	1	1	0	0	1	0	0	1	0	0
	**Eufriesea rufocauda* (Kimsey)	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	1	0	1	1	0	0	1	0	0	1	0	0
	**Eufriesea surinamensis* (Linnaeus)	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	1	0	1	1	0	0	1	0	0	1	0	0
	**Eulaema meriana* (Olivier)	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	1	0	1	1	0	0	1	0	0	1	0	0
	**Eulaema polychroma* (Mocsary)	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	1	0	1	1	0	0	1	0	0	1	0	0
Bombini	*Bombus mendax* Gerstaecker	0	1	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	1	1	0	2	1	1
	**Bombus waltoni* Cockerell	0	1	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	1	1	0	2	1	1
	**Bombus insularis* Smith	0	1	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	1	1	0	2	1	1
	Bombus dahlbomii Guérin-Méneville	0	1	1	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	1	1	0	2	1	1
	**Bombus impatiens* Cresson	0	1	1	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	1	1	0	2	1	1
Meliponini	*Cephalotrigona capitata* (Smith)	2	0	0	2	?	1	1	0	1	3	1	0	0	1	?	1	2	1	?	1	0	0	?	?	1	?	?	1	?	?	?
	**Melipona compressipes* (Fabricius)	2	0	0	2	?	1	1	0	1	3	1	0	0	1	?	1	2	1	?	1	0	0	?	?	1	?	?	1	?	?	?
	**Lestrimelitta limao* (Smith)	2	0	0	2	?	1	1	0	1	3	1	0	0	1	?	1	2	1	?	1	0	0	?	?	1	?	?	1	?	?	?
	Melipona melanopleura Cockerell	2	2	?	2	?	1	1	0	1	3	1	0	0	1	?	1	2	1	?	1	0	0	?	?	1	?	?	1	?	?	?
	**Hypotrigona gribodoi* (Magretti)	2	2	?	2	?	1	1	0	1	3	1	0	0	1	?	1	2	1	?	1	0	0	?	?	1	?	?	1	?	?	?
	**Trigona hypogea* Silverstri	2	2	?	2	?	1	1	0	1	3	1	0	0	1	?	1	2	1	?	1	0	0	?	?	1	?	?	1	?	?	?
	**Tetragona dorsalis* (*Smith*)	2	2	?	2	?	1	1	0	1	3	1	0	0	1	?	1	2	1	?	1	0	0	?	?	1	?	?	1	?	?	?
Apini	*Apis andreniformis* Smith	1	0	2	1	?	1	1	0	1	2	1	1	0	1	1	0	1	1	1	1	2	0	2	1	0	0	0	0	4	1	1
	**Apis florae* Fabricius	1	0	2	1	?	1	1	0	1	2	1	1	0	1	1	0	1	1	1	1	2	0	2	1	0	0	0	0	4	1	1
	Apis dorsata Fabricius	1	0	2	1	?	1	1	0	1	2	1	1	0	1	1	0	1	1	1	1	2	0	2	1	0	0	0	0	3	1	1
	**Apis mellifera* Fabricius	1	0	2	1	?	1	1	0	1	2	1	1	0	1	1	0	1	1	1	1	2	0	2	1	0	0	0	0	3	1	1
	**Apis cerana* Fabricius	1	0	2	1	?	1	1	0	1	2	1	1	0	1	1	0	1	1	1	1	2	0	2	1	0	0	0	0	3	1	1
Centridini	*Epicharis rustica* (Olivier)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
(outgroups)	*Centris trigonoides* Lepeletier	?	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 2: Character descriptions

Morphological terms follow those found in Packer (2003).

Hemitergite 7 (HT7)

0. Marginal ridge of HT7 (Fig. 3)

0: Complete or almost complete, HT7 entirely (Fig. 4a) or almost entirely (Fig. 4b) enclosed by well defined ridge

1: incomplete, posterior portion of HT7 not enclosed by ridge (Fig. 4c)

2: absent posteriorly; and absent or reduced laterally (Fig. 4d)

The margin of the plate where it was not enclosed by the ridge was markedly darkened in taxa coded as (0) for which the ridge was not entirely complete. *Centris trigonoides* was coded as (?) because there was a posterior incision in the lamina spiracularis. This incision precluded the marginal ridge being complete, although it was clearly at least 3/4 complete.

1. Lateral process—location

0: anteriorly positioned (Fig. 4a,c)

1: 1/2 way along lateral portion of marginal ridge (Fig. 4b)

2: absent or reduced to a nub (Fig. 4d)

The medially positioned lateral process (state 1) is a synapomorphy for the Bombini.

2. Lateral process—orientation

0: at a 30–110° angle from lateral ridge and largely straight (Fig. 4a)

1: at a 40–80° angle from lateral ridge and clearly sinuate (Fig. 4b)

2: at a 130–160° angle from lateral ridge and straight (Fig. 4c)

The lateral process in *Exaerete* was straight and at a 170° angle from the lateral ridge, but it was coded as

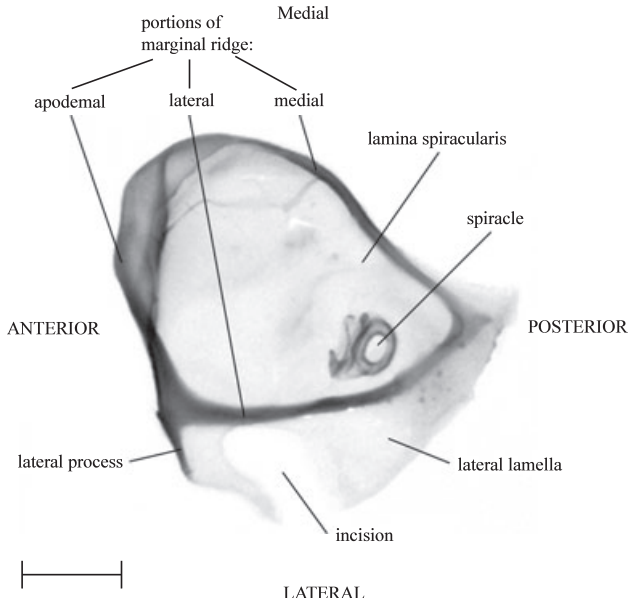

Fig. 3. Seventh hemitergite of *Eufriesea surinamensis*. Scale line represents 0.5 mm.

state (0). The reason for this is as follows. The lateral ridge in all other taxa was roughly perpendicular to the spiracular atrium, whereas in *Exaerete* it was at a 30° angle such that if the lateral process was at the same angle in *Exaerete* as in the other taxa, it would have been at a 100° angle. This character could not be coded for *Hypotrigona gribodi* and *Melipona melanopleura,* because the lateral process was not present. As a result, they were coded as (?). Although the range of the angle in the Bombini overlaps with that in the Euglossini and outgroups, the medial position and sinuate shape of the lateral process in Bombini clearly represents a different state from that in the Euglossini and outgroups. The lateral process being at a 130–160° angle (state 2) is a synapomorphy for the Apini.

3. Width of lamina spiracularis compared with that of the lateral lamella

0: < 2.9× (Fig. 4a,b)

1: ≥ ~4.6× (Fig. 4c)

2: lateral lamella absent (Fig. 4d)

Absence of the lateral lamella (state 2) is a synapomorphy for the Meliponini.

4. Incision of lateral lamella

0: anteriorly positioned, narrowed towards lateral margin of lamella (Fig. 4a)

1: posteriorly positioned, widest towards lateral margin of lamella (Fig. 4b)

2: anteriorly positioned, widest towards lateral margin of lamella

The Meliponini and Apini were coded as (?) because the lateral lamella was either absent or greatly reduced.

5. Position of spiracle in lamina spiracularis

0: posterolateral (Fig. 4a,b)

1: central (Fig. 4c,d)

In all of the outgroups, and the Euglossini, and Bombini, the spiracle was positioned posteriorly near the lateral ridge. In the Apini and Meliponini, it was positioned more medially. This character supports monophyly Apini + Meliponini.

Hemitergite 8 (HT8) (Fig. 5)

6. Anterior ridge of HT8

0: extended around medial margin of apodeme (Fig. 6a,b)

1: not extended (Fig. 6c)

The anterior ridge was extended medially (state 0) in all of the Euglossini, Bombini, and outgroups. The anterior ridge restricted to the anterior edge of hemitergite 8 (state 1) is a synapomorphy for the Apini and Meliponini.

7. Posterior ridge

0: weakly developed or absent (Fig. 6b,c)

1: well developed, complete (Fig. 6a)

The posterior ridge was only distinct and complete in the Euglossini.

8. Anteromedial portion of apodeme

0: not produced anteriorly (Fig. 6a,b)

1: produced anteriorly (Fig. 6c)

State (1) is a synapomorphy for the Apini and Meliponini.

9. Angle of the carina separating the apodeme from the plate of HT8 in relation to the anterior ridge

0: 45–70° (Fig. 6a)

1: 85° (Fig. 6b)

2: 20–25° (Fig. 6c)

3: 5–10°

The carina was between a 45° and 70° angle in the outgroups, Euglossini, *Bombus insularis, Bombus mendax* and *Bombus waltoni* and formed a moderate and consistent curve to the posterior edge of the hemitergite. This resulted in the apodemal area being slightly larger then the exposed portion of the plate. In *Bombus dahlbomii* and *B. impatiens*, the carina was at a 90° angle. It was initially directed ventrally along the anterior condyle before abruptly curving towards the posterior edge of the hemitergite. Consequently, the apodemal area was distinctly larger than the exposed portion of the plate. In the Apini, the apodeme was relatively smaller, because the carina was at a 20° angle. The apodeme was even more reduced in the Meliponini, because the carina runs almost parallel to the dorsal ridge.

10. Lateral margin of plate of HT8

0: extended laterally far beyond the apodeme (Fig. 6-a,b)

1: not extended (Fig. 6c)

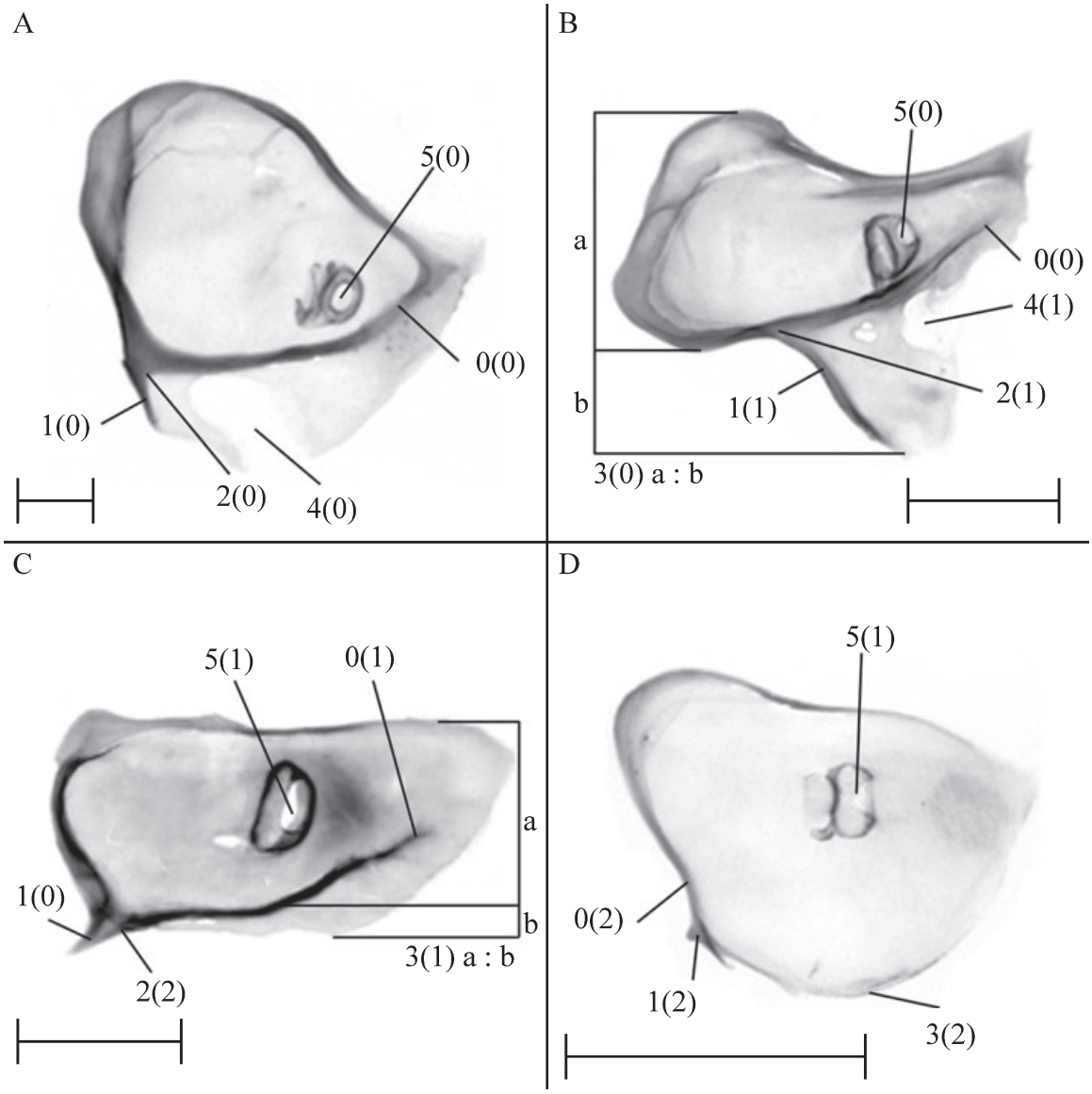

Fig. 4. Variation in the structure of seventh hemitergite of corbiculate bees labeled to show characters referred to in text. Views of morphologically dorsal surface. (A) *Eufriesea surinamensis*, (B) *Bombus waltoni*, (C) *Apis dorsata*, (D) *Melipona melanopleura*. Character states shown on figures. Scale lines represent 0.5 mm.

State (1) is a synapomorphy for the Apini and Meliponini.

11. Posteromedial edge of plate of HT8

0: narrowed towards posterior margin of apodeme (Fig. 6a,b)

1: expanded posteriorly away from margin of apodeme (Fig. 6c)

State (1) was only found in the Apini.

12. Shape of HT8 in anterior view

0: variable, but never distinctly sinuate

1: sinuate (Fig. 6d)

The sinuate shaped hemitergite was a synapomorphy for the Euglossini. The anterior portion of the apodeme was distinctly convex, whereas the posterior portion was distinctly concave.

First valvifer (Fig. 7)

13. Orientation of sting plates in body of insect

0: mostly dorsoventral (Fig. 8a)

1: mostly horizontal (Fig. 8c,d)

The angle between the anterior portions of the first rami was used as a point of reference for this character. The angle between the first rami for the Apini and Meliponini was near 180°. Consequently, the hemitergites are mostly horizontally oriented.

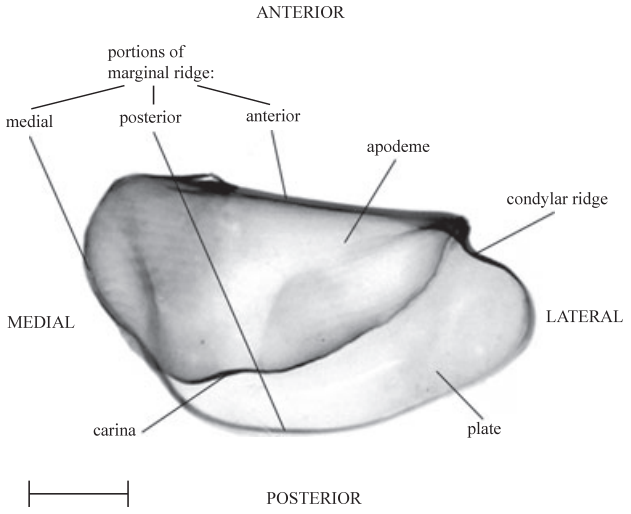

Fig. 5. Eighth hemitergite of *Eufriesea rufocauda* labeled to show characters referred to in text. Scale line represents 0.5 mm.

Conversely, the angle between the first rami of the Bombini, Euglossini, and outgroups was about 45°. Therefore, the hemitergites are oriented mostly dorsoventrally.

14. Length of anterior portion of first ramus (the part perpendicular to length of sting shaft) compared with height of sting shaft bulb

0: long, 5–7× height of bulb (**Fig. 9a)**

1: short, 2–3.5× height of bulb (**Fig. 9c)**

The first ramus of the outgroups, and the Euglossini, with the exception of the cleptoparasitic *Aglae coerulea*, is long compared to that of the other taxa examined. The Meliponini were coded as (?), because their sting shaft was extremely reduced. The first ramus is much longer than the height of the sting shaft bulb in these bees, but this was attributed more to the reduced height of the sting shaft bulb than to the length of the first ramus.

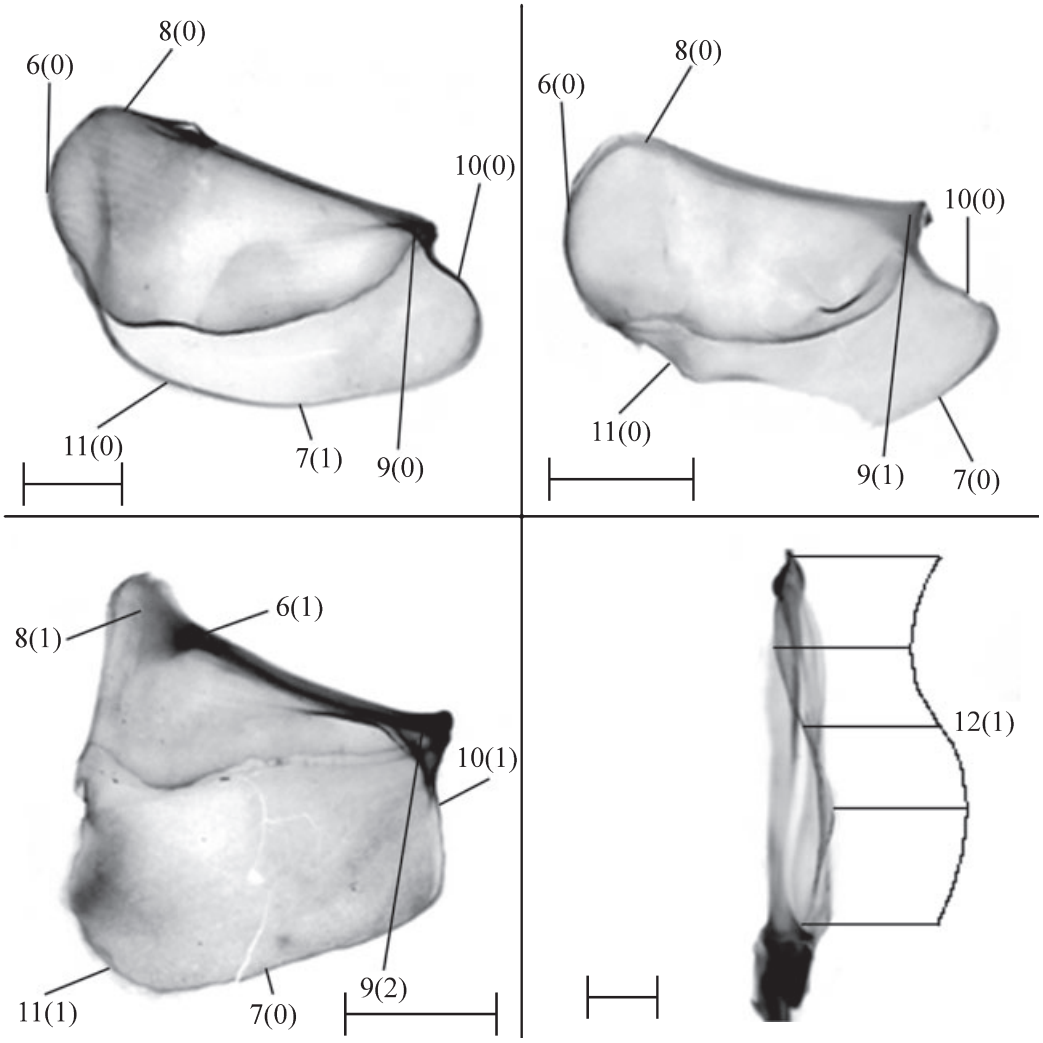

Fig. 6. Variation in structure of 8th hemitergites of corbiculate bees. (A) *Eufriesea rufocauda,* dorsal view, (B) *Bombus impatiens*, dorsal view, (C) *Apis dorsata,* dorsal view, (D) *Eulaema polychroma*, anterior view. Character states shown on figures. Scale lines represent 0.5 mm.

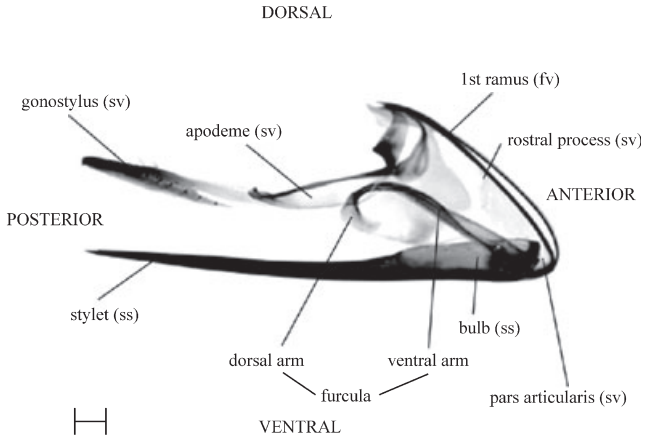

Fig. 7. Lateral view of the sting apparatus of *Eulaema polychroma* with the seventh and eighth hemitergites removed. Fv = first valvifer, sv = second valvifer, ss = sting shaft. Scale lines represent 0.5 mm.

15. Posterior margin of the first valvifer
0: concave (Fig. 9b–d)
1: straight (Fig. 8d)

State (1) is a synapomorphy for the Meliponini.

16. Ventral margin of the first valvifer
0: convex (Fig. 9a)
1: sinuate (Fig. 9d)
2: concave (Fig. 8d)

In the outgroups, Euglossini, and Bombini, the ventral edge was convex (state 0) and directed ventrally at an angle of ~70° from the dorsal edge, and then curved posteriorly so that it was parallel to the dorsal edge, making this portion of the first valvifer more elongate and slender. In the Apini, the ventral edge was directed ventrally at an angle of ~45° from the dorsal edge. The ventral edge maintained its sinuate and slight curve all the way to the ventral angle of the plate. Consequently, the ventral edge was never parallel to the

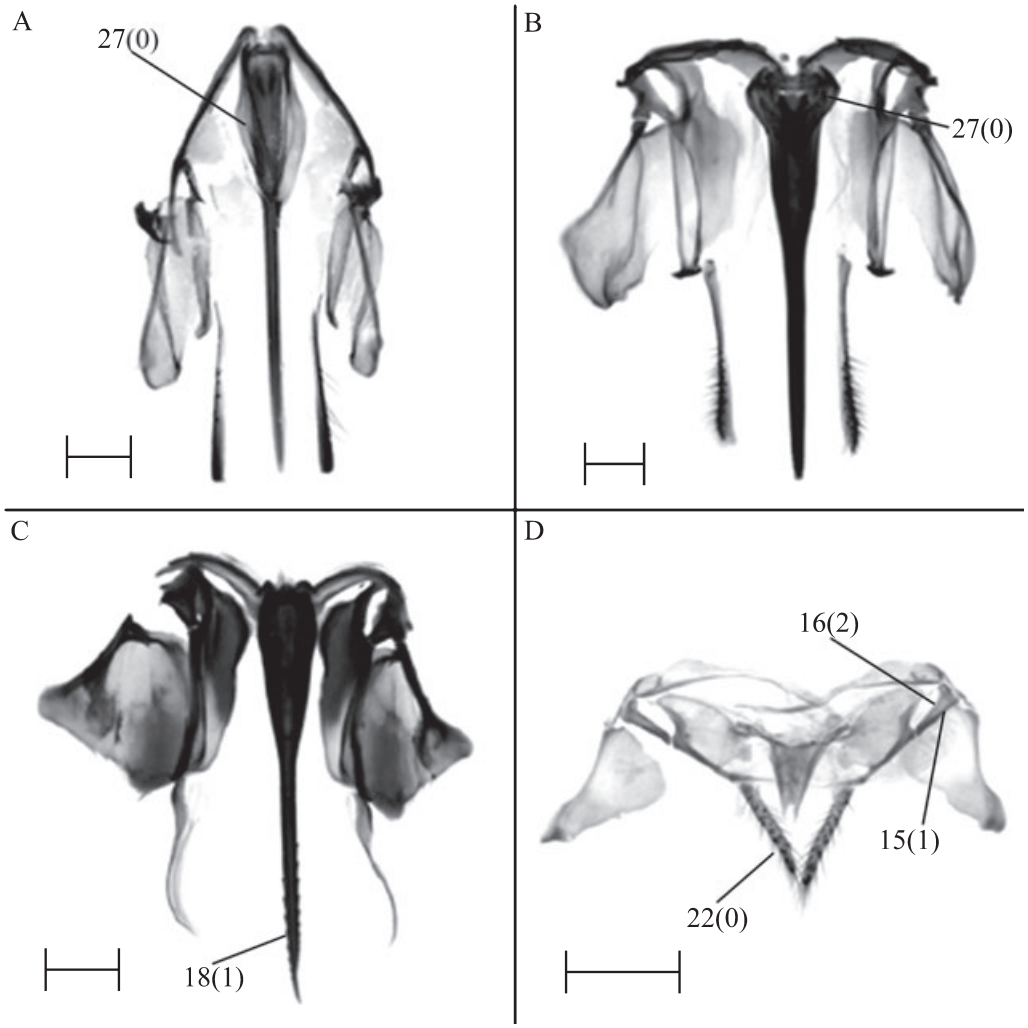

Fig. 8. Variation in the structure of the sting apparatus of corbiculate bees with the seventh hemitergites removed, view of dorsal surface. (A) *Euglossa piliventris*, (B) *Bombus* sp., (C) *Apis dorsata*, (D) *Melipona compressipes*. Character states shown on figures. Scale lines represent 0.5 mm.

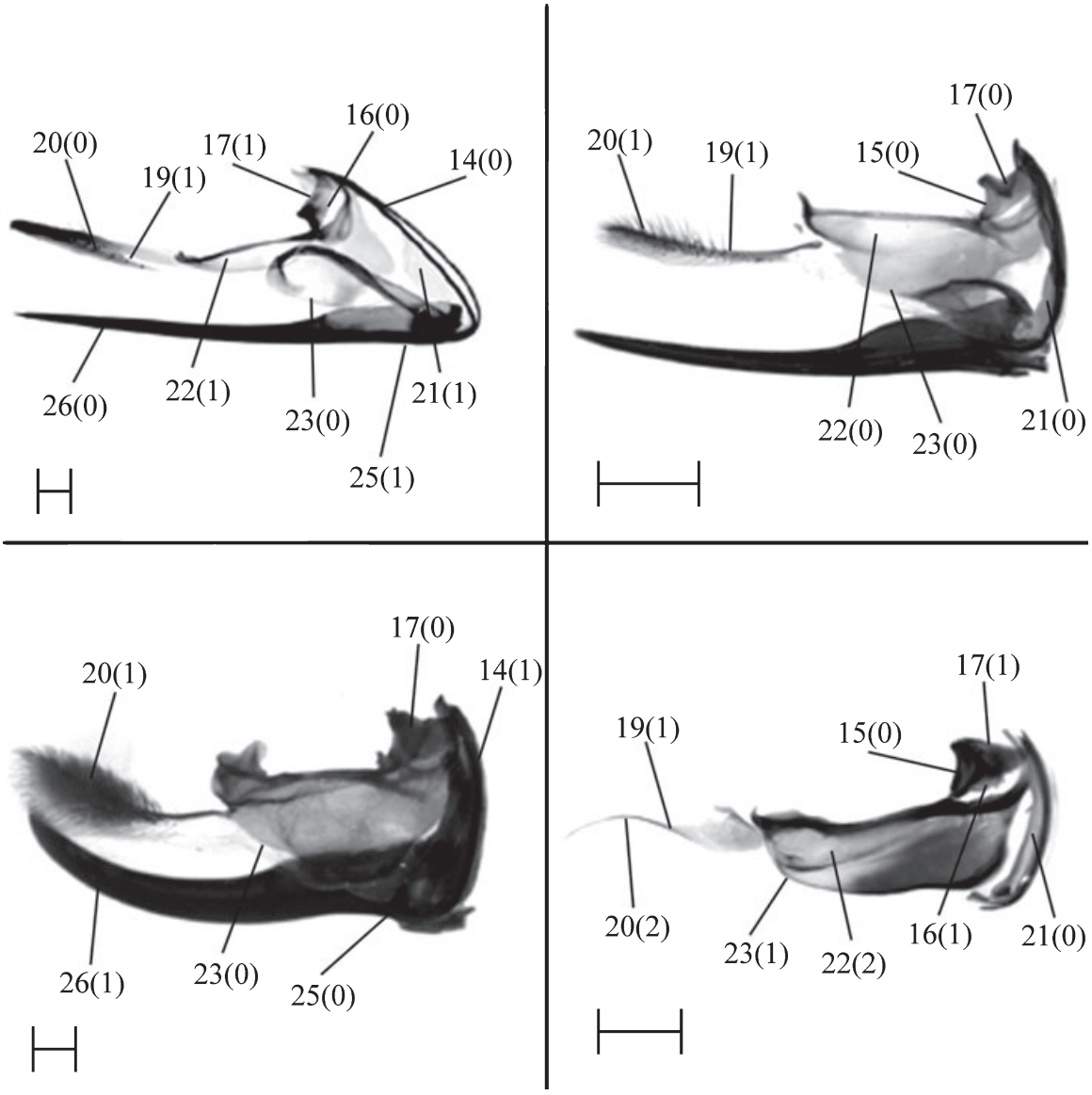

Fig. 9. Variation in structure of the sting apparatus of corbiculate bees with the seventh and eighth hemitergites removed, in lateral view. (A) *Eulaema polychroma*, (B) *Bombus* spp., (C) *Bombus insularis*, (D) *Apis dorsata*, with the sting shaft removed. Character states shown on figures. Scale lines represent 0.5 mm.

dorsal edge in the Apini, and therefore the first valvifer is more triangular shaped than in the other taxa (state 1). The ventral edge was also never parallel to the dorsal edge in the Meliponini, because of its concave form (state 2).

17. Longitudinal carina of the first valvifer

0: developed into a tooth (Fig. 9b,c)

1: not developed into a tooth (Fig. 9a,d)

The longitudinal carina was developed into a distinct tooth in the outgroups, the Bombini, and the cleptoparasitic *Aglae coerulea*.

18. Number of barbs on lancet

0: 2-4

1: 10-12 (Fig. 8c)

Having 10–12 barbs on the lancet is a synapomorphy for the Apini.

Second valvifer (Fig. 7)

19. Gonostylus

0: Clearly divided into two segments

1: undivided with at most an incomplete medial unsclerotized region (Fig. 9a–d)

All of the ingroup taxa had only 1 apparent segment. Some Euglossini had an unsclerotized v-shaped region, but it never made its way across the entire width of the gonostylus to separate it into two distinct segments.

20. Setae on gonostylus

0: distinct, long, dispersed and not forming brush (Figs 8d and 9a)

1: distinct, long, and forming brush (Fig. 9b,c)

2: indistinct, short (Fig. 9d)

The distinct brush found at the apical end of the gonostylus is a synapomorphy for the Bombini. The lack of distinct setae is a synapomorphy for the Apini.

21. Rostral process

0: gradually expanding towards pars articularis (Fig. 9b,d)

1: abruptly expanded towards pars articularis (Fig. 9a)

State (1) is a synapomorphy for the Euglossini.

22. Apodeme of the second valvifer

0: narrowly lunate, tapering evenly towards both ends (Fig. 9b)

1: basal portion wide, gradually narrowing apically (Fig. 9b)

2: basal portion narrow, gradually widening apically (Fig. 9d)

State (1) is a synapomorphy for the Euglossini and state (2) is a synapomorphy for the Apini. Meliponini

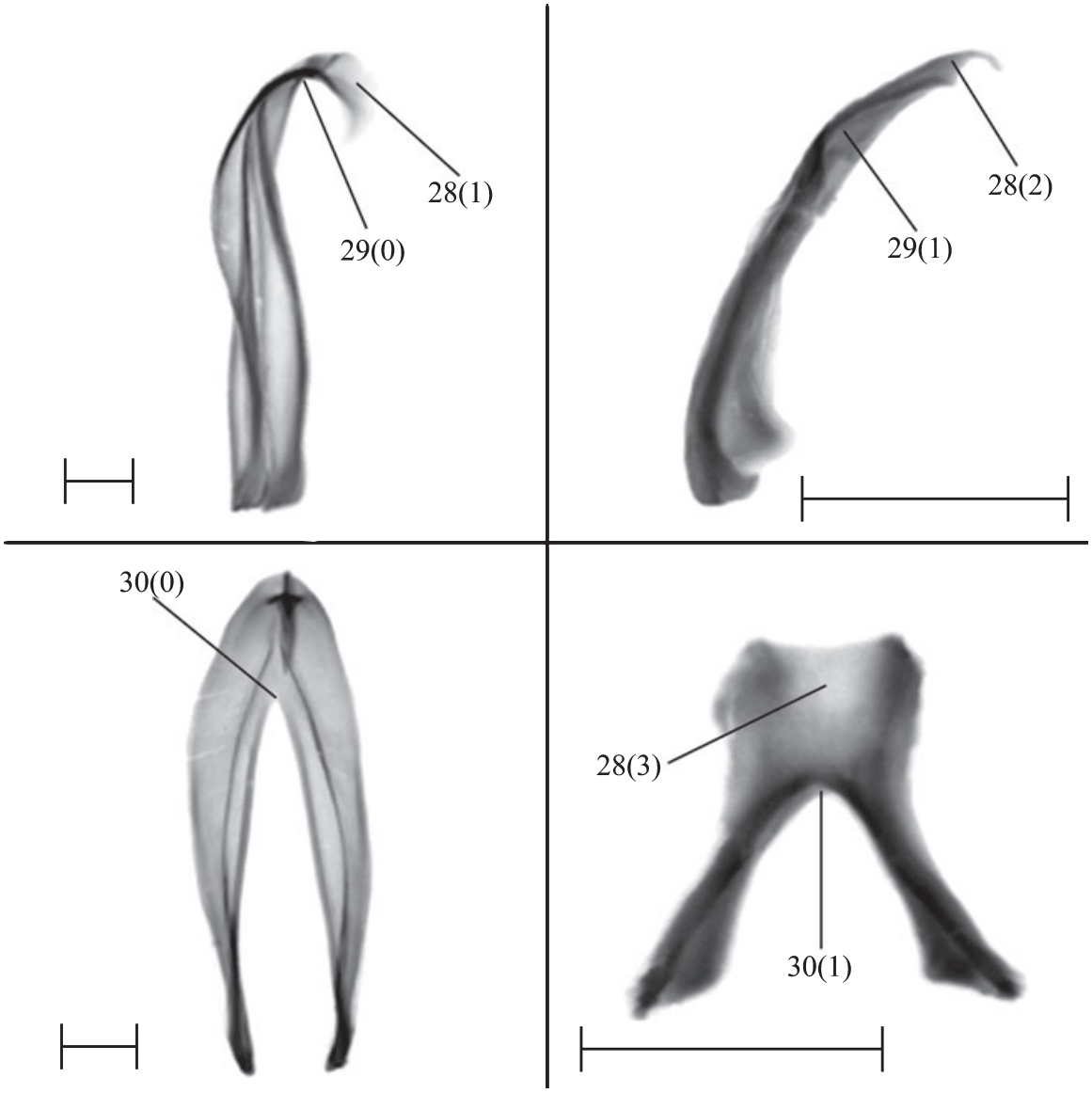

Fig. 10. Variation in structure of the furcula of corbiculate bees. (A) *Eulaema polychroma*, lateral view, (B) *Bombus impatiens*, lateral view, (C) *Eulaema polychroma*, anterior view, (D) *Apis dorsata,* anterior view. Character states shown on figures. Scale lines represent 0.5 mm.

had to be coded as (?) because of the reduced state of the second valvifer.

23. Posteroapical edge of the 2nd valvifer

0: evenly curved (Fig. 9a–c)

1: abruptly bent into a 110° angle (Fig. 9d)

The posteroapical edge of the second valvifer in the outgroups, Euglossini, and Bombini was straight and at an approximately 45° angle to the apodemal ridge throughout its length. In the Apini, it ran somewhat parallel to the apodemal ridge for most of its length, and then formed a 110° angle upwards. Meliponini was coded as (?) because of the reduced state of the second valvifer.

Sting shaft

24. Sting shaft

0: not reduced (Fig. 8a–c)

1: reduced (Fig. 8d)

The sting apparatus as a whole was reduced in the Meliponini, especially the sting shaft itself. Because of this character, the state "reduced" was never used as a state for any other character for the Meliponini. Instead, they were coded as (?) when a structure was too diminished to discern which state it possessed, as was also the case with the next two characters.

25. Bulb of the sting shaft

0: ventrally bent (Fig. 9c)

1: straight (Fig. 9a)

Homoplasious in Bombini and some Euglossini.

26. Stylet of the sting shaft

0: straight or slightly curved dorsad (Fig. 9a)

1: distinctly curved dorsad (Fig. 9c)

State (1) is a synapomorphy for the Bombini.

Furcula

27. Furcula

0: present (Fig. 8a,b)

1: absent (Fig. 8d)

The furcula was only absent in the Meliponini. This absence may be linked to the reduced state of the sting apparatus. Nonetheless, it was kept as a separate character because it is the only sclerite that has completely disappeared.

28. Dorsal arm of furcula

0: laterally compressed and about ½ length of ventral arms

1: laterally compressed and 1/10–1/5 length of ventral arms (Fig. 10a)

2: not distinctly compressed with apex pointed (Fig. 10b)

3: dorsoventrally compressed (Fig. 10d)

4: absent

The absence of the dorsal arm in *Apis florea* and *Apis andreniformis* suggests that these two species form a monophyletic clade. This character, and the next two, were coded as (?) in Meliponini because of the absence of the entire furcula in this tribe.

29. Shape of furcula in lateral view

0: angulate at junction of dorsal and ventral arms (Fig. 10a)

1: evenly curved (Fig. 10b)

The furcula of the outgroups and Euglossini was abruptly bent into a 90° angle at the point where the ventral arms fuse into the dorsal arm. *Euglossa imperialis* was an exception. Its furcula, along with that of the Apini and Bombini, was gently curved.

30. Angle subtended by ventral arms of furcula

0: acute (Fig. 10c)

1: rounded (Fig. 10d)

The ventral arms of the outgroups and Euglossini were long, and their inner edge formed a narrow "v" with an acute angle. The ventral arms of the Bombini and Apini did not appear to be as long, and their inner edge formed a wide rounded "u".

Appendix 3

Taxa that were included in the studies used for the simultaneous analyses. A "+" sign indicates that the taxon was coded in the matrix of the original study. The matrix of Engel (2001a) used genera as exemplars. Therefore a "+" sign for that study does not indicate that the particular species was examined, but that the species is included in a genus that was coded.

Species	Present study	Cameron and Mardulyn (2001)	Engel (2001a)	Noll (2002)
Aglae coerulea Lepeletier and Serville	+			
Eufriesea auriceps (Friese)			+	+
Eufriesea caerulescens Lepeletier and Serville		+	+	
Eufriesea lucifera (Kimsey)	+		+	
Eufriesea mariana (Mocsary)	+		+	
Eufriesea rufocauda (Kimsey)	+		+	
Eufriesea surinamensis (Linnaeus)	+		+	
Euglossa cordata (Linnaeus)			+	+
Euglossa decorata Smith	+		+	
Euglossa imperialis Cockerell	+	+	+	
Euglossa piliventris Guérin	+		+	

Appendix 3
Continued

Species	Present study	Cameron and Mardulyn (2001)	Engel (2001a)	Noll (2002)
Eulaema meriana (Olivier)	+	+	+	
Eulaema nigrita Lepeletier			+	+
Eulaema polychroma (Mocsary)	+		+	
Exaerete frontalis (Guérin)		+		
Exaerete smaragdina (Guérin)	+			
Bombus atratus Franklin			+	+
Bombus avinoviellus (Skorikov)		+	+	
Bombus dahlbomii Guérin-Méneville	+		+	
Bombus impatiens Cresson	+		+	
Bombus insularis Smith	+		+	
Bombus mendax Gerstaecker	+		+	
Bombus morio (Swederus)			+	+
Bombus nevadensis Cresson			+	+
Bombus pensylvanicus (DeGeer)		+	+	
Bombus terrestris (Linnaeus)		+	+	+
Bombus waltoni Cockerell	+		+	
Cephalotrigona capitata (Smith)	+			
Frieseomellita varia (Lepeletier)				+
Geotrigona mombuca (Smith)				+
Hypotrigona gribodoi (Magretti)	+			
†*Kelneriapis* Sakagami			+	
Lestrimelitta limao (Smith)	+	+		
Leurotrigona muelleri (Friese)				+
Liotrigona Moure			+	
†*Liotrigonopsis* Engel			+	
Melipona compressipes (Fabricius)	+	+	+	
Melipona melanopleura Cockerell	+		+	
Melipona quadrifasciata Lepeletier			+	+
Meliponula bocandei (Spinola)				+
Nannotrigona testaceicornis (Lepeletier)				+
Oxytrigona tataira Smith				+
Paratrigona lineata (Lepeletier)				+
Plebeia minima (Gribodo)				+
Scaptotrigona depilis (Moure)		+		
Scaptotrigona postica (Latreille)				+
Tetragona dorsalis (Smith)	+	+		
Trigona hypogea Silverstri	+	+		
Trigona spinipes (Fabricius)				+
Trigonisca duckei (Friese)				+
†*Electrobombus* Engel			+	
†*Electrapis* Cockerell			+	
†*Protobombus* Cockerell			+	
†*Thaumastobombus* Engel			+	
†*Melikertes* Engel			+	
†*Melissites* Engel			+	
†*Roussyana* Manning			+	
†*Succinapis* Engel			+	
Apis andreniformis Smith	+		+	
Apis cerana Fabricius	+		+	
Apis dorsata Fabricius	+	+	+	+
Apis florae Fabricius	+		+	
Apis mellifera Fabricius	+	+	+	+
Apis nigrocincta Smith		+	+	
Centris inermis Friese		+	+	
Centris pallida Fox			+	+
Centris trigonoides Lepeletier	+		+	
Epicharis rustica (Olivier)	+			
Epicharis zonata Smith				+
Melissodes druriella (Kirby)		+		
Anthophora pacifica Cresson		+	+	
Habropoda depressa Fowler		+		
Xylocopa virginica (Linnaeus)		+	+	

Appendix 4

Analyses done for combined analysis. T1D = taxa coded for only one data set removed.

Matrix fusion	Analysis	Commands	No. of trees	Length
Species	Heuristic	H 20 000; h/10; mu*20 000	20 000	2406
	Ratchet	Defaults except (hold 2, and 10% of characters sampled	2	2406
	Ratchet	Defaults except (hold 2, and 15% of characters sampled	4	2406
	Ratchet	Defaults except (hold 2, and 20% of characters sampled	6	2407
Genus	Heuristic	H 100 000; h/100; mu*10 000 Followed by max*	15 900	2146
	Ratchet	Defaults except (hold 2, and 10% of characters sampled	10	2146
	Ratchet	Defaults except (hold 2, and 15% of characters sampled	2	2149
	Ratchet	Defaults except (hold 2, and 20% of characters sampled	4	2146
Tribe	Heuristic	H 100 000; h/100; mu*10 000 Followed by max*	2	410
	Ratchet	Defaults except (hold 2, and 10% of characters sampled	3	410
	Ratchet	Defaults except (hold 2, and 15% of characters sampled	3	410
	Ratchet	Defaults except (hold 2, and 20% of characters sampled	2	410
Exemplar T1D	Heuristic	H 10 000:h/20; mu*1000	20 000	2392
	Ratchet	Defaults except (hold 2, and 10% of characters sampled)	2	2393
	Ratchet	Defaults except (hold 2, and 15% of characters sampled)	4	2392
	Ratchet	Defaults except (hold 2, and 20% of characters sampled)	8	2392
Genus T1D	Heuristic	H 2001:h/20; mu*1000	6	1675
	Ratchet	Defaults except (hold 2, and 10% of characters sampled)	4	1675
	Ratchet	Defaults except (hold 2, and 15% of characters sampled)	4	1675
	Ratchet	Defaults except (hold 2, and 20% of characters sampled)	5	1675
Tribe T1D	Heuristic	H 100 000; h/100; mu*10 000 Followed by max*	1	406
	Ratchet	Defaults except (hold 2, and 10% of characters sampled	1	406
	Ratchet	Defaults except (hold 2, and 15% of characters sampled	1	406
	Ratchet	Defaults except (hold 2, and 20% of characters sampled	1	406