

# Phylogeny of the bees of the family Apidae based on larval characters with focus on the origin of cleptoparasitism (Hymenoptera: Apiformes)

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**Abstract.** Fifty-four genera of the bee family Apidae comprising almost all tribes were analysed based on 77 traditional and one new character of the mature larvae. Nine, especially cleptoparasitic species, were newly added. Analyses were performed by maximum parsimony and Bayesian inference. Trees inferred from the analysis of the complete dataset were rooted by taxa from the families Melittidae and Megachilidae. Unrooted trees inferred from the analysis of the partial dataset (excluding outgroup taxa) are also presented to preclude possible negative effects of the outgroup on the topology of the ingroup. Only the subfamily Nomadinae was statistically well supported. The monophyly of the subfamilies Xylocopinae and Apinae was not topologically recovered. The monophyly of the tribe Tetrapediini was supported, and this tribe was found to be related to xylocopine taxa. At the very least, larval morphology suggests that *Tetrapedia* is not a member of the subfamily Apinae. Our analyses support the monophyly of the Eucerine line (Emphorini, Eucerini, Exomalopsini, Tapinotaspidini) and of the Apine line (Anthophorini, Apini, Bombini, Centridini, Euglossini, Meliponini). All analyses support the monophyly of totally cleptoparasitic tribes of the subfamily Apinae. We named this group the Melectine line (Ericrocidini, Isepeolini, Melectini, Osirini, Protepeolini, Rhathymini). In previous studies all these cleptoparasitic tribes were considered independent evolutionary lineages. Our results suggest that their similarities with hosts in morphology and pattern are probably the result of convergence and host–parasite co-evolution than phylogenetic affinity. According to the present analysis, the cleptoparasitism has evolved independently only six times within the family Apidae.

## Introduction

Bees (Apiformes) form a monophyletic group (Michener, 2000; Danforth *et al.*, 2006) comprising over 17 000 species (Michener, 2000). They are usually divided into two variously named groups (Michener, 2000): one consisting of short-tongued bees and the other of long-tongued bees (Roig-Alsina & Michener, 1993). Apidae belong to the group of long-tongued bees, whose monophyly is supported

by morphological and molecular analyses (Roig-Alsina & Michener, 1993; Danforth *et al.*, 2006).

Roig-Alsina & Michener (1993) reconstructed the phylogenetic relationships within the family Apidae using 131 morphological characters of adults and 77 morphological characters of mature larvae. This elaborate work produced the prevailing concept for the phylogeny and taxonomy of the family Apidae, supporting the monophyly of all three subfamilies of Apidae (Xylocopinae, Nomadinae and Apinae), each characterized by several apomorphies. This dominant and respected phylogenetic hypothesis stems only from the analysis of adult characters, although the analysis of larval characters and a combined analysis (adult and larval morphology) were included in this publication.

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Phylogenetic trees based on larval characters were highly ambiguous and less informative, because they did not recover the monophyly of the family Apidae. Furthermore, the character matrix was incomplete with respect to the number of species for which larval morphology was known. The results of the larval analysis, as well as the results of the combined analysis, were not useful and thus generally have not been accepted by other authors (e.g. Michener, 2000; Engel, 2001).

Species of the subfamily Apinae, the most diverse subfamily within the family Apidae, were previously divided into two phylogenetic lineages: the Eucerine line and the Apine line. Silveira (1993) established these terms for several tribes related to Eucerini and Apini in his cladistic work based on adult morphology and later Engel (2001) extended it over all the tribes of the subfamily. Silveira (1993) noted two synapomorphies of the Apine line: distinct stipital sclerite of the maxilla and small stigma of the forewing, although the latter is variable. The Eucerine line is also characterized by two adult characters: sternum 7 of the male with two or four apical lobes and the union of the anterior tentorial arm to the head wall, forming a large triangular subantennal area. However, these characters are somewhat variable as well. The clade commonly called corbiculate Apinae or the Apine clade represents the most studied group of the Apine line including four closely related and mostly eusocial tribes (Cameron & Mardulyn, 2001) with developed corbicula, a unique organ for collecting and transporting resin and pollen (Apini, Bombini, Euglossini, Meliponini) (Michener, 2000; Engel, 2001).

In contrast to the subfamily Apinae, all members of the subfamily Nomadinae are obligate cleptoparasites. The phylogenetic relationships among numerous genera of all tribes (Nomadinae s.str.) have recently been studied in several publications. The results of analyses based on adult morphology (see Roig-Alsina, 1991; Roig-Alsina & Michener, 1993) were incongruent with analyses based on larval morphology (see Alexander, 1990; Rozen, 1996). The largest discrepancy appeared after the analysis of many obligate cleptoparasitic tribes together with nonparasitic bees of all three subfamilies of Apidae. Several cleptoparasitic groups were transferred from the subfamily Nomadinae (s.l.) to the subfamily Apinae (*Coelioxoides*, Isepeolini, Parepeolini and Osirini) as separate evolutionary lineages (Roig-Alsina & Michener, 1993). These groups usually clustered in previous studies (e.g. Alexander, 1990). More recent results support the current conception that cleptoparasitism evolved independently 11 times within the family Apidae (Rozen, 2000; Michel-Salzat *et al.*, 2004). Cleptoparasitic bees of the subfamily Nomadinae might have a common cleptoparasitic ancestor, whereas other cleptoparasitic groups probably arose independently within the Eucerine line (*Ctenoplectrina*, Isepeolini, Protepeolini and Osirini) and within the Apine line of the subfamily Apinae (*Aglae*, *Coelioxoides*, Ericrocini, *Exaerete*, Melectini and Rhathymini) (Michener & Greenberg, 1980; Rozen, 2000). Surprisingly, no cleptoparasitic species have yet been found within the subfamily Xyllocopinae.

Michener (1953) reviewed descriptions of mature larvae of numerous, mostly pollen-collecting groups of bees. Later, Rozen (1966, 1969, 1996, 2001) described larvae of the majority of cleptoparasitic bees. Larvae of several phylogenetically important cleptoparasitic species have been described in the last few years: *Leiopodus lacertinus* (Protepeolini, Apinae) (Roig-Alsina & Rozen, 1994), *Melectoides bellus* (Isepeolini, Apinae) (Michelette *et al.*, 2000), *Tetrapedia diversipes* and *Coelioxoides waltheriae* (Tetrapediini, Apinae) (Alves-dos-Santos *et al.*, 2002), *Protosiris gigas* and *Parepeolus minutus* (Osirini, Apinae) and their hosts of the tribe Tapinotaspidiini (Rozen *et al.*, 2006), *Epeoloides coecutiens* (Osirini, Apinae) and *Leiopodus trochantericus* (Protepeolini, Apinae) (Straka & Bogusch, in press). Thus, with the exception of Manuelini and Ancylini tribes, at least one species from each tribe of the family Apidae has been studied.

Adult morphology is well known throughout all groups of bees; consequently, the phylogeny based on these morphological characters has been, until now, the only accepted phylogeny of the family Apidae. Knowledge of larval morphology can help in the reconstruction and understanding of the bee phylogeny. Analysis of characters of mature larvae can be expected to produce an alternative to the current opinion, which may support previous facts and warn of possible misconceptions.

The primary goal of the current study was to extend the knowledge of the phylogeny of the family Apidae using larval morphology of a higher number of analysed genera. We focused on the reconstruction of the phylogeny of cleptoparasitic bee lineages, which has never been studied in such a tribal sample. The main purpose of our work was to test the hypothesis of 11 independent origins of the cleptoparasitic way of life among bees of the family Apidae.

## Materials and methods

The character data matrix of mature larvae for our phylogenetic analysis was adopted in essence from Roig-Alsina & Michener (1993). McGinley (1981) had previously developed it for all families of bees, but he used less-dense sampling of the family Apidae. We also used the numbers of characters and state coding largely according to the later study (with modifications as described below), and extended the adopted matrix with one new character (78). Advanced state occurs in the newly added taxa *Epeoloides* and *Leiopodus*. Our sample consisted of 78 characters, 41 ingroup operational taxonomic units (OTU) (Apidae) and 13 outgroup OTUs (Melittidae, Megachilidae) (Table 1). Characters from *Epeoloides coecutiens* and five characters (previously missing) of the genus *Leiopodus* were adopted from Straka & Bogusch (in press), those of *Ericrocis lata*, *Melectoides bellus*, *Tetrapedia diversipes*, *Coelioxoides waltheriae*, *Parepeolus minutus* and its host *Lanthanomelissa betinae*, *Protosiris gigas* and its host *Monoeca haemorrhoidalis* were adopted from descriptions of their mature larvae (Rozen & Buchmann, 1990; Michelette *et al.*, 2000;

**Table 1.** Re-evaluated morphological data matrix of mature larvae. The first 13 taxa represent the outgroups. The symbol '?' indicates an unknown state. For specification of characters, see Roig-Alsina & Michener (1993).

Genus	1234567890	1	1111111112	2222222223	3333333334	4444444445	5555555556	6666666667	777777777
	(tribe)								
<i>Dasyoda</i>		0000000000	0000000000	0000100002	0000000000	0121201000	1000000000	0000001000	100000000
<i>Melitta</i>		0100100000	0000000000	0000100001	0000000010	2000011010	0001000000	0000001000	100000000
<i>Macropis</i>		0100100000	0000000000	0000100000	0000000010	2000011110	0000010000	0000011000	000000000
<i>Meganomia</i>		1000010000	0001000001	0000100000	0000000010	2000011010	0000001000	0000001000	000000020
<i>Pararhophites</i>		0011100010	0101011010	0000110002	1111100110	2100201110	1001000000	0000200100	10001010
<i>Parafidelia</i>		1000001010	0001011011	0100100012	0011101110	2100201110	1001001001	1000201101	00100120
<i>Neofidelia</i>		1011100010	0101001011	0100100002	0011101110	2100201110	1001011001	1000201100	00100020
<i>Fidelia</i>		1000000010	00010000?1	0100100002	0011101110	2100201110	1000100101	1000201101	00100120
<i>Lithurgus</i>		1011000010	0101000010	0100100002	0011101110	1100001110	1001001001	1000200000	00200?20
<i>Anthidium</i>		1011100010	1100010111	0100110002	0101000010	2100101110	0001011001	1100200100	01000?00
<i>Hoplitis</i>		1011100010	0101010010	0100100002	0101101100	0100201110	0001011000	1100000100	10010010
<i>Coeloxys</i>		1011100010	0100000011	0100100012	0101101010	2100201110	0001011001	1100200100	01100020
<i>Megachile</i>		1011100010	0101010011	0100110002	0001101110	0100201110	0001011001	1100200100	01200?20
<i>Exomalopsis</i>		1000000000	0002000000	000100101	0100000010	2100001010	1000000000	0001000100	00000000
<i>Paratrapedia</i>		000?00000	0002000000	0000100002	0100001110	112?001110	0001000000	0001001100	00001000
<i>Monoeca</i>		000010010	0102000000	0000100011	0100011110	10000?1110	0011000000	0000001100	01000020
<i>Lanthanomelissa</i>		0000?00010	0002000001	000010001?	1000111010	1000011110	0000000000	0000001100	1000?020
<i>Diadasia</i>		0000000000	0102000001	0000000001	0001101000	0100201010	0000001010	0001201100	00000020
<i>Melittoma</i>		1010000000	0002000001	0000000101	0001101010	0100201010	0000001010	0100001100	00000020
<i>Peponapis</i>		1101000010	0002000000	0000000101	0001101010	1100011110	0000001010	0000000101	00211000
<i>Xenoglossa</i>		1101000010	0002000000	0000000101	0001111010	1100011110	0000010000	0000000101	00211000
<i>Anthophora</i>		0010000000	0001000000	0000000010	0101001100	0121000001	1000010000	0000001000	01200100
<i>Habropoda</i>		0011000000	0001000000	0000000111	0101001100	0121000001	100?100000	0000001000	11200100
<i>Centris</i>		1001000000	0001000000	0000100011	0101001110	2000011010	1000011100	0000200100	01010000
<i>Epicharis</i>		0000000000	0001000000	0000100011	0100011100	0021011100	1001011000	0000200100	01100100
<i>Melecta</i>		0000000000	0001000000	0000100010	0000001100	2000201010	0001010010	0000001100	00200020
<i>Thyreus</i>		0100000000	0001001001	?001000010	0101101100	2?00200000	1011110000	0000000100	00210020
<i>Zacrosmia</i>		0010100000	0000000001	0000100000	000110112?	2000001110	00?1?10000	0000001100	00000020
<i>Rhathymus</i>		0000000100	0001000000	1000100010	0101101110	2001201110	0011000010	0000200100	00200020
<i>Acanthopus</i>		0010000010	0001001010	1000000010	0101101110	2001001010	0011100101	0000200100	00100020
<i>Ericrocis</i>		0001000010	0001001000	1?00100010	0101101110	2000001010	0011110100	0000000000	00100020
<i>Epeoloides</i>		1110000000	0001002001	1100100010	0001001100	1000001010	1011000000	0000001100	00001021
<i>Protosiris</i>		0100100000	0101001001	1100100000	0101001110	1001001010	1010?10001	0000001100	0000?000
<i>Parepeolus</i>		0000000000	0000000001	1?00100000	0101000010	1001001010	0010100000	0000001100	0000?020
<i>Leptodus</i>		1011000010	1001000001	0100101000	0101001100	1101001110	1000101000	0100001100	10100021
<i>Isepeolus</i>		0010100010	2000001100	1100200000	1101000010	2000001110	0001110000	0000000000	00001000
<i>Melectoides</i>		0010100000	1101000000	1101200010	0101000010	2000001010	0000110000	0000000100	00010020
<i>Ammobates</i>		0011110001	2011100010	0001001000	0101000021	2021200002	1010100000	1000000000	00000020
<i>Oreopasites</i>		0011100001	2011101110	0000000000	0101000021	2001000002	1010100000	0000100000	00200000
<i>Epeolus</i>		0000100001	1001101100	0001000000	0101100021	2021200002	1010110000	0000000010	10100020
<i>Nomada</i>		0000110001	2011100100	0001001000	0000000021	2001000002	1010100000	0000000000	10000020

Table 1. Continued.

Genus	(tribe)	1	111111112	222222223	333333334	444444445	555555556	666666667	777777777
<i>Paranomada</i>	(Brachynomadini)	000001101	2000100100	0000000000	1101000021	2000200002	1010001000	0000010100	112000000
<i>Holocaspites</i>	(Ammobatoideini)	000010001	2001100100	0001000000	0101000021	2021200002	1010110001	0000000000	100000000
<i>Neopasites</i>	(Blastini)	001100001	2011100100	0010000000	1101000021	201??00?02	1000100000	0001200000	000100000
<i>Neolarra</i>	(Neolarrini)	001100001	2011100100	0010000000	1101000021	201??00?02	1000100000	0001100000	002000000
<i>Coelioxoides</i>	(Tetrapediini)	101110000	0100000000	0000100000	1101000000	111??00?0?	1100110001	0000200000	100000000
<i>Tetrapedia</i>	(Tetrapediini)	001110000	0100000000	0000100011	0001101100	112000010?	1100110001	0000000000	000000000
<i>Xylocopa</i>	(Xylocopini)	101100010	0101000101	0000110001	0101001110	1120001002	1100100001	0000000201	001000100
<i>Ceratina</i>	(Ceratini)	101100010	0101000010	0000100001	1101001010	1020010002	1100110000	000000000?	??1??0?0
<i>Allodape</i>	(Allodapini)	101100010	1101100011	0000100010	1101100020	201??00?02	1100110000	010011010?	?1??010
<i>Bombus</i>	(Bombini)	101010010	0102000010	0000100012	0101101110	2000001110	0000001001	1010011101	011100100
<i>Apis</i>	(Apini)	111000000	1101101110	0000100000	0101100010	2020211110	0000110001	1000001001	010110100
<i>Melipona</i>	(Meliponini)	111000010	1100101110	0000100110	1101000110	2021011110	0000100000	0010000101	010100100
<i>Eufriesea</i>	(Euglossini)	100000000	0101000101	0000110012	0101101110	2000001010	0000101001	1010001101	010020100

Alves-dos-Santos *et al.*, 2002; Rozen *et al.*, 2006). Data from the most recently described cleptoparasitic tribes of the subfamily Nomadinae (Rozen, 1996) were not added because the data matrix presented consisted of differently defined characters (some characters missing, some divided into two or more characters), thus it was incompatible with the data matrix used in the current analysis.

For the list of larvae studied for re-evaluation of the data matrix in this study, see Table 2. All specimens are deposited in the collection of the first author. Drawings were prepared using a drawing tube (camera lucida) attached to an Olympus BX40 light microscope (from temporary glycerine slides).

All ingroup and outgroup OTUs were used in the first two analyses and a reduced OTU sample (excluding the outgroup) was used in two subsequent analyses.

Maximum parsimony (MP) analyses were carried out using PAUP\* 4.0b10 (Swofford, 2002) and MRBAYES 3.1.2 (Huelsenbeck, 2000) was used to generate a phylogeny by Bayesian inference (BI).

Characters were unordered and unweighted in all MP analyses (Fitch, 1971). The most-parsimonious trees were identified by a heuristic search with 1000 random stepwise additions of OTUs (TBR [tree bisection-reconnection] branch swapping, MulTrees option in effect). Bootstrap trees were calculated in 1000 replicates with 50 addition sequence replicates.

BI was performed by Metropolis Coupled Markov Chain Monte Carlo sampling for 5 100 000 generations (four simultaneous chains in one run, sample frequency 100, burn-in first 1001 trees) under the Mkv model (Lewis, 2001) with rate heterogeneity modelled by a gamma distribution with the shape parameter estimated from the data.

## Re-evaluation of characters

We re-evaluated states of several characters of mature larvae and added one character (Table 1).

18. *Pleurostomal ridge*: (0) well developed; (1) weak.

Rozen *et al.* (1978) presented this character in the description of the larva of *Leiopodus singularis* to be well developed (0). However, in the data matrix from Roig-Alsina & Michener (1993) it was coded as state 1. We changed the character state in our data matrix to the correct state 0.

22. *Clypeal length*: (0) moderate to long; (1) short.

State 1 was known only among long-tongued bees in the family Megachilidae (Roig-Alsina & Michener, 1993). The clypeus of *Epeoloides* is four times wider than the clypeal length in the middle. It is approximately the same clypeal length as in some larvae of megachilid bees and according to the conception of McGinley's (1981) scale it refers to the short clypeus. Considering the larval morphology of *Epeoloides*, we propose the homology of the clypeus with a short membranous area between the frons and the labrum in Isepeolini and Protepeolini (see Rozen, 1966; Rozen *et al.*,

**Table 2.** List of species studied for re-evaluation of the morphological data matrix of mature larvae.

Genus	Species	Family	Tribe	Origin
<i>Macropis</i>	<i>fulvipes</i> (F.)	Melittidae	Macropidini	Czech Republic
<i>Andrena</i>	sp.	Andrenidae	Andrenini	Czech Republic
<i>Lasioglossum</i>	<i>parvulum</i> (Schenck)	Halictidae	Halictini	Czech Republic
<i>Colletes</i>	<i>similis</i> Schenck	Colletidae	Colletini	Czech Republic
<i>Pararhophites</i>	<i>quadratus</i> Friese	Megachilidae	Pararhophitini	Tunisia
<i>Osmia</i>	sp.	Megachilidae	Osmiini	Czech Republic
<i>Megachile</i>	sp.	Megachilidae	Megachilini	Peru
<i>Xylocopa</i>	sp.	Apidae	Xylocopini	Peru
<i>Tetrapedia</i>	<i>diversipes</i> Klug	Apidae	Tetrapediini	Brazil
<i>Epeolus</i>	<i>variegatus</i> (L.)	Apidae	Epeolini	Czech Republic
<i>Nomada</i>	<i>flavopicta</i> (Kirby)	Apidae	Nomadini	Czech Republic
<i>Epeoloides</i>	<i>coecutiens</i> (F.)	Apidae	Osirini	Czech Republic
<i>Leiopodus</i>	<i>trochantericus</i> Ducke	Apidae	Protepeolini	Argentina
<i>Melecta</i>	<i>albifrons</i> (Förster)	Apidae	Melectini	Czech Republic
<i>Diadasina</i>	sp.	Apidae	Emphorini	Argentina
<i>Paratetrapedia</i>	sp.	Apidae	Tapinotaspidini	Peru
<i>Anthophora</i>	<i>plumipes</i> (Pallas)	Apidae	Anthophorini	Czech Republic
<i>Apis</i>	<i>mellifera</i> L.	Apidae	Apini	Czech Republic

1978; Roig-Alsina & Rozen, 1994; Michelette *et al.*, 2000; Straka & Bogusch, in press). For this reason, the states are re-evaluated to state 1 in both Isepeolini and Protepeolini. However, justification for these character states is tentative until the muscular location and the setal innervation are identified as homologous among numerous bee species. According to the illustration by Rozen *et al.* (2006), we also coded this clypeal character as state 1 for *Protosiris*.

25. *Labral tubercles*: (0) present, well defined; (1) absent or poorly defined.

We added an additional state: (2) only one, well-defined tubercle present in the middle of the labrum. This state is present in *Isepeolus* and *Melectoides* (Rozen, 1966; Michelette *et al.*, 2000).

27. *Epipharynx*: (0) not produced; (1) produced as a distinct lobe.

In *Leiopodus* larvae, the epipharynx is described as a large swollen area (Rozen *et al.*, 1978; Roig-Alsina & Rozen, 1994; Straka & Bogusch, in press). This state has been recoded as state 1 because it is conspicuously more similar to distinctly produced than to not produced. Roig-Alsina & Michener (1993) had coded it state 0 for *Leiopodus*.

67. *Dorsum of abdominal segment X*: (0) without transverse line or ridge; (1) with transverse ridge; (2) with transverse line.

We observed variability from an indistinct line to an indistinct ridge in *Epeoloides*. We suggest that line and ridge is one variable state. Therefore, we unite states 1 and 2 to one common state 1 in our data matrix.

78. *Extension of hypostomal ridge* [= lateral part of tentorial bridge between branching with anterior tentorial arm and branching with hypostomal ridge (in the point of

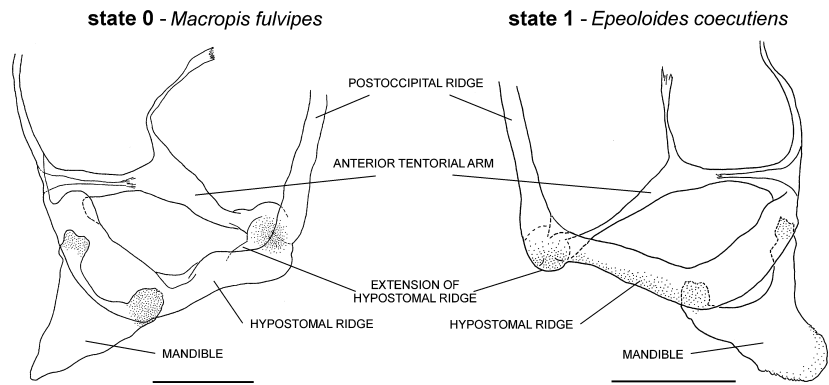
posterior tentorial pit) *sensu* Rozen (2001)]: (0) turns dorsomedially or medially, thus externally invisible in lateral view; (1) at least partly directed ventrally or posteroventrally; in lateral view externally visible at least as a ventral thickening of the hypostomal ridge (Fig. 1).

Character state 1 is developed in Protepeolini and some Osirini (see also Straka & Bogusch, in press).

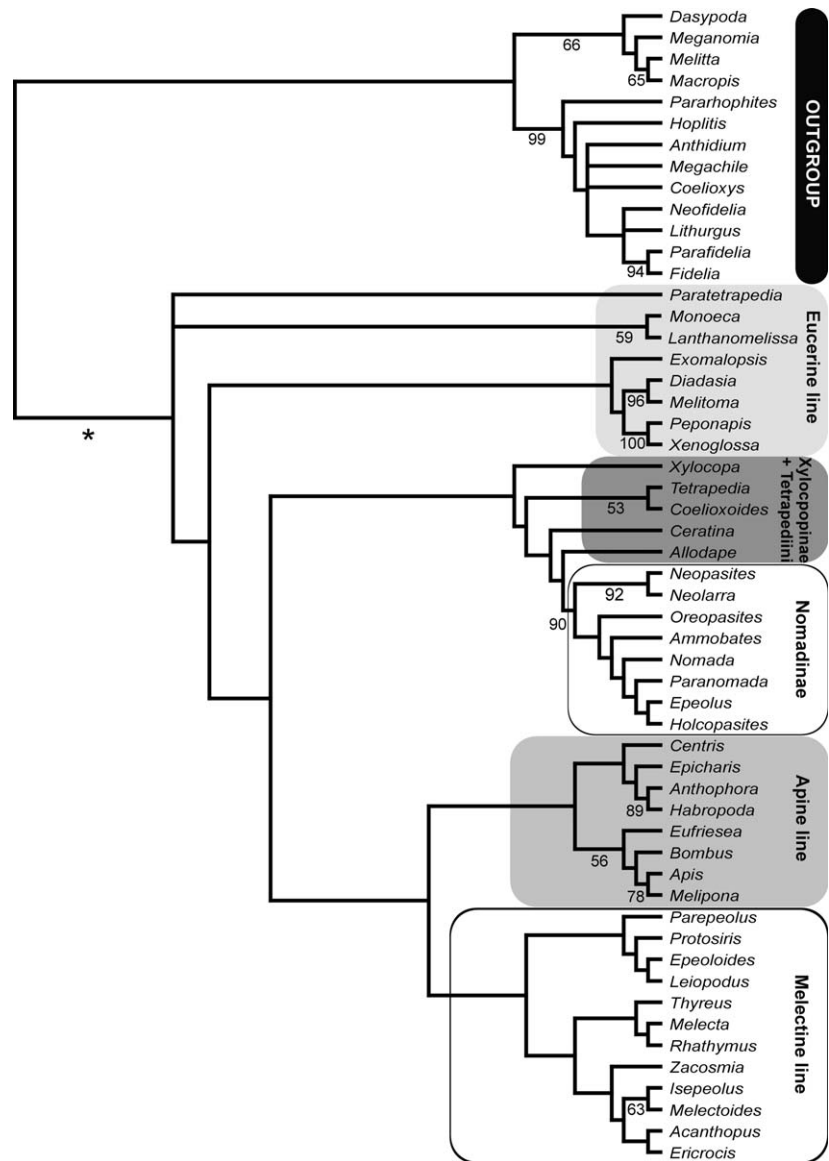
## Phylogenetic analysis

### Position of the root

The results obtained from both the MP and BI analyses including outgroup taxa were highly inconsistent and incongruent with the previous cladistic analysis (Roig-Alsina & Michener, 1993). OTUs of the family Melittidae tended to be positioned within the Eucerine line and OTUs of the family Megachilidae tended to be close to the Corbiculate bees within the subfamily Apinae. The outgroup was constrained for this reason. When the outgroup was constrained, its position was almost the same as for the family Melittidae itself (similar to previous analyses). The MP analysis led to eight equally parsimonious trees and the consensus tree was well resolved. It is presented with bootstrap support over 50% (Fig. 2) [length = 502, consistency index (CI) including all characters = 0.183, CI including only parsimony-informative characters = 0.182, retention index (RI) = 0.584]. In this tree, the monophyly of Nomadinae is strongly supported (90%). Corbiculate bees (56%), *Neopasites* and *Neolarra* (92%) and some couples or small groups of species that form their tribes (Anthophorini, Tetrapediini, Emphorini, Eucerini, Isepeolini and some Tapinotaspidini) show bootstrap support over 50% each.



**Fig. 1.** Character 78: extension of hypostomal ridge. *Macropis fulvipes* is a representative of state 0 and *Epeoloides coecutiens* is a representative of derived state 1; scale = 0.1 mm.



**Fig. 2.** The strict consensus of eight equally parsimonious trees (502 steps) obtained through the heuristic search analysis of the complete data matrix. The numbers below the branches indicate bootstrap values over 50%. Asterisks indicate constrained nodes. Totally cleptoparasitic clades are marked by white boxes.

The monophyly of the subfamily Apinae as well as the Eucerine line is not supported by this analysis. This MP analysis suggests that the majority of cleptoparasitic species of the subfamily Apinae (all except *Coelioxoides*) form a clade, the Melectine line, although this group is unsupported by bootstrap analysis. A similar situation is observed in the Apine line, whose monophyly similarly is not supported by bootstrap analysis. The subfamily Xylocopinae was not monophyletic in any MP tree.

Because the ingroup topology might be biased by the outgroup, we carried out additional analyses without the outgroup. The exclusion of outgroup taxa resulted in a loss of information about relationships among the subfamilies, but otherwise the relationships among tribes within each subfamily were made clearer (provided that these subfamilies or lines were truly monophyletic).

MP

The heuristic analysis of the modified data matrix, as mentioned above, resulted in a single MP tree (length = 392, CI including all characters = 0.227, CI including only parsimony-informative characters = 0.223, RI = 0.568). The unrooted tree is shown in Fig. 3 with displayed unambiguous character states supporting clades. Character states are polarized by the operative polarization point, the point representing the suppositional root (unreal). This tree

topology was almost identical to previous analyses except that the monophyly of the Eucerine line was recovered. Also, the clades supported by the bootstrap analysis were identical, thus the percentage is not indicated here.

BI

The BI tree based on the same reduced data matrix is presented with branch lengths and posterior probabilities over 0.50 (average likelihood = 1463.57; Fig. 4). We present a fully resolved tree, because it indicates the topological differences with the MP tree (cf. Fig. 3). The monophyly of Nomadinae is strongly supported (1.00), but the monophyly of Apinae (without Tetrapediini) has a rather low posterior probability value (0.73). *Tetrapedia* and *Coelioxoides*, along with OTUs of the subfamily Xylocopinae, form a paraphyletic group similar (but not identical) to the result of the MP analysis. The monophyly of Corbiculate bees (0.97) and the relationship of *Apis* to *Melipona* (0.99), *Neolarra* and *Neopasites* (0.96) as well as some pairs or small groups of species form clades with good support, corresponding to the tribal level (Anthophorini, Emphorini, Eucerini, Isepeolini and some Tapinotaspidiini). The BI analysis also recovered the monophyly of all totally cleptoparasitic tribes of the subfamily Apinae (Melectine line). The Apine line and the subfamily Xylocopinae were not recovered as monophyletic groups in the BI analysis.

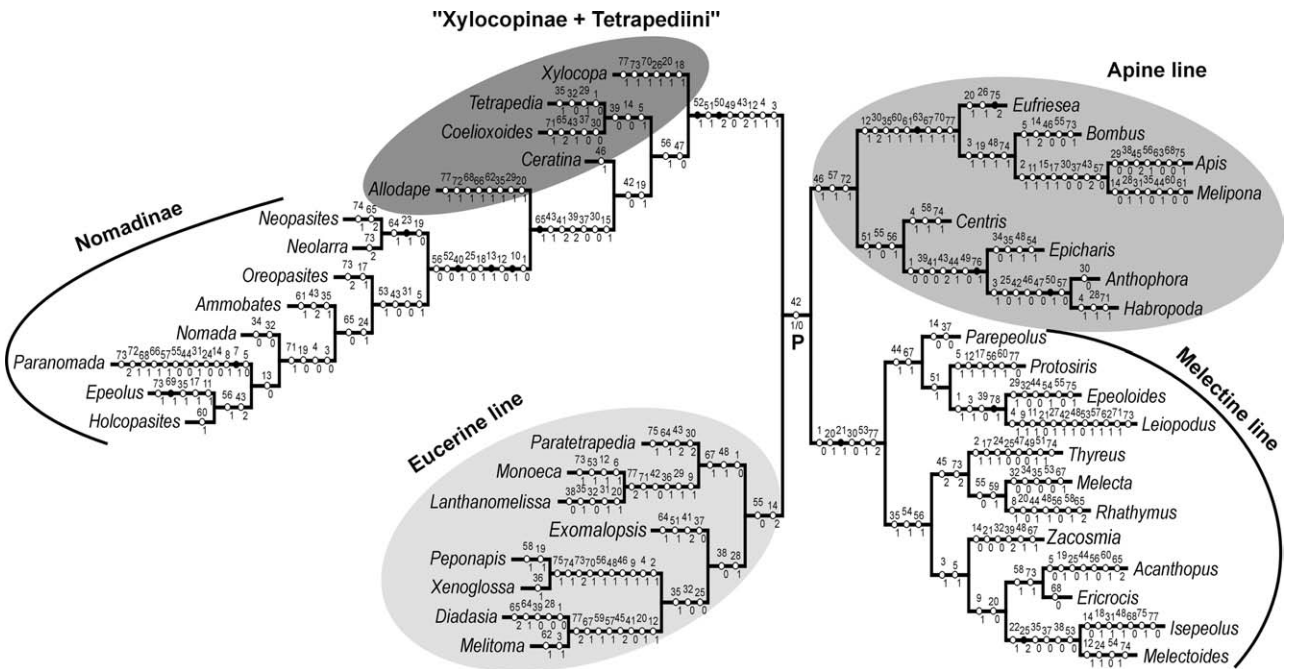
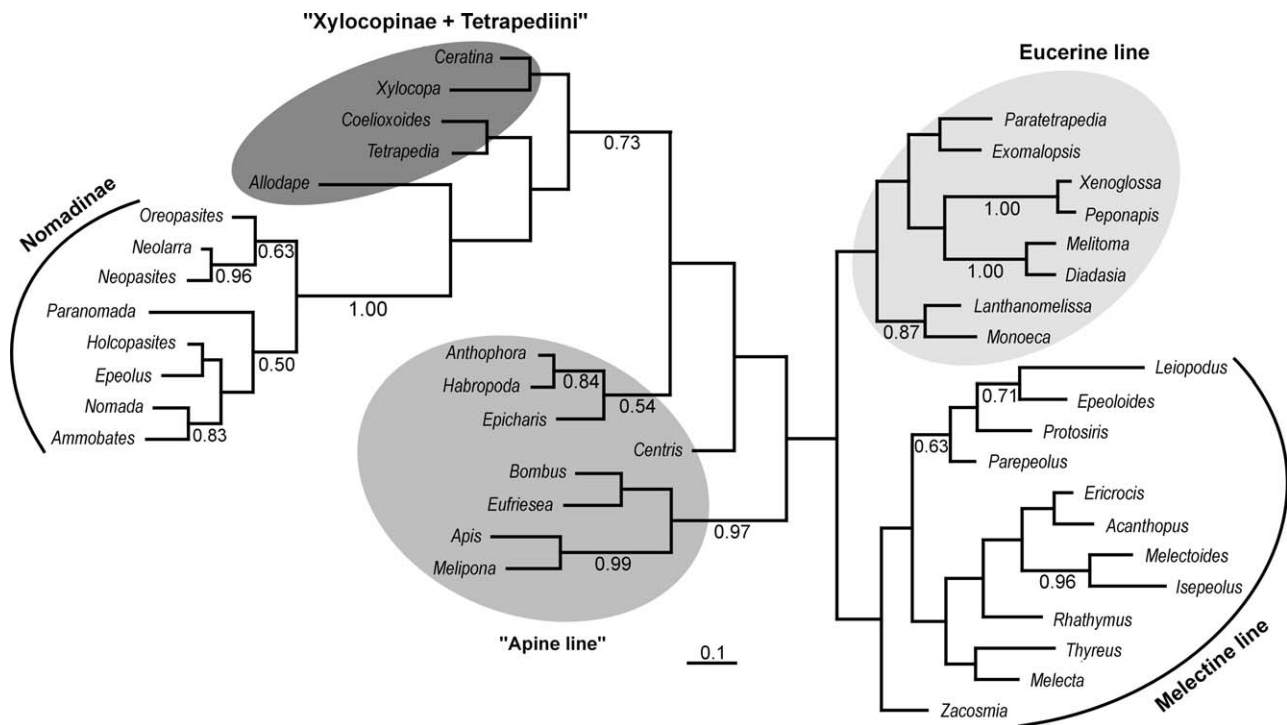


Fig. 3. Single unrooted most-parsimonious tree of the analysis excluding the outgroup taxa. The topology was recovered from a heuristic search. Characters are displayed on the branches. White dots indicate clade apomorphies; black dots indicate clade autapomorphies; the numbers of characters are above the dots and character states are below the dots. Characters are numbered according to Table 1. P indicates the operative polarization point. The names of paraphyletic groups are in quotation marks. Totally cleptoparasitic clades are marked by semioval lines.



**Fig. 4.** Unrooted tree of the Bayesian analysis of the reduced data matrix (excluding the outgroup taxa). Posterior probabilities over 0.50 are given below the nodes. The names of paraphyletic groups are in quotation marks. Totally cleptoparasitic groups are marked by semioval lines. The scale represents the proportions of character changes.

## Discussion

### *Topological ambiguity and utility of larval characters in bee phylogeny*

The tree obtained as a result of the complete analysis of larval characters was highly unstable in the position of the rooting groups (Melittidae and Megachilidae). It was necessary to constrain the outgroup, but the result was very different from the previous phylogenetic analysis based on adult morphology (Roig-Alsina & Michener, 1993). Thus, we removed the outgroup taxa from the data matrix, even though this reduced the information about the position of the root. We are convinced that it is unlikely that the root lies within the Eucerine or Apine lines (as the results of the analysis suggest). A previously published analysis of adult characters placed the root somewhere between Nomadinae and Xylocopinae (Roig-Alsina & Michener, 1993), but our analyses did not produce similar results.

We must support statements published by Alexander (1990) and Roig-Alsina & Michener (1993) that the morphological characters of mature larvae are not strong enough for the resolution of the topology at the base of the tree. Otherwise, these characters are useful in reconstructing phylogenetic relationships among tribes and species. There is a particular congruency with the analysis of morphological characters of adult bees (Roig-Alsina & Michener, 1993) in recovering the Nomadinae and main groups of Apine and Eucerine lines [*sensu* Silveira (1993), all pollen-collecting

bees]. Unrooted final trees seem to be phylogenetically informative and largely congruent with published data on the evolution of bees (see Michener, 2000). The exclusion of problematic OTUs (i.e. to eliminate topological inconsistency caused by OTUs used for rooting) resulted in an unambiguous topology in the MP analysis. Such a topological stability has not been previously observed (see Roig-Alsina & Michener, 1993; Silveira, 1993).

Larval characters might be very useful for phylogenetic purposes, because mature larvae do not have as many advanced adaptive characters in external morphology and anatomy as adult bees usually do. For example, cleptoparasitic adult bees are specifically adapted to the host-defending behaviour that varies among species from nonaggressivity to killing behaviour (Bogusch et al., 2006; see also Alexander, 1990; Rozen, 1991). On the contrary, the mature cleptoparasite larva is always hidden away from the strong selective pressure on active outdoor life and does not need to evolve a large number of strategies and counter-strategies, such as exposition to predators and cleptoparasites, competition, mating, reproduction, feeding behaviour, etc. Some adaptations of early instars surely persist to the mature larvae, but extreme character states are largely lost within two or three moultings (i.e. elongate mandibles adapted to kill competing larvae in the first instars of cleptoparasites). In general, phylogenetically informative characters of mature larvae have probably evolved at more or less similar rates among all groups of bees (selective pressure is



more balanced than in adults). Only the tendency to reduce the labiomaxillary area in some species or groups (Nomadinae, Xylocopinae, Tetrapediini, *Epicharis* and *Zacosmia*) connected with the origin of uncovered pupation (species without cocoon spinning) seems to be a problematic evolutionary change. The position of these species may be biased in our analysis, not only because of derived morphology, but also because of missing character states on the reduced labiomaxillary region (resulting in a loss of information). The aforementioned morphological changes are obviously phylogenetically informative; however, they result in differences in evolutionary rate and consequently impair the reconstruction of phylogeny. Similarly, the phylogeny of the bees based on adult morphology (Roig-Alsina & Michener, 1993) can also be biased. Selective pressure on the evolution of cleptoparasitic strategies may cause the main fluctuation in the evolutionary rate of adult morphological characters. For this reason, Roig-Alsina & Michener (1993) performed a separate analysis (Analysis D) for pollen-collecting bees only, because the cleptoparasitic bees were intuitively blamed for topological violation. Among all groups of bees, the cleptoparasitic strategy is the most derived way of life (as well as the eusociality), so that due to strong selective pressure and/or a small effective population size, the evolutionary rate differs compared with solitary pollen-collecting bees (Bromham & Leys, 2005). These facts probably caused some of the deviations from the true evolutionary history in the results of adult character-based analyses, as well as in our larval character analysis. Our analysis should be more accurate in some nodes than previous studies based on different characters. Which nodes are correct remains unclear, although we have noted here specific problems and topologies that should be tested in future studies. All mentioned tasks concerning adult and larval morphology could be answered by future DNA analysis and adjacent reconstruction of the evolution of morphological adaptations and bee behaviour.

#### *Apine and Eucerine lines*

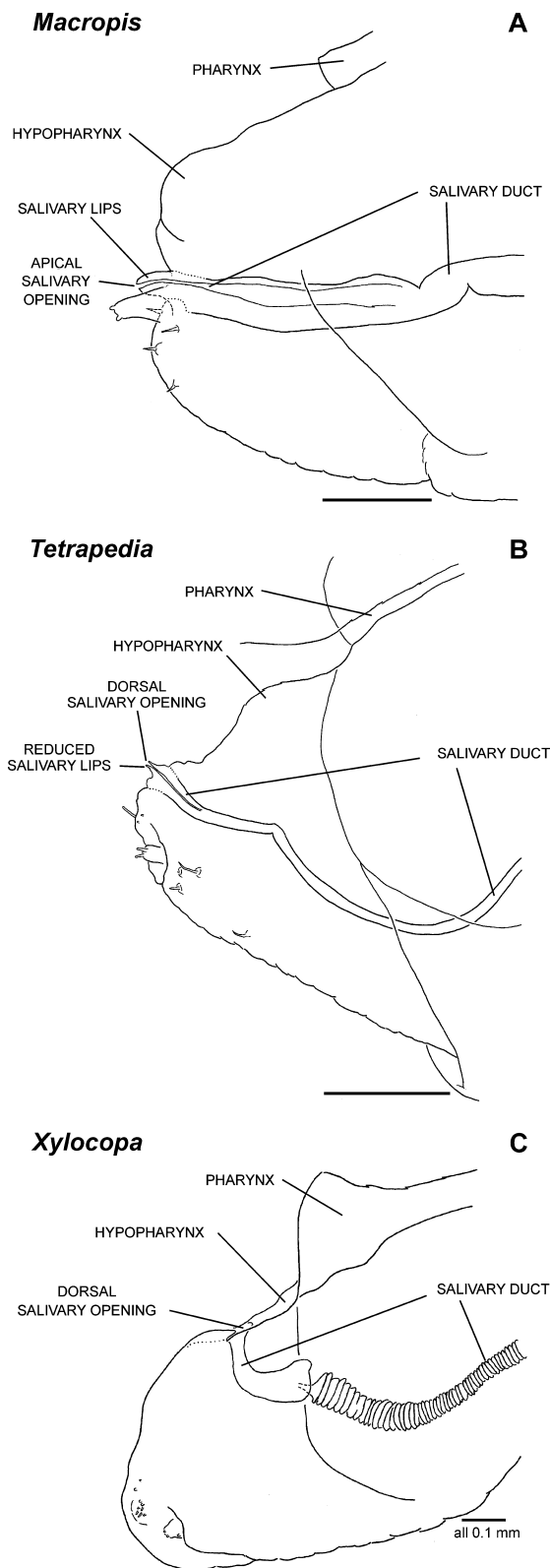
Our work, particularly the MP analysis, supports Silveira's (1993) systematic account based on adult morphology. We recognized Eucerine and Apine lines to be monophyletic when considering the same range of OTUs. One important and invariable larval synapomorphy currently supports the monophyly of the Apine line: the ridged spiracular atrial wall. The presence of galea is also a diagnostic character, but is missing in Anthophorini and Bombini (Fig. 3). The Eucerine line is characterized by an almost unique feature: the median longitudinal thickening of the head wall extending forward to the level of the epistomal suture (also present in *Bombus*). The distinct hypopharyngeal groove is the second characteristic feature of these bees, but it is also developed in some other tribes (see Fig. 3). Extension of the terms Eucerine and Apine lines over a wider range of species (Tetrapediini and cleptoparasitic tribes) (Engel, 2001) seems to be incorrect. The monophyly of the Apine line was

recovered in the MP analysis in a very similar topology, except for the positions of *Centris* and *Epicharis*. The inclusion of these genera results in the paraphyly of the tribe Centridini, but in the opposite branching order to that presented in Silveira (1993). We recovered the monophyly of the tribe Tapinotaspidini of the Eucerine line in one MP tree (Fig. 3) as Silveira (1993) and Roig-Alsina & Michener (1993) suggested. The proposed relationship of *Monoeca* to Centridini (Neff & Simpson, 1981) is not supported in our analysis. The phylogenetic position of *Ctenoplectra* remains unresolved. We could not include this bee in the analysis without the examination of any specimen because of its problematic morphology. We can hardly speculate about its relationship to other bees. We may infer that *Ctenoplectra* is not related to *Tetrapedia* as Alves-dos-Santos *et al.* (2002) suggested. *Ctenoplectra* seems to be a typical member of the subfamily Apinae [cocoon spinning and prominent salivary lips are present, see also the description of the mature larva (Rozen, 1978)], although it possesses neither an obvious synapomorphy with the Eucerine line nor with the Apine line.

#### *Origin of cleptoparasitic behaviour*

This is the first study presenting the results of an analysis of the phylogenetic relationships of the tribe Tetrapediini (both genera) as well as the tribe Osirini [three species of three main lineages according to Roig-Alsina (1989)] and three other cleptoparasitic species based on their mature larvae. The relationship of the cuckoo bee *Coelioxoides* with its host *Tetrapedia* (both genera of the tribe Tetrapediini) was discussed for the first time by Roig-Alsina (1990) and subsequently supported by Roig-Alsina & Michener (1993). We recovered the same relationship with bootstrap support higher than 50% (Fig. 2). Our result differs from all other studies of the relationship of the tribe Tetrapediini to other bees of the subfamily Apinae in that we cannot support their relationship with either the Apine line or the Apinae. The salivary opening of mature larvae of *Coelioxoides* and *Tetrapedia* is located in the upper part of the labiomaxillary region and the salivary opening and duct are directed dorsally (Fig. 5B), which may resemble the morphology in bees of the subfamily Xylocopinae (Fig. 5C). Even though the similarity is not large, the salivary duct and opening are directed straight towards the anterior in other analysed taxa (Fig. 5A). Xylocopinae + Tetrapediini are paraphyletic in all resulting trees. We consider that the relationship of the tribe Tetrapediini with species of Xylocopinae is probable; however, our analysis does not support the monophyly of this group. Our results suggest that *Coelioxoides* is the first known cleptoparasitic bee of the subfamily Xylocopinae, providing this subfamily is monophyletic.

Unlike the unclear position of *Coelioxoides* within the Xylocopinae, the monophyly of the lineage of all totally cleptoparasitic tribes of the subfamily Apinae was supported by five important synapomorphies: short spiracular subatrial length (twice the atrial length or less), short setae



**Fig. 5.** Labium and salivary duct. A, *Macropis*, a representative of the common plesiomorphic state; B, *Tetrapedia*; C, *Xylocopa*; spiculation and premental sclerites not indicated; scale = 0.1 mm.

on the head capsule, enlarged hypopharynx, well-developed antennal papilla, slender and projecting and with more than five antennal papillar sensilla. This bee group, the Melectine line, is the most surprising result of our analysis. Melittologists have divided cleptoparasitic bees among several unrelated lineages for almost 100 years (Börner, 1919; Michener, 2000). In particular, the Melectini have nearly always been considered to be closely related to their host tribe Anthophorini. Furthermore, Popov (1955) and Liefstinck (1968) both suggested that *Melecta* is related to its host genus *Anthophora* and *Thyreus* to its host *Amegilla*. Similarly, Rozen *et al.* (2006) speculated about the origin of some Osirini from their hosts of the tribe Tapinotaspini. However, the polyphyly of Melectini or Osirini has never been supported in any phylogenetic study in such a way as to suggest divergence from a common ancestor with the host. Our results suggest a new view of cuckoo bees and prove that similarities with hosts in morphology and pattern are probably the result of convergence and co-evolution than phylogenetic affinity.

Before the comprehensive morphological cladistic study based on adults by Roig-Alsina & Michener (1993), the phylogenetic position of the Osirini was unknown. However, results based on larval characters largely differ from their view. The monophyly of the Osirini was not supported; instead, it appears to be a paraphyletic tribe (*Parepeolus*, *Protosiris*, *Epeoloides*). The Osirini seem to be most closely related to the Protepeolini (*Leiopodus*), which probably forms an inner group of the osirine lineage, as both the MP and the BI analyses suggest (Figs 3, 4). The phylogenetic position of the tribe Protepeolini was previously as mysterious as the positions of the tribes Isepeolini or Osirini. One MP analysis of the adult morphology characters (Roig-Alsina & Michener, 1993) recovered a paraphyletic relationship among these three tribes, in which Isepeolini and Protepeolini appeared as sister groups. By contrast, the current MP analysis suggests a close relationship between Protepeolini and Osirini, although the relationship between Isepeolini and Protepeolini was not recovered. Both studies together indicate that all these three cleptoparasitic tribes may form a clade. Mature larvae of these cleptoparasitic bees have an extremely short clypeus (character 22: 1), sometimes reduced only to a membrane or a membrane adjoining area (Isepeolini and Protepeolini). Such a character is an extreme feature within bees of the family Apidae. However, character specification and homology are still tentative (see Roig-Alsina & Rozen, 1994; Michelette *et al.*, 2000; Straka & Bogusch, in press). Previously, some larvae of Megachilidae and Colletidae were the only known groups with a similarly short clypeus (McGinley, 1981).

This is the first phylogenetic study supporting the formerly uncommon opinion about the relationship between Melectini and Osirini, presented previously by Warncke (1977) (in regard to European species). His work was rather intuitive, but he was the first author to present the evolutionary lineage now called the Melectine line. This means that the cleptoparasitic life strategy evolved less frequently than formerly expected. Rozen (2000), who presented the

current conception of the evolution of cleptoparasitic bees, presented nine independent origins. With two other proved or predicted intertribal cleptoparasitic lineages (Michener & Greenberg, 1980; Michel-Salzat *et al.*, 2004), the number of unrelated cleptoparasitic groups increased to 11. Our results suggest only six independent evolutionary events within the family Apidae in total, namely *Coelioxoides*, *Ctenoplectrina*, Nomadinae, Melectine line, *Exaerete* and *Aglae*.

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