PHYLOGENETIC SYSTEMATICS AND THE EVOLUTION OF NESTING BEHAVIOR, HOST-PLANT PREFERENCE, AND CLEPTOPARASITISM IN THE BEE FAMILY MEGACHILIDAE (HYMENOPTERA, APOIDEA)

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PHYLOGENETIC SYSTEMATICS AND THE EVOLUTION OF NESTING BEHAVIOR, HOST-PLANT PREFERENCE, AND CLEPTOPARASITISM IN THE BEE FAMILY MEGACHILIDAE (HYMENOPTERA, APOIDEA)

Jessica Randi Litman, Ph.D.

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Members of the bee family Megachilidae exhibit fascinating behavior related to nesting, floral preference, and cleptoparasitic strategy. In order to explore the evolution of these behaviors, I assembled a large, multi-locus molecular data set for the bee family Megachilidae and used maximum likelihood-, Bayesian-, and maximum parsimony-based analytical methods to trace the evolutionary history of the family. I present the first molecular-based phylogenetic hypotheses of relationships within Megachilidae and use biogeographic analyses, ancestral state reconstructions, and divergence dating and diversification rate analyses to date the antiquity of Megachilidae and to explore patterns of diversification, nesting behavior and floral preferences in the family. I find that two ancient lineages of megachilid bees exhibit behavior and biology which reflect those of the earliest bees: they are solitary, restricted to deserts, build unlined nests, and are host-plant specialists. I suggest that the use of foreign material in nest construction allowed early megachilid bees to escape their ancestral desert habitat and colonize temperate, previously uninhabitable areas. In order to further examine phylogenetic relationships among tribes of the family Megachilidae, I develop a novel bootstrapping algorithm designed to balance the signal from combined molecular-morphological datasets; I use the results of all phylogenetic analyses to propose a new subfamilial- and tribal-level classification for Megachilidae and present a revised key to the tribes of Megachilidae.

I also reconstruct the evolutionary history of the tribe Anthidiini and offer the first molecular-based phylogenetic hypothesis of the generic and suprageneric relationships within Anthidiini based on maximum likelihood and Bayesian phylogenetic analyses. I trace the evolutionary history of nesting behavior, the origins of cleptoparasitism, and the evolution of cleptoparasitic strategy in the megachilid tribe Anthidiini using Bayesian ancestral state reconstructions. Our results indicate three suprageneric clades within Anthidiini: the *Trachusa* group, the *Anthidium* group, and the *Dianthidium* group; each of these groups shows a distinct preference for either plant fibers or plant resins as a primary nest-building material. Our phylogeny supports two origins of cleptoparasitism in Anthidiini and also supports the hypothesis that cleptoparasitic lineages with hospicidal adults are an evolutionary intermediate between nest-building bees and cleptoparasitic lineages with hospicidal larvae.

BIOGRAPHICAL SKETCH

Jessica Randi Litman was born on July 30, 1975 in Brooklyn, NY. She attended high school at Phillips Exeter Academy in Exeter, NH and graduated in 1993. Jessica received a BSc in biology from McGill University in Montreal, Quebec in 1998. After spending several years in South America, she attended Rutgers University in New Brunswick, NJ, where she received an MS in entomology in 2006.

for christophe

"...and this is the wonder that's keeping the stars apart..."

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CHAPTER 1

A REVIEW OF THE BIOLOGY OF THE BEE FAMILY MEGACHILIDAE (HYMENOPTERA), WITH A FOCUS ON THE EVOLUTION OF NESTING BEHAVIOR, HOST-PLANT PREFERENCE, AND CLEPTOPARASITISM

Abstract

The bee family Megachilidae is the second-largest family of bees, containing over 4000 species divided into seven tribes (Michener 2007). The family includes the alfalfa leafcutting bee, *Megachile rotundata*, which is intensively managed in North America as a pollinator of alfalfa (Pitts-Singer and Cane 2010). This charismatic family builds unique nests, exhibits a rich array of host-plant preferences, and includes a number of cleptoparasitic lineages, making it an ideal model group for understanding the evolution of nesting behavior, floral specialization, and cleptoparasitism. In this review I explore the nest-building behavior, host-plant preferences, and incidence of cleptoparasitism in bees belonging to the family Megachilidae and provide a brief introduction to the chapters that follow.

1.1 Introduction

Biologists have long marveled at the astounding variety of nest-building material favored by megachilids. The French naturalist, Jean-Henri Fabre, once remarked: “Each [megachilid] Bee has her art, her medium, to which she strictly confines herself. The first has her leaves; the second her wadding; the third her resin...”; according to Fabre, megachilid nests may be built of “bits of chalky gravel”, “particles of earth”, “fragments of sticks”, “scraps of moss and juniper-catkins”, “barriers of leaves”, “resinous putty”, and “felted cotton” (Fabre 1914). Unlike most

bees, which build their nest cells using glandular secretions, megachilids typically build their nests using foreign material.

The nests of megachilid bees, like those of other solitary bees, consist of a series of cells; each cell is provisioned with a mixture of pollen and nectar and sustains the growth of a single larva. Depending on the species of bee and the location of the nest, nest cells may be built in a linear series or grouped together in an irregular cluster. The number of cells varies greatly from nest to nest but usually numbers between one and twelve. Nesting sites are extremely variable; nests may be built in the ground, in dead wood, in plant stems, empty snail shells, fissures in rocks, or in the abandoned nests of other insects.

Pollen is the primary source of nutrition for developing bee larvae and megachilids exhibit a broad spectrum of host-plant associations. Some are oligolectic, gathering pollen from a single plant genus or family, while others are polylectic, gathering pollen from a variety of plant families. Regardless of host-plant preference, the female bee ultimately provisions her nest with a mixture of pollen and nectar. Not all megachilid bees, however, forage for pollen. Several lineages are cleptoparasites; they neither dig their own nests nor collect their own pollen. Instead, they usurp the nests and pollen provisions of other bees.

This thesis will focus on the phylogeny of Megachilidae and on behavioral evolution within the family. In this chapter, I will review the relevant background material for the chapters that follow, primarily: (a) nesting behavior and host-plant preference in the family Megachilidae and (b) nesting behavior and incidence of cleptoparasitism in the tribe Anthidiini. All information is presented according to tribe.

1.2 Fideliini and Pararhophitini

1.2.1 Fideliini

The tribe Fideliini consists of two genera, *Fidelia* and *Neofidelia*. The genus *Fidelia* is a small group of medium- to large-sized bees found exclusively in arid regions of southern and northern Africa. Eleven species inhabit the deserts of Namaqualand and the Kalahari, which encompass much of northeastern South Africa, central and southwestern Namibia, and southern Botswana (Michener 2007; Whitehead and Eardley 2003). The twelfth and final species is known only from the xeric regions south of the Atlas Mountains in Morocco (Warncke 1980; Whitehead and Eardley 2003). The genus *Neofidelia* contains two medium-sized species restricted to the Atacama Desert of northern Chile (Michener 2007).

Members of the tribe Fideliini are robust bees, ranging in size from approximately 7.5 to 17.5 mm (Whitehead and Eardley 2003). Their pilosity is often dense, ranging in color from white to yellow to orange; their integument is brown to black, except for yellow markings on the clypeus, labrum and mandibles of most species. A pygidial plate is present in all females and some males and varies in color from yellow to brown to black. The male seventh tergite is strongly modified in the genus *Fidelia*, forming either a simple or bifid apical process with lateral spines. The male seventh tergite is unmodified in *Neofidelia* and the hind femora of the males of this species are extremely expanded. Females have a metasomal scopa, as well as a brush of long hairs on their hind tibiae and basitarsi; in *Fidelia,* this brush of hairs is extremely dense, whereas in *Neofidelia* the hairs are more sparse. The basitarsus itself is flattened and greatly expanded in female members of the genus *Fidelia*; in female *Neofidelia*, the basitarsus is neither flattened nor expanded.

Fideliine bees are well known for their extreme host plant specificity. Although host-plant visitation records are difficult to interpret, particularly when they do not distinguish between floral visits for pollen and visits for nectar, the vast majority of visitation records for the tribe Fideliini suggest that most fideliines are oligolectic: *Fidelia fasciata*, *F. hessei*, *F. major* on *Grielum* sp. and *Neuradopsis* sp. (Neuradaceae;); *F. braunsiana* on *Berkheya* sp. (Asteraceae;); *F. friesei* on *Sesamum* sp. (Pedaliaceae;), *F. pallidula* on *Sisyndite* sp. (Zygophyllaceae;), *F. ulrikei* on *Convolvulus* sp. (Convolvulaceae;), *F. villosa* on various genera of Aizoaceae, and *Neofidelia longirostris* on *Nolana* sp. (Solanaceae) (Gess and Gess 2003; Whitehead and Eardley 2003).

The following species may have a slightly broader host plant spectrum: Although *F. kobrowi* and *F. paradoxa* have been collected on several genera of Aizoaceae and Asteraceae, most records are on Aizoaceae; these bees are either oligolectic on Aizoaceae or polylectic with a strong preference for Aizoaceae. *Fidelia ornata* is likely oligolectic on Aizoaceae (most visitation records are for *Tribulocarpus dimorphanthus*), although other hosts are possible (Gess and Gess 2003; Whitehead and Eardley 2003); Whitehead and Eardley (2003) list a single visitation record for *Tribulus* sp. (Zygophyllaceae). *Neofidelia profuga* collects pollen from *Trichocereus* sp, *Eulychnia* sp, (Cactaceae), and *Calandrinia* sp. (Portulacaceae). *Neofidelia profuga* has also been observed collecting pollen on *Encelia* sp. (Asteraceae) and *Calandrinia* (Moure and Michener 1955).

Regarding the host-plant preferences of Fideliini, Rozen (1977) reports that “all species apparently collect from large flowers with anthers well-exposed”. Rozen’s observation holds true even for those fideliines not discovered until after the publication of his 1970 work (e.g. *Fidelia*

ulrikei, not described until Warncke 1980a). In fact, the flowers of most fideliine host-plants are radially symmetrical and often have showy anthers with exposed pollen. This floral preference is markedly different from that of many of the higher megachilids (Osmiini, Anthidiini, and Megachilini), which often forage on bilaterally symmetrical flowers, such as those found in the families Fabaceae and Lamiaceae.

Like all megachilids, fideliine bees transport pollen by means of a metasomal scopa; after collection, the pollen is stored in nest cells to serve as food for developing larvae. The pollen provisions of fideliines are "mealy moist" and "slightly sweet to the taste"; females likely mix pollen with nectar in the process of provisioning their nests (Rozen 1970; Rozen 1977). The nests of fideliines are notably distinct from those of other megachilids. While the nests of most megachilids are built using foreign material, the nests of fideliine bees are built without the inclusion of any such material. Fideliine nests consist of shallow, branching tunnels in the sand; the nest cells are located at the distal ends of the tunnels. In some fideliines, such as *Fidelia villosa*, each nest consists of several branches, only one of which contains a provisioned cell (Rozen 1970); in others, such as *Neofidelia profuga*, more than one branch may contain a provisioned nest cell (Rozen 1973a). Females typically place the pollen mass toward the rear portion of the nest cell but the shape of the pollen mass and the position of the egg depends on the bee species. In *Neofidelia profuga*, the female sculpts the pollen provisions so that they cover the base and rear wall of the nest cell; the egg is laid on top of the pollen mass, such that the egg is elevated above the surface of the floor of the cell (Rozen 1973a). In *Fidelia villosa*, the female deposits a single egg in a cavity that she hollows inside the pollen mass (Rozen 1970); *Fidelia pallidula* deposits between one and three eggs within the pollen mass (Rozen 1977). After provisioning the cells and depositing eggs, females backfill the nest with loose sand.

Fideliini engage in another unusual behavior related to nesting: larvae incorporate sand in cocoon construction (Rozen 1970, 1973a, 1977). Final instar larvae ingest sand prior to cocoon-spinning; after the larva is completely encased in the cocoon, the larva applies its sand-laden feces to the inner walls of the cocoon, until the inside of the cocoon is completely coated with a layer of sand. Except for the genus *Pararhophites* (see below), this behavior is not known in any other bee. It is, however, seen in several species of sand-nesting apoid wasps (Evans 1966, 2007). Cocoons of *Neofidelia* have a single nipple at one end; cocoons of *Fidelia* are nippled at both ends (Rozen 1973a).

1.2.2 Pararhophitini

The tribe Pararhophitini consists of just three species belonging to the genus *Pararhophites*. This tiny group of desert-dwelling bees is found scattered throughout the arid habitat that extends from northern Africa, eastward to India, and northward into Kazakhstan. *Pararhophites quadratus* is found largely in northern Africa, while *Pararhophites orobinus* is found in Central Asia and India (Ascher and Pickering 2011; Michener 2007). *Pararhophites clavator* is found in Central Asia (Ascher and Pickering 2011).

Members of the genus *Pararhophites* are small, ranging in size from 5-9 mm. They are largely yellow; males have conspicuous black markings on head, thorax, legs and abdomen. Both sexes have sparse, white pilosity on thorax and abdomen. Unlike other megachilids, the metasomal scopa in the female is much reduced. Similar to *Neofidelia*, male *Pararhophites* have expanded hind femora.

Like many fideliines, members of the genus *Pararhophites* are extremely host-plant specific. In his original description of the genus *Pararhophites*, Friese (1898a) described *Pararhophites quadratus* flying on *Zygophyllum* (Zygophyllaceae) near Wadi Haff, Hellouan (Egypt). Both Popov (1949) and McGinley and Rozen (1987) record *Peganum harmala* (Nitrariaceae) as the pollen plant for *Pararhophites orobinus*. Although *Zygophyllum* and *Peganum* belong to plant families that are only distantly related, both genera produce flowers that are architecturally similar, with small, five-petaled white or yellow flowers with exposed anthers and easily accessible pollen.

The nests of *Pararhophites* are similar to those of Fideliini, yet differ markedly in one aspect. Like Fideliini, the nests of *Pararhophites* consist of shallow, branching tunnels in dry sand; brood cells are located at the distal ends of the tunnels and each nest may contain multiple brood cells, although only one brood cell is found per tunnel (McGinley and Rozen 1987). While there is generally no cell closure that separates the brood cell from the rest of the tunnel, McGinley and Rozen (1987) report that in one case, they observed "a moist area at the closure end of a newly completed cell, suggesting that a female may apply a moist stopper of soil and then fill the laterals". The walls of the nests of *Pararhophites*, like those of Fideliini, appear to be completely unlined. The manner in which the cells are provisioned, however, differs slightly from that of Fideliini. Instead of directly depositing the provisions in the nest cell, as do Fideliini, *Pararhophites* first constructs a "cuplike receptacle of sand", which is built into the rear portion of the nest cell; pollen provisions are subsequently packed into the receptacle. The sand with which the receptacle is built is of the same grain size as the surrounding substrate. The receptacle is described as "tarlike" and as having a "brown, wet sand color, distinctly darker"

than the sand of the surrounding substrate. The grains of the receptacle are glued together with an unknown substance, possibly nectar (McGinley and Rozen 1987).

The incorporation of sand into the larval cocoon in Fideliini and *Pararhophites* is unique among bees (described above, see section on Fideliini). In both tribes, the final instar larva ingests sand prior to cocoon-spinning; after spinning the outer cocoon layer, the larvae coats the inside of the cocoon with its sand-laden feces. The application of feces to the inner wall of the cocoon is not unusual among bees (Rozen 1970); the fact that the feces of Fideliini and Pararhophitini contain sand, on the other hand, is exceptional.

While both tribes incorporate sand into the cocoon, the *origin* of the sand that the larvae ingest differ between the two tribes. In Fideliini, larvae presumably ingest sand from the wall of the nest cell; the size of the sand grains lining the inner wall of the cocoon are much smaller than the average size of the sand grains of the substrate, which caused Rozen (1970) to conclude that "the grains had obviously been selected by the larvae". In contrast, the origin of the sand ingested by larvae of Pararhophites appears to be the receptacle surrounding the pollen provisions.

1.3 Dioxyini

The tribe Dioxyini is a small, largely Old World group consisting of 37 species divided into eight genera; only the genus *Dioxys* is present in the New World. Dioxyini are exclusively cleptoparasitic; hosts are other megachilid bees belonging to the tribes Anthidiini, Osmiini and Megachilini. Members of Dioxyini are likely not host-specific, given that individual species of Dioxyini have been reared from the nest cells of multiple megachilid species (Michener, 2007).

Friese (1923) and Popov (1936, 1953) report species of *Osmia*, *Hoplitis* (Osmiini) and *Megachile* (Megachilini) as hosts of *Dioxys cincta* (see Rozen and Özbek 2005). Grace (2010, as *Chalicodoma roeweri*) reports *Megachile (Chalicodoma) roeweri* (Megachilini) as a host of *Aglaoapis tridentata* and *Hoplitis wahrmani* (Osmiini) as a host of *Dioxys ammobius*.

Details regarding the manner in which dioxyine bees parasitize the nests of other bees are limited but several accounts have been made. Rozen and Favreau (1967) reported on the behavior of the New World species *Dioxys pomonae* and discuss finding "a small slit in the cell wall [in the nest of its host, *Osmia nigrobarbata*]...that apparently marked the spot through which the egg was inserted into the sealed cell." The first three larval instars of *Dioxys pomonae* are aggressive and armed with sickle-shaped, sclerotized mandibles; larval instars may kill either the host egg, or the first or second instars (Rozen and Favreau 1967). An account of the behavior of an Old World species of the same genus, *Dioxys cincta*, indicates a different mode of cleptoparasitic behavior: the female deposits her egg into open nest cells of *Osmia cerinthidis* (Rozen and Özbek 2005).

1.4 Lithurgini

The tribe Lithurgini contains approximately 55 species divided into three genera. The genus *Lithurgus* is distributed worldwide and is divided into two subgenera: *Lithurgus* and *Lithurgopsis*. The genus *Trichothurgus* is found in arid regions of Chile, Argentina, and Peru. *Microthurge* occurs in Buenos Aires Province, Argentina north to Ceara Province, Brazil (Michener 2007; Ascher and Pickering 2011).

The three genera are quite different, both in size and overall appearance. Bees of the genus *Microthurge* are small (5-8 mm; Michener 2007), slender and heriadiform (Michener 1983). Members of the genus *Lithurgus* are larger (8-19 mm) (Michener 2007), broad, and megachiliform (Michener 1983), with black integument and scant, light-colored pilosity; the female T6 is often densely covered by reddish-orange hairs. Bees of the genus *Trichothurgus* are the largest and most robust of the lithurgines (7-21 mm) (Michener 2007); they are megachiliform to euceriform (Michener 2007), with black integument covered by dense black and white pilosity.

Most New World species of the genus *Lithurgus* (those belonging to the subgenus *Lithurgopsis*) forage for pollen on Cactaceae (Parker and Potter 1973; Brach 1978; Krombein et al. 1979; Snelling 1983; Schlindwein 1997). In an article on North American species of *Lithurgus* (*Lithurgopsis*), Snelling (1983) reported *Opuntia* (Cactaceae) as the pollen plant of *Lithurgus apicalis*; various species of *Echinocactus* as pollen plants of *Lithurgus echinocacti*; *Opuntia* as the pollen plant of *Lithurgus gibbosa*; and *Opuntia* and *Echinocactus* as pollen plants of *Lithurgus littoralis*. Several genera of Cactaceae, including *Cereus, Echinocactus, Ferocactus,* and *Opuntia* are listed as host plants of *Lithurgus listrota*, although Snelling notes that floral records are so scant for this species that its floral preference is difficult to define. While floral records are scarce for South American species of *Lithurgus*, limited accounts suggest that at least some species forage on *Opuntia* (Schlindwein 1997).

Palaearctic members of the genus *Lithurgus*, including *Lithurgus chrysurus* and *Lithurgus cornutus*, are probably oligolectic on Cardueae (Asteraceae) (Malyshev 1930 ; Cros 1939 ; Roberts 1978; Müller et al. 1997; Pachinger 2004 ; but see also Güler and Sorkun 2007).

Lithurgus tibialis is a likely oligolege of *Chrozophora* (Euphorbiaceae) (Praz and Sedivy, personal observation, Iran).

Australian and Asian members of the subgenus *Lithurgus* appear to be largely polylectic. *Lithurgus atratiformis* and *Lithurgus collaris* gather pollen from both *Ipomoea* (Convolvulaceae) and *Hibiscus* (Malvaceae) (Houston 1971; Kitamura 2001; but see also Hannan and Maeta 2007). *Lithurgus atratus* gathers pollen from *Ipomoea* and *Sida* (Malvaceae) (Lieftinck 1939; Camillo et al. 1994). *Lithurgus rubricatus* has been reported on *Alyogyne* (Malvaceae ; Danforth, personal observation, Australia), although not enough is known about this species to establish a floral preference.

Floral records for African *Lithurgus* (*Lithurgus*) are less precise. Eardley and Urban (2010) report floral visits for *Lithurgus pullatus* on *Convolvulus*; *Lithurgus sparganotes* on several species of *Ipomoea*; and *Lithurgus spiniferus* on several species of Asteraceae, including *Athanasia*, *Lasiospermum*, and *Senecio*. These records do not distinguish between visits for pollen and visits for nectar. Gess and Gess (2003) also reported *Lithurgus spiniferus* on several species of Asteraceae.

Microthurge pygmaeus is oligolectic on Malvaceae, with recorded floral visits to *Abutilon*, *Krapovickasia*, *Modiolastrum*, *Sida*, and *Sphaeralcea* (Schlindwein 2004); other species of *Microthurge* are the prinicpal pollinators of both *Turnera sidoides* (Turneraceae) and *Modiolastrum malvifolium* (Malvaceae) (Benitez-Vieyra et al. 2007). *Microthurge friesei* is a visitor of *Sida* (Malvaceae) (Gaglianone 2000).

Trichothurgus aterrimus is oligolectic on *Cumulopuntia* (Cactaceae) (Packer et al. 2005); *Trichothurgus dubius* has also been observed foraging on Cactaceae (Claude-Joseph 1926, as *Lithurgus dubius*). Michener (2007) mentioned that "at least some" of the approximately thirteen species of *Trichothurgus* visit Cactaceae for pollen. In October 2009, I personally collected *Trichothurgus* foraging for pollen on cacti in the Atacama Desert of northern Chile (likely either *Trichocereus* or *Eulychnia*).

In brief, New World lithurgines are oligolectic on Cactaceae or Malvaceae. Old World species appear to be either oligolectic on Asteraceae, Euphorbiaceae, or Convolvulaceae; or narrowly polylectic on Malvaceae and Convolvulaceae. While these plant families are not closely related to one another, they share some remarkable similarities. Houston (1971) observes, "*Lithurge* [*Lithurgus*] *atratiformis*, like many other members of its genus, appears to depend exclusively on large-flowered plants with coarse-grained pollen for its food requirements." This observation is true not only for members of the genus *Lithurgus*, but for the tribe Lithurgini in general. Indeed, the floral architecture of lithurgine pollen plants is relatively conserved: the flowers are radially symmetrical and often large, with exposed anthers and easily accessible, large-grained (~ 100 μ in diameter), spiny pollen (Roberts 1978).

The nest architecture of Lithurgini is strikingly similar to that of Fideliini and Pararhophitini: nests typically consist of a series of branching burrows, each burrow containing one or more brood cells. In contrast to fideliine and pararhophitine bees, however, the nests of Lithurgini are excavated in soft or rotten wood rather than in sand. Hannan et al. (2007) found nests of *Lithurgus* (*Lithurgus*) *collaris* built in dead branches of *Hibiscus tiliaceus* on Iriomote Island, Japan, while Kitamura et al. (2001) observed *L. collaris* nesting in *Argusia*

(Boraginaceae) on Ishigaki Island, Japan; Roberts (1978) and Rust et al. (2004) reported nests of *Lithurgus chrysurus* built in exposed pine beams in southern France and the northeastern U.S.; Camillo et al. (1994) discovered nests of *Lithurgus huberi* in multiple substrates but most frequently in *Spathodea* (Bignoniaceae) and *Eucalyptus* (Myrtaceae) in Sao Paulo, Brazil; Garofalo et al. (1981) observed the nests of *Microthurge corumbae* in both *Eucalyptus* and *Cajanus indicus* (Fabaceae); Rozen (1973b) reported finding the nests of *Trichothurgus dubius* built in cactus (either *Trichocereus* or *Eulychnia*) in northern Chile; Houston (1971) reported nests of *Lithurgus atratiformis* built in rotten branches of *Banksia* (Proteaceae) in southeastern Queensland, Australia.

Regardless of the substrate, female lithurgines generally excavate their burrows using their mandibles. Brood cells are constructed end to end within nest tunnels; the walls of the brood cells are completely unlined. Each burrow may contain one or more nest cells (Brach 1978; Garofalo et al. 1981, 1992; Houston 1971; Rozen 1973; Roberts 1978; Camillo et al. 1983; Camillo et al. 1994, Rust et al. 2004); nest cells are either partitioned using particles of wood or wood dust, as seen in multiple species of *Lithurgus,* including *L. chrysurus* (Rust et al. 2004; Roberts 1978; Malyshev 1930), *L. atratiformis* (Houston 1971), *L. huberi* (Camillo et al. 1983; Camillo et al. 1994), *L. gibbosus* (Brach 1978), *L. cornutus* (Malyshev 1930; Lieftinck 1939), and *L. tibialis* (Cros 1939), or are unpartitioned, as reported in *L. chrysurus* (Roberts 1978) and *Microthurge corumbae* (Garafalo et al. 1992). Eggs may either be deposited in a chamber within the pollen mass, as in *Lithurgus gibbosus* (Brach 1978), *Lithurgus apicalis* (Parker and Potter 1973), *Microthurge corumbae* (Garofalo et al. 1981), *Lithurgus huberi* (some cases; Camillo et al. 1983); or in a small cavity at the rear of the cell, as in *Lithurgus chrysurus* (Roberts 1978), *Lithurgus atratiformis* (Houston 1971), and *Lithurgus huberi* (some cases; Camillo et al. 1983).

Although nest tunnels are sometimes backfilled with wood dust, nest entrances are usually left open. *Lithurgus apicalis*, however, seals its nest entrance with wood fibers, feces, and debris left by previous generations (the latter when nests are reused by successive generations; O'Toole and Raw 2004); Camillo et al. (1983) reported that the nest entrances of *Lithurgus huberi* are occasionally partially plugged with wood dust.

Claude-Joseph (1926) commented on the nesting behavior of *Trichothurgus dubius* (as *Lithurgus dubius*). He reported finding *Trichothurgus* nesting inside the abandoned nest cells of *Odynerus humeralis* (a mud-nesting eumenid wasp) in Valle de Maipo, Chile. He mentioned that the cells of *Trichothurgus* were made of resinous particles mixed with wood dust ("des particules résineuses mélangées à de la sciure de bois"); Rozen (1973b), however, disputed this claim and suggested that what may have appeared to be resinous particles to Claude-Joseph were in fact fecal pellets left by *Trichothurgus* attached to the surface of the cocoon. Rozen (1973b) described nests of *Trichothurgus* that he found in cactus; he reported that the nests were unlined.

1.5 Anthidiini

1.5.1 Introduction to Anthidiini

The tribe Anthidiini contains approximately 840 species distributed on all continents except Antarctica (Ascher and Pickering 2011). The assignment of these species to genera is still debated, with some authors preferring to divide the tribe into many smaller genera (Pasteels 1969; Pasteels 1977; Michener 2007) and other authors (Warncke 1980b) preferring fewer, but much larger, genera. Michener (2007) divides Anthidiini into 31 non-parasitic and six parasitic genera. In contrast, Warncke's (1980b) classification of the Palaearctic anthidiines recognizes

only two genera: *Anthidium*, which encompasses all non-cleptoparasitic anthidiines, and *Stelis*, which includes all cleptoparasitic anthidiines (Michener's (2007) classification recognizes at least six non-parasitic Palaearctic anthidiines). Although other authors (Pasteels 1977; Michener 2007) have defended the division of Anthidiini into many genera based on differences in morphology and ethology, Warncke (1980b) justified his classification by noting that "*Anthidium* differs in many groups but no group has developed a new characteristic. It seems not right to split it [the genus *Anthidium*] in many genera (for example Pasteels 1969)." Marked behavioral and morphological differences between species of Anthidiini indicate that it may be appropriate to divide the tribe Anthidiini into multiple, distinct genera. For this reason, I prefer to use the classification outlined by Michener (2007) for the remainder of this chapter. This classification, while excellent, is only partly satisfactory because in some cases, males belonging to different genera cannot be distinguished from one another.

Anthidiini are broad, robust bees that range in size from small (< 7mm) to large (> 20mm); they generally lack the pilosity typical of other megachilid bees. The integument of non-parasitic anthidiines is typically black or brown and marked by yellow, white or red maculations on head, thorax, and abdomen. Cleptoparasitic anthidiines are often black and frequently lack maculation; they are typically smaller and more slender than other anthidiines (Michener 2007).

Members of the tribe Anthidiini exhibit wonderfully diverse nesting behavior, both in terms of nesting substrate and nest-building materials. Some anthidiines build their nests in pre-existing cavities, such as pithy plant stems, empty snail shells, the abandoned nests of other insects, and cracks in stones; others build exposed nest cells which they affix to the surface of rocks, leaves, tree trunks, and twigs; several lineages are known to excavate their own burrows.

Regardless of nesting substrate, anthidiine bees typically build their nests out of either plant resins or plant fibers. The French naturalist, Jean-Henri Fabre, was among the first to divide the tribe Anthidiini into two broad groups based on primary nest-building material (Fabre 1923): *les résiniers*, or those anthidiines which use plant resins to build their nest cells, and *les cotonniers*, or those anthidiines which use plant fibers to build their nest cells. Both groups often supplement their nesting material with pebbles, leaf strips, grains of sand, animal fur, snail shell fragments, bits of bark, other bits of debris, and even small bird feathers (Michener 2007). Michener (2007) also divided Anthidiini into two groups: Series A includes those anthidiines in which the females have three or four mandibular teeth "joined by shallow or at least rounded concavities, so that, except frequently for the uppermost and lowermost teeth, the teeth are obtuse or rounded and often mere angles on the mandibular margin.", while Series B anthidiines are those having "five or more, commonly sharp teeth, separated by acute V-shaped notches" (Michener 2007).

1.5.2 The resin workers

Michener's (2007) Series A includes 23 genera of non-parasitic Anthidiini, all of which use plant resins to construct their brood cells. Plant resin used in nest construction may be gathered from a variety of sources. Many anthidiines gather resin from conifers: *Anthidiellum strigatum* collects resin from pine (Bischoff 1927; Westrich 1989); both *Dianthidium texanum* (Melander 1902) and *D. curvatum* (Michener 1975, as *Paranthidium jugatorium*) collect from red cedar (*Juniperus virginiana*). The nests of *Dianthidium simile* (O'Brien 2007), *Rhodanthidium septemdentatum* and *R. infusctaum* (Fabre 1923) also incorporate coniferous resin. Other anthidiines prefer resin from Asteraceae: *Paranthidium jugatorium* likely gathers

resin from *Grindelia squarrosa* (gumweed) (Evans 1993); *Dianthidium sayi* (now *D. curvatum sayi*) (Krombein et al. 1979) has been reported collecting resin from *Helianthus petiolaris* (Custer and Hicks 1927). Other plant families are also exploited: *Pachyanthidium micheneri* uses *Euphorbia angularis* (Euphorbiaceae) (Michener 1968), while *Notanthidium steloides* uses *Gymnocladus dioica* (Fabaceae) as a resin source (Claude-Joseph 1926).

Some anthidiines appear to gather resin from a single source, while others are more flexible in their choice of resin. *Trachusa larreae*, a bee native to the deserts of the southwestern United States, gathers resin exclusively from creosote bush, *Larrea tridentata* (Zygophyllaceae; Cane 1996). While it is not clear whether this exclusivity is a function of resource availability or is rather a behavioral constraint on the part of the bee, it is evident that the resin of *Larrea* confers undeniable benefits to nesting *Trachusa*. The nest cells of *Trachusa larreae* are composed of hard greyish-green resin; these cells are waterproof and impenetrable to other insects, such as ants (Cane 1996). Furthermore, *Larrea* resin has antimicrobial and antifungal properties, which may prevent spoilage of larval provisions; it is also unpalatable to many herbivores (Cane 1996). Not all anthidiines are so specific in their choice of resin, however. As reported above, *Dianthidium curvatum* may collect resin from both *Juniperus* and *Helianthus*. Other anthidiines may collect resin-like substances instead of actual resin. A nest of *Icteranthidium laterale* was found that had been lined with a material similar to machine grease (Pasteels 1977).

Regardless of the source, female anthidiines gather resin with their mandibles. If the resin is firm, the female uses her mandibles to cut off small chunks of resin; if the resin is softer, she uses her mandibles to mold the resin into a small ball. In some anthidiines, the resin is then

tucked between the mandibles and the labrum and carried back to the nest. *Trachusa larreae* has a unique tuft of hair on the labrum which may serve as an anchor point around which the collected resin ball is gathered (Cane 1996). In other species, such as *Anthodioctes megachiloides*, females carry resin in their mandibles (Alves-dos-Santos 2004); *Anthodioctes lunatus* transports resin using both her mandibles and front legs (Camarotti-de-Lima and Martins 2005). Females make multiple trips to gather resin; *Trachusa byssina* requires 20-30 bits of resin to complete a single nest cell (Müller et al. 1997).

Many resin workers build exposed nests that consist of one or more ovoid cells; in the case of multiple cells, the cells are not usually arranged linearly but are more often grouped together in small clusters. Nests of the genus *Anthidiellum* are typically found affixed to the surfaces of rock faces or leaves; the nest cells themselves are either built of pure resin or of resin mixed with other materials. The nests of *Anthidiellum strigatum*, for example, are often found on overhanging rock surfaces and are constructed of pure plant resin (Perez 1889; Ferton 1901; Friese 1915; Bischoff 1927; Pasteels 1977; Westrich 1989; Muller 1931; Müller et al. 1997); a single-celled nest was discovered attached to a branch of thyme (Ferton 1901). Nests of *A. strigatum* usually contain between two and three cells but may contain up to eight under optimal nesting conditions (Bischoff 1927). After nest cell construction is nearly complete, the entrance to each nest cell is crimped by the female into narrow, elongate "spout"; the "spout" is a slender opening that may serve to facilitate air exchange within the nest cell. Although these nests are built of pure resin, females may camouflage the outer surface of the nest cell with bits of bark (Müller et al. 1997). Other species within the genus *Anthidiellum* build similar nests. Nests of *A. notatum* have been found attached to the needles of *Pinus sylvestris* (Baker 1985); Baker (1985) notes that the nests of *A. notatum* bear likeness to those of *A. ehrhorni* described by Schwarz

(1928), *A. notatum rufimaculatum* and *A. notatum robertsoni* (Grigarick and Stange 1968). The nests of *A. notatum* have a small, bell-shaped projection on the lower surface; this projection bears a likeness to the spout on nest cells of *A. strigatum*, although it is not clear whether the projection is solid or allows for the passage of air into the cell (Baker 1985).

Other reports of the nests of *Anthidiellum* are considerably different. A nest of *A. strigatum* was found inside a fruit capsule of *Lychnis dioica* (Perez 1879). Nests of *A. apicepilosum* are self-excavated in rocky soil; nest cells built of pure resin are built in a linear series and the entrance to the nest is capped with a curved access tube built of resin ("cylindre d'acces") (Pagden 1934; Pasteels 1972; Pasteels 1977). *A. perplexum* has been reared from trap nests; its nests cells are built of resin mixed with pebbles (Baker 1985).

Anthodioctes is a Central and South American genus that nests in pre-existing cavities. Most accounts of the nesting behavior of *Anthodioctes* have been reported from trap nests in Brazil and each account describes the same general nesting strategy: multiple nest cells are constructed in linear series within the cylindrical cavity of a trap nest; an empty "vestibular" cell is built between the nest entrance and the first provisioned cell. Females line the inner walls of the trap nest, build cell partitions, and create their nest closures with viscous plant resin. Each species, however, appears to collect its resin from a different source. *A. lunatus* lines its nests with pure, red plant resin (Camarotti-de-Lima and Martins 2005), while *A. megachiloides* lines its nests with yellow resin mixed with bits of wood, grains of sand, and small pieces of paper (Alves-dos-Santos 2004, 2010); *A. moratoi* lines its nests with plant resin mixed with wood chips (Morato 2001). In none of these cases is the source of resin identified.

Anthodioctes megachiloides has also been reared from an abandoned nest of *Brachymenes dyscherus* (Eumenidae; Alves-dos-Santos 2010). Aside from the location in which the nest was built, the other details of nest cell construction were similar to that described above for *A. megachiloides*. A single male of *A. manauara* was reared from an abandoned wasp nest in the remains of a primary forest; the details of this nest were not described (Morato 2001).

All known species of *Dianthidium* build their nest cells using resin mixed with some combination of pebbles, sand grains, and plant debris. The substrate in which *Dianthidium* builds its nests, however, is highly variable between species. Several species build exposed nests. Nests of *Dianthidium texanum, Dianthidium cocinnum, Dianthidium macrurum* and *Dianthidium pudicum* are found affixed to tree branches, stones, and other surfaces (Melander 1902; Hicks 1927; Fischer 1951; Clement 1974; Parker 1977). Each species mixes resin with pebbles, although nest architecture may vary from species to species. The nests of *D. cocinnum* are built in linear series (Fischer 1951) while the nest cells of *D. micrurum* are arranged in a "two-storied" cluster. Nests contain up to ten cells. In some cases, such as *D. macrurum* and *D. pudicum*, completed nest cells are covered with an additional layer of resin (Hicks 1927; Parker 1977).

Dianthidium simile is the sole member of the genus known to excavate its own nests in soil (O'Brien 2007). Nests are built in vegetated sand dunes in clusters of three to 13 cells; cells consist of coniferous resin mixed with sand grains and are constructed at the base of dried clumps of grass, just below the surface of the ground, intermingled with dead rhizomes.

Other ground-nesting anthidiines build nests in pre-existing cavities in the ground. Nests of *Dianthidium curvatum* (as *Dianthidium sayi*) are found in sandy, vegetated soil; nest cells may be attached to plant roots such as *Bromus brizaeformis* (Custer and Hicks 1927) or built in the

abandoned nests of other insects (Michener 1975). Cells are built with resin taken from plants such as *Helianthus petiolaris* and *Juniperus virginiana* and mixed with pebbles, and, to some degree, dirt, small sticks, and chaff (Custer and Hicks 1927; Michener 1975). Nest cells may be built near the surface of the ground or may be clustered at the distal ends of branching burrows; cells are made of resin mixed with pebbles (Custer and Hicks 1927; Fischer 1951), although Michener (1975) reported that pebbles are used to fill empty space within nest cells but are "rarely incorporated in resin to form masonry". *D. curvatum* is found nesting in large aggregations.

A number of species of *Dianthidium* have been reared from trap nests bored in blocks of wood (Krombein 1967). *Dianthidium floridensis* was observed to line the entire inner surface of its nest with a thin coating of pure resin, while *D. platyurum* uses a mixture of resin and tiny pebbles. In contrast, *D. ulkei* and *D. heterulkei* "lined only the cell walls opposite the pollen nectar mass with a thin coating of resin" (as opposed to the entire inner surface of the nest). The cell partitions of *D. floridensis*, *D. platyurum*, and *D. ulkei* are multi-layered, consisting of a thin layer of resin, a layer of leaf bits, pebbles and bark, and then another thin layer of resin; the cell partitions of *D. heterulkei* are thinner and consist only of resin mixed with tiny pebbles (Krombein 1967). Another nest of *D. ulkei* was taken from a pre-existing cavity in the soil; the two-celled nest was constructed of plant debris and pebbles cemented together with a "resinous material" (Hicks 1933). The cell closures and the cell walls were constructed of pure resin (Hicks 1933).

Detailed observations were made on the nesting behavior *D. ulkei*, the details of which differ somewhat from those given by Krombein (1967) and Hicks (1933) for the same species

(see above) (Frohlich and Parker 1985). *D. ulkei* was reared in greenhouse trap nests consisting of elderberry sticks; the elderberry sticks were bored along the main axis, cut in half lengthwise and mounted on glass plates to allow for observation of nesting behavior (Frohlich and Parker 1985). Nest cells were built from resin impregnated with pebbles and pith powder. The trap nests were wide enough to accommodate the construction of two nest cells side by side; some females staggered their cells within the nest. Cell partitions consisted of pebbles mixed with resin, while nest closures consisted of resin mixed with a variety of foreign material, including pebbles, dirt pieces, vermiculite, paper, small sticks, pith and bark pieces. *D. ulkei* groomed her abdomen with her hind legs until "a small white droplet emerged from the tip"; she also "exuded a large amount of an opaque, whitish substance from the abdomen tip" (Frohlich and Parker 1985). The origin of these secretions was not clear. In both cases, the female daubed the secretions onto the walls of her nest and chewed the secretions into the resin. Chewing this secretion into the resin made the resin "more fluid and easier to manage" (Frohlich and Parker 1985).

Duckeanthidium thielei has been reared from trap nest blocks (Thiele 2002). Nests consist of one to three cells; each nest includes an empty, vestibular space between the last provisioned cell and the nest entrance. Cell partitions and nest closures are extremely hard; nest closures resemble "synthetic epoxy" and cell-cap material may be "made exclusively from secretions of the Dufour's and mandibular gland, or maybe a mixture of glandular and plant material" (Thiele 2002). Females deposit resin-like material within nests and at nest cell entrances and enclose themselves inside nests at night by building temporary nest closures out of resin-like material. It is not clear whether the walls of the nest are lined or not (Thiele 2002).

There is a single account of the nesting behavior of *Notanthidium steloides* from Chile (Claude-Joseph 1926, as *Anthidium steloides*). Nests are constructed in abandoned galleries dug by beetle larvae in gate posts and dead branches. Females clear any wood dust from the galleries, creating relatively cylindrical nests that they divide into cells using partitions of resin. Nests are also built in dry bamboo stems (Claude-Joseph 1926). A nest was reported in which *N. steloides* built cell partitions using the viscous, sweet material exuded by pods of *Gymnocladus*; nearly all larvae present in this nest died (Claude-Joseph 1926).

A nesting aggregation of *Paranthidium jugatorium* was found on a sandy slope littered with stones and plant roots near Livermore, Larimer County, Colorado (Evans 1993). *P. jugatorium* excavates either simple or branched burrows in the ground; nest cells are placed in closely spaced linear series at the ends of nest tunnels. The cell walls and cell partitions appear to be made from the same sticky, translucent plant gums; these gums are pure and are not mixed with other material. Evans (1993) believed that the source of these plant gums may have been gumweed (Asteraceae, most likely *Grindelia*) but was unable to confirm this hypothesis.

Plesianthidium volkmanni was found nesting in electricity boxes provided to campers in the Olifants River Valley, Western Cape Province, South Africa (Gess and Gess 2007). Females began nest cell construction by building a small "saucer"; this served as the foundation upon which nest cells were built and was of a different texture (and probably a different material) than the nest cells. The nest cells themselves were built of ochre-colored resin and were positioned horizontally. The closure of each individual cell consisted of a narrowed, tapering spout about half as long as the cell itself. The purpose of the spout may be to ventilate nest cells (Gess and Gess 2007). The inside of the spout itself is filled with small fragments of plant material

(although this material is not made of plant fibers or other "fluffy" material) (Gess and Gess 2007).

Icteranthidium is a largely Palaearctic genus that nests in pre-existing cavities. The nests of *Icteranthidium grohmanni* consist of a resinous cluster of cells typically found under stones or in abandoned ant nests (Fabre 1923; Pasteels 1977), although *I. grohmanni* is also known to excavate its own nests in the ground (Ferton 1914). The nests of *I. laterale* consist of up to twelve resinous cells clustered together; the cluster is found under stones, or in abandoned galleries left by beetles or ants (Ferton 1914; Pasteels 1977). Nest cell construction in *I. laterale* may be preceded by the construction of a small pedestal made of cypress resin; the function of this pedestal is not clear (Ferton 1914). In a single case, *I. laterale* was observed excavating its own nest; the nest consisted of a tunnel dug in the soil which lead to rounded chamber which housed the nest cells (Pasteels 1977).

Some members of the Palaearctic genus *Rhodanthidium* build nests in pre-existing cavities, while others excavate their own nests. The nests of *Rhodanthidium septemdentatum*, *R. sticticum* and *R. infuscatum* are built in abandoned snail shells (Xambeu 1896; Ferton 1911; Fabre 1923; Grandi 1934, 1961; Pasteels 1977). The nest architecture of these three species is extremely similar. One or two cells are provisioned deep in the whorls of the snail shell (Fabre 1923; Grandi 1961; Pasteels 1977). Although partitions between cells are built of resin, the inner walls of the snail shell are left unlined. A layer of sand grains, small pebbles and vegetable debris follows the final provisioned cell; this material in this layer is simply piled up and not cemented together. The entire nest is sealed with a plug of resin; the plug is often not flush with the opening of the shell but is rather farther inside the shell, so that the final whorl of the shell is

left empty (Fabre 1923). In smaller shells, the final whorl may be left empty and the final plug of resin omitted (Fabre 1923). The final nest closure may consist of a plug of shell fragments cemented together with resin (Grandi 1961), or of pebbles cemented together with resin (Fabre 1923). The nest-bearing snail shell is then hidden under stones or buried in the sand (Fabre 1923; Pasteels 1977); in rocky soil where burial is difficult, the nest may simply be left where it was built (Bischoff 1927).

The nests of *Rhodanthidium caturigense* are remarkable among anthidiines, for their nest cells are built of both plant resin and plant fibers. *R. caturigense* excavates its own nests in the soil and nests are often built in large aggregations (Micheli 1935; Maneval 1936; Pasteels 1977; Müller et al. 1997). Between three and six nest cells are located at the end of short burrow; cells are often oriented haphazardly to accommodate the presence of stones and roots in the soil. Each individual brood cell consists of two distinct layers. The outer layer is built of plant hairs; according to Pasteels (1977), the source of these hairs is *Verbascum thapsus*. The inner layer is built of resin. The nest entrance is sealed with a plug of resin coated with plant hairs (Pasteels 1977).

Like *Rhodanthidium caturigense*, at least some members of the genus *Pachyanthidium* are unusual among anthidiines for their ability to build nests from a combination of resin and plant fibers. A nest of *Pachyanthidium (Pachyanthidium) bicolor* was found attached to coffee leaves (Michener 1968). Individual cells were clearly distinct from one another and were connected together in linear series. Unlike the nest cells of *Rhodanthidium caturigense*, which consist of separate layers of resin and plant fibers, the nest cells of *P. bicolor* consist of plant resin mixed with plant fibers. The inner surfaces of the nest cells were smooth, while the outer

surfaces were roughened with projecting plant fibers. Another member of the same subgenus, *Pachyanthidium (Pachyanthidium) micheneri*, was observed collecting resin from *Euphorbia angularis* (Euphorbiaceae) (Michener 1968). The nests of *P. micheneri* are similar to those of *P. bicolor* (Pasteels 1977).

Members of the genus *Trachusa* typically dig their own nests in loose sand; nest cells are built from leaf strips and plant resin (Bischoff 1927). *Trachusa byssina* nests in large aggregations (Friese 1898; Friese 1923; Hachfeld 1926; Pasteels 1977). Nests are self-excavated (Müller et al. 1997) and consist of either simple or branching burrows in the soil; each nest contains between one and four cells (Pasteels 1977). The cells themselves are lined with overlapping pieces of leaves; the inner surface of the leaves is then coated with a layer of resin. Cell partitions and nest closures are made of resin mixed with pieces of leaves. Nests of *T. interrupta* are similar to those of *T. byssina*. The nests of *Trachusa interrupta* are built in the ground; nests are self-excavated, although females may use pre-existing cavities as the starting point for nest construction (Ferton 1920). Nest cells are built at the end of a deep burrow and are lined with long, narrow strips of leaves that wind around the inside of the cell like a "puttee" ("*les molletières de drap*") (Ferton 1920, as *Anthidium foliivolutor*). Leaf strips are taken from acacia and blackberry (*Rubus* spp.); the cut edges of the leaf strips are not straight, like those cut by *Megachile*, but are rather jagged (Ferton 1920). The inner surface of the leaves is then coated with a layer of resin (Ferton 1920; Pasteels 1977).

Trachusa perdita nests in small aggregations in loose, sandy soil (Michener 1941). Each nest consists of an unbranched tunnel with a linear series of brood cells at the distal end. Nest cells are built of leaf strips cut from *Rhamnus crocea* (Rhamnaceae) and are cemented together

with resin; leaf strips are arranged "with their long axes at right angles to the long axes of the cell". Michener (1941) describes the intercellular partitions as thin; he does not explicitly state what they are made of but seems to imply that they are made from resin.

The nests of *T. larreae* are built with resin but without leaves. *T. larreae* excavates its own nests in sandy, loamy soil; nests are often found in aggregations. The nests consist of an unbranched tunnel in the soil with three to four brood cells at the distal end. The nest cells and nest cell caps are made of hard, greyish green resin; the nest cells are fastened together by way of the cell caps (Cane 1996). Sand grains may be stuck to the outer surface of the cells but the inner surface of the cells are smooth and glassy. MacSwain (1946) describes a similar nest architecture but describes the nest cells as composed of both sand and resin; MacSwain does not identify the resin source.

The genus *Aspidosmia* includes just two species and is restricted to the deserts of South Africa and Namibia. Unlike other members of the subfamily Megachilinae, *Aspidosmia* has scopa-like hairs on its hind tibiae; in museum specimens, these hairs are full of pollen (Peters 1972). The nests of *Aspidosmia* consist of masticated leaf pulp mixed with small pebbles; nests are affixed to the surfaces of stones (Brauns 1926; Peters 1972).

1.5.3 The "cotton" workers

Michener (2007) includes eight genera in his Series B, all of which use plant fibers as the principle component in brood cell construction. At least two species belonging to Series A, however, are also known to incorporate plant fibers into nest cell construction: *Rhodanthidium caturigense* and *Pachyanthidium bicolor* (see above).

Female bees use their mandibles to scrape fibers from the stems and leaves of hairy plants (Custer and Hicks 1927; Masuda 1933; Grigarick and Stange 1968; Westrich 1989). The fibers are rolled into a small ball using the mandibles, legs and abdomen and then transported back to the nest (Westrich 1989), where they are used to construct brood cells. Plant fibers are gathered from multiple plant sources (see below).

The genus *Afranthidium* builds nests in pre-existing cavities. Nests of *Afranthidium concolor* are found on bare, sandy slopes. Nest entrances consist of a tube made of plant fibers which projects from the mouth of the nest. Nest burrows are oriented vertically; cells are located at the base of the burrow and are built from the same fibrous material as the entrance tube (Gess and Gess 2007). Fibers may be taken from *Eriocephalus* (Asteraceae). Nests of *A. junodi* have been reported from abandoned burrows of *Anthophora* (Michener 1968). Nest cells were made of plant fibers and were arranged in a linear series when the nest tunnel was narrow; in cases where the nest tunnel was wide, cells were arranged irregularly. Nests were filled to the entrance with "fine white plant down" (Michener 1968).

Afranthidium micrurum nests in plant stems (Pasteels 1977). The inside of the stem is lined with a cylinder of plant "cotton"; nest cells are built of plant fibers within this cylinder and are similar to "a rosary of cells made of cotton" ("chapelets des celules en coton") (Pasteels 1977). A nest of *Afranthidium micrurum* was found within the stem of a hollow weed in Pietermaritzburg, Natal (now KwaZulu-Natal); the nest consisted of five cells built in linear series made of "dense, fine, white plant down" (as *Anthidium micrurum*, Michener 1968).

Nests of *Afranthidium ablusum* have been found in abandoned snail shells (Mollusca: Gastropoda: Dorcasiidae: *Trigonephrus*) in the desertic areas north and south of the Orange

River, near Alexander Bay (Gess and Gess 1999; Gess and Gess 2007). Nests cells are built within a mass of densely packed "cotton-wool like plant fibres" that fill the shell. Another species of *Afranthidium*, "probably *odonturum*", also builds its nests in abandoned shells of *Trigonephrus* (Gess and Gess 1999). Nest architecture is similar to that of *A. ablusum*; nest cells are embedded in a mass of densely packed plant fibers (Gess and Gess 1999).

A massive nesting aggregation of *Afranthidium repetitum* was found in an electricity meter box in Estcourt, Natal (now KwaZulu-Natal) (Michener 1968). It was estimated that there were as many as 1750 nest and cocoons; nests were constructed of plant fibers (Michener 1968).

Bees of the genus *Anthidium* build nests in pre-existing cavities. *Anthidium maculosum, Anthidium formosum and Anthidium illustre* have been reared in trap nests (Krombein 1967; Parker 1987). Fibers used in nest cell construction may come from cottonwood or desert willow (*Anthidium maculosum*); from Asteraceae and tripetid galls (*Anthidium illustre*); or from other sources (Krombein 1967; Johnson 1904). Nest cells of *A. maculosum* are sealed with a partition of matted plant fibers; nest closures may consist of a thick wad of cotton or of a wad of cotton, a layer of debris, and then another wad of cotton. The nest entrances of *Anthidium formosum* are plugged with small pebbles stuck together with masticated leaf pulp; nests of *A. illustre* are similar. Nests of *A. illustre* have also been found in dead flower stalks of *Yucca whipplei* and in stumps of oak in California (Hicks 1929) and in the abandoned burrows of *Anthophora occidentalis* in Colorado (Johnson 1904).

Other members of the genus *Anthidium* build their nests in pre-existing cavities in the soil. The nests of *Anthidium paroselae* are built from plant fibers in sandy soil (Melander 1902; Newberry 1900). *A. porterae* has been observed nesting in vacant cavities in sparsely vegetated

areas near Boulder, Colorado (Custer and Hicks 1927). Nest cells are built of plant fibers gathered from *Cryptanthe gracilis* (now *Cryptantha gracilis*, Boraginaceae) and *Artemisia canadensis* (Asteraceae). The space between the nest entrance and the first provisioned cell was backfilled with pebbles. A brief description of the nests *Anthidium emarginatum* is given by Melander (1902); he states only that nests are constructed from plant fibers in dry sand banks.

Anthidium manicatum has been observed nesting in a variety of pre-existing cavities including rock crevices, old beams and posts, reeds, cracks in walls (Ferton 1909; Bischoff 1927; Pasteels 1977; Westrich 1989), and the abandoned nests of other bees such as *Anthophora plumipes* (Westrich 1989, as *Anthophora acervorum*). Nests are built with plant fibers taken from composites, Lamiaceae, and *Verbascum* (Scrophulariaceae) (Pasteels 1977); and *Helichrysum* (Asteraceae), *Stachys* (Lamiaceae), *Lychnis* (Caryophyllaceae), *Cydonia* (Rosaceae), and *Populus* (Saliceae) (Westrich 1989). The nest entrance is barricaded with pebbles, plant fibers, and bits of wood and earth (Pasteels 1977; Müller et al. 1997). *A. manicatum* impregnates the fibers with which it builds its nest with secretions harvested from *Pelargonium* (Geraniaceae), possibly as a means of protecting pollen provisions from microbial infection (Müller et al. 1996). Apparently the nesting behavior of *A. florentinum* and *A. diadema* is identical to that of *A. manicatum* (Pasteels 1977).

Anthidium punctatum builds its nests from plant fibers in abandoned nests or stone columns (Müller et al. 1997; Westrich 1989). Plant fibers may be collected from *Verbascum* (Scrophulariaceae)*, Onopordum* (Asteraceae), *Helichrysum* (Asteraceae), and *Antennaria dioica* (Asteraceae) (Westrich 1989). Like *Anthidium manicatum*, *A. punctatum* is known to impregnate the fibers of its nest with plant secretions (Müller et al. 1997). *A. punctatum* has also been

observed excavating its own nests in the ground; nests consist of three or four cells built of plant fibers and are woven together in linear series (Friese 1923; Muller 1931; Pasteels 1977). The nest closure was made from vegetable debris mixed with sand grains.

The nests of *Anthidium chilense* may be found in the ground, in wood, in walls, under stones, or in the abandoned galleries of other insects (Claude-Joseph 1926, as *Anthidium chilensis*). Nest cells are typically built in linear series if the nest is built in a narrow space; if the space is wider, nest cells may be built in a cluster (Claude-Joseph 1926). Plant fibers are harvested from *Populus*, *Platanus*, *Cydonia* and *Loasa*. Females weave together fibers to form small ovoid cells; between one and ten cells are built, depending on the size of the cavity (Claude-Joseph 1926). Nests of *Anthidium septemspinosum* were observed in bamboo stems and in holes dug by other insects (Masuda 1933, as *Anthidium japonicum*). Cells were composed of plant fibers taken from Asteraceae, i.e. *Artemisia vulgaris*. The outer surface of each nest was covered by green "paintic" material, which may have been derived from plant leaves (Masuda 1933).

The nest cells of *Anthidium oblongatum* are flattened and patty-shaped, often found in rock columns (Müller et al. 1997). Cells may be built in wall joints or crevices, between stones lying close together, even in the hollow stalks of thistle and umbellifers; nests may contain up to eight cells, which are built from plant fibers taken from *Stachys* (Lamiaceae), *Verbascum* (Scrophulariaceae), *Helichrysum* (Asteraceae), and *Echinops* (Asteraceae) (Xambeu 1896; Maneval 1929; Westrich 1989).

Nests of *Serapista denticulata* are found affixed to weedy stems (Michener 1968). Nests are built of plant fibers and are composed of two layers: the fibers of the outer layer are soft,

loose and grey due to the inclusion of animal hairs, while the inner layer is white, densely packed, firm, and extremely resistant to crushing or tearing. The outer layer is built first and then the individual nest cells are built within the outer layer (Michener 1968); up to fifteen cells have been found in completed nests of the same species (Friese 1916). Other descriptions of the nests of *S. denticulata* are similar (Gess and Gess 2007).

Until 2005, all known nests of *Serapista* were exposed aerial nests. Nests of *Serapista rufipes* had been found attached to the branches of *Lebeckia* (Fabaceae) (Gess and Gess 2007). In 2005, however, Gess and Gess (2007) were surprised to discover *S. rufipes* entering a tube made of plant fibers projecting from the sand near Lambert's Bay, Western Cape Province, South Africa. Upon excavating the nest, they found that *S. rufipes* had built a nest consisting of five brood cells built of plant fibers, presumably in a pre-existing cavity. Like *Anthidiellum strigatum* (mentioned above), *Serapista rufipes* appears capable of building both aerial nests and nests in pre-existing cavities, suggesting some degree of plasticity in the nesting behavior of these species.

The nests of *Pseudoanthidium* may be built in plant stems or other cavities, or on exposed surfaces. *Pseudoanthidium nanum* builds its nests in the stalks of various plants, in galls and in snail shells (Friese 1899, 1923; Enslin 1923; Bischoff 1927; Grandi 1934; Micheli 1934; Grandi 1961; Westrich 1989). Brood cells are constructed from plant fibers such as those of *Verbascum* (Scrophulariaceae) (Westrich 1989). Nests consist of a series of brood cells built within a cylinder of plant fibers; cell partitions are made of wads of the same fibers (Bischoff 1927; Pasteels 1977). While the outer walls of the nests are cottony, the inner walls of the brood cells are coated with a smooth film ("pellicule lisse"; Pasteels 1977) which is secreted by the larvae.

The nest entrance is closed with a plug of plant fibers woven in concentric layers. In narrow nests, cells are arranged linearly; in broader nests, such as those built in galls, cells may be built side by side (Bischoff 1927). Nests built in plant galls are of similar architecture but are built at the end of a winding tunnel that the female excavates in the gall with her mandibles (Pasteels 1977). The nests of *P. scapulare* are similar; nest cells are built of plant fibers in hollow or pithy stems, which *P. scapulare* is capable of hollowing out herself (Müller et al. 1997).

Nests of *Pseudoanthidium cribratum* have been found in stems of *Dorema* (Apiaceae) (Ponomareva 1958). Nests were self-excavated and consisted of a series of branching tunnels; each nest contained multiple cells. The inner surface of the nest was completely lined with a layer of felted plant fibers; the felted nature of this layer caused Ponomareva (1958) to speculate that the plant fibers had undergone some sort of "treatment" by the bee, although the nature of this "treatment" was not clear.

The nests of *Pseudoanthidium truncatum* consist of a mass of dull, white plant hairs containing cocoons of variable orientation (as *Micranthidium truncatum*, Michener 1968). Nests may be attached to banana leaves (Michener 1968), palm leaves (as *Anthidium truncatum*, Bischoff 1927) or other types of leaves (Pasteels 1977). Michener (1968) also described the nest of a species he referred to as *Micranthidium compactum*, although this species has since been synonymized with *P. truncatum*.

1.5.4 Cleptoparasites

Cleptoparasites do not build and provision their own nests but rather lay their eggs in nests that are built and provisioned by other bees; the offspring of the cleptoparasite develop on

the pollen provisions stored by the host bee. Seven genera of cleptoparasitic Anthidiini are currently recognized (Michener 2007).

In at least two cases, the adult cleptoparasite kills the host's offspring before depositing her own eggs. *Hoplostelis bilineolata* is a cleptoparasite of *Euglossa cordata* (Apidae: Apinae: Euglossini). *H. bilineolata* enters the host's nest while the nest is still being provisioned; the parasite repeatedly bites and stings the host, eventually driving her from the nest (Bennett 1966, as *Stelis bilineolata*). After chasing her from the nest, the parasite seals the nest entrance and opens the brood cells of *E. cordata*. She removes the host larvae from their cells and kills them; the parasite also kills older host pupae and adults by stinging or crushing their unopened nest cells. She then lays her own eggs in the nest cells and reseals them using material taken from other nest cells (Bennett 1966).

Euaspis basalis enters the sealed nests of resin-nesting *Megachile* (*Callomegachile*) (as *Chalicodoma*, Iwata 1976). The parasite chews her way through the resinous plug at the stem nest entrance, as well as through the cell partitions; she removes the host larvae from the nest using her mandibles and may eat the host eggs. She then starts at the farthest end of the nest and works her way toward the nest entrance, reforming the pollen masses and the cell partitions and depositing an egg in each of the cells. She only uses the nest cells deepest in the nest, leaving the nest cells closest to the nest entrance empty (Iwata 1976).

Stelis (*Dolichostelis*) *rudbeckiarum* parasitizes the nests of its host, *Megachile* (*Chelostomoides*) *subexilis*, by chewing away the resinous plug left by the host at the entrance of its stem nest (as *Chalicodoma subexilis*, Parker et al. 1987). The parasite deposits droplets of liquid, which it secretes from its abdomen, on the surface of the nest plug. The secretion softens

the resin and allows *Dolichostelis* to more effectively remove the plug; the secretion may also prevent the resin from sticking to the mouthparts of the parasite (Parker et al. 1987). Upon entering the nest, the parasite continues to remove resin from within the nest; she may also remove a small amount of the host's pollen provisions. The activities of the parasite within the nest are unknown but upon leaving the nest, the parasite reseals the nest with resin. (Parker et al. 1987).

In many cases, the cleptoparasitic larvae kill the eggs and larvae of the host. Several species exhibit similar behavior. Females of *Stelis* (*Stelis*) *lateralis* (host: *Hoplitis pilosifrons*), *Stelis* (*Stelis*) *ater* (host: *Osmia chalybea*), *Stelis* (*Stelis*) *chlorocyanea* (host: Osmia nigrifrons), *Stelis* (*Stelis*) *elongativentris* (host: *Ashmeadiella holtii*), and *Stelis* (*Stelis*) *montana* (hosts: *Osmia lignaria propinqua*, *O. californica*, and *O. montana montana*) lay their eggs in the brood cells of a host bee while the nest is still being provisioned. After hatching, the cleptoparasitic larvae eats its way through the pollen mass until it encounters the host larvae, which it immediately attacks and kills (Michener 1955; Rust and Thorp 1973; Torchio 1989; Rozen and Kamel 2009; Rozen and Hall 2011). In the case of *Stelis montana*, only the fifth larval instar attacks host larvae; in other species, multiple instars are capable of attack (Rozen 1966; Rozen 1987; Rozen and Kamel 2009; Rozen and Hall 2011).

Stelis nasuta parasitizes the mud nests of *Megachile* (*Chalicodoma*), including *Megachile parietina*, *M. pyrenaica*, and *M. sicula* (Fabre 1914; Friese 1923; Müller et al. 1997; Westrich 1989). Adult female *Stelis nasuta* chew their way through the hardened mud walls of the host's nest and deposit between two and twelve eggs per nest cell; after egg deposition, *S. nasuta* recloses the nest cells using a mortar made of saliva mixed with soil (Fabre 1914). The larvae of

S. nasuta are much smaller than those of the host (Fabre 1914; Friese 1923; Müller et al. 1997; Westrich 1989); it has been hypothesized that the larvae of *Stelis nasuta* consume all of the provisions intended for the host larva, thereby starving it to death (Fabre 1914). Sometimes the undersized, dried body of the host larva is still present in the nest cell after *S. nasuta* larvae have spun their cocoons, lending support to the theory that *S. nasuta* starves its host. Other times, however, the larva is not present, leaving Fabre (1914) to speculate that either the adult *S. nasuta* or her larvae may destroy or consume the host larva. The larvae of *S. nasuta* are not aggressive (Rozen and Kamel 2009); it seems more likely, then, that the larvae may be killed by the adult female.

1.5.5 Summary of anthidiine nesting behavior and cleptoparasitism

All nest-building Anthidiini use either resin or plant fibers in nest construction. It is clear that anthidiines specialized in the construction of resin nests have mandibular teeth which are unique from those anthidiines which build nests made of plant fibers (Pasteels 1977; Michener 2007). Pasteels' (1977) conviction that female mandibular morphology was correlated with primary nest-building material may be founded in light of the genera *Pachyanthidium* and *Rhodanthidium*. Many members of the genus *Pachyanthidium* exhibit reduced mandibular dentition consistent with Michener's Series A. Members of the subgenus *Pachyanthidium* (*Pachyanthidium*), however, build nests incorporating both resin and plant fibers; bees of this subgenus exhibit mandibular teeth consistent with Michener's (2007) Series B.

Rhodanthidium (*Asianthidium*) *caturigense* also builds its nest from both resin and plant fibers. While the genus *Rhodanthidium* belongs to Michener's (2007) Series A and most members of the genus have mandibular teeth which are barely distinguishable, *R. caturigense*

has four mandibular teeth which are clearly defined, if shallower than most Series B anthidiines. It is likely that mandibles with multiple, distinct teeth are adapted to scraping fibers from plants, while mandibles with less-defined teeth are adapted to the manipulation of plant resin. Michener (2007) supposes that anthidiines belonging to Series A are a paraphyletic group from which Series B arose one or more times. Thus far, however, relationships within Anthidiini are largely unknown. The only existing cladistic analysis of Anthidiini was presented by Müller (1996b); it included only Palaearctic anthidiines, however, making it impossible to assess relationships on a world-wide basis.

Anthidiini build three main types of nest: those which are self-excavated, those built in pre-existing cavities, and those built on exposed surfaces. Given that species within the same genus build different types of nests (e.g. *Dianthidium simile* digs its own nests, while *Dianthidium ulkei* nests in pre-existing cavities), and given that species belonging to apparently unrelated genera build similar types of nest (e.g. *Rhodanthidium septemdentatum* and *Afranthidium ablusum* both nest in snail shells), it appears that each type of nest construction has evolved independently multiple times. Of particular interest is that multiple species of Anthidiini are able to build more than one type of nest. Both *Anthidiellum strigatum* and *Serapista rufipes* build both exposed nests and nests within pre-existing cavities. *Anthidium punctatum* may nest in pre-existing cavities or excavate its own nests. This flexibility in nesting behavior suggests that, at least for some species, nest construction is not a rigidly constrained behavior but is rather a labile behavior that may reflect availability of suitable nesting sites.

There are at least two different cleptoparasitic strategies in Anthidiini. In some cases, seen in *Hoplostelis bilineolata*, *Euaspis basalis* and possibly *Stelis* (*Dolichostelis*) *rudbeckiarum*,

the adult female cleptoparasite enters the sealed nest of the host and destroys the host's eggs before depositing her own eggs. In most cases, however, the female cleptoparasite deposits her eggs in the host nest while the nest is still being provisioned (i.e. before the nest cells are closed); in these cases, it is not the adult cleptoparasite but rather her larvae which attack and destroy the host's larvae. This difference in cleptoparasitic strategy is accompanied by a difference in larval mandibular morphology. In those cleptoparasites with hospicidal (host-killing) adults, larval mandibles are bidentate, as seen *Hoplostelis bilineolata* and *Stelis* (*Stelidomorpha*) *nasuta* (Rozen 1966). In those cleptoparasites with hospicidal larvae, such as *Stelis* (*Stelis*) *lateralis*, *Stelis* (*Stelis*) *ater*, and *Stelis* (*Stelis*) *elongatoventris*, one or more of the larval instars is often armed with long, sharp, mandibles which it uses to kill the host's larvae (Michener 1955; Rozen and Hall 2011; Rozen 1987). *Hoplostelis* is not thought to be closely related to *Stelis*, however (Michener and Griswold 1994); this suggests that similarities in mandibular morphology between *Hoplostelis* and members of the genus *Stelis* may be either symplesiomorphies or convergence rather than synapomorphies for any particular clade.

A third cleptoparasitic strategy has been proposed for *Stelis* (*Stelidomorpha*) *nasuta*, although it remains speculative: the cleptoparasite deposits between two and twelve eggs in a single nest cell of its much-larger host (Fabre 1923; Müller et al. 1997). The cleptoparasitic larvae consume all of the provisions intended for the host larva, effectively starving it out (Fabre 1923). The provisions are apparently sufficient to sustain the development of more than one *S. nasuta* larva because Fabre (1923) reported finding multiple cocoons squeezed into a single brood cell.

The evolution of host-choice in cleptoparasitic Anthidiini is completely unknown. Several parasite-host associations appear to be clade-specific: members of the subgenus *Stelis* (*Dolichostelis*) are cleptoparasites of Group 2 *Megachile*; *Stelis* (*Heterostelis*) parasitizes nests of the genus *Trachusa*. *Hoplostelis* is the only anthidiine cleptoparasite with a non-megachilid host; both subgenera parasitize apid bees of the tribe Euglossini. Other host associations are broader; members of the subgenus *Stelis* (*Stelis*) are parasites of Lithurgini, Anthidiini, Osmiini, and Megachilini. There are many parasite-host associations for which behavioral details are unknown (Table 1).

1.6 Osmiini and Megachilini

The tribes Osmiini and Megachilini are not the focus of this thesis and I will not discuss them in detail. I will simply give a brief overview of the biology and behavior of both tribes.

1.6.1 Osmiini

The tribe Osmiini includes over 1000 species divided into approximately 20 genera. Osmiines are found on all continents except South America, Australia and Antarctica; they are most diverse, however, in Mediterranean-like climates of the Palaearctic (Praz et al. 2008). They are small- to medium-sized bees that are often slender and elongate, although many exhibit the broad, robust body form of other megachilids. The integument is often black or metallic blue and lacks the conspicuous white and yellow markings seen in Anthidiini (except the genus *Ochreriades*) (Michener 2007).

Most osmiines are cavity-nesters and construct their nests in plant stems, the abandoned burrows of other insects, snail shells, crevices in rocks or in the ground (Müller 2011). Some

Table 1.1: Reported anthidiine cleptoparasite-host associations for which behavioral details are unknown

Cleptoparasite	Host(s)	Reference
Afrostelis aethiopica	*Heriades freygessneri*	Taylor 1965
Euaspis abdominalis	*Lithurgus (Lithurgus) atratus*; *Megachile (Gronoceras) felina*	Lieftinck 1939; Gess and Gess 2007
Euaspis basalis	*Megachile (Callomegachile) disjuncta, Megachile (Callomegachile) disjunctiformis; Megachile (Callomegachile) sculpuralis;*	Iwata 1976
Hoplostelis (Rhynostelis) (suspected association)	*Eufriesea pulchra*	Michener 2007
Stelis (Dolichostelis) costalis floridana	*Megachile* (*Chelostomoides*) or a resin-nesting anthidiine	Krombein 1967
Stelis (Dolichostelis) costaricensis	*Megachile (Chalicodoma) otonita*	Parker et al. 1987
Stelis (Dolichostelis) louisae	*Megachile (Chelostomoides)* sp.; *Megachile* (*Chelostomoides*) *campanulae*	Parker and Bohart 1979; Parker et al. 1987
Stelis (Heterostelis) annulata	*Trachusa (Paraanthidium) interrupta*	Müller et al. 1997
Stelis (Heterostelis) anthidioides (suspected association)	*Trachusa (Heteranthidium) timberlakei*	Timberlake 1941
Stelis (Heterostelis) hurdi	*Trachusa (Trachusomimus) perdita*	Thorp 1966
Stelis (Heterostelis) manni (suspected association)	*Trachusa (Ulanthidium) manni*	Thorp 1966
Stelis (Protostelis) signata	*Anthidiellum strigatum*	Westrich 1989; Müller et al. 1997
Stelis (Stelis) depressa	*Osmia (Trichinosmia) latisulcata*	Parker 1984
Stelis (Stelis) franconica	*Osmia (Osmia) mustelina*	Westrich 1989; Müller et al. 1997
Stelis (Stelis) lateralis	*Osmia (Melanosmia) pumila*	Johnson 1986
Stelis minuta	*Hoplitis leucomelana; Hoplitis tridentata*	Müller et al. 1997
Stelis (Stelis) ornatula	*Pseudoanthidium (Pseudoanthidium) scapulare, Hoplitis leucomelana; Hoplitis tridentata, O. (Helicosmia) caerulescens*	Müller et al. 1997
Stelis (Stelis) phaeoptera	*Osmia (Helicosmia) leaiana; Osmia (Pyrosmia) submicans*	Müller et al. 1997; Rozen and Kamel 2009 (as Stelis murina)
Stelis (Stelis) punctulatissima	*Anthidium (Anthidium) manicatum, A. (Anthidium) oblongatum, Pseudoanthidium (Pseudoanthidium) scapulare, Megachile (Chalicodoma) parietina, and Hoplitis adunca; Lithurgus (Lithurgus) cornutus*	Müller et al. 1997; Malyshev 1930 (as *Stelis aterrima* on *Lithurgus fuscipennis*)
Stelis (Stelis) sexmaculata	*Osmia (Euthosmia) glauca*	Rust 1972
Stelis (Stelis) vernalis	*Heridaes carinata*	Matthews 1965
Stelis (Stelis) minima	*Heriades campanularum (Chelostoma??)*	Friese 1923; Enslin 1925
Stelis pygmaea	*Heriades truncorum*	Friese 1923
Stelis minuta	*Hoplitis leucomelana*	Verhoeff 1892
Stelis (Stelis) ornatula	*Osmia parvula; Hoplitis leucomelana;*	Höppner 1898, 1904a,1904b; Enslin 1925
Stelis (Stelis) phaeoptera	*Osmia fulviventris*	Asensio 1982
Stelis aethiopica (Afrostelis aethiopica)	*Heriades freygessneri*	Taylor 1965
Stelis odontopyga	*Osmia spinulosa*	Westrich 1989

species belonging to the genera *Osmia*, *Hoplitis*, *Heriades* and possibly *Haetosmia* are known to excavate their own nests, either in the ground or in pithy plant stems. Other species of the genera *Osmia* and *Hoplitis* build exposed nests.

Like most other megachilids, osmiines incorporate foreign material into nest construction. They may use masticated leaf pulp, plant resins, mud, salivary secretions, and bits of leaves to line their cells, construct cell partitions, and build nest closures. Several species

belonging to the genus *Osmia* and *Hoplitis* line their nests with fragments cut from flower petals (Rozen et al. 2010; Müller 2011).

Osmiines exhibit a broad range of host-plant specialization. Many species are host-plant specialists and gather pollen from a single genus or family of plants, while others are host-plant generalists, gathering pollen from multiple plant families (Müller 2011). Preferred families of host-plants include, but are not limited to, Fabaceae, Boraginaceae, Campanulaceae, and Dipsacaceae (Müller 2011). Many osmiines have evolved unique morphological and behavioral adaptations in order to fully exploit the pollen resources of their hosts. *Osmia aurulenta* and *O. caerulescens*, for example, have specially modified facial hairs that they use to harvest pollen from nototribic plants (e.g. certain species belonging to the families Lamiaceae and Scrophulariaceae) (Müller 1996a). Another osmiine, *Osmia ribifloris*, uses her forelegs to drum the stamens of highbush blueberry to release pollen from the poricidal anthers (Torchio 1990).

1.6.2 Megachilini

The tribe Megachilini is the largest of the megachilid tribes and includes over 2000 species divided into three genera: *Megachile*, *Coelioxys*, and *Radoszkowskiana*. The genera *Coelioxys* and *Radoszkowskiana* are cleptoparasitic; *Coelioxys* contains approximately 500 species and *Radoszkowskiana* contains four species. The remaining megachilines are nest-builders belonging to the genus *Megachile*. The tribe is widely distributed on all continents except Antarctica (Michener 2007). Members of the genus *Megachile* range from robust, thick-headed bees with wide metasoma to bees that are slender with parallel-sided metasoma. *Megachile* are often marked by dense white or grey pilosity. The genera *Coelioxys* and *Radoszskowskiana* are distinctly different: they are generally black and lack a scopa; the

abdomen often, although not always, tapers sharply to a point. The pilosity typical of other megachilines is much reduced in these genera and is generally limited to sparse, short, white hairs appressed to head, thorax and abdomen. Megachilines range in size from small to extremely large; the largest bee in the world, *Megachile pluto*, measures up to 39 mm (Messer 1983).

While there is an incredible amount of diversity among species of *Megachile*, the morphological characters that distinguish species are often difficult to discern without careful inspection, making subdivision into smaller groups challenging. Michener (2007) broadly divides the genus *Megachile* into three groups: Group 1 (*Megachile*, as defined by Michener 1962), Group 2 (*Chalicodoma*, as defined by Michener 1962), and Group 3 (*Creightonella*, as defined by Michener 1962). There are synapomorphies which define both Group 1 and Group 3; both groups are likely monophyletic (Michener 2007). Group 2, however, appears to lack synapomorphies and may ultimately require division into several genera (Michener 2007; Gonzalez 2008).

Females belonging to Group 1 have partial or complete cutting edges in mandibular tooth interspaces two or three (or both), although there are several genera that lack these cutting edges (Michener 2007). Females of this group use their sharp mandibles to cut leaves for nest construction. Round leaf pieces are used to create cell partitions and oblong pieces are used to form the bases and walls of the cells; leaves are cut with a smooth margin. Nests are usually built in pre-existing cavities, although some species excavate their own nests. Nest sites include plant stems, nests abandoned by other insects, and crevices in rocks or other structures. Other members of Group 1, namely those members whose mandibular teeth have reduced or absent

cutting edges, craft their cells using other materials, including flower petals, masticated leaf pulp, dirt or small pebbles (Michener 2007).

In Group 2 *Megachile*, females lack cutting edges on the mandibles, although in certain subgenera there is a partial cutting edge between teeth two and three (Michener 2007). Members of this group make their nests of mud or resin. The absence of cut leaves from the nests of Group 2 *Megachile* is likely associated with the absence of cutting edges on the mandible. There are several species, however, that incorporate irregularly cut leaf pieces into cell partitions or nest closures (Michener 2007).

Females belonging to Group 3 have five or six mandibular teeth and partial cutting edges in interspaces two, three and four. Similar to Group 1, members of Group 3 incorporate pieces of cut leaves into nest construction; these leaf pieces however, are irregularly shaped and are cut with ragged edges, unlike the smooth, rounded pieces cut by Group 1. Members of Group 3 also rely more heavily on the incorporation of resins, leaf pulp, mud, and possibly secretions to build their nests than Group 1 (Michener 2007).

1.7 Summary and later chapters

Nesting behavior within Megachilidae is extremely diverse: many species build elaborate nests using a variety of foreign material, while other species build exceedingly simple nests using no foreign material at all. The distinction between megachilid bees that use foreign material in their nests and those that do not is accompanied by differences in species richness, geographic distribution, and host-plant preference. The three megachilid tribes that use foreign material to line their nests, Anthidiini, Osmiini, and Megachilini, comprise nearly 98% of species

diversity within the family, are globally distributed and tend to favor bilaterally symmetrical flowers, while the two tribes that build unlined nests in sand, Fideliini and Pararhophitini, account for less than 1% of species diversity, are restricted to remote xeric regions of South America, Africa and Central Asia, and strongly favor flowers that are radially symmetrical. The tribe Lithurgini represents an intriguing intermediate: lithurgines build unlined nests, not in soil but in wood. They account for only 1.4% of megachilid diversity but are distributed worldwide; their floral preference is for large, radially symmetrical flowers. In Chapter 2, I present a comprehensive molecular phylogeny for the family Megachilidae based on five nuclear genes. I use biogeographic analyses, ancestral state reconstructions, and divergence dating and diversification rate analyses to date the antiquity of the family and explore patterns of diversification, nesting behavior and floral preferences in Megachilidae.

The phylogenetic relationships among the tribes of Megachilidae are still largely unclear. Several lineages, such as Fideliini and Pararhophitini, possess a baffling combination of ancestral and derived characters that have thus far obscured their phylogenetic placement. Other lineages, such as Osmiini, Anthidiini, and Megachilini, lack clearly defined tribal-level synapomorphies and depend instead on *combinations* of characters for the definition of each tribe, making it extremely difficult to choose meaningful characters for morphological cladistic analysis. The phylogenetic placement of many lineages, including Fideliini, Pararhophitini, Dioxyini, *Aspidosmia*, *Pseudoheriades*, *Afroheriades*, and *Ochreriades*, remains largely unknown. The phylogeny presented in Chapter 2 will be used in Chapter 3 to discuss the phylogenetic relationships among the tribes of Megachilidae and to determine the placement of lineages whose affinities are still unclear. The status of current tribes will be reassessed and a revised key to the tribes will be presented.

Members of the tribe Anthidiini exhibit fascinating behavior related to nest-building and cleptoparasitism. The evolution of such behavior is impossible to explore, however, in the absence of a molecular phylogeny. In Chapter 4, the first molecular phylogeny of the tribe Anthidiini is presented and is used to: (a) determine phylogenetic relationships among anthidiine genera and reassess the current classification of Anthidiini; (b) to discuss the evolution of nesting behavior in the tribe, namely the relationship between those anthidiines which use resin and those which use plant fibers in nest construction; and (c) to discuss the evolution of cleptoparasitism in Anthidiini, with a focus on the evolution of cleptoparasitic strategy and host-choice.

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CHAPTER 2

WHY DO LEAFCUTTER BEES CUT LEAVES? NEW INSIGHTS INTO THE EARLY EVOLUTION OF BEES*

Abstract

Stark contrasts in clade species diversity are reported across the tree of life and are especially conspicuous when observed in closely related lineages. The explanation for such disparity has often been attributed to the evolution of key innovations which facilitate colonization of new ecological niches. The factors underlying diversification in bees remain poorly explored. Bees are thought to have originated from apoid wasps during the mid-Cretaceous, a period that coincides with the appearance of angiosperm eudicot pollen grains in the fossil record. The reliance of bees on angiosperm pollen and their fundamental role as angiosperm pollinators have contributed to the idea that both groups may have undergone simultaneous radiations. We demonstrate that one key innovation - the inclusion of foreign material in nest construction - underlies both a massive range expansion and a significant increase in the rate of diversification within the second-largest bee family, Megachilidae. Basal clades within the family are restricted to deserts and exhibit plesiomorphic features rarely observed among modern bees but prevalent among apoid wasps. Our results suggest that early bees inherited a suite of behavioural traits that acted as powerful evolutionary constraints. While the transition to pollen as a larval food source opened an enormous ecological niche for the early

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bees, the exploitation of this niche and the subsequent diversification of bees only became possible after bees had evolved adaptations to overcome these constraints.

2.1 Introduction

Bees provide a mixture of pollen and nectar as food for their developing larvae. To protect these provisions from microbial infection or liquefaction that may result from exposure to moisture, most bees coat the inside of their brood cells with a hydrophobic lining secreted by the Dufour's gland (Cane 1983; Hefetz 1987). In contrast, megachilid bees use an eclectic array of foreign material to line their cells. The French naturalist, Jean-Henri Fabre, commented extensively on the nesting habits of megachilids and posed the following question: "...the Osmiae make their partitions with mud or with a paste of chewed leaves; the Mason-bees build with cement; ...the Megachiles made disks cut from leaves into urns; the Anthidia felt cotton into purses; the Resin-bees cement together little bits of gravel with gum;...Why all these different trades...?" (Fabre 1915).

It has been demonstrated that the foreign material used by megachilid bees is hydrophobic and shows antimicrobial activity (Messer 1985; Müller 1996), thus serving a similar function to the secreted cell lining in other bee groups. Not all megachilids, however, use foreign material in nest construction. Bees of the tribe Lithurgini do not line their nest cells at all; instead, they excavate burrows in wood or stems (Garófalo 1981; Roberts 1978). The absence of nest-lining in this group was originally attributed to a behavioural loss associated with above-ground nesting (Malyshev 1930), but the phylogenetic position of Lithurgini at the base of Megachilinae (Danforth 2006) suggests that it represents an ancestral trait (Eickwort 1981). Bees of the subfamily Fideliinae build unlined nests that they excavate in sandy soil (Rozen 1973;

Rozen 1977; McGinley 198; Rozen 1970). Two distinct tribes of fideliine bees are recognized, Fideliini and Pararhophitini, which are both entirely restricted to deserts; the absence of cell lining in these bees may be related to the arid conditions of their habitats, which may make nest-lining unnecessary (Michener 1964). It remains unclear, however, whether cell lining behaviour, using either secretions or foreign material, has been secondarily lost in these lineages or whether the absence of cell lining represents an ancestral state. To answer these questions, we present a robust molecular phylogeny of Megachilidae and trace the evolution of nesting biology within the family. We demonstrate that the use of foreign material in nest construction was a key innovation that triggered both range expansion and diversification in megachilid bees and also propose that the ancestral biology of this family, which is still reflected in several extant megachilid lineages, mirrors the ancestral behaviour of bees in general. Similarities in the biology of the early megachilid lineages pertaining to nesting and foraging behaviour are numerous, conspicuous and challenge our understanding of the evolution and diversification of bees.

2.2 Materials and methods

2.2.1 Taxon sample

We selected 98 ingroup taxa representing all seven tribes of the family Megachilidae. Our ingroup includes 12 Fideliini, two Pararhophitini, eight Lithurgini, three Dioxyini, 23 Anthidiini, 17 Osmiini, and 33 Megachilini. We chose 31 outgroup taxa to represent the diversity of the rest of the bees including one Colletidae, one Halictidae, one Andrenidae, five Melittidae, and 23 Apidae. Table 2.1 lists the DNA voucher numbers and collection localities for each of the specimens used in this study. We sampled more densely in the families Melittidae and Apidae to

Table 2.1 Complete taxon list, DNA voucher numbers, and collection localities for specimens used in this study

Taxon	Voucher #	Collection locality
Dasypoda argentata	973	FRANCE: Gard, Generac, 22.vi.2002
Macropis nuda	1272	NY: Rensselaer Co., Rensselaerville, 15.vii.2005
Melitta leporina	-	
Promelitta alboclypeata	1321	MOROCCO: Erfoud to Msissi road, 12.iv.2006
Meganomia binghami	1021	SOUTH AFRICA: Limpopo Prov.: 8.5 km N. Vivo, 07.i.2004
Ceratina calcarata	656	NY:Tompkins Co., Ithaca, 04.viii.1999
Exoneura bicolor	679	VIC: Flowerdale Forest, 20 xi 1999
Xylocopa pubescens	sc212	Tunisia: Blidette vill., 25-27.iii.2006
Xylocopa virginica	1153	NY: Tompkins Co., Ithaca 8.v.2001
Anthophora montana	633	AZ:Cochise Co., Chiricahua Mts.,14.ix.99
Pachymelus peringueyi	985	SOUTH AFRICA: NCP: Kamieskroon, 16.ix.2001
Apis cerana	-	
Apis dorsata	-	
Apis florea	-	
Apis mellifera	-	
Bombus ardens	-	
Bombus diversus	-	
Ctenoplectra albolimbata	983	SOUTH AFRICA: KZN: 20 km NE Hluhluwe, 09-12.iii. 2002
Eufriesea pulchra	-	
Euglossa imperialis	-	
Exaerete frontalis	-	
Cephalotrigona capitata	-	
Hypotrigona gribodoi	1040	SOUTH AFRICA: Limpopo Prov.: 27 km E Waterpoort 07.i. 2004
Meliponula brocandei	-	
Tetragonula carbonaria	685	NSW: Windsor, 02.xii.1999
Trigona fuscipennis	-	
Paranomada velutina	652	AZ:Cochise Co., Apachi, 2 mi E, 10.ix.1999
Thyreus delumbatus	987	SOUTH AFRICA: NP: 14 km E. Vivo, 17.iii.2002
Melitturga clavicornis	959	FRANCE: Herault, Causse de la Selle 17.vi.2002
Rophites algirus	968	FRANCE: Var, Entrecasteaux, 14.vi.2002
Colletes inaequalis	450	NY: Tompkins Co., Ithaca NY
Pararhophites orobinus	1424	UZ, Karakalpakstan, Mangit, 25.v.2008
Pararhophites quadratus	1547	Tunisia, Nefta, 28.iii.2006
Fidelia (Fidelia) kobrowi	JL007	SA: Richtersveld NP, 12.x.2008
Fidelia (Fidelia) paradoxa	JL002	SA: WCP: Vanrhynsdorp, 10.10.2002
Fidelia (Fidelia) villosa	JL008	SA: NCP: Nieuwoudtville, 18.x.2008
Fidelia (Fideliana) braunsiana	JL009	SA: NCP: Garies, x.2008
Fidelia (Fideliana) ulrikei	JL010	Morocco, Tansikht, 30.76194°N 6.05278°W, 12.iv.2006
Fidelia (Parafidelia) friesei	JL001	SA: NCP: Hotazel, 02.ii.2009
Fidelia (Parafidelia) pallidula	JL006	SA: Richtersveld NP, 11.x.2008
Fideliopsis (Fideliopsis) hessei	JL004	SA: NCP: Hotazel, 01.ii.2009
Fideliopsis (Fideliopsis) major	JL005	SA: NCP: Eksteenfontein, 09.x.2008
Fideliopsis (Fideliopsis) ornata	JL003	Angola: Namibe, 19.i.2009
Neofidelia longirostris	1543	Chile, Region 3, E. of Chanaral, 10.x.2001
Neofidelia profuga	802	Chile: Atacama Prov., Inca-havas, 5 km N. 03.x.1999
Lithurgus (Lithurgopsis) echinocacti	863	AZ: Pima Co., Tucson, 04.viii.2000
Lithurgus (Lithurgus) chrysurus	1545	Italy, Abruzzen, Massa, 20.viii.2002
Lithurgus (Lithurgus) pullatus	1028	SOUTH AFRICA: Gauteng Prov.: Roodeplaat 20 km N Pretoria, 05.i.2004
Lithurgus (Lithurgus) rubricatus	1352	Aust: WA 15 km S. Geraldton, 08.x.2005
Lithurgus (Lithurgus) scabrosus	CP1	New Caledonia, Noumea
Lithurgus (Lithurgus) tibialis	1520	Greece, Sparta, 15.vii.2006
Microthurge sp	sc207	Argentina: Jujuy Prov., Libertador General San Martín., 2-3.ii.2006
Trichothurgus herbsti	1275	CHILE: Region VIII, Las Trancas, 78 km E. Chillan, 12.xii. 2003
Aglaoapis tridentata	1576	Switzerland, Zeneggen, 22.vi.2005
Dioxys moesta	1546	Greece, Rhodos, Kamiros, 12.v.2005
Paradioxys pannonica	1581	Jordan, Jerash, 23.iv.2007
Afranthidium (Afranthidium) karooense	1588	NCP: 42 km S Eksteenfontein, 09.x.2008
Anthidium (Callanthidium) illustre	1384	NV: Clark Co. Lovell Cyn., 16.vi.2004
Anthodioctes (Anthodioctes) mapirense	1519	Bolivia, La Paz, Puente Villa, 11.iii.2001
Aspidosmia arnoldi	1570	South-Africa, Flower Reserve, Rondell, 26.ix.2006
Aspidosmia volkmanni	1579	SA, N. Cape, Richtersveld, near De Koci, 09.ix.2007
Bathanthidium (Manthidium) binghami	1536	Thailand, Petchabun Nam NP, 1-8.iii.2007
Benanthis madagascariensis	1518	Madagascar, Tulear, Androy, x.2002
Dianthidium (Adanthidium) arizonicum	1386	UT: Garfield Co. Escalante, 27.vi.2002
Duckeanthidium thielei	1607	bar code BBSL717389
Eoanthidium (Clistanthidium) turnericum	1589	NCP: Eksteenfontein, 09.x.2008
Epanthidium (Epanthidium) bicoloratum	1441	Argentina, Catamarca, Trampasacha, 25.x -12.xi.2003
Hypanthidioides (Saranthidium) marginata	CP2	Paraguay, Guaira, Res. de Recursos, Manejados 24.i.2007
Hypanthidium (Hypanthidium) obscurius	SC171	locality unknown

Table 2.1 (continued)

Taxon	Voucher #	Collection locality
Icteranthidium ferrugineum flavum	1432	UZ, Karakalpakstan, Beruni, 25.v.2008
Notanthidium (Notanthidium) steloides	1542	Chile, Region Metro, Farellones, 31.xii.2008
Pachyanthidium (Trichanthidium) bengualense	1434	SA: Limpopo Prov., 27 km E Waterpoort, 07.i.2004
Paranthidium (Paranthidium) jugatorium	495	NY: Tompkins Co., Ithaca, 31.vii.1997
Pseudoanthidium (Pseudoanthidium) scapulare	1601	ITALY: Toscana, Massa Maritima, 28.vii.2005
Rhodanthidium (Rhodanthidium) septemdentatum	1514	GR, Rhodos, Stegna, 08.v.2005
Serapista rufipes	1450	South Africa, NCP, Eksteenfontein, 09.x.2008
Stelis (Stelis) paiute	1394	NV: Clark Co. Jean Lake, 14.iv.2004
Trachusa (Archianthidium) pubescens	1533	Turkey, Erzurum, Akören, 15 km N Hinis, 19.vii.2003
Trachusa (Heteranthidium) larreae	1142	NV: Clark Co., Las Vegas Sand Dunes, 01.iv.2004
Coelioxys (Allocoelioxys) afra	1549	Switzerland, Weiach, 29.vi.2004
Megachile (Aethomegachile) sp	1515	Thailand, Chiang Mai, 22.iii.2007
Megachile (Amegachile) fimbriata	1523	S-Africa, 20 km E Waterpoort, 07.i.2004
Megachile (Argyropile) parallela	1522	AZ, Portal, Rucker Canyon, 31.viii.2008
Megachile (Austrochile) sp	1454	Australia, WA, Leonora, 27.ix.2005
Megachile (Callomegachile) sculpturalis	1423	USA,NY, Ithaca, vii.2008
Megachile (Chalicodoma) parietina	1555	Switzerland, Hohtenn, 26.v.2005
Megachile (Chalicodomoides) aethiops	1455	Australia, WA, Marble Bar, v.2003
Megachile (Chelostomoda) sp	1448	Thailand, Chiang Mai, 24.iii.2007
Megachile (Chelostomoides) angelarum	1283	NV: Clark Co., 2.5 mi S. Wheeler Well, 30.vi.2004
Megachile spinotulata	1435	USA, AZ, Portal, Rucker Canyon, 31.viii.2008
Megachile (Chrysosarus) sp	1442	Argentina, Jujuy Co., 2 km E Paso de Jama, 14.xi-21.xii.2003
Megachile (Creightonella) albisecta	1556	Italy, Toscana, Massa Maritima, 28.vii.2005
Megachile (Cressoniella) zapoteca	1439	USA, AZ, Cochise Co., Paradise Junction, 01.ix.2008
Megachile (Eutricharaea) mandibularis	1521	UZ, Bukara, 40 km N Gazli, 31.v.2008
Megachile patellimana	1453	Oman, Sur, 01.iii.2008
Megachile pilidens	1550	Switzerland, Weiach, 29.vi.2004
Megachile (Gronoceras) bombiformis	1531	South Africa, Limpopo Prov, 20 km E Waterpoort, 07.i.2004
Megachile (Hackeriapis) sp2	1447	Australia, WA, Coolgardie 25.ix.2005
Megachile (Largella) sp	1540	Thailand, Phetchabun Nam Nao NP, 8-15.iii.2007
Megachile (Lithomegachile) texana	1524	USA, NY, Ithaca, vii.2008
Megachile (Maximegachile) maxillosa	1532	South Africa, Mount Rupert, 08.ii.2008
Megachile (Megachile) melanopyga	1575	CH, Hohtenn, 26.v.2005
Megachile (Megachiloides) nevadensis	1427	USA, UT, Wayne Co, South Torrey, 05.viii.2008
Megachile (Mitchellapis) fabricator	1433	Australia, NSW, Wodonga, 09.xii.1999
Megachile (Pseudocentron) sidalceae	1429	USA, AZ, (County?), Wilcox, viii.2008
Megachile (Pseudomegachile) ericetorum	SC232	Czech Republic: Nový Brázdim. 17.vi.2000
Megachile (Ptilosarus) microsoma	1444	Trinidad, El Dorado, Caura Valley, 61 m, 10.iii.2008
Megachile (Rhodomegachile) sp	1443	Australia, W, Tom Price, iv.2003
Megachile (Sayapis) pugnata	595	NY: Schuyler Co., Valois gravel pit, 14.vii.1999
Megachile (Thaumatosoma) remeata	1445	Australia, WA, Laverton, 27.ix.2005
Megachile (Xanthosarus) maritima	1425	UZ, Bukara, 02.vi.2008
Noteriades sp	1580	Thailand, Chiang Mai, 24.iii.2007
Radoszkowskiana rufiventris	1587	Egypt, Tel el Kebir, 30°32'2"N 31°49'48"
Afroheriades primus	1585	SA, N. Cape, 6 km N Concordia, 14.ix.2007
Ashmeadiella (Ashmeadiella) aridula	1270	UT: Garfield Co., Long Canyon, 01.ix.2003
Atoposmia (Eremosmia) mirifica	1560	USA, NV, W Yucca Gap, 18.v.2005
Chelostoma (Chelostoma) florisomne	1553	Switzerland, Chur
Haetosmia brachyura	1428	UZ, Karakalpakstan, Beruni, 25.v.2008
Heriades (Neotrypetes) crucifer	1149	AZ: Coshise Co., Chiricahua Mts., 25.viii.2003
Hofferia schmiedeknechti	1586	Greece, Chimara, 26.v.2006
Hoplitis (Hoplitis) adunca	1552	Italy, Aosta, 30.viii.2004
Ochreriades fasciatus	1557	Jordan, 20 km W Amman, 24.iv.2007
Osmia (Osmia) lignaria	1265	locality unknown
Othinosmia (Megaloheriades) globicola	1569	South-Africa, W Cape Prov., Nieuwondtville, 09.x.2002
Othinosmia (Othinosmia) securicornis	1584	SA, N. Cape, Richtersveld, near De Koci, 09.ix.2007
Protosmia (Protosmia) humeralis	1559	Jordan, Wadi Shu'ayb, 22.iv.2007
Pseudoheriades moricei	1431	IL, Negev
Stenoheriades asiaticus	1578	Greece, Zachlorou, 22.v.2006
Wainia (Caposmia) eremoplana	1548	Jordan, Wadi el Hasa, 20.iv.2007

accommodate the placement of fossil calibration points. Voucher specimens are deposited in the Cornell University Insect Collection.

2.2.2 Datasets and alignment

We sequenced fragments from four protein-coding genes: CAD (882 bp), NAK (1489 bp), EF1-alpha (1111 bp), and LW rhodopsin (673 bp) and one ribosomal gene (28S; 1306 bp), following the DNA extraction and sequencing protocols outlined by Danforth et al. (Danforth 1999). All taxa and GenBank Accession numbers are listed in Table 2.2. PCR primers and conditions are listed in Table 2.3. The four protein coding genes were aligned using MAFFT (Katoh 2002) and then adjusted by eye in MacClade (Maddison 2005); all introns were removed. The ribosomal gene, 28S, was aligned via secondary structure according to the method described by Kjer (Kjer 1995); all unalignable regions were excluded. The secondary structure alignment was based on the 28S map of *Apis mellifera* (Gillespie 2006).

2.2.3 Data partitioning

We ran a preliminary Bayesian analysis to establish a partitioning regime: we concatenated the four protein-coding genes and partitioned each gene into first, second and third codon positions; the resulting dataset contained 12 partitions. We then ran a short analysis in MrBayes v.3.1.2 (Huelsenbeck 2001; Ronquist 2003) (5,000,000 generations using a GTR model) and examined the parameter files in Tracer (Rambaut 2007). After eliminating an appropriate burnin, we used Tracer to determine the substitution rate and nucleotide composition for each of the twelve partitions. We grouped similar partitions together and selected the following partitioning regime: Partition 1 included the first codon position of CAD and LW

Table 2.2 GenBank accession numbers for all sequences used in this study

Taxa	EF1a	Opsin	CAD	NAK	28S
Dasypoda argentata	AY585148	DQ116680	DQ067161	EF646418	AY654518
Macropis nuda	AY585155	DQ116686	DQ067171	HQ995917	HQ996008
Melitta leporina	AY585158	DQ116688	DQ067174	EF646394	AY654529
Promelitta alboclypeata	EF594330	EF594379	Missing	HQ995918	HQ996009
Meganomia binghami	DQ141114	DQ116689	DQ067169	EF646406	HQ996010
Ceratina calcarata	AY585108	AF344620	DQ067190	GU245213	HQ996011
Exoneura bicolor	GU245041	GU245337	Missing	GU245212	GU244896
Xylocopa pubescens	GU245052	GU245347	Missing	GU245225	GU244908
Xylocopa virginica	GU245047	GU245343	Missing	GU245220	GU244903
Anthophora montana	AY585107	AF344616	DQ067177	HQ995919	HQ996012
Pachymelus peringueyi	AY585114	DQ116678	DQ067182	GU245061	AY654544
Apis cerana	EU184774	EU184839	EU184808	EU184750	Missing
Apis dorsata	AY208277	AF091733	EU184807	EU184749	FJ042186
Apis florea	EU184773	EU184838	EU184806	EU184748	Missing
Apis mellifera	AF015267	AMU26026	DQ067178	XM_623142	AY703551
Bombus ardens	AF492964	AF493031	EU184803	EU184741	Missing
Bombus diversus	AF492961	AF493028	EU184804	EU184742	Missing
Ctenoplectra albolimbata	AY585118	DQ116677	EU122060	EF646391	HQ996013
Eufriesea pulchra	EU421377	EU184834	EU184802	EU184740	Missing
Euglossa imperialis	EU421408	AY267160	EU184800	EU184738	FJ042183
Exaerete frontalis	AY208286	AY267159	EU184801	EU184739	AF181602
Cephalotrigona capitata	EU184771	EU184836	EU184805	EU184745	FJ042015
Hypotrigona gribodoi	GU244957	GU245280	Missing	GU245121	GU244811
Meliponula brocandei	AY267145	AY267161	Missing	EU184746	FJ042177
Tetragonula carbonaria	GU244960	GU245282	Missing	GU245124	GU244814
Trigona fuscipennis	EU184770	EU184835	Missing	EU184744	EU049733
Paranomada velutina	AY585115	AF344627	DQ067188	GU245190	AY654545
Thyreus delumbatus	AY585119	DQ116679	DQ067184	GU245118	HQ996014
Melitturga clavicornis	AY585104	DQ116703	DQ067134	HQ995920	HQ996015
Rophites algirus	AY585144	DQ116675	DQ067159	HQ995921	HQ996016
Colletes inaequalis	AY363004	DQ115542	DQ067139	EF646387	HQ996017
Pararhophites orobinus	HQ995679	HQ995749	HQ995823	HQ995922	HQ996018
Pararhophites quadratus	EU851522	EU851627	HQ995824	GU245153	GU244841
Fidelia kobrowi	HQ995680	HQ995750	HQ995825	HQ995923	HQ996019
Fidelia paradoxa	HQ995681	HQ995751	HQ995826	HQ995924	HQ996020
Fidelia villosa	HQ995682	HQ995752	HQ995827	HQ995925	HQ996021
Fidelia braunsiana	HQ995683	HQ995753	HQ995828	HQ995926	HQ996022
Fidelia ulrikei	HQ995684	HQ995754	HQ995829	HQ995927	HQ996023
Fidelia friesei	HQ995685	HQ995755	HQ995830	HQ995928	HQ996024
Fidelia pallidula	HQ995686	HQ995756	HQ995831	HQ995929	HQ996025
Fideliopsis hessei	HQ995687	HQ995757	HQ995832	HQ995930	HQ996026
Fideliopsis major	DQ141113	EU851628	HQ995833	HQ995931	HQ996027
Fideliopsis ornata	HQ995688	HQ995758	HQ995834	HQ995932	HQ996028
Neofidelia longirostris	HQ995689	HQ995759	HQ995835	HQ995933	HQ996029
Neofidelia profuga	GU244990	HQ995760	HQ995836	GU245151	HQ996030

Table 2.2 (continued)

Taxa	EF1a	Opsin	CAD	NAK	28S
Lithurgus echinocacti	DQ141116	HQ995761	DQ067195	EF646390	AY654541
Lithurgus pullatus	HQ995690	HQ995762	HQ995838	HQ995935	HQ996032
Lithurgus rubricatus	HQ995691	HQ995763	HQ995839	HQ995936	HQ996033
Lithurgus scabrosus	HQ995692	HQ995764	HQ995840	HQ995937	HQ996034
Lithurgus tibialis	HQ995693	HQ995765	HQ995841	HQ995938	HQ996035
Microthurge sp	HQ995694	HQ995766	HQ995842	GU245161	GU244849
Trichothurgus herbsti	HQ995695	HQ995767	HQ995843	GU245160	GU244848
Aglaoapis tridentata	EU851524	EU851630	HQ995844	HQ995939	HQ996036
Dioxys moesta	HQ995696	HQ995768	HQ995845	HQ995940	HQ996037
Paradioxys pannonica	HQ995697	HQ995769	HQ995846	HQ995941	HQ996038
Afranthidium karooense	HQ995698	HQ995770	HQ995847	HQ995942	HQ996039
Anthidium illustre	HQ995699	HQ995771	HQ995848	HQ995943	HQ996040
Anthodioctes mapirense	HQ995700	HQ995772	HQ995849	HQ995944	HQ996041
Aspidosmia arnoldi	HQ995701	HQ995773	HQ995850	HQ995945	HQ996042
Aspidosmia volkmanni	HQ995702	HQ995774	HQ995851	HQ995946	HQ996043
Bathanthidium binghami	HQ995703	HQ995775	HQ995852	HQ995947	HQ996044
Benanthis madagascariensis	HQ995704	HQ995776	HQ995853	HQ995948	HQ996045
Dianthidium arizonicum	HQ995705	HQ995777	HQ995854	HQ995949	HQ996046
Duckeanthidium thielei	HQ995706	HQ995778	HQ995855	HQ995950	HQ996047
Eoanthidium turnericum	HQ995707	HQ995779	HQ995856	HQ995951	HQ996048
Epanthidium bicoloratum	HQ995708	HQ995780	HQ995857	HQ995952	HQ996049
Hypanthidioides marginata	HQ995709	HQ995781	HQ995858	HQ995953	HQ996050
Hypanthidium obscurius	HQ995710	HQ995782	HQ995859	HQ995954	HQ996051
Icteranthidium ferrugineum flavum	HQ995711	HQ995783	HQ995860	HQ995955	HQ996052
Notanthidium steloides	HQ995712	HQ995784	HQ995861	HQ995956	HQ996053
Pachyanthidium bengualense	HQ995713	HQ995785	HQ995862	HQ995957	HQ996054
Paranthidium jugatorium	GU244994	HQ995786	HQ995863	GU245156	GU244844
Pseudoanthidium scapulare	HQ995714	HQ995787	HQ995864	HQ995958	HQ996055
Rhodanthidium septemdentatum	HQ995715	HQ995788	HQ995865	HQ995959	HQ996056
Serapista rufipes	HQ995716	HQ995789	HQ995866	HQ995960	HQ996057
Stelis paiute	HQ995717	HQ995790	HQ995867	HQ995961	HQ996058
Trachusa pubescens	HQ995718	HQ995791	HQ995868	HQ995962	HQ996059
Trachusa larreae	HQ995719	HQ995792	HQ995869	GU245154	GU244842
Coelioxys afra	EU851528	EU851634	HQ995870	HQ995963	HQ996060
Megachile (Aethomegachile) sp	HQ995720	HQ995793	HQ995871	HQ995964	HQ996061
Megachile fimbriata	HQ995721	HQ995794	HQ995872	HQ995965	HQ996062
Megachile parallela	HQ995722	HQ995795	HQ995873	HQ995966	HQ996063
Megachile (Austrochile) sp	HQ995723	HQ995796	HQ995874	HQ995967	HQ996064
Megachile sculpturalis	HQ995724	HQ995797	HQ995875	HQ995968	HQ996065
Megachile parietina	EU851530	EU851636	HQ995876	HQ995969	HQ996066
Megachile aethiops	HQ995725	HQ995798	HQ995877	HQ995970	HQ996067
Megachile (Chelostomoda) sp	HQ995726	HQ995799	Missing	HQ995971	HQ996068
Megachile angelarum	HQ995727	HQ995800	HQ995878	GU245163	GU244851
Megachile spinotulata	HQ995728	HQ995801	HQ995879	HQ995972	HQ996069

Table 2.2 (continued)

Taxa	EF1a	Opsin	CAD	NAK	28S
Megachile (Chrysosarus) sp	HQ995729	HQ995802	HQ995880	HQ995973	HQ996070
Megachile albisecta	EU851529	EU851635	HQ995881	HQ995974	HQ996071
Megachile zapoteca	HQ995730	HQ995803	HQ995882	HQ995975	HQ996072
Megachile mandibularis	HQ995731	HQ995804	HQ995883	HQ995976	HQ996073
Megachile patellimana	HQ995732	HQ995805	HQ995884	HQ995977	HQ996074
Megachile pilidens	EU851531	EU851637	HQ995885	HQ995978	HQ996075
Megachile bombiformis	HQ995733	HQ995806	HQ995886	HQ995979	HQ996076
Megachile (Hackeriapis) sp	HQ995734	HQ995807	HQ995887	HQ995980	HQ996077
Megachile (Largella) sp	HQ995735	HQ995808	HQ995888	HQ995981	HQ996078
Megachile texana	HQ995736	HQ995809	HQ995889	HQ995982	HQ996079
Megachile maxillosa	HQ995737	HQ995810	HQ995890	HQ995983	HQ996080
Megachile melanopyga	HQ995738	HQ995811	HQ995891	HQ995984	HQ996081
Megachile nevadensis	HQ995739	HQ995812	HQ995892	HQ995985	HQ996082
Megachile fabricator	HQ995740	HQ995813	HQ995893	HQ995986	HQ996083
Megachile sidalceae	HQ995741	HQ995814	HQ995894	HQ995987	HQ996084
Megachile ericetorum	HQ995742	HQ995815	HQ995895	GU245165	GU244853
Megachile microsoma	HQ995743	HQ995816	HQ995896	HQ995988	HQ996085
Megachile (Rhodomegachile) sp	HQ995744	HQ995817	HQ995897	HQ995989	HQ996086
Megachile pugnata	AY585147	HQ995818	DQ067196	HQ995990	HQ996087
Megachile remeata	HQ995745	HQ995819	HQ995898	HQ995991	HQ996088
Megachile maritima	HQ995746	HQ995820	HQ995899	HQ995992	HQ996089
Noteriades sp	EU851589	EU851695	HQ995900	HQ995993	HQ996090
Radoszkowskiana rufiventris	HQ995747	HQ995821	HQ995901	HQ995994	HQ996091
Afroheriades primus	EU851532	EU851638	HQ995902	HQ995995	HQ996092
Ashmeadiella aridula	EU851535	EU851641	HQ995903	GU245171	GU244858
Atoposmia mirifica	EU851541	EU851647	HQ995904	HQ995996	HQ996093
Chelostoma florisomne	EU851546	EU851652	HQ995905	HQ995997	HQ996094
Haetosmia brachyura	HQ995748	HQ995822	HQ995906	HQ995998	HQ996095
Heriades crucifer	EU851555	EU851661	DQ067194	GU245168	GU244855
Hofferia schmiedeknechti	EU851556	EU851662	HQ995907	HQ995999	HQ996096
Hoplitis adunca	EU851572	EU851678	HQ995908	HQ996000	HQ996097
Ochreriades fasciatus	EU851590	EU851696	HQ995909	HQ996001	HQ996098
Osmia lignaria	EU851610	EU851715	HQ995910	GU245169	GU244856
Othinosmia globicola	EU851616	EU851721	HQ995911	HQ996002	HQ996099
Othinosmia securicornis	EU851617	EU851722	HQ995912	HQ996003	HQ996100
Protosmia humeralis	EU851621	EU851726	HQ995913	HQ996004	HQ996101
Pseudoheriades moricei	EU851622	EU851727	HQ995914	HQ996005	HQ996102
Stenoheriades asiaticus	EU851623	EU851728	HQ995915	HQ996006	HQ996103
Wainia eremoplana	EU851626	EU851731	HQ995916	HQ996007	HQ996104

Table 2.3 PCR primer sequences and conditions for the five nuclear genes sequenced in this study

Primer	Sequence		
28S			
A (Ward 2003)	5' CCC CCT GAA TTT AAG CAT AT 3'		
Mar (Mardulyn 1999)	5' TAG TTC ACC ATC TTT CGG GTC CC 3'		
Bel (Belshaw 1997)	5' AGA GAG AGT TCA AGA GTA CGT G 3'		
D4 (Danforth 2006)	5' GTT ACA CAC TCC TTA GCG GA 3'		
PCR conditions[a]: A/Mar: 1m@94°C /1m@58°C /1m30s@72°C, Bel/D4, 1m@94°C /1m@58°C /1m30s@72°C.			
LW Rhodopsin			
Opsin fora	5' AAT TGY TAY TWY GAG ACA TGG GT 3'		
Opsin rev3y	5' GCC AAT TTA CAC TCG GCR CT 3'		
Opsinfor5a (Praz,2008)	5' GCG TGY GGC ACM GAY TAC TTC 3'		
Opsinrev5a (Praz 2008)	5' RGC GCA YGC CAR YGA YGG 3'		
PCR conditions: Opsin fora/Opsin rev3y: 45s@94°C /45s@54°C /45s@72°C, Opsinfor5a/Opsinrev5a: 45s@94°C /45s@58°C /45s@72°C.			
Ef1-alpha (F2 copy)			
Haf2for1 (Danforth 1999)	5' GGG YAA AGG WTC CTT CAA RTA TGC 3'		
F2revmeg (Praz 2008)	5' AAT CAG CAG CAC CCT TGG GTG G 3'		
For4y	5' AGC TCT GCA AGA GGC TGT YC 3'		
Cho10(mod)	5' ACR GCV ACK GTY TGH CKC ATG TC 3'		
PCR conditions: Haf2for1/F2revmeg: 45s@94°C /45s@58°C /1m@72°C, For4y/Cho10(mod) 45s@94°C /45s@58°C /45s@72°C.			
NAK			
Nakfor1 (Cardinal 2010)	5' GGY GGT TTC GCS WTG YTG YTG TGG ATC GG 3'		
Nakrev1a (Cardinal 2010)	5' CCG ATN ARR AAG ATR TGM GCG TCN AGC CAA TG 3'		
Nakfor2 (Cardinal 2010)	5' GCS TTC TTC TCB ACS AAC GCC GTY GAR GG 3'		
Nakrev2 (Cardinal 2010)	5' ACC TTG ATR CCG GCY GAW CGG CAC TTG GC 3'		
PCR conditions: Nakfor1/Nakrev1a: 45s@94°C /45s@54°C /45s@72°C, Nakfor2/Nakrev2 45s@94°C /45s@58°C /1m15s@72°C.			
CAD			
Cadfor4 (Danforth 2006)	5' TGG AAR GAR GTB GAR TAC GAR GTG GTY CG 3'		
Cadrev1meg (Praz 2008)	5' GCC ATC ACT TCY CCT AYG CTC TTC AT 3'		
Cadmegfor1	5' GAR CCY AGY CTC GAT TAY TG 3'		
Cadrev4a	5' GGC CAY TGN GCN GCC ACY GTG TCT ATY TGY TTN ACC 3'		
PCR conditions: Cadfor4/Cadrev1meg 30s@94°C /30s@55°C /30s@72°C, Cadmegfor1/Cadrev4a 30s@94°C /30s@56°C /30s@72°C.			

[a] All PCR reactions included an initial step at 94°C for 5 minutes, then 35 cycles under the indicated conditions, and finally a step at 72°C for 7 minutes.

rhodopsin (518 bp); partition 2 included the first positions of EF1-alpha and NAK, and the second codon positions of CAD, EF1-alpha, NAK and LW rhodopsin (2250 bp); partition 3 included the third codon positions of CAD and NAK (791 bp); and partition 4 included the third position of EF1-alpha and LW rhodopsin (596 bp). The ribosomal gene, 28S, was divided into two partitions, a stem partition, consisting of nucleotides hydrogen-bound in paired strands (767 bp) and a loop partition, consisting of unpaired nucleotides (539 bp). The resulting dataset therefore contained six partitions (5461 total base pairs).

2.2.4 Model testing

Models of nucleotide substitution were selected based on the Akaike Information Criterion (AIC) as determined by MrModelTest 2.3 (Akaike 1974; Nylander 2008). MrModelTest calculates AIC values for each of 24 models of nucleotide substitution; the model associated with the lowest AIC score is selected as the best-fit model. Independent model tests were performed on each data partition. For each partition, the best-fit model was a general time reversible model with a gamma correction for among site rate variation and an allowance for invariant sites (GTR+I+Γ).

2.2.5 Phylogenetic analyses

Phylogenetic analyses were performed using both Bayesian and maximum likelihood methods. Bayesian analyses were performed using MrBayes v.3.1.2 (Huelsenbeck 2001; Ronquist 2003). A GTR+I+Γ model was used for all partitions except for the stem partition of 28S, which was analyzed using the doublet model. All parameters were unlinked between partitions. Preliminary analyses resulted in poor mixing of chains, so the default temperature

setting of 0.2 was adjusted to 0.03, which improved mixing and increased the chain swap acceptance rate to within the range recommended by the MrBayes users' manual. We ran six independent analyses, for a total of 180,000,000 generations. Sampling was performed every 2000 generations. An appropriate burn-in was discarded from each analysis using Tracer (Rambaut 2007), leaving 96,956,000 post-burn-in generations; these were further sampled using LogCombiner v.1.6.1 (Drummond 2007) to ensure independent sampling of trees. The final combined posterior distribution of 25,239 trees was used to build a maximum clade credibility tree using TreeAnnotator v. 1.6.1 (Drummond 2007) (Fig. 3.1).

Maximum likelihood analyses were performed using RAxML v. 7.0.4 (sequential version raxmlHPC; (Stamatakis 2005)). We used the rapid bootstrapping algorithm with a GTR+CAT approximation to perform 1000 bootstrap replicates. The maximum likelihood bootstrap tree is shown in Fig. 3.1.

2.2.6 Divergence dating analysis using BEAST

We used BEAST v.1.6.1 to perform a Bayesian divergence dating analysis. (Drummond 2007). Each partition was analyzed using a GTR+I+Γ model; substitution models were unlinked across partitions. We used an uncorrelated lognormal relaxed-clock model with a Yule tree prior. Trees were sampled every 2000 generations. We randomly chose a starting tree from the posterior distribution of trees from the MrBayes analysis; we used TreeEdit v.1.0 (Rambaut 2001) to scale the root height to 130 my in order to conform to the constraints imposed by prior distributions on divergence times. Ten independent analyses were run for a total of 300,000,000 generations. An appropriate burn-in was discarded from each analysis using Tracer (Rambaut 2007), leaving 217,068,000 total post-burnin generations. In order to ensure independent

sampling of trees, we sampled every third tree from the post-burn-in posterior distribution of trees using LogCombiner v.1.6.1 (Drummond 2007) and then used TreeAnnotator v.1.6.1 (Drummond 2007) to build a maximum clade credibility tree from this posterior distribution of trees (Figure 2.1, 2.2A).

2.2.7 Calibration of internal nodes and root node in BEAST

We used fossils to time-calibrate seven internal nodes on our tree. Five of these calibration points were assigned a lognormal prior distribution, while two were assigned a normal prior distribution. Here we present the details of these calibration points, as well as a discussion of fossils that were unusable for the purposes of calibrating our phylogeny.

For each fossil used to time-calibrate our phylogeny, we outline our reasoning and list the parameters used in BEAST to set the prior distribution and the 95% upper, median and lower bounds on *a priori* ages. All zero-offset values correspond to the most recent boundary of the geological epoch to which the fossil has been assigned. The placement of each fossil on our phylogeny is shown in Fig. 2.1.

1. *Apis lithohermaea*

   This fossil is recorded from the middle Miocene deposits of Iki Island, Japan and has been assigned to the *Apis dorsata* species-group based on its enlarged body size, elongate metabasitarsus, and infuscated wing membranes (Engel 2006). We therefore consider this fossil as a member of the stem group for *Apis dorsata* and use it to set a minimum age on the node uniting *A. dorsata* and its sister group (*A. cerana* + *A. mellifera*). We calibrated this node using a lognormal prior distribution; the

corresponding parameters used in BEAST were a zero-offset of 11.2 my, a log(mean) of 0.11, and a standard deviation of 1.0. The 95% upper bound, median and lower bound on our *a priori* ages were 17.0, 12.3, and 11.4 my.

2. *Palaeomacropis eocenicus*

This specimen was found in early Eocene (Sparnacian) amber in Oise, France. A cladistic analysis (Michez 2007) based on seventeen morphological characters places it as the sister taxon to the melittid genus *Macropis*. The absence of other macropidine genera in the cladistic analysis of Michez et al., namely *Promelitta*, makes it unclear whether *Palaeomacropis* belongs to the crown or stem group for Macropidini. We prefer the conservative option and consider *Paleomacropis* as a member of the stem group. We use it to place a minimum age on the node uniting Macropidini (represented by *Macropis nuda* and *Promelitta alboclypeata* in our phylogeny) to its sister taxon, *Melitta leporina*. We calibrated this node using a lognormal prior distribution; the corresponding parameters used in BEAST were a zero-offset of 49.0 my, a log(mean) of 1.6, and a standard deviation of 1.0. The 95% upper bound, median and lower bound on our *a priori* ages were 74.7, 54.0, and 50.0 my.

3. *Paleohabropoda oudardi*

Paleohabropoda oudardi is a compression fossil recorded from the Paleocene of Menat, Puy-de-Dome, France (Michez 2009). While the fossil is assigned to the apid tribe Anthophorini, two conflicting analyses present different phylogenetic positions for *Paleohabropoda oudardi*. A cladistic analysis based on seventeen morphological characters (Michez 2009) places the fossil as sister to the extant Anthophorini; in our

phylogeny, this corresponds to a calibration point at the node uniting Anthophorini (represented in our phylogeny by *Pachymelus peringueyi* and *Anthophora montana*) with the rest of the apids. A separate analysis based on wing morphometry (Michez 2009), however, places this fossil within the extant Anthophorini, more closely related to *Pachymelus* than to *Anthophora*; in our phylogeny, this corresponds to a calibration point at the node uniting *Pachymelus* to its sister taxon, *Anthophora*. In order to accommodate this uncertainty in phylogenetic position, we used the fossil to place a mean age on the node uniting *Pachymelus* and *Anthophora*. We used a normal prior distribution at this node, thereby allowing the node to be either older or younger than the age of the fossil. The normal distribution was assigned a mean of 60 my and a standard deviation of 6.0. The 95% upper bound, median and lower bound on our *a priori* ages were 69.9, 60.0, and 50.1 my.

4. Kelneriapis eocenica

This specimen is from middle Eocene Baltic amber. Based on morphological characters, Engel (Engel 2001) assigns this fossil to the extant tribe Meliponini and indicates that *Kelneriapis* is likely sister to the extant genus *Hypotrigona*, due to the rounded posterior apical corner of the metatibia in both genera. We therefore consider this fossil as a member of the stem group for the genus *Hypotrigona*. In our phylogeny, however, the relationship between *Hypotrigona* and its sister taxon, *Tetragonula*, is not strongly supported in either Bayesian or maximum likelihood analyses (Bayesian analysis shown in Figure 2.1; maximum likelihood analysis not shown). A recent molecular phylogeny (Cardinal 2010) also recovered low branch support for the sister group

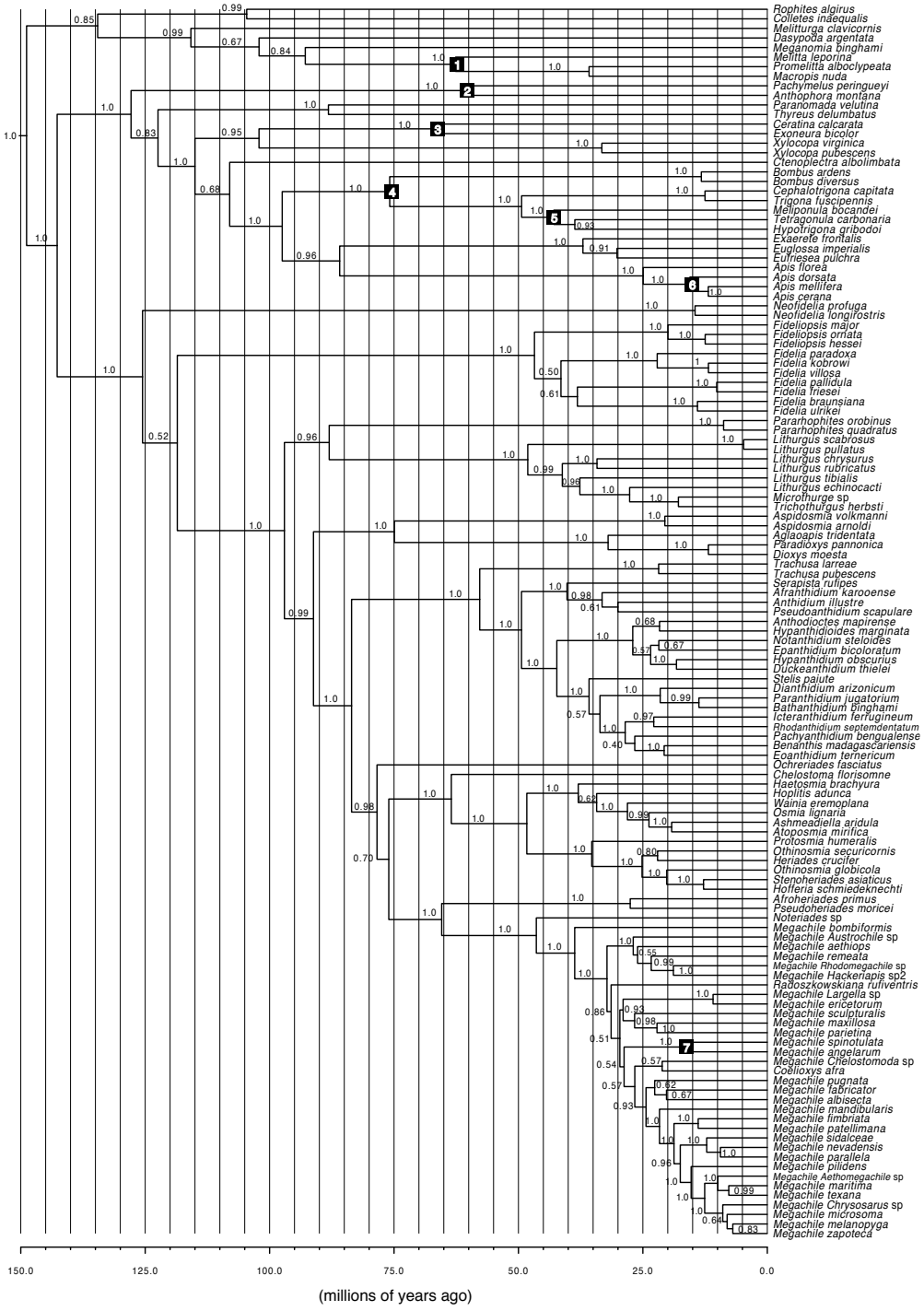


Figure 2.1 Maximum clade credibility tree from BEAST analysis. Fossil-calibrated phylogeny based on ~217,000,000 post-burnin generations in BEAST v.1.6.1. Numbers shown at nodes are Bayesian posterior probabilities. Numbered black squares correspond to fossil calibration points. 1. *Palaemacropis eocenicus*; 2. *Paleohabropoda oudardi*; 3. *Boreallodapae* sp.; 4. *Cretotrigona prisca*; 5. *Kelneriapis eocenica*; 6. *Apis lithohermaea*; 7. *Megachile glaesaria*.

relationship between *Hypotrigona* and *Tetragonula* in both Bayesian and maximum likelihood analyses. The sister taxon to *Hypotrigona+Tetragonula* is the genus *Meliponula*; these three taxa for a well-supported clade in both Bayesian and maximum likelihood analyses (Bayesian analysis shown in Figure 2.1; maximum likelihood analysis not shown). It remains unclear, however, what the relationship is between *Hypotrigona*, *Tetragonula* and *Meliponula*; for this reason, we use this fossil to place a minimum age on the node uniting *Hypotrigona*, *Tetragonula*, and *Meliponula*. We calibrated this node using a lognormal prior distribution; the corresponding parameters used in BEAST were a zero-offset of 41 my, a log(mean) of 1.4, and a standard deviation of 1.0. The 95% upper bound, median and lower bound on our *a priori* ages were 66.7, 46.0, and 42.0 my.

5. *Boreallodape* sp.

At least three species of the Baltic amber genus *Boreallodape* have been discovered: *B. baltica*, *B. mollyae*, and *B. striebichi* (Engel 2001). A fourth species may exist but key attributes of the specimen are not visible and the species remains undetermined. This genus has been assigned to the apid tribe Boreallodapini. Engel suggests that this tribe is closely related to Ceratinini and Allodapini; in a cladistic analysis based on fourteen morphological characters, Engel (Engel 2001) demonstrates that Boreallodapini is more closely related to Allodapini than to Ceratinini. We therefore use this fossil to place a minimum age on the node uniting Allodapini (represented in our phylogeny by *Exoneura bicolor*) and Ceratinini (represented by *Ceratina calcarata*). Due to the presence of at least three unique species of *Boreallodape*, we consider it likely that this genus arose 5-

10 million years earlier than the age of the fossil. We calibrated this node using a lognormal prior distribution; the corresponding parameters used in BEAST are a zero-offset of 41 my, a log(mean) of 2.0, and a standard deviation of 1.0. The 95% upper bound, median and lower bound on our *a priori* ages were 79.3, 48.4, and 42.4 my.

6. Megachile glaesaria

This specimen was recovered from Miocene Dominican amber. Engel (Engel 1999) proposes that *M. glaesaria* is most similar to the subgenus *Chelostomoides* and probably closely related to the extant species *Megachile manni*. Our phylogeny includes two members of the subgenus *Chelostomoides*: *Megachile spinotulata* and *Megachile angelarum*. The phylogenetic position of *Megachile manni* within the subgenus *Chelostomoides* is unknown, which makes placement of this fossil difficult. *M. glaesaria* is placed in its own subgenus, *Chalicodomopsis*; therefore we did not place it as a crown member of *Chelostomoides*. Placing it as a stem group fossil for *Chelostomoides* also proved difficult, however, as the position of this subgenus within *Megachile* is uncertain. Given the close morphological similarity of *M. glaesaria* with extant members of the subgenus *Chelostomoides*, and given that this fossil and the extant subgenus *Chelostomoides* are the only new world representatives of the *Chalicodoma*-group of subgenera, we used this fossil to place a mean age on the node uniting both species of *Chelostomoides*. We used a normal prior distribution at this node, thereby allowing the node to be either older or younger than the age of the fossil. The normal distribution was assigned a mean of 17.5 my and a standard deviation of 1.6. The 95% upper bound, median and lower bound on our *a priori* ages were 20.1, 17.5, and 14.9 my.

7. Cretotrigona prisca

Cretotrigona prisca was recovered from late Cretaceous amber (Maastrichtian) from New Jersey. It has alternately been placed as the sister taxon to *Trigona* (Michener 1988) and to *Dactylurina* (Engel 2000). While we agree that this fossil is correctly assigned to the apid tribe Meliponini, we are not confident that it is a member of the crown group for Meliponini. For this reason, we consider this fossil as a member of the stem group for Meliponini and use it to place a minimum age on the node uniting Meliponini (represented in our phylogeny by *Cephalotrigona capitata*, *Hypotrigona gribodoi*,*Meliponula bocandei*, *Tetragonula carbonaria*, and *Trigona fuscipennis*) with its sister group (*Bombus ardens*+*Bombus diversus*). We calibrated this node using a lognormal prior distribution; the corresponding parameters used in BEAST are a zero-offset of 65 my, a log(mean) of 2.3, and a standard deviation of 1.0. The 95% upper bound, median and lower bound on our *a priori* ages were 116.7, 75.0, and 66.9 my.

Our dating analysis is in agreement with several fossil-calibrated phylogenies of different groups of bees (Cardinal 2010; Rasmussen 2010) and consistent with fossil data that we did not use to calibrate our phylogeny. We obtain an age for the corbiculate bees of around 95 my, which corresponds well to Turonian (89-93 mya) fossils of resin-producing Clusiaceae flowers that were likely visited by corbiculate bees (Crepet 1998). The absence from Baltic amber of both the crown *Heriades*-group of genera in the tribe Osmiini and the *Chalicodoma*-group of subgenera in the genus *Megachile* may appear surprising, given that members of both groups collect tree resin for nest construction. However, the inferred ages of both groups (35 my and 32 my, respectively) are in keeping with the complete absence of these bees from Baltic amber (age

of Baltic amber ~ 40 my). While megachilids are well-represented in Baltic amber (Engel 2001), these species have been attributed to extinct tribes with little affinity to extant lineages.

Unused fossils

There are a number of fossils that have been assigned to the family Megachilidae whose phylogenetic relationship to extant megachilid taxa is largely unclear. The following fossils may only be interpreted as stem group members for clades consisting of multiple tribes; the uncertainty regarding the phylogenetic position of each of these fossils, as well as the fact that they must be placed deeply in the phylogeny, render them unusable for the purposes of calibrating our phylogeny.

Probombus hirsutus is a compression fossil recorded from a volcanic paleolake deposit in Menat, Puy-de-Dôme, France (late Paleocene, ~ 60 mya). Initially described as a bumblebee, this genus was later transferred to the family Megachilidae based on "the presence of a metasomal sternal scopa and the absence of a clearly differentiated scopa on metathoracic leg" (Nel 2003). Within Megachilidae, the presence of two submarginal wing cells and several other morphological characters ally *Probombus* more closely with the subfamily Megachilinae than the subfamily Fideliinae. Its position within Megachilinae, however, is unclear. Nel and Petrulevicius (2003) exclude affinities with Lithurgini, Dioxyini, and Anthidiini, ultimately concluding that *Probombus* is probably closely related to either the tribe Osmiini or the tribe Megachilini. Their conclusion, however, is based on the elimination of relationships between *Probombus* and other tribes, rather than on morphological synapomorphies that unite *Probombus* to either Osmiini or Megachilini. Furthermore, characters that could ally *Probombus* to either Osmiini or Megachilini are not visible in the fossil. We therefore consider this fossil as member

of the subfamily Megachilinae, *incertae sedis*; this fossil can only be used to calibrate the node uniting the subfamily Megachilinae with its sister taxa (*Pararhophites*, Lithurgini).

Engel (Engel 2001) refers to the genus *Glyptapis* as "an enigmatic lineage of megachilines", initially placing the four species of *Glyptapis* (Baltic amber, ~ 40 mya) in the subtribe Glyptapina within the megachilid tribe Osmiini. The subtribe Glyptapina later became the tribe Glyptapini (Engel 2006; Engel 2005). The phylogenetic position of *Glyptapis* within Megachilidae is uncertain: some characters suggest a close relationship with the tribe Anthidiini, while others suggest a closer relationship with the tribe Osmiini. The only interpretation possible for this fossil is as a member of the stem group for Anthidiini, Osmiini, and Megachilini; the genus *Glyptapis* may therefore only be used to calibrate the node uniting Anthidiini, Osmiini, and Megachilini to its sister group (Dioxyini+*Aspidomia*).

Protolithurgus ditomeus is recorded from Baltic amber. Engel (Engel 2001) states that "*Protolithurgus* seems to possess an enigmatic combination of characters unique among megachilids" and that "the genus does share with other Lithurginae the distinctive flattened, first metasomal tergum with a rounded apical margin, a feature found only in lithurgines". While this feature may indeed reveal a close relationship between *Protolithurgus* and the tribe Lithurgini, it remains unclear whether this genus is a member of the crown Lithurgini or is better placed as a stem group member. Nel and Petrulevicius (Nel 2003) note the absence of coarse tubercles on the outer tibial surface of *Protolithurgus*, the presence of which is a synapomorphy for the extant Lithurgini (Michener 1983). For this reason, *Protolithurgus* may only be interpreted as a member of the stem group for Lithurgini; the most appropriate placement for this fossil is therefore at the node uniting Lithurgini with its sister group. It must be noted, however, that in our phylogeny,

the relationship between Lithurgini and its sister taxon, the genus *Pararhophites*, is poorly supported in maximum likelihood analyses (Figure 2.2). Therefore the only valid placement for this fossil is at the node uniting the subfamily Megachilinae.

The Baltic amber fossil genera *Ctenoplectrella* and *Glaesosmia* were initially placed in the subtribe Ctenoplectrellina, within the megachilid tribe Osmiini (Engel 2001). The subtribe Ctenoplectrellina later became the tribe Ctenoplectrellini (Engel 2005; Engel 200). Wedmann et al. (Wedmann 2009) added *Friccomelissa schopowi*, an Eocene fossil from the Messel Pit Fossil Site (Fossillagerstätte Grube Messel, Germany), to the tribe Ctenoplectrellini. Wedmann et al. (Wedmann 2009) state that a number of plesiomorphic traits relative to Osmiini and Megachilini indicate that Ctenoplectrellini may belong to the stem group of either Osmiini or Osmiini+Megachilini. The confluent position of wing veins 2rs-m and 2 m-cu in Ctenoplectrellini suggest a phylogenetic position between Anthidiini and Osmiini+Megachilini. We therefore interpret the tribe Ctenoplectrellini as a member of the stem group for Osmiini+Megachilini; this fossil may only be used to calibrate the node uniting Anthidiini, Osmiini, and Megachilini.

We have personally examined the megachilid fossils from the Florisssant, Colorado fossil beds deposited in the Museum of Comparative Zoology at Harvard University. While a number of these taxa have been assigned to extant genera, we agree with Engel (Engel 1999) that these assignments are tenuous and that these fossils should be relegated to "Apoidea incertae sedis".

Lastly, several authors (reviewed in (Wedmann 2009)) have reported trace fossils (Eocene to Miocene) supposedly left by leafcutting bees (genus *Megachile*). We did not include these fossils for two reasons. Firstly, attribution to leafcutting bees remains hypothetical

(Labandeira 2002). Secondly, even if these leaf cuts had been left by members of the genus *Megachile*, they would be very difficult to place on the phylogeny, given that many distantly related *Megachile* cut leaves. According to our dating analysis, the genus *Megachile* originated approximately 40 mya, strongly suggesting that at least the earliest of these trace fossils may not have been left by bees of the genus *Megachile*.

Bees are thought to be the sister group to the apoid wasps (Melo 1999). Apoids first appear in the fossil record during the Cretaceous (Engel 2001); Engel (Engel 2001) proposes that bees originated sometime after this and gives an uppermost boundary for their age of 125 my. There is no direct fossil evidence to suggest that bees arose at this time, however, and we believe that the age of the bees may be older than previously estimated. The late Cretaceous (~65 mya) origin of *Cretotrigona prisca*, a highly derived eusocial meliponine bee, indicates that a significant amount of bee diversification had already taken place by the late Cretaceous. Furthermore, it has been widely speculated that the origin of bees happened after the origin of the angiosperms (Michener 1979; Grimaldi 1999; Engel 2000; Engel 2001); recent molecular evidence (Smith 2010) places the origin of the angiosperms in the late Triassic, 30-80 my earlier than previously estimated. We find both of these arguments compelling reasons to explore the possibility that bees arose earlier than current estimates suggest.

We assign a uniform prior distribution to the root node. While other studies have favoured more informative root priors, such as the lognormal (Ward 2010) or the normal (Cardinal 2010), we feel that the only way to obtain an objective estimate for the origin of Megachilidae is to impose a relatively uninformative prior on the root. The lower bound of the root prior is assigned a value of 100 mya and is based on an extremely conservative estimate for

the origin of bees based on the fossil record (Engel 2001). The upper bound is assigned a value of 217 mya and is based on a recent molecular estimate for the age of crown angiosperms (Smith 2010). Our use of a fairly broad uniform prior causes the 95% HPD for divergence date estimates to be larger than those associated with other types of prior distributions. Our dating analyses, however, were run to stationarity and age estimates from multiple, independent runs converged to a single, stable value; we accept the broad 95% HPD as a necessary consequence of using a uniform prior distribution.

2.2.8 Biogeographic reconstruction

Biogeographic reconstructions were performed using both S-Diva (Yu 2010) and Lagrange (Ree 2008). Most of our terminal taxa represent genera; for this reason, the most plausible ancestral range for each terminal was coded based on the current distribution of the species represented by the terminal (based on (Michener 2007)). In both S-Diva and Lagrange analyses, the following areas were considered: Afrotropic, Palaearctic, Southeast Asia, Australia, Nearctic and Neotropic; in case of ambiguity, polymorphism was allowed. Given our near-complete sampling of the basal-most branches, such polymorphisms only concerned the higher megachilid tribes Anthidiini, Osmiini and Megachilini and did not affect inference at the base of the family.

For biogeography inference using S-Diva (Yu 2010), we sampled 1010 trees from the posterior distribution of post-burnin trees from the BEAST analysis. To further distinguish between alternative biogeographical scenarios in a maximum likelihood framework, we used the software Lagrange (Ree 2008). We used the consensus chronogram from the BEAST analysis and the same ancestral range coding as in the S-Diva analysis. The maximum number of areas

occupied by a single taxon was set to two. Analyses where Africa and South America were allowed to be adjacent resulted in ancestral range reconstructions that strongly favoured vicariance between South America and Africa (relative probability 0.87, likelihood values -251.2 to -252.7) over alternative scenarios (relative probability 0.08, likelihood -253.5 to -254.6). A difference of two log-units can be taken as evidence for a significant difference (25).

2.2.9 Ancestral state reconstruction

We used BayesTraits (Pagel 1997) to reconstruct the ancestral nesting biology of Megachilidae. Cell lining behaviour was coded for each terminal (including the outgroup) as: totally unlined (0), in *Dasypoda*, fideliine and lithurgine bees; lined with glandular secretion (1), in all members of the families Andrenidae, Halictidae and Colletidae, as well as in several lineages of Apidae and in the genus *Melitta*; lined with foreign material (2), in the oil-collecting bees, some Apidae and all higher Megachilidae; or as cleptoparasitic (3). We coded the corbiculate apidae, as well as all lineages for which no information was available, as (012). *Meganomia* was coded (02), as Rozen (Rozen 1977) states that cells of *Meganomia* contained "no built-in lining, i.e., consisting of soil mixed with secretions", but have a waterproof lining, possibly consisting of nectar. Information on nesting biology was found in Michener (Michener 2007) and references therein.

In BayesTraits (Pagel 1997; Pagel 1999), we ran both maximum likelihood and Bayesian ancestral state reconstructions using the same 1010 trees sampled in the biogeography analyses. In maximum likelihood analyses, we restricted all rates to be equal (command "restrictall"), except for the reversions from cleptoparasitism to other states, which were constrained to zero. We successively constrained nodes of interest to different states ("fossil" command) to test for

differences in log-likelihoods. In the Bayesian analyses, we applied a reverse-jump hyperprior (command "rjhp exp 0 10") and a "ratedev" value of 5 to obtain acceptance rates between 20 and 40%, as recommended in the BayesTraits manual. The same 1010 trees used in the biogeography analyses were used as input trees. Each Bayesian chain was run for 5 million generations (burnin 50000). We successively constrained the ancestor of Megachilidae to states 0, 1 and 2 using the "fossil" command. We repeated each analysis 5 times and averaged the harmonic means to calculate Bayes Factors, which equal twice the difference in harmonic mean. Values above 6 are commonly taken as strong evidence for significance (Kass 1995).

2.2.10 Correlated trait evolution

We used BayesTraits (Pagel 1997) to test for correlated evolution between the total geographic area occupied by a taxonomic group and diversification rate. We calculated diversification rate using the function *lambda.stem.ms01* in the Laser package in R (Rabosky 2006; R Development Core Team 2010) and the total geographic range for each terminal taxon using the area calculator provided by the website "Free Map Tools" (Free Map Tools 2010).

In order to explore the relationship between the total geographic area occupied by a taxonomic group and diversification rate, we pruned our original dataset of 98 ingroup terminals to a smaller dataset of 69 clearly defined monophyletic groups and calculated diversification rate and total geographic range for each terminal; pruning the dataset was necessary to determine clade size and geographic range for each terminal. Data on the distribution of each species was obtained from revisionary works on Megachilidae cited in Michener (Michener 2007) and from the following websites: "Discover Life" (Ascher 2010) *and the* "Palaearctic Osmiine Bees" website (Müller 2011).

To test for correlation between total area occupied and diversification rate, we performed two sets of Bayesian analyses using the software BayesTraits. In the first set of analyses, total geographic range and diversification rate evolved independently of one another; in the second, the traits were allowed to evolve in a correlated fashion. The same 1010 trees used in the biogeography and ancestral state reconstruction analyses were used as input trees. The "ratedev" value was adjusted to 0.2 to obtain acceptance rates between 20 and 40%. Each set of analyses consisted of five independent Bayesian chains, each run for 5,050,000 generations (burnin = 50000). We took the harmonic means of the likelihood scores from each set of analyses to calculate Bayes Factors. The value of lambda (where lambda represents the degree to which phylogeny predicts patterns of covariance) was estimated from the data. Analyses where the two variables were allowed to co-evolve exhibited significantly better likelihood scores (lambda=0.40; harmonic mean of LH = -207.7) than those analyses in which the variables evolved independently (lambda=0.40, harmonic mean of LH = -220.6; BF = 25.8).

2.2.11 Diversification rate analysis

We used MEDUSA (Modeling Evolutionary Diversification Using Stepwise Akaike Information Criterion; (Alfaro 2009)) to test for changes in the tempo of diversification among the branches of the megachilid phylogeny. We used the final consensus tree from our BEAST analysis and removed the outgroup using Mesquite (Maddison 2010). We collapsed several taxa into single terminals and calculated the total number of species represented by each terminal; terminals were collapsed in order to more easily quantify the number of species represented. The resulting phylogeny contained 82 taxa. We chose to use corrected AIC scores (AICc) instead of AIC scores in order to account for the small sample size of our phylogeny. We used MEDUSA

to fit a series of 20 models and used a strict cut-off value of 10 as our ΔAICc threshold. A model with two rate shifts (three sets of birth and death rates) was chosen as the best-fit model.

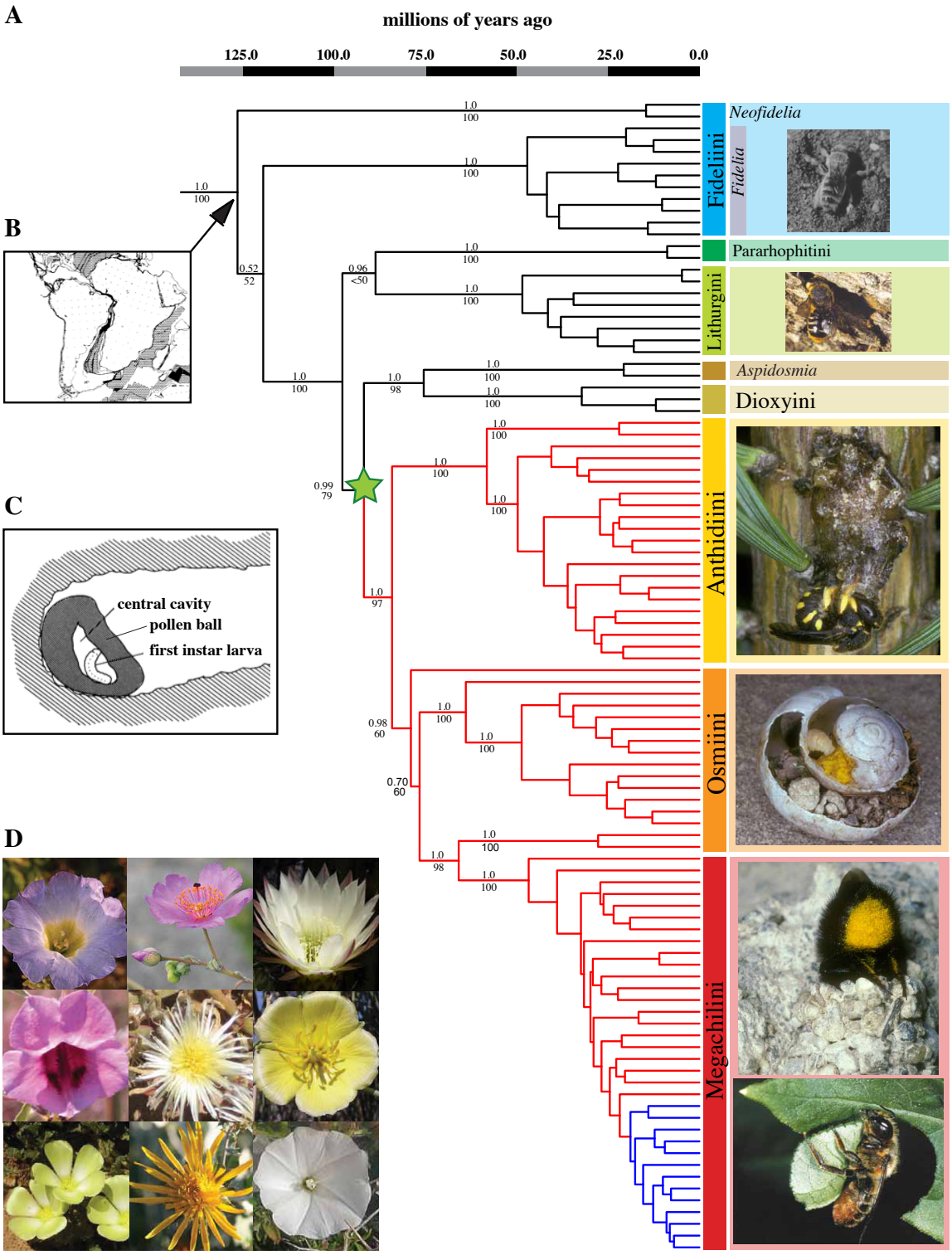
2.3 Results and Discussion

2.3.1 Biogeography and diversification

The results of both maximum likelihood and Bayesian analyses support a non-traditional interpretation of early megachilid phylogeny (Figure 2.2A). According to our phylogenetic hypothesis, the small palaearctic tribe Pararhophitini is not closely related to the largely austral tribe Fideliini but appears more closely related to the subfamily Megachilinae; this result is strongly supported in all analyses (Figures 2.2A and 3.1). Furthermore, the two lineages of Fideliini (the genera *Fidelia* and *Neofidelia*) constitute a weakly supported grade at the base of Megachilidae. Further tests using Bayes Factors (Kass 1995) strongly support the non-monophyly of both the subfamily Fideliinae (Bayes Factor: hereafter BF = 260.36) and the tribe Fideliini (BF = 33.68).

The first two branches in our phylogeny are thus the South American genus *Neofidelia* and the primarily southern African genus *Fidelia*. The geographical distribution and phylogenetic placement of these lineages reveal an austral disjunction between the Old and the New World, suggestive of a Gondwanan origin. We find the age of Megachilidae, and thus of the divergence between the South American and African fideliine bees, to be 126 mya (95% HPD 100-154), pre-dating the separation of the African and South American continental plates (Figure 2.2B). Our estimate of the age of Megachilidae is older than anticipated, given that bees are generally thought to have originated around 125 mya (Engel 2001). Our results indicate an origin

Figure 2.2 Fossil-calibrated maximum clade credibility tree for bee family Megachilidae. A. Bayesian posterior probabilities and maximum likelihood bootstrap values shown above and below nodes, respectively, for all clades older than 50 million years. Terminals are labelled to tribe according to present taxonomic assignment, even if determined to be paraphyletic in the current analysis. Branch colours correspond to significant changes in diversification rate (Black: diversification rate = 0.0164, relative extinction = 0.885; Red: diversification rate = 0.0867, relative extinction = 0.848; Blue: diversification rate = 0.315, relative extinction = 0.518). Node marked with green star corresponds to the transition between building unlined nests and building nests using foreign material. There is no reversion to building unlined nests after this point. Photographs to right of phylogeny from top to bottom: (1) Tribe Fideliini: *Fidelia villosa* using hind legs to excavate sand from a burrow (photo: Jerome G. Rozen (Rozen 1970), courtesy of the American Museum of Natural History); (2) Tribe Lithurgini: *Lithurgus chrysurus* entering nest in dead tree trunk (photo: Andreas Müller); (3) Tribe Anthidiini: *Anthidium strigatum* closing a nest cell of resin (photo: Albert Krebs); (4) Tribe Osmiini: Nest of *Osmia bicolor* built in an abandoned snail shell (photo: Albert Krebs); (5) Tribe Megachilini: (top) *Megachile parietina* entering her nest made of mud (photo: Albert Krebs); (bottom) *Megachile ligniseca* using her mandibles to cut a leaf disc (photo: Andreas Müller); B. Biogeographical reconstructions indicate a Gondwanan origin for Megachilidae, approximately 126 mya (photo from Scotese et al. 1988, Figure 10, p. 37); C. The ancestor of all Megachilidae built unlined nests in sandy soil, much like extant lineages *Fidelia*, *Neofidelia*, and *Pararhophites* (nest of *Fidelia villosa* shown; picture: Jerome G. Rozen (Rozen 1970), courtesy of the American Museum of Natural History); D. Host-plants of Fideliini (see Table S4). Top row (L-R): *Nolana* sp. (Solanaceae; host of *Neofidelia longirostris*; photo: Michael O. Dillon), *Calandrinia* sp., *Trichocereus* sp. (Portulacaceae and Cactaceae, respectively; hosts of *N. profuga*; photos: Joshua R. McDill, Scott Zona); centre row (L-R): *Sesamum* sp. (Pedaliaceae; host of *Fidelia friesei*; photo: Jessica Litman), *Psilocaulon* sp. (Aizoaceae; host of *F. villosa*, *F. kobrowi*, *F. paradoxa*; photo: Jessica Litman), *Sisyndite spartea* (Zygophyllaceae; host of *F. pallidula*; photo: Tomas Hajek); bottom row (L-R): *Grielum* sp. (Neuradaceae; host of *F. hessei*, *F. major*, *F. fasciata*; photo: Serban Proches), *Berkheya fruticosa* (Asteraceae; host of *F. braunsiana*; photo: Henry Brisse), *Convolvulus trabutianus* (Convolvulaceae; host of *F. ulrikei*; photo: Pierre-Marie Roux). Not shown: *Tribulocarpus dimorphanthus* (Aizoaceae; host of *F. ornata*). Note that all flowers are characterized by radial symmetry and exposed anthers.


A
millions of years ago
125.0
100.0
75.0
50.0
25.0
0.0
B
C
central cavity
pollen ball
first instar larva
D
Neofidelia
Fidelia
Fideliini
Pararhophitini
Lithurgini
Aspidosmia
Dioxyini
Anthidiini
Osmiini
Megachilini

for the bees (the root height of our tree) of 149 mya (95% HPD = 119-182). We ran another analysis where the root was constrained to 120 mya; even under this conservative estimate for the age of the bees (Engel 2001), the age of Megachilidae is 104 mya (95% HPD 95-113), which is still in keeping with a Gondwanan origin, as the last connections between Africa and South America are thought to have disappeared 100-110 mya (Sanmartin 2002). Both analyses indicate that the Megachilidae arose relatively rapidly after the origin of the bees.

A Gondwanan origin for Megachilidae is further supported by biogeographical reconstructions. S-Diva results favour a South-American/African vicariance (75.0 % of reconstructions) over scenarios involving either African (12.6%) or African/Palaearctic (12.4%) origins and subsequent dispersal to South America. Similarly, in biogeographical inferences using Lagrange (Ree 2008), analyses where Africa and South America were allowed to be adjacent strongly supported Gondwanan vicariance at the root node (global maximum likelihood -250.4; electronic supplementary material). Analyses where Africaand South America were not adjacent (thus precluding vicariance as a possible outcome and implying northern hemisphere migrations) had significantly worse overall likelihood scores (global maximum likelihood -252.3). Dispersal from Africa to South America via Australia and Antarctica (achieved by allowing dispersal between Australia and South America) was even less likely (global maximum likelihood -295.9). However, we agree with Rozen (Rozen 1973) that the most convincing support for vicariance over migration comes from biological evidence. The brood cells of fideliine bees consist of unlined cavities in the sand (Figure 2.2C); for this reason these bees are entirely restricted to strongly seasonal deserts where annual rainfall is not only low but also extremely unlikely during their nesting season (Rozen 1973). Alternative biogeographical scenarios to explain their present-day distribution necessarily involve migrations through the

northern hemisphere or via Antarctica; both of these scenarios imply adaptations to temperate habitats, which we consider extremely unlikely. Indeed, ancestral state reconstructions performed using BayesTraits (Pagel 1997) reveal that the ancestor of Megachilidae built nests that were neither lined with foreign material nor with glandular secretions (average maximum likelihood probability 0.99, average difference in likelihood 3.6 and 5.4, respectively; posterior probability 0.98, BF 6.0 and 14.4, respectively). All species using foreign material in nest construction form a monophyletic group. The use of foreign material in nest construction has a single origin at the base of the tribes Anthidiini, Dioxyini, Osmiini and Megachilini (average maximum likelihood probability 0.99, average difference in likelihood 2.5 and 7.3; posterior probability 0.99, BF 4.4 and 10.3).

The use of foreign material in nest construction underlies the ability of megachilid bees to colonize temperate regions and appears to be associated with a dramatic increase in clade species diversity. The lineages *Fidelia*, *Neofidelia*, and *Pararhophites* together number seventeen species, while the tribes Anthidiini, Osmiini, and Megachilini collectively include over 3900 species and exhibit a worldwide distribution. MEDUSA (Alfaro 2009) results provide evidence for two significant increases in diversification rate in our phylogeny, the first at the base of the higher megachilids and the second nested within the genus *Megachile* (Figure 2.2A).

The larger of the two rate shifts increases from 0.0164 to 0.0867 and occurs approximately seven million years after the advent of nest construction using foreign material, a behaviour that is first observed in the enigmatic genus *Aspidosmia* (Michener 2007), the first branch within the subfamily Megachilinae. The increase in diversification rate that occurs after

the divergence between *Aspidosmia* and the rest of Megachilinae suggests that the use of foreign material in nesting may have driven diversification but was not the only factor underlying it.

The second shift in diversification rate occurs within the genus *Megachile*, from 0.087 to 0.315. The increase in diversification tempo happens approximately eight million years after the origin of the true leafcutters (Michener's group I) from the paraphyletic assemblage of the *Chalicodoma*-group of sub-genera (Michener's group II) (Michener 2007). Despite their relatively recent origin, (22 mya; 95% HPD 16-27), leafcutter bees are extremely diverse and abundant on all continents. The explanation for such species richness may be related to their high reproductive output (Pitts-Singer 2011) and their ability to colonize an extremely broad range of habitats, from moist tropics to extreme deserts.

In association with the ancestral state reconstructions of nesting biology, the diversification rate analysis reveals an intimate association between nesting biology, distribution, and diversification. The single origin of nest-lining behaviour in Megachilidae makes it difficult to test for correlated evolution between nesting and other traits of interest. In contrast, the total geographic area occupied by the terminal taxa varies from lineage to lineage throughout the phylogeny, allowing us to test for an association between area and diversification rate. The results of BayesTraits analyses (Pagel 1997) indicate strongly correlated evolution between geographic area and diversification rate (BF = 25.8). In keeping with other studies where geographic area has been correlated with diversification (Parent 2006), we envision a scenario where nest-lining behaviour promoted the widespread colonization of temperate habitats, which in turn drove the diversification seen in the higher megachilids.

Ancestral state reconstructions strongly indicate that the three fideliine lineages are restricted to deserts due to their plesiomorphic nesting biology, rather than as a secondary adaptation. The use of foreign material in nest construction has a single origin at the base of the tribes Anthidiini, Dioxyini, Osmiini and Megachilini. It has enabled these bees to repeatedly colonize temperate habitats and catalyzed a massive shift in diversification rate. Surprisingly, Lithurgini manage to survive in temperate and tropical conditions, although they do not line their brood cells. All Lithurgines dig burrows in wood or stems and their pollen provisions are protected from humidity in these above-ground substrates (Garófalo 1981; Roberts 1978).

The identification of nest-lining behaviour as a key innovation also offers an explanation for the behavioural conservatism seen in the early megachilids. The two basal lineages, *Fidelia* and *Neofidelia*, which emerged prior to the advent of this innovation, have retained highly similar and comparatively unusual behaviours on two different continents for more than 100 million years, suggesting powerful evolutionary constraints on these behaviours. A comparison of their nesting biology and host-plant associations provides a unique glimpse into the biology of early megachilids over 120 million years ago, early in bee evolution.

2.3.2 Nesting

Unlined nests similar to those observed in fideliine bees are rare among bees. All members of the species-rich short-tongued bee families Andrenidae, Halictidae and Colletidae, which likely form a monophyletic group (Danforth 2006), apply secreted lining to their brood cells (Cane 1983; Hefetz 1987). Curiously, some desert andrenids apply a secreted lining not to the walls of their nests but to the pollen provisions themselves (Rozen 1967). In the family Apidae, the evolution of nest-lining behaviour is obscured by three probable origins of oil- or

resin- collection, the unknown phylogenetic positions of lineages which apparently do not line their brood cells (e. g. *Eremapis*; (Neff 1984)), four independent origins of cleptoparasitism and the evolution of social behaviour (Cardinal 2010). Lastly, unlined nests are known in several members of the melittid bees (Michener 2007), a species-poor group that may represent the earliest lineages of extant bees (Danforth 2006). Many melittids are restricted to xeric areas, especially several species-poor genera for which the nesting biology is not documented (e. g., *Eremaphanta*, *Afrodasypoda*, *Promelitta*). The few genera that are present in temperate regions either collect floral oil (*Macropis* and *Rediviva*), have evolved secreted cell lining (*Melitta*) or shape their pollen balls into peculiar, tripod-like structures that reduce contact between the provisions and the cell wall (*Dasypoda*). In fact, according to the most comprehensive phylogenetic hypothesis currently available for bees (Danforth 2006), the construction of unlined nests is a behaviour restricted to a few primitive lineages; among all bees, there is not a single documented instance of a reversion to building unlined nests after the evolution of nest-lining behaviour occurs. These observations strongly suggest that the ancestor of bees did not line its nest cells (Radchenko 1996) and that cell lining, using either glandular secretions or foreign material, has multiple origins in bees.

In contrast, unlined nests are prevalent among apoid wasps (Bohart 1976; Evans, 1966), the paraphyletic group from which bees arose. In fact, the nesting biology of fideliine bees is reminiscent of that of many sand-nesting apoids (Eickwort 1981) whose nests consist of unlined burrows in the sand. Apoid wasps store paralyzed prey that may stay alive for several weeks before being consumed by their larvae. While stored provisions are always susceptible to spoilage (Kaltenpoth 2005), the transition from prey-hunting to pollen-collecting in the early bees may have dramatically exacerbated the problems associated with the storage of provisions,

given the hygroscopic properties of pollen and its susceptibility to fungal infection, and driven selective pressure to protect provisions from moisture.

2.3.3 Foraging behaviour and host-plant associations

Interactions with angiosperms have often been cited as important driving factors underlying diversification in phytophagous insects (Farrell 1998). Our results, however, suggest that the shift to pollen collection in early bees did not simply open a vast new ecological niche. First, if the biology of the earliest extant megachilids indeed mirrors the biology of ancestral bees, early bees were constrained to xeric and strongly seasonal habitats and highly limited in their phenology. Second, another aspect of the behaviour of early bees may have seriously hampered them from fully utilizing all available angiosperm hosts: a pronounced floral specificity (oligolecty). Comparisons of the well-documented foraging behaviour of the basal members of Megachilidae (Table 2.4) provides unique insights into bee-flower relationships prevalent more than 100 million years ago. Fideliine bees, both in South America and South Africa, are notorious oligoleges. Rozen (Rozen 1977) states that on both continents, fideliine bees tend to forage on large flowers with well-exposed anthers (Figure 2.2D); even the narrowly polylectic *Neofidelia profuga* appears to restrict pollen collection to a few hosts with similar flower architecture, namely large flowers with radial symmetry and well-exposed stamens. The same appears to be true for many lithurgine bees: distantly related species of the genera *Lithurgus* and *Microthurge* in Australia, Africa and South America forage exclusively or predominantly on Malvaceae with large flowers, such as *Hibiscus*, *Sida,* and *Turnera* (Table 2.4); Asian species appear polylectic but restrict pollen collection to flowers of Malvaceae and Convolvulaceae; and two lineages, the subgenus *Lithurgopsis* and the genus *Trichothurgus* have

Table 2.4 Host-plant data for tribes Fideliini, Pararhophitini, and Lithurgini. Shown are individual taxa and their preferred host-plant/s based on collection and literature records.

Subfamily/ tribe	Genus	Subgenus	Further grouping	Species	Host-plant
Fideliinae					
Fideliiini	*Neofidelia*			*longirostris*	Oligolectic on *Nolana* sp (Solanaceae) (Rozen 1970; Rozen 1977) (Litman pers. obs. in Chile)
				profuga	Polylectic with pollen collection records for Cactaceae *(Trichocereus, Eulychnia*), Portulacaceae *(Calandrinia)* and floral visits (possibly for nectar) on Asteraceae *(Encelia)* (Rozen 1970; Rozen 1977; Moure 1955) (Litman pers. obs. in Chile)
	Fidelia	*Fidelia*		*kobrowi*	As *F. paradoxa* (Whitehead 2003)
				paradoxa	The species was found on several genera of Aizoaceae and Asteraceae; pollen and nectar visits were not distinguished. Most records are on Aizoaceae, so the species is either oligolectic on Aizoaceae or polylectic with a strong preference on this plant family (Rozen 1970; Whitehead 2003; Gess 2003)
				villosa	Probably oligolectic on Aizoaceae (Rozen 1970; Whitehead 2003; Gess 2003)
		Parafidelia		*friesei*	Probably oligolectic on *Sesamum* (Pedaliaceae), although the species has been collected on flowers from other plant families (Whitehead 2003; Gess 2003) (Litman pers. obs. in South Africa)
				pallidula	Probably oligolectic on Sisyndite (Zygophyllaceae) (Rozen 1977; Whitehead 2003) (Litman pers. obs. in South Africa)
		Fideliopsis		*fasciata*	Probably oligolectic on Neuradaceae (*Grielum* and *Neuradopsis*) (Whitehead 2003)
				hessei	Oligolectic on Neuradaceae (*Grielum* and *Neuradopsis*) (Whitehead, 2003 #37;Gess, 2003 #38) (Litman pers. observ in South Africa)
				major	Oligolectic on Neuradaceae (*Grielum*) (Whitehead 2003) (Litman pers. obs. in South Africa)
				ornata	Most records on Aizoaceae; other hosts possible (Whitehead 2003; Gess 2003)
		Fideliana		*braunsiana*	Probably oligolectic on *Berkheya* (Asteraceae) (Whitehead 2003; Gess 2003)
				ulrikei	Floral records on *Convolvulvus* (Whitehead 2003; Warncke 1980)
Pararhophitini	*Pararhophites*			*orobinus*	Probably oligolectic on *Peganum harmala* (Nitrariaceae) (McGinley 1987) (Praz pers. obs. in Uzbekistan, Iran)
				quadratus	probably oligolectic on *Zygophyllum* (Zygophyllaceae) (Popov 1949; Roche 1981) (Praz pers. obs. in Tunisia)
Lithurgini	*Lithurgus*	*Lithurgopsis*		*apicalis*	Oligolectic on *Opuntia* (Cactaceae) (Parker 1973; Krombein 1979)
				echinocacti	Presumably oligolectic on *Echinocactus* (Cactaceae) (Krombein 1979)

Table 2.4 (continued)

Subfamily/ tribe	Genus	Subgenus	Further grouping	Species	Host-plant
				gibbosus	Probably oligolectic on *Opuntia rigida* (Cactaceae) (Brach 1978)
				rufiventris	Oligolectic on Opuntia (Cactaceae) (Schlindwein 1997)
		Lithurgus	Palaearctic species	*chrysurus*	Oligolectic on Carduaceae (Roberts 1978; Müller 1997; Pachinger 2004)
				cornutus	Probably oligolectic on Carduaceae (Pachinger 2004; Malyshev 1930) (Praz pers. obs. in Uzbekistan, Spain)
				tibialis	Probably oligolectic on *Chrozophora* (Euphorbiaceae) (Praz pers. obs. in Iran)
			Australian and Asian species	*atratiformis*	Polylectic, collects pollen from *lpomoea* and *Hibiscus;* like many other members of its genus, appears to depend exclusively on large-flowered plants with coarse-grained pollen (Houston 1971)
				atratus	Polylectic, collects pollen mainly from *Ipomoea* (Convolvulaceae) and *Sida* (Malvaceae) (Lieftinck 1939; Camillo 1994)
				collaris	Polylectic with preference for Malvaceae (Kitamura 2001; Hannan 2007)
				rubricatus	Floral records: *Alyogyne* (Malvaceae) (Danforth pers. obs in Australia)
			African species	*pullatus*	Floral record: *Convolvulus* (Eardley 2010)
				spiniferus	Several flower records on Asteraceae (Gess 2003; Eardley 2010)
	Microthurge			*pygmaeus*	Oligolectic on Malvaceae (Schlindwein 1998; Schlindwein 2004)
				sp	Main pollinator of *Turnera sidoides* (Malvaceae) (Benitez-Vieyra 2007)
	Trichothurgus			*aterrimus*	Oligolectic on Cactaceae (Packer 2005)
				dubius	Visits *Cactus* flowers (Cactaceae) (Claude-Joseph 1925)
				spp	At least some species visit Cactaceae for pollen (Michener 2007)

maintained a close association with the large flowers of Cacteaceae in both South and North America. Lastly, the two species of *Pararhophites* for which host-plant information is available restrict their foraging to morphologically similar but phylogenetically unrelated flowers that have exposed stamens and five white petals (Table 2.4). In summary, a narrow host-range clearly appears to be the plesiomorphic condition in Megachilidae. Moreover, there is a striking lack of bilaterally symmetrical flowers among the hosts of the basal megachilid lineages. In contrast,

bilaterally symmetrical flowers, such as Fabaceae and Lamiaceae, which are typical bee-pollinated flowers, are common hosts of a significant proportion of the higher megachilids.

These observations strongly support the view that host-choices in bees are evolutionarily constrained (Sedivy 2008), as well as the widely discussed assertion that oligolecty is a primitive, rather than a derived state in bees (reviewed in (Sedivy 2008)). Müller (Müller 1996) suggested that oligolecty might be a behavioural constraint related to flower manipulation, pollen collecting or pollen digestion, rather than a secondary specialization. Interestingly, Müller (Müller 1996) notes that most apoid wasps are specialized hunters. In fact, the foraging behaviour of apoid wasps is similar in many ways to that of primitive bees. It is evolutionarily conserved, with related species exhibiting similar behaviour on different continents. Most species restrict their host-range to distantly related prey belonging to the same order (e.g. grasshoppers, spiders, or leafhoppers) that are often similar in size and appearance (Bohart 1976; Evans 1966) and co-occur in the same habitat. Evans (Evans 1971) elegantly summarizes the foraging behaviour of the philanthine wasp tribe Cercerini as follows: "I suggest that these wasps are not necessarily "good taxonomists," but that they are programmed to hunt in certain situations and to respond to prey of a certain size and behaviour". We hypothesize that early bees inherited foraging specificity as a behavioural constraint from their apoid wasp ancestors.

2.4 Conclusion

Our work reveals that two extant lineages are "living fossils" among the bees. The mid-Cretaceous origin of *Fidelia* and *Neofidelia* and their bizarre, plesiomorphic biology strongly support the possibility that these bees reflect the biology of the earliest bees more closely than any other extant lineage. The evolutionary patterns we report in Megachilidae lay the initial

framework for understanding patterns of nesting behaviour, distribution, host-plant preference and diversification in all bees.

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CHAPTER 3

PHYLOGENETIC SYSTEMATICS OF THE BEE FAMILY MEGACHILIDAE, INCLUDING A NOVEL METHOD FOR BOOTSTRAPPING COMBINED MOLECULAR AND MORPHOLOGICAL DATA

Abstract

Combining molecular and morphological data is a common practice in phylogenetic analysis; analyses of combined data, however, are potentially biased toward signal from molecular data, given that a single dataset often contains much more molecular data than morphological data. In an effort to balance the contribution of molecular and morphological data in combined analyses, I develop a novel bootstrapping algorithm which samples an equal number of characters from molecular and morphological datasets. I use this algorithm to explore phylogenetic relationships within the family Megachilidae using the megachilid molecular dataset presented in Chapter 2 and a pre-existing megachilid morphological dataset. I use the results of these bootstrap analyses, together with the results of the phylogenetic analyses from Chapter 2, to create a revised subfamilial- and tribal-level classification for the family Megachilidae. I propose two new megachilid subfamilies (Lithurginae and Pararhophitinae) and two new megachilid tribes (Ochreriadini and Pseuodheriadini) and present a revised key to the tribes of Megachilidae.

3.1 Introduction

In his seminal 1966 work, *Phylogenetic Systematics*, Willi Hennig outlined the central principles of cladistic analysis and asserted that shared ancestry should form the basis for the

classification of organisms and not “successive degrees of resemblance in a single category of characters”; Hennig believed that “the fundamental difference between the method of morphological and phylogenetic systematics is that the latter breaks up the simple concept of ‘resemblance’" (Hennig 1965, 1966). From this moment forward, morphological data have been analyzed within the cladistic framework of Hennig to assess evolutionary relationships among organisms.

The advent of the use of molecular sequence data in phylogenetic analysis radically changed the face of phylogenetics because the number of characters that could be used to establish phylogenetic relationships among taxa increased by many orders of magnitude, from the tens or hundreds of characters typical of morphological data matrices to the thousands or tens of thousands of characters possible in molecular data matrices. While some researchers prefer to work exclusively with either morphological or molecular data, many are of the opinion that an approach that combines molecular data with morphological data may offer the most comprehensive overview of phylogenetic relationships among taxa. The use of morphological data in tandem with molecular data may be particularly useful in cases where molecular data are unavailable, e.g. for fossils or rare taxa for which molecular data are difficult or impossible to obtain.

Studies combining molecular and morphological data have been performed on a broad range of organisms, including bees (Danforth et al. 2006), spiders (Agnarsson et al. 2007), cetaceans (O’Leary and Gatesy 2007), and molluscs (Lindgren et al. 2004). In each of the studies cited above, the molecular data are far more abundant than the morphological data: in the dataset of Danforth et al. (2006), there are 4299 molecular and 109 morphological characters; in the

dataset of Agnarsson et al (2007), there are approximately 4500 molecular and 148 morphological characters; the dataset of O'Leary and Gatesey (2007) includes 40,000 molecular and 635 morphological characters; and the dataset of Lindgren et al. (2004) includes approximately 4000 molecular and 101 morphological characters. The extremely skewed ratio of molecular to morphological data seen in virtually all analyses using combined data raises the question of whether such analyses truly provide a vision of phylogenetic relationships based on both molecules and morphology, or whether these relationships are biased toward signal coming from the molecular data. Indeed, many such studies reveal that an analysis of combined data results in a tree topology more similar to the topology based on molecular data than to the topology based on morphological data (Danforth et al. 2006; Agnarsson et al. 2007).

In this chapter, I present the systematic results from the molecular phylogeny introduced in Chapter 2 and use these results as the basis for a revised subfamilial- and tribal-level classification of the bee family Megachilidae. I also present a revised key to the tribes of Megachilidae. I introduce a novel bootstrapping method which samples equal numbers of characters from molecular and morphological datasets and which is designed to balance the signal from molecular and morphological data. I test this method using the megachilid molecular dataset presented in Chapter 2 in conjunction with the megachilid morphological character matrix of Gonzalez et al. (in review). I present the results of these bootstrap analyses and discuss the potential utility of this method in future analyses combining molecular and morphological data.

3.2 Methods and Materials

3.2.1 Molecular dataset

The molecular dataset that I present here is identical to the one presented in Chapter 2. This dataset includes 98 ingroup taxa representing all seven families of Megachilidae and 31 outgroup taxa representing the rest of the bees (see Table 2.1 for the species, DNA voucher numbers and collection localities for each of the specimens used in this study). Four thousand one hundred fifty-six base pairs were sequenced from four nuclear protein-coding genes (CAD, NAK, LW-rhodopsin, and EF1-alpha) and 1246 base pairs were sequenced from one nuclear ribosomal gene (28S), for total of 5402 base pairs (see Table 2.2 for the GenBank accession numbers for all sequences used in this study). Protein-coding genes were aligned using MAFFT (Katoh et al. 2002) and then adjusted manually in MacClade (Maddison and Maddison 2005); all introns were excluded. The 28S ribosomal RNA gene was aligned by secondary structure using a 28S map of *Apis mellifera* (Gillespie et al. 2006); all unalignable regions were excluded.

3.2.2 Combined datasets

To generate bootstrapped datasets of combined data (molecular + morphological), I used the molecular dataset from Chapter 2 and the morphological dataset from Gonzalez et al. (in review). This morphological dataset includes 198 characters coded for 61 megachilid taxa from all seven megachilid tribes and ten outgroup taxa representing the bee families Melittidae and Apidae.

I ran phylogenetic analyses on the combined datasets using three different taxon sets: (1) a 31-taxon dataset that included only the *species* that were shared between the molecular and morphological datasets; this dataset contained no missing data; (2) a 54-taxon dataset that included only the *genera* that were shared between the molecular and morphological datasets; this dataset contained no missing data; and (3) a 73-taxon dataset, consisting of all the genera

present in both molecular and morphological datasets. In both the 54- and 73-taxon datasets, data were pooled for a genus in cases where the same genus was represented by different species in the molecular and morphological datasets.

3.2.3 Phylogenetic analyses

3.2.3.1 Molecular dataset

Details regarding partitioning regime, model-testing and phylogenetic analyses are described in Chapter 2, Materials and Methods, Section 2.2. Bayesian phylogenetic analyses were performed on the partitioned dataset using MrBayes v.3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003); six independent analyses were run, for a total of 180,000,000 generations. I used Tracer (Rambaut and Drummond 2007) to discard the burnin from each analysis, leaving a total of 96,956,000 post-burn-in generations. I used this posterior distribution of trees to create a maximum clade credibility tree in TreeAnnotator v.1.6.1 (Drummond and Rambaut 2007). Maximum likelihood analyses were performed using RAxML v.7.0.4 (sequential version raxmlHPC; Stamatakis 2005). I used the rapid bootstrapping algorithm with a GTRCAT approximation on the partitioned dataset to perform 1000 bootstrap replicates.

3.2.3.2 Combined data: "Balanced" bootstrap analyses

In a standard bootstrap analysis, characters are sampled with replacement from a data matrix; the "bootstrapped" data matrix is the same length as the original data matrix, although some characters may be represented more than once and some may be entirely absent. In most analyses that combine molecular with morphological data, both types of data are concatenated

into a single matrix and then analyzed. In order to explore the effects of using equal amounts of molecular and morphological data, I modified the number of characters sampled from the molecular and morphological data matrices to create "balanced" bootstrap matrices, in which each bootstrapped matrix contains the same number of molecular and morphological characters. For each of the three taxon sets, I excluded all parsimony non-informative sites from both the molecular and morphological matrices using PAUP* (Swofford 2011). This resulted in a molecular dataset of 1383 characters and a morphological dataset of 177 characters for the 31-taxon dataset; a molecular dataset of 1595 characters and a morphological dataset of 197 characters for the 54-taxon dataset; and a molecular dataset of 1650 characters and a morphological dataset of 197 characters for the 73-taxon dataset (Table 3.1). Using a script written in R (R Development Core Team 2010; Appendix 3.1), I sampled an equal number of characters from the molecular and morphological data matrices for each taxon set, thereby generating 100 bootstrapped matrices of 354 characters for the 31-taxon dataset (177 characters from the molecular dataset and 177 from the morphological dataset); 100 matrices of 394 characters for the 54-taxon dataset; and 100 matrices of 396 characters for the 73-taxon dataset (Table 3.1). For each taxon set, characters were sampled randomly with replacement; for the molecular data matrix, the number of characters sampled from each partition was proportional to the size of that partition.

Analyses of the 100 bootstrapped matrices for each taxon set were performed using maximum parsimony in PAUP* v.4.0a122 (Swofford 2011), applying an unweighted heuristic search using 20 random sequence additions, with four trees held at each step. The analysis of each bootstrap matrix in PAUP* was automated using the Perl script shown in Appendix 3.2. For

Table 3.1 Numbers of parsimony-informative characters in each of the three taxon sets (31, 54 and 73 taxa; see text). Shown are the number of characters in the molecular matrix, the morphological matrix, and the bootstrapped matrices that result from sampling equal numbers of molecular and morphological characters. The size of the bootstrapped matrices is limited by the size of the morphological matrix. Differences in the sizes of the matrices between different taxon sets are a function of the fact that the number of parsimony informative sites changes as taxa are added or removed.

	Number of parsimony informative characters		
Number of taxa	Molecular matrix	Morphological matrix	Bootstrapped matrices
31-taxon dataset	1383	177	354
54-taxon dataset	1595	197	394
73-taxon dataset	1650	197	394

each taxon set, the output trees from the analysis of each bootstrap matrix were saved to a single file. In order to compute the consensus of all trees obtained for each set of 100 bootstrap matrices, I wrote a script in R to parse the tree file and export all of the resulting trees to a file readable by PAUP* (Appendix 3.3).

For the remainder of this chapter, these analyses will be referred to as "balanced bootstrap analyses".

3.2.3.3 Combined data: Concatenated bootstrap analyses

In order to compare the performance of the "balanced" bootstrap analyses to the performance of conventional combined analyses where all molecular and morphological data are analyzed together, I concatenated the molecular data matrix with the morphological matrix of Gonzalez et al. (in review) to create a 5600-character combined molecular-morphological data matrix. For each of the three taxon sets described above, I analyzed this concatenated dataset using a maximum parsimony bootstrap analysis in PAUP*. I applied an unweighted heuristic search using 20 random sequence additions, with four trees held at each step. For the remainder of this chapter, these analyses will be referred to as "concatentated bootstrap analyses".

3.3 Results and Discussion

The results of these phylogenetic analyses reveal eleven suprageneric lineages within Megachilidae (Figure 3.1): 1) the genus *Fidelia*; 2) the genus *Neofidelia*; 3) the genus *Pararhophites*; 4) the tribe Lithurgini; 5) the genus *Aspidosmia*; 6) the tribe Dioxyini; 7) the tribe Anthidiini; 8) the genus *Ochreriades*; 9) the tribe Osmiini; 10) the genera *Afroheriades* + *Pseudoheriades*; and 11) the tribe Megachilini. These lineages correspond to tribal-level groups and I use them to present a revised tribal- and subfamilial-level classification for Megachilidae.

3.3.1 Neofidelia and Fidelia

Analyses of the molecular dataset do not recover the monophyly of the tribe Fideliini but rather indicate that *Neofidelia* and *Fidelia* form a paraphyletic grade at the base of Megachilidae; this suggests that these genera may not be as closely related as implied by current megachilid classification. The paraphyletic relationship between *Neofidelia* and *Fidelia* is only weakly supported (53% posterior probability; 52% ML bootstrap support) but tests using BayesFactors strongly support the non-monophyly of the tribe Fideliini (*Neofidelia* + *Fidelia*; BF = 33.68). In contrast, analyses of both the morphological dataset (Figure 3.8) and the 31-, 54, and 73-taxon "balanced" bootstrapped datasets consistently recover relatively high support (between 73%-100%) for a monophyletic Fideliini (Figures 3.2, 3.3, and 3.4). Analyses of the concatenated dataset result either in weak support for the monophyly of Fideliini (54- and 73- taxon datasets, 51% and 59% parsimony bootstrap support, respectively) (Figures 3.6 and 3.7), or in weak support for the non-monophyly of Fideliini (as seen in the 31-taxon dataset, 53% parsimony bootstrap support) (Figure 3.5).

Neofidelia and *Fidelia* share a puzzling combination of morphological characters that have long obscured their phylogenetic relationship to other bees. This strange combination of characters, however, has led taxonomists to believe that these two genera may be closely related to one another. Both *Fidelia* and *Neofidelia* lack basitibial plates and possess a metasomal sternal scopa, both synapomorphies for the family Megachilidae (Moure and Michener 1955). Unlike other megachilids, however, Fideliini have three submarginal wing cells, a distinct episternal groove, and subantennal sutures directed toward the middle of the lower margin of the antennal socket (Michener 2007; Moure and Michener 1955).The brush of long, dense hairs on the hind legs of *Fidelia* (and also, to some degree, on the hind legs of *Neofidelia*) is also unusual in Megachilidae; it is, however, similar to the scopa on the hind legs of other bees (Michener 2007).

If *Neofidelia* and *Fidelia* do not constitute a monophyletic clade, as indicated by molecular data, then some of the unusual characters shared by both genera may in fact be plesiomorphic within Megachilidae. The presence of three submarginal wing cells seen in both of these genera and also in most bees, for example, is likely the plesiomorphic condition in Megachilidae; the presence of two submarginal wing cells first evolved after the appearance of *Neofidelia* and *Fidelia* and is retained in all other members of the family. The construction of unlined nests by *Neofidelia* and *Fidelia* is an extremely rare behavior in bees but is seen in other basal lineages of Megachilidae (including *Pararhophites* and members of the tribe Lithurgini; Roberts 1978; McGinley and Rozen 1987; Rust et al. 2004; Hannan and Maeta 2007), basal lineages of all bees (such as *Dasypoda*; Michener 1964), and apoid wasps (Bohart and Mencke 1976; Evans 1966); this behavior is likely not only the plesiomorphic condition in Megachilidae but in bees in general (see chapter 2). Finally, the long brush of hair on the hind legs of fideliine bees is not used to transport pollen but is rather a modification to aid in nest excavation (Brauns

1905; Rozen 1970); some melittid bees (such as *Dasypoda*) use long hairs present on their hind legs for a similar purpose, implying that this condition may be the ancestral trait in bees, also. The results of these phylogenetic analyses yield conflicting results regarding the monophyly of Fideliini. Sufficient evidence does not exist to change the current taxonomic status of Fideliini and I thus retain the tribe Fideliini in my proposed classification until further evidence suggests otherwise.

3.3.2 Pararhophites

The genus *Pararhophites* is strongly supported as monophyletic in the molecular analyses (100% posterior probability; 100% ML bootstrap support) and is the sister group to the tribe Lithurgini in Bayesian analyses (97% posterior probability); the relationship between *Pararhophites*, Lithurgini and the remaining members of the family (except Fideliini) is unresolved in maximum likelihood analyses (Figure 3.1). In the 31- and 73-taxon "balanced" bootstrap analyses (Figures 3.2 and 3.4), and in the 31- and 54-taxon concatenated analyses (Figures 3.5 and 3.6), *Pararhophites* is supported as the sister taxon to the rest of Megachilidae (except Fideliini); in the 54-taxon "balanced" bootstrap analysis, *Pararhophites* is supported as the sister taxon to Fideliini (61% parsimony bootstrap support) (Figure 3.3) and in the 73-taxon concatenated dataset, *Pararhophites* is sister to the tribe Lithurgini (63% parsimony bootstrap

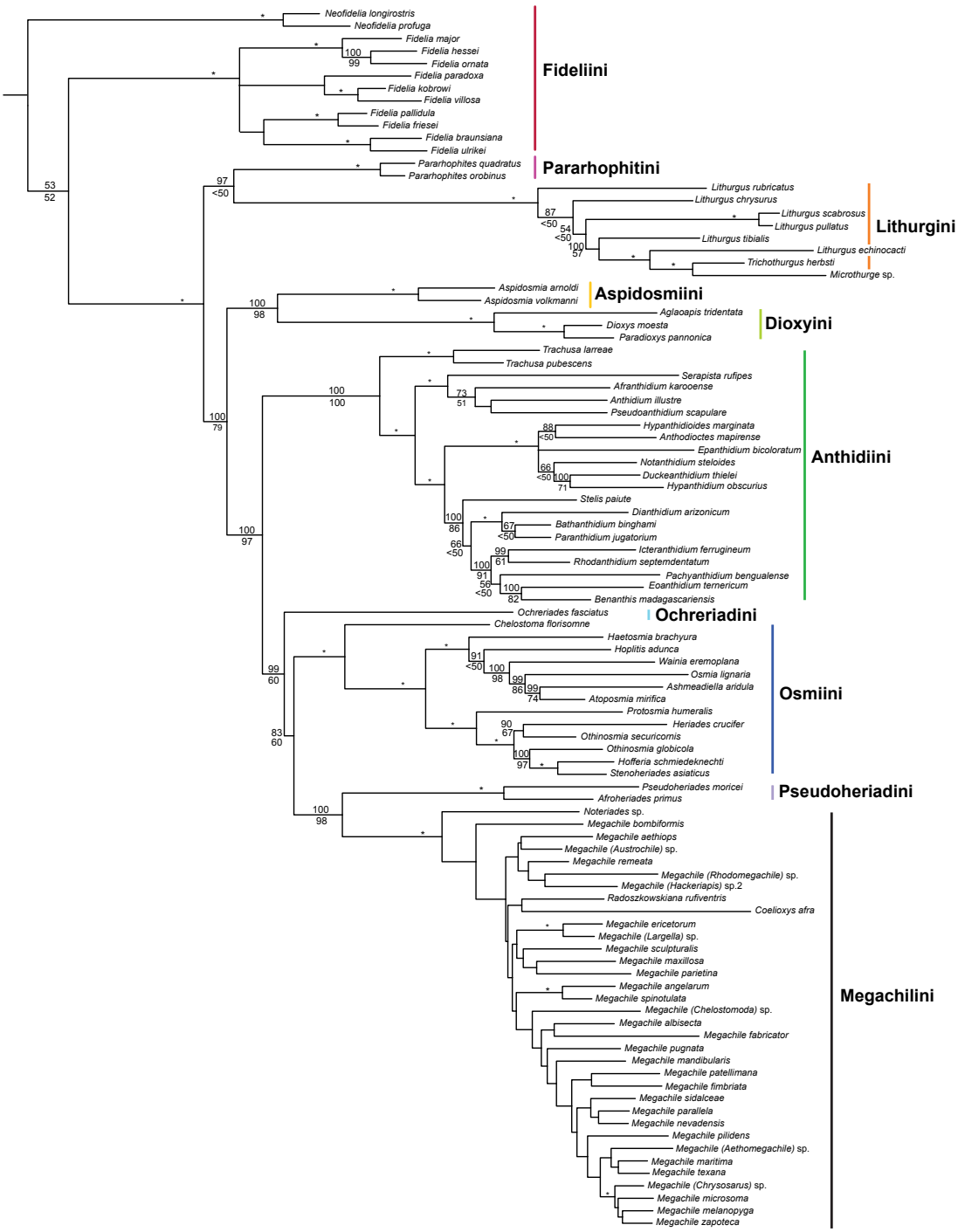

Figure 3.1 Phylogeny of Megachilidae based on Bayesian and maximum likelihood analyses of the molecular dataset. Numbers above nodes are Bayesian posterior probabilities; numbers below nodes are maximum likelihood bootstrap values. An asterisk ("*") marks those nodes supported by 100% posterior probability and 100% ML bootstrap support. In order to save space, node support has been omitted within the tribe Megachilini, except at nodes supported by 100% posterior probability and 100% ML bootstrap support. Tribes are indicated to the right of the phylogeny.

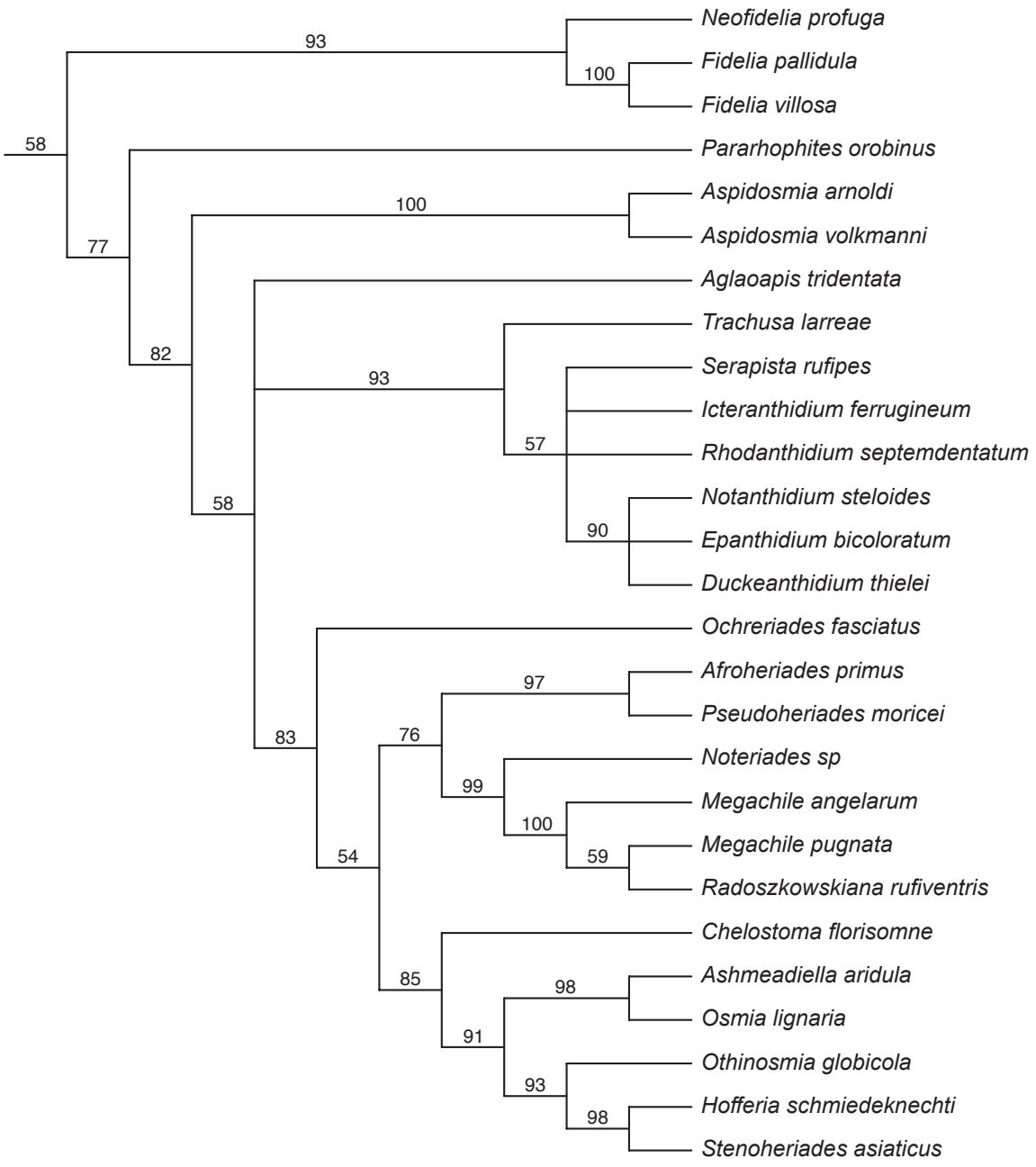

Figure 3.2 Parsimony bootstrap consensus tree for "balanced" bootstrap analysis with 31 taxa. Bootstrap values are indicated above the nodes. Outgroups have been pruned out.

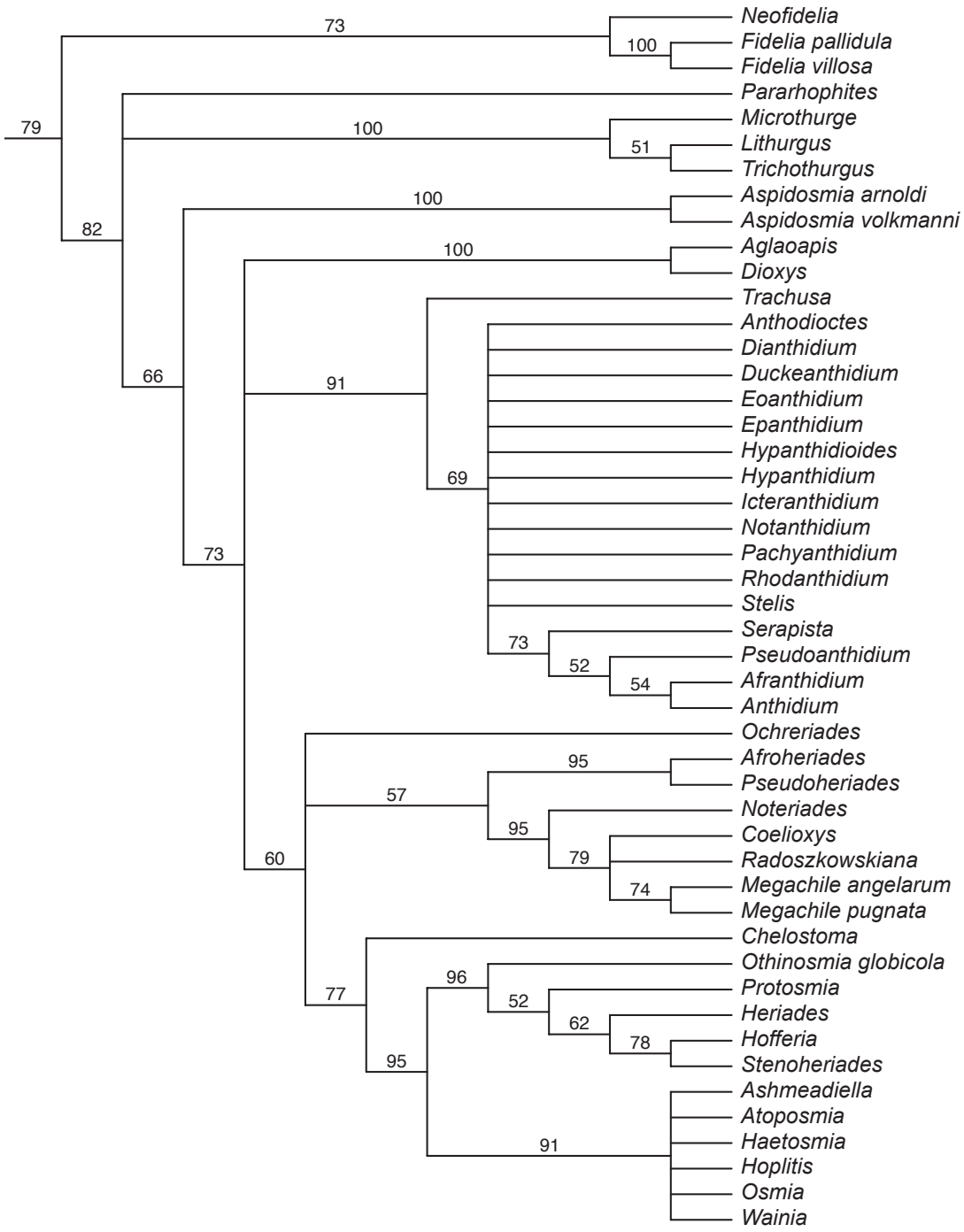

Figure 3.3 Parsimony bootstrap consensus tree for "balanced" bootstrap analysis with 54 taxa. Bootstrap values are indicated above the nodes. Outgroups have been pruned out.

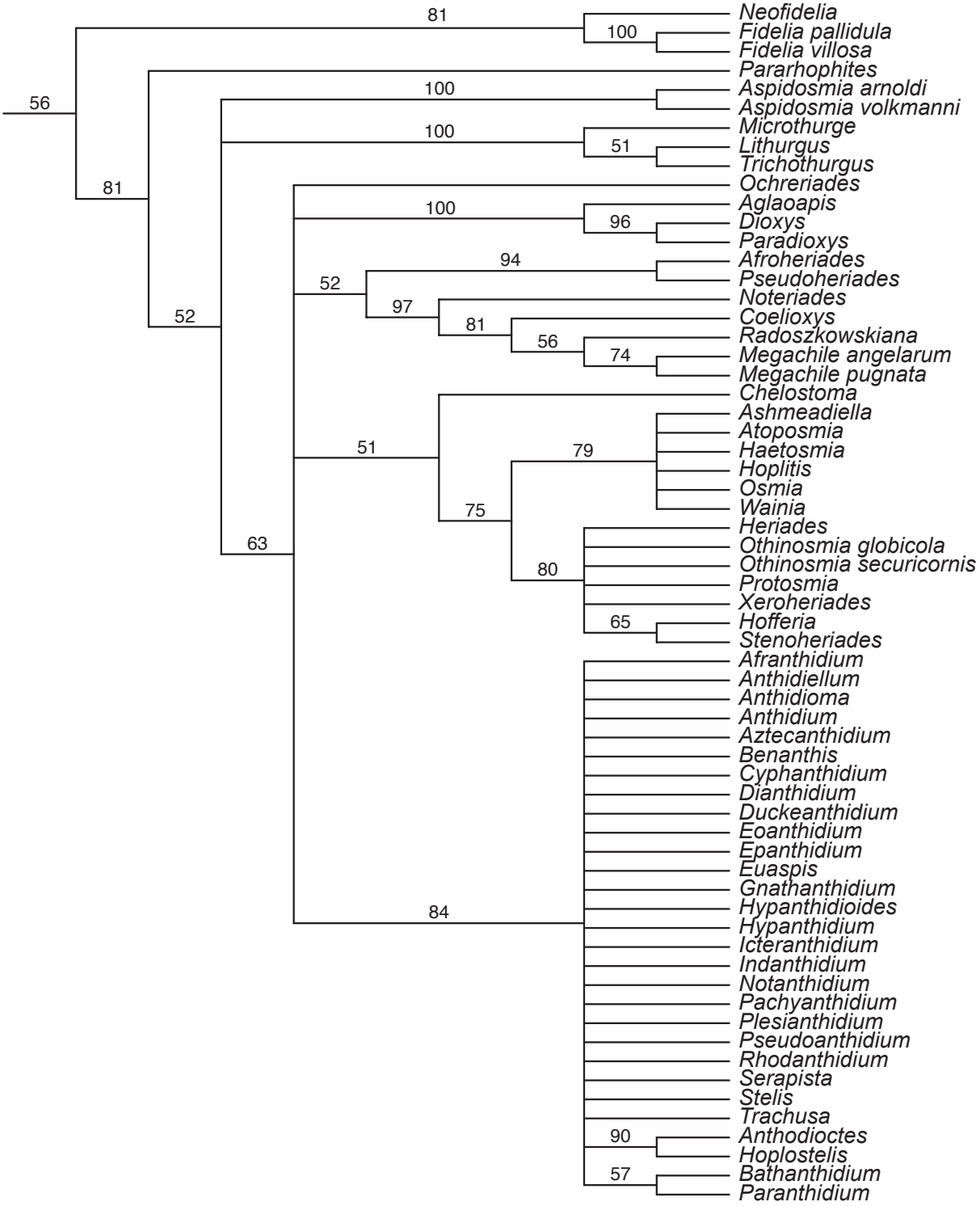

Figure 3.4 Parsimony bootstrap consensus tree for "balanced" bootstrap analysis with 73 taxa. Bootstrap values are indicated above the nodes. Outgroups have been pruned out.

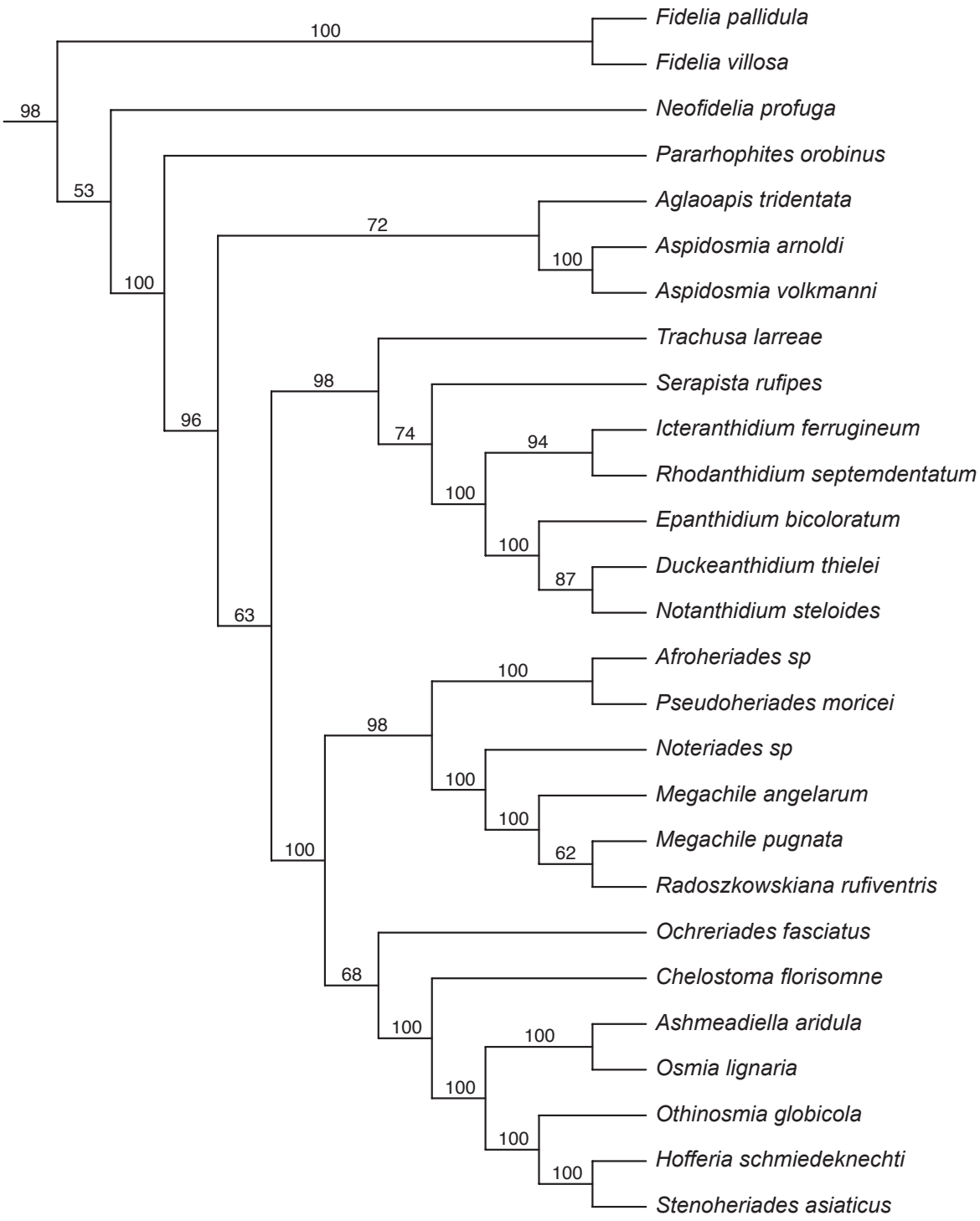

Figure 3.5 Parsimony bootstrap consensus tree for concatenated bootstrap analysis with 31 taxa. Bootstrap values are indicated above the nodes. Outgroups have been pruned out.

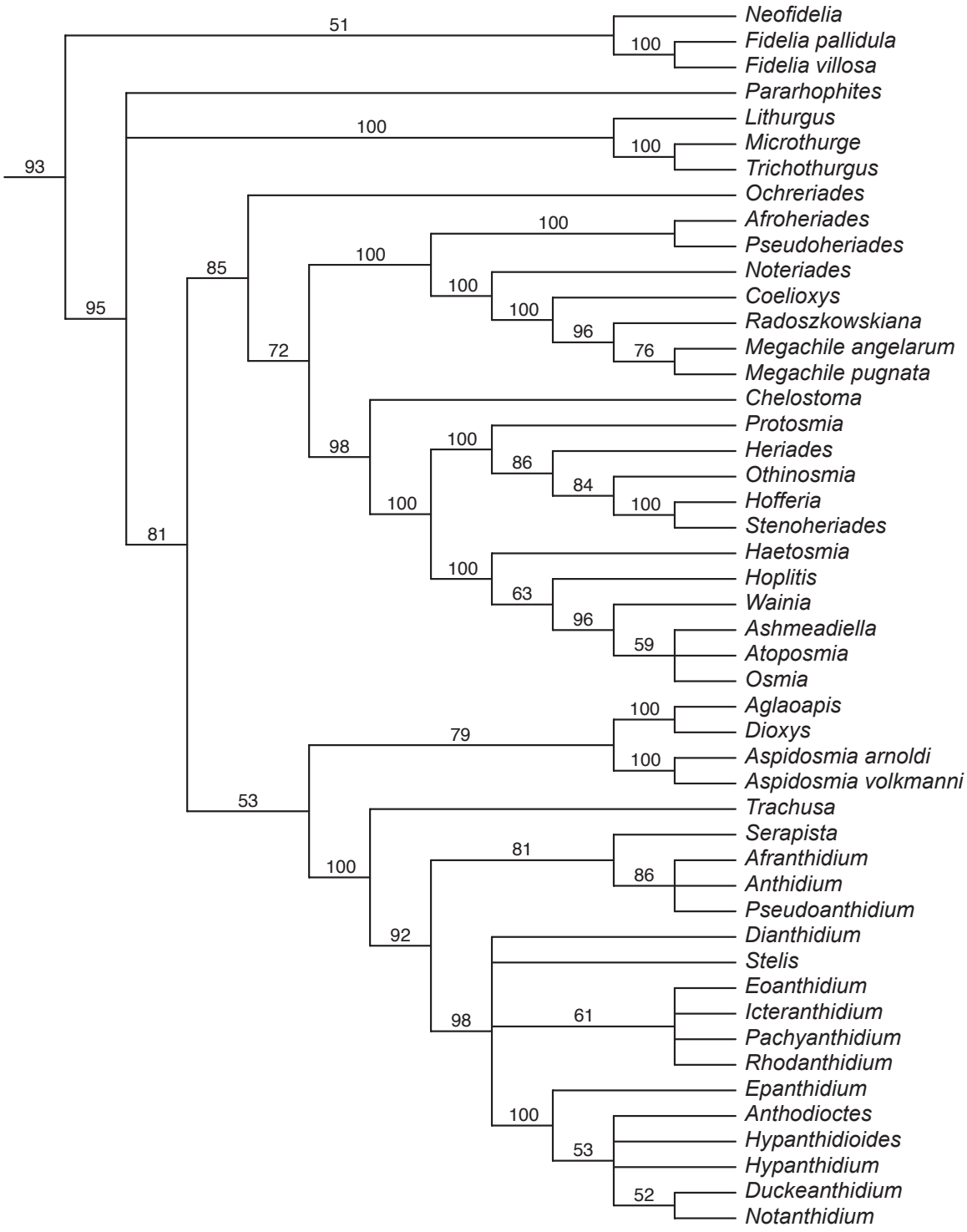

Figure 3.6 Parsimony bootstrap consensus tree for concatenated bootstrap analysis with 54 taxa. Bootstrap values are indicated above the nodes. Outgroups have been pruned out.

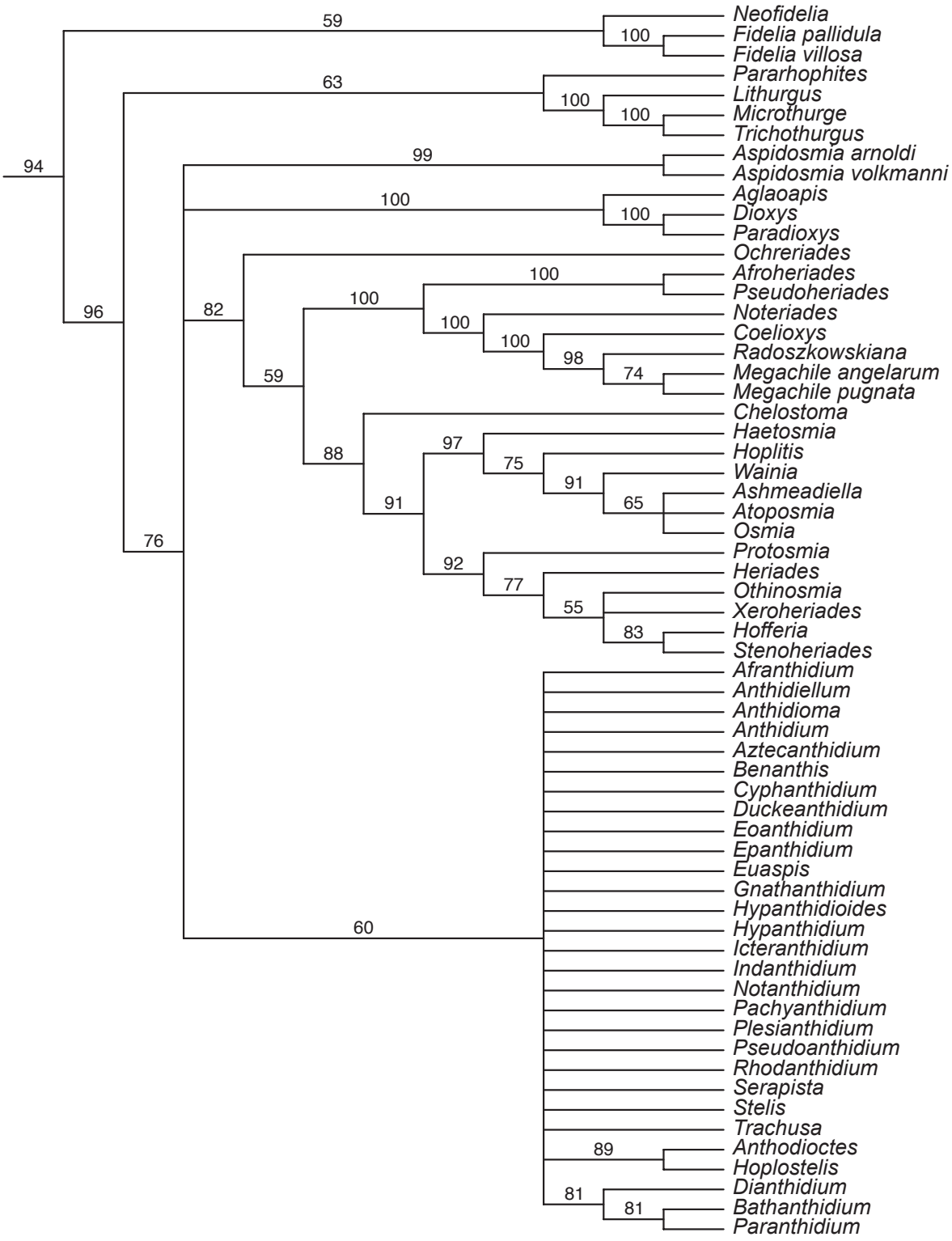

Figure 3.7 Parsimony bootstrap consensus tree for concatenated bootstrap analysis with 73 taxa. Bootstrap values are indicated above the nodes. Outgroups have been pruned out.

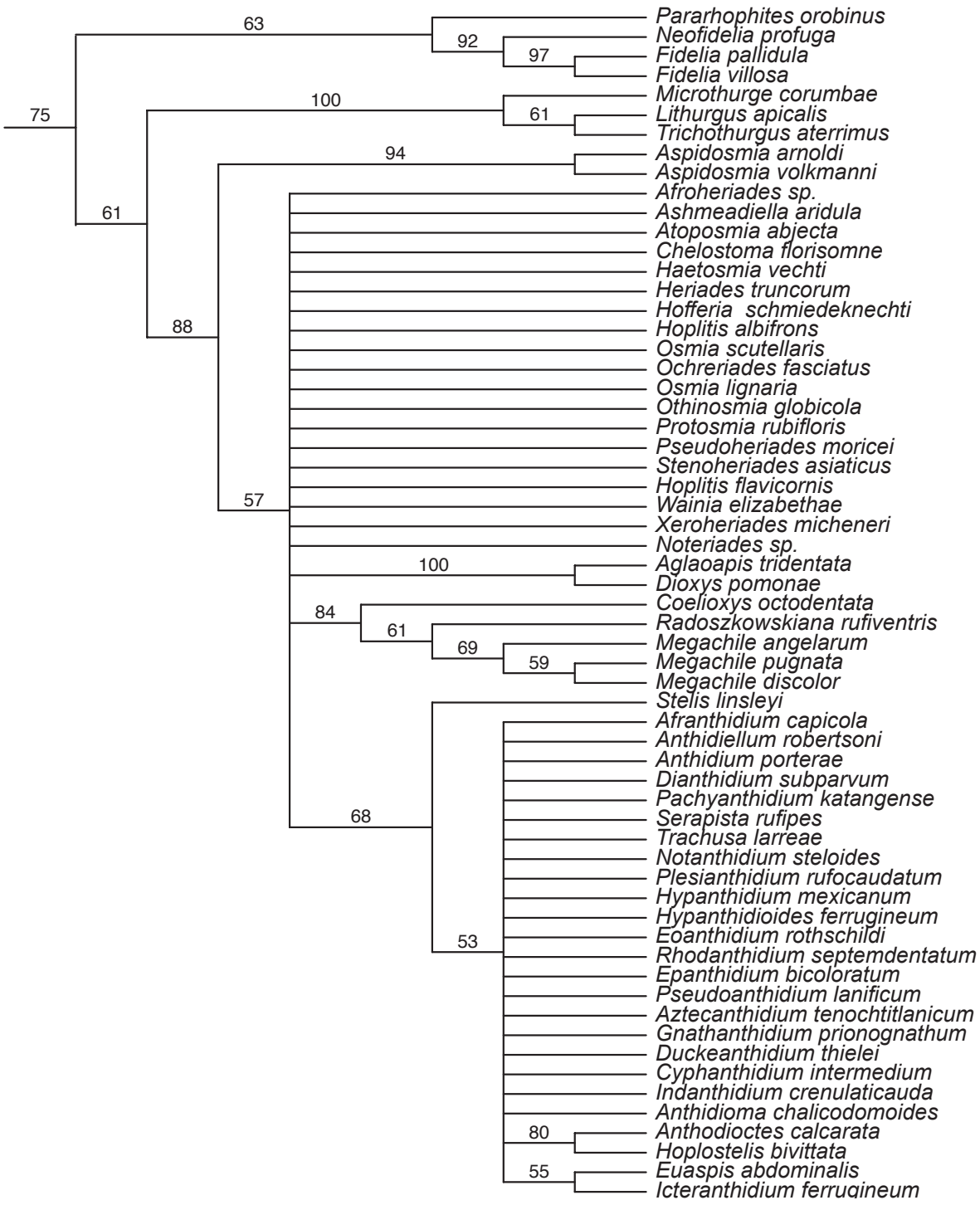

Figure 3.8 Parsimony bootstrap consensus tree for morphological bootstrap analysis with 71 taxa. Bootstrap values are indicated above the nodes. Outgroups have been pruned out.

support) (Figure 3.7). The morphological bootstrap analysis shows *Pararhophites* as the sister taxon to Fideliini (63% parsimony bootstrap support) (Figure 3.8).

Like members of the tribe Fideliini, *Pararhophites* possesses a strange combination of morphological characters that has rendered its taxonomic placement difficult. Like most members of the family Megachilidae except Fideliini, members of the tribe Pararhophitini have two submarginal wing cells and a metasomal scopa; the metasomal scopa is, however, extremely reduced, although still denser than the scopa of parasitic Megachilinae (Michener 2007). In contrast to other megachilids, pollen is transported not only on the metasomal scopa but also on the legs and head, making "a restricted, well-defined scopal area" difficult to identify (McGinley and Rozen 1987). In male pararhophitines, the volsellae are well-developed, a character not seen in other megachilids besides Fideliini and the genus *Noteriades*. Distinct from other megachilids but shared with Fideliini is the papillate T6 of the female. The peculiar morphology of *Pararhophites* has caused taxonomists to suggest phylogenetic affinities with Megachilidae (Rozen's opinion; McGinley and Rozen 1987), the subfamily Anthophorinae in Apidae (McGinley's opinion, McGinley and Rozen 1987), the genus *Dasypoda* (Warncke 1977), and the apid tribe Exomalopsini (Michener 1944).

According to each of these analyses, the phylogenetic affinity of *Pararhophites* clearly lies with the family Megachilidae. In contrast to current megachilid classification, however, which places the tribes Fideliini and Pararhophitini together in the subfamily Fideliinae, the molecular, "balanced", and concatenated bootstrap analyses indicate that *Pararhophites* is not closely related to either *Neofidelia* or *Fidelia*; in the morphological bootstrap analysis, *Pararhophites* is recovered as the sister taxon to Fideliini with low bootstrap support (63%

parsimony bootstrap support) (Figure 3.8). I recommend that the tribe Pararhophitini be removed from the megachilid subfamily Fideliinae. The unique morphology and behavior of *Pararhophites* (see Chapter 1), in combination with its distant phylogenetic relationship to other major megachilid groups, suggest that it may be appropriate to place *Pararhophites* into a unique megachilid subfamily, Pararhophitinae; this recommendation is in agreement with the proposed classification system of Gonzalez et al. (in review).

3.3.3 Lithurgini

The tribe Lithurgini is strongly supported as monophyletic in all analyses (although it is absent in both of the 31-taxon datasets) (Figures 3.2-3.4, 3.6-3.8). Its phylogenetic relationship to other megachilids, however, remains unresolved. The Bayesian and concatenated 73-taxon analyses indicate that Lithurgini is the sister group to *Pararhophites* (Figures 3.1 and 3.7), the morphological analysis shows Lithurgini as the sister taxon to the rest of Megachilidae (except for *Fidelia*, *Neofidelia*, and *Pararhophites*) (Figure 3.8), and the maximum likelihood, "balanced" 54- and 73-taxon, and concatenated 54-taxon bootstrap analyses show that the phylogenetic position of Lithurgini is unclear (Figures 3.1, 3.3, 3.4, and 3.6). While the results of the molecular and concatenated analyses support a sister-group relationship between the lithurgine genera *Microthurge* and *Trichothurgus* (Figures 3.1, 3.6, 3.7), the results of the 54- and 73-taxon "balanced" and morphological bootstrap analyses recover weak support for a sister group relationship between *Lithurgus* and *Trichothurgus* (Figure 3.3, 3.4, and 3.8). Both of these relationships are contrary to the classical phylogenetic hypothesis based on morphology, which suggests, based on several morphological "synapomorphies", that *Microthurge* is the sister taxon to the genus *Lithurgus* (Michener 1983).

A number of morphological synapomorphies define Lithurgini as a monophyletic group; the following characters are taken from Michener (1983). The proboscis is long, often reaching the metasoma when fully extended. The first three segments of the labial palpus are produced on the same axis, while the fourth segment extends laterally at a right angle to the first three; in contrast, both the third and fourth segments are produced on the same axis and extend laterally at a right angle to the first two segments in all other long-tongued bees (except *Chelostoma*). The outer tibial surface is covered by hairless spicules or tubercles (although this character is absent on the hind tibiae of some males). The hind basitarsi are slender and rounded rather than flattened, as seen in most other bees. The posterior margin of the first metasomal tergite is rounded; the tergite itself is small and flattened. In contrast, the first metasomal tergite in other bees is convex and the posterior margin transverse. In female lithurgines, the posterior margin of the sixth metasomal tergite is produced as an apical spine, a character unseen in other bees. In male lithurgines, the genitalia and hidden sternites are extremely small compared to those of other bees.

The tribe Lithurgini is strongly supported as monophyletic in the phylogenetic analyses presented here but questions are raised regarding the placement of the tribe Lithurgini in the subfamily Megachilinae. While the phylogenetic position of Lithurgini within Megachilidae is unclear, none of the analyses support a close relationship between Lithurgini and other members of the subfamily Megachilinae. Although I retain the tribe Lithurgini in my proposed classification, I remove Lithurgini from the subfamily Megachilinae and place it in a new subfamily, Lithurginae; I believe that the lack of phylogenetic affinity between Lithurgini and other megachilines, in conjunction with the the unique biology and distinct morphology of

Lithurgini (see Chapter 1), justifies this transfer. The placement of Lithurgini in a separate subfamily, Lithurginae, has also been proposed by Engel (2005) and Gonzalez et al. (in review).

3.3.4 Aspidosmia

The genus *Aspidosmia* is strongly supported as a monophyletic group in all analyses (Figures 3.1-3.8). It is recovered as the sister group to Dioxyini in molecular analyses (100% posterior probability; 98% ML bootstrap support) (Figure 3.4) and in the 31- and 54-taxon concatenated analyses (72% parsimony bootstrap and 79% parsimony bootstrap, respectively) (Figures 3.5 and 3.6). Its phylogenetic position is unresolved in the "balanced" 73-taxon and concatenated 73-taxon analyses (Figures 3.4 and 3.7). It is recovered as the sister group to the rest of Megachilidae (except *Neofidelia*, *Fidelia* and *Pararhophites*) in the 31-taxon "balanced" and morphological analyses (82% parsimony bootstrap support and 88% parsimony bootstrap support, respectively) (Figures 3.2 and 3.8). In the 54-taxon "balanced" analysis, *Aspidosmia* is the sister group to the remaining Megachilidae (except *Neofidelia*, *Fidelia*, *Pararhophites* and Lithurgini) (Figure 3.3).

The genus *Aspidosmia* was originally placed in the tribe Osmiini (Brauns 1926): like other members of Osmiini, the second recurrent wing vein of *Aspidosmia* enters the second submarginal wing cell. Peters (1972), however, transferred *Aspidosmia* to the tribe Anthidiini, based on the yellow clypeal markings on the face of the males, the width of the stigma, and the shape of the thorax. The prestigma of *Aspidosmia* is longer than the stigma and the claws of the female are cleft, characters seen in both Osmiini and Anthidiini (Michener 2007). Other morphological characters suggest a relationship with other megachilid tribes, including Megachilini and Lithurgini.

While these results do not clearly reveal the phylogenetic placement of *Aspidosmia* within Megachilidae, they do reveal that *Aspidosmia* is not closely related to Anthidiini, nor to Osmiini. I agree with the proposed classification of Gonzalez et al. (in review), which removes *Aspidosmia* from the tribe Anthidiini and places it in a new tribe, Aspidosmiini.

3.3.5 Dioxyini

The cleptoparasitic tribe Dioxyini is recovered as monophyletic in all analyses in which multiple dioxyine species were present (in both 31-taxon analyses, Dioxyini was represented only by *Aglaoapis tridentata*) (Figures 3.1, 3.3, 3.4, 3.6-3.8). Molecular analyses show Dioxyini as the sister tribe to *Aspidosmia* (100% posterior probability; 98% ML bootstrap support) (Figure 3.1), as do the 31- and 54-taxon concatenated analyses (72% parsimony bootstrap and 79% parsimony bootstrap, respectively) (Figures 3.5 and 3.6). In all other analyses, the phylogenetic position of Dioxyini within Megachilidae in unclear.

A number of morphological characters strongly support the monophyly of the tribe Dioxyini: an elongate, parallel-sided labrum; a strong preoccipital carina; metanotum with median tooth (although this character is absent in *Ensliniana* and *Allodioxys*); females with sting greatly reduced; and a pronotum with a prominent obtuse or right-angular dorsolateral angle, from which a vertical ridge descends (although this character is absent in *Prodioxys*) (Michener 2007). While the phylogenetic position of Dioxyini remains unresolved, all analyses place Dioxyini within the same clade as other members of the subfamily Megachilinae. Therefore these results support the current classification of Dioxyini as a tribe within the subfamily Megachilinae; I retain this placement in my proposed classification.

3.3.6 Anthidiini

The results of all of these analyses support the monophyly of the tribe Anthidiini (*sensu* Gonzalez et al., in review, thus without the genus *Aspidosmia*). The molecular analyses recover a sister group relationship between Anthidiini and a clade consisting of the "core" Osmiini (the genus *Chelostoma*, the *Heriades* group and the *Osmia* group; Praz et al. 2008), the tribe Megachilini, and the genera *Ochreriades*, *Pseudoheriades*, and *Afroheriades* (100% posterior probability; 100% ML bootstrap support) (Figure 3.1); the concatenated 31-taxon analysis recovers the same relationship (61% parsimony bootstrap support) (Figure 3.5). The relationship between Anthidiini and other megachilids is unresolved in all "balanced" analyses, as well as in the 73-taxon concatenated and morphological analyses (Figures 3.2-3.4, 3.7, and 3.8). In the 54-taxon concatenated analysis, Anthidiini is weakly supported as the sister taxon to a clade consisting of Dioxyini and *Aspidosmia* (53% parsimony bootstrap support) (Figure 3.6).

Many morphological characters have been used to describe the tribe Anthidiini. There are no unique tribal synapomorphies, however, and it is rather a *combination* of characters that typifies the tribe. Some of the characters used to define Anthidiini include: both stigma and prestigma are short, typically less than twice as long as wide; the claws of the females are cleft or have an inner tooth (except in *Trachusoides*); the outer surface of the hind tibia is covered by dense, simple bristles (this character is absent in *Aspidosmia*); the preaxilla is vertical and nearly hairless; the dorsal lamella of the metapleuron is usually absent; and the second recurrent vein usually inserts distally on the second submarginal crossvein; the absence of a basal polished area on anterior surface of the female labrum; gonostylus articulated to the gonocoxite, although often partially fused; the rounded, almost spherical shape of the thorax; and the nearly vertical

alignment of the propodeum and metanotum (characters taken from Peters 1972; Roig-Alsina and Michener 1993; Michener 2007).

I retain the tribe Anthidiini in my proposed classification. Current megachilid classification recognizes Anthidiini as part of the subfamily Megachilinae; given that the results of all phylogenetic analyses place Anthidiini in the same clade as other members of the subfamily Megachilinae, I also retain the current subfamilial placement of Anthidiini.

3.3.7 Ochreriades

The results of the molecular analyses recover the genus *Ochreriades* as the sister lineage to a clade consisting of the "core" Osmiini, the tribe Megachilini and the genera *Pseudoheriades* and *Afroheriades* (99% posterior probability; 60% ML bootstrap support) (Figure 3.1); this relationship is also recovered in the 54- and 73-taxon concatenated analyses (85% and 82% parsimony bootstrap support, respectively) (Figures 3.6 and 3.7) and in the 31-taxon "balanced" analyses (83% parsimony bootstrap support) (Figure 3.2). The 31-taxon concatenated analysis places *Ochreriades* as the sister taxon to the "core" Osmiini (68% parsimony bootstrap support) (Figure 3.5). The results of the 54- and 73-taxon "balanced" analyses, as well as the results of the morphology analysis, do not clearly reveal the phylogenetic position of *Ochreriades*, although all three analyses place *Ochreriades* in the same clade as other members of the subfamily Megachilinae (Figures 3.3, 3.4, and 3.8).

The tiny genus *Ochreriades* contains just two species which exhibit a disjunct geographical distribution: *Ocheriades fasciatus* is restricted to the deserts of the Middle East and *Ochreriades rozeni* is limited to the deserts of southern Africa (Michener 2007; Ascher and

Pickering 2011). This unusual genus is currently placed in the tribe Osmiini yet exhibits a number of characters which distinguish it from other osmiines, including the presence of yellow or white integumental markings and an enlarged pronotum which eliminates both the preomaular surface and the anterior surface of the scutum (Michener 2007).

The results of these phylogenetic analyses demonstrate that the genus *Ochreriades* is not closely related to other osmiines or to any other tribe but rather constitutes a unique lineage. These results corroborate the findings of a recent molecular analysis of the tribe Osmiini, which also found *Ochreriades* to be only distantly related to other osmiines (Praz et al. 2008). I hereby remove the genus *Ochreriades* from the tribe Osmiini and transfer it to a new tribe, Ochreriadini, in the subfamily Megachilinae.

The similarity of *Ochreriades* to other osmiines is largely manifested in the heriadiform (slender and elongate) shape of its body, which is reminiscent of that of the osmiine genera *Chelostoma* and *Heriades*. Like *Chelostoma* and *Heriades*, *Ochreriades* exhibits a long narrow abdomen; in contrast to these two genera, however, *Ochreriades* also exhibits an elongate thorax. Although the body form of *Ochreriades* may be a plesiomorphy within the clade *Ochreriades* + (("core" Osmiini) + ((*Pseudoheriades* + *Afroheriades*) + Megachilini)), the strangely elongate thorax of *Ochreriades* suggests that the long narrow body of *Ochreriades* may not be homologous with that of other osmiines. A more likely explanation is that the slender, elongate body shape seen in *Chelostoma*, *Heriades* and *Ochreriades* is an adaptation to nesting in narrow openings, such as abandoned insect burrows or plant stems (Müller 2011).

3.3.8 Osmiini

The results of all of these phylogenetic analyses confirm the paraphyly of the tribe Osmiini (*sensu* Michener 2007). The "core" Osmiini are supported as monophyletic in the molecular analyses (100% posterior probability; 100% ML bootstrap support) (Figure 3.1), in each of the "balanced" analyses (85%, 77%, and 51% parsimony bootstrap support for the 31-, 54- and 73-taxon datasets) (Figures 3.2-3.4), and in each of the concatenated datasets (100%, 98%, and 88% parsimony bootstrap support for the 31-, 54-, and 73-taxon datasets) (Figures 3.5-3.7). All phylogenetic analyses except for the morphological analysis show support for three core osmiine lineages: the genus *Chelostoma*, the *Heriades* group, and the *Osmia* group. All analyses (except morphology) indicate, however, that two small genera currently placed in the tribe Osmiini, *Afroheriades* and *Pseudoheriades*, are not closely related to Osmiini.

Like the tribe Anthidiini, the tribe Osmiini is not characterized by the presence of any one character but rather by a combination of characters. Characters used to define the tribe include the presence of arolia, females with simple tarsal claws (although claws are cleft in *Osmia (Metallinella)*), a second recurrent vein which inserts basally to the second submarginal cross-vein, a long stigma and prestigma, a vertical, nearly hairless preaxilla, the absence of the dorsal lamella of the metapleuron and the presence of a longitudinal ridge extending downward to the marginal area of the propodeum on the lower extremity of the metapostnotum (internally) (characters taken from Roig-Alsina and Michener 1993; Michener 2007). While these characters are used to characterize members of Osmiini, all are likely plesiomorphic within Megachilinae. The apparent absence of concrete morphological synapomorphies for the tribe may explain why several genera unrelated to Osmiini, including *Pseudoheriades*, *Afroheriades*, *Ochreriades* and *Noteriades*, have nevertheless been classified as such.

I retain the tribe Osmiini is my proposed classification but limit its members to the "core" Osmiini. I formally remove *Afroheriades* and *Pseudoheriade*s from Osmiini and classify them as discussed below.

3.3.9 Afroheriades and Pseudoheriades

The genera *Afroheriades* and *Pseudoheriades* together constitute a monophyletic clade in all analyses (Figures 3.1-3.7) except morphology (Figure 3.8), in which their relationship to each other and to other taxa is unresolved. Furthermore, all analyses except morphology recover *Afroheriades* + *Pseudoheriades* as the sister group to the tribe Megachilini.

Afroheriades and *Pseudoheriades* are small genera restricted to the Old World: *Afroheriades* is found in the deserts of southern Africa, while *Pseudoheriades* is found scattered throughout Africa and the Middle East. These two small genera superficially resemble the osmiine genus *Heriades* and exhibit a number of morphological characters which ostensibly ally them to the tribe Osmiini, where they are currently placed.

The results of these phylogenetic analyses, however, indicate that *Afroheriades* and *Pseudoheriades* are more closely related to the tribe Megachilini than to Osmiini. These analyses confirm the results of a recent molecular phylogenetic analysis of the tribe Osmiini, which also demonstrated that *Afroheriades* and *Pseudoheriades* were not included in the monophyletic group of "core" Osmiini (Praz et al. 2008). I hereby remove both *Afroheriades* and *Pseudoheriades* from the tribe Osmiini and transfer them both to a new tribe, Pseudoheriadini. The monophyly of this tribe is clearly supported by two morphological characters: the reduction in the number of maxillary palpi to two (most other megachilids have four maxillary palpi,

except for some members of the *Proteriades*-group of *Hoplitis*, which also have two; Michener 2007); and the quadrate shape of the seventh tergite of the males, which lies in an emargination in the sixth tergite.

3.3.10 Megachilini

All analyses except the morphological analysis recover the monophyly of the tribe Megachilini (*sensu* Gonzalez et al., in review) (Figures 3.1-3.7); the morphological analysis failed to recover the genus *Noteriades* within the same clade as the rest of Megachilini (Figure 3.8).

A number of morphological characters have been used to define the tribe Megachilini. Some of these characters include: a long stigma and prestigma; the presence of a second recurrent cross vein which inserts basally to the second submarginal vein; a sloping preaxilla with long hairs; the absence of arolia (except in the subgenera *Heriadopsis* and *Matangapis*); the absence of a basal polished area on the anterior surface of the labrum in females; and the pronotum with ventrolateral extensions fused mid-ventrally, usually on the internal surfaces of extensions (characters taken from Roig-Alsina and Michener 1993; Michener 2007).

A recent cladistic analysis based on adult morphological data was grounds for the recent transfer of the genus *Noteriades*, formerly placed in the tribe Osmiini, into the tribe Megachilini (Gonzalez et al. (in review)). These results soundly confirm that *Noteriades* is the sister taxon to the remaining Megachilini and I agree with the placement of *Noteriades* in this tribe. The placement of *Noteriades* in the tribe Megachilini was initially proposed by Griswold (1985); the presence of an anterior angle or spine followed by a notch on the apical margin of the outer surfaces of both fore and mid tibiae may be a synapomorphy which supports Megachilini +

Noteriades (Gonzalez et al. in review). I retain the tribe Megachilini in my proposed classification.

3.4.2 Comparison of analytical methods

The results of all phylogenetic analyses are summarized in Table 3.2. The "balanced" analyses recover support for the same tribal lineages as the molecular and concatenated analyses; the only tribal-level conflict between the molecular and the "balanced" analyses was the tribe Fideliini, recovered as paraphyletic in molecular analyses but monophyletic in "balanced" analyses. In concatenated analyses, Fideliini is alternately recovered as paraphyletic (31-taxon dataset) and monophyletic (54- and 73-taxon datasets). It is possible that the absence of strong support for the relationship between *Fidelia* and *Neofidelia* in the molecular dataset, in combination with the strong signal for the monophyly of Fideliini from the morphological dataset, results in phylogenetic relationships that are driven by morphology, thus explaining the monophyly of Fideliini in the "balanced" analyses.

Phylogenetic support for inter-tribal relationships, however, decreases in the "balanced" analyses as the number of taxa present in the dataset increases. Inter-tribal relationships are well-resolved in the 31-taxon datset and reflect those of the molecular analyses; only the relationships among Dioxyini, Aspidosmiini and the rest of the higher megachilids is unresolved. In the 54-taxon dataset, these same relationships remain unresolved, as do the phylogenetic position of *Ochreriades*, *Pararhophites*, and Lithurgini. In the 73-taxon dataset, these relationships are still unresolved, as are the relationships between Megachilini and Osmiini. This decrease in resolution may be a function of significantly increasing the number of taxa (by 74%, between the 31- and 54-taxon datasets, and by 35%, between the 54- and the 73-taxon datasets) while only

Table 3.2 Summary of phylogenetic results Node support for each of the proposed megachilid tribes from the "balanced", concatenated, morphological, and molecular analyses. In analyses where a tribe was only represented by a single taxon or not represented at all, node support is labeled "N/A". Unsupported nodes are labeled "U/S". Node support for each of the "balanced", concatenated, and morphological analyses are given as parsimony bootstrap values, while node support for the all-molecular dataset are given as Bayesian posterior probabilities for Bayesian analyses and as maximum likelihood bootstrap values for ML analyses.

	Fideliini	**Pararhophitini**	**Aspidosmiini**	**Lithurgini**	**Dioxyini**	**Anthidiini**	**Ochreriadini**	**Osmiini**	**Pseudoheriadini**	**Megachilini**
31-taxon balanced	93%	N/A	100%	N/A	N/A	93%	N/A	85%	97%	99%
54-taxon balanced	73%	N/A	100%	100%	100%	91%	N/A	77%	95%	95%
73-taxon balanced	81%	N/A	100%	100%	100%	84%	N/A	51%	94%	97%
31-taxon concatenated	U/S	N/A	100%	N/A	N/A	98%	N/A	100%	100%	100%
54-taxon concatenated	51%	N/A	100%	100%	100%	100%	N/A	98%	100%	100%
73-taxon concatenated	59%	N/A	99%	100%	100%	60%	N/A	88%	100%	100%
All morphology	92%	N/A	94%	100%	100%	68%	N/A	U/S	U/S	U/S
All molecular	U/S (in both Bayesian and ML)	100% (in both Bayesian and ML)	100% (in both Bayesian and ML)	100% (in both Bayesian and ML)	100% (in both Bayesian and ML)	100% (in both Bayesian and ML)	N/A	100% (in both Bayesian and ML)	100% (in both Bayesian and ML)	100% (in both Bayesian and ML)

marginally increasing the number of characters in the dataset (by 11%, between the 31- and 54-taxon datasets, and by 0%, between the 54- and 73-taxon datasets), possibly resulting in too few characters per taxon to resolve relationships (see Graybeal 1998). The decrease in resolution with increasing number of taxa may also be exacerbated by the greater amount of missing data present in the 73-taxon dataset. Each of the concatenated analyses yields tree topologies that are more resolved than those of the "balanced" analyses using the same number of taxa; this is probably due to the greater number of characters present in the concatenated analyses.

In general, the topology of the concatenated analyses is more reflective of the molecular topology than the topology of the "balanced" analyses. The tribes Aspidosmiini and Dioxyini, for example, consistently emerge as sister taxa in molecular and concatenated analyses, a relationship never seen in the "balanced" datasets. The tribe Fideliini is supported as monophyletic with relatively strong support in the "balanced" analyses but is paraphyletic in the molecular and 31-taxon concatenated analyses and is only weakly supported as monophyletic in the 54- and 73-taxon concatenated analyses. Despite the slightly lower resolution inherent in the "balanced" analyses, I believe that such analyses may still provide a unique overview of phylogenetic relationships that may be lost if molecular and morphological data are simply concatenated and analyzed.

One of the drawbacks to the use of "balanced" data matrices is that the number of morphological characters available for analysis generally places the upper limit on the total size of the "balanced" dataset: if the morphological dataset is smaller in size than the molecular dataset (as is almost always the case) and contains x characters, then the size of the "balanced" bootstrapped matrices contain $2x$ characters. Given that morphological data are often measured in hundreds of characters, the maximum size of the "balanced" dataset is often only several hundred characters long. This may make analyses of datasets with many taxa particularly difficult.

Another potential drawback to the use of "balanced" data matrices is that each morphological character has a much greater chance than any molecular character of being represented one or more times in any given bootstrap replicate. This necessarily changes the

sampling properties between the morphological and molecular datasets and may in fact give more weight to phylogenetic relationships supported by the morphological dataset.

Perhaps the greatest utility of combining molecular with morphological data is the phylogenetic placement of taxa for which no molecular data is available. I present the osmiine genus *Xeroheriades* as an example. *Xeroheriades* is absent from the molecular dataset yet is present in the morphological dataset. Although the phylogenetic position of *Xeroheriades* is completely unresolved in the analysis of morphological data, both "balanced" and concatenated 73-taxon analyses (the only analyses of combined data which contain *Xeroheriades*) place *Xeroheriades* in the tribe Osmiini, within the suprageneric *Heriades* group; this placement is consistent with the current taxonomic classification of *Xeroheriades* (Michener 2007). In this respect, both "balanced" and concatenated analyses performed equally well. While I employed this method to determine the phylogenetic position of an extant taxon for which I was missing molecular sequence data, this method may also be useful for the placement of fossil taxa.

3.4.3 Summary of proposed classification

In summary, I propose the following taxonomic changes to the subfamilial and tribal level classification of the bee family Megachilidae (summarized in Table 3.3):

1. These results support the recognition of four distinct subfamilies: Fideliinae (including the tribe Fideliini), Pararhophitinae (including the tribe Pararhophitini), Lithurginae (including the tribe Lithurgini), and Megachilinae (including the tribes Dioxyini, Aspidosmiini, Anthidiini, Osmiini, Ochreriadini, Pseudoheriadini, and Megachilini).

2. I create two new tribes to accomodate three genera formerly placed in Osmiini but whose phylogenetic affinities clearly lie outside Osmiini: Ochreriadini (including the genus *Ochreriades*) and Pseudoheriadini (including the genera *Pseudoheriades* and *Afroheriades*)

3. These results support the placement of the genus *Aspidosmia* in a new tribe Aspidosmiini, as initially proposed by Gonzalez et al. (in review). Furthermore, I concur with their placement of the genus *Noteriades* in the tribe Megachilini.

4. These results suggest that the tribe Fideliini may not be monophyletic and may thus warrant division into two separate tribes, Fideliini (including the genus *Fidelia*) and Neofideliini (including the genus *Neofidelia*; as initially proposed by Engel 2004, 2005). This would also require the formation of a new subfamily, Neofideliinae, to accomodate the tribe Neofideliini. While these changes may become necessary in the future, I await further evidence before changing the current status of Fideliini.

Table 3.3 Revised subfamilial- and tribal-level classification for Megachilidae. Shown are the proposed classification of Michener (2007), the proposed classification of Gonzalez et al. (in review) based on their preferred Proposal 2, and my revised classification. My revised classification does not yet include fossil taxa.

Michener 2007	**Gonzalez et al. (in review)**	**My revised classification**
Subfamily/ tribe	**Subfamily/ tribe**	**Subfamily/ tribe**
Fideliinae	Fideliinae	Fideliinae
Fideliini	Fideliini	Fideliini
Pararhophitini	Pararhophitinae	Pararhophitinae
Megachilinae	Pararhophitini	Pararhophitini
Lithurgini	Lithurginae	Lithurginae
Dioxyini	Lithurgini	Lithurgini
Anthidiini	Megachilinae	Megachilinae
Osmiini	Dioxyini	Dioxyini
Megachilini	Aspidosmiini	Aspidosmiini
†Protolithurgini	Anthidiini	Anthidiini
	Osmiini	Osmiini
	Megachilini	Ochreriadini
	†Ctenoplectrellini	Pseudoheriadini
	†Glyptapini	Megachilini

3.4.3 Revised key to the extant tribes of Megachilidae

The following is a revised key to the extant tribes of Megachilidae (modified from Michener 2007 and Gonzalez et al. (in review)).

1. Metanotum with median spine or tubercle (except in *Allodioxys* and *Ensliniana*); mandible of female slender apically, bidentate, similar to that of male; pronotum (except in *Prodioxys*) with prominent obtuse or right-angular dorsolateral angle, below which a vertical ridge extends downward; sting and associated structures greatly reduced (scopa absent).....**Dioxyini**

—. Metanotum without median spine or tubercle; mandible of female usually wider apically, with three or more teeth, except rarely bidentate when mandible is greatly enlarged and porrect and clypeus is also modified; pronotum with dorsolateral angle weak or absent (or produced to a tooth in some *Chelostoma* but without vertical ridge below it); sting and associated structures well developed.....**2**

2(1). Stigma less than twice as long as broad, inner margin basal to vein r usually little if any longer than width, rarely about 1.5 times width; claws of female cleft or with an inner tooth (except in *Trachusoides*); body commonly with yellow or white integumental marks.....**3**

—. Stigma over twice as long as broad, inner margin basal to vein r longer than width; claws of female simple (except in *Osmia* subgenus *Metalinella*, Palaearctic); body without light-colored integumental markings (except in *Ochreriades*).....**4**

3(2). Outer surface of hind tibia with long hairs forming a distinct scopa; prestigma much more than twice as long as broad; preaxilla, below posterolateral angle of scutum, sloping and with small patch of hairs, these as long as those of adjacent sclerites (Fig. 10).....**Aspidosmiini**

—. Outer surface of hind tibia usually with abundant simple bristles, not forming a distinct scopa; prestigma commonly short, usually less than twice as long as broad; preaxilla vertical, smooth and shining, usually without hairs (Fig. 11) .....**Anthidiini**

4(2). Outer surfaces of fore and mid tibiae apically with an acute angle (usually projecting into a spine) and distinct notch anteriorly (Fig. 14); arolia absent, except in a few tropical Old World taxa (*Noteriades*, *Matangapis* and *Megachile* subgenus *Heriadopsis*); body nonmetallic or nearly so.....**Megachilini**

—. Outer surfaces of fore and mid tibiae apically without an acute angle (usually projecting into a spine) and distinct notch anteriorly; arolia present; body sometimes metallic green, blue, or brassy.....**5**

5(4). White or yellow integumental markings on thorax, abdomen, and legs, although markings on legs of females less conspicuous than those of males; male clypeus white or yellow; thorax extremely elongate, with pronotum projecting anteriorly such that it is visible when seen from above; dorsal surface of metanotum extending posteriorly in roughly the same plane as scutellum and not forming part of the vertical surface of the propodeum; male with one shiny tubercle on other side of the midline of S2; mouthparts extremely elongate, nearly the same length as body when fully extended.....**Ochreriadini**

—. All tagmata without white or yellow markings; thorax not overly elongate but *if* so (as in *Chelostoma*), *then* pronotum not visible when seen from above; S2 of males without shiny tubercles.....**6**

6(5). Males with large, exposed, quadrate T7 which fits inside an emargination in T6, such that the anterior lateral margins of T7 are overlapped by the posterior lateral margins of T6; maxillary palpi two-segmented; S1 of female *Pseudoheriades* with slender, erect spine; anterior margin of clypeus of female *Afroheriades* rounded, strongly dentate, and overlapping base of labrum.....**Pseuodoheriadini**

—. Males with T6 and T7 not as above; maxillary palpi three- to five- segmented but *if* two-segmented (as in some members of *Hoplitis (Proteriades)*), *then* T6 and T7 of males not as above, S1 of female without slender, erect spine, and anterior margin of clypeus of female not as above.....**Osmiini**

Appendix 3.1 R-script used to generate bootstrapped datasets. Script shown corresponds to the 31-taxon dataset. Script is written in black. Italicized comments in red placed after a pound sign (#) explain the various elements of the script.

```
x<-read.csv("megs_31_pi.csv", header=FALSE) # reads in molecular dataset as a .csv file
y<-read.csv("victors_test.csv",header=FALSE) # reads in morphological dataset as a .csv file

# WARNING: you will see NO error message if the taxa in both the molecular and the morphological datasets are not in the same order. Make sure they are!

taxa<-x[,1] # creates a single-column matrix whose elements represent the names of the taxa in the analysis

x_no_taxa<-x[,-1] # these two lines remove the first column of values from the molecular
y_no_taxa<-y[,-1] # and morphological data matrices - this column corresponds to the names of the taxa.

i<-1 # starts the counter at one - script will continue to loop until the number specified in the line below is reached
while(i<101)
  {s_stems<-sample(1:56,size=7,replace=TRUE) # creates a vector of seven numbers randomly chosen with replacement on the interval from 1 to 56 - corresponds to partition “stems”
  s_loops<-sample(57:101,size=6,replace=TRUE) # creates a vector of six numbers randomly chosen with replacement on the interval from 57 to 101 - corresponds to partition “loops”
  s_one<-sample(102:193,size=12,replace=TRUE) # creates a vector of twelve numbers randomly chosen with replacement on the interval from 102 to 193 - corresponds to partition “one”
  s_two<-sample(194:320,size=16,replace=TRUE) # creates a vector of sixteen numbers randomly chosen with replacement on the interval from 194 to 320 - corresponds to partition “two”
  s_three<-sample(321:932,size=78,replace=TRUE) # creates a vector of sventy-eight numbers randomly chosen with replacement on the interval from 321 to 932 - corresponds to partition “three”
  s_four<-sample(933:1384,size=58,replace=TRUE) # creates a vector of fifty-eight numbers randomly chosen with replacement on the interval from 933-1384 - corresponds to partition “four”

  matrix_stems<-x_no_taxa[,s_stems] # uses the vector “s_stems” generated above to sample from the stems partition of the molecular matrix
```

```
  matrix_loops<-x_no_taxa[,s_loops] # uses the vector “s_loops” generated above to sample from the loops partition of the molecular matrix
  matrix_one<-x_no_taxa[,s_one] # uses the vector “s_one” generated above to sample from the first partition of the molecular matrix
  matrix_two<-x_no_taxa[,s_two] # uses the vector “s_two” generated above to sample from the second partition of the molecular matrix
  matrix_three<-x_no_taxa[,s_three] # uses the vector “s_three” generated above to sample from the third partition of the molecular matrix
  matrix_four<-x_no_taxa[,s_four] # uses the vector “s_four” generated above to sample from the fourth partition of the molecular matrix

  mol_matrix<-
cbind(matrix_stems,matrix_loops,matrix_one,matrix_two,matrix_three,matrix_four)
# binds all of the characters sampled in the block above into a single matrix
  final_mol_matrix<-as.data.frame(t(mol_matrix)) # converts this matrix into a dataframe where rows represent taxa and columns represent individual charcters

  boots_morph<-sample(1:177,size=177,replace=TRUE) # creates a vector of 177 numbers randomly chosen with replacement on the interval from 1 to 177
  matrix_1_morph<-y_no_taxa[,boots_morph] # uses the vector “boots_morph” created in the line above to sample from the morphological matrix, thereby creating a new matrix
  matrix_2_morph<-as.data.frame(t(matrix_1_morph))   # converts the matrix generated in the line above into a data frame where rows represent taxa and columns represent individual characters

  super<-rbind(final_mol_matrix,matrix_2_morph) # binds the bootstrapped molecular matrix (“final_mol_matrix”) and the bootstrapped morphological matrix (“matrix_2_morph”) into a single matrix
  colnames(super)<-taxa # reattaches the list of taxa to the final bootstrapped matrix

  write.nexus.data(super,file=paste("matrix_",i,".nex",sep=""),format="dna",datablo
ck=TRUE, interleaved=FALSE,charsperline=NULL,gap=NULL,missing=NULL) #
converts the bootstrapped matrix into a Nexus file; to use the “write.nexus.data” function, you need to load the “ape” library in R
  cat(paste("begin paup;\n","set autoclose=yes warnreset=no
increase=auto;\n","set criterion = parsimony;\n",sep="","set
rootmethod=outgroup;\n","outgroup Melitta_leporina Apis_mellifera
Ceratina_calcarata Macropis_nuda/Only;\n","set
outroot=polytomy;\n","HSearch/addseq=random hold=4 nreps=20;\n","SaveTrees
BrLens=yes File=megs_mol_morph.tre replace = no
append=yes;\n","quit;\n","end;\n"), file = paste("matrix_",i,".nex",sep=""), sep = "",
fill = FALSE, labels = NULL,append = TRUE) # pastes all the parameters of the parsimony analysis for use in PAUP* to the bottom of the newly created Nexus file
```

```
  x<-readLines(paste("matrix_",i,".nex",sep="")) # the "write.nexus.data" function
used above creates a Nexus file only partially suitable for our purposes; the file
must therefore be modified. this line of script assigns the Nexus file that we've just
produced to the variable "x" and allows us to modify it..
 symbols<-"abcdefghijklmnopqrstuvwxyz0123456789" # will be used to specify the
symbols used in our modified Nexus file for PAUP*
 options(useFancyQuotes=FALSE) # makes sure the quotation marks used in our
Nexus file are not "fancy" and will be legible by PAUP*
 y<-sub("DNA",paste("standard symbols=",dQuote(symbols)),x) # pastes the
necessary modifications at the top of the Nexus file
 cat(y,file=paste("matrix_",i,".nex",sep=""),sep="\n") # renames the new Nexus file

  i<-i+1 # bumps the counter up one and returns to the top of the "while" loop
  }
```

Appendix 3.2 Perl script used to automate analyses of bootstrap replicates in PAUP*. This script sends each of the 100 bootstrapped matrices generated by the script in Figure 3.1 to PAUP* for analysis.

```
#!/usr/bin/perl
 $N=101;
 for($i=1; $i<$N;$i++){
        $nexFile= ("matrix_" . $i . ".nex");
        system("./paup_x86 $nexFile");
}
```

Appendix 3.3 R-script used to parse file produced by script in Figure 3.2 to produce a tree file readable in PAUP*. Script is written in black. Italicized comments in red placed after a pound sign (#) explain the various elements of the script.

```
tree_file<-readLines("megs_mol_morph.tre"); # reads in the tree file produced by scripts
in Figures 3 and 4
write(paste("#NEXUS\n","begin trees;\n"),"output_file.txt") # pastes in a Nexus block at
the top of the file
      i<-1; # starts the counter at one - script will continue to loop until the number
      specified in the line below is reached
while (i<=length(tree_file)) { # these lines parse through the tree file and export all of the
lines that correspond to trees to a file called "output_file.txt"; all other lines are ignored
      if (grepl("tree PAUP_",tree_file[i])==TRUE)
        write(tree_file[i],"output_file.txt",append=TRUE);
      i<-i+1
      }
      cat("end;",file="output_file.txt",append=TRUE); # adds "end;" to the end of the file
so the file can be read by PAUP*
```

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CHAPTER 4

THE EVOLUTION OF NESTING BEHAVIOR AND CLEPTOPARASITISM IN THE BEE TRIBE ANTHIDIINI (HYMENOPTERA: MEGACHILIDAE)

Abstract

Members of the megachilid tribe Anthidiini are colorful and easily recognizable bees that exhibit fascinating behavior related to nest-building and cleptoparasitism. Although the behavior of anthidiine bees has intrigued scientists for well over a century, the phylogenetic history of the tribe remains a mystery and the evolutionary origins of nest building behavior and cleptoparasitism in Anthidiini are entirely unknown. In order to reconstruct the evolutionary history of the tribe, I assembled a four-gene dataset and performed phylogenetic analyses using Bayesian- and maximum likelihood-based methods. I discuss the evolutionary history of nesting behavior and trace the origins of cleptoparasitism and the evolution of cleptoparasitic strategy in Anthidiini using Bayesian ancestral state reconstructions. My results strongly support the presence of three suprageneric groups within Anthidiini: the *Trachusa* group, the *Anthidium* group, and the *Dianthidium* group. Each of these groups favors different materials in nest construction: the *Trachusa* group builds nests with plant resins and, in several subgenera, strips of leaves; the *Dianthidium* group builds nests with plant resins and sometimes sand or pebbles but without leaves; and the *Anthidium* group favors plant fibers in nest construction. My results further imply a correlation between primary nest building material and female mandibular morphology. In contrast to the hypotheses of other authors, my phylogeny supports two independent origins of cleptoparasitism in Anthidiini and suggests that cleptoparasitic lineages with hospicidal adults are an evolutionary intermediate between nest-building bees and

cleptoparasitic lineages with hospicidal larvae. I also present a revised suprageneric classification for Anthidiini.

4.1 Introduction

The megachilid tribe Anthidiini is one of the most diverse bee tribes in the world, containing over 800 species and currently divided into thirty-seven genera (Michener 2007). Anthidiines are found on all continents except Antarctica and are often easily distinguishable from other megachilids by their dark cuticula and striking yellow, white, or red integumental markings (Ascher and Pickering 2011; Michener 2007). Anthidiine nesting behavior is unusual among megachilids and the French entomologist, Jean-Henri Fabre, noted the "resinous putty" and "felted cotton" that characterizes the nests of anthidiine bees (Fabre 1914). Fabre's descriptions of anthidiine nesting material highlight one of the more intriguing aspects of their nesting biology: unlike members of the megachilid tribes Osmiini and Megachilini, whose primary nest-building materials may include leaf pieces, mud, pebbles, resin, flower petals, and masticated leaf pulp, the preferred *materia prima* of Anthidiini is almost exclusively limited to one of two principal sources: plant resins and plant fibers.

Fabre was among the first to divide the tribe Anthidiini into two broad groups based on primary nest-building material (Fabre 1923). He recognized *les résiniers*, those anthidiines which use plant resins to build their nest cells, as a separate group from *les cotonniers*, which include those anthidiines which use plant fibers to build their nest cells. Michener's (2007) suprageneric classification also divided Anthidiini into two groups: Series A includes those anthidiines in which the females have three or four rounded or blunt mandibular teeth separated by shallow concavities, while Series B anthidiines have five or more sharp teeth separated by

acute V-shaped notches (Michener 2007). While Michener's groups are based on mandibular morphology and Fabre's groups are based on nest-building material, Michener's Series A coincides with *les résiniers* of Fabre and his Series B coincides with *les cotonniers* of Fabre, implying a relationship between female mandibular morphology and choice of nesting material (Perez 1879, 1889; Pasteels 1977).

Lepeletier (1841) assigned the generic name *Diphysis* (from Greek: "two natures") to members of the anthidiine genus *Trachusa*: he considered the overall appearance of *Trachusa* to resemble the apid genera *Anthophora* and *Eucera* but noted that female *Trachusa* carry pollen on a metasomal scopa and male *Trachusa* lack the long antennae typical of male *Eucera*. Although the name *Diphysis* was later synonymized with *Trachusa*, Schenck (1861) used the name *Diphysis* as the basis for the modern German vernacular term for *Trachusa*: *Bastardbiene* (from German: "hybrid bee"). Friese (1923) also favored the term *Bastardbiene* but apparently for different reasons than those of Schenck: he considered the short broad abdomen and the yellow clypeus of males to be *Anthidium*-like and the black cuticle and the absence of abdominal spines to be *Megachile*-like. While the number of species included in the genus *Trachusa* has increased substantially since the original publications of Lepeletier, Schenck and Friese, and the phylogenetic affinity of this genus now clearly lies with the tribe Anthidiini, *Bastardbiene* may still be an appropriate nickname for the genus: most members of *Trachusa* build their nests with a strange combination of resin and long strips of cut leaves, a behavior not seen in other anthidiines. The mandibular morphology of *Trachusa* is consistent with that of Michener's (2007) Series A but its unique choice of nesting materials makes it difficult to assign to either of the groups discussed by Fabre (1923).

Not all anthidiines, however, are nest-building. Seven genera are recognized as cleptoparasites (Michener 2007). Cleptoparasitism is defined as the theft by one organism of the provisions or resources of another organism; such behavior has been reported extensively in a broad range of animals and may take the form of food theft, as seen in sharks (Klimley 2001), hyaenas (Höner et al. 2002), frigatebirds (Vickery and Brooke 1994) and snails (Iyengar 2005), or resource theft, as seen in silk-stealing spiders (Theridiidae, *Agyrodes*; Tso and Severinghaus 1998) and hay-stealing pikas (Ochotonidae, *Ochotona*; McKechnie et al. 1994). Cleptoparasitism in bees is most similar to the brood parasitism seen in the common cuckoo (Cuculidae, *Cuculus canorus*): cleptoparasitic bees, like the common cuckoo, neither build nor provision their own nests but instead lay their eggs in nests that are built and provisioned by a host. In contrast to the common cuckoo, whose offspring are fed directly by a host bird, the offspring of a cleptoparasitic bee never comes into contact with the host bee. Instead, the offspring of the cleptoparasite develops on the pollen provisions stored by the host for her own offspring.

Solitary bees provision their nest cells with just enough pollen to sustain the growth of a single larva (Minckley et al. 1994; Schlindwein and Martins 2000; Schlindwein et al. 2005; Müller et al. 2006). The provisions in one brood cell, therefore, are insufficient to nourish both the cleptoparasite and the host larva. In order to eliminate the competition for resources presented by the hosts' larvae, cleptoparasitic bees have evolved two distinct strategies (Rozen and Kamel 2009). In some cleptoparasitic bees, the adult female cleptoparasite removes or kills the host eggs and larvae (hospicidal[1] adult); in others, one or more cleptoparasitic larval instars, not the adult female, kill the host eggs and larvae (hospicidal larvae). Hospicidal larvae are

[1] Rozen (1989) introduced the term "hospicidal" (from Latin: "host killer") to describe those cleptoparasitic bee larvae with special modifications (such as modified mandibles) used to destroy the host eggs or larvae. In this chapter, I apply the term more broadly and use it to specify which life stage of the cleptoparasite (adult or larva) kills the host.

acutely aggressive and usually endowed with long, sharp mandibles which they use to destroy the eggs or larvae of their host (Rozen 1966; Rust and Thorp 1973; Rozen 1987; Torchio 1989; Rozen 2001; Rozen and Hall 2011). Larvae exhibiting such mandibles have evolved independently in multiple cleptoparasitic bee lineages, including members of the megachilid tribes Anthidiini (e.g. the subgenus *Stelis* (*Stelis)*) and Megachilini (e.g. *Coelioxys*) (Rozen et al. 2010), as well as members of the apid subfamily Nomadinae and their close relatives (Rozen and Michener 1968; Rozen et al. 1978). In contrast, the larvae of hospicidal adults are usually nonhospicidal; such larvae are unaggressive and lack mandibular modifications.

Four independent origins of cleptoparasitism are known in the bee family Apidae (Cardinal et al. 2010); multiple other independent origins are postulated in the bee families Colletidae (one origin), Andrenidae (one origin), Halictidae (nine origins), and Megachilidae (ten origins) (Rozen 2003; Michener 2007). Of the ten origins in the family Megachilidae, six are proposed in the tribe Anthidiini. Cleptoparasitic lineages typically display either hospicidal larvae or hospicidal adults; the tribe Anthidiini is exceptional because certain lineages appear to contain both modes of cleptoparasitism. This, in combination with the well-documented nesting behavior of anthidiine bees in general, makes Anthidiini an outstanding model group for understanding the evolutionary transition from nest-building to cleptoparasitism, as well as the evolution of cleptoparasitic strategy in bees.

Despite the fascinating natural history of Anthidiini, there has been no treatment of the phylogenetic relationships among lineages of anthidiine bees and the evolutionary history of Anthidiini remains largely unknown. The only existing cladistic analysis of Anthidiini was presented by Müller (1996); it included only non-cleptoparasitic Palaearctic anthidiines,

however, making it difficult to assess relationships on a world-wide basis. In the following paper I reconstruct the evolutionary history of the tribe Anthidiini using model-based methods and a large, multi-locus dataset. I offer the first molecular-based phylogenetic hypothesis of the generic and suprageneric relationships within Anthidiini based on maximum likelihood and Bayesian phylogenetic analyses and trace the evolutionary history of nesting behavior, the origins of cleptoparasitism, and the evolution of cleptoparasitic strategy in Anthidiini using Bayesian ancestral state reconstructions. I discuss the correlation between anthidiine mandibular morphology and primary nest-building material, the evolution of host choice in cleptoparasitic Anthidiini, the significance of my results on modern anthidiine classification and the impact of my results on understanding the evolution of cleptoparasitism and cleptoparasitic strategy in all bees.

4.2 Methods and Materials

4.2.1 Taxon sample

For molecular phylogenetic analyses, I sampled extensively in the tribe Anthidiini, choosing 105 representative species from 27 genera and 63 subgenera (Table 4.1). I included five of seven cleptoparasitic genera and sampled densely within the genus *Stelis*. I chose fifteen outgroup taxa representing the megachilid tribes Osmiini, Megachilini, and Aspidosmiini (Gonzales et al., submitted), as well as the genera *Pseudoheriades* and *Ochreriades* (Table 4.1). Collection localities and DNA voucher numbers are listed in Table 4.1. Voucher specimens are deposited in the Cornell University Insect Collection.

The tribe Anthidiini contains many species-poor genera exhibiting limited geographic

distributions; as a consequence, a number of taxa could not be included here. My taxon sample represents 73% of the total generic-level diversity of the tribe; many of the missing taxa are rare monotypic genera, at least two of which are known only from single specimens. Nevertheless, this taxon sample provides a robust backbone for the first phylogenetic hypothesis of relationships within Anthidiini and serves as an excellent framework on which to study the evolution of nesting behavior and cleptoparasitism in Anthidiini and in bees in general.

4.2.2 Dataset and alignment

I sequenced a total of 3769 base pairs from four nuclear protein-coding genes: CAD (880 base pairs), NAK (1489 base pairs), LW-rhodopsin (673 base pairs) and EF1-alpha, F2 copy (727 base pairs). All DNA extraction and sequencing protocols follow Danforth et al. (1999). PCR primers and conditions for CAD, NAK, and LW-rhodopsin were identical to those listed in Chapter 2. For EF1-alpha, we used the forward primer Haf2for1 (5' GGG YAA AGG WTC CTT CAA RTA TGC 3') together with an anthidiine-specific reverse primer, F2RevAnth (5' AAT CAG CAG CRC CYT TCG GTG G 3'). The PCR conditions for this set of primers were 45s@94°C /45s@58°C /1m@72°C, run for 36 cycles; the PCR runs were preceded by 5 minutes at 94°C and followed by 7 minutes at 72°C.

Sequencing was performed at the Cornell University Life Sciences Core Laboratories Center using an Applied BioSystems 3730xl DNA analyzer. Sequences were edited using Sequencher version 5.0 sequence analysis software (Gene Codes Corporation 2011). Alignments were performed using MAFFT (Katoh et al. 2002) and then adjusted by eye in MacClade (Maddison and Maddison 2005); all introns were removed.

4.2.3 Partitioning regime

In order to establish a partitioning regime, I ran a preliminary Bayesian analysis in which each of the three codon positions from each gene represented a unique partition, amounting to twelve total partitions. I used MrBayes v.3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) to run three independent analyses of 10,000,000 generations each under a GTR model. I then used Tracer (Rambaut and Drummond 2007) to eliminate an appropriate burnin and to summarize the substitution rates for each of the twelve partitions. I grouped similar partitions together and chose the following partitioning regime: Partition 1 contained the first codon positions of CAD and LW-rhodopsin (517 base pairs); partition 2 contained the first codon positions of NAK and EF1-alpha and the second codon positions of CAD and LW-rhodopsin (1255 base pairs); partition 3 contained the second codon positions of NAK and EF1-alpha (738 base pairs); partition 4 contained the third positions of CAD and NAK (791 base pairs); and partition 5 contained the third codon positions of EF1-alpha and LW-opsin (468 base pairs).

I experimented with two alternative partitioning regimes. In the first, each individual gene constituted a unique partition, resulting in four total partitions. In the second, the three codon positions of each gene were combined, resulting in three total partitions. For all three partitioning regimes, I ran four independent analyses of 15,000,000 generations each (60,000,000 total generations for each of the three partitioning regimes) under a GTR+I+Γ model (see the section on *Model Testing* below). After eliminating an appropriate burnin, I used AICc and BIC scores to evaluate the performance of all three partitioning regimes (McGuire et al. 2007). The AIC and BIC scores associated with the partitioning regime based on substitution rate (AICc = 96674.5, BIC = 99029.9) were lower than those corresponding to the partitioning regime by gene (AICc = 99536.2, BIC = 101817.9) and those corresponding to the partitioning regime by codon position

Table 4.1: Complete taxon list, DNA voucher numbers, collection localities and dates for specimens used in this study

Taxon	Voucher number	Collection locality
Afranthidium (Afranthidium) karooense	1588	NCP: 42 km S Eksteenfontein, 9.x.2008
Afranthidium (Branthidium) micrurum	1592	NCP: Richtersveld National Park, 13.x.2008
Afranthidium (Branthidium) minutulum	1593	NCP: Richtersveld National Park, 13.x.2008
Afranthidium (Capanthidium) capicola	1594	WCP: Clanwilliam, 19.x.2008
Afranthidium (Capanthidium) rubellulum	1610	SOUTH AFRICA: NCP. 42 km S Eeksteenfontein, 9.x.2008
Afranthidium (Domanthdium) abdominale	1644	SOUTH AFRICA: EC, 42 km NW Cradock, 02.iii.2010
Afranthidium (Immanthidium) immaculatum	1629	SOUTH AFRICA: EC,74 km E Barkly East, 04.iii.2002
Afranthidium (Immanthidium) junodi	1634	SOUTH AFRICA: EC,74 km E Barkly East, 04.iii.2002
Afranthidium (Immanthidium) repetitum	1632	SOUTH AFRICA: NP, 30 km W Sibasa, 30.iii.2002
Afranthidium (Immanthidium) sjoestedti	1633	SOUTH AFRICA: FS, Tussen Die Riviere NR (near Bethulie, 1474m), 02.iii.2002
Afranthidium (Mesanthidium) carduele	1596	GREECE: Lesvos, vic Pyrra, Kalloni Bay, 16.vi.2007
Afranthidium (Nigranthidium) sp. nov. 1	1611	SOUTH AFRICA: NCP. 10 km E Nabapeep, 15.x.2008
Afranthidium (Nigranthidium) poecilodontum	1612	SOUTH AFRICA: NCP. 10 km E Nabapeep, 15.x.2008
Afranthidium (Nigranthidium) sp.	1452	South Africa, NCP, Nabapeep, 15.x.2008
Afranthidium (Oranthidium) folliculosum	1640	SOUTH AFRICA: NC, 12km N Kuruman, 09.iii. 2010
Anthidiellum (Loyolanthidium) notatum	1387	NV: Clark Co. E. CC Spring, 19.vii.2004
Anthidiellum (Chloranthidiellum) sp. nov. 1	1608	Tanzania: Dodoma, Region: 62km E Dodoma, 03.i.2003
Anthidiellum (Pycnanthidium) absonulum	1635	SOUTH AFRICA: KZN,Kulene Experimental Farm, 20 km N Hluhluwe, 09-12.iii.2002
Anthidiellum (Pycnanthidium) sp.	1647	SOUTH AFRICA: EC,W Baviaanskloof P.P., 27.ii.2010
Anthidium (Anthidium) cockerelli	1385	NV: Clark Co. Yucca Gap, 17.v.2004
Anthidium (Anthidium) chilense	1625	CHILE: Coquimbo Province,13 km E Vicuna, Rt.41, 21.x.2009
Anthidium (Anthidium) colliguayanum	1624	CHILE: Coquimbo Province,13 km E Vicuna, Rt.41, 21.x.2009
Anthidium (Anthidium) deceptum	1642	PERU: Ica, E of Nazca, 15 km marker on Hwy 30A, 02.iv.2010
Anthidium (Anthidium) porterae	645	NM:Hidalgo Co., 20 mi S Animas, 17.ix.1999
Anthidium (Anthidium) punctatum	1554	Switzerland, Weiach, 29.vi.2004
Anthidium (Callanthidium) illustre	1384	NV: Clark Co. Lovell Cyn.,16.vi.2004
Anthidium (Gulanthidium) sp.	1637	IRAN: 13 km E Kalameh, road Busher-Shiraz, 03.vi.2009
Anthidium (Proanthidium) oblongatum	505	NY: Tompkins Co., Ithaca, 01.vii.1999
Anthidium (Severanthidium) cordiforme	1628	SOUTH AFRICA: NP, 26 km W Messina, 18.iii.2002
Anthidium (Turkanthidium) gratum	1598	UZBEKISTAN: Bukara Prov,40 km NE Gazli, 31.v. 2008
Anthidium (Turkanthidium) unicum	1597	UZBEKISTAN: Qarschi Prov, 25 km SE Muborak, 02.vi.2008
Anthodioctes (Anthodioctes) mapirense	1519	Bolivia, La Paz, Puente Villa, 11.iii.2011
Anthodioctes (Bothranthidium) lauroi	1649	BOLIVIA: La Paz, 5 km W Mapiri, 16-18.iii.2001
Austrostelis catamarcensis	1599	ARGENTINA: Salta Prov, Carayete, 10 km S, 24.x - 13.xi.2003
Bathanthidium (Manthidium) binghami	1536	Thailand, Petchabun Nam NP, 1-8.iii.2007
Benanthis madagascariensis	1518	Madagascar, Tulear, Androy, x.2002
Dianthidium (Adanthidium) arizonicum	1386	UT: Garfield Co. Escalante, 27.vi.2002
Dianthidium (Dianthidium) subparvum	1268	UT: Cache Co. BSFC, Left hand fork, 05.viii.2003
Dianthidium (Mecanthidium) sonorum	1648	MEXICO: Sonora, 40 km E Alamos, Rancho Palo Injerto, 30.ix.2006
Duckeanthidium thielei	1607	Costa Rica, Heredia, La Selva Biol. Stn., Puerto Viejo de Sarapiquí, 05.1.1999
Eoanthidium (Clistanthidium) turnericum	1589	NCP: Eksteenfontein, 9.x.2008
Eoanthidium (Eoanthidium) clypeare	1436	Jordan, Wadi Shuyab, vi.2007
Epanthidium (Epanthidium) bicoloratum	1441	Argentina, Catamarca, Trampasacha, 25.x -12.xi.2003
Euaspis abdominalis	1627	SOUTH AFRICA: NP,14 km E Vivo, 17.iii.2002
Euaspis polynesia	1426	Thailand, Prachup Kiri Khan Province, Pranburi District, 24.vi.2003
Hoplostelis bivittata	1636	Panama, Veraguas Province, Rancheria Island, 18.vii.2009
Hypanthidioides (Saranthidium) marginata	CP2	Paraguay, Guaira, Res. de Recursos, Manejados 24.i.2007
Hypanthidium (Hypanthidium) obscurius	SC171	Paraguay, Paraguarí, M. Nat. Acahay, 17.i.2007
Icteranthidium ferrugineum flavum	1432	UZ, Karakalpakstan, Beruni, 25.v.2008
Notanthidium (Allanthidium) rodolfi	1623	CHILE: Coquimbo Province,13 km E Vicuna, Rt.41, 21.x.2009
Notanthidium (Notanthidium) steloides	1542	Chile, Region Metro, Farellones, 31.xii.2008
Pachyanthidium (Ausanthidium) ausense	1591	NCP: Richtersveld National Park, 11.x.2008
Pachyanthidium (Pachyanthidium) bicolor	1606	Kenya, Coast Province, Taita Hill Discovery Centre, 13-14.xii.2002
Pachyanthidium (Pachyanthidium) cordatum	1631	SOUTH AFRICA: KZN,Ithala Nature Reserve, near Louwsburg, 07.iii.2002
Pachyanthidium (Trichanthidium) benguelense	1434	SA: Limpopo Prov., 27 km E Waterpoort, 07.i.2004
Pachyanthidium (Trichanthidium) sp.	1646	SOUTH AFRICA: EC,W Baviaanskloof P.P., 27.ii.2010
Paranthidium (Paranthidium) jugatorium	495	NY: Tompkins Co., Ithaca, 31.vii.1997
Paranthidium (Rapanthidium) sp. nov. 2	1604	Mexico, Clima, San Antonio, La Becarrera, 10.x.2008
Plesianathidium (Carinanthidium) cariniventre	1595	WCP: Hoek se Berg, E Clanwilliam, 20.x.2008
Plesianthidium (Spinanthidiellum) rufocaudatum	1609	SOUTH AFRICA: NCP. Nieuwoudtville Wildflower Preserve, 18.x.2008
Plesianthidium (Spinanthidiellum) volkmanni	1449	South Africa, NCP, Eksteenfontein, 09.x.2008
Plesianthidium (Spinanthidium) calescens	1279	SOUTH AFRICA: WCP, 7 km W. Nieuwoudtville, 9.x. 2002
Plesianthidium (Spinanthidium) trachusiforme	1613	SOUTH AFRICA: NCP. 10 km E Nabapeep, 15.x.2008
Pseudoanthidium (Exanthidium) eximium	1600	ITALY: Piemonte, Susa, Salbertrand, 01.vii. 2006
Pseudoanthidium (Micranthidium) sp. nov. 3	1605	Tanzania, Kilimanjaro Region, 19km SE Same, 14.i.2003
Pseudoanthidium (Micranthidium) sp.	1641	GHANA: Central UCC, Valco Gardens, 01.xi.2008

Table 4.1 (continued)

Taxon	Voucher number	Collection locality
Pseudoanthidium (Pseudoanthidium) scapulare	1601	ITALY: Toscana, Massa Maritima, 28.vii.2005
Pseudoanthidium (Royanthidium) melanurum	1438	Greece, Kalogria, 25.v.2006
Rhodanthidium (Meganthidium) superbum	1638	IRAN: Yasuj Region,Margoon Waterfall, 29.v.2009
Rhodanthidium (Rhodanthidium) septemdentatum	1514	GR, Rhodos, Stegna, 08.v.2005
Serapista rufipes	1450	South Africa, NCP, Eksteenfontein, 09.x.2008
Serapista soni	1626	SOUTH AFRICA: NP, 1 km N Vivo,17.iii.2002
Serapista sp.	1643	SOUTH AFRICA: NC, N of Salt Lake, S. Douglas, 06.iii.2010
Afrostelis sp.	1645	SOUTH AFRICA: NC,1km S Campbell, 07.iii.2010
Stelis (Dolichostelis) laticincta	1389	CA: Mariposa Co. Yosem. Valley, 27.vi.2005
Stelis (Heterostelis) hurdi	1409	CA: San Benito Co. Pinnacles, 22.v.1999
Stelis (Protostelis) signata	1440	CH, Hohtenn, 26.v.2005
Stelis (Stelidomorpha) nasuta	1614	GREECE: Atiiki, Athens pref., 20km S Athens, 15.vi.2006
Stelis (Stelis) aff. *robertsoni*	1408	CA: Mariposa Co. Yosem. Valley, 05.vii.2006
Stelis (Stelis) anthocopae	1392	NV: Clark Co. St. Thomas Gap, 12.v.2005
Stelis (Stelis) anthracina	1396	NV: Clark Co. St. Thomas Gap, 15.iv.2005
Stelis (Stelis) broemelingi	1391	AZ: Cochise Co. E. Apache, 29.viii.2003
Stelis (Stelis) calliphorina	1403	CA: Mariposa Co. Moraine Dome, 06.vii.2005
Stelis (Stelis) carnifex	1395	CA: Mariposa Co. Eagle Peak, 19.v.2006
Stelis (Stelis) foederalis Gv. sp. B	1398	CA: Maripsoa Co. Ostrander Rocks
Stelis (Stelis) holocyanea	1405	CA: Mariposa Co. Deer Camp, 09.viii.2006
Stelis (Stelis) interrupta	1406	CA: Mariposa Co. Ostrander Rocks, 03.vii.2005
Stelis (Stelis) joanae	1410	NV: Clark Co. St. Thomas Gap, 21.iv.2004
Stelis (Stelis) lamelliterga	1400	UT: Kane Co. Kitchen Corral Sp., 29.v.2002
Stelis (Stelis) lateralis	1401	UT: Washington Co. Rimrock Sp., 11.v.2006
Stelis (Stelis) linsleyi	1271	CA: Madera Co., Yosemite National Park, 11.vii.2005
Stelis (Stelis) monticola	1402	CA: Mariposa Co. Moraine Dome, 05.vi.2006
Stelis (Stelis) occidentalis	1399	CA: Tuolumne Co. White Wolf, 08.vii.2006
Stelis (Stelis) paiute	1394	NV: Clark Co. Jean Lake, 14.iv.2004
Stelis (Stelis) palmarum	1393	UT: Kane Co. Paradise Cyn., 26.v.2003
Stelis (Stelis) pavonina	1404	CA: Tuolumne Co. Mammoth Peak, 29.vii.2004
Stelis (Stelis) punctulatissima	1551	Switzerland, Hohtenn, 26.v.2005
Stelis (Stelis) semirubia	1407	CA: Madera Co. Parsons Peak, 02.viii.2004
Stelis (Stelis) subcaerulea	1397	CA: Mariposa Co. Bernice Lake, 09.viii.2006
Stelis rozeni	1603	bar code BBSL761312
Trachusa (Archianthidium) pubescens	1533	Turkey, Erzurum, Akören, 15 km N Hinis, 19.vii.2003
Trachusa (Congotrachusa) schoutedeni	1538	Rep. of Congo, Dept. Pool, Iboubikro, Lesio-Loun, 9-15.ix.2008
Trachusa (Heteranthidium) larreae	1142	NV: Clark Co., Las Vegas Sand Dunes, 1.iv.2004
Trachusa (Paraanthidium) interrupta	1602	ITALY: Piemonte, Susa, 2.vii.2006
Trachusa (Trachusa) byssina	1558	Switzerland, Splügen, 23.vii.2005
Afroheriades primus	1585	SA, N. Cape, 6 km N Concordia, 14.ix.2007
Aspidosmia arnoldi	1544	South Africa, NCP, Eksteenfontain, 09.x.2008
Aspidosmia volkmanni	1579	SA, N. Cape, Richtersveld, near De Koci, 09.ix.2007
Chelostoma (Chelostoma) florisomne	1553	Switzerland, Chur
Hoplitis (Hoplitis) adunca	1552	Italy, Aosta, 30.08.2004
Megachile (Chelostomoides) spinotulata	1435	USA, AZ, Portal, Rucker Canyon, 31.viii.2008
Megachile (Gronoceras) bombiformis	1531	South Africa, Limpopo Prov, 20 km E Waterpoort, 07.i.2004
Megachile (Lithomegachile) texana	1524	USA, NY, Ithaca, x.2008
Noteriades sp.	1580	Thailand, Chiang Mai, 24.iii.2007
Ochreriades fasciatus	1557	Jordan, 20 km W Amman, 24.iv.2007
Osmia (Osmia) lignaria	1265	no locality data
Othinosmia (Othinosmia) securicornis	1584	SA, N. Cape, Richtersveld, near De Koci, 09.ix.2007
Protosmia (Protosmia) humeralis	1559	Jordan, Wadi Shu'ayb, 22.iv.2007
Pseudoheriades moricei	1431	IL, Negev
Stenoheriades asiaticus	1578	Greece, Zachlorou, 22.v.2006

Table 4.2 AICc and BIC scores for three different partitioning regimes. For each partitioning regime tested, we show the total number of partitions included in each analysis; the harmonic mean of the -lnL score associated with each partitioning regime, as calculated from four independent Bayesian analyses; the number of parameters estimated from each partitioning regime, based on a GTR+I+Γ model; the length of the dataset (i.e. total number of nucleotides); and the total number of taxa in the analysis. The final two columns show the AICc and BIC score associated with each partitioning regime, calculated as a function of columns 2-5. Calculations for both AICc and BIC were performed following McGuire et al. 2007. The AICc and BIC scores shown in bold correspond to the preferred partitioning regime.

No. partitions, partitioning regime	**Harmonic mean (-lnL)**	**No. parameters**	**No. nucleotide sites**	**No. included taxa**	**AICc**	**BIC**
3 partitions, by codon	-48594.8	37	3679	122	97264.37	99472.09
4 partitions, by gene	-49718.4	49	3679	122	99536.23	101817.89
5 partitions, by rate	-48275.2	61	3679	122	**96674.47**	**99029.92**

(AICc = 97264.4, BIC = 99472.1) (Table 4.2). The partitioning regime based on substitution rate was thus implemented in all subsequent analyses.

4.2.4 Model testing

Best-fit models of nucleotide substitution were chosen using MrModelTest 2.3 (Nylander 2008). MrModelTest 2.3 employs both the hierarchical likelihood ratio test (hLRT) and the Akaike Information Criterion (AIC) (Akaike 1974) to select a best-fit model from 24 models of nucleotide substitution; I chose to assess model performance using AIC scores, based on the reasoning outlined by Posada and Buckley (2004). Independent model tests were run on each of the data partitions described above. The best-fit model for each partition was a general time reversible model with a gamma correction for among site rate variation and a proportion of invariant sites (GTR+I+Γ).

4.2.5 Bayesian analyses

Using the partitioning regime based on substitution rate described above, I used MrBayes v.3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) to run eight

independent phylogenetic analyses, each consisting of two independent runs using four exploratory chains. Each analysis was run between 26 and 36 million generations, resulting in a total of 448,072,000 generations. Each partition was analyzed using a GTR+I+Γ model. All parameters were unlinked across partitions and sampling was performed every 2000 generations. The default "temp" setting in MrBayes, which controls the swap frequency among the four exploratory chains, resulted in chain swap frequencies that were lower than those recommended by the user's manual; I adjusted the "temp" setting from the default of 0.2 to 0.05, which improved chain mixing and increased the proportion of successful chain swaps to within the recommended range of 10%-70%.

I used two methods to determine whether analyses had reached stationarity: (1) I compared the average standard deviation of split frequencies between both independent runs in each analysis and only accepted those analyses where this value had dropped below 0.01; (2) I visualized the parameter files from all analyses using Tracer (Rambaut and Drummond 2007), which allowed me to eliminate an appropriate burnin and determine whether all parameters had converged to stationarity. I eliminated 190,000,000 generations as the burnin, resulting in 258,072,000 total post-burnin generations; these were further subsampled to ensure independent sampling of trees. The final combined posterior distribution of 25,807 trees was used to build a maximum clade credibility tree using Tree Annotator v.1.6.1 (Drummond and Rambaut 2007) (Figure 4.1).

4.2.6 Maximum likelihood analyses

I also performed phylogenetic analyses using maximum likelihood-based methods with the software RAxML v.7.2.8 (sequential version raxmlHPC, Stamatakis 2005, 2006). I used the

rapid bootstrapping algorithm to run 1000 bootstrap replicates using the GTRCAT approximation. I employed the same partitioning regime as for the Bayesian analysis and used a maximum parsimony starting tree to begin each bootstrap replicate. The maximum likelihood bootstrap tree is shown as part of Figure 4.1.

4.2.7 Ancestral state reconstructions

I used BayesTraits (Pagel 1997, 1999) to reconstruct the number of origins of cleptoparasitism in the tribe Anthidiini. Each terminal taxon was coded as either nest-building (0) or cleptoparasitic (1); information was taken from the literature and all taxa were successfully coded as either 0 or 1 (Table 4.3). I used 1000 randomly selected trees from the posterior distribution of trees from my Bayesian analysis. I ran a preliminary maximum likelihood analysis to obtain values for the rates of transition from one state to another and used these rates to set the boundaries of the starting priors for Bayesian ancestral state reconstruction. The rate of transition from nest-making (0) to cleptoparasitic (1) was between 1.0 and 2.0 for each tree analyzed; the reverse rate was consistently zero. I therefore used a reversible jump model with an exponential prior seeded from a uniform distribution on the interval 0 to 5. Acceptance rates in preliminary analyses were lower than recommended; for this reason, I changed the "ratedev" value from the default setting of 2.0 to 16.0, which resulted in acceptance rates that fell within the recommended range of 20%-40%. I ran five independent analyses for 10,000,000 generations each and eliminated 2,000,000 generations as the burnin, resulting in 40,000,000 total generations.

I also tested for the possibility of a single origin of cleptoparasitism with multiple reversals to nest-building behavior. I used the BayesTraits command "fossil" to constrain the common ancestor of all cleptoparasitic Anthidiini to state (1). Using the same set of 1000 trees as

above, I ran five independent analyses for 10,000,000 generations each, using a reversible jump model with an exponential prior seeded from a uniform distribution on the interval 0 to 5 and a "ratedev" value set to 16.0. In order to determine if there was significant statistical support for one hypothesis over the other (multiple origins of cleptoparasitism vs. a single origin and multiple reversals), I compared the harmonic means of the likelihood scores from both analyses and used them to calculate a BayesFactor, which is defined as twice the difference in the harmonic means of the likelihood scores from both analyses (Kass and Raftery 1995).

I also used BayesTraits to reconstruct the evolution of cleptoparasitic strategy in Anthidiini. Terminal taxa were coded as nest-building (0), cleptoparasitic with hospicidal adults (1), or cleptoparasitic with hospicidal larvae (2) (Table 4.3). All known members of the subgenus *Stelis (Stelis)* are cleptoparasites with hospicidal larvae. In cases where the behavior of terminal taxa belonging to this subgenus was unknown, species were coded as (2). The behavior of the subgenus *Stelis (Heterostelis)* is unknown. Its final instar larva, however, has bidentate, unmodified mandibles (Thorp 1966), a feature characteristic of nonhospicidal larvae; the sole member of this subgenus in my dataset, *Stelis (Heterostelis) hurdi*, was therefore coded as (1). Other cleptoparasitic lineages for which no information was available were coded as (12). Preliminary ML-based analyses showed that transition rates between the three states ranged between zero and six; these values were used to set the priors for Bayesian ancestral state reconstruction. I used a reversible jump model with an exponential prior seeded from a uniform distribution on the interval zero to ten. In order to obtain acceptance rates within the recommended range of values, the "ratedev" value was adjusted to 11.0. I again ran five independent analyses for 10,000,000 generations each and eliminated 2,000,000 generations as the burnin, resulting in 40,000,000 total generations.

Table 4.3: Character coding for BayesTraits analysis. In Column A, each taxon included in my analysis is coded as either nest-building (0) or cleptoparasitic (1). In Column B, each taxon is coded as nest-building (0), cleptoparasitic with hospicidal adults (1), or cleptoparasitic with hospicidal larvae (2). In Column B, cleptoparasites whose mode of cleptoparasitism is unknown are coded as (12). See Chapter 1 for references regarding the behavior of individual species.

Taxon	(A)	(B)	Taxon	(A)	(B)
Afranthidium (Afranthidium) karooense	0	0	*Pachyanthidium (Pachyanthidium) bicolor*	0	0
Afranthidium (Branthidium) micrurum	0	0	*Pachyanthidium (Pachyanthidium) cordatum*	0	0
Afranthidium (Branthidium) minutulum	0	0	*Pachyanthidium (Trichanthidium) bengualense*	0	0
Afranthidium (Capanthidium) capicola	0	0	*Pachyanthidium Trichanthidium* sp.	0	0
Afranthidium (Capanthidium) poecilodontum	0	0	*Paranthidium (Paranthidium) jugatorium*	0	0
Afranthidium (Capanthidium) rubellulum	0	0	*Paranthidium (Rapanthidium)* new sp. 2	0	0
Afranthidium (Domanthidium) abdominale	0	0	*Plesianthidium (Carinanthidium) carniventre*	0	0
Afranthidium (Immanthidium) immaculatum	0	0	*Plesianthidium (Spinanthidiellum) ruficaudatum*	0	0
Afranthidium (Immanthidium) junodi	0	0	*Plesianthidium (Spinanthidium) calescens*	0	0
Afranthidium (Immanthidium) repetitum	0	0	*Plesianthidium (Spinanthidium) trachusiforme*	0	0
Afranthidium (Immanthidium) sjoestedti	0	0	*Plesianthidium (Spinanthidium)* sp.	0	0
Afranthidium (Mesanthidium) carduele	0	0	*Pseudoanthidium (Exanthidium) eximium*	0	0
Afranthidium (Nigranthidium) sp.	0	0	*Pseudoanthidium (Micranthidium)* sp.	0	0
Afranthidium (Oranthidium) folliculosum	0	0	*Pseudoanthidium (Micranthidium)* new sp. 3	0	0
Afranthidium new sp. 1	0	0	*Pseudoanthidium (Pseudoanthidium) scapulare*	0	0
Afrostelis sp.	1	12	*Pseudoanthidium (Royanthidium) melanurum*	0	0
Anthidiellum (*Chloranthidiellum)* new sp. 1	0	0	*Rhodanthidium (Meganthidium) superbum*	0	0
Anthidiellum (Loyolanthidium) notatum	0	0	*Rhodanthidium (Rhodanthidium) septemdentatum*	0	0
Anthidiellum (Pycnanthidium) absonulum	0	0	*Serapista rufipes*	0	0
Anthidiellum (Pycnanthidium) sp.	0	0	*Serapista soni*	0	0
Anthidium (Anthidium) chilense	0	0	*Serapista* sp.	0	0
Anthidium (Anthidium) cockerelli	0	0	*Stelis (Dolichostelis) laticincta*	1	1
Anthidium (Anthidium) colliguayanum	0	0	*Stelis (Heterostelis) hurdi*	1	1
Anthidium (Anthidium) deceptum	0	0	*Stelis (Protostelis) signata*	1	12
Anthidium (Anthidium) porterae	0	0	*Stelis (Stelidomorpha) nasuta*	1	1
Anthidium (Anthidium) punctatum	0	0	*Stelis (Stelis) anthocopae*	1	2
Anthidium (Callanthidium) illustre	0	0	*Stelis (Stelis) anthracina*	1	2
Anthidium (Gulanthidium) sp.	0	0	*Stelis (Stelis) broemelingi*	1	2
Anthidium (Proanthidium) oblongatum	0	0	*Stelis (Stelis) calliphorina*	1	2
Anthidium (Severanthidium) cordiforme	0	0	*Stelis (Stelis) carnifex*	1	2
Anthidium (Turkanthidium) gratum	0	0	*Stelis (Stelis) foederalis* Gv sp. B	1	2
Anthidium (Turkanthidium) unicum	0	0	*Stelis (Stelis) holocyanea*	1	2
Anthodioctes (Anthodioctes) mapirensis	0	0	*Stelis (Stelis) interrupta*	1	2
Anthodioctes (Bothranthidium) lauroi	0	0	*Stelis (Stelis) joanae*	1	2
Bathanthidium binghami	0	0	*Stelis (Stelis) lamelliterga*	1	2
Benanthis madagascariensis	0	0	*Stelis (Stelis) lateralis*	1	2
Dianthidium (Adanthidium) arizonicum	0	0	*Stelis (Stelis) linsleyi*	1	2
Dianthidium (Dianthidium) subparvum	0	0	*Stelis (Stelis) monticola*	1	2
Dianthidium (Mecanthidium) sonorum	0	0	*Stelis (Stelis) occidentalis*	1	2
Duckeanthidium thielei	0	0	*Stelis (Stelis) paiute*	1	2
Eoanthidium (Clistanthidium) turnericum	0	0	*Stelis (Stelis) palmarum*	1	2
Eoanthidium (Eoanthidium) clypeare	0	0	*Stelis (Stelis) pavonina*	1	2
Epanthidium (Epanthidium) bicoloratum	0	0	*Stelis (Stelis) punctulatissima*	1	2
Euaspis abdominalis	1	1	*Stelis (Stelis)* aff *robertsoni*	1	2
Euaspis polynesia	1	1	*Stelis (Stelis) semirubia*	1	2
Hoplostelis (Hoplostelis) bivittata	1	1	*Stelis (Stelis) subcaerulea*	1	2
Austrostelis catamarcensis	1	12	*Stelis* (unplaced) *rozeni*	1	12
Hypanthidioides (Saranthidium) marginata	0	0	*Trachusa (Archianthidium) pubescens*	0	0
Hypanthidium (Hypanthidium) obscurius	0	0	*Trachusa (Congotrachusa) schoutedeni*	0	0
Icteranthidium ferrugineum	0	0	*Trachusa (Heteranthidium) larreae*	0	0
Notanthidium (Allanthidium) rodolfi	0	0	*Trachusa (Paraanthidium) interrupta*	0	0
Notanthidium (Notanthidium) steloides	0	0	*Trachusa (Trachusa) byssina*	0	0
Pachyanthidium (Ausanthidium) ausense	0	0			

4.3 Results and Discussion

4.3.1 Suprageneric and generic relationships

Both Bayesian and ML analyses yield congruent, well-resolved phylogenies (Figure 4.1). my results clearly reveal three major suprageneric clades within Anthidiini; I present these clades as a replacement for Michener's Series A and Series B (Table 4.4). These clades are: (1) the *Trachusa* group; (2) the *Dianthidium* group; and (3) the *Anthidium* group. The descriptions of two genera not included in the phylogeny, *Trachusoides* and *Apianthidium*, suggest a close phylogenetic relationship with *Trachusa*; in order to accommodate the future addition of other genera to this group, I choose to refer to the genus *Trachusa* as the *Trachusa* group. The genus name *Stelis* Panzer (1806) is the oldest in the *Dianthidium* group; I prefer to name the group, however, for a genus whose behavior is representative of the group in general. For this reason, I name the group after the oldest available non-cleptoparasitic genus name in the group, *Dianthidium* Cockerell (1900).

The genus *Trachusa* forms a strongly supported monophyletic clade (100% posterior probability; 100% ML bootstrap support) which is sister to the *Anthidium* group + the *Dianthidium* group (100% posterior probability; 100% ML bootstrap support). Both the *Anthidium* group and the *Dianthidium* group are strongly supported as monophyletic (*Anthidium* group: 100% posterior probability, 99% ML bootstrap support; *Dianthidium* group: 100% posterior probability, 100% ML bootstrap support); the sister group relationship between these two groups is strongly supported in Bayesian and ML analyses (100% posterior probability; 99% ML bootstrap support).

The results of these phylogenetic analyses challenge the current classification of anthidiine bees, primarily because many genera and subgenera emerge as paraphyletic (Figure 4.1). The central and southern African genus *Serapista* is strongly supported as monophyletic (100% posterior probability; 100% ML bootstrap support). While Bayesian analyses support the placement of *Serapista* at the base of the *Anthidium* group (85% posterior probability), ML analyses do not resolve the phylogenetic position of *Serapista* within the *Anthidium* group

The Central and South American genera *Notanthidium*, *Epanthidium*, *Anthodioctes*, *Hoplostelis*, *Austrostelis*, *Hypanthidium*, *Hypanthidioides*, and *Duckeanthidium* form a strongly supported monophyletic clade (100% posterior probability; 100% ML bootstrap support) at the base of the *Dianthidium* group. *Hoplostelis* is strongly supported as the sister taxon to *Austrostelis* in both Bayesian and ML analyses (100% posterior probability; 100% ML bootstrap support). A sister-group relationship between *Hypanthidium* and *Duckeanthidium* is strongly supported in Bayesian analyses and moderately supported in ML analyses (65% ML bootstrap support). Phylogenetic relationships among the other genera in this clade are unclear. The genera *Anthodioctes* and *Notanthidium* are both paraphyletic in Bayesian analyses; in ML analyses, the monophyly of these genera is uncertain.

The southeast Asian genus *Bathanthidium* is the sister taxon to a clade consisting of the Central and North American genera *Paranthidium* and *Dianthidium*; this relationship is strongly supported in both Bayesian and ML analyses (100% posterior probability; 99% bootstrap support). These three genera form a monophyletic group (100% posterior probability; 99% bootstrap support) which is sister to a clade containing the genera *Rhodanthidium*, *Icteranthidium*, *Eoanthidium*, *Anthidiellum*, *Pachyanthidium*, *Benanthis* and *Plesianthidium*

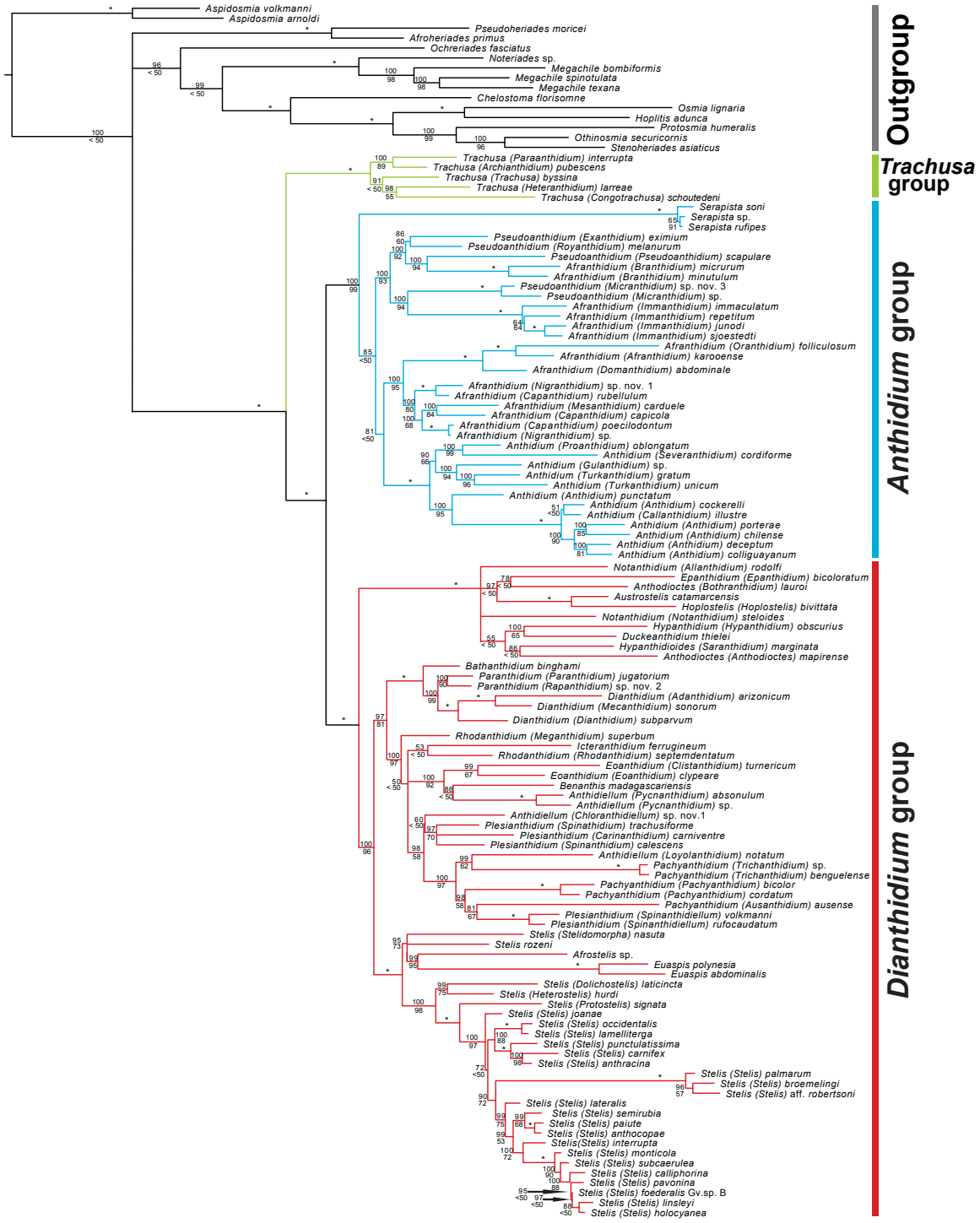

Figure 4.1 Phylogeny of Anthidiini based on Bayesian and maximum likelihood analyses. Numbers above nodes are Bayesian posterior probabilities; numbers below nodes are maximum likelihood bootstrap values. An asterisk ("*") marks those nodes supported by 100% posterior probability and 100% ML bootstrap support. The *Trachusa*-group is marked in green, the *Anthidium* group in blue and the *Dianthidium* group in red. Nest-building members of the *Trachusa* and *Dianthidium* groups incorporate resin into nest construction; members of the *Anthidium* group build nests using plant fibers.

Table 4.4 Revised suprageneric classification for the tribe Anthidiini. (a) The suprageneric classification of Anthidiini proposed by Michener (2007), based on female mandibular morphology; (b) My revised classification based on the results of Bayesian and maximum likelihood phylogenetic analyses presented in Figure 4.1. Genera included in my phylogeny are marked in bold typeface. Genera not included in my phylogeny are proposed affiliations and are marked in regular typeface. Cleptoparasitic genera are marked with an asterisk.

(a) Michener's (2007) suprageneric classification of Anthidiini

Series A		Series B
Acedanthidium	*Hoplostelis*	*Afranthidium*
Afrostelis	*Hypanthidioides*	*Anthidioma*
Anthidiellum	*Hypanthidium*	*Anthidium*
Anthodioctes	*Icteranthidium*	*Gnathanthidium*
Apianthidium	*Larinostelis*	*Indanthidium*
Austrostelis	*Notanthidium*	*Neanthidium*
Aztecanthidium	*Pachyanthidium*	*Pseudoanthidium*
Bathanthidium	*Paranthidium*	*Serapista*
Benanthis	*Plesianthidium*	
Cyphanthidium	*Rhodanthidium*	
Dianthidium	*Stelis*	
Duckeanthidium	*Trachusa*	
Eoanthidium	*Trachusoides*	
Epanthidium	*Xenostelis*	
Euaspis		

(b) My revised suprageneric classification of Anthidiini

Trachusa group	*Anthidium* group	*Dianthidium* group	
Apianthidium	***Afranthidium***	*Acedanthidium*	***Euaspis****
Trachusa	*Anthidioma*	***Afrostelis****	***Hoplostelis****
Trachusoides	***Anthidium***	***Anthidiellum***	***Hypanthidioides***
	Gnathanthidium	***Anthodioctes***	***Hypanthidium***
	Indanthidium	***Austrostelis****	***Icteranthidium***
	Neanthidium	*Aztecanthidium*	*Larinostelis**
	Pseudoanthidium	***Bathanthidium***	***Notanthidium***
	Serapista	***Benanthis***	***Pachyanthidium***
		Cyphanthidium	***Paranthidium***
		Dianthidium	***Plesianthidium***
		Duckeanthidium	***Rhodanthidium***
		Eoanthidium	***Stelis****
		Epanthidium	*Xenostelis**

(100% posterior probability; 97% ML bootstrap support); this sister group relationship is strongly supported in Bayesian analyses (97% posterior probability) and well-supported in ML

analyses (81% ML bootstrap support). The subgenus *Anthidiellum (Pycnanthidium)* is strongly supported as monophyletic in both Bayesian and ML analyses (100% posterior probability; 100% ML bootstrap support); this subgenus, together with the genera *Eoanthidium* and *Benanthis*, are strongly supported as a monophyletic group in both analyses (100% posterior probability; 92% ML bootstrap support). The subgenera *Pachyanthidium (Trichanthidium)* and *Pachyanthidium (Pachyanthidium)* are each strongly supported as monophyletic in both Bayesian and ML analyses (100% posterior probability; 100% ML bootstrap support), although they do not emerge together as part of the same clade. The phylogenetic relationships among other members of this clade are unclear. The genera *Anthidiellum*, *Pachyanthidium* and *Plesianthidium* are paraphyletic in both Bayesian and ML analyses. The genus *Rhodanthidium* is paraphyletic in Bayesian analyses; its status in ML analyses is unresolved.

The genera *Stelis*, *Euaspis* and *Afrostelis* form a strongly supported monophyletic group (hereafter the "*Stelis* clade", 100% posterior probability; 100% ML bootstrap support). The lineages *Stelis (Stelidomorpha) nasuta*, *Stelis* (unassigned to subgenus) *rozeni*, *Afrostelis*, and *Euaspis* form a monophyletic clade at the base of the *Stelis* clade (100% posterior probability; 73% ML bootstrap support). The subgenera *Stelis (Dolichostelis)* and *Stelis (Heterostelis)* form a strongly supported clade (100% posterior probability; 75% ML bootstrap support) which is sister to *Stelis (Protostelis)* + *Stelis (Stelis)* (100% posterior probability; 100% ML bootstrap support). The subgenus *Stelis (Stelis)* is strongly supported as monophyletic (100% posterior probability; 97% ML bootstrap support).

4.3.2 Taxonomic conclusions

The generic subdivision of the Anthidiini is still debated, with some authors preferring to divide the tribe into many smaller genera (Pasteels 1969) and other authors preferring fewer, but much larger, genera (Warncke 1980). Pasteels' (1969) revision of Old World Anthidiini recognizes 46 genera, while Warncke's (1980) classification of the same fauna recognizes only two genera. Michener's (2007) classification is the only one to include worldwide Anthidiini; he recognizes 38 genera.

Marked behavioral and morphological differences between species of Anthidiini suggest that it may be appropriate to divide the tribe into more groups than proposed by Warncke (1980), while the results of the phylogenetic analyses that I present here indicate the abundant paraphyly of genera and subgenera classified according to the system outlined by Michener (2007). These results clearly call for a radical revision of the tribe Anthidiini. While a comprehensive evaluation of current anthidiine classification will only be possible with the inclusion of missing genera and subgenera, I offer the following observations regarding anthidiine classification.

These results show that the bees defined as Series A in Michener's (2007) classification (the *Trachusa* and *Dianthidium* groups) are a paraphyletic group from which Series B (the *Anthidium* group) arose. The position of *Trachusa* at the base of the tribe is supported by a number of characters that are plesiomorphic with respect to other anthidiines, including fine punctation and the general absence of carinae, sulci, and propodeal pits (Michener 1948; but see subgenera *Orthanthidium* and *Paraanthidium*, Michener 2007). Systematic treatments of *Trachusa* have often focused on either Old World (Pasteels 1969, 1984) or New World (Griswold and Michener 1988; Thorp and Brooks 1994) members of the genus and no study has clearly elucidated morphological characters that define *Trachusa* in a global sense. Possible

synapomorphies of *Trachusa* include a lateral ocellus which is closer to the eye than to the posterior margin of the vertex (although equidistant in *Trachusa* (*Heteranthidium*) *larreae* and *bequaerti*, and *Trachusa (Metatrachusa)*) (Griswold and Michener 1988; Michener 2007); a median ocellus whose anterior margin is closer to the antennal bases than to the posterior margin of the vertex (or equidistant) (Griswold and Michener 1988); fore- and mid-tibial spines which are produced as blunt, obtuse projections which extend along the tibial surface as carinae (Griswold and Michener 1988); and vein cu-v of the hind wing oblique and one-half the length of the second abscissa of M+Cu (vein cu-v oblique but less than one-half the length of M+Cu in *Trachusa (Metatrachusa)*) (Michener 2007).

Given that Michener's (2007) Series A is paraphyletic, the character used to separate this group in Michener's (2007) key, namely the presence of few, blunt mandibular teeth, is likely plesiomorphic within Anthidiini. The characters used to describe Series B, however, may be synapomorphies of the *Anthidium* group. These characters include female mandibles with five or more sharp teeth which are separated by acute notches (Michener 2007) and maxillary palpi reduced to two segments. These characters are not entirely exclusive to the *Anthidium* group, however, and appear to a limited degree in genera belonging to the *Dianthidium* group. The genus *Pachyanthidium* emerges within the *Dianthidium* group, yet members of the subgenus *Pachyanthidium (Pachyanthidium)* exhibit mandibular dentition consistent with the *Anthidium* group. The resin-nesting subgenus *Plesianthidium (Spinanthidium)* belongs to the *Dianthidium* group yet has two-segmented maxillary palpi; other members of the *Dianthidium* group have three- or four-segmented maxillary palpi. The absence of tarsal arolia is used to distinguish the *Anthidium* group (Series B) in Michener's (2007) key, although there are members of the *Dianthidium* group, including *Icteranthidium*, *Apianthidium*, *Eoanthidium (Salemanthidium)* and

Larinostelis (arolia absent in female, male unknown), that also lack arolia.

Many characters appear in multiple anthidiine genera and subgenera, apparently without phylogenetic pattern. Males from unrelated genera, for example, exhibit apical marginal combs on sternites three, four or five. Such combs are present in genera from all three suprageneric anthidiine groups, including members of *Trachusa* (on S4 and S5, depending on subgenera), *Pseudoanthidium* (on S5 in *Pseudoanthidium (Pseudoanthidium)* and *P. (Royanthidium)*), *Pachyanthidium* (on S4 and S5), *Hypanthidioides* (S3, S4, and S5, depending on subgenus), *Plesianthidium* (S4 and S5, depending on subgenus), *Bathanthidium* (S4 and S5, depending on subgenus), Dianthidium (S5), and *Stelis* (S4); sternal combs are absent in members of the following genera: *Trachusa, Afranthidium*, *Anthidium*, *Pseudoanthidium*, *Plesianthidium*, *Dianthidium*, *Duckeanthidium*, *Cyphanthidium*, and *Hypanthidioides*. The appearance of sternal combs not only varies within genera (as in *Trachusa*, *Pseudoanthidium, Dianthidium, Plesianthidium and Rhodanthidium*) but also within species: male *Trachusa (Heteranthidium) occidentalis* have been found both with and without combs on the fourth sternite (Brooks and Griswold 1988).

The presence or absence of arolia is another character which varies from genus to genus and within genera: each of the genera *Trachusa*, *Pachyanthidium*, *Hypanthidioides*, and *Dianthidium* contains species both with and without arolia. Other characters that appear in multiple, unrelated lineages include a strongly carinate or lamellate omaulus, seen in *Anthidiellum*, *Pachyanthidium*, *Pseudoanthidium (Micranthidium)*, and *Gnathanthidium*; juxtantennal carinae, seen in *Duckeanthidium*, *Hoplostelis*, *Eoanthidium*, *Epanthidium*, *Euaspis*, *Hypanthidioides*, and *Larinostelis*; and a complete or partial preoccipital carina, seen in

Afranthidium (Mesanthidiellum), *Aztecanthidium*, *Anthodioctes*, *Afrostelis*, *Euaspis*, *Gnathanthidium*, *Icteranthidium*, *Pachyanthidium*, *Plesianthidium (Spinanthidiellum)*, and various subgenera of *Pseudoanthidium* and *Anthidiellum*.

The appearance of male sternal combs in many unrelated genera from all three suprageneric groups suggests that such combs may be plesiomorphic within Anthidiini. Arolia are present in most bees, although a number of lineages from several bee families including Colletidae, Apidae and Megachilidae have secondarily lost arolia. The presence of arolia is likely plesiomorphic in bees and in Apoidea in general, while the loss of arolia appears to have occurred in parallel in many, unrelated lineages. Omaular and preoccipital carinae appear in multiple, unrelated lineages of bees (preoccipital carinae are found in members of Apidae, Halictidae, Megachilidae and Colletidae; omaular carinae are found in members of Colletidae, Megachilidae and Apidae), while juxtantennal carinae are found in unrelated lineages of Megachilidae (Osmiini and Anthidiini). These various carinae have likely evolved in parallel and may serve to protect vulnerable areas of the body, such as the neck and antennal bases (Michener 2007). Each of the above-mentioned characters is either a plesiomorphy or a convergence. While such characters may be generally useful for identifying species within the context of a key, they are clearly not synapomorphies and are therefore inappropriate for defining monophyletic groups within the context of a phylogeny-based classification system.

The inclusion of both *Afrostelis* and *Euaspis* in the *Stelis* clade renders the genus *Stelis* paraphyletic. The genera *Afrostelis* and *Euaspis* have morphologically distinct characters which separate them from *Stelis* s.s. *Afrostelis* has a highly modified thorax and unique male genitalia (Michener 2007). Like *Stelis*, however, it exhibits two apical spines on its fore- and mid-tibiae

and some have theorized that *Afrostelis* may be a specialized derivative of *Stelis* (Michener 2007). The genitalia of male *Euaspis* differ from those of *Stelis*: although the male gonostylus is slender at the base (as in *Stelis*), the distal end of the gonostylus is rounded and flattened. *Euaspis* has, however, two apical spines on its fore- and mid-tibiae and it has been suggested that *Euaspis* and *Stelis* may best be placed in the same genus (Alfken 1926). In keeping with the theories of Michener (2007) and Alfken (1926), my results demonstrate that *Afrostelis* and *Euaspis* are derived from within *Stelis*; both genera may deserve subgeneric rank within the genus *Stelis*. The taxonomic assignment of *Stelis rozeni* to the genus *Stelis* was described as "provisional" by Griswold and Parker (2003), based on the numerous morphological differences between *S. rozeni* and other members of the genus, the difficulty associated with its assignment to subgenus, and the fact that males of the species are unknown. My results soundly confirm the affinity of *Stelis rozeni* with the *Stelis* clade; its generic assignment, however, will await further clarification of the generic status of other members of this clade, including *Euaspis*, *Stelis (Stelidomorpha)*, and *Afrostelis*.

While *Hoplostelis* was originally proposed as a subgenus of *Stelis* (see Michener and Griswold 1994), it has long been recognized as a distinct genus (Griswold and Michener 1988; Michener and Griswold 1994); it differs from other New World *Stelis* by the presence of distinct foveae between the scutum and scutellum, the short, transverse scutellum, the presence of a single spine on fore- and mid-tibiae, the apicolateral tooth of the male sixth sternite, and the quadridentate female mandible. *Hoplostelis* also lacks the distinctive clubbed genitalia of male *Stelis*. While Michener (2007) speculates that *Hoplostelis* may be closely related to the Central and South American genus *Hypanthidioides*, my results indicate that the closest relatives of *Hoplostelis* and *Austrostelis* are *Epanthidium (Epanthidium) bicoloratum* and *Anthodioctes*

(Bothranthidium) lauroi.

The genus *Austrostelis* was originally recognized as a subgenus of *Hoplostelis* (Michener and Griswold 1994); the presence, however, of unique morphological characters which clearly distinguish it from other members of *Hoplostelis*, including the sparse punctation of T1 and T6, the robust body form, and the unmodified female mandible, have caused it to be recognized as a unique genus (Michener 2007). Although my analyses support the possibility that *Austrostelis* and *Hoplostelis* are two distinct genera, only the inclusion of other species from both groups will clarify whether the genera are reciprocally monophyletic.

4.3.3 Evolution of nesting behavior

A clear phylogenetic pattern may be discerned in the primary nest-building materials of anthidiine bees (Figure 4.1). The genus *Trachusa*, positioned at the base of the tribe, uses plant resin as its primary nest-building material. Most members of this genus, including members of the subgenera *Trachusa*, *Trachusomimus* and *Paraanthidium*, incorporate both resin and long, narrow strips of leaves into nest construction (Ferton 1920; Michener 1941; Pasteels 1977; personal observation for *T. interrupta*). Other subgenera of *Trachusa*, such as *Heteranthidium*, build nests with resin and possibly sand but without leaves (MacSwain 1946; Cane 1996). Members of the *Dianthidium* group also build nests of resin, often mixed with sand, pebbles and other debris; unlike members of the genus *Trachusa*, however, species belonging to the *Dianthidium* group do not incorporate leaves into nest construction. In contrast to the other two clades, nests built by members of the *Anthidium* group are constructed of plant fibers.

The mandibular morphology of anthidiines using resin in nest construction differs markedly from that of anthidiines using plant fibers and it has long been suggested that there may be a correlation between nest-building material and mandibular dentition (Perez 1879, 1889; Pasteels 1977). Female anthidiines building nests of resin, including both the *Trachusa* group and the *Dianthidium* group, have three or four obtuse or rounded mandibular teeth joined by shallow concavities; they use these wide, blunt teeth to gather resin into small chunks, which they transport back to their nests. In contrast, female anthidiines using plant fibers in nest construction have five or more sharp teeth separated by acute notches (Pasteels 1977; Michener 2007); they use their teeth as serrated edges, sawing through plant fibers in order to collect them.

The theory that mandibles with multiple, distinct teeth are adapted to cutting fibers from plants, while mandibles with less-defined teeth are adapted to the manipulation of plant resin is supported by the unusual nesting behavior of some members of the genera *Rhodanthidium* and *Pachyanthidium* (in the *Dianthidium* group). The nest cells of *Rhodanthidium (Asianthidium) caturigense* are built of two distinct layers: an outer layer woven of plant fibers and an inner layer built of resin. While most female members of the genus *Rhodanthidium* do not include plant fibers in their nests and exhibit extremely reduced mandibular teeth consistent with those of other resin-nesting anthidiines, *R. caturigense* has four mandibular teeth which are clearly defined, if somewhat shallower than those described above for plant fiber-nesting bees. Some members of the genus *Pachyanthidium*, including *Pachyanthidium bicolor* (Michener 1968) and *Pachyanthidium cordatum* (Gueinzius 1958; Steiner and Whitehead 1991), also build nests of both resin and plant fibers; brood cells are not two-layered, as in *Rhodanthidium caturigense*, but rather consist of resin mixed together with plant fibers. While many members of *Pachyanthidium* exhibit reduced mandibular dentition consistent with that of resin-nesting anthidiines, members

of the subgenus *Pachyanthidium* (*Pachyanthidium*), which includes *Pachyanthidium bicolor* and *Pachyanthidium cordatum*, exhibit multiple mandibular teeth that are consistent with those of plant fiber-nesting anthidiines. If numerous sharp teeth are an adaptation for gathering plant fibers and fewer rounded teeth are an adaptation for gathering resin, then the intermediate dentition seen in *Rhodanthidium caturigense*, *Pachyanthidium bicolor* and *Pachyanthidium cordatum* may be an adaptation which may facilitate the manipulation of both resin and plant fibers in nest construction.

The *Trachusa* group and the *Dianthidium* group do not belong to the same monophyletic clade, yet both nest using resin and exhibit similar mandibular teeth. This, in conjunction with the basal position of *Trachusa*, suggests that resin-nesting and reduced mandibular dentition may be plesiomorphic in Anthidiini. My results strongly support a transition to mandibles with multiple, sharp teeth at the base of the *Anthidium* group; this transition is presumably an adaptation to the use of plant fibers in nest building which characterizes this group. *Pachyanthidium bicolor* and *Pachyanthidium cordatum* are strongly supported as a monophyletic group in both Bayesian and ML analyses (100% posterior probability; 100% ML bootstrap support); the position of these two species, nested deeply within the *Dianthidium* group, implies that their inclusion of plant fibers in nest construction and their mandibles with multiple, sharp teeth are a convergence shared with members of the *Anthidium* group. The phylogenetic position of *Rhodanthidium caturigense* is unknown; given that the genus *Rhodanthidium* is paraphyletic in the phylogeny, it is difficult to offer a hypothesis regarding the phylogenetic affinity of *Rhodanthidium caturigense*. The future inclusion of this species in the phylogeny will allow for a more comprehensive understanding of the evolution of nesting behavior in Anthidiini.

The use of plant resin as a nesting material confers certain advantages to nesting bees. Resin is hydrophobic, unpalatable to potential predators, and exhibits anti-microbial and anti-fungal properties; its use in brood cell construction, therefore, protects both pollen provisions and developing larvae from infection by pathogens, predation, and damage caused by moisture (Ghisalberti 1979; Cane 1996; Müller et al. 1996; see also Chapter 2). The use of plant resin in nest construction is not limited to Anthidiini and is seen in many lineages of megachilid bees. The anthidiine genus *Trachusa* (Ferton 1920; Michener 1941; Pasteels 1977; Brooks and Griswold 1988; Cane 1996), the megachiline subgenera *Austrochile*, *Gronoceras*, *Hackeriapis*, *Rhodomegachile*, and *Chalicodomoides* (Michener 2007), the osmiine *Heriades* group (Praz 2008; Müller 2011), and the genus *Pseudoheriades* (Müller 2011) all build nests using resin. The phylogenetic position of all these lineages at the bases of major megachilid clades allows for the possibility that resin may have been the first foreign material used by the higher megachilids (the clade consisting of the tribes Anthidiini, Osmiini, and Megachilini, and the genera *Ochreriades*, *Pseudoheriades* and *Afroheriades*) in nest construction. *Aspidosmia*, the most basally positioned lineage of Megachilidae to incorporate foreign material in nest construction, builds nests of masticated leaf pulp, probably resinous, and small pebbles (Brauns 1926; Peters 1972; Michener 2007). The use of resinous leaf pulp in nest construction may have been an evolutionary stepping stone between the unlined nests that are apparently plesiomorphic in Megachilidae (see Chapter 2) and the use of plant resin seen in other megachilid lineages. *Aspidosmia*, perhaps unable for behavioral or morphological reasons to collect resin, may have instead chewed leaves and incorporated both the leaf pulp and the associated resin into its nests. This may also have set an evolutionary precedent for a behavior thus far unreported in Anthidiini but known in other resin-

nesting bees such as the osmiine genus *Heriades* (Michener 1968): the biting of resinous plants in order to collect the sticky material exuded by the wounded plant.

4.3.4 Origins of cleptoparasitism

The results of my BayesTraits analyses provide decisive support for two independent origins of cleptoparasitism in Anthidiini with no reversals to nest-building behavior, over a single origin of cleptoparasitism and multiple reversals (Bayes Factor = 19.2; values above 10 indicate decisive support, Kass and Raftery 1995). Two Central and South American cleptoparasitic lineages, *Hoplostelis* and *Austrostelis*, form a strongly-supported monophyletic clade (100% posterior probability; 100% ML bootstrap support) unrelated to the genus *Stelis*. Ancestral state reconstructions reveal that the common ancestor of *Hoplostelis* and *Austrostelis* was cleptoparasitic with a probability of 99%. The genera *Stelis*, *Afrostelis*, and *Euaspis* constitute the second clade of cleptoparasites; this clade is strongly supported in both Bayesian and ML analyses (100% posterior probability; 100% ML bootstrap support). The common ancestor of these three genera was cleptoparasitic with a probability of 99%. The most recent common ancestor of all cleptoparasitic anthidiines, represented by the node at the base of the *Dianthidium* group, was reconstructed as nest-building with a probability of 99%.

Six independent origins of cleptoparasitism were proposed within Anthidiini (Michener 2007). It was assumed that the genera *Austrostelis* and *Hoplostelis* together represented a single origin of cleptoparasitism and that the remaining cleptoparasitic genera, *Afrostelis*, *Euaspis*, *Larinostelis*, *Stelis* and *Xenostelis* represented one origin each. These results strongly support a single origin of cleptoparasitism in the common ancestor of *Afrostelis*, *Euaspis*, and *Stelis*, implying a dramatic reduction in the number of origins of cleptoparasitism in the tribe.

The phylogenetic placement of two extremely rare cleptoparasitic genera remains unknown. The genera *Larinostelis* and *Xenostelis* are both known from single female specimens from Kenya and Yemen, respectively. If these two genera are unrelated to *Stelis* and to each other and truly represent two independent origins of cleptoparasitism, the total number of independent origins of cleptoparasitism in Anthidiini is four. I believe, however, that *Larinostelis* and *Xenostelis* share morphological similarities with the genus *Stelis*, implying a close relationship with the *Stelis* clade and a common cleptoparasitic origin. Both *Larinostelis* and *Xenostelis* exhibit two apical spines on fore- and mid-tibiae, a character shared with *Afrostelis*, *Euaspis*, and *Stelis* but rare in other anthidiines. The shape of the thorax in *Xenostelis* is shared with *Stelis* and the enlarged tegulae are similar to those of *Afrostelis*. *Larinostelis* also exhibits two apical fore- and mid-tibial spines, although these spines are much reduced (Michener and Griswold 1994). I predict that *Larinostelis* and *Xenostelis* are derived from within the *Stelis* clade; the non-*Stelis*-like features exhibited by both genera suggest a phylogenetic placement at the base of the *Stelis* clade, closely related to *Afrostelis*, *Stelis rozeni*, *Stelis nasuta*, and *Euaspis*. My results, in conjunction with morphological evidence from taxa not present in my phylogeny, imply that the number of origins of cleptoparasitism in Anthidiini is two. This reduces the number of origins of cleptoparasitism in Megachilidae to six and the number of origins in bees to twenty (after modification of the number of cleptoparasitic apid lineages from Cardinal et al. 2010).

4.3.5 Evolution of cleptoparasitic strategy

The results of Bayesian ancestral state reconstructions strongly indicate that both origins of cleptoparasitism in Anthidiini are associated with hospicidal adults. The common ancestor of

the cleptoparasitic genera *Hoplostelis* and *Austrostelis* and the common ancestor of the *Stelis* clade are both reconstructed as having hospicidal adults, with a probability of 97% and 100%, respectively. Members of *Stelis (Stelis)* have hospicidal larvae and my results imply a single transition from hospicidal adult to hospicidal larvae within the *Stelis* clade; the exact placement of this transition, however, is unclear. The cleptoparasitic strategy of *Stelis (Protostelis) signata* is unknown and its phylogenetic position, nested between cleptoparasitic lineages with hospicidal adults and lineages with hospicidal larvae, allows for two possible transition points: (1) in the common ancestor of *Stelis (Stelis)*, or (2) in the common ancestor of *Stelis (Stelis)* + *Stelis (Protostelis)*. Only further understanding of the cleptoparasitic behavior of *Stelis (Protostelis)* will allow us to resolve the precise position of the transition from cleptoparasitic lineages with hospicidal adults to lineages with hospicidal larvae.

Adult female members of *Hoplostelis* and the basally positioned lineages in the *Stelis* clade are hospicidal. *Hoplostelis* and *Euaspis* are known to enter the sealed nests of their hosts, eject or kill the host eggs and larvae, deposit their own eggs, and then reform the cell partitions and nest closures (Bennett 1966; Iwata 1976). A similar behavior is seen in *Stelis (Dolichostelis)* (Parker et al. 1987). While the cleptoparasitic strategy of *Stelis (Heterostelis)* is unknown, the mandibles of at least one larval instar are bidentate and have been likened to the mandibles of *Dianthidium curvatum*, suggesting that they are unmodified for killing and therefore consistent with lineages which have hospicidal adults (Thorp 1966). In contrast, all studied members of the subgenus *Stelis (Stelis)* have hospicidal larvae. After hatching on the pollen provisions left by the host bee, the larvae belonging to the subgenus *Stelis (Stelis)* eat their way through the pollen mass until they encounter the host larvae, which they immediately attack and kill (Michener

1955; Rozen 1966; Rust and Thorp 1973; Torchio 1989; Rozen and Kamel 2009; Rozen and Hall 2011).

In some members of the subgenus *Stelis (Stelis)*, the hospicidal instar is armed with unidentate, apically tapering mandibles (*Stelis lateralis*, *Stelis montana*), while in other members of the same subgenus the hospicidal larval instar has mandibles which taper to a narrowly bidentate apex (*Stelis elongativentris*, *Stelis ornatula*) (Michener 1955; Rozen 1987; Torchio 1989; Rozen and Kamel 2009). In *Stelis (Stelis) chlorocyanea*, early larval instars have tapering bidentate mandibles, while the final instar has unidentate mandibles; all instars are hospicidal (Rust and Thorp 1973). In *Hoplostelis*, a lineage in which the adult female kills the host, the mandibles of all larval instars are similar to those seen in other nest-building anthidiines: bidentate and untapered (Rozen 1966). Mandibles modified to a tapering point, regardless of whether unidentate or bidentate, appear to be an adaptation in *Stelis* for killing host eggs and larvae. In lineages such as *Hoplostelis*, larvae are nonhospicidal and have not evolved the specialized mandibular modifications seen in hospicidal larvae.

Mandibular morphology, however, may not be the only factor contributing to cleptoparasitic strategy. In *Stelis (Stelis) lateralis*, all larval instars have unidentate, tapered mandibles but only the final instar appears to be hospicidal (Michener 1955; Rozen and Kamel 2009). A defining feature of all hospicidal *Stelis* larvae is an extremely aggressive temperament: when prodded gently with forceps, hospicidal instars of *Stelis chlorocyanea*, *Stelis elongativentris, Stelis montana*, and *Stelis ater* respond by rearing up, gnashing their mandibles, and lunging at the forceps (Rust and Thorp 1973; Rozen 1987; Torchio 1989; Rozen and Hall 2011); in contrast, nonhospicidal instars, like nest-building anthidiine larvae, are unaggressive,

even “sessile” (Rozen 1987; Torchio 1989). Although the mandibular morphology of *Stelis (Stelidomorpha) nasuta* is unclear (Rozen and Kamel 2009), none of its larval instars are aggressive; this suggests that at least some lineages at the base of the *Stelis* clade have larvae that are nonhospicidal. It seems likely that the transition between lineages with hospicidal adults and lineages with hospicidal larvae may have been precipitated not only by the evolution of specialized larval mandibles but also by a change in larval behavior.

4.3.6 Implications for the evolution of cleptoparasitism in bees

A number of factors have been cited as potential drivers of cleptoparasitic behavior in bees, including competition for floral resources (Wcislo 1981), competition for adequate nesting sites (Michener 2007), and the synchronization of the ontogenies of a particular organism and its potential host, such that the period of host-nest seeking by a potential parasite coincides with the greatest availability of host nests (Wcislo 1981). A cleptoparasitic lineage arises when some combination of these factors contributes to an environment where parasitizing the nests of other bees results in greater reproductive success for a cleptoparasite than building and provisioning a nest of its own.

The larva of a lineage recently transitioned from nest-building to cleptoparasitic would likely still exhibit the non-aggressive behavior and non-specialized mandibular morphology inherited from its nest-building ancestors. Thus in the earliest forms of cleptoparasitism, the elimination of the host’s offspring must have been carried out by the adult female. The adult females of such a lineage would not yet have evolved the adaptive behaviors seen in other cleptoparasitic lineages, such as the concealment of eggs within the pollen mass or cell walls of a host nest. Each independent origin of cleptoparasitism in bees, therefore, was likely one in which

the adult female entered a closed, unguarded nest cell, removed the host offspring herself and then laid her own egg, thereby avoiding confrontation with the host, protecting her eggs from discovery, and removing competition for resources presented by the host offspring. This strategy is still seen in members of the genus *Dialictus* (Halictidae), *Sphecodes* (Halictidae), *Exaerete* (Apidae), *Hoplostelis* (Megachilidae), *Euaspis* (Megachilidae) and members of the genus *Stelis* (Megachilidae) (Michener 2007).

This type of cleptoparasitism, however, requires the female to oviposit in nest cells that may not contain enough pollen to sustain her offspring: if the host larva has consumed much of the pollen mass, there may not be enough pollen left to support the growth of the cleptoparasitic larva (Wcislo 1981). The cleptoparasite *Euaspis basalis* chews and reforms the pollen provisions of its host, *Megachile (Callomegachile)*, possibly in order to destroy any host eggs or larvae present in the provisions (Iwata 1976). While the nests of *Megachile (Callomegachile)* typically contain three or four completed cells, nests that have been parasitized by *Euaspis basalis* often contain only one or two cells; this may be a function of the fact that much of the pollen originally present in the cells is already consumed by the host's offspring at the moment of parasitization, thus leaving a limited amount for the cleptoparasite. Some cleptoparasites, such as *Hoplostelis bilineolata*, may be able to assess the suitability of a closed nest cell (Bennett 1966). Others, such as *Exaerete smaragdina* (Apidae, Euglossini) (Garófalo and Rozen 2001), are not; in the absence of newly finished nest cells, *Exaerete smaragdina* will deposit eggs in much older cells, even those that have already been parasitized by other bees (Garófalo and Rozen 2001). The only way to ensure that cleptoparasitic larvae have the entire pollen contents of a nest cell at their disposal is for the cleptoparasitic female to deposit her eggs in nest cells that are still in the

process of being provisioned by the host. This strategy, however, presents several new challenges for both the cleptoparasitic adult and its larva.

Often a host egg is laid only after nest cell provisioning is complete, making it probable that the host egg may not yet be present at the moment that the cleptoparasite parasitizes an open nest cell. In this case, the adult cleptoparasite cannot destroy the host's offspring; their destruction, therefore, may only be carried out by the cleptoparasitic larvae. Furthermore, parasitizing an open nest cell means that the cleptoparasitic adult not only runs a greater risk of encountering a returning host but also that her eggs run a greater risk of detection and destruction by the host. Despite these challenges, cleptoparasitic lineages with hospicidal larvae are far more common than those with hospicidal adults.

I argue that in cleptoparasitic bees, hospicidal adults are a necessary evolutionary transition between nest-building bees and lineages with hospicidal larvae. A bee recently transitioned from nest-building to cleptoparasitic could only have had hospicidal adults, given that it would have been difficult for such larvae to kill the larvae of its host. Lineages with hospicidal larvae have evolved multiple times independently in bees, implying a tremendous selective pressure for the evolution of aggressive larvae with sharp, pointed mandibles and strongly suggesting an evolutionary advantage associated with hospicidal larvae. Lineages at the bases of major cleptoparasitic clades, including the *Stelis* clade and the apid clade consisting of the subfamily Nomadinae and its close relatives, tend to parasitize closed nest cells, whereas more derived lineages in both clades parasitize open nest cells, which may also imply an evolutionary advantage associated with parasitizing open nest cells.

The dated phylogeny presented in Chapter 2 indicates a stem clade age for the *Stelis* clade of 36 my (95% HPD 27-45 my) and an age for the clade *Austrostelis+Hoplostelis* of < 27 my (95% HPD 19-36 my) (Litman et al. 2011). The cleptoparasitic anthidiine clades are relatively young and lineages representing the progression from nest-building to hospicidal adult to hospicidal larva are still present. Other large cleptoparasitic clades, such as the apid clade consisting of the subfamily Nomadinae and its close relatives are considerably older (95 mya, 95% HPD 87-103 my; Cardinal et al. 2010). A number of basal lineages within this clade, including the tribes Melectini, Ericrocidini, Rhathymini, Tetrapediini and Osirini, as well as the genus *Coelioxoides*, all parasitize closed nest cells, while other lineages in the same clade, including the subfamily Nomadinae and the tribes Protepeolini and Isepeolini, parasitize open nest cells that are still in the process of being provisioned (Rozen 2003). This large apid clade, however, consists entirely of cleptoparasites with hospicidal larvae (but see *Protosiris*; Rozen 2006). Lineages with hospicidal adults which may have been present at the base of the clade and which may have represented the transition between nest-building lineages and lineages with hospicidal larva have likely since disappeared.

The only bee which may exhibit a third cleptoparasitic strategy is *Stelis (Stelidomorpha) nasuta*. This bee parasitizes the mud nests of *Megachile* (*Chalicodoma*), including *M. parietina*, *M. pyrenaica*, and *M. siculum* (Fabre 1914; Friese 1923; Müller et al. 1997; Westrich 1989). *Stelis nasuta* may lay eggs in either open or closed nests and deposits between two and twelve eggs per nest cell (Fabre 1914). The larvae of *S. nasuta* are much smaller than those of its host (Fabre 1914; Friese 1923; Müller et al. 1997; Westrich 1989) and the pollen mass present in a single cell of *Megachile (Chalicodoma)* is sufficient to sustain the development of multiple larvae of *S. nasuta*. The larvae of *Stelis nasuta* are thought to consume all of the provisions

intended for the host larva, thereby starving it to death (Fabre 1914). The underdeveloped dead body of the host larva is sometimes still present in the nest cell after *S. nasuta* larvae have spun their cocoons, lending support to the theory that *S. nasuta* starves its host (Fabre 1914). Like other nonhospicidal larvae, the larvae of *S. nasuta* are unaggressive (Rozen 1966; Rozen and Kamel 2009). The preference for a host whose pollen provisions are adequate to sustain the development of multiple parasitic larvae may make it unnecessary for *S. nasuta* to kill its host directly: *S. nasuta* is the only cleptoparasitic bee in which neither the adult female nor any of the larval instars appear to kill the host.

4.3.7 Evolution of host preference in cleptoparasitic Anthidiini

In 1909, Carlo Emery offered the observation that the hosts of parasitic organisms are often species to which they are closely related. If "Emery's rule" were indeed true, I would expect to find that cleptoparasitic anthidiines, particularly the early branches of cleptoparasitic clades such as those at the base of the *Stelis* clade, would display a host-preference for anthidiine bees. I find no evidence, however, that either clade of cleptoparasitic anthidiines shows a preference for closely related hosts: *Hoplostelis* is cleptoparasitic on the family Apidae and the hosts of the *Stelis* clade are largely members of the megachilid tribes Megachilini and Osmiini, with comparatively few host records for Anthidiini (Table 1.1). Instead, I find that *Hoplostelis* and the earliest branches of the *Stelis* clade show a preference for *the nesting materials* of their closest relatives: *Hoplostelis*, *Euaspis*, *Afrostelis*, *Stelis (Dolichostelis)*, *Stelis (Heterostelis)*, and *Stelis (Protostelis)* are all derived from within the resin-nesting *Dianthidium* group and all are cleptoparasites of bees using resin in nest construction. In contrast, members of the subgenus

Stelis (Stelis) show a marked preference for osmiine hosts, often belonging to the genera *Osmia* and *Hoplitis*, which use masticated leaf pulp in nest construction.

The transition in preferred host-nesting material occurs at the base of the subgenus *Stelis (Stelis)*, either co-occurring with, or just after, the transition in cleptoparasitic strategy. The explanation for these near-simultaneous transitions may lie in the curious behavior of *Stelis (Stelis) montana*, a western North American cleptoparasite of *Osmia montana*, *Osmia lignaria propinqua*, and *Osmia californica*, all cavity-nesting megachilid bees that build their cell partitions and nest plugs from masticated leaf material (Torchio 1989). All three species of *Osmia* react violently upon finding the adult cleptoparasite in their nests: the osmiines forcibly drag *Stelis* from the nest and, in the case of *O. californica*, furiously rechew all of the pollen provisions, thereby destroying any eggs that may have been left by the cleptoparasite (Torchio 1989). In contrast, none of the three osmiine species appears to notice when their nests have been visited by *Stelis* while they are out foraging, if they do not encounter the adult cleptoparasite upon arriving back at the nest. This may be because *Stelis (Stelis) montana*, a cleptoparasite with hospicidal larvae, covers its body with plant-derived fluid which it extracts from the same leaves used in nest construction by the three species of *Osmia*; *S. montana* also spreads pollen-nectar provisions, which it steals from its hosts, on all body surfaces (Torchio 1989). In covering itself with the nesting material used by its host, *Stelis (Stelis) montana* may conceal olfactory evidence of its visit (Torchio 1989).

The shift from hospicidal adults to hospicidal larvae greatly changed the stakes for marauding cleptoparasitic females. In parasitizing nests that have already been sealed by the host, hospicidal adult females are able to largely avoid contact with host females. Bees that

parasitize nests that are still being provisioned, on the other hand, are required to enter open nests, scout for suitable cells, deposit eggs, and leave the nest, all undetected by the host female. If bees such as *Stelis (Stelis)* use the nesting material of their hosts to mask their host-nest visits, the tractability of the nesting material used by hosts may be a critical factor in their success as cleptoparasites. Juices chewed from leaf material are more amenable to grooming over the cuticle than resin, perhaps making such nest-building material easier to manipulate for certain cleptoparasites.

4.4 Conclusions

The phylogenetic hypothesis that I present here not only clarifies evolutionary relationships among anthidiine bees but should also serve as a framework on which to base an improved classification of the tribe. My results yield new insights into the evolution of nesting behavior and nesting material in anthidiine bees and allow us to speculate on the origins of nesting material in Megachilidae. Finally, my phylogeny reveals the origins of cleptoparasitism and the evolution of cleptoparasitic strategy in Anthidiini. My results support the hypothesis that cleptoparasitic lineages with hospicidal adults are an evolutionary intermediate between nest-building bees and cleptoparasitic lineages with hospicidal larvae, which facilitates our understanding of the evolution of cleptoparasitism in all bees.

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