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Why do leafcutter bees cut leaves? New insights into the early evolution of bees

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

Keywords: bees; key innovation; diversification; Megachilidae; nesting biology; bee–flower relationships

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 Dufour's gland [1,2]. By 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...?' [3].

It has been demonstrated that the foreign material used by megachilid bees is hydrophobic and shows antimicrobial activity [4,5], 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 [6,7]. The absence of nest-lining in this group was originally attributed to a behavioural loss associated with above-ground nesting [8], but the phylogenetic position of Lithurgini at the base of Megachilinae [9] suggests that it represents an ancestral trait [10]. Bees of the subfamily Fideliinae build unlined nests that they excavate in sandy soil [11–14]. Two distinct tribes of fidelini 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 [15]. 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.

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2. MATERIAL AND METHODS

(a) *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. Electronic supplementary material, table S1 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 accommodate the placement of fossil calibration points. Voucher specimens are deposited in the Cornell University Insect Collection.

(b) *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.* [16]. All taxa and GenBank accession numbers are listed in electronic supplementary material, table S2. PCR primers and conditions are listed in electronic supplementary material, table S3. The four protein-coding genes were aligned using MAFFT [17] and then adjusted by eye in MacClade [18]; all introns were removed. The ribosomal gene, 28S, was aligned via secondary structure according to the method described by Kjer [19]; all unalignable regions were excluded. The secondary structure alignment was based on the 28S map of *Apis mellifera* [20]. Details regarding data partitioning and model-testing are included as the electronic supplementary material.

(c) *Phylogenetic analyses*

Phylogenetic analyses were performed using both Bayesian and maximum-likelihood methods. Bayesian analyses were performed using MRBAYES v. 3.1.2 [21,22]. A GTR + I + Γ model was used for all partitions except for the stem partition of 28S, which was analysed 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 [23], leaving 96 956 000 post-burn-in generations; these were further sampled using LOGCOMBINER v. 1.6.1 [24] 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 [24] (electronic supplementary material, figure S1).

Maximum-likelihood analyses were performed using RAxML v. 7.0.4 (sequential version raxmlHPC; [25]). We used the rapid bootstrapping algorithm with a GTR + CAT approximation to perform 1000 bootstrap replicates. The maximum-likelihood bootstrap tree is shown in electronic supplementary material, figure S2.

(d) *Divergence dating analysis using BEAST*

We used BEAST v. 1.6.1 to perform a Bayesian divergence dating analysis [24]. Each partition was analysed 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 [26] to scale the root height to 130 Myr in order to conform to the constraints imposed by prior distributions on divergence times. Q1 Ten independent analyses were run for a total of 300 000 000 generations. An appropriate burn-in was discarded from each analysis using TRACER [23], 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 [24] and then used TREEANNOTATOR v. 1.6.1 [24] to build a maximum clade credibility tree from this posterior distribution of trees (electronic supplementary material, figure S3).

(e) *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. 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, as the electronic supplementary material.

Bees are thought to be the sister group to the apoid wasps [27]. Apoids first appear in the fossil record during the Cretaceous [28]; Engel [28] proposes that bees originated sometime after this and gives an uppermost boundary for their age of 125 Myr. 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 (approx. 65 Ma) origin of Q1 *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 [28–31]; recent molecular evidence [32] places the origin of the angiosperms in the Late Triassic, 30–80 Myr 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 [33] or the normal [34], 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 Ma and is based on an extremely conservative estimate for the origin of bees based on the fossil record [28]. The upper bound is assigned a value of 217 Ma and is based on a recent molecular estimate for the age of crown angiosperms [32]. Our use of a fairly broad uniform prior causes the 95 per cent 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 per cent HPD as a necessary consequence of using a uniform prior distribution.

(f) Biogeographic reconstruction

Biogeographic reconstructions were performed using both S-Diva [35] and Lagrange [36]. 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 [37]). 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. We present the details of both biogeographic analyses as the electronic supplementary material.

(g) Ancestral state reconstruction

We used BAYESTRAITS [38] to reconstruct the ancestral nesting biology of Megachilidae. Cell-lining behaviour was coded for each terminal (including the outgroup) as: totally unlined (0), in *Dasyroda*, fideline 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 [39] 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 [37] and references therein. We present the details of our Bayesian ancestral state reconstructions as the electronic supplementary material.

(h) Correlated trait evolution

We used BAYESTRAITS [38] to test for correlated evolution between the total geographical 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 [40,41] and the total geographical range for each terminal taxon using the area calculator provided by the website 'Free Map Tools' [42]. We present the details of this analysis, as well as specific information regarding species distribution, as the electronic supplementary material.

(i) Diversification rate analysis

We used MEDUSA (Modelling Evolutionary Diversification Using Stepwise Akaike Information Criterion; [43]) 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 [44]. 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 Akaike information criterion (AICc) scores 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 Δ AICc threshold. A model with two rate shifts (three sets of birth and death rates) was chosen as the best-fit model.

3. RESULTS AND DISCUSSION

The results of both maximum-likelihood and Bayesian analyses support a non-traditional interpretation of early megachilid phylogeny (figure 1a). According to our phylogenetic hypothesis, the small palaeartic tribe Parahopitini 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 (figure 1a and electronic supplementary material, figures S1, S2 and S3). 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 [46] 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 fideline bees, to be 126 Ma (95% HPD 100–154), pre-dating the separation of the African and South American continental plates (figure 1b). Our estimate of the age of Megachilidae is older than anticipated, given that bees are generally thought to have originated around 125 Ma [28]. Our results indicate an origin for the bees (the root height of our tree) of 149 Ma (95% HPD = 119–182). We ran another analysis where the root was constrained to 120 Ma; even under this conservative estimate for the age of the bees [28], the age of Megachilidae is 104 Ma (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 Ma [47]. Both analyses indicate that the Megachilidae arose relatively rapidly after the origin of the bees.

A Gondwanan origin for Megachilidae is further supported by biogeographic reconstructions. S-Diva results favour a South American/African vicariance (75% of reconstructions) over scenarios involving either African (12.6%) or African/Palaearctic (12.4%) origins and subsequent dispersal to South America. Similarly, in biogeographic inferences using Lagrange [36], 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 Africa and 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 [11] that the most convincing support for vicariance over migration comes from biological evidence. The brood cells of fideline bees consist of unlined cavities in the sand (figure 1c); for this reason, these bees are entirely restricted to strongly seasonal

513 Figure 1. (*Opposite.*) 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 Myr. 577
 514 Terminals are labelled to tribe according to present taxonomic assignment, even if determined to be paraphyletic in the current 578
 515 analysis. Branch colours correspond to significant changes in diversification rate (black: diversification rate = 0.0164, relative 579
 516 extinction = 0.885; red: diversification rate = 0.0867, relative extinction = 0.848; blue: diversification rate = 0.315, relative 580
 517 extinction = 0.518). Node marked with green star corresponds to the transition between building unlined nests and 581
 518 building nests using foreign material. There is no reversion to building unlined nests after this point. Photographs to the right 582
 519 of phylogeny from top to bottom: (1) Tribe Fideiini: *Fidelia villosa* using hind legs to excavate sand from a burrow (photo: 583
 520 Jerome G. Rozen [14], courtesy of the American Museum of Natural History); (2) Tribe Lithurgini: *Lithurgus chrysurus* entering 584
 521 nest in dead tree trunk (photo: Andreas Müller); (3) Tribe Anthidiini: *Anthidium strigatum* closing a nest cell of resin (photo: 585
 522 Albert Krebs); (4) Tribe Osmiini: nest of *Osmia bicolor* built in an abandoned snail shell (photo: Albert Krebs); (5) Tribe Mega- 586
 523 chilini: (top) *Megachile parietina* entering her nest made of mud (photo: Albert Krebs); (bottom) *Megachile lignisecca* using her 587
 524 mandibles to cut a leaf disc (photo: Felix Amiet). (b) Biogeographic reconstructions indicate a Gondwanan origin for Megachi- 588
 525 lidae, approximately 126 Ma (figure reprinted from Scotese et al. [45], copyright 1988, with permission from Elsevier). (c) The 589
 526 ancestor of all Megachilidae built unlined nests in sandy soil, much like extant lineages *Fidelia*, *Neofidelia* and *Pararhophites* (nest 590
 527 of *Fidelia villosa* shown; picture: Jerome G. Rozen [14], courtesy of the American Museum of Natural History). (d) Host plants of 591
 528 Fideiini (electronic supplementary material, table S4). Top row (left to right): *Nolana* sp. (Solanaceae; host of *Neofidelia* 592
 529 *longirostris*; photo: Michael O. Dillon), *Calandrinia* sp., *Trichocereus* sp. (Portulacaceae and Cactaceae, respectively; hosts of 593
 530 *N. profuga*; photos: Joshua R. McDill, Scott Zona); centre row (L–R): *Sesamum* sp. (Pedaliaceae; host of *Fidelia friesei*; photo: 594
 531 Jessica Litman), *Psilocalon* sp. (Aizoaceae; host of *F. villosa*, *F. kobrowi*, *F. paradoxa*; photo: Jessica Litman), *Sisyndite sparteae* 595
 532 (Zygophyllaceae; host of *F. pallidula*; photo: Tomas Hajek); bottom row (left to right): *Griehum* sp. (Neuradaceae; host of 596
 533 *F. hessei*, *F. major*, *F. fasciata*; photo: Serban Proches), *Berkheya fruticosa* (Asteraceae; host of *F. braunsiana*; photo: Henry 597
 534 Brisse), *Convolvulus trabutianus* (Convolvulaceae; host of *F. ulrikei*; photo: Pierre-Marie Roux). Not shown: *Tribulocarpus* 598
 535 *dimorphanthus* (Aizoaceae; host of *F. ornata*). Note that all flowers are characterized by radial symmetry and exposed anthers. 599
 536

537 has a single origin at the base of the tribes Anthidiini, 601
 538 Dioxyini, Osmiini and Megachilini (average maximum- 602
 539 likelihood probability 0.99, average difference in likeli- 603
 540 hood 2.5 and 7.3; posterior probability 0.99, BF 4.4 604
 541 and 10.3). 605

542 The use of foreign material in nest construction 606
 543 underlies the ability of megachilid bees to colonize tem- 607
 544 perate regions and appears to be associated with a 608
 545 dramatic increase in clade species diversity. The lineages 609
 546 *Fidelia*, *Neofidelia* and *Pararhophites* together number 17 610
 547 species, while the tribes Anthidiini, Osmiini and Mega- 611
 548 chilini collectively include over 3900 species and exhibit 612
 549 a worldwide distribution. MEDUSA [43] results provide 613
 550 evidence for two significant increases in diversification 614
 551 rate in our phylogeny, the first at the base of the higher 615
 552 megachilids and the second nested within the genus 616
 553 *Megachile* (figure 1). 617

554 The larger of the two rate shifts increases from 0.0164 618
 555 to 0.0867 and occurs approximately 7 Myr after the 619
 556 advent of nest construction using foreign material, a be- 620
 557 haviour that is first observed in the enigmatic genus 621
 558 *Aspidosmia* [37], the first branch within the subfamily 622
 559 Megachilinae. The increase in diversification rate that 623
 560 occurs after the divergence between *Aspidosmia* and the 624
 561 rest of Megachilinae suggests that the use of foreign 625
 562 material in nesting may have driven diversification but 626
 563 was not the only factor underlying it. 627

564 The second shift in diversification rate occurs within 628
 565 the genus *Megachile*, from 0.087 to 0.315. The increase 629
 566 in diversification tempo happens approximately 8 Myr 630
 567 after the origin of the true leafcutting *Megachile* (Mich- 631
 568 ener's group I) from the paraphyletic assemblage of the 632
 569 *Chalicodoma* group of subgenera (Michener's group II) 633
 570 [37]. Despite their relatively recent origin (22 Ma; 95% 634
 571 HPD 16–27), leafcutting *Megachile* are extremely diverse 635
 572 and abundant on all continents. The explanation for such 636
 573 species richness may be related to their high reproductive 637
 574 output [48] and their ability to colonize an extremely 638
 575 broad range of habitats, from moist tropics to extreme 639
 576 deserts. 640

601 In association with the ancestral state reconstructions 602
 603 of nesting biology, the diversification rate analysis reveals 604
 605 an intimate association between nesting biology, 606
 607 distribution and diversification. The single origin of 608
 609 nest-lining behaviour in Megachilidae makes it difficult 610
 611 to test for correlated evolution between nesting and 612
 613 other traits of interest. In contrast, the total geographical 614
 615 area occupied by the terminal taxa varies from lineage to 616
 617 lineage throughout the phylogeny, allowing us to test for 618
 619 an association between area and diversification rate. The 620
 621 results of BAYES TRAITs analyses [38] indicate strongly cor- 622
 623 related evolution between geographical area and 624
 625 diversification rate (BF = 25.8). In keeping with other 626
 627 studies where geographical area has been correlated 628
 629 with diversification [49], we envision a scenario where 630
 631 nest-lining behaviour promoted the widespread coloniza- 632
 633 tion of temperate habitats, which in turn drove the 634
 635 diversification seen in the higher megachilids. 636
 637

638 Ancestral state reconstructions strongly indicate that 639
 640 the three fideiine lineages are restricted to deserts 641
 642 owing to their plesiomorphic nesting biology, rather 643
 644 than as a secondary adaptation. The use of foreign 645
 646 material in nest construction has a single origin at the 646
 647 base of the tribes Anthidiini, Dioxyini, Osmiini and 647
 648 Megachilini. It has enabled these bees to repeatedly colo- 648
 649 nize temperate habitats and catalysed a massive shift in 649
 650 diversification rate. Surprisingly, Lithurgini manage to 650
 651 survive in temperate and tropical conditions, although 651
 652 they do not line their brood cells. All Lithurgines dig bur- 652
 653 rows in wood or stems and their pollen provisions are 653
 654 protected from humidity in these above-ground sub- 654
 655 strates. In other respects, the pollen provisions and nest 655
 656 architecture of lithurgine bees are very similar to those 656
 657 of fideiine bees. The pollen mass is neither worked nor 657
 658 manipulated by the female; it does not form a spherical 658
 659 mass but rather occupies the entire rear portion of the 659
 660 nest cell. Their burrows are mostly branched and the 660
 661 cells are either not partitioned or partitioned using saw- 661
 662 dust or wood particles obtained from the excavation of 662
 663 brood cells. These bees then fill their completed burrows 663
 664

with sawdust, in much the same way that fideliine bees do with sand [6,7].

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 Myr, 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 Ma, early in bee evolution.

(a) 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 probably form a monophyletic group [9], apply secreted lining to their brood cells [1,2]. Curiously, some desert andrenids apply a secreted lining not to the walls of their nests but to the pollen provisions themselves [50]. 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 that apparently do not line their brood cells (e.g. *Eremapis*; [51]), four independent origins of cleptoparasitism and the evolution of social behaviour [34]. Lastly, unlined nests are known in several members of the melittid bees [37], a species-poor group that may represent the earliest lineages of extant bees [9]. Many melittids are restricted to xeric areas, especially several species-poor genera for which the nesting biology is not documented (e.g. *Eremaphanta*, *Afrodasyopoda*, *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 (*Dasyopoda*). In fact, according to the most comprehensive phylogenetic hypothesis currently available for bees [9], 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 [52] and that cell lining, using either glandular secretions or foreign material, has multiple origins in bees.

By contrast, unlined nests are prevalent among apoid wasps [53,54], the paraphyletic group from which bees arose. In fact, the nesting biology of fideliine bees is reminiscent of that of many sand-nesting apoids [10] 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 [55], 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.

(b) Foraging behaviour and host-plant associations

Interactions with angiosperms have often been cited as important driving factors underlying diversification in phytophagous insects [56]. 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 using all available angiosperm hosts: a pronounced floral specificity (oligolecty). Comparisons of the well-documented foraging behaviour of the basal members of Megachilidae (figure 1d and electronic supplementary material, table S4) provide unique insights into bee–flower relationships prevalent more than 100 Ma. Fideliine bees, both in South America and South Africa, are notorious oligolectes. Rozen [12] states that on both continents, fideliine bees tend to forage on large flowers with well-exposed anthers (figure 1d); 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 *Microthurga* in Australia, Africa and South America forage exclusively or predominantly on Malvaceae with large flowers, such as *Hibiscus*, *Sida* and *Turnera* (electronic supplementary material, table S4); 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 maintained a close association with the large flowers of Cactaceae 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 (electronic supplementary material, table S4). 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. By 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 [57], as well as the widely discussed assertion that oligolecty is a primitive, rather than a derived state in bees (reviewed in [57]). Müller [58] suggested that oligolecty might be a behavioural constraint related to flower manipulation, pollen collecting or pollen digestion, rather than a secondary specialization. Interestingly, Müller [58] 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 [53,54] and co-occur in the

769 same habitat. Evans [59] elegantly summarizes the foraging
770 behaviour of the philanthine wasp tribe Cercerini
771 as follows: 'I suggest that these wasps are not necessarily
772 "good taxonomists", but that they are programmed to
773 hunt in certain situations and to respond to prey of a certain
774 size and behaviour'. We hypothesize that early bees
775 inherited foraging specificity as a behavioural constraint
776 from their apoid wasp ancestors.

777 4. CONCLUSION

780 Our work reveals that two extant lineages are 'living fossils'
781 among the bees. The Mid-Cretaceous origin of
782 *Fidelia* and *Neofidelia* and their bizarre, plesiomorphic
783 biology strongly support the possibility that these bees
784 reflect the biology of the earliest bees more closely than
785 any other extant lineage. The evolutionary patterns we
786 report in Megachilidae lay the initial framework for
787 understanding patterns of nesting behaviour, distribution,
788 host-plant preference and diversification in all bees.

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1 **Electronic supplementary material for:**

2

3 **Why do leafcutter bees cut leaves? New insights into the early evolution of bees**

4

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26 **Table of contents:**

27

28	Data partitioning	Page 2
29	Model testing	Page 2
30	Fossil calibration points	Pages 2 - 7
31	Unused fossils	Pages 7 - 9
32	Biogeographic analyses	Pages 9 - 10
33	Ancestral state reconstructions	Page 10
34	Correlated trait evolution	Pages 10 - 11
35	Table legends	Pages 11 - 12
36	Figure legends	Page 12
37	Table S1	Pages 13 - 14
38	Table S2	Pages 15 - 16
39	Table S3	Page 17
40	Table S4	Page 18 - 19
41	References	Pages 20 - 23

42

43

44 **Data partitioning**

45 We ran a preliminary Bayesian analysis to establish a partitioning regime: we
46 concatenated the four protein-coding genes and partitioned each gene into first, second
47 and third codon positions; the resulting dataset contained 12 partitions. We then ran a
48 short analysis in MrBayes v.3.1.2 [1, 2] (5,000,000 generations using a GTR model) and
49 examined the parameter files in Tracer [3]. After eliminating an appropriate burnin, we
50 used Tracer to determine the substitution rate and nucleotide composition for each of the
51 twelve partitions. We grouped similar partitions together and selected the following
52 partitioning regime: Partition 1 included the first codon position of CAD and LW
53 rhodopsin (518 bp); partition 2 included the first positions of EF1-alpha and NAK, and
54 the second codon positions of CAD, EF1-alpha, NAK and LW rhodopsin (2250 bp);
55 partition 3 included the third codon positions of CAD and NAK (791 bp); and partition 4
56 included the third position of EF1-alpha and LW rhodopsin (596 bp). The ribosomal
57 gene, 28S, was divided into two partitions, a stem partition, consisting of nucleotides
58 hydrogen-bound in paired strands (767 bp) and a loop partition, consisting of unpaired
59 nucleotides (539 bp). The resulting dataset therefore contained six partitions (5461 total
60 base pairs).

61

62 **Model testing**

63 Models of nucleotide substitution were selected based on the Akaike Information
64 Criterion (AIC) as determined by MrModelTest 2.3 [4, 5]. MrModelTest calculates AIC
65 values for each of 24 models of nucleotide substitution; the model associated with the
66 lowest AIC score is selected as the best-fit model. Independent model tests were
67 performed on each data partition. For each partition, the best-fit model was a general time
68 reversible model with a gamma correction for among site rate variation and an allowance
69 for invariant sites (GTR+I+ Γ).

70

71 **Fossil calibration points**

72 For each fossil used to time-calibrate our phylogeny, we outline our reasoning and list
73 the parameters used in BEAST to set the prior distribution and the 95% upper, median
74 and lower bounds on *a priori* ages. All zero-offset values correspond to the most recent

75 boundary of the geological epoch to which the fossil has been assigned. The placement of
76 each fossil on our phylogeny is shown in Fig. S3.

77

78 1. *Apis lithohermaea*

79 This fossil is recorded from the middle Miocene deposits of Iki Island,
80 Japan and has been assigned to the *Apis dorsata* species-group based on its
81 enlarged body size, elongate metabasitarsus, and infuscated wing membranes [6].
82 We therefore consider this fossil as a member of the stem group for *Apis dorsata*
83 and use it to set a minimum age on the node uniting *A. dorsata* and its sister group
84 (*A. cerana* + *A. mellifera*). We calibrated this node using a lognormal prior
85 distribution; the corresponding parameters used in BEAST were a zero-offset of
86 11.2 my, a log(mean) of 0.11, and a standard deviation of 1.0. The 95% upper
87 bound, median and lower bound on our *a priori* ages were 17.0, 12.3, and 11.4
88 my.

89

90 2. *Palaeomacropis eocenicus*

91 This specimen was found in early Eocene (Sparnacian) amber in Oise,
92 France. A cladistic analysis [7] based on seventeen morphological characters
93 places it as the sister taxon to the melittid genus *Macropis*. The absence of other
94 macropidine genera in the cladistic analysis of Michez et al., namely *Promelitta*,
95 makes it unclear whether *Palaeomacropis* belongs to the crown or stem group for
96 Macropidini. We prefer the conservative option and consider *Paleomacropis* as a
97 member of the stem group. We use it to place a minimum age on the node uniting
98 Macropidini (represented by *Macropis nuda* and *Promelitta alboclypeata* in our
99 phylogeny) to its sister taxon, *Melitta leporina*. We calibrated this node using a
100 lognormal prior distribution; the corresponding parameters used in BEAST were a
101 zero-offset of 49.0 my, a log(mean) of 1.6, and a standard deviation of 1.0. The
102 95% upper bound, median and lower bound on our *a priori* ages were 74.7, 54.0,
103 and 50.0 my.

104

105 3. *Paleohabropoda oudardi*

106 *Paleohabropoda oudardi* is a compression fossil recorded from the Paleocene
107 of Menat, Puy-de-Dome, France [8]. While the fossil is assigned to the apid tribe
108 Anthophorini, two conflicting analyses present different phylogenetic positions
109 for *Paleohabropoda oudardi*. A cladistic analysis based on seventeen
110 morphological characters [8] places the fossil as sister to the extant Anthophorini;
111 in our phylogeny, this corresponds to a calibration point at the node uniting
112 Anthophorini (represented in our phylogeny by *Pachymelus peringueyi* and
113 *Anthophora montana*) with the rest of the apids. A separate analysis based on
114 wing morphometry [8], however, places this fossil within the extant
115 Anthophorini, more closely related to *Pachymelus* than to *Anthophora*; in our
116 phylogeny, this corresponds to a calibration point at the node uniting *Pachymelus*
117 to its sister taxon, *Anthophora*. In order to accommodate this uncertainty in
118 phylogenetic position, we used the fossil to place a mean age on the node uniting
119 *Pachymelus* and *Anthophora*. We used a normal prior distribution at this node,
120 thereby allowing the node to be either older or younger than the age of the fossil.
121 The normal distribution was assigned a mean of 60 my and a standard deviation
122 of 6.0. The 95% upper bound, median and lower bound on our *a priori* ages were
123 69.9, 60.0, and 50.1 my.

124

125 4. *Kelneriapis eocenica*

126 This specimen is from middle Eocene Baltic amber. Based on
127 morphological characters, Engel [9] assigns this fossil to the extant tribe
128 Meliponini and indicates that *Kelneriapis* is likely sister to the extant genus
129 *Hypotrigona*, due to the rounded posterior apical corner of the metatibia in both
130 genera. We therefore consider this fossil as a member of the stem group for the
131 genus *Hypotrigona*. In our phylogeny, however, the relationship between
132 *Hypotrigona* and its sister taxon, *Tetragonula*, is not strongly supported in either
133 Bayesian or maximum likelihood analyses (Figs. S1, S2, S3). A recent molecular
134 phylogeny [10] also recovered low branch support for the sister group relationship
135 between *Hypotrigona* and *Tetragonula* in both Bayesian and maximum likelihood
136 analyses. The sister taxon to *Hypotrigona*+*Tetragonula* is the genus *Meliponula*;

137 these three taxa for a well-supported clade in both Bayesian and maximum
138 likelihood analyses (Figs. S1, S2, S3). It remains unclear, however, what the
139 relationship is between *Hypotrigona*, *Tetragonula* and *Meliponula*; for this
140 reason, we use this fossil to place a minimum age on the node uniting
141 *Hypotrigona*, *Tetragonula*, and *Meliponula*. We calibrated this node using a
142 lognormal prior distribution; the corresponding parameters used in BEAST were a
143 zero-offset of 41 my, a log(mean) of 1.4, and a standard deviation of 1.0. The
144 95% upper bound, median and lower bound on our *a priori* ages were 66.7, 46.0,
145 and 42.0 my.

146

147 5. *Boreallodape* sp.

148 At least three species of the Baltic amber genus *Boreallodape* have been
149 discovered: *B. baltica*, *B. mollyae*, and *B. striebichi* [9]. A fourth species may
150 exist but key attributes of the specimen are not visible and the species remains
151 undetermined. This genus has been assigned to the apid tribe Boreallodapini.
152 Engel [9] suggests that this tribe is closely related to Ceratinini and Allodapini; in
153 a cladistic analysis based on fourteen morphological characters, Engel
154 demonstrates that Boreallodapini is more closely related to Allodapini than to
155 Ceratinini. We therefore use this fossil to place a minimum age on the node
156 uniting Allodapini (represented in our phylogeny by *Exoneura bicolor*) and
157 Ceratinini (represented by *Ceratina calcarata*). Due to the presence of at least
158 three unique species of *Boreallodape*, we consider it likely that this genus arose 5-
159 10 million years earlier than the age of the fossil. We calibrated this node using a
160 lognormal prior distribution; the corresponding parameters used in BEAST are a
161 zero-offset of 41 my, a log(mean) of 2.0, and a standard deviation of 1.0. The
162 95% upper bound, median and lower bound on our *a priori* ages were 79.3, 48.4,
163 and 42.4 my.

164

165 6. *Megachile glaesaria*

166 This specimen was recovered from Miocene Dominican amber. Engel [11]
167 proposes that *M. glaesaria* is most similar to the subgenus *Chelostomoides* and

168 probably closely related to the extant species *Megachile manni*. Our phylogeny
169 includes two members of the subgenus *Chelostomoides*: *Megachile spinotulata*
170 and *Megachile angelarum*. The phylogenetic position of *Megachile manni* within
171 the subgenus *Chelostomoides* is unknown, which makes placement of this fossil
172 difficult. *M. glaesaria* is placed in its own subgenus, *Chalicodomopsis*; therefore
173 we did not place it as a crown member of *Chelostomoides*. Placing it as a stem
174 group fossil for *Chelostomoides* also proved difficult, however, as the position of
175 this subgenus within *Megachile* is uncertain. Given the close morphological
176 similarity of *M. glaesaria* with extant members of the subgenus *Chelostomoides*,
177 and given that this fossil and the extant subgenus *Chelostomoides* are the only
178 new world representatives of the *Chalicodoma*-group of subgenera, we used this
179 fossil to place a mean age on the node uniting both species of *Chelostomoides*.
180 We used a normal prior distribution at this node, thereby allowing the node to be
181 either older or younger than the age of the fossil. The normal distribution was
182 assigned a mean of 17.5 my and a standard deviation of 1.6. The 95% upper
183 bound, median and lower bound on our *a priori* ages were 20.1, 17.5, and 14.9
184 my.

185

186 7. *Cretotrigona prisca*

187 *Cretotrigona prisca* was recovered from late Cretaceous amber
188 (Maastrichtian) from New Jersey. It has alternately been placed as the sister taxon
189 to *Trigona* [12] and to *Dactylurina* [13]. While we agree that this fossil is
190 correctly assigned to the apid tribe Meliponini, we are not confident that it is a
191 member of the crown group for Meliponini. For this reason, we consider this
192 fossil as a member of the stem group for Meliponini and use it to place a
193 minimum age on the node uniting Meliponini (represented in our phylogeny by
194 *Cephalotrigona capitata*, *Hypotrigona gribodoi*, *Meliponula bocandei*,
195 *Tetragonula carbonaria*, and *Trigona fuscipennis*) with its sister group (*Bombus*
196 *ardens*+*Bombus diversus*). We calibrated this node using a lognormal prior
197 distribution; the corresponding parameters used in BEAST are a zero-offset of 65
198 my, a log(mean) of 2.3, and a standard deviation of 1.0. The 95% upper bound,

199 median and lower bound on our *a priori* ages were 116.7, 75.0, and 66.9 my.

200

201 Our dating analysis is in agreement with several fossil-calibrated phylogenies of
202 different groups of bees [14, 15] and consistent with fossil data that we did not use to
203 calibrate our phylogeny. We obtain an age for the corbiculate bees of around 95 my,
204 which corresponds well to Turonian (89-93 mya) fossils of resin-producing Clusiaceae
205 flowers that were likely visited by corbiculate bees [16]. The absence from Baltic amber
206 of both the crown *Heriades*-group of genera in the tribe Osmiini and the *Chalicodoma*-
207 group of subgenera in the genus *Megachile* may appear surprising, given that members of
208 both groups collect tree resin for nest construction. However, the inferred ages of both
209 groups (35 my and 32 my, respectively) are in keeping with the complete absence of
210 these bees from Baltic amber (age of Baltic amber ~ 40 my). While megachilids are well-
211 represented in Baltic amber [9], these species have been attributed to extinct tribes with
212 little affinity to extant lineages.

213

214 **Unused fossils**

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

221

222 *Probombus hirsutus* is a compression fossil recorded from a volcanic paleolake
223 deposit in Menat, Puy-de-Dôme, France (late Paleocene, ~ 60 mya). Initially described as
224 a bumblebee, this genus was later transferred to the family Megachilidae based on “the
225 presence of a metasomal sternal scopa and the absence of a clearly differentiated scopa
226 on metathoracic leg” [17]. Within Megachilidae, the presence of two submarginal wing
227 cells and several other morphological characters ally *Probombus* more closely with the
228 subfamily Megachilinae than the subfamily Fideliinae. Its position within Megachilinae,
229 however, is unclear. Nel and Petrulevicius [17] exclude affinities with Lithurgini,

230 Dioxyini, and Anthidiini, ultimately concluding that *Probombus* is probably closely
231 related to either the tribe Osmiini or the tribe Megachilini. Their conclusion, however, is
232 based on the elimination of relationships between *Probombus* and other tribes, rather than
233 on morphological synapomorphies that unite *Probombus* to either Osmiini or
234 Megachilini. Furthermore, characters that could ally *Probombus* to either Osmiini or
235 Megachilini are not visible in the fossil. We therefore consider this fossil as member of
236 the subfamily Megachilinae, *incertae sedis*; this fossil can only be used to calibrate the
237 node uniting the subfamily Megachilinae with its sister taxa (*Pararhophites*, Lithurgini).

238

239 Engel [9] refers to the genus *Glyptapis* as "an enigmatic lineage of megachilines",
240 initially placing the four species of *Glyptapis* (Baltic amber, ~ 40 mya) in the subtribe
241 Glyptapina within the megachilid tribe Osmiini. The subtribe Glyptapina later became the
242 tribe Glyptapini [18, 19]. The phylogenetic position of *Glyptapis* within Megachilidae is
243 uncertain: some characters suggest a close relationship with the tribe Anthidiini, while
244 others suggest a closer relationship with the tribe Osmiini. The only interpretation
245 possible for this fossil is as a member of the stem group for Anthidiini, Osmiini, and
246 Megachilini; the genus *Glyptapis* may therefore only be used to calibrate the node uniting
247 Anthidiini, Osmiini, and Megachilini to its sister group (*Dioxyini+Aspidomia*).

248

249 *Protolithurgus ditomeus* is recorded from Baltic amber. Engel [9] states that
250 "*Protolithurgus* seems to possess an enigmatic combination of characters unique among
251 megachilids" and that "the genus does share with other Lithurginae the distinctive
252 flattened, first metasomal tergum with a rounded apical margin, a feature found only in
253 lithurgines". While this feature may indeed reveal a close relationship between
254 *Protolithurgus* and the tribe Lithurgini, it remains unclear whether this genus is a member
255 of the crown Lithurgini or is better placed as a stem group member. Nel and Petrulevicius
256 [17] note the absence of coarse tubercles on the outer tibial surface of *Protolithurgus*, the
257 presence of which is a synapomorphy for the extant Lithurgini [20]. For this reason,
258 *Protolithurgus* may only be interpreted as a member of the stem group for Lithurgini; the
259 most appropriate placement for this fossil is therefore at the node uniting Lithurgini with
260 its sister group. It must be noted, however, that in our phylogeny, the relationship

261 between Lithurgini and its sister taxon, the genus *Pararhophites*, is poorly supported in
262 maximum likelihood analyses (Figure S3). Therefore the only valid placement for this
263 fossil is at the node uniting the subfamily Megachilinae.

264

265 The Baltic amber fossil genera *Ctenoplectrella* and *Glaesosmia* were initially
266 placed in the subtribe Ctenoplectrellina, within the megachilid tribe Osmiini [9]. The
267 subtribe Ctenoplectrellina later became the tribe Ctenoplectrellini [18, 19]. Wedmann et
268 al. [21] added *Friccomelissa schopowi*, an Eocene fossil from the Messel Pit Fossil Site
269 (Fossilagerstätte Grube Messel, Germany), to the tribe Ctenoplectrellini. Wedmann et al.
270 [21] state that a number of plesiomorphic traits relative to Osmiini and Megachilini
271 indicate that Ctenoplectrellini may belong to the stem group of either Osmiini or
272 Osmiini+Megachilini. The confluent position of wing veins 2rs-m and 2 m-cu in
273 Ctenoplectrellini suggest a phylogenetic position between Anthidiini and
274 Osmiini+Megachilini. We therefore interpret the tribe Ctenoplectrellini as a member of
275 the stem group for Osmiini+Megachilini; this fossil may only be used to calibrate the
276 node uniting Anthidiini, Osmiini, and Megachilini.

277

278 We have personally examined the megachilid fossils from the Florissant,
279 Colorado fossil beds deposited in the Museum of Comparative Zoology at Harvard
280 University. While a number of these taxa have been assigned to extant genera, we agree
281 with Engel [11] that these assignments are tenuous and that these fossils should be
282 relegated to "Apoidea incertae sedis".

283

284 Lastly, several authors (reviewed in [21]) have reported trace fossils (Eocene to
285 Miocene) supposedly left by leafcutting bees (genus *Megachile*). We did not include
286 these fossils for two reasons. Firstly, attribution to leafcutting bees remains hypothetical
287 [22]. Secondly, even if these leaf cuts had been left by members of the genus *Megachile*,
288 they would be very difficult to place on the phylogeny, given that many distantly related
289 *Megachile* cut leaves. According to our dating analysis, the genus *Megachile* originated
290 approximately 40 mya, strongly suggesting that at least the earliest of these trace fossils
291 may not have been left by bees of the genus *Megachile*.

292

293 **Biogeographic analyses**

294 For biogeography inference using S-Diva [23], we sampled 1010 trees from the
295 posterior distribution of post-burnin trees from the BEAST analysis. To further
296 distinguish between alternative biogeographical scenarios in a maximum likelihood
297 framework, we used the software Lagrange [24]. We used the consensus chronogram
298 from the BEAST analysis and the same ancestral range coding as in the S-Diva analysis.
299 The maximum number of areas occupied by a single taxon was set to two. Analyses
300 where Africa and South America were allowed to be adjacent resulted in ancestral range
301 reconstructions that strongly favoured vicariance between South America and Africa
302 (relative probability 0.87, likelihood values -251.2 to -252.7) over alternative scenarios
303 (relative probability 0.08, likelihood -253.5 to -254.6). A difference of two log-units can
304 be taken as evidence for a significant difference (25).

305

306 **Ancestral state reconstructions**

307 In BayesTraits [25, 26], we ran both maximum likelihood and Bayesian ancestral
308 state reconstructions using the same 1010 trees sampled in the biogeography analyses. In
309 maximum likelihood analyses, we restricted all rates to be equal (command "restrictall"),
310 except for the reversions from cleptoparasitism to other states, which were constrained to
311 zero. We successively constrained nodes of interest to different states ("fossil" command)
312 to test for differences in log-likelihoods. In the Bayesian analyses, we applied a reverse-
313 jump hyperprior (command "rjhp exp 0 10") and a "ratedev" value of 5 to obtain
314 acceptance rates between 20 and 40%, as recommended in the BayesTraits manual. The
315 same 1010 trees used in the biogeography analyses were used as input trees. Each
316 Bayesian chain was run for 5 million generations (burnin 50000). We successively
317 constrained the ancestor of Megachilidae to states 0, 1 and 2 using the "fossil" command.
318 We repeated each analysis 5 times and averaged the harmonic means to calculate Bayes
319 Factors, which equal twice the difference in harmonic mean. Values above 6 are
320 commonly taken as strong evidence for significance [27].

321

322 **Correlated trait evolution**

323 In order to explore the relationship between the total geographic area occupied by
324 a taxonomic group and diversification rate, we pruned our original dataset of 98 ingroup
325 terminals to a smaller dataset of 69 clearly defined monophyletic groups and calculated
326 diversification rate and total geographic range for each terminal; pruning the dataset was
327 necessary to determine clade size and geographic range for each terminal. Data on the
328 distribution of each species was obtained from revisionary works on Megachilidae cited
329 in Michener [28] and from the following websites: “Discover Life” [29] and the
330 “Palaeartic Osmiine Bees” website [30].

331

332 To test for correlation between total area occupied and diversification rate, we
333 performed two sets of Bayesian analyses using the software BayesTraits. In the first set
334 of analyses, total geographic range and diversification rate evolved independently of one
335 another; in the second, the traits were allowed to evolve in a correlated fashion. The same
336 1010 trees used in the biogeography and ancestral state reconstruction analyses were used
337 as input trees. The “ratedev” value was adjusted to 0.2 to obtain acceptance rates between
338 20 and 40%. Each set of analyses consisted of five independent Bayesian chains, each run
339 for 5,050,000 generations (burnin = 50000). We took the harmonic means of the
340 likelihood scores from each set of analyses to calculate Bayes Factors. The value of
341 lambda (where lambda represents the degree to which phylogeny predicts patterns of
342 covariance) was estimated from the data. Analyses where the two variables were allowed
343 to co-evolve exhibited significantly better likelihood scores (lambda=0.40; harmonic
344 mean of LH = -207.7) than those analyses in which the variables evolved independently
345 (lambda=0.40, harmonic mean of LH = -220.6; BF = 25.8).

346

347 **Table legends**

348

349 **Table S1.** Complete taxon list, DNA voucher numbers, and collection localities for
350 specimens used in this study

351

352 **Table S2:** GenBank accession numbers for all sequences used in this study

353

354 **Table S3:** PCR primer sequences and conditions for the five nuclear genes sequenced in
355 this study

356

357 **Table S4:** Host-plant data for tribes Fideliini, Pararhophitini, and Lithurgini. Shown are
358 individual taxa and their preferred host-plant/s based on collection and literature records.

359

360 **Figure legends**

361

362 **Figure S1. Maximum clade credibility tree from MrBayes analysis.** Tree based on
363 ~100,000,000 post-burnin generations in MrBayes v.3.2.1. Numbers shown at nodes are
364 Bayesian posterior probabilities.

365

366 **Figure S2. Maximum clade credibility tree from BEAST analysis.** Fossil-calibrated
367 phylogeny based on ~217,000,000 post-burnin generations in BEAST v.1.6.1. Numbers
368 shown at nodes are Bayesian posterior probabilities. Numbered black squares correspond
369 to fossil calibration points. 1. *Palaemacropis eocenicus*; 2. *Paleohabropoda oudardi*; 3.
370 *Boreallodape* sp.; 4. *Cretotrigona prisca*; 5. *Kelneriapis eocenica*; 6. *Apis lithohermaea*;
371 7. *Megachile glaesaria*.

372

373 **Figure S3. Bootstrap tree from RAxML analysis.** Phylogeny based on 1000 maximum
374 likelihood bootstrap replicates in RAxML v.7.0.4. Numbers shown at nodes are ML
375 bootstrap values.

376

377

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381

382

383

384

385 **Table S1**

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

<i>Eoanthidium (Clistanthidium) tenericum</i>	1589	NCP: Eksteenfontein, 09.x.2008
<i>Epanthidium (Epanthidium) bicoloratum</i>	1441	Argentina, Catamarca, Trampasacha, 25.x -12.xi.2003
<i>Hypanthidioides (Saranthidium) marginata</i>	CP2	Paraguay, Guaira, Res. de Recursos, Manejados 24.i.2007
<i>Hypanthidium (Hypanthidium) obscurius</i>	SC171	locality unknown
<i>Icteranthidium ferrugineum flavum</i>	1432	UZ, Karakalpakstan, Beruni, 25.v.2008
<i>Notanthidium (Notanthidium) steloides</i>	1542	Chile, Region Metro, Farellones, 31.xii.2008
<i>Pachyanthidium (Trichanthidium) bengualense</i>	1434	SA: Limpopo Prov., 27 km E Waterpoort, 07.i.2004
<i>Paranthidium (Paranthidium) jugatorium</i>	495	NY: Tompkins Co., Ithaca, 31.vii.1997
<i>Pseudoanthidium (Pseudoanthidium) scapulare</i>	1601	ITALY: Toscana, Massa Maritima, 28.vii.2005
<i>Rhodanthidium (Rhodanthidium) septemdentatum</i>	1514	GR, Rhodos, Stegna, 08.v.2005
<i>Serapista rufipes</i>	1450	South Africa, NCP, Eksteenfontein, 09.x.2008
<i>Stelis (Stelis) paiute</i>	1394	NV: Clark Co. Jean Lake, 14.iv.2004
<i>Trachusa (Archianthidium) pubescens</i>	1533	Turkey, Erzurum, Akören, 15 km N Hınıs, 19.vii.2003
<i>Trachusa (Heteranthidium) larreae</i>	1142	NV: Clark Co., Las Vegas Sand Dunes, 01.iv.2004
<i>Coelioxys (Allocoelioxys) afra</i>	1549	Switzerland, Weiach, 29.vi.2004
<i>Megachile (Aethomegachile) sp</i>	1515	Thailand, Chiang Mai, 22.iii.2007
<i>Megachile (Amegachile) fimbriata</i>	1523	S-Africa, 20 km E Waterpoort, 07.i.2004
<i>Megachile (Argyropile) parallela</i>	1522	AZ, Portal, Rucker Canyon, 31.viii.2008
<i>Megachile (Austrochile) sp</i>	1454	Australia, WA, Leonora, 27.ix.2005
<i>Megachile (Callomegachile) sculpturalis</i>	1423	USA,NY, Ithaca, vii.2008
<i>Megachile (Chalicodoma) parietina</i>	1555	Switzerland, Hohtenn, 26.v.2005
<i>Megachile (Chalicodomoides) aethiops</i>	1455	Australia, WA, Marble Bar, v.2003
<i>Megachile (Chelostomoda) sp</i>	1448	Thailand, Chiang Mai, 24.iii.2007
<i>Megachile (Chelostomoides) angelarum</i>	1283	NV: Clark Co., 2.5 mi S. Wheeler Well, 30.vi.2004
<i>Megachile spinotulata</i>	1435	USA, AZ, Portal, Rucker Canyon, 31.viii.2008
<i>Megachile (Chrysosarus) sp</i>	1442	Argentina, Jujuy Co., 2 km E Paso de Jama, 14.xi-21.xii.2003
<i>Megachile (Creightonella) albisecta</i>	1556	Italy, Toscana, Massa Maritima, 28.vii.2005
<i>Megachile (Cressoniella) zapoteca</i>	1439	USA, AZ, Cochise Co., Paradise Junction, 01.ix.2008
<i>Megachile (Eutricharaea) mandibularis</i>	1521	UZ, Bukara, 40 km N Gazli, 31.v.2008
<i>Megachile patellimana</i>	1453	Oman, Sur, 01.iii.2008
<i>Megachile pilidens</i>	1550	Switzerland, Weiach, 29.vi.2004
<i>Megachile (Gronoceras) bombiformis</i>	1531	South Africa, Limpopo Prov, 20 km E Waterpoort, 07.i.2004
<i>Megachile (Hackeriapis) sp2</i>	1447	Australia, WA, Coolgardie 25.ix.2005
<i>Megachile (Largella) sp</i>	1540	Thailand, Phetchabun Nam Nao NP, 8-15.iii.2007
<i>Megachile (Lithomegachile) texana</i>	1524	USA, NY, Ithaca, vii.2008
<i>Megachile (Maximegachile) maxillosa</i>	1532	South Africa, Mount Rupert, 08.ii.2008
<i>Megachile (Megachile) melanopyga</i>	1575	CH, Hohtenn, 26.v.2005
<i>Megachile (Megachiloides) nevadensis</i>	1427	USA, UT, Wayne Co, South Torrey, 05.viii.2008
<i>Megachile (Mitchellapis) fabricator</i>	1433	Australia, NSW, Wodonga, 09.xii.1999
<i>Megachile (Pseudocentron) sidalceae</i>	1429	USA, AZ, (County?), Wilcox, viii.2008
<i>Megachile (Pseudomegachile) ericetorum</i>	SC232	Czech Republic: Nový Brázdım. 17.vi.2000
<i>Megachile (Ptilosarus) microsoma</i>	1444	Trinidad, El Dorado, Caura Valley, 61 m, 10.iii.2008
<i>Megachile (Rhodomegachile) sp</i>	1443	Australia, W, Tom Price, iv.2003
<i>Megachile (Sayapis) pugnata</i>	595	NY: Schuyler Co., Valois gravel pit, 14.vii.1999
<i>Megachile (Thaumatostoma) remeata</i>	1445	Australia, WA, Laverton, 27.ix.2005
<i>Megachile (Xanthosarus) maritima</i>	1425	UZ, Bukara, 02.vi.2008
<i>Noteriades sp</i>	1580	Thailand, Chiang Mai, 24.iii.2007
<i>Radoszkowskiana rufiventris</i>	1587	Egypt, Tel el Kebir, 30°32'2"N 31°49'48"
<i>Afroheriades primus</i>	1585	SA, N. Cape, 6 km N Concordia, 14.ix.2007
<i>Ashmeadiella (Ashmeadiella) aridula</i>	1270	UT: Garfield Co., Long Canyon, 01.ix.2003
<i>Atoposmia (Eremosmia) mirifica</i>	1560	USA, NV, W Yucca Gap, 18.v.2005
<i>Chelostoma (Chelostoma) florissomme</i>	1553	Switzerland, Chur
<i>Haetosmia brachyura</i>	1428	UZ, Karakalpakstan, Beruni, 25.v.2008
<i>Heriades (Neotrypetes) crucifer</i>	1149	AZ: Coshise Co., Chiricahua Mts., 25.viii.2003
<i>Hofferia schmiedeknechti</i>	1586	Greece, Chimara, 26.v.2006
<i>Hoplitis (Hoplitis) adunca</i>	1552	Italy, Aosta, 30.viii.2004
<i>Ochreheriades fasciatus</i>	1557	Jordan, 20 km W Amman, 24.iv.2007
<i>Osmia (Osmia) lignaria</i>	1265	locality unknown
<i>Othinosmia (Megaloheriades) globicola</i>	1569	South-Africa, W Cape Prov., Nieuwoudtville, 09.x.2002
<i>Othinosmia (Othinosmia) securicornis</i>	1584	SA, N. Cape, Richtersveld, near De Koci, 09.ix.2007
<i>Protosmia (Protosmia) humeralis</i>	1559	Jordan, Wadi Shu'ayb, 22.iv.2007
<i>Pseudoheriades moricei</i>	1431	IL, Negev
<i>Stenoheriades asiaticus</i>	1578	Greece, Zachlorou, 22.v.2006
<i>Wainia (Caposmia) eremoplana</i>	1548	Jordan, Wadi el Hasa, 20.iv.2007

386
387
388
389

Table S2

Taxa	EF1a	Opsin	CAD	NAK	28S
<i>Dasygaster argentata</i>	AY585148	DQ116680	DQ067161	EF646418	AY654518
<i>Macropis nuda</i>	AY585155	DQ116686	DQ067171	HQ995917	HQ996008
<i>Melitta leporina</i>	AY585158	DQ116688	DQ067174	EF646394	AY654529
<i>Promelitta alboclypeata</i>	EF594330	EF594379	Missing	HQ995918	HQ996009
<i>Meganomia binghami</i>	DQ141114	DQ116689	DQ067169	EF646406	HQ996010
<i>Ceratina calcarata</i>	AY585108	AF344620	DQ067190	GU245213	HQ996011
<i>Exoneura bicolor</i>	GU245041	GU245337	Missing	GU245212	GU244896
<i>Xylocopa pubescens</i>	GU245052	GU245347	Missing	GU245225	GU244908
<i>Xylocopa virginica</i>	GU245047	GU245343	Missing	GU245220	GU244903
<i>Anthophora montana</i>	AY585107	AF344616	DQ067177	HQ995919	HQ996012
<i>Pachymelus peringueyi</i>	AY585114	DQ116678	DQ067182	GU245061	AY654544
<i>Apis cerana</i>	EU184774	EU184839	EU184808	EU184750	Missing
<i>Apis dorsata</i>	AY208277	AF091733	EU184807	EU184749	FJ042186
<i>Apis florea</i>	EU184773	EU184838	EU184806	EU184748	Missing
<i>Apis mellifera</i>	AF015267	AMU26026	DQ067178	XM_623142	AY703551
<i>Bombus ardens</i>	AF492964	AF493031	EU184803	EU184741	Missing
<i>Bombus diversus</i>	AF492961	AF493028	EU184804	EU184742	Missing
<i>Ctenoplectra albolimbata</i>	AY585118	DQ116677	EU122060	EF646391	HQ996013
<i>Eufriesea pulchra</i>	EU421377	EU184834	EU184802	EU184740	Missing
<i>Euglossa imperialis</i>	EU421408	AY267160	EU184800	EU184738	FJ042183
<i>Exaerete frontalis</i>	AY208286	AY267159	EU184801	EU184739	AF181602
<i>Cephalotrigona capitata</i>	EU184771	EU184836	EU184805	EU184745	FJ042015
<i>Hypotrigona gribodoi</i>	GU244957	GU245280	Missing	GU245121	GU244811
<i>Meliponula brocadei</i>	AY267145	AY267161	Missing	EU184746	FJ042177
<i>Tetragonula carbonaria</i>	GU244960	GU245282	Missing	GU245124	GU244814
<i>Trigona fuscipennis</i>	EU184770	EU184835	Missing	EU184744	EU049733
<i>Paranomada velutina</i>	AY585115	AF344627	DQ067188	GU245190	AY654545
<i>Thyreus delumbatus</i>	AY585119	DQ116679	DQ067184	GU245118	HQ996014
<i>Melitturga clavicornis</i>	AY585104	DQ116703	DQ067134	HQ995920	HQ996015
<i>Rophites algeris</i>	AY585144	DQ116675	DQ067159	HQ995921	HQ996016
<i>Colletes inaequalis</i>	AY363004	DQ115542	DQ067139	EF646387	HQ996017
<i>Pararhophites orobinus</i>	HQ995679	HQ995749	HQ995823	HQ995922	HQ996018
<i>Pararhophites quadratus</i>	EU851522	EU851627	HQ995824	GU245153	GU244841
<i>Fidelia kobrowi</i>	HQ995680	HQ995750	HQ995825	HQ995923	HQ996019
<i>Fidelia paradoxa</i>	HQ995681	HQ995751	HQ995826	HQ995924	HQ996020
<i>Fidelia villosa</i>	HQ995682	HQ995752	HQ995827	HQ995925	HQ996021
<i>Fidelia braunsiana</i>	HQ995683	HQ995753	HQ995828	HQ995926	HQ996022
<i>Fidelia ulrikei</i>	HQ995684	HQ995754	HQ995829	HQ995927	HQ996023
<i>Fidelia friesei</i>	HQ995685	HQ995755	HQ995830	HQ995928	HQ996024
<i>Fidelia pallidula</i>	HQ995686	HQ995756	HQ995831	HQ995929	HQ996025
<i>Fideliopsis hessei</i>	HQ995687	HQ995757	HQ995832	HQ995930	HQ996026
<i>Fideliopsis major</i>	DQ141113	EU851628	HQ995833	HQ995931	HQ996027
<i>Fideliopsis ornata</i>	HQ995688	HQ995758	HQ995834	HQ995932	HQ996028
<i>Neofidelia longirostris</i>	HQ995689	HQ995759	HQ995835	HQ995933	HQ996029
<i>Neofidelia profuga</i>	GU244990	HQ995760	HQ995836	GU245151	HQ996030
<i>Lithurgus echinocacti</i>	DQ141116	HQ995761	DQ067195	EF646390	AY654541
<i>Lithurgus chrysurus</i>	EU851523	EU851629	HQ995837	HQ995934	HQ996031
<i>Lithurgus pullatus</i>	HQ995690	HQ995762	HQ995838	HQ995935	HQ996032
<i>Lithurgus rubricatus</i>	HQ995691	HQ995763	HQ995839	HQ995936	HQ996033
<i>Lithurgus scabrosus</i>	HQ995692	HQ995764	HQ995840	HQ995937	HQ996034
<i>Lithurgus tibialis</i>	HQ995693	HQ995765	HQ995841	HQ995938	HQ996035
<i>Microthurga sp</i>	HQ995694	HQ995766	HQ995842	GU245161	GU244849
<i>Trichothurgus herbsti</i>	HQ995695	HQ995767	HQ995843	GU245160	GU244848
<i>Aglaoapis tridentata</i>	EU851524	EU851630	HQ995844	HQ995939	HQ996036
<i>Dioxys moesta</i>	HQ995696	HQ995768	HQ995845	HQ995940	HQ996037
<i>Paradoxys pannonica</i>	HQ995697	HQ995769	HQ995846	HQ995941	HQ996038
<i>Afranthidium karoense</i>	HQ995698	HQ995770	HQ995847	HQ995942	HQ996039
<i>Anthidium illustre</i>	HQ995699	HQ995771	HQ995848	HQ995943	HQ996040
<i>Anthodioctes mapirensis</i>	HQ995700	HQ995772	HQ995849	HQ995944	HQ996041
<i>Aspidosmia arnoldi</i>	HQ995701	HQ995773	HQ995850	HQ995945	HQ996042
<i>Aspidosmia volkmanni</i>	HQ995702	HQ995774	HQ995851	HQ995946	HQ996043
<i>Bathanthidium binghami</i>	HQ995703	HQ995775	HQ995852	HQ995947	HQ996044
<i>Benanthis madagascariensis</i>	HQ995704	HQ995776	HQ995853	HQ995948	HQ996045
<i>Dianthidium arizonicum</i>	HQ995705	HQ995777	HQ995854	HQ995949	HQ996046
<i>Duckeanthidium thielei</i>	HQ995706	HQ995778	HQ995855	HQ995950	HQ996047
<i>Eoanthidium ternericum</i>	HQ995707	HQ995779	HQ995856	HQ995951	HQ996048

Taxa	EF1a	Opsin	CAD	NAK	28S
<i>Epanthidium bicoloratum</i>	HQ995708	HQ995780	HQ995857	HQ995952	HQ996049
<i>Hypanthidioides marginata</i>	HQ995709	HQ995781	HQ995858	HQ995953	HQ996050
<i>Hypanthidium obscurius</i>	HQ995710	HQ995782	HQ995859	HQ995954	HQ996051
<i>Icteranthisium ferrugineum</i>	HQ995711	HQ995783	HQ995860	HQ995955	HQ996052
<i>Notanthidium steloides</i>	HQ995712	HQ995784	HQ995861	HQ995956	HQ996053
<i>Pachyanthidium bengualense</i>	HQ995713	HQ995785	HQ995862	HQ995957	HQ996054
<i>Paranthidium jugatorium</i>	GU244994	HQ995786	HQ995863	GU245156	GU244844
<i>Pseudoanthidium scapulare</i>	HQ995714	HQ995787	HQ995864	HQ995958	HQ996055
<i>Rhodanthidium septemdentatum</i>	HQ995715	HQ995788	HQ995865	HQ995959	HQ996056
<i>Serapista rufipes</i>	HQ995716	HQ995789	HQ995866	HQ995960	HQ996057
<i>Stelis paiute</i>	HQ995717	HQ995790	HQ995867	HQ995961	HQ996058
<i>Trachusa pubescens</i>	HQ995718	HQ995791	HQ995868	HQ995962	HQ996059
<i>Trachusa larreae</i>	HQ995719	HQ995792	HQ995869	GU245154	GU244842
<i>Coelioxys afra</i>	EU851528	EU851634	HQ995870	HQ995963	HQ996060
<i>Megachile (Aethomegachile) sp</i>	HQ995720	HQ995793	HQ995871	HQ995964	HQ996061
<i>Megachile fimbriata</i>	HQ995721	HQ995794	HQ995872	HQ995965	HQ996062
<i>Megachile parallela</i>	HQ995722	HQ995795	HQ995873	HQ995966	HQ996063
<i>Megachile (Austrochile) sp</i>	HQ995723	HQ995796	HQ995874	HQ995967	HQ996064
<i>Megachile sculpturalis</i>	HQ995724	HQ995797	HQ995875	HQ995968	HQ996065
<i>Megachile parietina</i>	EU851530	EU851636	HQ995876	HQ995969	HQ996066
<i>Megachile aethiops</i>	HQ995725	HQ995798	HQ995877	HQ995970	HQ996067
<i>Megachile (Chelostomoda) sp</i>	HQ995726	HQ995799	Missing	HQ995971	HQ996068
<i>Megachile angularum</i>	HQ995727	HQ995800	HQ995878	GU245163	GU244851
<i>Megachile spinotulata</i>	HQ995728	HQ995801	HQ995879	HQ995972	HQ996069
<i>Megachile (Chrysosarus) sp</i>	HQ995729	HQ995802	HQ995880	HQ995973	HQ996070
<i>Megachile albisecta</i>	EU851529	EU851635	HQ995881	HQ995974	HQ996071
<i>Megachile zapoteca</i>	HQ995730	HQ995803	HQ995882	HQ995975	HQ996072
<i>Megachile mandibularis</i>	HQ995731	HQ995804	HQ995883	HQ995976	HQ996073
<i>Megachile patellimana</i>	HQ995732	HQ995805	HQ995884	HQ995977	HQ996074
<i>Megachile pilidens</i>	EU851531	EU851637	HQ995885	HQ995978	HQ996075
<i>Megachile bombiformis</i>	HQ995733	HQ995806	HQ995886	HQ995979	HQ996076
<i>Megachile (Hackeriapis) sp</i>	HQ995734	HQ995807	HQ995887	HQ995980	HQ996077
<i>Megachile (Largella) sp</i>	HQ995735	HQ995808	HQ995888	HQ995981	HQ996078
<i>Megachile texana</i>	HQ995736	HQ995809	HQ995889	HQ995982	HQ996079
<i>Megachile maxillosa</i>	HQ995737	HQ995810	HQ995890	HQ995983	HQ996080
<i>Megachile melanopyga</i>	HQ995738	HQ995811	HQ995891	HQ995984	HQ996081
<i>Megachile nevadensis</i>	HQ995739	HQ995812	HQ995892	HQ995985	HQ996082
<i>Megachile fabricator</i>	HQ995740	HQ995813	HQ995893	HQ995986	HQ996083
<i>Megachile sidalceae</i>	HQ995741	HQ995814	HQ995894	HQ995987	HQ996084
<i>Megachile ericetorum</i>	HQ995742	HQ995815	HQ995895	GU245165	GU244853
<i>Megachile microsoma</i>	HQ995743	HQ995816	HQ995896	HQ995988	HQ996085
<i>Megachile (Rhodomegachile) sp</i>	HQ995744	HQ995817	HQ995897	HQ995989	HQ996086
<i>Megachile pugnata</i>	AY585147	HQ995818	DQ067196	HQ995990	HQ996087
<i>Megachile remeata</i>	HQ995745	HQ995819	HQ995898	HQ995991	HQ996088
<i>Megachile maritima</i>	HQ995746	HQ995820	HQ995899	HQ995992	HQ996089
<i>Noteriades sp</i>	EU851589	EU851695	HQ995900	HQ995993	HQ996090
<i>Radoszkowskiana rufiventris</i>	HQ995747	HQ995821	HQ995901	HQ995994	HQ996091
<i>Afroheriades primus</i>	EU851532	EU851638	HQ995902	HQ995995	HQ996092
<i>Ashmeadiella aridula</i>	EU851535	EU851641	HQ995903	GU245171	GU244858
<i>Atoposmia mirifica</i>	EU851541	EU851647	HQ995904	HQ995996	HQ996093
<i>Chelostoma florissomne</i>	EU851546	EU851652	HQ995905	HQ995997	HQ996094
<i>Haetosmia brachyura</i>	HQ995748	HQ995822	HQ995906	HQ995998	HQ996095
<i>Heriades crucifer</i>	EU851555	EU851661	DQ067194	GU245168	GU244855
<i>Hofferia schmidedeknehti</i>	EU851556	EU851662	HQ995907	HQ995999	HQ996096
<i>Hoplitis adunca</i>	EU851572	EU851678	HQ995908	HQ996000	HQ996097
<i>Ochreeriades fasciatus</i>	EU851590	EU851696	HQ995909	HQ996001	HQ996098
<i>Osmia lignaria</i>	EU851610	EU851715	HQ995910	GU245169	GU244856
<i>Othinosmia globicola</i>	EU851616	EU851721	HQ995911	HQ996002	HQ996099
<i>Othinosmia securicornis</i>	EU851617	EU851722	HQ995912	HQ996003	HQ996100
<i>Protosmia humeralis</i>	EU851621	EU851726	HQ995913	HQ996004	HQ996101
<i>Pseudoheriades moricei</i>	EU851622	EU851727	HQ995914	HQ996005	HQ996102
<i>Stenoheriades asiaticus</i>	EU851623	EU851728	HQ995915	HQ996006	HQ996103
<i>Wainia eremoplana</i>	EU851626	EU851731	HQ995916	HQ996007	HQ996104

391
392
393

Table S3

Primer	Sequence
28S	
A [31]	5' CCC CCT GAA TTT AAG CAT AT 3'
Mar [32]	5' TAG TTC ACC ATC TTT CGG GTC CC 3'
Bel [33]	5' AGA GAG AGT TCA AGA GTA CGT G 3'
D4 [34]	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 [35]	5' GCG TGY GGC ACM GAY TAC TTC 3'
Opsinrev5a [35]	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 [36]	5' GGG YAA AGG WTC CTT CAA RTA TGC 3'
F2revmeg [35]	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 [10]	5' GGY GGT TTC GCS WTG YTG YTG TGG ATC GG 3'
Nakrev1a [10]	5' CCG ATN ARR AAG ATR TGM GCG TCN AGC CAA TG 3'
Nakfor2 [10]	5' GCS TTC TTC TCB ACS AAC GCC GTY GAR GG 3'
Nakrev2 [10]	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 [34]	5' TGG AAR GAR GTB GAR TAC GAR GTG GTY CG 3'
Cadrev1meg [35]	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.

395
396
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Table S4

Subfamily/ tribe	Genus	Subgenus	Further grouping	Species	Host-plant		
Fideliinae							
Fideliini	<i>Neofidelia</i>			<i>longirostris</i>	Oligolectic on <i>Nolana</i> sp (Solanaceae) [37, 38] (Litman pers. obs. in Chile)		
				<i>profuga</i>	Polylectic with pollen collection records for Cactaceae (<i>Trichocereus</i> , <i>Eulychnia</i>), Portulacaceae (<i>Calandrinia</i>) and floral visits (possibly for nectar) on Asteraceae (<i>Encelia</i>) [37-39] (Litman pers. obs. in Chile)		
	<i>Fidelia</i>	<i>Fidelia</i>			<i>kobrowi</i>	As <i>F. paradoxa</i> [40]	
					<i>paradoxa</i>	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 [37, 40, 41]	
					<i>villosa</i>	Probably oligolectic on Aizoaceae [37, 40, 41]	
			<i>Parafidelia</i>			<i>friesei</i>	Probably oligolectic on <i>Sesamum</i> (Pedaliaceae), although the species has been collected on flowers from other plant families [40, 41] (Litman pers. obs. in South Africa)
						<i>pallidula</i>	Probably oligolectic on Sisymbrium (Zygophyllaceae) [38, 40] (Litman pers. obs. in South Africa)
			<i>Fideliopsis</i>			<i>fasciata</i>	Probably oligolectic on Neuradaceae (<i>Grielum</i> and <i>Neuradopsis</i>) [40]
						<i>hessei</i>	Oligolectic on Neuradaceae (<i>Grielum</i> and <i>Neuradopsis</i>) [40, 41] (Litman pers. observ in South Africa)
						<i>major</i>	Oligolectic on Neuradaceae (<i>Grielum</i>) [40] (Litman pers. obs. in South Africa)
						<i>ornata</i>	Most records on Aizoaceae; other hosts possible [40, 41]
			<i>Fideliana</i>			<i>braunsiana</i>	Probably oligolectic on <i>Berkheya</i> (Asteraceae) [40, 41]
						<i>ulrikei</i>	Floral records on <i>Convolvulus</i> [40, 42]
		Pararhophitini	<i>Pararhophites</i>			<i>orobinus</i>	Probably oligolectic on <i>Peganum harmala</i> (Nitrariaceae) [43] (Praz pers. obs. in Uzbekistan, Iran)
				<i>quadratus</i>	probably oligolectic on <i>Zygophyllum</i> (Zygophyllaceae) [44, 45] (Praz pers. obs. in Tunisia)		
Lithurgini	<i>Lithurgus</i>	<i>Lithurgopsis</i>			<i>apicalis</i>	Oligolectic on <i>Opuntia</i> (Cactaceae) [46, 47]	
					<i>echinocacti</i>	Presumably oligolectic on <i>Echinocactus</i> (Cactaceae) [47]	
					<i>gibbosus</i>	Probably oligolectic on <i>Opuntia rigida</i> (Cactaceae) [48]	
					<i>rufiventris</i>	Oligolectic on <i>Opuntia</i> (Cactaceae) [49]	

	<i>Lithurgus</i>	Palearctic species	<i>chrysurus</i>	Oligolectic on Carduaceae [50-52]
			<i>cornutus</i>	Probably oligolectic on Carduaceae [52, 53] (Praz pers. obs. in Uzbekistan, Spain)
			<i>tibialis</i>	Probably oligolectic on <i>Chrozophora</i> (Euphorbiaceae) (Praz pers. obs. in Iran)
		Australian and Asian species	<i>atriformis</i>	Polylectic, collects pollen from <i>Ipomoea</i> and <i>Hibiscus</i> ; like many other members of its genus, appears to depend exclusively on large-flowered plants with coarse-grained pollen [54]
			<i>atratus</i>	Polylectic, collects pollen mainly from <i>Ipomoea</i> (Convolvulaceae) and <i>Sida</i> (Malvaceae) [55, 56]
			<i>collaris</i>	Polylectic with preference for Malvaceae [57, 58]
			<i>rubricatus</i>	Floral records: <i>Alyogyne</i> (Malvaceae) (Danforth pers. obs in Australia)
		African species	<i>pullatus</i>	Floral record: <i>Convolvulus</i> [59]
			<i>spiniferus</i>	Several flower records on Asteraceae [41, 59]
	<i>Microthurge</i>		<i>pygmaeus</i>	Oligolectic on Malvaceae [60, 61]
			<i>sp</i>	Main pollinator of <i>Turnera sidoides</i> (Malvaceae) [62]
	<i>Trichothurgus</i>		<i>aterrimus</i>	Oligolectic on Cactaceae [63]
			<i>dubius</i>	Visits <i>Cactus</i> flowers (Cactaceae) [64]
			<i>spp</i>	At least some species visit Cactaceae for pollen [28]

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Figure S1

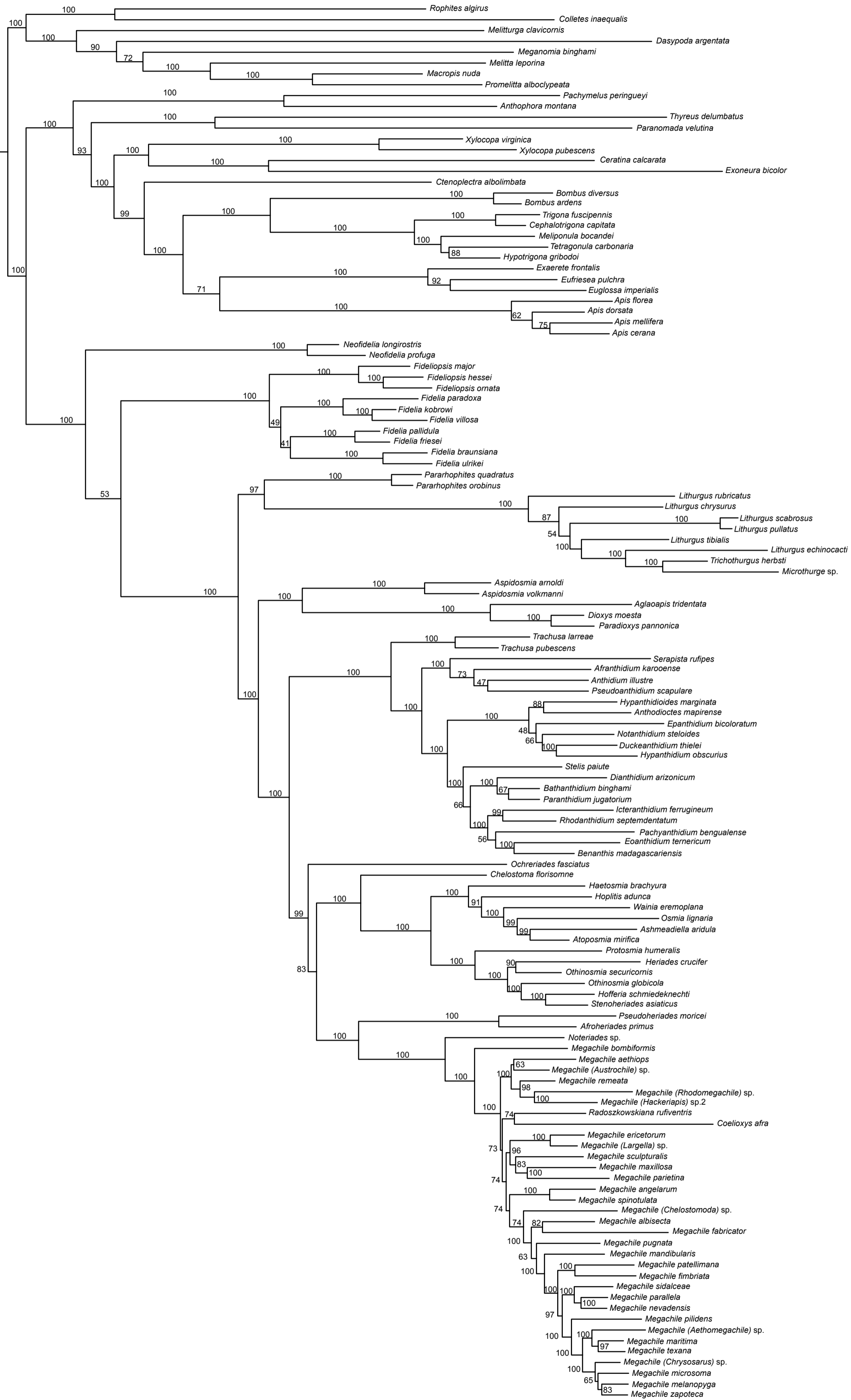


Figure S2

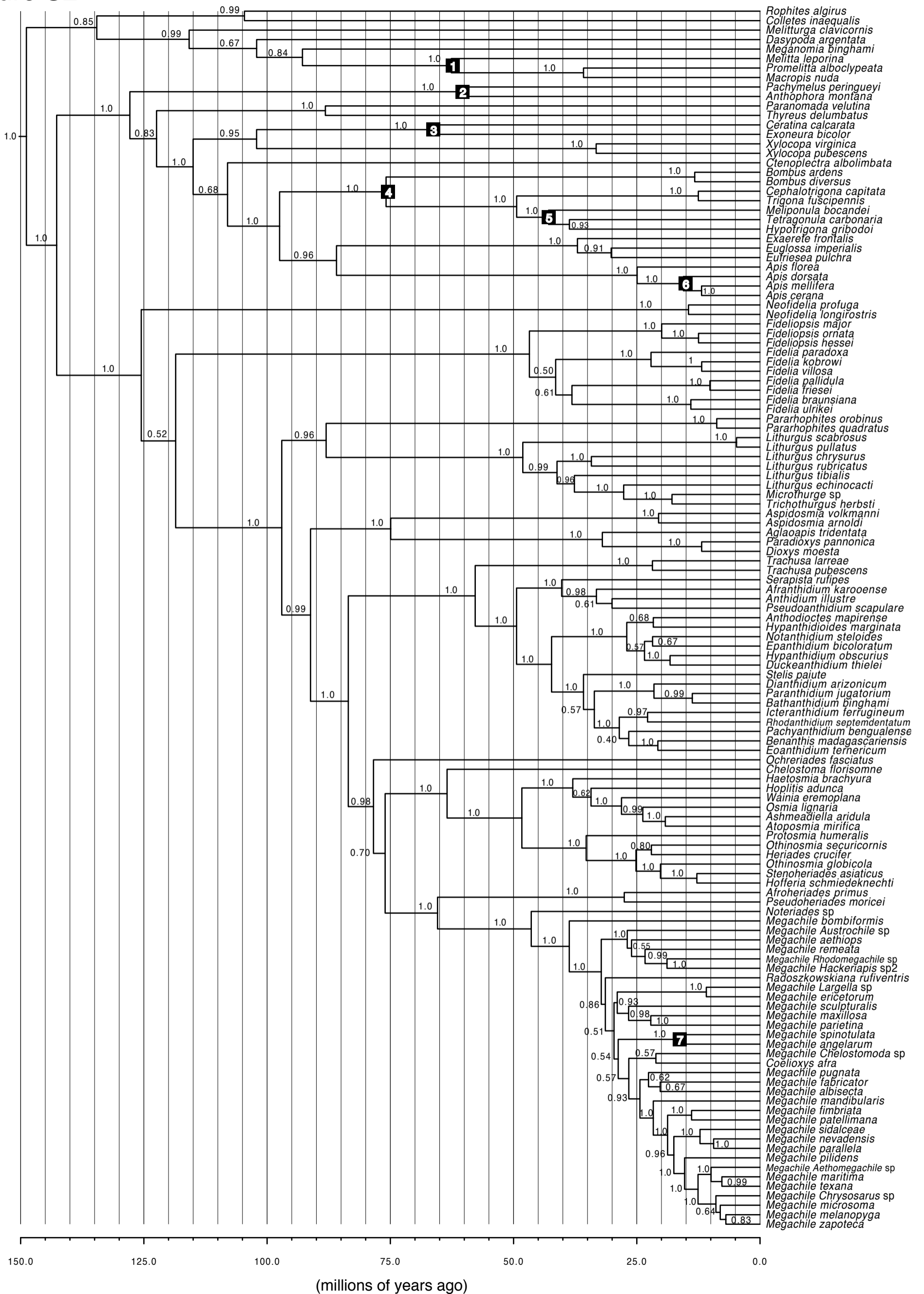


Figure S3

