



The corbiculate bees arose from New World oil-collecting bees: Implications for the origin of pollen baskets



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ABSTRACT

The economically most important group of bees is the “corbiculates”, or pollen basket bees, some 890 species of honeybees (*Apis*), bumblebees (*Bombus*), stingless bees (Meliponini), and orchid bees (Euglossini). Molecular studies have indicated that the corbiculates are closest to the New World genera *Centris*, with 230 species, and *Epicharis*, with 35, albeit without resolving the precise relationships. Instead of concave baskets, these bees have hairy hind legs on which they transport pollen mixed with floral oil, collected with setae on the anterior and middle legs. We sampled two-thirds of all *Epicharis*, a third of all *Centris*, and representatives of the four lineages of corbiculates for four nuclear gene regions, obtaining a well-supported phylogeny that has the corbiculate bees nested inside the *Centris*/*Epicharis* clade. Fossil-calibrated molecular clocks, combined with a biogeographic reconstruction incorporating insights from the fossil record, indicate that the corbiculate clade arose in the New World and diverged from *Centris* 84 (72–95) mya. The ancestral state preceding corbiculae thus was a hairy hind leg, perhaps adapted for oil transport as in *Epicharis* and *Centris* bees. Its replacement by glabrous, concave baskets represents a key innovation, allowing efficient transport of plant resins and large pollen/nectar loads and freeing the corbiculate clade from dependence on oil-offering flowers. The transformation could have involved a novel function of *Ubx*, the gene known to change hairy into smooth pollen baskets in *Apis* and *Bombus*.

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1. Introduction

Most of the ca. 20,000 species of bees provision their larvae with a mix of pollen and nectar (Danforth et al., 2013; Michener, 2007). Some 450 species in 18 genera and a few families instead provision with a mix of pollen and fatty oils that the females obtain from the flowers of about 2000 species in 11 families on all continents except Antarctica (Buchmann, 1987; Renner and Schaefer, 2010). By far the largest oil-collecting genus is *Centris* with 230 species, traditionally considered as sister to *Epicharis* (35 species), with both together making up the tribe Centridini (Michener, 2007; Moure et al., 2012). *Centris* occurs in dry and humid areas of South America, Central America, and southern North America, spanning a latitudinal range from 47° South to 39° North. *Epicharis* occurs only in humid areas from 34° South to 23° North in Mexico.

Species in both groups are solitary medium-sized to large bees that build their nests in the soil, either in inclined banks or flat ground or in pre-existing holes in dead trees (Frankie et al., 1993; Gaglianone, 2005). All *Epicharis* and most *Centris* feed their larvae with a mix of pollen and floral oils instead of, or in addition to, nectar (Vinson et al., 1995, 2006). They collect the oil with combs of rigid setae on the anterior and middle pair of legs (Neff and Simpson, 1981; Vogel, 1974). In some *Centris*, the oil-collecting apparatus is restricted to the anterior pair of legs and consists of soft, absorptive hairs. A few have no oil-collecting setae, but all of them have large bristly hind legs (scopae) (Neff and Simpson, 1981; Simpson et al., 1990; Vivallo and Melo, 2009). Based on the morphology of their oil-collecting structures, *Centris* and *Epicharis* are very similar and unique among oil-collecting bees. The only other bees with oil-collecting apparatuses on four legs are the ten species of *Monoeca* (Tapinotaspidini), but their basi-tarsal combs are different from those of *Centris* and *Epicharis* (Neff and Simpson, 1981). Combs on two pairs of legs as present in all *Epicharis* and most *Centris* are associated with the exploitation of New World Malpighiaceae flowers, which have four pairs of oil

Abbreviation: mya, million years ago.

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glands located on the abaxial side of the calyx sepals such that a bee sitting on the flower can exploit them with its four legs while touching the flower's male and female sexual organs (Anderson, 1979; Vogel, 1974). Malpighiaceae are the only oil source for *Epicharis* (Machado, 2004), while species of *Centris* exploit a wider array of oil-offering flowers (Martins et al., 2013; Simpson et al., 1990, 1977; Vogel, 1974).

Adult morphology has traditionally been seen as supporting a sister group relationship between *Centris* and *Epicharis*, i.e., the tribe Centridini (Roig-Alsina and Michener, 1993), but molecular trees have neither consistently nor strongly supported this tribe. Instead, DNA trees that included one or two species of *Epicharis*, up to six of *Centris*, and representatives of other Apidae showed the so-called corbiculates as sister to *Centris* albeit without statistical support (Cardinal et al., 2010; Hedtke et al., 2013; with 58% and 42% maximum likelihood bootstrap values). Cardinal and Danforth (2013), however, recovered *Epicharis* and *Centris* as sister to the corbiculate bees, supporting the topology of Roig-Alsina and Michener (1993).

The corbiculates, or pollen-basket bees, consist of ca. 890 species, namely the honeybees (Apini, *Apis* with 7–10 species), bumblebees (Bombini, *Bombus* with 240 species), stingless bees (Meliponini, with ca. 450 species), and orchid bees (Euglossini, with 187 species), all with glabrous, concave pollen-carrying hind tibiae. This is the commercially most important clade of bees (Gallai et al., 2009; Klatt et al., 2014). Resolving their sister group and likely time and place of origin thus is important for interpreting geographic, physiological and genomic evolution of apine bees (Medved et al., 2014). If the corbiculate bees are the sister clade to a monophyletic Centridini, this would imply that oil-collecting setae arose in the common ancestor of the latter, because the next-closest clades do not collect oil. However, if instead the genus *Centris* alone is sister to the corbiculate bees, with *Epicharis* sister to both, this implies that the corbiculates arose from an oil-collecting New World ancestor, with implications for the likely precursor state of the corbicula (Fig. 1).

To answer these questions, we sequenced one ribosomal and three protein-coding nuclear gene regions for 72 of 230 species of *Centris*, 22 of the 35 species of *Epicharis*, and a representative sample of corbiculate bees as well as further outgroups. We then carried out a statistical biogeographic reconstruction on fossil-calibrated versions of the phylogeny of *Epicharis*, *Centris*, and the common ancestor of corbiculates to have a time frame for the gain or loss of oil-collecting setae on two or one pairs of legs.

2. Materials and methods

2.1. Taxon sampling

We sampled 72 of the 230 species of *Centris* and 22 of the 35 species of *Epicharis* (see Table A1 for species names, collection site, and voucher information), representing all 12 subgenera of *Centris* and all eight of *Epicharis* (Moure et al., 2012), mostly with >2 species, but in the case of subgenera *Ptilocentris* and *Parepicharis* with only 1 species; subgenera *Anepicharis*, *Cyphepicharis* and *Triepicharis* are monospecific. More than 300 new sequences have been submitted the GenBank (Table A3 – a lists all GenBank accession numbers). Other Apinae (Michener's Apidae) representatives are 20 species from the corbiculate tribes Apini, Meliponini, Euglossini, and Bombini and 56 species representing 22 of 33 tribes of Apinae sensu lato (Allodapini, Ammobatini, Ancylini, Anthophorini, Caenoprosopidini, Ceratinini, Ctenoplectrini, Emphorini, Epeolini, Ericrocidiini, Eucerini, Exomalopsini, Iseoepolini, Manuelliini, Melectini, Nomadini, Osirini, Protepeolini, Rhathymini, Tapinotaspidini, Tetrapediini, Xylocopini). As more distant outgroups, with included eight species from subfamily Megachilinae sensu lato (Michener's Megachilidae) (Fideliini, Pararhophitini, Anthidiini, Lithurgini, Osmiini). In total, our matrix thus comprised 174 species of Apinae and Megachilinae, mostly with sequences from Cardinal et al. (2010). Voucher specimens for our new sequences are housed in the DZUP – Jesus Santiago Moure Entomological Collection at Federal University of Paraná, Brazil, or at the institutions that provided specimens for DNA extractions (Table A1).

2.2. Sequence data, alignments, and phylogenetic analyses

Most newly sequenced specimens were field-collected and preserved in EtOH, but some pinned specimens (up to twelve years old) were also used. DNA was extracted using the Qiagen DNeasy blood and tissue extraction kit, following the manufacturer's protocol. We sequenced part of the ribosomal 28S gene (1400 base pairs) and three nuclear protein-coding genes: LW-Rhodopsin (800 base pairs), Elongation factor 1 α – F2 copy (1000 base pairs), and RNA-polymerase (900 base pairs). Primers sequences and specific conditions are listed in Table A4. Most PCR products were purified and sequenced by Macrogen Inc., South Korea; some were purified with ExoSAP-IT (USB Corporation, Cleveland, OH, USA), for removing leftover primers and dNTPs, using a denaturation temperature of 37 °C and an inactivation temperature of 80 °C. A mixture of 1.0 μ l BigDye[®] (Applied Biosystems, Foster City, CA, USA),



Fig. 1. Comparative morphology of hindlegs in *Centris*, *Epicharis* and corbiculates. (A) Hindleg of female of *Centris* (*Aphemisia*) *plumipes*, showing the scopa. (B) Same, inner view of leg. (C) Hindleg of *Epicharis* (*Anepicharis*) *dejeanii*, outer view. (D) Same, inner view. (E) Hindleg of an corbiculate bee, *Eufriesea violacea*, showing the tibial corbicula.

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