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Comprehensive phylogeny, biogeography and new classification of the diverse bee tribe Megachilini: Can we use DNA barcodes in phylogenies of large genera?

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ABSTRACT

Classification and evolutionary studies of particularly speciose clades pose important challenges, as phylogenetic analyses typically sample a small proportion of the existing diversity. We examine here one of the largest bee genera, the genus *Megachile* – the dauber and leafcutting bees. Besides presenting a phylogeny based on five nuclear genes (5480 aligned nucleotide positions), we attempt to use the phylogenetic signal of mitochondrial DNA barcodes, which are rapidly accumulating and already include a substantial proportion of the known species diversity in the genus. We used barcodes in two ways: first, to identify particularly divergent lineages and thus to guide taxon sampling in our nuclear phylogeny; second, to augment taxon sampling by combining nuclear markers (as backbone for ancient divergences) with DNA barcodes. Our results indicate that DNA barcodes bear phylogenetic signal limited to very recent divergences (3–4 my before present). Sampling within clades of very closely related species may be augmented using this technique, but our results also suggest statistically supported, but incongruent placements of some taxa. However, the addition of one single nuclear gene (LW-rhodopsin) to the DNA barcode data was enough to recover meaningful placement with high clade support values for nodes up to 15 million years old. We discuss different proposals for the generic classification of the tribe Megachilini. Finding a classification that is both in agreement with our phylogenetic hypotheses and practical in terms of diagnosability is particularly challenging as our analyses recover several well-supported clades that include morphologically heterogeneous lineages. We favour a classification that recognizes seven morphologically well-delimited genera in Megachilini*:* *Coelioxys*, *Gronoceras*, *Heriadopsis*, *Matangapis*, *Megachile*, *Noteriades* and *Radoszkowskiana*. Our results also lead to the following classification changes: the groups known as *Dinavis*, *Neglectella*, *Eurymella* and *Phaenosarus* are reestablished as valid subgenera of the genus *Megachile*, while the subgenus *Alocanthedon* is placed in synonymy with *M. (Callomegachile)*, the subgenera *Parachalicodoma* and *Largella* with *M. (Pseudomegachile)*, *Anodonteutricharaea* with *M. (Paracella)*, *Platysta* with *M. (Eurymella)*, and *Grosapis* and *Eumegachile* with *M. (Megachile)* (new synonymies). In addition, we use maximum likelihood reconstructions of ancestral geographic ranges to infer the origin of the tribe and reconstruct the main dispersal routes explaining the current, cosmopolitan distribution of this genus.

1. Introduction

Molecular phylogenies are available for a number of bee clades (reviewed in Danforth et al., 2013) and have contributed to the resolution of many long-standing controversies in bee systematics (e.g. Danforth and Ji, 2001; Praz et al., 2008; Almeida and Danforth, 2009; Cardinal et al., 2010; Rasmussen and Cameron, 2010; Praz and Packer, 2014; Romiguier et al., 2016). Together, these studies are contributing to the development of stable classifications and provide a framework for examining patterns of diversification (e.g., Hines, 2008; Litman et al., 2011, 2013), plant-bee coevolution (e.g., Sedivy et al., 2008), social evolution (e.g., Schwarz et al., 2007) and historical biogeography (e.g., Almeida et al., 2011; Chenoweth and Schwarz, 2011; Praz and Packer,

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2014). For a few large bee clades, including most of the largest bee genera, however, no phylogenetic hypothesis is yet available, and classifications remain largely conjectural. Nearly ten years ago, Michener (2007: 120) listed five large complexes of problematic taxa, "for which the current generic classification is arbitrary and will probably be revised in the near future". Of these five groups, three have been examined using molecular markers (Praz et al., 2008 for the osmiine complex; Almeida and Danforth, 2009 for the *Leioproctus* and *Lonchopria*-group; and, among others, Gibbs et al., 2012 for the various lineages of the genus *Lasioglossum*). The present paper examines a fourth problematic group, the bees currently placed in the genus *Megachile* in the tribe Megachilini.

Four genera are currently recognized in this speciose tribe: *Coelioxys*, *Megachile*, *Noteriades* and *Radoszkowskiana*. The genus *Noteriades* includes only a few little-known African and Oriental species (Griswold and Gonzalez, 2011). *Coelioxys* and *Radoszkowskiana* are cleptoparasites, principally of species belonging to the genus *Megachile* (Michener, 2007). The former is distributed worldwide and includes more than 400 species (Ascher and Pickering, 2016; see Rocha-Filho and Packer, in press, for a subgeneric treatment of this genus), while the latter includes only four species limited to the Palearctic (Schwarz, 2001). With more than 1400 species (Ascher and Pickering, 2016), the cosmopolitan genus *Megachile* is among the largest bee genera worldwide and represents a significant proportion of most bee faunas, from tropical to temperate regions. Unlike most bee lineages, *Megachile* have their maximal diversity in tropical regions. Numerous species cut leaf discs that they use to line their brood cells in the ground or in existing cavities, and are commonly referred to as the leafcutter bees. Other *Megachile* species do not cut leaves but rather build brood cells with resin or mud mixed with salivary secretions (Kronenberg and Hefetz, 1984) in cavities, in the ground, or sometimes exposed on the surfaces of stone or wood (references in Michener, 2007). While some authors have referred to these species as either resin or mason bees (e. g., O'Toole and Raw, 1991), we find these terms confusing as both are also used for Anthidiini and Osmiini, respectively. We prefer to follow Eardley (2012) and refer to these species as "dauber" bees.

The relationships among the main megachiline lineages remain largely unclear, with the exception of the genus *Noteriades*, which has been demonstrated to be the sister group to all other Megachilini based on analyses of molecular (Praz et al., 2008; Litman et al., 2011) and morphological data (Gonzalez et al., 2012). The phylogenetic position of the two cleptoparasitic genera *Coelioxys* and *Radoszkowskiana* is unclear both in terms of whether they are derived from within the genus *Megachile* (Litman et al., 2011, 2013) and whether they are sister taxa (Litman et al., 2011, 2013; Rocha-Filho and Packer, in press). Thus, it remains unknown whether cleptoparasitism has evolved once or twice within the tribe (Rozen and Kamel, 2007, 2008; Litman et al., 2013).

Michener (2007) recognizes 56 subgenera within *Megachile*. While acknowledging that the diversity observed in this genus is larger than that seen in other tribes that are divided into numerous genera (e.g. the Eucerini, Osmiini and Anthidiini), Michener refrains from recognizing distinct genera because of the morphological intergradation among the diverse lineages. Instead, he assembles the subgenera in three groups, which correspond to the genera recognized by some authors, and broadly mirror the species' nesting biology. Michener's Group 1 includes the leafcutter species, in which the female mandible mostly has a "cutting edge", a blade-like structure between some mandibular teeth. Group 2 is made of the dauber lineages, which mostly lack mandibular cutting edges and use resin or mud to build their brood cells. Group 3 only includes the subgenus *Creightonella*, whose species exhibit an intermediate morphology between the other two groups and use a combination of resin or mud and leaf discs as nesting materials. The morphological separation of these three groups is difficult (Michener, 2007): Group 2 is likely a paraphyletic assemblage from which Group 1 and possibly the cleptoparasitic genera arose (Litman et al., 2011); and distinction between *Creightonella* and the other groups is "about as weak as that between Groups 1 and 2" (Michener, 2007: 554). Nesting biology and associated mandibular structure (presence or absence of cutting edges) do not always differ clearly by group. Most members of Groups 1 and 3 are leafcutters but cutting edges are reduced or absent in some lineages of Group 1; moreover, some subgenera included in Michener's Group 2 have distinct cutting edges, such as *Chelostomoda* and *Mitchellapis*. At least the former uses leaf discs to close its nests (Iwata, 1976). Other morphological characters used to segregate these three groups are equally ambiguous (Michener, 2007: 556).

Group 1, 2 and 3 correspond to the genera *Megachile*, *Chalicodoma* and *Creightonella* of some authors (Michener, 1965; Pasteels, 1965; Tkalců, 1969). In addition, some group 2 subgenera such as *Gronoceras* and *Thaumatosoma* have sometimes been given generic rank (e. g., Cockerell, 1935; King and Exley, 1985). The proposal of Mitchell (1980) to further divide Michener's group 1 into five genera does not appear practical (Michener, 2007: 555) and is not further discussed here. Michener (2007) suggested splitting his heterogeneous Group 2 into the following five genera: *Matangapis*, *Mitchellapis*, *Megella*, *Chelostomoides* ("including [as subgenera] *Callomegachile*, perhaps *Gronoceras* and *Thaumatosoma*"), and *Chalicodoma*. Based on cladistic analyses of morphological characters, Gonzalez (2008) (see also Engel and Gonzalez, 2011; Gonzalez and Engel, 2012) suggested a classification that breaks up Group 2 into three genera: *Matangapis*, *Chalicodoma* (including as subgenera, among others, *Pseudomegachile*, *Gronoceras* and *Callomegachile*) and *Thaumatosoma* (including the heriadiform subgenera, such as *Hackeriapis*, *Chelostomoides* and *Maximegachile*).

The purpose of the present study is to unravel the relationships within Megachilini, especially among the subgenera of the diverse genus *Megachile*, lay the foundations for a sound classification of the group and obtain insights into their biogeographic history. We sequenced five nuclear genes for more than 100 species of *Megachile* representing most subgenera, members of all other genera of Megachilini and representatives of all other megachiline tribes. To further refine our taxon sampling in the genus *Megachile*, we also use information from trees based on DNA-barcodes (the 658 bp fragment of the mitochondrial gene cytochrome oxidase I; Hebert et al., 2003) to pinpoint particularly divergent species and to break-up long branches within subgenera. We examine the phylogenetic signal of DNA barcodes and evaluate the possibility of combining our nuclear dataset with DNA barcodes (see Kjer et al., 2014, for a similar approach applied to the caddisfly genus *Chimarra*). We use our nuclear dataset as a backbone to infer old divergences (e.g., between subgenera and species-groups) and DNA barcodes to augment taxon sampling within more recent clades. We discuss the potential benefits and pitfalls of using DNA barcodes in combination with nuclear genes to reconstruct densely sampled phylogenies.

2. Materials and methods

2.1. Taxon sampling

In total, 127 species were included in our phylogenetic analyses of nuclear genes (Table S1). As outgroup taxa, we included representatives of the subfamilies Lithurginae and Pararhophitinae (following the classification of Gonzalez et al., 2012), as well as representatives of all tribes of the subfamily Megachilinae (Aspidosmiini, Dioxyini, Anthidiini, Osmiini) and of the genera *Ochreri-*