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Molecular phylogeny, biogeography, and host plant shifts in the bee genus *Melitta* (Hymenoptera: Anthophila)

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ABSTRACT

New molecular studies suggested that the family Melittidae is either a paraphyletic group from which all the other bees are derived, or the sister clade to all other existing bees. Studying the historical biogeography and evolution of each major lineage within this group is a key step to understand the origin and early radiation of bees. *Melitta* is the largest genus of melittid bees, for which a robust molecular phylogeny and a biogeographic analysis are still lacking. Here, we derive a phylogenetic hypothesis from the sequences of seven independent DNA fragments of mitochondrial and nuclear origin. This phylogenetic hypothesis is then used to infer the evolution of the species range and of the host-plant shifts in *Melitta*. Our results confirmed the monophyly of *Melitta*, but did not recover all previously defined clades within the genus. We propose new taxa by splitting the genus in three subgenera (including two new subgenera described in the Appendix: *Afromelitta* subgen. nov., *Plesiomelitta* subgen. nov.) and describe two new species: *Melitta avontuurensis* sp. n. and *M. richtersveldensis* sp. n. Regarding the evolution of host-plant use, our analysis suggests that all species currently specialized on one plant family originated from an ancestor that was specialized on Fabaceae plants. The inferred biogeographic history for the genus supported an African origin. In concordance with previous studies identifying Africa as the geographic origin for many clades of bees, our data bring new evidence for an African origin of melittid bees.

1. Introduction

Bees form a monophyletic group of pollen eaters derived from predatory wasps (Danforth et al., 2013), with more than 19,000 species described worldwide, and are found in most ecosystems (Ascher, 2009). They are usually among the most important pollinators, and therefore play a key role in agricultural and natural ecosystems (Ollerton et al., 2011). Because of their importance both in fundamental and applied research, a clear understanding of bee diversity, its evolution, and its origin, is essential.

Currently seven bee families are recognized: Andrenidae, Apidae, Colletidae, Halictidae, Megachilidae, Melittidae and Stenotritidae (Michener, 2007). New molecular studies suggested that the family Melittidae (about 200 species; Michez et al., 2009) is either a paraphyletic group from which all the other bees are derived, or the sister clade to all other existing bees (phylogeny summarized in Fig. 1; Danforth et al., 2006a,b, 2013). While reliable phylogeny estimations are available for most of the non-melittid families (Danforth et al., 2008; Almeida and Danforth, 2009; Cardinal et al., 2010; Gonzalez et al., 2012; Hedtke et al., 2013), a detailed phylogeny and biogeographic analysis is still lacking for the Melittidae family (Danforth et al., 2013). An important step to better understand the evolutionary relationships and biogeographical history of this family is to infer and study the phylogenies of the 14 melittid genera. Recent phylogenetic studies were conducted for most of these genera: *Capicola* Friese 1911 (Michez and Kuhlmann, 2007), *Dasypoda* Latreille 1802 (Michez et al., 2004a,b), *Eremaphanta* Popov 1940 (Michez and Patiny, 2006), *Hesperapis* Cockerell 1898 (Stage, 1966; Michener, 1981), *Promelitta* Warncke, 1977 (Michez et al., 2007), *Macropis* Panzer 1909 (Michez and Patiny, 2005), *Meganomia* Cockerell 1898 (Michener, 1981; Michez et al., 2010), *Samba* Friese 1908 (Michez et al., 2010), *Rediviva* Friese 1911 (Whitehead and Steiner, 2001; Whitehead et al., 2008; Kuhlmann, 2012a) and *Redivivoides* (Kuhlmann, 2012b). Yet, a robust molecular phylogeny is still lacking for the largest (around 50 species) and most widespread genus, *Melitta* Kirby 1802.

Melitta belongs to the subfamily Melittinae and the tribe Melittini that also includes the genera *Rediviva* and *Redivivoides* (Fig. 1; Michez et al., 2009). Species of *Melitta* differ from other melittid bees by several plesiomorphic features such as the structure of the sternum 7 in males, which has a large disc and weakly developed lateral process. *Melitta* also shows a few synapomorphies,

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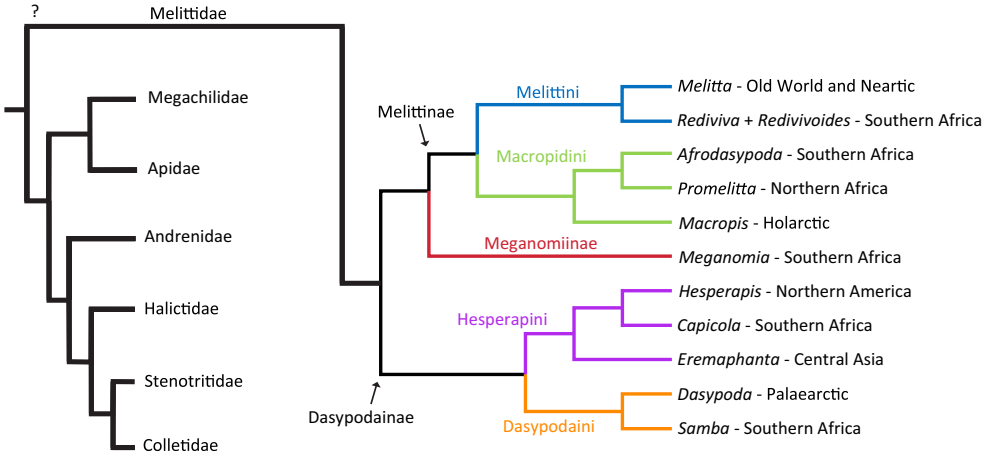

Fig. 1. (A) Family-level phylogeny of bees based on Danforth et al. (2013). (B) Phylogeny of the subfamilies, tribes, and genera of Melittidae *sensu lato* according to Michez et al. (2009) ("?" indicates that the Melittidae family is either a paraphyletic group from which all the other bees are derived, or the sister clade to all other existing bees).

such as lateral tubercles on the labrum, apical projection on the posterior basitarsus and volsella with elongated digitus (Michener, 1981). Michez and Eardley (2007) recognized two subgenera of *Melitta* (*Cilissa* and *Melitta s. str.*) based on a comprehensive taxonomic revision and a phylogeny based on morphological characters. An updated list of 48 valid names was provided by Michez et al. (2012). *Melitta* bees nest in the ground and most species are specialist pollen foragers (i.e. oligolectic) (Michez et al., 2008). The host-plants associated with the genus are morphologically and phylogenetically diverse, including both the bilateral flowers of the Scrophulariaceae or Fabaceae (Lamiales and Fabales respectively; APG III, 2009), and the radiate flowers of the Campanulaceae and Lythraceae (Asterales and Myrtales respectively; APG III, 2009). This high diversity in flower morphology is unusual for clades of specialist bees, more often associated with a group of similar flowers (Sipes and Wolf, 2001; Sedivy et al., 2008).

While the two sister genera *Rediviva* and *Redivivoides* are restricted to South Africa and Lesotho, *Melitta* also occurs in temperate areas of the Holartic and sub-Saharan Africa (Warncke, 1973; Michener, 1979, 1981; Snelling and Stage, 1995; Wu, 2000; Eardley and Kuhlmann, 2006; Kuhlmann, 2009; Michez et al., 2009, 2012). Previous studies inferred that *Melitta* species from southern Africa and North America belong to derived clades, suggesting a Palaearctic origin for the genus, although this pattern was only weakly supported (Michez and Eardley, 2007). Because the sister clade of *Melitta* (grouping the genera *Rediviva* and *Redivivoides*) is endemic to southern Africa, the geographic origin of the tribe Melittini is uncertain.

Here, we present new sequence data from one mitochondrial and six nuclear genes for a total of ~5500 bp, collected for 23 species of *Melitta*. With these data, we aim: (i) to infer the phylogenetic relationships among these sampled species of *Melitta*; (ii) to explore if host-plant shifts can explain diversification of *Melitta*; (iii) to determine the most likely geographical origin of the genus and of the tribe Melittini.

2. Material and methods

2.1. Studied material

All 23 sampled species belong to the Melittidae *sensu lato*. Their names and countries of origin are listed in Table 1. Our sampling spans all biogeographic regions where *Melitta* occurs: Afrotropical, Nearctic, East Palaearctic and West Palaearctic. In addition, we selected the following species as outgroups: six species of the sister group formed by *Rediviva* and *Redivivoides*, and two additional species, *Dasypoda hirtipes* and *Macropis europaea* from outside the Melittini. Voucher specimens are housed in the collections of the University of Mons (Belgium) or those of Cornell University (USA).

2.2. Molecular data

Genomic DNA was extracted using the Qiagen DNeasy® Blood & Tissue kit. A half thorax per specimen was ground in the Qiagen ATL buffer and incubated overnight with proteinase K at 56 °C. The remaining DNA-extraction steps were conducted as described in the manufacturer's protocol. For one specimen per species, we sequenced seven loci: an 800 base pair (bp) long fragment of the ribosomal RNA 28S gene, an 850 bp long fragment of the mitochondrial cytochrome oxydase I (COI) gene, an 950 bp long fragment of the F2 copy of elongation factor-1α (EF-1α) gene, a 1000 bp long fragment of the sodium potassium adenosine triphosphatase (NaK) gene, a 600 bp long fragment of the *long-wavelength rhodopsin* (Opsin) gene, a 850 bp long fragment of the *RNA polymerase II* (RNAp) gene, and a 450 bp long fragment of the *Wingless* (WgL) gene. All fragments were PCR-amplified following the TrueStart Hot Start Taq DNA polymerase manufacturer's protocol (Fermentas International Inc.). The 28S fragment was amplified (annealing temperature of 53.5 °C) using primers Bel and Mar (Belshaw and Quicke, 1997; Mardulyn and Whitfield, 1999), the COI fragment (annealing temperature of 51 °C) with primers Jerry and Pat (Simon et al., 1994), the EF-1α fragment with primers For1deg (annealing temperature of 54.9 °C) or HaF2for1 (annealing temperature of 56.2 °C) and F2-rev1 (Danforth and Ji, 1998), the NaK fragment (annealing temperature of 66 °C) with primers NaKfor2 and NaKrev2 (Michez et al., 2009), the Opsin fragment with primers For (annealing temperature of 58.1 °C) or For3 (annealing temperature of 59 °C) and Rev4a (Danforth et al., 2004), the RNAp fragment (annealing temperature of 57 °C) with primers Polfor2a and Polrev2a (Danforth et al., 2006a) and the WgL fragment (annealing temperature of 63.5 °C) with primers Bee-wg-For1 or Bee-wg-For2 and Lep-Wg2a-Rev (Brower and DeSalle, 1998; Danforth et al., 2004; Almeida and Danforth, 2009). Sequences were aligned using the MUSCLE algorithm (Edgar, 2004) implemented in CODONCODE ALIGNER (v. 3.7.1.1, Codon Code Corporation). Multiple alignments were then checked manually and pruned at both 5′- and 3′-ends to ensure that all sequences were of identical length.

2.3. Phylogenetic analyses

We analyzed each gene independently and in combination using maximum likelihood (ML) and Bayesian methods (MB). All