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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., 2010a), *Samba* Friese 1908 (Michez et al., 2010b), *Rediviva* Friese 1911 (Whitehead and Steiner, 2001; Whitehead et al., 2008; Kuhlmann, 2012a) and *Redivivoides* Michener 1981 (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, such as lateral tubercles on the labrum, apical projection on the

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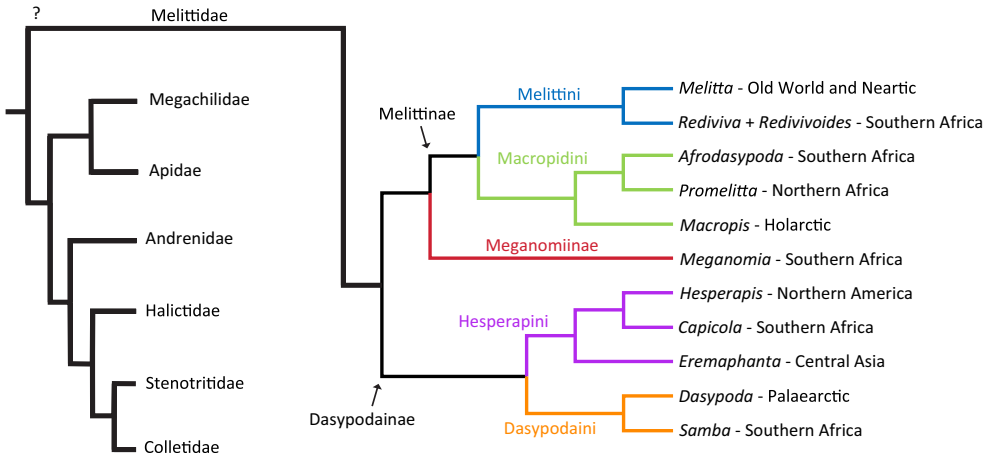

Fig. 1. Family-level phylogeny of bees based on Danforth et al. (2013) and phylogeny of the subfamilies, tribes, and genera of Melittidae *sensu lato* according to Michez et al. (2009) ("?" indicates that the Melittidae family is either a paraphyletic group from which all the other bees are derived,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

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 24 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 24 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: a 800 base pair (bp) long fragment of the ribosomal RNA 28S gene, a 850 bp long fragment of the mitochondrial cytochrome oxydase I (COI) gene, a 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

Table 1
Samples description. (*) refers to sequences already published and available in Genbank prior to this study (na = not available).

Taxon	Distribution	Collected in	GenBank accession numbers						
			28S	COI	EF1α	NaK	Opsin	RNAp	WgL
Outgroups									
Macropis europaea	Europe	Belgium	*AY654525**	*KC253158*	*KC253129*	*KC253105*	*KC253081*	*KC253207*	*KC253053*
Dasypoda hirtipes	Palaearctic	Ukraine	*AY654519**	*KC253149*	*KC253123*	*KC253099*	*KC253075*	*KC253201*	*KC253044*
Rediviva emdeorum	Southern Africa	South Africa	*KC253138*	*KC253175*	*KC253146*	*KC253120*	*KC253096*	*KC253221*	*KC253070*
R. saetigera	Southern Africa	South Africa	*EF594347**	*KC253177*	*EF594322**	*EF646402**	*EF594371**	*AY945201**	*KC253071*
R. macgregori	Southern Africa	South Africa	*AY654531**	*KC253176*	*JF806355**	*na*	*DQ116690**	*AY945159**	*GU320189**
Redivivoides capensis	Southern Africa	South Africa	*KC253199*	*KC253179*	*KC253147*	*KC253121*	*KC253097*	*KC253222*	*KC253073*
Re. namaquensis	Southern Africa	South Africa	*KC253100*	*KC253180*	*KC253148*	*KC253122*	*KC253098*	*KC253223*	*KC253074*
Re. simulans	Southern Africa	South Africa	*AY654532**	*KC253178*	*JF806356**	*EF646401**	*DQ116691**	*AY654532**	*KC253072*
Ingroups									
Melitta aegyptiaca	Northern Africa	Israel	*KC253182*	*KC253151*	*KC253125*	*KC253101*	*KC253077*	*KC253203*	*KC253046*
M. arrogans	South Africa	South Africa	*GU244854**	*KC253152*	*GU245005**	*GU245167**	*GU245314**	*GU245451**	*KC253047*
M. avontu. sp. nov.	Southern Africa	South Africa	*KC253181*	*KC253150*	*KC253124*	*KC253100*	*KC253076*	*KC253202*	*KC253045*
M. bicollaris	Turkey	Turkey	*KC253183*	*KC253153*	*KC253126*	*KC253102*	*KC253078*	*KC253204*	*KC253048*
M. budashkini	Ukraine (Krim)	Ukraine	*KC253184*	*KC253154*	*KC253127*	*KC253103*	*KC253079*	*KC253205*	*KC253049*
M. budensis	West-Palaearctic	Ukraine	*KC253185*	*KC253155*	*KC253128*	*KC253104*	*KC253080*	*KC253206*	*KC253050*
M. dimidiata	West-Palaearctic	France	*EF594342**	*KC253156*	*EF594317**	*EF646395**	*EF594367**	*EF599266**	*KC253051*
M. eickworti	North America	USA	*AY654527**	*KC253157*	*AY585157**	*EF646393**	*AF344604**	*AY945141**	*KC253052*
M. ezoana	East-Palaearctic	South Korea	*EF594346**	*KC253159*	*KC253130*	*EF646399**	*EF594370**	*EF599270**	*KC253054*
M. haemorrhoidalis	Europe	France	*EF594345*	*KC253160*	*KC253131*	*EF646398**	*EF594369**	*EF599269**	*KC253055*
M. hispanica	Spain	Spain	*KC253186*	*KC253161*	*KC253132*	*KC253106*	*KC253082*	*KC253208*	*KC253056*
M. japonica	East-Palaearctic	Russia	*KC253187*	*KC253162*	*KC253133*	*KC253107*	*KC253083*	*KC253209*	*KC253057*
M. leporina	Palaearctic	Spain	*AY654529**	*KC253163*	*KC253134*	*KC253108*	*KC253084*	*KC253210*	*KC253058*
M. maura	Northern Africa	Israel	*KC253188*	*KC253164*	*KC253135*	*KC253109*	*KC253085*	na	*KC253059*
M. melanura	Palaearctic	Russia	*KC253189*	*KC253165*	*KC253136*	*KC253110*	*KC253086*	*KC253211*	*KC253060*
M. melittoides	North America	USA	*KC253190*	*KC253166*	*KC253137*	*KC253111*	*KC253087*	*KC253212*	*KC253061*
M. nigricans	Europe	Spain	*KC253191*	*KC253167*	*KC253138*	*KC253112*	*KC253088*	*KC253213*	*KC253062*
M. richtersvel. sp. nov.	Southern Africa	South Africa	*KC253192*	*KC253168*	*KC253139*	*KC253113*	*KC253089*	*KC253214*	*KC253063*
M. schmiedeknechti	Northern Africa	Israel	*KC253193*	*KC253169*	*KC253140*	*KC253114*	*KC253090*	*KC253215*	*KC253064*
M. schultzei	Southern Africa	South Africa	*KC253194*	*KC253170*	*KC253141*	*KC253115*	*KC253091*	*KC253216*	*KC253065*
M. sibirica	East-Palaearctic	Mongolia	*KC253195*	*KC253171*	*KC253142*	*KC253116*	*KC253092*	*KC253217*	*KC253066*
M. tomentosa	Istria	Slovenia	*KC253196*	*KC253172*	*KC253143*	*KC253117*	*KC253093*	*KC253218*	*KC253067*
M. tricincta	West-Palaearctic	France	*EF594344**	*KC253173*	*KC253144*	*KC253118*	*KC253094*	*KC253219*	*KC253068*
M. udmurtica	West-Palaearctic	Russia	*KC253197*	*KC253174*	*KC253145*	*KC253119*	*KC253095*	*KC253220*	*KC253069*

ML and MB analyses were performed on the computer cluster HYDRA available at the *Université Libre de Bruxelles*. Prior to combining all seven loci in a single data set, we assessed congruence among genes by comparing the well-supported clades among trees inferred for each locus separately.

Single gene datasets were further partitioned as follows, prior to the identification of the most appropriate nucleotide substitution model: (i) the Opsin fragment was partitioned into two introns and one exon, (ii) EF-1α into one exon and one intron, (iii) COI, RNAp, WgL and the Opsin and EF-1α exons by base position (1st, 2nd and 3rd). The best fitting substitution model for each partition was chosen with jMODELTEST (Posada, 2008) using the Akaike information criterion (Akaike, 1974), corrected for small samples sizes (Hurvich and Tsai, 1989). The chosen models were the following: COI: TPM2uf + G (1st), F81 + G (2nd), HKY + G (3rd); 28S: TVM + I + G; EF-1α: JC + I (1st), JC (2nd), HKY (3rd), HKY + G (intron); NaK: K80 (1st), HKY + I (2nd), HKY + I + G (3rd); Opsin: TVMeF + G (intron1), JC (1st and 2nd), K80 (3rd and intron2); RNAp: TrNef (1st), JC (2nd), TPM1+G (3rd); WgL: GTR + I (1st), TVMeF (2nd), SYM (3rd).

ML analyses were conducted in GARLI 2.0 (Zwickl, 2006). Each run was started from a random starting tree and used the automated stopping criterion (stop when the *ln* score remained constant for 20,000 consecutive generations). Ten independent runs were carried out for each gene separately as well as for the combined data set. Only the highest likelihood tree of those 10 runs was retained. Statistical confidence in nodes was evaluated using 1000 non-parametric bootstrap pseudoreplicates (Felsenstein, 1985) using the automated stopping criteria set at 10,000 generations. Clades whose bootstrap values were >70% were considered to be well supported (Hillis and Bull, 1993).

MB analyses were conducted with MrBAYES 3.1.2 (Ronquist and Huelsenbeck, 2003). The models selected with jMODELTEST but not implemented in MrBayes were substituted by the closest over-parameterized model available (Huelsenbeck and Rannala, 2004). The TPM1, TrNef and TVMef substitution models were replaced by the SYM model, and the TPM2uf and TVM substitution models were replaced by the GTR model. Ten independent runs were carried out for each gene and for the combined data set (15 millions generations, four chains with mixed-models, default priors, saving trees every 500 generations, discarding 25% of sampled trees as burn-in). Convergence among MCMC chains was checked by plotting likelihood values across generations using TRACER 1.2 (Rambaut and Drummond, 2007). When convergence occurred, one independent run was randomly selected for further analysis. The phylogeny and posterior probabilities were estimated from the sampled trees and summarized in a majority-rule 50% consensus tree. Clades with associated posterior probabilities $\geqslant 0.95$ were considered to be well supported (Wilcox et al., 2002).

2.4. Evolution of host plants choices

Host-plants for the genus *Melitta* were determined from records found in the literature. These records included observations of floral visits (Sitdikov, 1986; Celary, 2005; Eardley and Kuhlmann, 2006; Michez and Eardley, 2007; Michez et al., 2012) and palynological analyses (Michez et al., 2008). Host plants for the ancestral nodes were inferred using the BBM (Bayesian binary MCMC) method implemented in the program RASP 2.0 (Yu et al., 2011), a character mapping approach taking phylogenetic uncertainty into account. The host plant family was coded as character state for each species. In the case of generalist species, the generalist

behavior was simply considered an additional character state (i.e. as a specific host-plant choice, not as a combination of several character states). Species for which no host-plant data were available were discarded from the analysis. As input tree for RASP, we used all the non burn-in trees sampled by the MCMC chain of the MB analysis (i.e. 22,500 trees after excluding burn-in) on all genes combined (see above). For the BBM analysis, ten independent MCMC chains were run for ten million generations, sampling every 1000 generations and discarding the first 10% as burn-in. Finally, we also used the R package "Picante" (Kembel et al., 2010) to measure the phylogenetic signal associated with the evolution of host-plant choice. We used the *K* statistic that measures the phylogenetic signal of a given trait by comparing the observed signal in this trait to the signal under a Brownian motion model of trait evolution on a phylogeny (Blomberg et al., 2003; Kembel et al., 2010). We assessed the level of significance of *K* by permuting host-plant choice across the species at the tips of the tree (Kembel et al., 2010).

2.5. Historical biogeography

To infer the biogeographic history of the genus *Melitta*, we used the S-DIVA (Statistical Dispersal-Vicariance) analysis also implemented in the program RASP 2.0 (Yu et al., 2011). This method reconstructs the ancestral geographic distribution of a clade by optimizing a three-dimensional cost matrix. The optimal ancestral distributions are those that minimize the number of implied dispersal and extinction events (Ronquist, 1997). In this method, the frequencies of an ancestral range at a node are averaged over all trees in order to account for uncertainties in the phylogeny (Yu et al., 2011). As for the evolution of host-plant choices inferred with the BBM method, we used all the non burn-in trees sampled by the MCMC chain of the MB analysis on all genes combined. The species distributions were categorized into nine regions: (1) East Palaearctic; (2) West Palaearctic; (3) Palaearctic (West and East Palaearctic); (4) Nearctic; (5) southern Africa; (6) West Palaearctic and North America; (7) West Palaearctic and southern Africa; (8) East Palaearctic and southern Africa; (9) Palaearctic and southern Africa. The geographic range of each species was defined according to Michez and Eardley (2007) and Michez et al. (2012).

3. Results

3.1. Phylogeny and taxonomic implication

ML and MB analyses performed on each gene independently resulted in similar topologies, at least when focussing on well-supported clades (i.e., those associated with bootstrap supports >70% and/or posterior probabilities >0.95). The trees inferred from each gene fragment separately are given in Supplementary Files (Figs. S1–S14). Fig. 2 presents the Bayesian majority-rule consensus tree inferred from all genes combined, along with the few ML clades that contradict it with a high bootstrap support. In general,

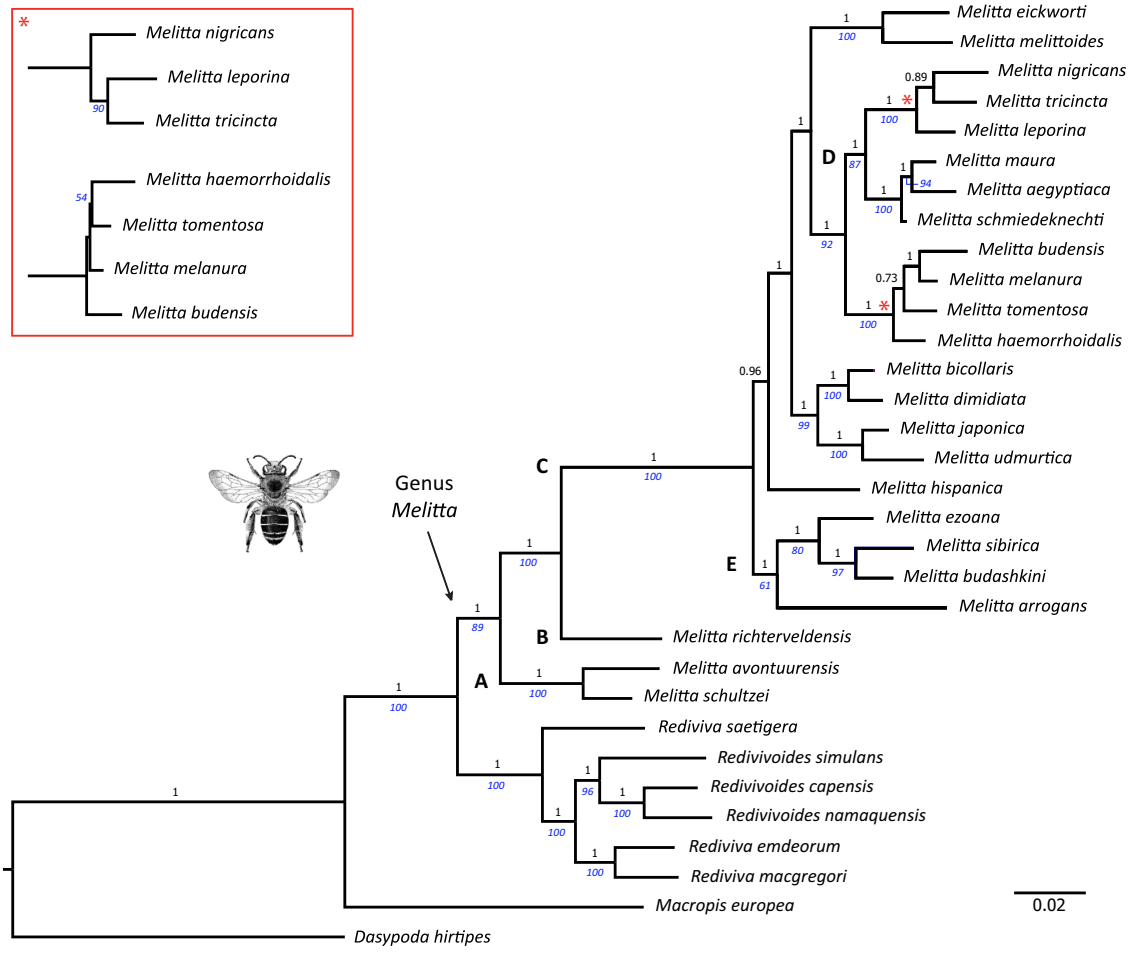

Fig. 2. Majority-rule consensus of the trees sampled by the Bayesian analysis conducted on the combined molecular data matrix (28S, COI, EF-1α, NaK, Opsin, RNAp and WgL). Values above the branches are Bayesian posterior probabilities; italicized blue values below the branches are maximum likelihood bootstrap supports (values <50% are not reported on the tree). An asterisk indicates a clade whose internal branches are contradicted by the maximum likelihood analysis. The alternative ML clades are shown inside a red box. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

branch support values were high in both combined analyses (ML and MB). The topologies of two well-supported clades on the Bayesian tree, identified by an asterisk on Fig. 2, are contradicted by the maximum likelihood analysis (with bootstrap support $\geqslant$70%). The alternative tree topologies for these clades are also shown in Fig. 2.

Note that some well-supported clades in the trees inferred from each gene separately are not included in the MB and ML trees based on the full dataset. These clades are identified by red asterisks on Figs. S1–S14. However, these alternative high-supported clades (that contradict the phylogenetic hypothesis of Fig. 2) were each found only in a single gene tree.

The inferred phylogeny is compatible with some aspects of the current classification of the genus: *Rediviva* + *Redivivoides* is a sister group to *Melitta*, which confirms the monophyly of the tribe Melittini; the monophyly of the genus *Melitta* and of the former subgenus *Melitta s. str.* (clade D in Fig. 2) are well supported. All species included in the former subgenera *Cilissa* and *Melitta s. str.* are gathered in clade C, in which only Holartic species are found, with the exception of *M. arrogans* from southern Africa. The previously defined subgenus *Cilissa* (that corresponds to clade C minus clade D) is paraphyletic. The South African endemic species *M. richtersveldensis* sp. nov. is sister to clade C. Clade A (*M. avontuurensis* sp. nov. + *M. schultzei*) is sister to all other *Melitta*.

The taxonomic implications of our phylogenetic hypothesis are detailed in the Supplementary material. In summary, we suggest to synonymise the subgenus *Cilissa* with the subgenus *Melitta s. str.*, and describe two new subgenera: *Afromelitta* subgen. nov. (defined by clade B) with the new species *Melitta* (*Afromelitta*) *richtersveldensis* sp. nov. and *Plesiomelitta* subgen. nov. (defined by clade A) with the new species *Melitta* (*Plesiomelitta*) *avontuurensis* sp. nov.

3.2. Evolution of flower specialization in Melitta

Host-plant specialization appears rather conserved in the genus *Melitta*. For example, a clade of four species (*M. budensis*, *M. melanura*, *M. tomentosa*, *M. haemorrhoidalis*; Fig. 3) is exclusively associated with the Campanulaceae plant family, and another clade of four species (*M. bicollaris*, *M. dimidiata*, *M. japonica*, *M. udmurtica*) feed exclusively on Fabaceae flowers. The analysis based on the *K* statistic confirmed the presence of a significant phylogenetic signal associated to host-plant choice ($K = 1.308$, one-tailed test p-value = 0.001). On the other hand, when a host-plant switch does occur, it often occurs between plants that are phylogenetically unrelated, and morphologically different. For example, the host-plant of *M. tricincta* (genus *Odontites*, Scrophulariaceae, Lamiales) characterized by a bilateral flower, is morphologically completely different from that of its sister species, *M. nigricans* (genus *Lythrum*, Lythraceae, Myrtales) having radiate flowers.

Regarding host-plant shifts inferred by the BBM analysis (Fig. 3), the Fabaceae family appears to be an important ancestral floral ressource for the genus *Melitta*, with the identification of several shifts from the Fabaceae family. On the other hand, this character mapping analysis failed to clearly identify if the ancestor of the *Melitta* genus had a generalist foraging behavior or a Fabaceae-specialized diet.

3.3. Historical biogeography of Melitta and Melittini

The historical biogeographic hypothesis inferred by S-DIVA (Statistical Dispersal-Vicariance) is summarized in Fig. 3. The result suggests a southern African origin for the genus (red asterisk on Fig. 3) and at least one subsequent dispersal event from Africa to the Palaearctic. Also, because the two North American species, *M. eickworti* and *M. melittoides*, form a clade, a single migration from Palaearctic to Nearctic is likely.

4. Discussion

4.1. Systematics of Melitta

The only phylogenetic study of *Melitta* to date, by Michez and Eardley (2007), used morphological characters and a restricted number of southern African species. It failed to resolve some of the most important phylogenetic relationships within the genus, e.g. those between the African and Palaearctic species. Some of the clades supported by this morphological study are strongly contradicted by our results. For example, the subgenus *Cilissa* is clearly paraphyletic in our study while presented as monophyletic by Michez and Eardley (2007). This discrepancy may be the result of an erroneous coding of morphological characters. Michez and Eardley (2007) identified a particular feature of the male genitalia (short gonostylus) as an apomorphy defining the *Cilissa* clade, but in view of our DNA-based phylogenetic hypothesis, it is more likely a synplesiomorphy within the genus *Melitta*. Our sampling of key southern African species, *M. avontuurensis* sp. nov., *M. richtersveldensis* sp. nov. and *M. schultzei*, also allowed us to propose a new subgeneric classification, strongly differing from that of Warncke (1973), Michener (1981, 2007) and Michez and Eardley (2007). The two subgenera that had been described based on Palaearctic and Neartic species, *Cilissa* and *Dolichochile*, are here synonymised with *Melitta s. str.* While the species diversity within *Melitta* is higher in the Palaearctic region than in southern Africa, the phylogenetic diversity is still highest in southern Africa (two endemic subgenera).

4.2. Speciation and host-plant shifts

Interactions with angiosperms have often been cited as important driving factors underlying diversification in phytophagous insects because host-plant shifts can be associated with speciation events (e.g. Farrell, 1998; Calcagno et al., 2007; Borer et al., 2011). However, most bee genera include either exclusively generalist species (e.g. genus *Bombus*; e.g. Kleijn and Raemakers, 2008) or specialist species (e.g. genus *Macropis*; e.g. Michez et al., 2008), with only a few genera including both generalists and specialists (e.g. genus *Colletes*; Müller and Kuhlmann, 2008) (Wcislo and Cane, 1996). In specialist bees, host-plant shifts rarely occur and new host-plants are usually morphologically similar to the ancestral host-plants (Müller, 1996; Michez et al., 2004b; Sipes and Tepedino, 2005; Sedivy et al., 2008). The genus *Melitta* is quite rare in comprising both generalists (e.g. *Melitta arrogans*) and specialists (e.g. *Melitta tricincta*) species, foraging on morphologically diverse host-plants.

As in other bee genera (e.g. Müller, 1996; Larkin et al., 2008), our results show that speciation events in the genus *Melitta* are not systematically associated with inter-family host-plant shifts (seven shifts and 24 species). However, inter-family host-plant switches occured mainly from Fabaceae to hosts that are not morphologically similar (e.g. host-plants of *M. leporina*, *M. tricincta* and *M. nigricans*, Fig. 2). Additionally, pollen nutritive content is highly variable among the Fabaceae, Campanulaceae, Lythraceae and Scrophulariaceae that are the preferred host-plant families of *Melitta* (Roulston et al., 2000). Selection of chemically divergent host-pollens suggests that nutritional profile of pollen does not influence the host-plant choice in the genus *Melitta*. Host-plant family shifts in *Melitta* therefore appear unconstrained by the morphology or chemistry of pollen, which is quite unique in the evolution of bees. This plasticity might be explained by the existence of preadaptations such as an ancestral flexible physiology (Janz et al., 2001), symbiosis with particular microorganisms (Clayton, 1964; Kok et al., 1970; Mondy and Corio-Costet, 2000; Janson et al., 2009) or

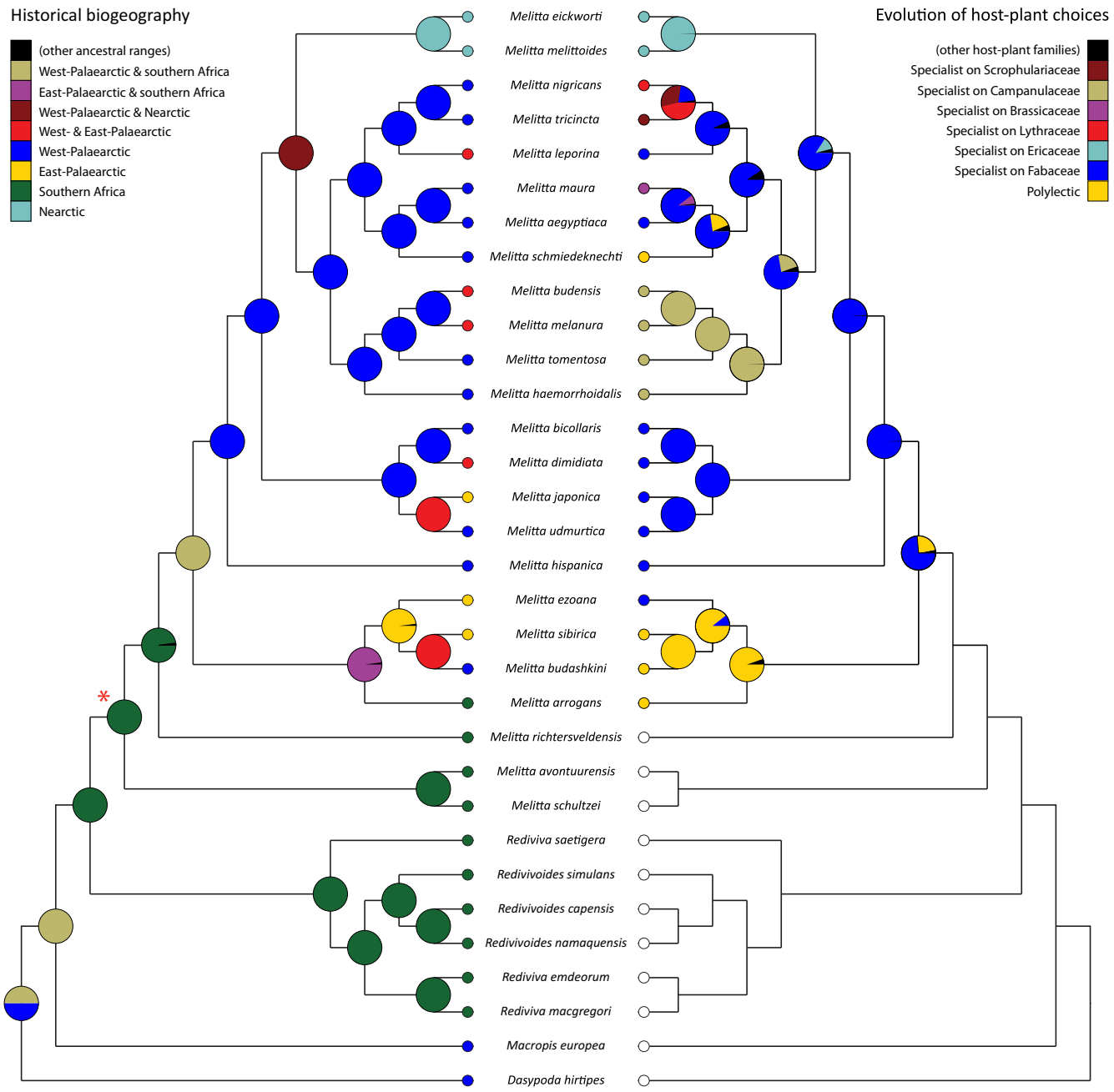

Fig. 3. (1) Historical biogeography inferred with the S-DIVA (Statistical Dispersal-Vicariance) method implemented in the program RASP 2.0. (2) Host-plant choice evolution inferred with the BBM (Bayesian binary MCMC) method implemented in RASP 2.0. Alternative ancestral geographic ranges and host-plant choices are both indicated as colored pie charts, with the surface of each color proportional to the relative likelihood of each ancestral geographic range or host-plant choice.

Dufour's gland secretions (Hefetz, 1987). In particular, Dufour's gland of bees is known to be an extremely rich source of diverse natural products and this gland is hypertrophied in the genus *Melitta* (Hefetz, 1987; Cane, 1983). The hypothesis that hypertrophied Dufour's gland may be involved in melittid tolerance to nutritional variations is supported by the larval nutritional function of Dufour's gland secretions described in the bee genera *Anthophora, Emphoropsis* and *Megachile* (Cane and Carlson, 1984; Duffield et al., 1984).

4.3. African origin and subsequent dispersals

Danforth et al. (2006b) already hypothesized an African origin for the melittid bees, but their hypothesis was mostly based on distributional evidence (most of melittid lineages are African endemics), as a robust phylogeny was lacking at the time. Our results confirm that Melittini probably originated from Africa with subsequent dispersal of *Melitta* to the Palaearctic region. This, in turn, brings additional evidence in favor of an African origin for the melittid bees. However, even if this result is well supported in our analysis, only slightly more than half of the known *Melitta* species are included in our study (24 out of 42 known species). Because several non-African species are not included, we could conceive a potential sampling bias influencing the outcome of the biogeographic analysis.

Africa seems to be the origin of many clades of bees. Halictidae probably originated from Africa and dispersed to South America and North Africa (Danforth et al., 2004). Molecular studies of

allodapine and ceratinine bees suggested also an African origin for these xylocopine tribes with subsequent dispersal to Australia for Allodapini and quickly around the world for Ceratinini (Schwarz et al., 2006; Chenoweth et al., 2007; Rehan et al., 2010). Finally, Schaefer and Renner (2008) also inferred an African origin of the Ctenoplectrine bees, followed by a dispersal into Asia. These studies combined suggest multiple dispersal out of Africa. The range expansion of *Melitta* is probably limited by their requirement of a temperate climate and their nesting behavior (ground nesting) (Celary, 2006). Also, *Melitta* seems unable to cross tropical regions or oceans, unlike Ctenoplectrini (tropical taxa) or Ceratinini (stem nesting). This could explain why *Melitta* is not present in isolated but climatically suitable areas in Western Australia and South America.

Although the RASP analysis identified a single dispersion from the West-Palaearctic to the Nearctic, other species from the Palaearctic or Est-Palaearctic, not included in this study, could also be genetically close to some Nearctic species. As a consequence, a dispersion from the East-Palaearctic side cannot be formally excluded at this stage.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2013.08.013.

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