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Phylogeny and biogeography of bees of the tribe Osmiini (Hymenoptera: Megachilidae)

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ABSTRACT

The Osmiini (Megachilidae) constitute a taxonomically and biologically diverse tribe of bees. To resolve their generic and suprageneric relationships, we inferred a phylogeny based on three nuclear genes (Elongation factor 1-α, LW-rhodopsin and CAD) applying both parsimony and Bayesian methods. Our phylogeny, which includes 95 osmiine species representing 18 of the 19 currently recognized genera, is well resolved with high support for most basal nodes. The core osmiine genera were found to form a well-supported monophyletic group, but four small genera, *Noteriades*, *Afroheriades, Pseudoheriades* and possibly *Ochreriades*, formerly included in the Osmiini, do not appear to belong within this tribe. Our phylogeny results in the following taxonomic changes: *Stenosmia* and *Hoplosmia* are reduced to subgeneric rank in *Hoplitis* and *Osmia*, respectively, *Micreriades* is recognized as a subgenus in *Hoplitis* and the subgenus *Nasutosmia* is transferred from *Hoplitis* to *Osmia.* We inferred a biogeographic scenario for the Osmiini applying maximum likelihood inference and models of character evolution. We provide evidence that the Osmiini originated in the Palearctic, and that extensive exchanges occurred between the Palearctic and the Nearctic. The latter finding may relate to the fact that many osmiine species nest in wood or in stems, facilitating dispersal by overseas transport of the nests.

1. Introduction

The Osmiini, commonly referred to as the mason bees, constitute a very diverse tribe within the megachilid bees, with over 1000 species currently recognized (Michener, 2007; Ungricht et al., in press). They occur on all continents except Australia and South America, being especially diverse and numerous in regions with Mediterranean and xeric climates of the Palearctic (southern Europe, northern Africa, middle east and central Asia), North America (southwestern deserts, California) and southern Africa (Cape Province, Namibia).

The biology of the Osmiini is astonishingly diverse and has fascinated entomologists for well over a century (Réaumur, 1742; Fabre, 1886; Ferton, 1923). In particular, the nesting biology of the Osmiini is highly varied and encompasses much of the diversity observed in other bees (Malyshev, 1937; Westrich, 1989; Müller et al., 1997; Cane et al., 2007). Depending on the species, osmiine bees build their nests in holes in the ground, below stones, on rock surfaces, in pithy stems, galls or in beetle borings in dead wood. Many species are known to nest exclusively in abandoned snail shells (Müller, 1994; Bellmann, 1997; Haeseler, 1997). Diversity in nesting site is matched by materials used in nest construction, such as sand or mud, masticated plant material, bright-coloured petals or resin. The Osmiini are thus model organisms to trace the evolution of nesting behavior in bees in general (Bosch et al., 2001).

The Osmiini are also famous for their specific relationships with flowers. Several osmiine genera and subgenera are predominantly composed of pollen specialists (e. g., *Chelostoma:* Westrich, 1989; Michener, 2007; Sedivy et al., in press; *Atoposmia* and *Hoplitis* subgenus *Proteriades*: Michener, 2007, and references therein), while other taxa are mainly composed of generalists (e. g., *Protosmia* and *Osmia* subgenus *Pyrosmia*: A. Müller, unpublished). The Osmiini are of particular interest for studies of the evolution of floral relationships because some species can easily be reared in trap nests or under caged conditions. This feature has enabled studies on the acceptance of novel pollen hosts by adult bees (Strickler, 1979; Williams, 2003; Praz et al., in press) and on pollen digestion by larvae (Levin and Haydak, 1957; Suarez-Cervera et al., 1994; Dobson and Peng, 1997; Williams, 2003; Praz et al., 2008). Studies combining experiments on the physiological capacities of bees with a phylogenetic approach are most promising to elucidate underlying mechanisms of bee-flower relationships in general (Williams, 2003).

Unfortunately, the phylogeny of the Osmiini remains controversial. First, the monophyly of this tribe is contentious. Its traditional

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recognition has relied on symplesiomorphies relative to the other tribes of the Megachilidae (Michener, 1941, 2007), which has led several authors to suspect that the Osmiini are paraphyletic (Engel, 1999, 2001; Ascher et al., 2001; Michener, 2007). Second, the suprageneric subdivision of the Osmiini is much debated. The Osmiini have long been divided into two groups, the *Heriades* group and the *Osmia* group. However, species with combinations of characters from both groups exist, leading to the view that these two groups may merge (Michener, 2007). For instance, the genus *Chelostoma* was long included in the *Heriades* group, but is currently placed in the *Osmia* group due to its similarity to some members of the genus *Hoplitis* (Michener, 2007). Lastly, important controversies exist on the generic classification of the *Osmia* group. Griswold and Michener (1997) recognized eight genera within the *Osmia* group. Michener (2007) retained this classification, but acknowledged that the *Osmia* group is one of the most problematic bee taxa due to the diffuse boundaries between the genera. In contrast, most European authors recognize only one large genus *Osmia* sensu lato for all members of the *Osmia* group (Westrich, 1989; Schwarz et al., 1996; Amiet et al., 2004) or even for all osmiine bees (Warncke, 1991; Westrich and Dathe, 1997; Westrich, 2006). This important difference in the treatment of the Osmiini by American and European authors may relate to the high diversity of osmiine bees in the Old World, rendering the clear segregation of taxonomic groups in the Palearctic more difficult than in North America. Indeed, most American members of *Osmia* and *Hoplitis* can unambiguously be assigned to one of the two genera by a typical combination of morphological characters (Michener, 2007). American *Osmia* species are rather robust (chalicodomiform), possess punctiform parapsidal lines and are mostly metallic blue or green in coloration, whereas the species of *Hoplitis* are more slender (hoplitiform), have linear parapsidal lines and are non-metallic. In the Palearctic, however, many species exist, which present intermediate combinations of these traits, such as *Osmia cephalotes*, *O. rufohirta* or *O. andrenoides*, which have linear parapsidal lines. Moreover, many Palearctic species of *Hoplitis* have a chalicodomiform rather than a hoplitiform body.

In the present study, we provide the first molecular phylogeny of the Osmiini. It is based on three nuclear genes and includes 95 osmiine species. Our aim is to assess the monophyly of the osmiine bees and to clarify their suprageneric and generic classification. We further use our phylogenetic framework to develop a hypothesis of the biogeographic history of the Osmiini.

2. Methods

2.1. Taxon sampling

The 95 osmiine species included in our phylogeny (Table 1) represent 18 of the 19 genera worldwide and 61 of the 82 subgenera currently recognized (Michener, 2007). The only genus not included is *Xeroheriades*, a monotypic genus that has been assigned to the *Heriades* group (Griswold, 1986a). For widespread subgenera (those present in more than one of the three main biogeographic zones [Palearctic, Nearctic and Afrotropical]), we included species from each continent whenever possible. The nomenclature and classification of the tribe Osmiini follows Michener (2007) and Ungricht et al. (in press). As outgroups, we selected 12 species representing all other megachilid tribes, namely the Fideliini and Pararhophitini (subfamily Fideliinae), and the Lithurgini, Anthidiini, Dioxyini and Megachilini (subfamily Megachilinae). According to phylogenies based on morphology (Roig-Alsina and Michener, 1993) and DNA sequences (Danforth et al., 2006b), the Fideliinae and the Megachilinae are sister groups, and the Lithurgini are sister to the other tribes of the Megachilinae, including the Osmiini.

2.2. DNA sequencing

DNA was extracted from bees preserved in 100% ethanol and from a few pinned specimens up to 5 years old. Whenever possible, we preferred males because they are haploid, which simplifies sequencing of nuclear markers. We extracted DNA from the head and conserved the rest of the specimen as voucher as osmiine males can usually be identified by the examination of their metasoma. For females, we extracted DNA from the thorax, keeping the other parts as a voucher. For minute species, we used the entire body for DNA extraction selecting another specimen of the same species as voucher. Vouchers are deposited in the Entomological Collection of the ETH Zurich or in the Cornell University Insect Collection as indicated in Table 1. Our DNA extraction protocol follows Danforth (1999), except that we did not use RNase.

PCR amplifications were performed in 50 μl reactions with 2.5 mM $MgCl_2$, 200 μM dNTP, 0.4 μM of each primer and Taq polymerase (Promega™, GoTaq) using a thermocycler (GeneAmp®, Applied Biosystems). We used nested PCR with specific primers for some pinned specimens with highly degraded DNA. We always added a blank sample to each PCR and used this blank sample in the second, nested PCR. In those rare cases where PCR products were detected in these controls, we discarded all samples. PCR conditions are given in Table 2. PCR products were purified using DNA-purification kits (GFX™) or MultiscreenTM 96-wells plates when processing more than 10 samples at the same time. In the latter case, PCR products were bound with HCL-Guanidine buffer (7 M Guanidine–HCL; 200 mM MES Buffer, pH 5.6), then washed twice with 80% ethanol and eluted with TE buffer (10 mM Tris–HCL, pH 8; 0.1 mM Na_2EDTA). Prior to sequencing, the PCR products were filtered through Sephadex™ columns. Automated sequencing of the PCR products was performed on an ABI Prism® 3130xl sequencer using BigDye technology. We used internal primers to sequence Elongation factor 1-α and the PCR primers for the other genes.

2.3. Genes analyzed

2.3.1. Elongation factor 1-α

Elongation factor 1-α (hereafter EF) has been widely used to infer bee phylogeny (e. g., Danforth et al., 2004, 2006b; Larkin et al., 2006; Schwarz et al., 2006; Cameron et al., 2007; Patiny et al., 2007). We amplified two overlapping fragments of the F2 copy of this gene (Danforth and Ji, 1998). For the large fragment (approximately 1200 bp), we used the primer pair HaF2For1 and F2Rev1 (Danforth et al., 1999). Based on initial sequences, we designed a modified reverse primer specific to the Megachilidae, F2Rev1-Meg (Table 2). For the short fragment (approximately 600 bp), we replaced For3 (Danforth et al. 1999) by the F2-specific forward primers For4 and For4a (Table 2). This F2-specific primer site was chosen in a region showing a highly differing amino acid composition between the F1 and F2 copies in *Apis mellifera* (GenBank Accession Nos.: F1, X52884; F2, AF015267). For4 and For4a yield together with Cho10 (Danforth et al., 1999) one single bright band corresponding to the F2 paralog. We designed F2-specific sequencing primers (F2-Intron-Rev and F2-Intron-For, Table 2) in a highly conserved region found in both introns 2 and 3, thus appropriate for the large and the small fragment. Additionally, we used the primers Exon2Rev, Exon2For, Exon3Rev, For3-Meg, Exon4For and EF-Rev (Table 2) as sequencing primers or primers for nested PCR. The assembled fragment has a length of approximately 1600 bp.

2.3.2. LW-rhodopsin

The phylogenetic utility of LW-rhodopsin (hereafter opsin) to infer bee phylogeny has been widely discussed (Mardulyn and Cameron, 1999; Ascher et al., 2001; Cameron and Mardulyn,

Table 1
Locality information, voucher numbers and GenBank Accession Nos. for sequences used in this study

Taxon	Locality	Collector	Voucher	Genbank Accession Nos.		
				EF 1−α	Opsin	CAD
Outgroup						
Pararhophites quadratus	Tunisia, Nefta	CP	ETHZ 61	EU851522	EU851627	EU851416
Fideliopsis major	South Africa, Clanwilliam	BD	CUIC 948	DQ141113	EU851628	EU851417
Lithurgus chrysurus	Italy, Abruzzen, Massa	AM	ETHZ 3	EU851523	EU851629	EU851418
Aglaoapis tridentata	Switzerland, Zeneggen	CP	ETHZ 27	EU851524	EU851630	EU851419
Anthidium punctatum	Switzerland, Weiach	AM	ETHZ 4	EU851525	EU851631	EU851420
Stelis punctulatissima	Switzerland, Hohtenn	AM	ETHZ 28	EU851526	EU851632	EU851421
Trachusa byssina	Switzerland, Splügen	AM	ETHZ 55	EU851527	EU851633	EU851422
Coelioxys afra	Switzerland, Weiach	AM	ETHZ 11	EU851528	EU851634	EU851423
Megachile albisecta	Italy, Massa Maritima	AM	ETHZ 47	EU851529	EU851635	EU851424
Megachile parietina	Switzerland, Hohtenn	AM	ETHZ 122	EU851530	EU851636	EU851425
Megachile pilidens	Switzerland, Weiach	AM	ETHZ 12	EU851531	EU851637	EU851426
Ingroup						
Afroheriades primus	South Africa, North Cape	TG	ETHZ 153	EU851532	EU851638	EU851427
Afroheriades sp.n.	South Africa, Nieuwoudtville	TG	CUIC 1281	EU851533	EU851639	EU851428
Ashmeadiella (Arogochila) timberlakei	USA, CA, Mariposa Co.	H. Ikerd	ETHZ 101	EU851534	EU851640	EU851429
Ashmeadiella (Ashmeadiella) aridula	USA, UT, Garfield Co.	TG	CUIC 1270	EU851535	EU851641	EU851430
Ashmeadiella (Ashmeadiella) aff. gillettei	Mexico, Sonora	R. Minckley	ETHZ 92	EU851536	EU851642	EU851431
Ashmeadiella (Chilosima) holtii	Mexico, Sonora	R. Minckley	ETHZ 114	EU851537	EU851643	EU851432
Ashmeadiella (Cubitognatha) xenomastax	USA, UT, Cane Co.	K. Huntzinger	ETHZ 86	EU851538	EU851644	EU851433
Atoposmia (Atoposmia) elongata	USA, CA, Mariposa Co.	E. Stephens	ETHZ 155	EU851539	EU851645	EU851434
Atoposmia (Atoposmia) hebitis	USA, CA, Tuolumne Co.	TG	ETHZ 90	EU851540	EU851646	EU851435
Atoposmia (Eremosmia) mirifica	USA, NV, Clark Co.	S. Higbee	ETHZ 88	EU851541	EU851647	EU851436
Atoposmia (Eremosmia) sp. n. aff. daleae	USA, UT, Kane Co.	H. Ikerd	ETHZ 158	EU851542	EU851648	EU851437
Atoposmia (Eremosmia) timberlakei	USA, NV, Clark Co.	S. Higbee	ETHZ 112	EU851543	EU851649	EU851438
Atoposmia (Hexosmia) copelandica	USA, CA, Mariposa Co.	TG	CUIC 1286	EU851544	EU851650	EU851439
Chelostoma (Ceraheriades) lamellum	China, Yunan Province	C. Sedivy	ETHZ 149	EU851545	EU851651	EU851440
Chelostoma (Chelostoma) florisomne	Switzerland, Chur	E. Steinmann	ETHZ 13	EU851546	EU851652	EU851441
Chelostoma (Eochelostoma) aureocinctum	Thailand, Chiang Mai	C. Sedivy	ETHZ 126	EU851547	EU851653	EU851442
Chelostoma (Foveosmia) californicum	USA, CA, Mariposa Co.	TG	CUIC 1269	EU851548	EU851654	EU851443
Chelostoma (Foveosmia) campanularum	Switzerland, Winterthur	AM	ETHZ 5	EU851549	EU851655	EU851444
Chelostoma (Gyrodromella) rapunculi	Switzerland, Fully	CP	ETHZ 14	EU851550	EU851656	EU851445
Chelostoma (Prochelostoma) philadelphi	USA, MD, Pr. George's Co.	S. Droege	ETHZ 38	EU851551	EU851657	EU851446
Haetosmia circumventa	UEA, Sharjah Desert Park	T. van Harten	ETHZ 97	EU851552	EU851658	EU851447
Heriades (Heriades) truncorum	Switzerland, Winterthur	AM	ETHZ 6	EU851553	EU851659	EU851448
Heriades (Michenerella) punctulifera	Greece, Rhodos, Stegna	AM	ETHZ 45	EU851554	EU851660	EU851449
Heriades (Neotrypetes) crucifer	USA, AZ, Chiricahua Mts	TG	CUIC 1149	EU851555	EU851661	EU851450
Hofferia schmiedeknechti	Greece, Chimara	CP	ETHZ 68	EU851556	EU851662	EU851451
Hoplitis (Alcidamea) leucomelana	Switzerland, Hohtenn	AM	ETHZ 16	EU851557	EU851663	EU851452
Hoplitis (Alcidamea) mitis	Switzerland, Zeneggen	CP	ETHZ 30	EU851558	EU851664	EU851453
Hoplitis (Alcidamea) pilosifrons	USA, NY, Thompkins Co.	J. Ascher	CUIC 506	EU851559	EU851665	EU851454
Hoplitis (Alcidamea) tridentata	Italy, Aosta, St-Pierre	CP	ETHZ 15	EU851560	EU851666	EU851455
Hoplitis (Annosmia) annulata crenulata	Greece, Rhodos, Afandou	AM	ETHZ 31	EU851561	EU851667	EU851456
Hoplitis (Anthocopa) bisulca	Greece, Rhodos, Stegna	AM	ETHZ 32	EU851562	EU851668	EU851457
Hoplitis (Anthocopa) hemisphaerica	Jordan, Wadi Mujib	CP, AM, C. Sedivy	ETHZ 143	EU851563	EU851669	EU851458
Hoplitis (Anthocopa) sp.	South Africa, Nieuwoudtville	K. Timmermann	ETHZ 108	EU851564	EU851670	EU851459
Hoplitis (Anthocopa) villosa	Switzerland, Säntis, Fälalp	D. Dietiker	ETHZ 17	EU851565	EU851671	EU851460
Hoplitis (Chlidoplitis) illustris	Turkey, Ankara	E. Scheuchl	ETHZ 99	EU851566	EU851672	EU851461
Hoplitis (Chlidoplitis) sp. n. aff. onychophora	Jordan, Wadi el Hasa	CP, AM, C. Sedivy	ETHZ 133	EU851567	EU851673	EU851462
Hoplitis (Cyrtosmia) hypocrita	USA, CA, Tuolumne Co.	TG	ETHZ 94	EU851568	EU851674	EU851463
Hoplitis (Dasyosmia) biscutellae	USA, CA, Riverside Co.	J. Ascher	CUIC 493	EU851569	EU851675	EU851464
Hoplitis (Formicapis) robusta	Switzerland, Visperterminen	CP	ETHZ 54	EU851570	EU851676	EU851465
Hoplitis (Hoplitina) mojavensis	USA, NV, Clark Co.	S. Higbee	ETHZ 102	EU851571	EU851677	EU851466
Hoplitis (Hoplitis) adunca	Italy, Aosta	AM	ETHZ 9	EU851572	EU851678	EU851467
Hoplitis (Megahoplitis) tigrina	Turkey, Ankara	E. Scheuchl	ETHZ 146	EU851573	EU851679	EU851468
Hoplitis (Micreriades) antalyae	Greece, Rhodos, Afandou	AM	ETHZ 41	EU851574	EU851680	EU851469
Hoplitis (Micreriades) lebanotica	Jordan, Wadi Mujib	CP, AM, C. Sedivy	ETHZ 147	EU851575	EU851681	EU851470
Hoplitis (Microhoplitis) paralias	Tunisia, Nefta	CP	ETHZ 137	EU851576	EU851682	EU851471
Hoplitis (Monumetha) albifrons	USA, CA, Contra Costa Co.	J. Ascher	CUIC 507	EU851577	EU851683	EU851472
Hoplitis (Monumetha) tuberculata	Switzerland, Wasserauen	AM	ETHZ 33	EU851578	EU851684	EU851473
Hoplitis (Nasutosmia) nasuta	France, Aureilles	S. Roffler	ETHZ 131	EU851579	EU851685	EU851474
Hoplitis (Pentadentosmia) moricei	Morocco	D. Michez	ETHZ 120	EU851580	EU851686	EU851475
Hoplitis (Pentadentosmia) villiersi	Tunisia, Nefta	CP	ETHZ 67	EU851581	EU851687	EU851476
Hoplitis (Penteriades) incanescens	USA, CA, Inyo Co.	A. Menke	ETHZ 104	EU851582	EU851688	EU851477
Hoplitis (Platosmia) platalea	Morocco	D. Michez	ETHZ 129	EU851583	EU851689	EU851478
Hoplitis (Prionohoplitis) brachypogon	Italy, Aosta, St-Pierre	CP	ETHZ 42	EU851584	EU851690	EU851479
Hoplitis (Proteriades) zuni	USA, UT, Garfield Co.	K. Huntzinger	ETHZ 91	EU851585	EU851691	EU851480
Hoplosmia (Hoplosmia) spinulosa	Switzerland, Merishausen	AM	ETHZ 8	EU851586	EU851692	EU851481
Hoplosmia (Odontanthocopa) scutellaris	Greece, Rhodos, Malonas	AM	ETHZ 34	EU851587	EU851693	EU851482
Hoplosmia (Paranthocopa) pinguis	Tunisia, Gafsa	CP	ETHZ 65	EU851588	EU851694	EU851483
Noteriades sp.	Thailand, Chiang Mai	C. Sedivy	ETHZ 132	EU851589	EU851695	EU851484
Ochreriades fasciatus	Jordan, Wadi Shu'ayb	CP, AM, C. Sedivy	ETHZ 124	EU851590	EU851696	EU851485
Osmia (Acanthosmioides) integra	USA, Utah, Kane Co.	K. Huntzinger	ETHZ 87	EU851591	EU851697	EU851486
Osmia (Allosmia) rufohirta	Italy, Aosta, St-Pierre	CP	ETHZ 18	EU851592	EU851698	EU851487

(*continued on next page*)

Table 1 (*continued*)

Taxon	Locality	Collector	Voucher	Genbank Accession Nos.		
				EF 1−α	Opsin	CAD
Osmia (Allosmia) sybarita	Greece, Kalogria	CP	ETHZ 63	EU851593	EU851699	EU851488
Osmia (Cephalosmia) montana	USA, WA, Okanogan Co.	J. Wilson	ETHZ 117	EU851594	n. a.	EU851489
Osmia (Erythrosmia) andrenoides	Switzerland, Hohtenn	AM	ETHZ 19	EU851595	EU851700	EU851490
Osmia (Euthosmia) glauca	USA, California, Madera Co.	H. Ikerd	ETHZ 103	EU851596	EU851701	EU851491
Osmia (Helicosmia) aurulenta	Switzerland, Wasserauen	AM	ETHZ 20	EU851597	EU851702	EU851492
Osmia (Helicosmia) coloradensis	USA, WA, Okanogan Co.	L. Wilson	ETHZ 111	EU851598	EU851703	EU851493
Osmia (Helicosmia) niveata	Switzerland, Hohtenn	AM	ETHZ 21	EU851599	EU851704	EU851494
Osmia (Hemiosmia) anceps	Tunisia, Nefta	CP	ETHZ 70	EU851600	EU851705	EU851495
Osmia (Hemiosmia) difficilis	Turkey, Ankara	E. Scheuchl	ETHZ 96	EU851601	EU851706	EU851496
Osmia (Melanosmia) juxta	USA, WA, Okanogan Co.	L. Wilson	ETHZ 93	EU851602	EU851707	EU851497
Osmia (Melanosmia) xanthomelana	Switzerland, Hohtenn	AM	ETHZ 51	EU851603	EU851708	EU851498
Osmia (Metallinella) brevicornis	Germany, Konstanz	M. Hermann	ETHZ 53	EU851604	EU851709	EU851499
Osmia (Monosmia) apicata	Greece, Platania	K. Standfuss	ETHZ 62	EU851605	EU851710	EU851500
Osmia (Mystacosmia) nemoris	USA, CA, Contra Costa Co.	G. Frankie	ETHZ 98	EU851606	EU851711	EU851501
Osmia (Neosmia) bicolor	Switzerland, Rüdlingen	AM	ETHZ 23	EU851607	EU851712	EU851502
Osmia (Neosmia) tingitana	Tunisia, Gafsa	CP	ETHZ 128	EU851608	EU851713	EU851503
Osmia (Osmia) cornuta	Switzerland, Zürich	AM	ETHZ 2	EU851609	EU851714	EU851504
Osmia (Osmia) lignaria	USA, UT, Kane Co.	TG	CUIC 1265	EU851610	EU851715	EU851505
Osmia (Pyrosmia) ferruginea	Tunisia, Bir el Hafey	CP	ETHZ 139	EU851611	EU851716	EU851506
Osmia (Pyrosmia) gallarum	Switzerland, Hohtenn	AM	ETHZ 24	EU851612	EU851717	EU851507
Osmia (Tergosmia) rhodoensis	Turkey, Ankeara, Güvem	E. Scheuchl	ETHZ 138	EU851613	EU851718	EU851508
Osmia (Tergosmia) tergestensis	Switzerland, Hohtenn	AM	ETHZ 36	EU851614	EU851719	EU851509
Osmia (Trichinosmia) latisulcata	USA, Utah, Kane Co.	O. Messinger	ETHZ 89	EU851615	EU851720	EU851510
Othinosmia (Megaloheriades) globicola	South Africa, Nieuwoudtville	F. Parker/M. Irwin	ETHZ 119	EU851616	EU851721	EU851511
Othinosmia (Othinosmia) sp. aff. securicornis	South Africa, Richtersveld	TG	ETHZ 156	EU851617	EU851722	EU851512
Protosmia (Chelostomopsis) longiceps	Greece, Rhodos, Afandou	AM	ETHZ 46	EU851618	EU851723	EU851513
Protosmia (Chelostomopsis) rubifloris	USA, CA, Tuolumne Co.	TG	CUIC 1145	EU851619	EU851724	EU851514
Protosmia (Nanosmia) minutula	Switzerland, Embd	CP	ETHZ 25	EU851620	EU851725	EU851515
Protosmia (Protosmia) humeralis	Jordan, Wadi Shu'ayb	CP, AM, C. Sedivy	ETHZ 135	EU851621	EU851726	EU851516
Pseudoheriades moricei	UEA, Al-Ajban	T. van Harten	ETHZ 100	EU851622	EU851727	EU851517
Stenoheriades asiaticus	Greece, Zachlorou	CP	ETHZ 66	EU851623	EU851728	EU851518
Stenosmia sp. aff. aravensis	Armenia, Nalbandyan	O. Berg	ETHZ 140	EU851624	EU851729	EU851519
Stenosmia minima	Tunisia, Nefta	CP	ETHZ 64	EU851625	EU851730	EU851520
Wainia (Caposmia) eremoplana	Jordan, Wadi al Hasa	CP, AM, C. Sedivy	ETHZ 125	EU851626	EU851731	EU851521

Names of the authors of the present study are abbreviated. Vouchers are deposited in the Entomological Collection of the ETHZ ("ETHZ") or in the Cornell University Insect Collection ("CUIC").

2003; Danforth et al., 2004). This gene shows a comparatively high rate of non-synonymous substitutions (Danforth et al., 2004). Ascher et al. (2001) suggested opsin to be suitable for resolving low-level taxonomic relationships, namely within tribes and genera. Based on the published opsin sequence of *Osmia bicornis* (=*O. rufa*; GenBank Accession No. AY572828; Spaethe and Briscoe, 2004), we modified the widely applied primer OpsinFor (=LW-RhF; Mardulyn and Cameron, 1999) by the more specific Opsin-For-Osm (Table 2), which we used in combination with specific reverse primers designed for the Osmiini, OpsinRev3 and OpsinRev3b (Table 2). As the primers OpsinRev4 and OpsinRev4a (Danforth et al., 2004) failed to amplify the 3′ end of opsin in the Megachilidae, we designed two new reverse primers OpsinRev5 and OpsinRev5a by aligning published sequences of short-tongued bee species (Danforth et al., 2004). These primers were used with specific forward primers designed for the Megachilidae, OpsinFor5 and OpsinFor5a (Table 2). The two fragments overlap and yield together a fragment of approximately 1200 bp. We performed BLAST searches in GenBank to verify that all sequences included in our data set represent the first of two LW opsin genes described in bees by Spaethe and Briscoe (2004).

2.3.3. Conserved ATPase domain (CAD)

CAD has recently been used in analyzes of family-level relationships in bees (Danforth et al., 2006a) and yielded promising results. We sequenced a 450-bp fragment of the approximately 1200-bp fragment used by Danforth et al. (2006a). We applied the primers CADFor4 (=ApCADFor4; Danforth et al., 2006a) with the modified reverse primer CADRev1-Meg (Table 2), which we designed by aligning published megachilid sequences (Danforth et al., 2006a). This primer pair yielded one bright band in all megachilid species investigated, corresponding to the exon 6 of CAD (Danforth et al., 2006a).

2.4. Sequence editing

The sequences were trimmed and assembled using the software Sequencher 4.7 for Macintosh (Gene Codes Corp.), and aligned applying Clustal X 1.83 (Thompson et al., 1997). The alignments were corrected visually in MacClade 4.08 for Macintosh OSX (Maddison and Maddison, 2005). Reading frame and intron/exon boundaries were determined by comparison with published sequences for *Apis mellifera* (GenBank Accession Nos.: AF015267, AMU26026 and DQ067178 for EF, opsin and CAD, respectively). All introns were removed prior to analysis in MacClade. The coding sequences of the three genes were converted into amino acid sequences to ensure that the correct reading frame had been found. No stop codons were detected in any of the three genes. Alignments of the translated proteins further confirmed that our data sets consist of orthologous sequences for all three genes.

2.5. Phylogenetic analyzes

2.5.1. Parsimony analyzes

We first performed parsimony analyzes of the coding sequences of each gene separately using Paup 4.0b for Macintosh (Swofford, 2002) with the following parameter settings: unweighted analysis, heuristic search, 100 random sequence additions with 4 trees held at each step, maximum of 500 trees retained, and TBR branch swapping. We performed 100 bootstrap replicates with 10 random sequence additions to assess the robustness of the clades. We then combined the three gene sequences into a single matrix and ana-

Table 2
Primers used for the three genes Elongation factor 1-α, LW-rhodopsin and Conserved ATPase domain

Primer	Sequence 3′–5′	Position on *Apis*
Elongation factor 1-α		
HaF2For1	GGG YAA AGG WTC CAA RTA TGC	508
F2Rev1-Meg	AAT CAG CAG CAC CCT TGG GTG G	1620
For4	AGC TCT GCA AGA GGC TGT CC	1592
For4a	AGC TTT GCA AGA GGC TGT TC	1592
Cho10	ACR GCV ACK GTY TGH CKC ATG TC	2185
F2-Intron-Rev	AAA AAT CCT CCG GTG GAA AC	1247
F2-Intron-For	CGT ATA AAC TTC ATT CGC GGT TTC	≈1210
Exon2Rev	GGG AAG ACG GAG AGC TTT GT	1108
Exon2For	CCG ACT AGA CCT ACA GAC AAA GCT C	1073
Exon3Rev	GAG CAA ATG TGA CAA CCA TAC C	1445
For3-Meg	GGT GAC AAT GTT GGT TTC AAC G	1514
Exon4For	YAA YGG RTA YAC RCC AGT GTT G	1943
EF-Rev	ARA GGH GGR AAT TCT TGG AAA G	2143
PCR conditions:	HaF2For1/F2Rev1-meg: 30″ 94 °C, 30 ″ 58 °C, 40″ 72 °C For4/Cho10: 30″ 94 °C, 30″ 58 °C, 30″ 72 °C	
LW-Rhodopsin		
OpsFor-Osm	AAT TGY TAY TWY GAG ACA TGG GT	398
OpsinRev3	GCC AAT TTA CAC TCG GCA CT	894
OspinRev3b	GCC AAT TTA CAC TCG GCG CT	894
OpsinFor5	GCG TGY GGC ACC GAT TAC TTC	665
OpsinFor5a	GCG TGY GGY ACM GAY TAY TTC	665
OpsinRev5	RGC GCA YGC CAA YGA TGG	1120
OpsinRev5a	RGC GCA YGC CAR YGA YGG	1120
PCR conditions:	OpsinFor-osm/OpsinRev3: 30″ 94 °C, 30″ 55 °C, 30″ 72 °C OpsinFor5/OpsinRev5: 30″ 94 °C, 30″ 58 °C, 30″ 72 °C	
CAD		
CADFor4	TGG AAR GAR GTB GAR TAC GAR GTG GTY CG	118
CADRev1-Meg	GCC ATC ACT TCY CCT AYG CTC TTC AT	632
PCR conditions:	CADFor4/CADRev1-Meg: 30″ 94 °C, 30″ 55 °C, 30″ 72 °C	

Primer positions on published sequences for *Apis mellifera* is given (GenBank Accession Nos. AF015267, AMU26026 and DQ067178 for EF, opsin and CAD, respectively).

Table 3
Alternative partitioning regimes and DNA substitution models for the five Bayesian analyzes performed

Three partitions	Criterion for model selection	
	HLRT Analysis 1	AIC Analysis 2
EF	GTR + I + G	GTR + I + G
Opsin	GTR + I + G	HKY + I + G
CAD	GTR + I + G	K80 + I + G

Nine partitions	Criterion for model selection		
	HLRT Analysis 3	AIC Analysis 4	Analysis 5
EF nt1	HKY + I + G	HKY + I + G	GTR
EF nt2	HKY + I	GTR + I	GTR
EF nt3	HKY + I + G	HKY + I + G	GTR
Opsin nt1	GTR + I + G	HKY + I + G	GTR
Opsin nt2	GTR + I + G	GTR + I + G	GTR
Opsin nt3	GTR + I + G	HKY + I + G	GTR
CAD nt1	GTR + I	GTR + I + G	GTR
CAD nt2	JC + I + G	SYM + I	GTR
CAD nt3	K80 + G	GTR + I + G	GTR

Substitution models were selected according to the two criteria implemented in MrModeltest: the hierarchical likelihood ratio test (HLRT) and the Akaike information criterion (AIC).

lyzed the combined dataset, performing 1000 bootstrap replicates with 10 random sequence additions (settings as above).

2.5.2. Bayesian analyzes

For the Bayesian analyzes, the three genes were analyzed collectively under two different partitioning regimes. First, we partitioned the data set by gene, resulting in three partitions. Second, we partitioned the data set by gene and codon position, resulting in a total of nine partitions. We ran MrModeltest 2.2 (Nylander, 2004) to determine the best model of sequence evolution for each partition (Table 3). Two approaches are implemented in MrModeltest, the hierarchical likelihood ratio test (HLRT) and the Akaike information criterion (AIC). As the HLRT approach may prefer different substitution models according to the hierarchy used, we choose in these cases the most complex model, i.e., the one with the most parameters. We ran two analyzes for each partitioning regime, one with the models suggested by the HLRT approach and one with those suggested by the AIC approach. Additionally, we performed a fifth analysis applying a separate GTR model, without gamma distribution or proportion of invariant sites, to each codon position (corresponding to the GTR + SSR model of some authors, e.g., Danforth et al., 2004, 2006a, 2006b; Patiny et al., 2007). We calculated AIC_c and BIC scores as described in McGuire et al. (2007) to select for the best of these five analyzes.

Markov Chain Monte Carlo analyzes were undertaken using MrBayes 2.1.1 (Huelsenbeck and Ronquist, 2001). We ran two simultaneous, completely independent runs starting from different random trees, and stopped the analysis only once convergence between both runs has occurred (standard deviation of split frequency < 0.01). We ran 4 million generations for the analyzes with three partitions, sampling trees and parameters every 100 generations, and 10 million generations for the analyzes with nine partitions, sampling every 1000 generations. Preliminary analyzes indicated that the default temperature regime for heating ($T = 0.2$) resulted in infrequent swapping between the cold and the three heated chains. Hence, we followed McGuire et al. (2007) and fixed the temperature parameter to 0.02, which resulted in appropriate values of state swap frequency but in virtually identical trees. In all analyzes, we allowed the rates to vary between partitions with the "prset ratepr=variable" command in MrBayes. To determine the burn-in region, we checked for stationarity of all model parameters using the software Tracer (Rambaut and Drummond, 2003). Stationarity of all parameters was reached after 10–20% of the generations in all analyzes, and we therefore used a burn-in of 25% in all analyzes. We discarded the trees saved during the burn-in period, combined the remaining trees of both runs and computed majority-rule consensus trees using Paup 4.0b.

2.6. Reconstruction of ancestral geographic range

We inferred ancestral geographic ranges using maximum likelihood inference and models of character evolution. The use of character models to infer ancestral geographic ranges suffers from some limitations, especially because state transitions often have immediate effects on speciation if states are geographic ranges (Ronquist, 1997; Ree et al., 2005; Ree and Smith, 2008). However, the use of character models is widely applied for biogeography reconstruction (Nepokroeff et al., 2003; Dubey et al., 2007; McGuire et al., 2007; Pereira et al., 2007; Pereira and Baker, 2008) as it presents many advantages over parsimony-based methods. Character models take phylogenetic uncertainty and branch lengths into account and allow distinct rates between different states. Their current use in biogeography inference clearly represents a transitional step until more complex models (e. g., Ree and Smith, 2008) become routinely available.

We scored biogeography as a three-state categorical character: "0" for Palearctic, "1" for Nearctic and "2" for Afrotropical. Although a few osmiine species are present in tropical Asia, we did not apply a fourth state corresponding to the Oriental zone but coded these taxa as Palearctic. The distribution data were drawn from Michener (2007). In cases in which terminal taxa were present in more than one geographic zone we allowed polymorphisms. We also ran the same analyzes without polymorphisms by attributing to such taxa the geographic range where the highest

species diversity is found. As such a scoring had no substantial influence on the results, we allowed polymorphisms in all analyzes. The subgenera *Pentadentosmia* and *Annosmia* were scored as Palearctic although few species of these taxa enter the Afrotropical zone in Sudan. In addition, we assigned state "1" to one of the two included Palearctic species of the subgenus *Pyrosmia* as the morphologically highly similar subgenus *Diceratosmia*, not available for our study, occurs in North America.

We inferred ancestral states with the software BayesTraits (Pagel et al., 2004; Pagel and Meade 2006) at nine highly supported nodes (all with posterior probabilities of 100% in the Bayesian analysis) corresponding to important lineages within the Osmiini. We used a sample of 1000 trees saved in the favoured Bayesian analysis. This analysis was allowed to run for 1 million generations after convergence. We sampled trees every 2000 generations and combined the trees from both runs. We explored two models of character evolution applying the maximum likelihood approach implemented in BayesTraits. First, rates were all constrained to be equal, corresponding to the simple model Mk1 (Lewis, 2001) usually used in biogeography reconstruction (Dubey et al., 2007; McGuire et al., 2007). Second, we allowed three free rates of biogeographic exchange: Afrotropical versus Palearctic, Palearctic versus Nearctic, Afrotropical versus Nearctic. The rates between two geographic regions were constrained to be equal in both directions using the command "restrict". Preliminary analyzes with six free rates failed to correctly estimate the rates and parameters, suggesting that our data did not contain enough information to estimate as many rates.

To assess the robustness of the ancestral reconstructions, we successively constrained ancestral states at each node to each of the three states using the "fossil" command in BayesTraits. We calculated the differences in ln-likelihood for each tree and averaged them over all trees. A difference of two log-units is conventionally taken as evidence for a "significant" difference (Pagel, 1999).

3. Results

3.1. DNA sequences

Our dataset is complete except for the opsin sequences of *Osmia montana* (entirely missing) and of *Protosmia rubifloris* (400 bp fragment only, sequenced with OpsinFor5/OpsinRev5). After removal of the introns, our dataset comprised 1111 bp for EF, 682 bp for opsin and 448 bp for CAD, of which 333 (30.0%), 275 (40.3%) and 163 (36.4%) were parsimony informative, respectively. Only EF showed significant among-species heterogeneity in third position base composition.

3.2. Parsimony analyzes

Parsimony analyzes performed for each of the three genes separately (data not shown) indicated that opsin was the most useful gene, followed by EF and CAD, as judged by the number of nodes with a bootstrap support of over 50%. We noticed very little incongruence among the genes (Table 4). First, EF indicated that the *Heriades* group was derived from within the *Osmia* group (59% bootstrap support), whereas the other genes suggested a monophyletic *Osmia* group. Second, CAD was the only gene supporting the monophyly of *Othinosmia* (63% bootstrap support). Third, *Haetosmia* was sister group of a clade comprising *Wainia*, *Atoposmia*, *Ashmeadiella*, *Hoplosmia* and *Osmia* in the analysis with opsin alone (67% bootstrap support), whereas the two other genes placed it as sister to the whole *Osmia* group (CAD: <50% bootstrap support; EF: 55%). These conflicting topologies are minor and no incongruence was supported by bootstrap values above 70%. We therefore combined the three genes into a single matrix for all subsequent analyzes.

Parsimony analysis of the combined dataset yielded one island with 134 most parsimonious trees. The 50%-bootstrap consensus tree is highly resolved with most basal nodes supported by bootstrap values >70% (Fig. 1). The Osmiini appeared monophyletic with a bootstrap support of 100% (Table 4) with the exclusion of the four small genera *Ochreriades*, *Afroheriades*, *Pseudoheriades* and *Noteriades*. The *Heriades* group and the *Osmia* group were each recovered as monophyletic with bootstrap supports of 100% and 70%, respectively (Table 4), with the exception of the four genera mentioned above and the genus *Chelostoma*. This genus turned out to be the sister to all other Osmiini with a bootstrap support of 100%.

3.3. Bayesian analyzes

AIC_c and BIC scores both favoured the models with nine partitions with a proportion of invariant sites and/or a gamma distribution (analyzes 3 and 4; Table 5). However, examination of the parameter estimations for these two favoured models with the software Tracer indicated that the two parameters I and G did not converge for several partitions, which suggests overparametri-

Table 4
Summary of support measures for the main lineages and genera of the Osmiini

	Individual genes			Combined analyzes		
	EF 1−α Parsimony	Opsin Parsimony	CAD Parsimony	Parsimony	Bayesian analyzes	
					Analysis 1	Analysis 5
Osmiini[a]	<50	93	57	100	100	100
Noteriades + Megachilini	77	91	84	100	100	100
Heriades group[b]	84	86	94	100	100	100
Osmia group[c]	paraphyletic	63	<50	72	100	100
Protosmia	100	100	96	100	100	100
Heriades	97	95	<50	100	100	100
Othinosmia	paraphyletic	paraphyletic	63	paraphyletic	paraphyletic	paraphyletic
Atoposmia	paraphyletic	<50	paraphyletic	–	–	–
Ashmeadiella	100	100	95	100	100	100
Osmia	paraphyletic	paraphyletic	paraphyletic	paraphyletic	paraphyletic	paraphyletic
Osmia + *Hoplosmia*	paraphyletic	paraphyletic	<50	90	100	100
Hoplitis[d]	87	99	84	100	100	100

[a] Genera *Afroheriades*, *Noteriades*, *Ochreriades* and *Pseudoheriades* excluded.
[b] Genera *Afroheriades*, *Noteriades* and *Pseudoheriades* excluded.
[c] Genera *Chelostoma* and *Ochreriades* excluded.
[d] Including genus *Stenosmia*.

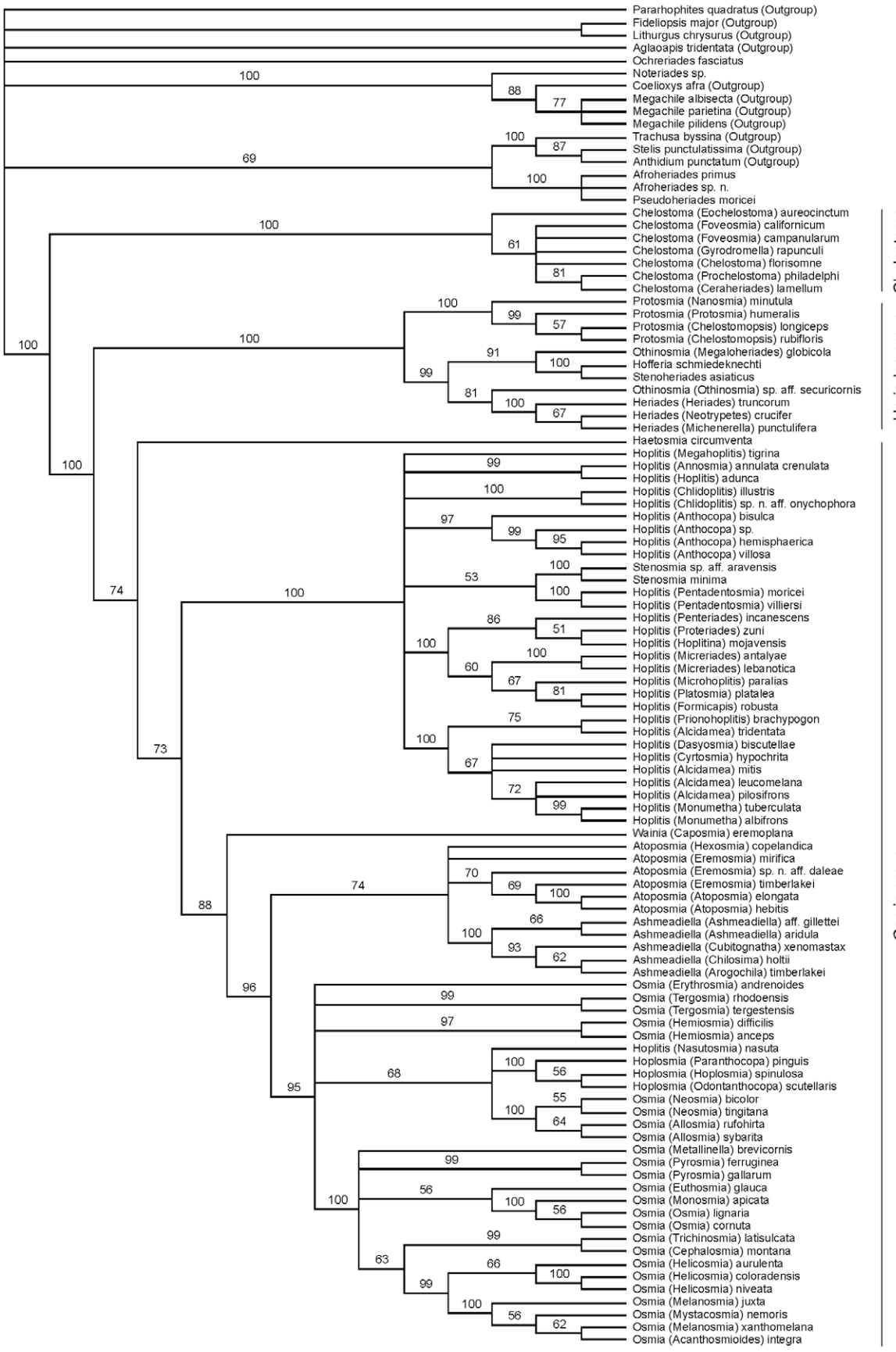

Fig. 1. Parsimony bootstrap consensus tree of the combined dataset. All nodes with less than 50% bootstrap support were collapsed. Bootstrap values are indicated above the nodes.

Table 5
Ln-likelihood values for the five different Bayesian analyzes of the combined dataset

Analysis	Model likelihood (harmonic mean)	Number of parameters	AIC_c	BIC
1 (3 partitions, HLRT)	−29432.72	41	58949.01	60794.11
2 (3 partitions, AIC)	−29669.39	27	59393.46	61159.44
3 (9 partitions, HLRT)	−28539.31	82	57248.93	59323.59
4 (9 partitions, AIC)	−28458.93	93	57112.00	59247.69
5 (9 partitions, GTR)	−29973.20	100	60155.84	62330.24

Model selection criteria (AIC_c and BIC values) were calculated following McGuire et al. (2007).

zation (Nylander et al., 2004). The analysis of the nine partitions applying a GTR model with neither gamma distribution nor proportion of invariant sites (analysis 5) showed the lowest ln-likelihood of all analyzes (Table 5). In contrast, every parameter in this analysis converged and differed from the priors, suggesting no overparametrization. Combining these facts with the AIC_c and BIC criterion, we selected the analysis with a different GTR + I + G model applied to each of the three gene partitions (analysis 1) as the favoured analysis (Fig. 2). This is also the model we used in the biogeographic reconstruction.

In spite of these differences in likelihood, trees yielded by the five Bayesian analyzes (Table 5) were almost identical topologically. In Fig. 2, all conflicting topologies in the 50%-majority rule consensus trees are indicated by black squares. The following relationships at the generic level varied among the five different Bayesian analyzes. *Ochreriades* was sister group of all other Osmiini in the three analyzes under nine partitions (98% posterior probability in the analysis 5), but not related to the Osmiini in the analyzes with three partitions. *Haetosmia* was sister to the *Osmia* group under both three-partition regimes, but sister group of the clade *Wainia*, *Atoposmia*, *Ashmeadiella*, *Osmia* and *Hoplosmia* in the three analyzes under nine partitions. Lastly, all analyzes failed to recover the monophyly of the genus *Atoposmia*. However, all representatives of *Atoposmia* except *Atoposmia* (*Hexosmia*) *copelandica* formed a monophyletic group in all analyzes (posterior probability 82–89%) except in the analysis 5, where there was a polytomy including *Atoposmia* (*Eremosmia*) *mirifica*, *A.* (*Hexosmia*) *copelandica*, the rest of *Atoposmia*, and *Ashmeadiella*.

3.4. Biogeography

We inferred the ancestral geographic range for nine selected nodes in the Bayesian tree (A–I in Fig. 3): The analysis allowing for three free rates of biogeographic exchange had a higher average ln-likelihood (−64.4) than the analysis under the Mk1 model with only one rate (−69.9), this difference being significant (ln-likelihood ratio = 10.9, df = 2, $p = 0.004$; Pagel, 1999). The estimations of the rates in the three-rate model suggest higher exchanges between the Palearctic and the Nearctic than between the Palearctic and the Afrotropical zones. Ancestral state reconstruction differed little between both models (Fig. 3). Both analyzes indicated a Palearctic origin for nodes A–C, F, G and I, and an African origin for node E. Pairwise comparisons with each node successively constrained to each of the three states significantly supported one state over the others (asterisks in Fig. 3) for all nodes, except E, either under both models (nodes B, C, F, G and I) or only under one model (A, D and H).

4. Discussion

In combination, the three selected markers provided a strong phylogenetic signal for the taxonomic level considered in our study. Opsin was the most useful gene and our results confirm the prediction of Ascher et al. (2001) that this gene is particularly suitable to resolve recent divergences in bees, namely within tribes and genera. CAD was surprisingly informative in spite of the small size of the fragment included. It shows comparable properties as opsin, such as unbiased base composition and substantial nt1 and nt2 variation (Danforth et al., 2006a) and thus appears highly promising for the reconstruction of bee phylogeny. EF performed comparatively poorly, even for the basal nodes, considering its high number of informative sites. This marker is highly conserved at the amino acid level, and much of the silent nucleotide variation shows relatively high levels of homoplasy. Nevertheless, these three genes complemented each other to yield a well-supported phylogeny. Our results resolve many issues on the systematics and taxonomy of the Osmiini. They constitute an important first step towards the development of a stable classification of this tribe and towards the evolutionary reconstruction of the biological diversity observed among these bees.

4.1. Systematics

4.1.1. Monophyly and suprageneric classification of the Osmiini

Based on our study, the monophyly of the Osmiini can be soundly assessed for the first time. Four small and rare genera do not appear to be related to the Osmiini. The genus *Noteriades* was found to be the sister group of the Megachilini in all analyzes. This placement was revealed by each of the three genes (Table 4) and highly supported in both parsimony (100% bootstrap support) and Bayesian analyzes (100% posterior probability) of the combined dataset. Based on the morphology, Griswold (1985) already suspected a close relationship between *Noteriades* and the Megachilini. Most Megachilini are characterized by the absence of arolia between the claws, although two exceptions exist (Peters, 1970; Baker and Engel, 2006). *Noteriades* is thus a further example of a Megachilini with an arolium. Its basal position within the Megachilini makes it a suitable outgroup for phylogenetic studies of this tribe. In contrast, the placement of the two small genera *Afroheriades* and *Pseudoheriades* remains unclear. These two genera formed a well-supported monophyletic group in all analyzes. However, they are not allied to the *Heriades* group as previously assumed (Michener, 2007). Though they never appeared to be related to the Osmiini, we refrain from excluding them definitively from this tribe because their phylogenetic position varied in our analyzes (Parsimony analysis: sister to the Anthidiini; Bayesian analysis: sister to the Megachilini). Similarly, the position of the genus *Ochreriades* remains unsolved, being alternatively sister to the Osmiini (nine partitions regime) or sister to the clade Megachilini, Anthidiini and Osmiini (three partitions regime). These three genera do not seem to be closely related to any other tribe of the Megachilidae. To elucidate their position, analyzes with other markers, additional megachilid species and an expanded sample of outgroups are currently underway (B. Danforth, T. Griswold and C. Praz, unpublished data).

Apart from these four small taxa, the remaining genera of the Osmiini, comprising approximately 99% of all known osmiine species, formed a well-supported monophyletic group in all analyzes. Our data enable a clearer suprageneric subdivision of the Osmiini (Table 6) than that suggested by the current classification (Michener, 2007). The genus *Chelostoma*, whose placement switched from the *Heriades* group to the *Osmia* group in the past, did not appear to be related to any of these two groups but clearly emerged as the sister group of all other Osmiini. Hence, it would deserve the same rank as the *Heriades* and the *Osmia* group, often recognized as subtribes Heriadina and Osmiina, respectively (Engel, 2005; Ungricht et al., in press). The monophyly of the genus *Chelostoma* is also supported by several unique morphological traits such as the orientation of the third and fourth labial palpi, the nonfringed labrum of females as well

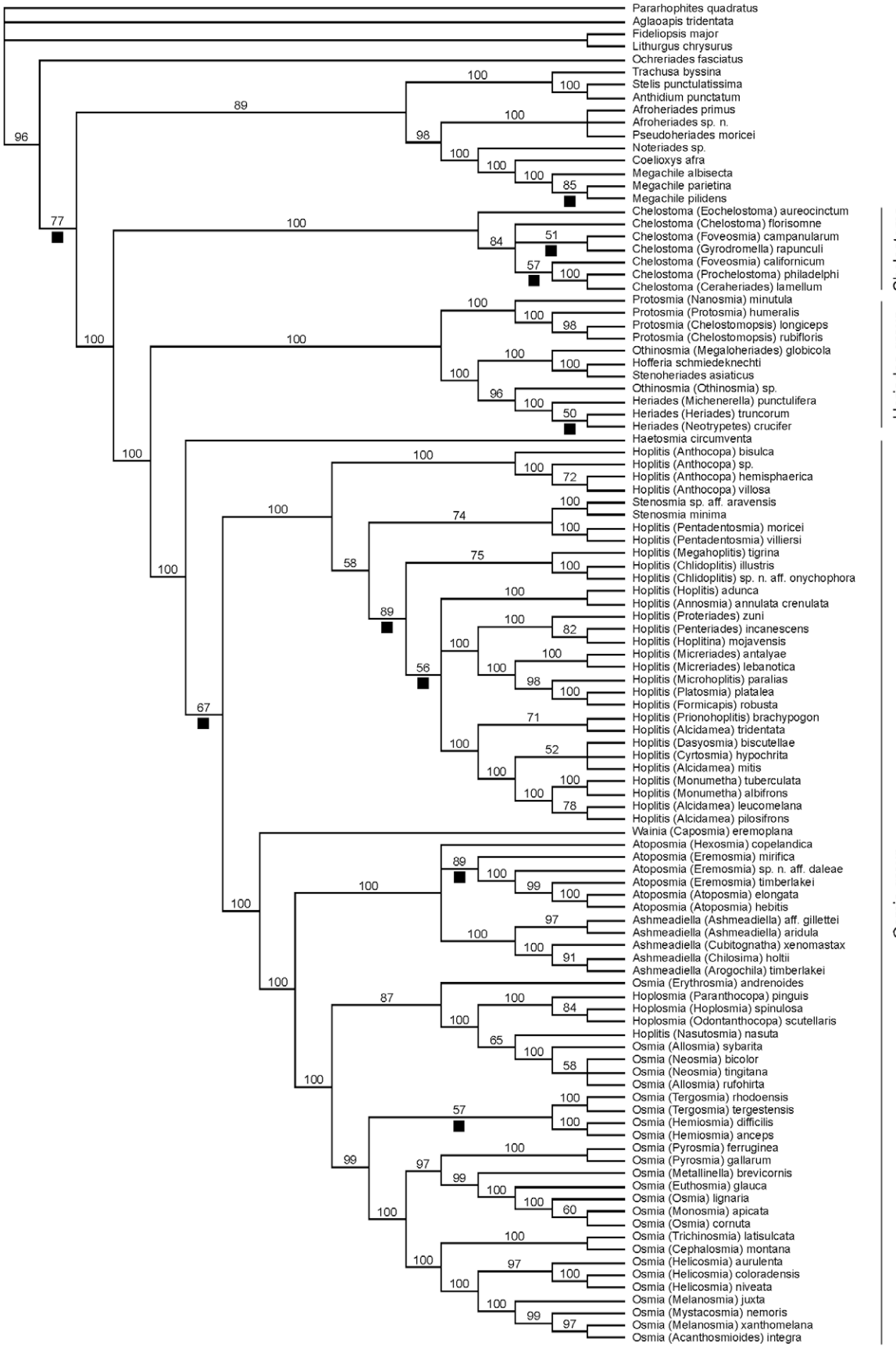

Fig. 2. Majority rule consensus of trees 10,000–40,000 in the Bayesian analysis applying a separate GTR + I + G model to each of the three genes. Nodes incongruent between this and any of the four other Bayesian analyzes are indicated with solid squares.

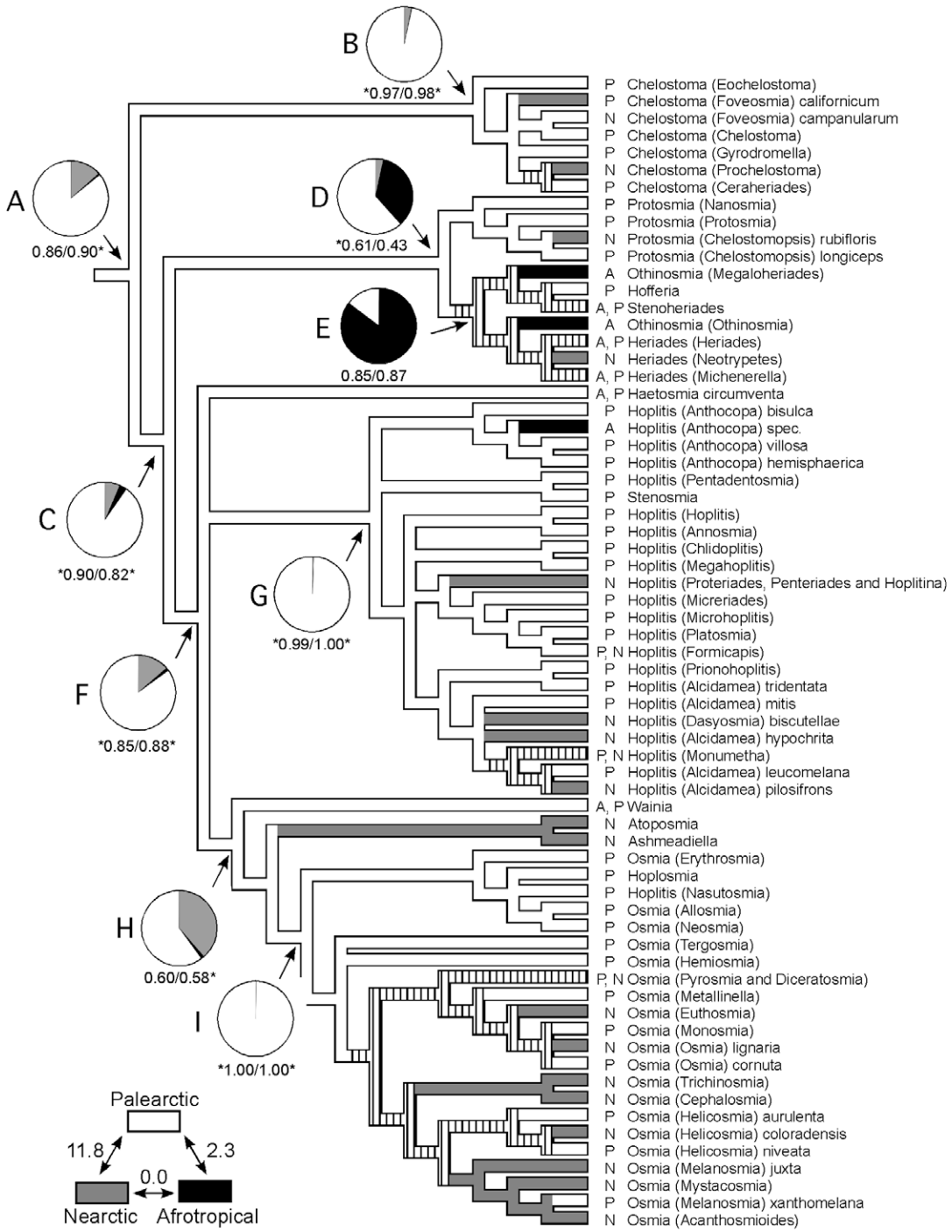

Fig. 3. Maximum likelihood reconstruction of ancestral geographic range for nine selected clades of osmiine bees. Three geographic zones were recognized: Palearctic (P; white), Nearctic (N; grey) and Afrotropical (A; black). The values at the nine selected nodes give the probability of having the most likely state at that node under two models of character evolution: three independent rates (left value) or all rates equal (right value). Pie diagrams represent the probability of each of the three states under the three-rate model. The asterisks indicate that analyzes constraining the most likely state had significantly higher ln-likelihood values than analyzes with both alternative states constrained. For visualization, the tree was drawn in MacClade with parsimony reconstruction of ancestral ranges. The tree topology corresponds to the 70%-majority rule consensus tree in the favoured Bayesian analysis. Genera and subgenera entirely restricted to one geographic zone were summarized to one terminal taxon.

as the presence of a comb-like structure on sternum 5 of males (Michener, 2007; Sedivy et al., in press). The exclusion of *Chelostoma* and *Ochreriades* from the *Osmia* group, and of *Afroheriades*, *Noteriades* and *Pseudoheriades* from the *Heriades* group, sharpens the distinction between these two groups. In the *Heriades* group, the structure of the male sterna, especially of sternum 5, is highly similar in *Protosmia*, *Othinosmia*, *Stenoheriades* and *Heriades*. Additionally, females of most species of the *Heriades* group bear a tuft of hairs medially on the labrum. These traits are found neither in the *Osmia* group nor in *Chelostoma*. Finding consistent morphological synapomorphies for the *Osmia* group is more difficult as this group is most diverse and very species rich. A probable synapomorphy observed in most females is the orientation of the bristles of yellowish hairs at the apical margin of the clypeus. In megachilid bees, including the *Heriades* group and *Chelostoma*, the bristles are parallel and straight, whereas in

Table 6
New supra-generic grouping of Osmiini (modifies Michener 2007, Table 81–1)

Chelostoma group	*Heriades* group	*Osmia* group
Chelostoma	*Heriades*	*Ashmeadiella*
	Hofferia	*Atoposmia*
	Othinosmia	*Haetosmia*
	Protosmia	*Hoplitis* (including *Stenosmia*)
	Stenoheriades	*Osmia* (including *Hoplosmia*)
	Xeroheriades	*Wainia*

The tribal position of the three genera *Afroheriades*, *Ochreriades* and *Pseudoheriades* remains unsolved, but they probably do not belong to the three groupings proposed here.

most genera of the *Osmia* group they converge towards the center (Gogala, 1995; Michener, 2007). Another possible synapomorphy might be the lateral teeth on male tergum 6 observed in most *Hoplitis*, *Atoposmia*, *Ashmeadiella*, *Haetosmia* and some *Osmia*.

4.1.2. Generic classification

At the generic level, our phylogeny reflects surprisingly well the classification of Michener (2007) except for the following taxa. First, the genus *Stenosmia* appears to be derived from within the genus *Hoplitis* and should be given subgeneric rank. The main characters that led to its recognition as a genus, namely the absence of lateral teeth on male tergum 6, the small size and the large stigma (Michener, 1941), clearly appear to be reversions within *Hoplitis*. Second, the genus *Hoplosmia* appeared to be derived from within the genus *Osmia* in all analyzes rendering the latter genus paraphyletic. Therefore, *Hoplosmia* should be included in *Osmia* though it possesses linear parapsidal lines in contrast to most members of the genus *Osmia*, which have punctiform parapsidal lines. Third, the recognition of *Micreriades* as a valid subgenus of *Hoplitis* rather than a synonym of *Alcidamea* is supported. Lastly, the subgenus *Nasutosmia* does not belong to the genus *Hoplitis* as expected (Michener, 2007), but turned out to be a member of the genus *Osmia*. *Nasutosmia* has linear parapsidal lines as the species of the genus *Hoplitis*, but it does not have the other characters of *Hoplitis*. Further, the structure of male sternum 3 in *Nasutosmia* is much like that of other *Osmia* species. The phylogenetic position of *Hoplosmia* and *Nasutosmia*, as suggested by our analyzes, shows that the form of the parapsidal lines is more variable than previously thought. Indeed, there are several other species within the genus *Osmia* which have linear parapsidal lines: the subgenera *Allosmia* and *Erythrosmia*, in *Osmia* (*Pyrosmia*) *cephalotes* as well as in several undescribed species of different subgenera of *Osmia* (A. Müller, unpublished data). These species, especially in the female sex, cannot easily be distinguished from species of *Hoplitis*. Michener (2007) suggested removal of the subgenera *Allosmia* and *Erythrosmia* from the genus *Osmia* to sharpen its definition. Our phylogeny does not support such exclusion, and further points out the vague morphological boundaries between the genera of the *Osmia* group. We acknowledge that merging all currently recognized genera of the *Osmia* group into one single genus *Osmia*, as is done by many European authors (Westrich, 1989; Schwarz et al., 1996; Amiet et al., 2004), is not incongruent with our phylogeny. However, we suggest the retention of the generic classification of Michener (2007) with the three necessary alterations detailed above. His classification, which is based on the detailed study of Griswold and Michener (1997), has already gained wide acceptance and certainly will remain the standard reference in the future. Therefore, any substantial changes would threaten nomenclatural stability. Indeed, the identification keys in Michener (2007) allow the unambiguous assignment of over 95% of all osmiine species to the generic and subgeneric level.

4.2. Biogeography

Our data indicate a Palearctic origin for the Osmiini (Fig. 3), a result largely supported by the high generic and species diversity observed in the Old World osmiine bees. Only the *Heriades* group seems to be equally diverse in sub-Saharan Africa and the Palearctic (Griswold, 1985). Our data indeed suggest an African origin for one clade of this group. Further, we found significantly more exchanges between the Palearctic and the Nearctic than between the Palearctic and the Afrotropical zones (Fig. 3), a result that has important implications for the understanding of the biogeography of the Osmiini and of bees in general. The comparatively low dispersal between the Palearctic and sub-Saharan Africa might reflect barriers formed by both deserts and tropical ecosystems. Indeed, the Osmiini are rare in tropical regions (Michener, 2007). The extended tropical areas of both Central America and the Indo-Malaysian region might have prevented the Osmiini from colonizing South America and Australia, respectively, where this group of bees is completely lacking. On both continents, regions with mediterranean climates exist that would provide suitable habitats for the osmiine bees.

Our phylogeny reveals at least 15 exchanges between the Old and the New World, and this value will certainly increase once detailed phylogenies of holarctic subgenera are available (e. g., *Monumetha*, *Osmia* s. str, *Melanosmia*, *Alcidamea*). For the majority of these exchanges, our data imply colonization of North America from the Palearctic, but the reverse pattern of colonization is possible in some holarctic groups equally diverse in North America and the Palearctic (e. g., *Melanosmia*). The high number of exchanges between the Palearctic and North America calls for an explanation as this dispersal rate is clearly higher than that observed in other groups of bees (Leys et al., 2002; Danforth et al., 2004; Patiny et al., 2007, but see Cameron et al., 2007; Hines, 2008). Some cold-adapted holarctic osmiine lineages, which have a boreal distribution both in Eurasia and North America (e. g., *Melanosmia*, *Monumetha*, *Formicapis*), may have been able to cross the Bering Strait in relatively recent times. In bumble bees, most dispersal events between Palearctic and Nearctic have occurred in clades of cold-adapted species, most likely during the last 5 million years (Hines, 2008). Similarly, several mammalian lineages probably crossed a land bridge between Alaska and Siberia 4–6 million years ago (Hunt, 2004; Van der Made et al., 2002). In contrast to the boreal osmiine lineages, many North American osmiine taxa are restricted to or have their center of distribution in arid regions of the southwestern USA, especially the Madrean Region (Michener, 2007), e. g., *Chelostoma* (*Foveosmia*), *Xeroheriades*, *Hoplitis* (*Proteriades*), *Atoposmia* and *Ashmeadiella*. This distribution pattern might indicate older dispersal events from the eastern hemisphere, e. g., during the Middle Miocene as observed in two clades of less cold-adapted bumblebees (10–15 Myr and 12–18 Myr, respectively; Hines 2008) and in shrews (~14–12 Myr; Dubey et al., 2007), or even around the Oligocene–Miocene boundary (~23–20 Myr; Hunt, 2004).

Interestingly, many North American osmiine taxa build nests in dead wood or in stems, whereas their Palearctic relatives show a higher diversity of nesting behaviors, including nesting in the ground. This is best exemplified in the genus *Hoplitis*, where the speciose ground-nesting subgenera of the Old World are absent from North America (e. g., *Anthocopa*, *Pentadentosmia*, *Annosmia*) in spite of their wide distribution in the Palearctic. The majority of the American species of *Hoplitis* nest in wood or stems (Michener, 2007). The same pattern is observed in *Protosmia* and *Osmia* (*Helicosmia*). The few species of these taxa present in the New

World nest in wood as far as known (Griswold, 1986b; Cane et al., 2007; Michener, 2007), whereas their Old World relatives, most likely the group from which they have evolved, show a much more variable nesting behavior (Michener, 2007; A. Müller and C. Praz, unpublished). As suggested by Michener (1979, 2007), wood-nesting bees are more likely to cross water barriers than ground-nesting bees, because wood nests can serve as "rafts" for long-distance, oceanic dispersal. The high percentage of wood-nesting taxa among the osmiine bees might explain why the number of exchanges between the Palearctic and North America is higher than that observed in other groups of bees, which primarily nest in the ground (Danforth et al., 2004; Patiny et al., 2007). Similarly, disjunct distributions observed in other above-ground nesting bee taxa, e. g., in the *Chelostomoides* group of subgenera of *Megachile* (Michener, 1979) or in Allodapini (Schwarz et al., 2006), might also be partly explained by overseas dispersal of nests.

4.3. Future research

The following phylogenetic relationships remain unsolved and call for further analyzes: (i) The genus *Othinosmia* appeared paraphyletic in all analyzes, but more species should be included before any classificatory changes are made. (ii) Our phylogeny failed to recover the monophyly of the genus *Atoposmia*. In particular, the position of the subgenus *Hexosmia* remains unclear. (iii) In contrast to parsimony that placed the genus *Haetosmia* at the base of the *Osmia* group, some Bayesian analyzes placed it as sister to the clade *Wainia-Atoposmia-Ashmeadiella-Osmia*. The latter placement is supported by the structure of the male sterna, which are similar in *Haetosmia*, *Wainia*, *Atoposmia* and *Osmia*. (iv) Only one subgenus of *Wainia* was included in our study. As the other subgenera differ substantially from it, the monophyly of this genus should be confirmed by including additional species. (v) Similarly, confirmation of the monophyly of *Stenoheriades* is needed. Our study includes a representative of only one of the four species groups within *Stenoheriades*. These groups span morphologies that otherwise are used to differentiate genera (Griswold, 1985). (vi) Representatives of the rare central Asian taxa *Kumobia* and *Jaxartinula*, presently placed as subgenera of *Hoplitis* (Michener, 2007), could not be included in our study. Some species of these taxa show an odd combination of morphological characters rendering them intermediate between *Osmia* and *Hoplitis*.

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