Blackwell Publishing

Cladistics 23 (2007) 1–15

Cladistics

10.1111/j.1096-0031.2007.00182.x

Phylogenetic relationships and host-plant evolution within the basal clade of Halictidae (Hymenoptera, Apoidea)

S. Patiny[1]*, D. Michez[2] and B. N. Danforth[3]

[1]Faculté universitaire des Sciences agronomiques de Gembloux, Entomologie fonctionnelle et évolutive, Passage des Déportés 2, 5030 Gembloux, Belgique; [2]Université de Mons Hainaut, Laboratoire de Zoologie, Avenue du champs de Mars 6, 7000 Mons, Belgique; [3]Department of Entomology, Comstock Hall, Cornell University, Ithaca, NY 14853-0901, USA

Accepted 26 June 2007

Abstract

Bees are among the most important pollinators of angiosperm plants. Many bee species show narrow host-plant preferences, reflected both in behavioral and morphological adaptations to particular attributes of host-plant pollen or floral morphology. Whether bee host-plant associations reflect co-cladogenesis of bees and their host plants or host-switches to unrelated host plants is not clear. Rophitinae is a basal subfamily of Halictidae in which most species show narrow host-plant preferences (oligolecty). We reconstructed the phylogenetic relationships among the rophitine genera using a combination of adult morphology (24 characters) and DNA sequence data (EF-1α, LW rhodopsin, *wingless*; 2700 bp total). The data set was analyzed by parsimony, maximum likelihood and Bayesian methods. All methods yielded highly congruent results. Using the phylogeny, we investigated the pattern of host-plant association as well as the historical biogeography of Rophitinae. Our biogeographical analysis suggests a number of dispersal/vicariance events: (1) a basal split between North America and South America (most likely a dispersal from South America to North America), and (2) at least two subsequent interchanges between North America and Eurasia (presumably via the northern hemisphere land bridges). Our analysis of host-plant associations indicates that Rophitinae specialized on a closely related group of angiosperm orders in the Euasterid I clade (mainly Gentianales, Lamiales and Solanales). However, there is little evidence of cocladogenesis between bees and plants and strong evidence of host switches to unrelated host plants. Based on our phylogenetic results we describe two new tribes of Rophitinae: Conanthalictini new tribe (including the genus *Conanthalictus*) and Xeralictini new tribe (including *Xeralictus* and *Protodufourea*).

Bees constitute a monophyletic group of > 16 000 described species feeding exclusively on the pollen and nectar of flowers (Michener, 2000). Like many groups of herbivorous insects (Schoonhoven et al., 1998), they show enormous variation in host-plant breadth. Some species, such as *Apis mellifera* and *Halictus ligatus*, are widespread and visit many different host plants. Many other species show much narrower host-plant associations. These oligoleges (host-plant specialists) tend to restrict their pollen foraging to phylogenetically closely related host plants (within the same family, tribe, or genus) (Robertson, 1925; Cane and Sipes, 2006). Bee species restricted to a single host-plant species are rare, but some examples exist (e.g., *Andrena florea* on *Bryonia dioica*) (Cane et al., 1996; Cane and Sipes, 2006; Schlindwein and Medeiros, 2006).

Oligolecty in bees appears to be driven by several factors and is most common in solitary bees with short life cycles. On the contrary, eusocial species tend to be polylectic. Resource abundance is likewise closely related with the evolution of host-plant specificity in bees. Abundant and widespread plants [e.g., *Larrea* (Zygophyllaceae); Minckley et al., 1999) are known to support many specialist pollinators. Arid regions with highly seasonal rainfall patterns (Minckley et al., 1999) and Mediterranean climate regions (Pekkarinen, 1997) also host large proportions of oligolectic species.

---

*Corresponding author:
E-mail address: patiny.s@fsagx.ac.be

Oligolectic bees often show phenologies tightly coupled to host-plant flowering, suggesting that bees may be tracking the same abiotic factors (such as rainfall) as the host plants (Hurd, 1957; Danforth, 1999b). Host-plant specialization itself appears to be conserved within lineages of bees such that members of some bee genera, tribes or subfamilies are almost exclusively specialists. Such lineages include Melittidae, Fideliini (Megachilidae), Emphorini and Eucerini (Apidae), Panurginae and Andreninae (Andrenidae), Paracolletinae (Colletidae), and Rophitinae (Halictidae) (see Westrich, 1989a,b; Sipes and Wolf, 2001; Michez and Patiny, 2005; Sipes and Tepedino, 2005). Oligolectic bees often show morphological adaptations to collecting and manipulating pollen of their preferred host-plant (Müller, 1996a).

Understanding evolutionary patterns of host-plant selection in bees requires that we know (1) the host-plant associations of the bee taxa; (2) the phylogenetic relationships among the bee taxa; and (3) the phylogenetic relationships among the host-plant taxa. Previous studies have analyzed phylogenetic patterns in host-plant usage in two other bee taxa: Müller (1996b) on western Palearctic Anthidiini (Megachilinae: Megachilidae) and Sipes and Wolf (2001) and Sipes and Tepedino (2005) on *Diadasia* (Emphorini: Apidae). The Sipes and Tepedino (2005) study is exemplary because their study combined accurate, quantitative estimates of host-plant preferences (derived from the analysis of bee pollen loads). Their study demonstrated both that host-plant usage may be highly constrained (i.e., host-plant associations may persist over several speciation events) and that when host-switching occurs, the bees do not necessarily switch to phylogenetically related host plants. Results of the Müller (1996b) study are more difficult to interpret because the western Palearctic Anthidiini are not necessarily a monophyletic group.

Because few studies of host-plant evolution in bees have been conducted, it is difficult to know if the patterns detected by Sipes and Wolf (2001) and Sipes and Tepedino (2005) are general patterns applicable to other oligolectic bee clades. In this study we analyzed phylogenetic relationships and host-plant associations among a monophyletic group of oligolectic bees: Rophitinae (Halictidae). Rophitinae is one of the four subfamilies of Halictidae (Michener, 2000; Danforth et al., 2004). Rophitinae appears to be the sister group to the remaining three subfamilies (Nomiinae, Nomioidinae, Halictinae) based on morphology (Pesenko, 1999) and molecular data (Danforth et al., 2004). All species are solitary and they occur primarily in warm xeric regions. The 208 described species of Rophitinae are unique among halictids in that most species are narrow oligoleges (Ebmer, 1984, 1993, 1994; Westrich, 1989a; Baker, 1996; Rozen, 1997; Patiny and Michez, 2006). Many species restrict their pollen collecting to closely related species or genera of host plants [e.g., *Rophites algirus* is a specialist on *Stachys recta* and *Clinopodium vulgare* (closely related within the family Lamiaceae)], whereas other species are apparently monolectic [e.g., *Conanthalictus conanthi* is a specialist on *Nama hispidum* (Hydrophyllaceae)]. We know of no species that would be described as a "generalist" or "polylege". Another particularity of the subfamily is its large number of genera (13; ≈ 17.5% of the Halictidae) but a rather small number of species (208; ≈ 6% of the species), mostly grouped in two genera (*Dufourea* and *Systropha*). This pattern may be due to the antiquity of the group, which is estimated to have arisen over 90 My BP (Danforth et al., 2004). A similar pattern is also observed in Andrenidae (for example), in which generic diversity is far higher in Panurginae than in the other two subfamilies (Andreninae and Oxaeinae).

In order to elucidate the evolutionary history of host-plant usage in Rophitinae (as well as to analyze the historical biogeography of the group) we conducted a phylogenetic analysis of the 13 recognized genera based on a combined analysis of morphological and molecular data. We analyzed a data set of three single-copy, nuclear genes that have proven phylogenetically informative in a previous study of generic relationships in Halictidae: EF-1α, LW-rhodopsin and *wingless* (Danforth et al., 2004). The phylogeny provides the basis for an historical analysis of floral host use in Rophitinae.

Materials and methods

Our analyses are based on a data set including four partitions corresponding to the sequences of three single-copy nuclear genes plus a set of morphological characters for 34 species (Table 1; Appendix 2). The phylogeographic analysis and the mapping of floral choices refer additionally to an area matrix (describing spatial distributions), a distribution block (describing bee/host-plant associations) and a topology of the plant relationships at the ordinal level (Fig. 5 adapted from Stevens, 2001).

A list of the taxa studied is given in Table 1. *Apis mellifera* was used to determine the reading frame for the sequence alignment and for the delimitation of intron/exon boundaries. This taxon was included in the outgroup together with nine other bee species belonging to the genera *Andrena* (Andrenidae), *Hesperapis* (Dasypodaidae), *Nomioides*, *Dieunomia*, *Curvinomia*, *Hoplonomia*, *Lipotriches* and *Pseudapis* (Halictidae) (Table 1).

Molecular data

The molecular data set includes nearly complete sequences for three nuclear genes: EF-1α, LW-rhodopsin

Table 1
Description of the data set. First column lists the species included in the analysis. Second column indicates what species were treated as outgroups ([H] indicates that the species belongs to Halictidae). The third column provides the collecting information of the specimens used for DNA extraction. NA (not applicable) indicates species described in the morphological partition only (several series of specimens have usually been studied). Columns 4, 5 and 6 give the GenBank access numbers for the studied taxa, respectively, for the genes EF-1α, LW-rhodopsin and *wingless*

Taxon	Classification	Sampling locality (voucher specimen for sequencing)	Molecular partitions—GenBank accession numbers		
			EF-1α	LW-rhodopsin	Wingless
Apis mellifera	Outgroup	USA: New York, Tompkins Co.	AF015267	U26026	AY222546
Andrena brooksi	Outgroup	USA: New Mexico, Hidalgo Co.	AY230129	EF416861	AY222551
Curvinomia ridleyi	[H] Outgroup	Malaysia: Sabah, Kianson waterfall (C. Rasmussen)	EF411173	EF416857	EF411182
Dieunomia nevadensis	[H] Outgroup	USA: Arizona, Cochise Co.	AF435396	AY227931	AY222568
Dieunomia triangulifera	[H] Outgroup	USA: Kansas, Douglas Co.	AF435397	AY227932	AY222569
Hesperapis larreae	[H] Outgroup	USA: California, Los Angeles Co. (J. Ascher)	AY230131	EF416862	AY222552
Hoplonomia amboinensis	[H] Outgroup	Malaysia: Sabah, n.Sek Keb Labang (C. Rasmussen)	EF411174	EF416858	EF411183
Lipotriches australica	[H] Outgroup	Australia: S. Australia, Cowell.	AF435395	AY227930	AY222567
Nomioides facilis	[H] Outgroup	Spain: Granada Prov.	AF435394	AY227929	AY222566
Pseudapis unidentata	[H] Outgroup	Spain: Almeria Prov.	AF435404	AY227933	AY222570
Ceblurgus longipalpis	[H] Rophitinae	Brazil: São João do Cariri (C.M.L. Aguiar)	None	None	None
Conanthalictus wilmattae	[H] Rophitinae	USA: California (J.Neff)	AF435378	AY227916	AY222553
Conanthalictus conanthi	[H] Rophitinae	USA: Arizona, Cochize Co.	EF411175	EF416856	EF411185
Dufourea holocyanea	[H] Rophitinae	USA: California, Tulare Co.	EF411181	None	EF411190
Dufourea malacothricis	[H] Rophitinae	USA: Michigan (P.Lincoln)	AF435382	AY227917	AY222554
Dufourea mulleri	[H] Rophitinae	USA: Michigan (P.Lincoln)	AF435383	AY227918	AY222555
Dufourea novaeangliae	[H] Rophitinae	USA: New York, Cayuga Co.	EF411178	AY227919	AY222556
Dufourea spirula	[H] Rophitinae	USA: California, Tulare Co.	EF411179	EF416863	EF411188
Dufourea spinifera	[H] Rophitinae	USA: California, Tulare Co.	EF411180	EF416864	EF411189
Goeletapis peruensis	[H] Rophitinae	Peru: Lima Dept. (J.G. Rozen, Jr.)	AF435386	AY227923	AY222560
Micralictoides quadriceps	[H] Rophitinae	NA	None	None	None
Morawitzella nana	[H] Rophitinae	China: Ordos (Type material, St Petersburg Museum)	None	None	None
Morawitzia fuscescens	[H] Rophitinae	NA	None	None	None
Penapis moldenkei	[H] Rophitinae	Chile: Huasco Prov. (J.G. Rozen, Jr.)	AF435401	AY227921	AY222558
Penapis toroi	[H] Rophitinae	Chile: Region III (L.Packer)	AF435402	AY227922	AY222559
Protodufourea parca	[H] Rophitinae	USA: Arizona, Pima Co. (L. Packer)	AF435399	AY227920	AY222557
Rophites algirus	[H] Rophitinae	France: Var, Entrecasteaux.	AY585144	DQ116675	EF411184
Sphecodosoma beameri	[H] Rophitinae	USA: Texas, Presidio Co.	EF411177	EF416860	EF411187
Sphecodosoma pratti	[H] Rophitinae	USA: Texas, Mason Co. (J.Neff)	AF435410	AY227924	AY222561
Systropha curvicornis	[H] Rophitinae	Austria: Vienna (M. Ayasse)	AF435411	AY227925	AY222562
Systropha glabriventris	[H] Rophitinae	South Africa: Gauteng Prov.	EF411176	EF416859	EF411186
Systropha planidens	[H] Rophitinae	Austria: Vienna (M. Ayasse)	AF435412	AY227926	AY222563
Xeralictus bicuspidariae	[H] Rophitinae	USA: California, San Diego Co. (R. Snelling)	AF435413	AY227927	AY222564
Xeralictus timberlakei	[H] Rophitinae	USA: California, Riverside Co. (R. Snelling)	AF435414	AY227928	AY222565

and *wingless*. Partial sequences were included for just four species: *Dufourea holocyanea*, *D. spinifera*, *Goeletapis peruensis*, *Sphecodosoma beameri*. The relevance of these regions of the nuclear genome for phylogeny inference in bees has been demonstrated in previous studies (Danforth et al., 1999; Mardulyn and Cameron, 1999; Leys et al., 2000, 2002; Ascher et al., 2001; Cameron and Mardulyn, 2001, 2003; Danforth and Ji, 2001; Danforth et al., 2003, 2004, 2006; Danforth, 2002). Specific details about the structures of these genes were published previously (Danforth et al., 2004). The three genes span 2742 bp in total: 1616 bp in EF-1α, 719 bp in LW-rhodopsin (introns excluded) and 405 bp in *wingless*.

Owing to a lack of suitable specimens, the molecular data set does not include four genera: *Ceblurgus*, *Morawitzia*, *Morawitzella* and *Micralictoides*. The phylogenetic relationships between these latter taxa and the nine other genera of Rophitinae are inferred based on the morphological data only.

DNA was extracted from specimens preserved in 95% EtOH, following a phenol/chloroform protocol adapted from Saghai-Maroof et al. (1984; Danforth, 1999a). PCR products were gel purified and sequenced on an automated 3730 DNA Analyzer (Applied Biosystems, Foster City, CA). Big Dye Terminator was used for the sequencing reaction. In order to obtain the complete sequences, several primers (forward and reverse) were used for each gene amplification (Table 2). The sequences were trimmed and assembled using Sequencher (Gene Codes). Initial alignments were done in Megalign (DNA*, Lasergene, Madison, WI) using the ClustalW alignment algorithm. Alignments were checked and altered slightly by eye. The data set was additionally edited and formatted in MacClade (Maddison and Maddison, 2000) and exported as a Nexus file.

Morphological data

Morphological data were obtained by study of museum specimens in the following collections: AMNH (New York, USA), CU (Ithaca, USA), FuSaGx (Gembloux, Belgium) and OöL (Linz, Austria). Seventeen characters were obtained from previous studies (Alexander and Michener, 1995; Rozen, 1997; Pesenko, 1999) and 32 characters were added for the first time. An initial data set including 49 characters was constructed. After a series of preliminary analyses, yielding topologies globally congruent with the ones displayed herein, 25 characters that were uninformative or ambiguously coded were excluded. Exclusion of these characters did not affect the overall topology obtained, but reduced the number of equally parsimonious trees in the analysis of the morphological partition.

Phylogenetic analysis

Parsimony. Morphological data were first analyzed separately using maximum parsimony (MP) as implemented in PAUP 4.0b10 (Swofford, 2002). This first analysis used a data set including representatives of the 13 genera in Rophitinae (25 species (outgroup = *A. brooksi*); Table 1). One thousand random sequence additions with equal weights were performed. The tree-bisection-reconnection (TBR) algorithm was used for branch swapping. Trees were summarized in a strict and a 50% majority-rule consensus. Ten cycles of successive weighting-reweighting-heuristic searches were also applied and the last trees yielded were summarized in a strict consensus. Bootstrap support was computed (150 replicates) in each case (weighted and unweighted analyses).

MP was also used for the analysis of the molecular and molecular + morphological data, subdivided into partitions corresponding to each nuclear gene (EF-1α, opsin, wingless) and morphological characters. Gaps in the nucleotide alignment were considered as a fifth state

Table 2
PCR conditions and sequences of the primers used

Ef-1α:			
For1deg	F2rev1	94 °C/45 s; 94 °C/1 min; 52 °C/1 min; 72 °C/1min 30 s.	35 cycles
For1deg	EF1intron1	94 °C/45 s; 94 °C/1 min; 50 °C/1 min; 72 °C/1 min	35 cycles
HaF2For1	F2rev1	94 °C/45 s; 94 °C/1 min; 54 °C/1 min; 72 °C/1 min 30 s.	35 cycles
F3rho	Cho10	94 °C/45 s; 94 °C/1 min; 58 °C/1 min; 72 °C/1 min 30 s.	35 cycles
LW-rhodopsin:			
For3mod	Revmod	94 °C/45 s; 94 °C/1 min; 56 °C/1 min; 72 °C/1 min	35 cycles
For5	Rev4	94 °C/45 s; 94 °C/1 min; 56 °C/1 min; 72 °C/1 min	35 cycles
Wingless:			
BeewgFor	Lepwg2a	94 °C/45 s; 94 °C/45 s; 54 °C/45 s; 72 °C/45 s.	35 cycles

Primers	Sequences from 5′ to 3′
Ef-1α	
For1deg	5′-G[C/T] ATC GAC AA[A/G] CGT AC[C/G] AT[C/T]G-3′
HaF2For1	5′-G GG[T/C] AAA GG[A/T] TCC TTC AA[A/G] TAT GC-3′
For3rho	5′-GGY GAC AA[C/T] GTT GTT TT[C/T] AA[C/T] G-3′
F2rev1	5′-A ATC AGC AGC ACC TTT AGG TGG –3′
EF1intron1(rev)	5′-GTA ATC ATG TT[C/T] TTG AT[A/G] AAA TCT CT-3′
Cho10(mod)	5′-AC [A/G]GC [A/G/C]AC [G/T]GT [T/C]TG [A/T/C]C[T/G] CAT GTC-3′
LW-rhodopsin	
Opsin For3 (mod)	5′-TTC GAY AGA TAC AAC GTR ATC GTN AAR GG-3′
Opsin For5	5′-ATG CGN GAR CAR GCN AAR AAR ATG AA-3′
Opsin Rev (mod)	5′-ATA NGG NGT CCA NGC CAT GAA CCA-3′
Opsin Rev4	5′-GGT GGT GGT RCC GGA RAC GGT G-3′
Wingless	
beewgFor	5′-TGC ACN GTS AAG ACC TGY TGG ATG AG-3′
Lepwg2a	5′-ACT ICG CAR CAC CAR TGG AAT GTR CA-3′

(coding gaps as missing data yielded congruent topologies). The combined morphological + molecular data set was analyzed as above.

Maximum likelihood. Likelihood scores were computed for 20 models, based on a parsimony starting tree (Table 3, Fig. 1). Trees were searched using the GTR + SSR model (scoring the best likelihood value; Table 2) in combination with the three branch-swapping methods implemented in PAUP 4.0b10 (Swofford, 2002): TBR (tree bisection-reconnection), SPR (subtree pruning-regrafting), NNI (nearest neighbor interchange).

Bayesian analyses. These were carried out using MrBayes 3.1.1 (Huelsenbeck and Ronquist, 2001). A first analysis of the molecular data was made using a GTR + SSR model (similar to that used in the ML analysis). Ten discrete rates were used, corresponding to each codon position in the three genes, plus the introns. Four simultaneous chains were run for 1000 000 generations. Trees were sampled every 100 generations for a total of 10 000 trees over the entire run. Examination of the likelihood scores indicated that the first 2000 generations could be considered as "burnin." Trees and parameter estimates were based on the last 8000 trees sampled.

Parallel analysis was performed using a combined data set including the morphological data. Settings for the molecular data were kept as above. The morphology partition was analyzed under the standard model implemented in MrBayes 3.1.1. Longer runs, up to

Table 3
Description of the likelihood parameters enforced in the 20 models tested. The last column gives the likelihood scores. The GTR + SSR model gave the highest –ln likelihood score and was therefore used for ML and Bayesian tree searches

Model	Base frequency	Rates across sites	Shape	No. of categories	Proportion of invariant	–ln Likelihood
JC	Equal	Equal			0	18697,08481
JC + G	Equal	Gamma	Estimate	4	0	17438,23496
JC + I	Equal	Equal			Estimate	17500,72539
JC + I + G	Equal	Gamma	Estimate	4	Estimate	17430,23771
JC + SSR	Equal	Site specific				17158,21489
K2P	Equal	Equal			0	18127,5887
K2P + G	Equal	Gamma	Estimate	4	0	16824,59939
K2P + I	Equal	Equal			Estimate	16899,40883
K2P + I + G	Equal	Gamma	Estimate	4	Estimate	16812,99652
K2P + SSR	Equal	Site specific				16570,41465
HKY	Empirical	Equal			0	18116,4519
HKY + G	Empirical	Gamma	Estimate	4	0	16814,80197
HKY + I	Empirical	Equal			Estimate	16890,70439
HKY + I + G	Empirical	Gamma	Estimate	4	Estimate	16803,65828
HKY + SSR	Empirical	Site specific				16564,58936
GTR	Empirical	Equal			0	18046,57427
GTR + G	Empirical	Gamma	Estimate	4	0	16801,80013
GTR + I	Empirical	Equal			Estimate	16880,05467
GTR + I + G	Empirical	Gamma	Estimate	4	Estimate	16789,11359
GTR + SSR	Empirical	Site specific				16536,01525

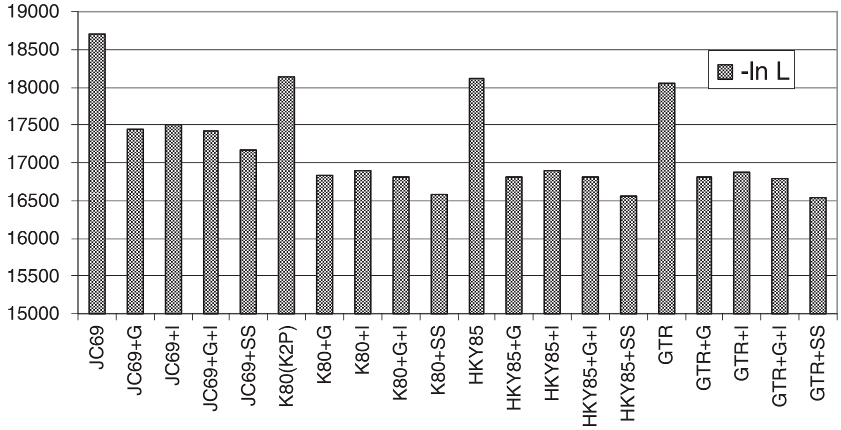


Fig. 1. Likelihood scores for the 20 models tested (Table 2).

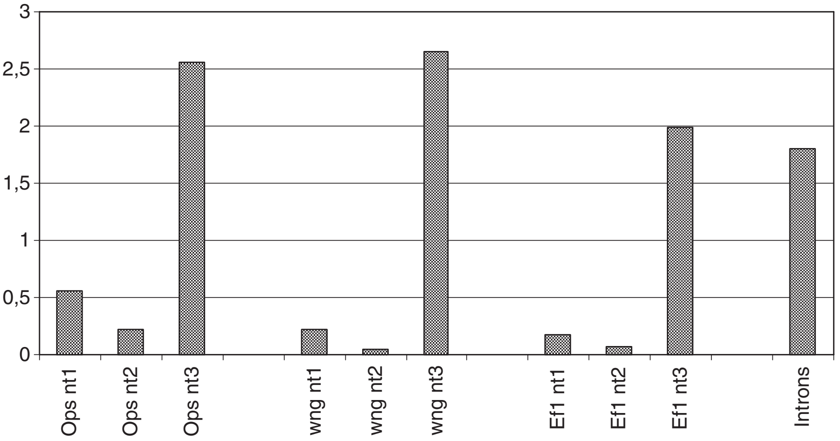


Fig. 2. Relative rates in each codon position for the three genes and within the intron partition of EF-1α.

3000 000 generations, were tested in this latter analysis. The tree displayed in Fig. 3(d) is a majority rule consensus of the 29 001 trees sampled from the 3000 000 of generations (the 1000 first trees were considered as "burnin").

Complementary analyses

DIVA (Ronquist, 1996) was used to infer the ancestral distributions of the studied taxa based on an area-matrix including 10 areas defined across the known distribution of rophitines. We used the topology obtained from the combined data set in Bayesian analysis. The results of this biogeographical analysis were mapped on to the tree (Fig. 4).

The evolution of floral associations was analyzed using Component 2.0 (Page, 1993). A distribution block, giving the floral choices (at the level of order) of the Rophitinae genera was constituted based on literature (Ebmer, 1984, 1993, 1994; Baker, 1996; Rozen, 1997; Patiny and Michez, 2006; Westrich, 1989a,b). Plants were considered as hosts and the bees as users. The topology of the host-plant phylogeny was taken from Stevens (2001).

Results

According to the likelihood values computed for the 20 models tested (Table 3), GTR + SSR is the best model for the combined molecular data set (Fig. 1). It is noteworthy that the site-specific mutation rate yields the best likelihood score independent of the other parameters enforced in the models. Based on these results, GTR + SSR was chosen for both the maximum likelihood tree search (Fig. 3c) and the Bayesian analysis (Fig. 3d).

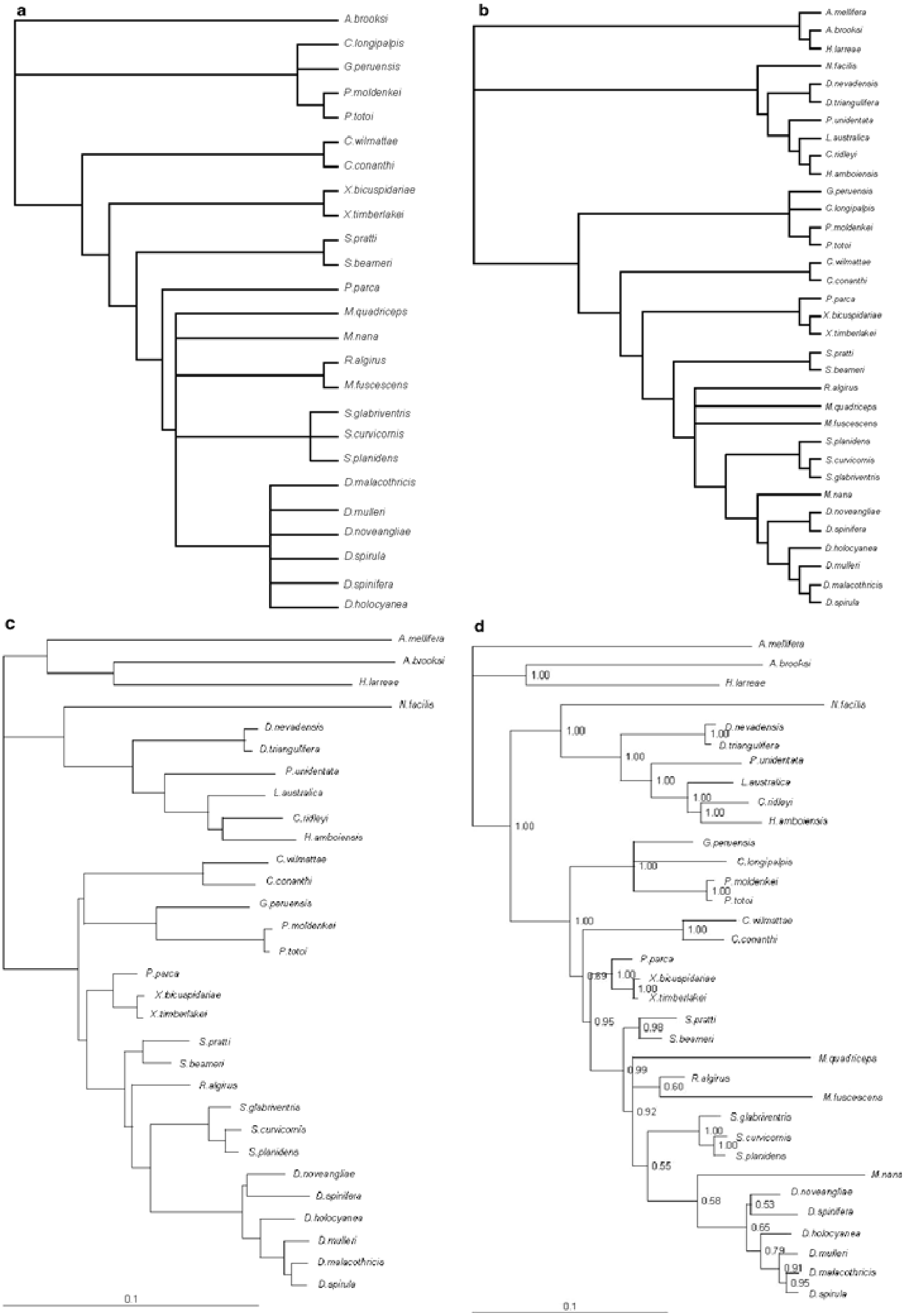
The rates of substitution among codon positions were rather uniform between the three genes (Fig. 2). A much higher rate is observed in the third positions of each gene, as expected. In EF-1 α the substitution rate in third positions is close to that computed for introns (Fig. 2).

Phylogeny of Rophitinae

The MP analysis of the morphological data yielded 264 most parsimonious trees (L = 56; Fig. 3a). When cycles of successive weighting are applied to the data set, 66 MP trees with similar topologies are obtained. Figure 3(a) is a strict consensus of trees from the unweighted analysis. Except for some details, this topology is very similar to the one produced using other data sets and analytical methods (see below). One main monophyletic group is composed of the Old World taxa (*Dufourea*, *Morawitzella*, *Morawitzia*, *Rophites* and *Systropha*) + *Micralictoides* (a small genus restricted to southern California). We refer to this group as the "OW + *Micralictoides*" group below. The position of *Protodufourea* as sister group to OW + *Micralictoides* is the main difference observed between this topology and the ones obtained based on molecular data (see below). The South American taxa (*C. longipalpis*, *G. peruensis*, *P. moldenkei*, *P. toroi*) form the sister group to the other genera in the subfamily based on the morphological data.

Parsimony analysis of the combined morphological and molecular data set produced almost the same

Fig. 3. (a) Strict consensus of the 264 MP trees based on morphological characters (L: 56, RI: 0.8271, CI: 0.5893); (b) strict consensus of the six MP trees based on combined molecular + morphological data (L: 2872, RI: 0.6514, CI: 0.5223); (c) ML tree based on GTR + SSR model, using TBR as the branch swapping algorithm; (d) 50% majority rule consensus of the 29 001 trees produced by the Bayesian analysis of the mixed data set (posterior probabilities are indicated on branches). Trees were edited using Treeview (Page, 1996).


a
A.brooksi
C.longipalpis
G.peruensis
P.moldenkei
P.totoi
C.wilmattae
C.conanthi
X.bicuspidariae
X.timberlakei
S.pratti
S.beameri
P.parca
M.quadriceps
M.nana
R.algirus
M.fuscescens
S.glabriventris
S.curvicornis
S.planidens
D.malacothricis
D.mulleri
D.noveangliae
D.spirula
D.spinifera
D.holocyanea
b
A.mellifera
A.brooksi
H.larreae
N.facilis
D.nevadensis
D.triangulifera
P.unidentata
L.australica
C.ridleyi
H.amboiensis
G.peruensis
C.longipalpis
P.moldenkei
P.totoi
C.wilmattae
C.conanthi
P.parca
X.bicuspidariae
X.timberlakei
S.pratti
S.beameri
R.algirus
M.quadriceps
M.fuscescens
S.planidens
S.curvicornis
S.glabriventris
M.nana
D.noveangliae
D.spinifera
D.holocyanea
D.mulleri
D.malacothricis
D.spirula
c
A.mellifera
A.brooksi
H.larreae
N.facilis
D.nevadensis
D.triangulifera
P.unidentata
L.australica
C.ridleyi
H.amboiensis
C.wilmattae
C.conanthi
G.peruensis
P.moldenkei
P.totoi
P.parca
X.bicuspidariae
X.timberlakei
S.pratti
S.beameri
R.algirus
S.glabriventris
S.curvicornis
S.planidens
D.noveangliae
D.spinifera
D.holocyanea
D.mulleri
D.malacothricis
D.spirula
0.1
d
A.mellifera
A.brooksi
1.00
H.larreae
N.facilis
1.00
1.00
D.nevadensis
D.triangulifera
1.00
P.unidentata
1.00
L.australica
1.00
C.ridleyi
1.00
H.amboiensis
1.00
G.peruensis
C.longipalpis
1.00
P.moldenkei
1.00
P.totoi
1.00
C.wilmattae
1.00
C.conanthi
P.parca
0.89
1.00
X.bicuspidariae
1.00
X.timberlakei
0.95
S.pratti
0.98
S.beameri
M.quadriceps
0.99
R.algirus
0.60
M.fuscescens
0.92
S.glabriventris
1.00
S.curvicornis
1.00
S.planidens
0.55
M.nana
0.58
D.noveangliae
0.53
D.spinifera
0.65
D.holocyanea
0.79
D.mulleri
0.91
D.malacothricis
0.95
D.spirula
0.1

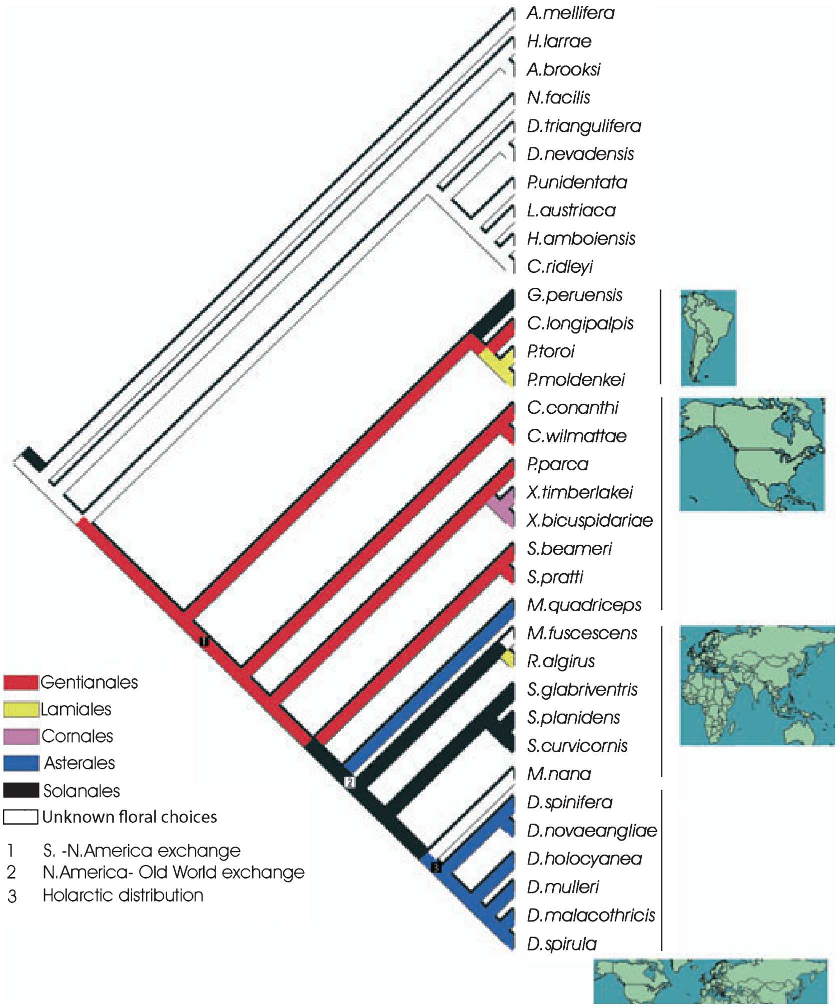


Fig. 4. Host-plant associations and historical biogeography of Rophitinae. Host-plant associations are mapped on to the tree topology based on the combined Bayesian analysis of morphological plus molecular data. Important nodes for the biogeographical analysis are labeled: node 1: vicariance between South America and North America; node 2: vicariance between North America and Eurasia; node 3: interchange between Eurasia and North America within the genus *Dufourea*.

topology as when morphology was analyzed alone (Fig. 3b). We recovered a monophyletic group comprising OW + *Micralictoides*. However, unlike in the MP analysis of the morphological data, we recovered a monophyletic group consisting of *Protodufourea* + *Xeralictus*. This analysis again recovers the South American taxa (Penapini) as sister to the remaining genera.

ML analysis produced one single topology irrespective of the branch swapping method used (Fig. 3c). The genera *Dufourea*, *Systropha* and *Rophites* again form a clade similar to the OW + *Micralictoides* clade observed in the MP analysis. No molecular data are available for *Ceblurgus*, *Micralictoides*, *Morawitzia* and *Morawitzella*, and their phylogenetic affinities cannot be assessed based on the ML analysis. The ML analysis also recovered a monophyletic group consisting of *Protodufourea* + *Xeralictus*. Unlike the parsimony analyses, ML recovered a monophyletic group consisting of the South American genera (Penapini) + *Conanthalictus*, although this clade is not well supported (Fig. 3c).

The Bayesian trees display almost the same topology for the terminal groups (Fig. 3d). The main difference between the ML and Bayesian trees involves the placement of the South American genera (Penapini). In the ML analysis, this group formed the sister group to *Conanthalictus* while in the Bayesian analysis Penapini forms the sister group to the remaining rophitine genera.

The positions of the taxa for which only the morphological partition is available are robust and conserved independent of the analysis method. *C. longipalpis* branches with the other South American taxa in all topologies (Fig. 3a,b,d). Likewise,

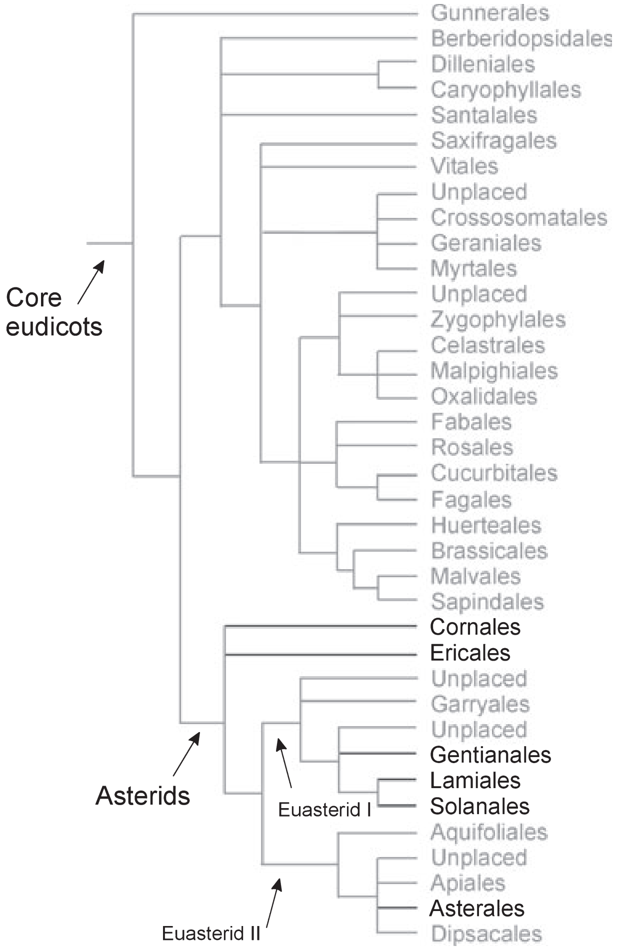


Fig. 5. Angiosperm phylogeny adapted from Stevens (2001). The main groups of rophitine host plants are displayed in bold.

Micralictoides quadriceps is always placed within the group referred to as OW + *Micralictoides*. *M. fuscescens* and *M. nana* display a bit more variation in the assessed relationships. These two species are, respectively, grouped with *R. algirus* and *Dufourea* or branch basally within Old World genera + *Micralictoides* (Fig. 3a,b,d). Figure 4 summarizes our phylogenetic results based on the Bayesian analysis of morphological + molecular data. This tree represents our best estimate of the phylogeny of the rophitine genera.

There are some important morphological characters that support the trees obtained in our combined molecular + morphological analyses. The South American clade (Penapini), including *Ceblurgus*, *Goeletapis* and *Penapis*, is united by the relative position of tentorial pit on the subantennal suture (Rozen, 1997; character 8) as well as development of the apodemes of S8 (characters 20, 21). Like in many other groups of bees, the male genital structures and the associated characters provide important phylogenetic information. Most of the clades we obtained in the combined analysis are united by one or more genital characters. The development of the apex of S7 is a synapomorphy for the northern Hemisphere taxa (character 18). The bilobed apodemes of S8 (character 22) supports monophyly of the Old World genera.

Ancestral distribution and floral choices

The results of the DIVA reconstruction of the ancestral distributions support a number of radiation–isolation events within Rophitinae (Fig. 4). First, the basal split between Penapini (*Ceblurgus* + *Goeletapis* + *Penapis*; Engel, 2001) and the remaining genera likely represents a northern/southern hemisphere vicariance (node labeled "1" in Fig. 4). Second (and later), there is an apparent vicariance between the New World (North American) and Old World taxa, likely representing dispersal from North America to Eurasia via one of the northern hemisphere land bridges (node labeled "2" in Fig. 4). Finally, *Dufourea* represents a terminal Old World/New World interchange because *Dufourea* has a Holarctic distribution with an equally diverse fauna in both the Nearctic and Palearctic regions (node labeled "3" in Fig. 4).

Appendix 3 summarizes the host-plant preferences among the genera of Rophitinae. There is a clear tendency for rophitine bees to specialize on certain groups of plants, including Asterales, Gentianales, Lamiales, Solanales and Cornales. Most host associations in Rophitinae center on asterids, particularly the Euasterid I clade (Stevens, 2001; Fig. 5). Mapping host-plant associations on to the tree (Fig. 4) suggests that the ancestral host association was with Gentianales (primarily Boraginaceae and Hydrophyllaceae). Subsequent host shifts to Lamiales, Cornales, Solanales and Asterales are indicated and there is evidence of parallel host shifts to the same host-plant orders (e.g., Lamialies, Solanales, Asterales). Our Component analysis based on the same data implies a high number of duplications (seven when considering the floral choices of 10 bee genera), indicating an absence of cocladogenesis between bees and plants. Perfect congruence between host-plant and bee phylogeny would have shown no duplications. Overall, our mapping and Component analyses indicate that host-plant evolution in rophitine bees shows little correlation with the phylogeny of the host plants.

Interestingly, we observe that the Old World species display floral choices closer to the South American species than to the North American ones. For instance, both *Systropha* (Old World) and *Goeletapis* (South America) forage on Solanales, while the preferred host plants for North American taxa (Cornales and Gentianales) are not used by the Old World or South American taxa (Fig. 4).

Discussion

Systematics and phylogeny of the Rophitinae

Although the monophyly of Rophitinae has been questioned based on morphological studies (Alexander and Michener, 1995; Pesenko, 1999), previous molecular results (Danforth et al., 2004) and our current molecular plus morphological results strongly support monophyly of Rophitinae. Despite efforts to identify additional morphological synapomorphies, the only morphological character supporting subfamily monophyly remains the relative position of the antennal sockets in the lower half of the face (Pesenko, 1999; Michener, 2000).

Our tree topologies support the monophyly of the tribe Penapini, including the three South American genera: *Ceblurgus*, *Goeletapis* and *Penapis* (= Penapini, Engel, 2001) (Fig. 3a–d). The monophyletic clade formed by these three genera is supported in all analyses, scoring high bootstrap and posterior probability values. Placement of *Conanthalictus*, however, is somewhat unstable. The ML analyses place Penapini as sister to *Conanthalictus* (Fig. 3c), while the parsimony and Bayesian analyses place Penapini as sister to the remaining rophitine genera (Fig. 3a,b,d). However, there is weak support for the ML result and we believe it is more likely that *Conanthalictus* falls within the clade including the remaining North American genera. Our results indicate that *Conanthalictus* is a distinct lineage of Rophitinae and we propose a new tribe for *Conanthalictus*, Conanthalictini trib. nov. (description in Appendix 1).

As also suggested but not formalized by Engel (2001), we propose a new tribe, namely Xeralictini trib. nov. (description in Appendix 1), for *Protodufourea* + *Xeralictus*. In the topologies we obtained, Xeralictini branch at the base of a wider clade grouping *Sphecodosoma* + *Micralictoides* + Old World genera. The latter group can be identified as Rophitini s.str. The subfamily is therefore best subdivided into four monophyletic tribes: Conanthalictini trib. nov., Penapini, Rophitini and Xeralictini trib. nov.

Within Rophitini, our topologies group *Morawitzia* and *Rophites* (morphology MP and combined characters Bayesian trees), as well as *Dufourea* and *Morawitzella*. These groups are particularly consistent from the morphological point of view. *Rophites* and *Morawitzia* display similarities in many morphological characters, notably in male genitalia and associated structures. *Morawitzella* is poorly known, the description being based on a unique specimen. However, the morphology of that specimen is astonishingly close in some ways to *Flavodufourea* Ebmer, which is regarded as a subgenus of *Dufourea* (Patiny, 2003).

Finally, only three species of *Systropha* are included in the present study. However, they belong to two of the three described subgenera (Patiny and Michez, 2006). The relationships we obtained for the species of *Systropha* match the topologies obtained in a more extensive study of the genus (Patiny and Michez, 2006).

Historical biogeography

Assuming the phylogeny presented in Fig. 4 is correct, we hypothesize three intercontinental exchanges or vicariance events within Rophitinae. First, there is a basal interchange between northern and southern hemispheres implied by the sister group relationship of the South American taxa (Penapini) to the remaining (northern hemisphere) genera. According to the time scale suggested in Danforth et al. (2004), this interchange is likely to have happened close to the K/T boundary. At that time, North America was nearly completely subdivided into its eastern and western parts by the Western Interior Seaway. Populations having settled in the western part of North America in this period would have been isolated from the eastern part of the continent since the early Tertiary. Second, there was a subsequent interchange between the New World and the Old World implied by the placement of the predominantly Old World taxa as arising from the paraphyletic New World group. Finally, there was a likely dispersal of *Dufourea* back to the New World. The latter two interchanges are likely to have occurred via the Old World—New World land bridges (available during most of the Cenozoic despite temporary breaks).

The role of land bridges in biogeographical exchanges between North America and Eurasia have been well documented in plants (Qian, 1999; Coleman et al., 2003). This is also plausible hypothesis for varied groups of bees including several genera and subgenera of Andrenidae and Halictidae show Holarctic distributions suggestive of North American/Eurasian interchange. Based on that hypothesis, the repeated periods of global warming during the Tertiary and Quaternary could have provided opportunities for biogeographical exchanges for other bee taxa (Qian, 1999; Coleman et al., 2003; Kaufman et al., 2004).

Given our analysis, it is impossible to say whether the interchange between northern and southern hemispheres represents dispersal or vicariance, one can infer from previous studies that the common ancestor of Halictidae was most likely in the southern hemisphere (Danforth et al., 2006). Recent studies of family-level bee phylogeny (Danforth et al., 2006) have strongly supported a sister group relationship between Halictidae and Colletidae + Stenotridae. Colletidae are primarily, and Stenotritidae exclusively southern hemisphere groups (Colletidae on Australia and South America and Stenotritidae endemic to Australia), suggesting that the most parsimonious hypothesis is for the common ancestor of Halictidae to be a southern hemisphere group. Given this information it seems likely that the northern/southern hemisphere interchange represents a dispersal event from the south to the north. Danforth et al. (2004) considered this a likely scenario given a much smaller sample of rophitine genera.

The phylogenetic relationships among the species of *Systropha* exemplify a frequent biogeographical pattern in bees. As in several other groups of Apoidea (e.g., Andrenidae and Melittidae), the basal group in *Systropha* is endemic to south–western Africa and forms the sister to a group that is widespread in the northern hemisphere (Patiny, 2001; Patiny and Michez, 2006, 2007).

Floral choices

Our analysis of floral association data in Rophitinae (Appendix 3) indicates that the majority of species (and even genera) show restricted host-plant associations. For most genera of Rophitinae there is a narrow group of preferred host plants. For example, *Ceblurgus* is restricted to *Cordia* (Boraginaceae), *Xeralictus* is restricted to two closely related genera of Loasaceae (*Eucnide* and *Mentzelia*), and *Systropha* is a narrow host-plant specialist on Convolvulaceae (*Convolvulus*, *Ipomoea*, *Merremia*). Some species may even be monolectic [e.g., *Conanthalictus conanthi* on *Nama hispidum* (Hydrophylaceae)]. *Dufourea* shows the widest diversity of host-plant preferences, but this is largely due to the fact that this genus is the largest of the rophitine genera. In spite of diversity in host-plant choice at the genus level, the subgenera of *Dufourea* are virtually all host-plant specialists with preferences for Asteraceae, Campanulaceae and Lamiaceae (Appendix 3). Understanding the evolution of host-plant associations in *Dufourea* may require an analysis at the subgeneric and species levels, which was not our goal here.

When the phylogeny of the preferred host plants is considered (Fig. 5), it is evident that the plant groups specialized upon by Rophitinae fall into a few, closely related orders (Cornales, Ericales, Gentianales, Lamiales, Solanales and Asterales) all within the Euasterid I and II clades (Stevens, 2001). Most of these latter plant groups are diverse and widespread.

While there is a tendency for the Rophitinae to specialize on a relatively closely related group of angiosperm families, our analysis of host-plant evolution using Component (Page, 1993) revealed that frequent host switching has occurred. Our results are thus largely congruent with those of Sipes and Tepedino (2005) in that we found little evidence that host-switching in bees is constrained by host-plant phylogeny. This may be a common pattern in bees because host-plant switching may be driven more by floral abundance and availability than host-plant phylogenetic affinities (Sipes and Tepedino, 2005).

Host-plant specialization in Rophitinae is reflected to some extent in morphological specialization on the part of the bees. In *Rophites*, for example, there are specialized morphological structures (spines located on the face) for extracting pollen from the narrow nototribic flowers of Lamiaceae. Likewise, in *Systropha* there is a specialized metasomal scopa apparently modified for collecting the large and sticky pollen grains from *Convolvulus* (Thorp, 1979). *Conanthalictus* females visiting narrow, tubular flowers of *Nama* have extremely long heads and mouthparts for reaching the nectar and pollen within. The existence of such morphological adaptations to host-plant morphology implies that host-plant specialization in rophitines may be relatively old.

Acknowledgments

This project was supported by an OECD co-operative fellowship, as well as ARB and FNRS travel grants. This project was also partially supported by a National Science Foundation Research Grant in Systematic Biology to B.N.D. (DEB-0412176). S. Patiny is a FNRS postdoctoral researcher. The authors are grateful to J. Ascher, F. Gusenleitner, L. Packer, Y. Pesenko, J.G. Rozen, F.A. Silveira for the facilitated access to collections.

References

Alexander, B.A., Michener, C.D., 1995. Phylogenetic studies of the families of short-tongued bees (Hymenoptera: Apoidea). Univ. Kansas Sci. Bull. 55, 377–424.

Ascher, J.S., Danforth, B.N., Ji. S., 2001. Phylogenetic utility of the major opsin in bees (Hymenoptera: Apoidea): a reassessment. Mol. Phylogenet. Evol. 19, 76–93.

Baker, D.B., 1996. Notes on some palaearctic and oriental *Systropha*, with descriptions of new species and a key to the species (Hymenoptera: Apoidea: Halictidae). J. Nat. Hist. 30, 1527–1547.

Bohart, G.E., 1947. New North American bees of the genus *Dufourea* (Apoidea: Halictidae). Ann. Entomol. Soc. Am. 40, 692–704.

Bohart, G.E., 1948. New North American Bees of the Genus *Dufourea* (Hymenoptera: Halictidae), Part II. Ann. Entomol. Soc. Am. 41, 119–136.

Bohart, G.E., 1949. New North American Bees of the Genus *Dufourea* (Apoidea: Halictidae), Part III. Ann. Entomol. Soc. Am. 42, 55–62.

Bohart, G.E., 1965. A new genus of Dufoureine bee from Texas (Hymenoptera: Halictidae). Ann. Entomol. Soc. Am. 58, 319–321.

Bohart, G.E., Griswold, T.L., 1987. A revision of the Dufoureine genus *Micralictoides* Timberlake. Pan-Pac. Entomol. 63, 178–193.

Bohart, G.E., Griswold, T.L., 1997. A revision of the rophitine genus *Protodufourea*. J. Kansas Entomol. Soc. 69, 177–184.

Cameron, S.A., Mardulyn, P., 2001. Multiple molecular data sets suggest independent origins of highly eusocial behavior in bees (Hymenoptera: Apinae). Syst. Biol. 50, 194–214.

Cameron, S.A., Mardulyn, P., 2003. The major opsin gene is useful for inferring higher level phylogenetic relationships of the corbiculate bees. Mol. Phylogenet. Evol. 28, 610–613.

Cane, J.H., Sipes, S.D., 2006. Characterizing floral specialization by bees: analytical methods and a revised lexicon for oligolecty. In: Waser N.M., Ollerton J. (Eds.), Specialization and Generalization in Plant–Pollinator Interactions. University of Chicago Press, Chicago, IL.

Cane, J., Snelling, R.R., Kervin, L.J., 1996. A new monolectic coastal bee, *Hesperapis oraria* Snelling and Stage (Hymenoptera: Melittidae), with a review of desert and neotropical disjunctives in the Southeastern U.S. J. Kansas Entomol. Soc. 69, 238–247.

Coleman, M., Liston, A., Kadereit, J.W., Abbotts, R.J., 2003. Repeat intercontinental dispersal and Pleistocene speciation in disjunct Mediterranean and desert *Senecio* (Asteraceae). Am. J. Bot. 90, 1446–1454.

Danforth, B.N., 1999a. Phylogeny of the bee genus *Lasioglossum* (Hymenoptera: Halictidae) based on mitochondrial COI sequence data. Syst. Entomol. 24, 377–393.

Danforth, B.N., 1999b. Emergence dynamics and bet hedging in a desert bee, *Perdita portalis*. Proc. R. Soc. B–Biol. Sc. 266, 1985–1994.

Danforth, B.N., 2002. Evolution of sociality in a primitively eusocial lineage of bees. Proc. Natl Acad. Sci. USA 99, 286–290.

Danforth, B.N., Ji, S., 2001. Australian *Lasioglossum* + *Homalictus* form a monophyletic group: resolving the "Australian Enigma". Syst. Biol. 50, 268–283.

Danforth, B.N., Sauquet, H., Packer, L., 1999. Phylogeny of the bee genus *Halictus* (Hymenoptera: Halictidae) based on parsimony and likelihood analyses of nuclear EF-1a sequence data. Mol. Phylogenet. Evol. 13, 605–618.

Danforth, B.N., Conway, L., Ji, S., 2003. Phylogeny of eusocial *Lasioglossum* reveals multiple losses of eusociality within a primitively eusocial clade of bees (Hymenoptera: Halictidae). Syst. Biol. 52, 23–36.

Danforth, B.N., Brady, S.G., Sipes, S.D., Pearson, A., 2004. Single-copy nuclear genes recover Cretaceous-age divergences in bees. Syst. Biol. 53, 309–326.

Danforth, B.N., Sipes, S., Fang, J., Brady, S.G., 2006. The history of early bee diversification based on five genes plus morphology. Proc. Natl Acad. Sci. USA 103, 15118–15123.

Ebmer, A.W., 1978. *Halictus, Lasioglossum, Rophites* und *Systropha* aus den Iran. (Halictidae, Apoidea) sowie neue Arten aus der Paläaktis. Linz. Biol. Beit. 10, 1–109.

Ebmer, A.W., 1984. Die westpaläarktischen Arten der Gattung *Dufourea* Lepeletier 1841 mit illustrierten Bestimmungstabellen (Insecta: Hymenoptera: Apoidea: Halictidae: Dufoureinae). Senckenbergiana Biol. 64, 313–379.

Ebmer, A.W., 1993. Die westpaläarktischen Arten der Gattung *Dufourea* Lepeletier 1841 mit illustrierten Bestimmungstabellen (Insecta: Hymenoptera: Apoidea: Halictidae: Rophitinae). Dritter Nachtrag. Linz. Biol. Beit. 25, 15–42.

Ebmer, A.W., 1994. *Systropha difformis* Smith, 1879 und *Systropha inexspectata* n.sp., die beiden östlichen Vertreter der altweltlichen Gattung *Systropha* Illiger 1806 (Insecta: Hymenoptera: Apoidea: Halictidae: Rophitinae). Linz. Biol. Beit. 26, 807–821.

Engel, M.S., 2001. A monograph of the Baltic amber bees and evolution of the Apoidea (Hymenoptera). Bull. Am. Mus. Nat. Hist. 259, 1–192.

Ebmer, W.A., Schwammberger, K.H., 1986. Die Bienengattung *Rophites* Spinola 1808 (Insecta: Hymenoptera: Apoidea: Halictidae: Rophitinae). Illustrierte Bestimmungstabellen. Senckenbergiana Biol. 66, 271–304.

Friese, H., 1901. Die Bienen Europa's (Apidae europaeae) nach ihren Gattungen, Arten und Varietäten auf vergleichend morphologisch-biologischer Grundlage. Teil VI Solitäre Apiden: subfam. Panurginae, Melittinae, Xylocopinae. Selbstverlag - C.Lampe.

Herbert, F., 1982. Die Hymenopteren des NordostAlpengebietes und seines vorlandes. I Teil. Österreichische Akademie der Wissenschaften. Mathematisch-Naturwissenschaftliche Klasse.

Huelsenbeck, J.P., Ronquist, F., 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Hurd, P.D. Jr, 1957. Notes on the autumnal emergence of the vernal desert bee, *Hesperapis fulvipes* Crawford (Hymenoptera, Apoidea). J. Kansas Entomol. Soc. 30, 10.

Kaufman, D.S., Ager, T.A., Anderson, N.J., Anderson, P.M., Andrews, J.T., Bartlein, P.J., Brubaker, L.B., Coats, L.L., Cwynar, L.C., Duvall, M.L., Dyke, A.S., Edwards, M.E., Eisner, W.R., Gajewski, K., Geirsdottir, A., Hu, F.S., Jennings, A.E., Kaplan, M.R., Kerwin, M.W., Lozhkin, A.V., MacDonald, G.M., Miller, G.H., Mock, C.J., Oswald, W.W., Otto-Bliesner, B.L., Porinchu, D.F., Ruhland, K., Smol, J.P., Steig, E.J., Wolfe, B.B., 2004. Holocene thermal maximum in the western Arctic (0–180W). Quat. Sci. Rev. 23, 529–560.

Leys, R., Cooper, S.J.B., Schwarz, M.P., 2000. Molecular phylogeny of the large carpenter bees, genus *Xylocopa* (Hymenoptera: Apidae), based on mitochondrial DNA sequences. Mol. Phylogenet. Evol. 17, 407–418.

Leys, R., Cooper, S.J.B., Schwarz, M.P., 2002. Molecular phylogeny and historical Formulaire de candidature—biogeography of the large carpenter bees, genus Xylocopa (Hymenoptera: Apidae). Biol. J. Linn. Soc. 77, 249–266.

Maddison, D., Maddison, W., 2000. MacClade 4.0 Analysis of Phylogeny and Characters Evolution. Sinauer Associates, Sunderland, MA.

Mardulyn, P., Cameron, S.A., 1999. The major Opsin in bees (Insecta: Hymenoptera): a promising nuclear gene for higher level phylogenetics. Mol. Phylogenet. Evol. 12, 168–176.

Michener, C.D., 2000. The Bees of the World. Johns Hopkins University Press, Baltimore, MD.

Michez, D., Patiny, S., 2005. World catalogue, biogeography and floral choices of the oil-collecting bee genus *Macropis* Panzer 1809 (Hymenoptera, Apoidea, Melittidae). Ann. Soc. Entomol. Fr. (N.S.) 45, 15–28.

Minckley, R.L., Cane, J.H., Kervin, L., Roulston, T.H., 1999. Spatial predictability and resource specialization of bees (Hymenoptera: Apoidea) at a superabundant, widespread resource. Biol. J. Linn. Soc. 67, 119–147.

Müller, A., 1996a. Convergent evolution of morphological specializations in central European bee and honey wasp species as an adaptation to the uptake of pollen from nototribic flowers. Biol. J. Linn. Soc. 57, 235–252.

Müller, A., 1996b. Host-plant specialization in western Palearctic anthidiine bees (Hymenoptera: Apoidea: Megachilidae). Ecol. Monogr. 66, 235–257.

Page, R.D.M., 1993. Component, Tree comparison software for Microsoft Windows, Version 2.0. The Natural History Museum, London.

Page, R.D.M., 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. Comput. Appl. Biosci. 12, 357–358.

Patiny, S., 2001. Monographie des Panurginae de l'ancien monde (Hymenoptera: Apoidea, Andrenidae). PhD. Faculté universitaire des Sciences agronomiques de Gembloux.

Patiny, S., 2003. Revision of the subgenus *Dufourea* (*Flavodufourea*) Ebmer, 1984 (Hymenoptera, Halictidae, Rophitinae) and description of a new species *D.*(*Flavodufourea*) *ulkenkalkana* sp.nov. from Kazakhstan. Zootaxa, 255, 1–8.

Patiny, S., Michez, D., 2006. Phylogenetic analysis of the *Systropha* Illiger, 1806 (Hymenoptera: Halictidae) and description of a new subgenus. Ann. Soc. Entomol. Fr. 42, 27–44.

Patiny, S., Michez, D., 2007. Biogeography of bees (Hymenoptera, Apoidea) in Sahara and the Arabian deserts. Insect Syst. Evol. 38, 19–34.

Pekkarinen, A., 1997. Oligolectic bee species in Northern Europe (Hymenoptera, Apoidea). Entomol. Fennica 8, 205–214.

Pesenko, Y.A., 1999. Phylogeny and classification of the family Halictidae revised (Hymenoptera: Apoidea). J. Kansas Entomol. Soc. 72, 104–123.

Qian, H., 1999. Spatial pattern of vascular plant diversity in North America north of Mexico and its floristic relationship with Eurasia. Ann. Bot. 83, 271–283.

Robertson, C., 1925. Heterotropic bees. Ecology 6, 412–436.

Ronquist, F., 1996. DIVA, version 1.1. computer program available by anonymous ftp. Uppsala University, Uppsala.

Rozen, J.G. Jr, 1997. South American rophitine bees (Hymenoptera: Halictidae: Rophitinae). Am. Mus. Novit. 3206, 1–27.

Saghai-Maroof, M.A., Soliman, K.M., Jorgensen, R.A., Allard, R.W., 1984. Ribosomal DNA spacer-length polymorphism in barley. Proc. Natl Acad. Sci. USA, 81, 8014–8018.

Schlindwein, C., Medeiros, P.C.R., 2006. Pollination in *Turnera subulata* (Turneraceae): unilateral reproductive dependence of a narrowly oligolectic bee *Protomeliturga turnerae* (Hymenoptera, Andrenidae). Flora 201, 178–188.

Schoonhoven, L.M., Jermy, T., van Loon, J.J.A., 1998. Insect-plant biology: from physiology to evolution. Chapman & Hall, London.

Sipes, S.D., Tepedino, V., 2005. Pollen-host specificity and evolutionary patterns of host switching in a clade of specialist bees (Apoidea: *Diadasia*). Biol. J. Linn. Soc. 86, 487–505.

Sipes, S.D., Wolf, P.G., 2001. Phylogenetic relationships within *Diadasia*, a group of specialist bees. Mol. Phylogenet. Evol. 19, 144–156.

Snelling, R.R., Stage, G.I., 1995. Systematics and biology of the bee genus *Xeralictus*. Contrib. Sci. Nat. Hist. Mus. Los Angeles County 451, 1–17.

Stevens, P.F., 2001. Angiosperm Phylogeny Website, Version 7. May 2006. http://www.mobot.org/MOBOT/research/APweb/.

Stöckhert, F.K., 1933. Die Bienen Frankens (Hym. Apid.). Eine Ökologische-tiergeographische Unterschung. Deutschen Entomologischen Zeitschrift. Berlin.

Swofford, D.L., 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4. Sinauer Associates, Sunderland, MA.

Thorp, R.W., 1979. Structural, behavioral, and physiological adaptations of bees (Apoidea) for collecting pollen. Ann. Mo. Bot. Gard. 66, 788–812.

Timberlake, P.H., 1939. New species of bees of the genus *Dufourea* from California (Hymenoptera, Apoidea). Ann. Entomol. Soc. Am. 32, 395–414.

Timberlake, P.H., 1941. Three new Dufoureine bees from California (Hymenoptera, Apoidea). Ann. Entomol. Soc. Am. 34, 38–42.

Timberlake, P.H., 1961. A review of the genus *Conanthalictus*. Pan Pac. Entomol. 37, 145–160.

Urban, D., Moure, J.S., 1993. *Ceblurgus longipalpis* gen. e sp. N. Primeiro representante de Dufoureinae do Brasil (Hymenoptera, Halictidae). An. Acad. Bras. Ciênc. 65, 101–106.

Westrich, P., 1989a. Die Wildbienen Baden-Württembergs. Spezieller Teil. Ulmer, Stuttgart.

Westrich, P., 1989b. Die Wildbienen Baden-Württembergs. Allgemeiner Teil. Ulmer, Stuttgart.

Appendix 1: Description of the new tribes Conanthalictini and Xeralictini

Conanthalictini trib. nov.

This is proposed as a new tribe name for *Conanthalictus* Cockerell, 1901. In its current definition, the latter genus includes the subgenera *Conanthalictus* Cockerell & *Phaceliapis* Michener, 1942 containing a total of 13 described species ranging in south-western North-America. *Conanthalictus* was reviewed by Timberlake (1961).

Diagnosis. Small species (3–7 mm). Three submarginal cells in forewings. Subantennal area generally well identifiable. Labial palpus not flattened. Propodeum dorsal area longer than the posterior area. Head of females appearing conspicuously enlarged ventrally due to the truncation of clypeus. S7 of males with short bilobate apical processes. S8 with pointed apical process.

Floral choices. The floral choices of the species are conspicuously broad. A typical orientation toward Hydrophyllaceae and the genera *Nama* and *Phacelia* appears common.

Xeralictini trib. nov.

This is proposed as a new tribe name including the genera *Protodufourea* Timberlake, 1955 and *Xeralictus* Cockerell, 1927. The tribe includes seven species occurring in south-western North-America. *Protodufourea* was reviewed by Bohart and Griswold (1997) and *Xeralictus* was reviewed by Snelling and Stage (1995).

Diagnosis. Medium sized species (5–12 mm). Three submarginal cells in forewings. Subantennal area generally well identifiable. In *Xeralictus*, mandibles very large and strong. Labial palpus not flattened. Dorsal surface of the propodeum equal to one half or less of the propodeum length. S6 long with short apodemes; apex shallowed or slightly emarginated (short lateral lobes in *Xeralictus*). S7 with strong basal lobes; apical lobes short (paired in *Protodufourea*). S8 with straight apical process (slightly emarginated in some species of *Protodufourea*). Gonostyli long.

Floral choices. The floral choices of the species included in the two genera are diverse. However, *Protodufourea* is thought to be oligolectic on Hydrophyllaceae (*Emmenanthe* and *Phacelia*) and *Xeralictus* is oligolectic on Loasaceae (*Mentzelia*), respectively.

Appendix 2: Morphological data set

A. brooksi	`010000000000000000000000`
C. wilmattae	`000000100000010001021000`
G. peruensis	`010001121000001010032000`
P. moldenkei	`110001121001021010032000`
P. toroi	`110001121001021010032000`
P. parca	`100000101100000001020001`
R. algirus	`101000100111000101110101`
S. pratti	`000010100100000102021001`
S. curvicornis	`100100110101100002010111`
S. planidens	`100100110101100002010111`
X. bicuspidariae	`000000101100001001021000`
X. timberlakei	`000000101100001001021000`
C. conanthi	`000000100000010001021000`
S. glabriventris	`100200110101100002010111`
S. beameri	`000010100110000102021001`
D. malacothricis	`11001010001000.02010101`
D. mulleri	`11001010001000.02010101`
D. novaeangliae	`110010100010000202.10101`
D. spirula	`110010100010000002010101`
D. spinifera	`110010100010000002010101`
D. holocyanea	`111010100010000202010101`
C. longipalpis	`0.00.112..01.00010032000`
M. nana	`101002100010.00002010.01`
M. fuscescens	`001010100101000102110101`
M. quadriceps	`000000100010000102110000`

01 Eye inner margins. 0, subparallel-diverging slightly below; 1, converging ventrally.

02 Paraocular area.
0, undifferentiated; 1, cuticle displaying a distinct sculpture.

03 Male flagellum terminal segment (A13).
0, short, bud-like; 1, longer and slender.

04 Male flagellum apex.
0, straight; 1, several (usually 4) segments in a spiral; 2, less than 13 segments, flagellum ending in a short hook.

05 Flagellar segment.
0, without ventro-basal depression; 1, with a semicircular ventro-basal depression.

06 Subantennal suture.
0, reaching the socket ventral margin; 1, reaching the socket outer side; 2, reaching the socket inner side.

07 Position of antennal sockets.
0, level with the mid-point of eyes; 1, below to the mid-point of eyes.

08 Position of tentorial pits.
0, on the epistomal suture; 1, on the subantennal suture; 2, at basis of antennal socket.

09 Proportion clypeus/frons.
0, < 1; 1, > 1

10 Propodeum declivity.
0, propodeum with an subhorizontal area behind postscutellum; 1, propodeum directly declivous after postscutellum.

11 Number of submarginal cells.
0, three; 1, two.

12 Male pygidial plate.
0, absent; 1, present.

13 Female sterna.
0, without conspicuous pilosity; 1, with hairs on undersurface of metasoma forming a scopa for pollen collection.

14 S6 apex.
0, no lateral emargination; 1, lateral emargination shallow; 2, deep lateral emargination.

15 S5 apical margin in males.
0, no medio-apical process; 1, with a medio-apical process.

16 S6 apical margin in males.
0, without process; 1, S6 with a short process; 2, with a large, well differentiated process.

17 S7 termination in males.
0, no single apical processes; 1, with one single apical process.

18 S7 apical processes in males.
0, no paired processes; 1, short paired processes; 2, paired processes as long as apodemes

19 S7 secondary inner processes in males.
0, without secondary inner expansions; 1, with individualized secondary inner expansions.

20 S8 in males.
0, with one single apodeme; 1, apodeme truncated; 2, two well individualized apodemes; 3, single apodeme with apex enlarged

21 S8 lateral apodemes in males.
0, well-developed; 1, obviously reduced; 2, nearly absent.

22 S8 basal apodeme in males.
0, unilobate; 1 bilobate.

23 S8 apex.
0, straight not enlarged; 1, forming a large apical plate.

24 Gonostyli and gonocoxites.
0, fused; 1, not fused.

Appendix 3: Floral choices reports for Rophitinae

Rophitinae genera	Host plants Family and Orders Asparagales [As], Asterales [A], Brassicales [B], Caryophyllales [Ca], Cornales [C], Ericales [E], Fabales [F], Gentianales [G], Lamiales [L], Liliales [Li], Linaceae [LN], Malvales [Ma], Myrtales [M], Ranunculales [Ra], Rosales [R], Solanales [S]	Main references
Ceblurgus Urban and Moure	**[G] Boraginaceae: *Cordia***	Urban & Moure (1993)
Conanthalictus Cockerell	[A] Asteraceae: *Malacothrix*; [C] Loasaceae: *Mentzelia* **[G] Boraginaceae: *Cryptantha*; [G] Hydrophyllaceae: *Emmenanthe, Phacelia, Nama*** [M] Onagraceae: *Oenothera* [R] Rhamnaceae: *Rhamnus*	Timberlake (1961)
Dufourea Lepeletier, 1841	**[A] Asteraceae: *Anisocoma, Coreopsis, Hieracium, Lactuca, Leontodon, Malacothrix, Solidago*; [A] Campanulaceae: *Campanula, Jasione, Phyteuma*;** [Ca] Caryophyllaceae: *Silene*; [Ca] Cactaceae: *Echinocactus*; [E] Ericaceae: *Calluna*; [E] Polemoniaceae: *Linanthus*; [E] Polemoniaceae: *Gilia* [G] Boraginaceae: *Cryptantha*; [G] Hydrophyllaceae: *Phacelia* **[L] Lamiaceae: *Acinos, Mentha, Stachys, Thymus*; [L] Scrophulariaceae: *Euphrasia, Mimulus, Veronica*** [Li] Liliaceae: *Calochortus* [M] Onagraceae: *Oenothera* [Ma] Malvaceae: *Sphaeralcea* [R] Rosaceae: *Dryas* [Ra] Papaveraceae: *Eschscholtzia*	Bohart (1947, 1948, 1949), Ebmer (1984), Timberlake (1939, 1941)
Goeletapis Rozen	**[S] Solanaceae: *Exodeconus***	Rozen (1997)
Micralictoides Timberlake	**[A] Asteraceae: *Acamptopappus, Baeria, Chaenactis, Eriophyllum, Malacothrix*;** [As] Alliaceae: *Allium* [E] Polemoniaceae: *Gilia* [F] Fabaceae: *Astragulus* **[G] Boraginaceae: *Amsinckia, Cryptantha*; [G] Hydrophyllaceae: *Phacelia*** [Ra] Papaveraceae: *Eschscholtzia*	Bohart & Griswold (1987)
Penapis Michener (1965)	**[L] Bignoniaceae: *Argylia***; [?] Portulaceae: *Calandrinia* [S] Solanaceae: *Nolana* (L.Packer, comm.pers.)	Rozen (1997)
Protodufourea Timberlake	[B] Brassicaceae: *Lesquerella*; **[G] Hydrophyllaceae: *Emmenanthe, Phacelia*** [Ma] Malvaceae: *Sphaeralcea*	Bohart and Griswold (1997)
Rophites Spinola, 1808	[A] Campanulaceae: *Campanula*; [F] Fabaceae: *Melilotus* **[L] Lamiaceae: *Ballota, Betonica, Clinopodium, Lamium, Nepeta, Origanum, Stachys***	Ebmer & Schwammberger (1986)
Sphecodosoma Crawford	[B] Brassicaceae: *Lesquerella*; **[G] Hydrophyllaceae: *Nama*; [G] Boraginaceae: *Coldenia***	Timberlake (1961)Bohart (1965),
Systropha Illiger	[A] Asteraceae: *Cichorium* [F] Fabaceae: *Melilotus*; [Ln] Linaceae: *Linum*; **[S] Convolvulaceae: *Calystegia, Convolvulus***	Friese (1901), Stoeckhert (1933), Herbert (1982), Baker (1996), Friese (1901), Ebmer (1978), Herbert (1982), Stoeckhert (1933) Patiny and Michez (2006)
Xeralictus Cockerell	[A] Asteraceae: *Encelia, Geraea, Malacothricx* **[C] Loasaceae: *Eucnide, Mentzelia*** [Ca] Cactaceae: *Echinocactus*, *Opuntia basilaris* [L] Scrophulariaceae: *Mohavea* [S] Solanaceae: *Datura* [May not all be pollen-hosts (L.Packer, pers.comm.)]	Snelling and Stage (1995)

Bold type families represent preferred host plants based on collecting records.