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Phylogeny of halictine bees supports a shared origin of eusociality for *Halictus* and *Lasioglossum* (Apoidea: Anthophila: Halictidae)

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ABSTRACT

The halictid bees are excellent models for the study of social evolution because greater social diversity and plasticity are observed in the tribe Halictini than in any other comparable taxonomic group. We examine the evolutionary relationships within the subfamily Halictinae (“sweat bees”) to investigate the origins of social behaviour within the tribe Halictini. We present a new phylogeny of the subfamily Halictinae based on three nuclear genes (elongation factor-1 alpha, wingless, and long-wavelength rhodopsin) and one mitochondrial gene (cytochrome *c* oxidase 1) sequenced for 206 halictine bees. We use model-based character reconstruction to infer the probability of a shared eusocial ancestor for the genera *Halictus* and *Lasioglossum*, the two genera of Halictini which display eusociality. Our results suggest a high probability for a single origin of eusociality for these two genera, contradicting earlier views of separate origins within each taxon. Fossil-calibrated divergence estimates place this ancestor at approximately 35 million years ago, about 14 million years earlier than previous estimates of eusocial origins in the halictid bees.

1. Introduction

Eusociality – including cooperative brood care, reproductive division of labour, and overlap of generations – is a remarkable evolutionary innovation that has allowed some clades of insects (ants, termites, paper wasps, and corbiculate bees) to become ecologically and numerically dominant in terrestrial environments (Wilson and Hölldobler, 2005). However, for each of these major eusocial clades, the origins of eusociality are ancient: ants, >115 mya (Brady et al., 2006a; Moreau et al., 2006); termites, >180 mya (Ware et al., 2010); paper wasps, >65 mya (Wenzel, 1990), corbiculate bees, >68 mya (Cardinal and Danforth, 2011). Furthermore, extant closely related solitary taxa are not necessarily available for comparison (e.g., in ants; Brady et al., 2006a). This places a major constraint on studies of the evolutionary origin of eusocial behaviour in these groups.

The bee-subfamily Halictinae (sweat bees), on the other hand, provides an exceptional model for the study of social evolution (Schwarz et al., 2007). First, eusociality is thought to have arisen as many as three times independently within Halictinae (Danforth, 2002) and more origins are possible (see Michener, 1968, 1990; Packer, 1997, 1998). Second, unlike the more ancient lineages of advanced eusocial insects, such as corbiculate bees, there are repeated reversals to solitary nesting within each of the eusocial halictine clades (Danforth, 2002; Danforth et al., 1999, 2003). Third, eusociality is thought to have arisen relatively recently in halictine bees, based on fossil-calibrated phylogenetic studies (Brady et al., 2006b). Finally, intra-specific variation in social behaviour has been documented for several halictine bees (Eickwort et al., 1996; Field et al., 2010; Packer, 1990; Plateaux-Quénu, 1989; Richards et al., 2003; Soucy and Danforth, 2002). Social behaviour in these species appears to be facultatively expressed and largely determined by the length of the season of adult activity associated with either altitude or latitude (Field et al., 2010; reviewed in Purcell, 2011). This combination of social diversity and plasticity makes halictine bees ideal candidates for studying the genetic and environmental factors involved in the development and maintenance of eusociality.

1.1. Phylogenetic background

Molecular phylogenetic studies of bee family-level relationships place the Halictidae as sister to Colletidae + Stenotritidae (Brady et al., 2011; Danforth et al., 2006b). Monophyly of Halictidae and relationships among the four halictid subfamilies (Rophitinae, Nomiinae, Nomioidinae, and Halictinae; Fig. 1) are strongly supported by molecular data (Danforth et al., 2004, 2006a,b, 2008) and morphological synapomorphies (Alexander and Michener,

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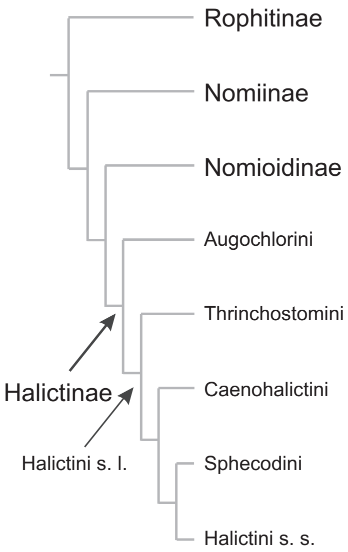

Fig. 1. Summary of higher-level halictid bee phylogenetic relationships (modified from Danforth et al., 2008).

1995; Pesenko, 1999; Rozen, 2008). The Halictinae has often been subdivided into two tribes: Augochlorini and Halictini *sensu lato* (Michener, 2007), which are reciprocally monophyletic (Brady et al., 2006b; Danforth et al., 2004, 2008; Pesenko, 1999). Halictini *s.l.* has since been subdivided into as many as four tribes (Caenohalictini, Halictini *sensu stricto*, Sphecodini, and Thrinchostomini) (see Danforth et al., 2004, 2008; Engel, 2005).

1.2. Social diversity in the Halictidae

Among halictids, eusocial behaviour has only been recorded from the Halictinae. The remaining subfamilies (Rophitinae, Nomiinae, and Nomioidinae) display only solitary and communal nesting (Batra, 1966; Eickwort et al., 1986; Michener, 1968; Patiny et al., 2008; Rozen, 1993; Rust et al., 2004; Wcislo, 1993, 1997). The Halictinae includes species with a diverse array of behavioural repertoires, including solitary nesting and subsocial, communal, semisocial, annual eusocial, delayed eusocial, and perennial eusocial colonies (reviewed in Michener, 1974; Packer, 1993; Schwarz et al., 2007; Yanega, 1997; Table 1). Cleptoparasitism and social parasitism also occur in the Halictinae (Michener, 1978; Wcislo, 1997). The halictine tribes Caenohalictini and Thrinchostomini are all believed to be solitary or communal (Eickwort, 1981; Michener et al., 1979; Packer, 2006; Roberts and Brooks, 1987), with the exception of two cleptoparasitic species in the Thrinchostomini (genus *Parathrinchostoma*; Michener, 2007). The tribe Sphecodini is entirely composed of cleptoparasitic and socially parasitic species. Augochlorini is more diverse with solitary, semisocial, eusocial, and cleptoparasitic species included (reviewed in Danforth and Eickwort, 1997). The most behaviourally diverse tribe of sweat bees is certainly Halictini *s. s.*

Species richness and behavioural diversity of the Halictidae is most heavily concentrated in the tribe Halictini *s. s.* (Appendix 1), which includes six genera (*Glossodialictus*, *Halictus*, *Lasioglossum s.l.*, *Mexalictus*, *Patellapis*, and *Thrincohalictus*) and nearly 2200 described species (Ascher and Pickering, 2012). Solitary and communal nesting has been recorded from *Thrincohalictus* (Packer, 2000) and *Patellapis* (Timmermann and Kuhlmann, 2008), respectively. The nesting biologies of *Mexalictus*, *Glossodialictus* and most species of *Patellapis* remain unstudied. Eusociality is only known to occur in *Halictus* and *Lasioglossum* (Michener, 1974). *Halictus* is a widespread genus, with most species displaying at least facultative eusociality. Some *Halictus* are strictly solitary or cleptoparasitic (Pauly, 1997; Sitdikov, 1988) and many are known to be socially polymorphic (Eickwort et al., 1996; Richards, 1994; Richards et al., 2003). For example, the Holarctic species *Halictus rubicundus* (Christ) is known to be solitary at high altitudes and latitudes, but eusocial at low elevations and in its southern range (Eickwort et al., 1996; Field et al., 2010). Rather than remain as workers, some first brood females of *H. rubicundus* immediately enter diapause; founding nests the following year (Yanega, 1988). Social plasticity in *H. rubicundus* has been related to both environmental (Field et al., 2010) and possibly underlying genetic factors (Soucy and Danforth, 2002; but see Soro et al., 2010).

Lasioglossum is the largest (>1750 described spp.) and most widely-distributed bee genus. Michener (2007) divided the genus *Lasioglossum* into two informal groups: the "*Lasioglossum* series" and the "*Hemihalictus* series", and molecular studies (Danforth and Ji, 2001; Danforth et al., 2003) have indicated that these two groups are reciprocally monophyletic. The *Lasioglossum* series is composed of primarily solitary or communal species (Michener, 1960; Packer, 1998), but there is evidence for eusociality occurring in as many as three subgenera: *Leuchalictus*, *Rubrihalictus*, and *Ctenonomia* (Batra, 1966; Knerer and Schwarz, 1976; Michener, 1968; Packer, 1997). Some primarily solitary species in the subgenus *Leuchalictus* have been shown to display facultative social nesting with rudimentary division of labour indicative of eusociality (Miyanaga et al., 1998, 2000). Cleptoparasitism has likely arisen independently within the subgenera *Homalictus* (*i.e., Echthralictus*; see Michener, 1978) and *Chilalictus* (A. Pauly, *in litt.*). The *Hemihalictus* series are even more behaviourally diverse than the *Lasioglossum* series, with species forming annual, delayed, or perennial eusocial colonies, and multiple known occurrences of solitary and communal behaviour (Danforth, 1999; Danforth et al., 2003). Social parasitism has evolved twice in North American *L. (Dialictus)* (Gibbs et al., 2012) and there are also cleptoparasitic or socially parasitic species of *Dialictus* in the *Afrodialictus* group of central Africa (Gibbs, 2009; Pauly, 1984).

1.3. Previous studies of social evolution in the Halictini

In previous phylogenetic studies of *Halictus* (Danforth et al., 1999; Richards, 1994), *Lasioglossum s.l.* (Danforth et al., 2003; Packer, 1991, 1997), and the subfamily Halictinae as a whole (Danforth, 2002), patterns of social evolution (origins and reversals) were identified through mapping of social behaviour onto phylogenies using equal-weights parsimony. The predominant topology obtained for the tribe Halictini placed *Lasioglossum* as sister to *Halictus* + *Thrincohalictus* (Fig. 2A). Based on simple parsimony mapping

Table 1
Types of social organisation among halictine bees used in this study.

Degree of sociality	Multiple broods per year	Adult nest sharing	Reproductive division of labour	Co-operative brood care	Overlap of generations
Solitary	+	–	–	–	–
Communal	+	+	–	–	–
Semisocial	+	+	+	+	–
Eusocial	+	+	+	+	+

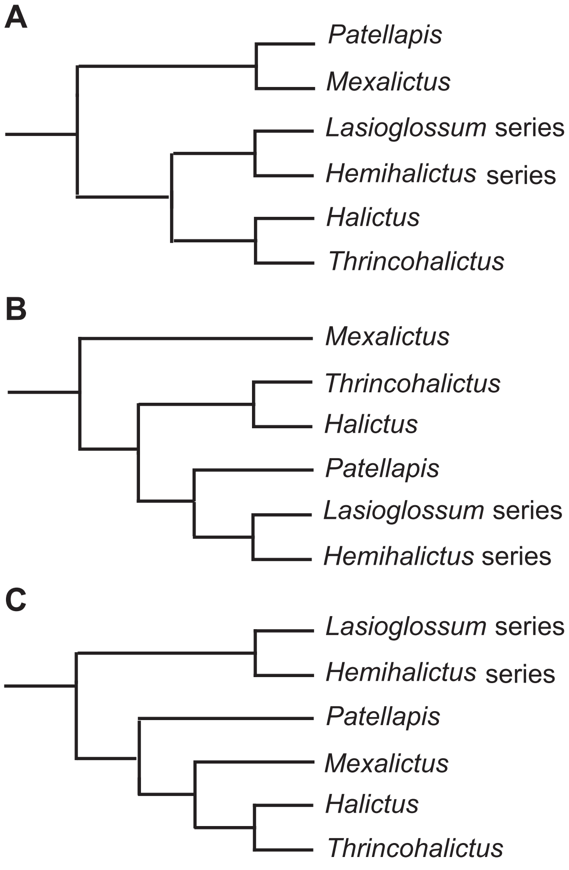

Fig. 2. Summary of genus level relationships in the bee tribe Halictini sensu stricto. (A) Topology supported in higher level phylogenies, Danforth et al. (2004); Brady et al. (2006a, 2006b). (B) Topology recovered based on our 4-gene data set with data partitioned by genome/codon/intron. (C) Topology recovered based on our 4-gene data set with data partitioned by gene/codon/intron.

of eusociality, this topology would imply dual origins of eusociality in (1) the predominantly eusocial clades of the *Hemihalictus* series and (2) the genus *Halictus* (Danforth, 2002). In addition, Brady et al. (2006b), using fossil-calibrated phylogenies and parsimony mapping, inferred dual origins of eusociality at approximately the mid to late Miocene (20–25 Ma). In both Danforth (2002) and Brady et al. (2006b), the hypothesis of a single origin of eusociality in the common ancestor of *Halictus* and *Lasioglossum* was not fully explored, in spite of the fact that single and dual origins differed by just one evolutionary step.

The hypothesis of single vs. dual origins of eusociality in Halictini could be examined further through (1) a more comprehensive analysis of generic, subgeneric, and species-level relationships within Halictinae, and (2) the use of model based methods of ancestral state reconstruction. Model-based methods calculate a probability for the ancestral state while allowing for uncertainty in tree topology, variation in branch lengths, and unequal rates of gains/losses to be incorporated into the reconstruction of ancestral states (Cunningham et al., 1998; Huelsenbeck and Bollback, 2001; Pagel et al., 2004; Ronquist, 2004), and therefore provide major advantages over reconstructions based on equal-weights parsimony.

In this study, we employed model-based approaches in order to examine more carefully the evolutionary history of eusociality in Halictinae. We generated an expanded phylogeny for the group and used Bayesian and maximum-likelihood methods of ancestral state reconstruction to re-examine the possibility of a single origin of eusociality within Halictini. Our results indicate the origins of eusociality are less frequent in the Halictinae than previously supposed (e.g. Brady et al., 2006b) and continues a trend that began with pre-phylogenetic estimates of many (e.g. Eickwort, 1986; Packer, 1993) or even dozens or hundreds of origins (Michener, 1990) to more recent estimates of three origins or, as we suggest below, only two.

2. Methods

2.1. Taxon sampling

We generated a large data set for the tribe Halictini, including all major genera, by merging and expanding previously published data sets for *Halictus* (Brady et al., 2006b; Danforth et al., 1999), *Lasioglossum* (Brady et al., 2006b; Danforth, 1999; Danforth and Ji, 2001; Danforth et al., 2003; Gibbs et al., 2012), *Mexalictus, Thrincohalictus*, and *Patellapis* (Danforth et al., 1999, 2008). No previous study has combined the taxa from all these studies into a single analysis. The taxon sampling was further increased by including 39 additional representatives of the massive genus *Lasioglossum s.l.* Our taxon sampling of *Lasioglossum* includes 6 nominal subgenera of *Lasioglossum* not included in previous studies, *Callalictus*, *Ipomalictus*, *Oxyhalictus*, *Pseudochilalictus*, *Rubrihalictus*, and *Sellalictus*. Michener (2007) considered *Ipomalictus, Oxyhalictus,* and *Rubrihalictus* to be synonymous with *Ctenonomia.* Representatives from each tribe of Halictinae were also included in the data set as outgroups with representatives of the tribe Augochlorini used to root the tree.

2.2. Genes analysed

We generated a data set comprising three nuclear genes (wingless: *wnt*-1, long-wavelength rhodopsin: LW *rh*, and elongation factor-1 alpha: *ef*-1α) and one mitochondrial gene (cytochrome *c* oxidase subunit 1: *co1*). Sequences from previous studies (see Brady et al., 2006b; Danforth, 1999; Danforth et al., 1999, 2003, 2004, 2008; Gibbs et al., 2012) were downloaded from GenBank (Table 2). Additional sequences were generated using standard protocols to make all four genes available for the taxa included in our data set, when possible.

2.3. DNA extraction, sequencing, and alignment

DNA extractions followed the protocol used by Danforth (1999). Primers and PCR conditions used for all the genes in this paper are summarised in Table S1. PCR products were sequenced in both directions. For most genes, more than one primer pair was used. These often overlapped extensively giving us fourfold coverage for some areas of the sequence. Sequencing was performed at the Cornell University Life Sciences Core Laboratories Center and the Laboratories of Analytical Biology (National Museum of Natural History) using Applied Biosystems Automated 3730 DNA analyzers. Big Dye Terminator was used for the sequencing reaction. The sequences were assembled and trimmed using Sequencher (Gene Codes). Alignments were initially made using MegAlign (DNA*, LaserGene) and then modified by eye to remove ambiguously aligned regions. Codon position and intron/exon boundaries were determined by reference to *Apis mellifera* L. using Mesquite v. 2.75 (Maddison and Maddison, 2010).

2.4. Phylogenetic methods

2.4.1. Partitions and models

We partitioned the data in multiple ways (Table S2) including by (i) codon position, (ii) by genome (mitochondrial and nucle-

Table 2
Halictine species included in the data set with locality information and GenBank accessions (na = not available).

Taxon	Locality data	LW *rh*	*wnt*-1	*ef*1-α	*co1*
Augochlora (Augochlora) pura	USA: New York, Tompkins Co.	AY455893	AY455897	AF140314	JQ266383
Augochlorella pomoniella	USA: California, Inyo Co.	AY227935	AY222572	AF435373	JQ266382
Augochloropsis metallica	USA: New York, Tompkins Co.	AY227934	AY222571	AF140315	JQ266379
Corynura patagonica	Chile: Region IX, near Temuco	AY227936	AY222573	na	JQ266406
Megalopta genalis	Republic of Panama: Panama Prov.	AY227937	AY222574	AF140316	JQ266453
Neocorynura discolor	Colombia	AY227938	AY222575	AF140317	JQ266454
Agapostemon leunculus	Republic of Panama: Panama Prov.	AY227939	AY222576	AF435371	JQ266373
Agapostemon sericeus	USA: New York, Ithaca	JQ342157	na	AF140319	JQ266374
Agapostemon tyleri	USA: Arizona, Cochise Co.	AY227940	AY222577	AF140320	JQ266375
Agapostemon virescens	USA: New York, Ithaca	na	na	AF140321	JQ266376
Caenohalictus sp. 2	Chile: Region I, Socoroma	AY227941	AY222578	AF435376	JQ266404
Caenohalictus sp. 3	Brazil: Minas Gerais	AY227942	AY222579	AF435377	JQ266405
Dinagapostemon sp. 1	Republic of Panama: Chiriqui Prov.	AY227943	AY222580	AF435380	JQ266413
Dinagapostemon sp. 2	Republic of Panama: Cocle Prov.	AY227944	AY222581	AF435381	JQ266414
Habralictus sp.	Brazil: Minas Gerais	AY227945	AY222582	AF435387	JQ266438
Pseudagapostemon braziliensis	Brazil: Minas Gerais	AY227946	AY222583	AF140323	JQ266468
Pseudagapostemon pissisi	Chile: Region VIII (nr. Temuco)	AY227947	AY222584	AF435403	JQ266470
Rhinetula denticrus	Republic of Panama: Panama Prov.	AY227948	AY222585	AF435405	JQ266472
Ruizanthedella mutabilis	Chile: Region VIII (nr. Temuco)	AY227949	AY222586	AF435406	JQ266473
Ruizantheda proxima	Chile: Region VIII (nr. Temuco)	AY227950	AY222587	AF435407	JQ266474
Eupetersia (Eupetersia) seyrigi	Madagascar: Parc National Ranomafana	EU203287	EU203228	EU203259	JQ266415
Sphecodes autumnalis	USA: NY: Cortland Co., Cortlandville	na	EU203225	EU203256	JQ266477
Sphecodes clematidis	USA: NY: Tompkins Co., Salmon Creek	na	EU203226	EU203257	JQ266478
Sphecodes confertus	USA: NY: Tompkins Co., Salmon Creek	na	EU203227	EU203258	JQ266479
Sphecodes ruficrus	No locality data	AY227960	AY222596	AF140324	AF102844
Sphecodes ranunculi	Canada: Nova Scotia	AY227961	AY222597	AF140325	JQ266480
Parathrinchostoma seyrigi	Madagascar: Parc National Ranomafana	EU203287	EU203224	EU203255	JQ266415
Thrinchostoma (Diagonozus) sp.	Borneo: Sarawak	AY227951	na	na	na
Thrinchostoma (Eothrinchostoma) torridum	South Africa: Kwazulu-Natal, Hluhluwe	EU203283	EU203221	EU203252	JQ266485
Thrinchostoma (Thrinchostoma) conjugens	Madagascar: Parc National Ranomafana	EU203284	EU203222	EU203253	JQ266481
Thrinchostoma (Thrinchostoma) kandti	South Africa: Kwazulu-Natal, Hluhluwe	EU203282	EU203220	EU203251	JQ266482
Thrinchostoma (Thrinchostoma) lemuriae	Madagascar: Parc National Ranomafana	EU203285	EU203223	EU203254	JQ266483
Halictus (Halictus) quadricinctus	France: Dordogne	AY227956	AY222592	AF140334	JQ266429
Halictus (Hexataenites) fulvipes	Spain: Trevelez	JQ266574	JQ266696	AF140298	JQ266420
Halictus (Hexataenites) scabiosae	France: Dordogne	JQ266587	JQ266709	AF140329	JQ266434
Halictus (Hexataenites) scabiosae	Spain: Granada Prov.	AY455896	AY455900	AF140330	JQ266436
Halictus (Hexataenites) sexcinctus	France: Mont Serein	JQ266590	JQ266712	AF140331	JQ266439
Halictus (Monilapis) crenicornis	Spain: Caratauna	JQ266572	JQ266694	AF140296	JQ266419
Halictus (Monilapis) simplex	France: Dordogne	JQ266588	JQ266710	AF140297	JQ266435
Halictus (Nealictus) farinosus	No locality data	JQ266573	JQ266695	AF140332	AF102838
Halictus (Nealictus) parallelus	USA: North Carolina, Chatham Co.	JQ266578	JQ266700	AF140333	JQ266425
Halictus (Odontalictus) ligatus	USA: New York, Tompkins Co.	AY455895	AY455899	AF140299	JQ266423
Halictus (Odontalictus) ligatus	USA: South Carolina, Rock Hill	JQ266577	na	AF140300	AF102840
Halictus (Odontalictus) poeyi	USA: North Carolina, Hoke county	JQ266580	JQ266702	AF140327	JQ266427
Halictus (Odontalictus) poeyi	USA: South Carolina, Rock Hill	JQ266581	JQ266703	AF140303	AF102841
Halictus (Protohalictus) rubicundus	USA: Montana, Missoula	JQ266583	JQ266705	AF140335	JQ266430
Halictus (Protohalictus) rubicundus	USA: North Carolina, Moore Co.	JQ266584	JQ266706	AF140336	JQ266431
Halictus (Tytthalictus) maculatus	France: Nancy	JQ861245	JQ266699	AF140301	JQ266424
Halictus (Tytthalictus) palustris	Israel: Mount Hermon	JQ266582	JQ266704	AF140328	JQ266428
Halictus (Seladonia) confusus	USA: New York, Junius Ponds	JQ266571	JQ266693	AF140304	JQ266418
Halictus (Seladonia) gemmeus	Spain: Laujar de Andarax	JQ266575	JQ266697	AF140305	JQ266421
Halictus (Seladonia) kessleri	Austria: Vienna	AY455894	AY455898	AF140306	JQ266422
Halictus (Seladonia) smaragdulus	Spain: Caratauna	JQ266589	JQ266711	AF140307	JQ266437
Halictus (Seladonia) sp.	France: Les Eyzies	JQ266586	JQ266708	AF140308	JQ266433
Halictus (Seladonia) subauratus	Spain: Trevelez	JQ266585	JQ266707	AF140309	JQ266432
Halictus (Seladonia) tripartitus	USA: Arizona, Cochise Co.	AY227957	AY222593	AF140310	JQ266440
Halictus (Seladonia) tumulorum	France: Paris	JQ266591	JQ266713	AF140311	JQ266441
Halictus (Vestitohalictus) pollinosus	Spain: Trevelez	JQ266579	JQ266701	AF140312	JQ266426
Halictus (Vestitohalictus) vestitus	Spain: Granada Prov.	AY227958*	AY222594	AF140313	JQ266486
Lasioglossum (Australictus) lithuscum	Australia: Victoria	AY227962	AY222598	AF435372	JQ266378
Lasioglossum (Australictus) peraustrale	Australia: VIC, Colquhuon State Forest	JQ266536	JQ266621	JQ266493	JQ266380
Lasioglossum (Australictus) plorator	Australia: VIC, Anglesea, 2 km S.	JQ266537	JQ266622	JQ266494	JQ266381
Lasioglossum (Australictus) rufipes	Australia: VIC, Colquhuon State Forest	JQ266538	JQ266623	JQ266495	JQ266384
Lasioglossum (Callalictus) callomelittinum	Australia: VIC, Toolangi	JQ266539	JQ266624	JQ266496	JQ266385
Lasioglossum (Chilalictus) baudini	Australia: VIC, Mt. Hotham (1749 m)	JQ266541	JQ266626	JQ266497	JQ266386
Lasioglossum (Chilalictus) bicingulatum	Australia: VIC, Melbourne	JQ266543	JQ266628	JQ266498	na
Lasioglossum (Chilalictus) calophyllae	Australia: NSW: Rugby, 10 km W.	JQ266547	JQ266632	JQ266501	JQ266390
Lasioglossum (Chilalictus) chapmanni	Australia: SA: Burra	JQ266545	JQ266630	JQ266499	JQ266387
Lasioglossum (Chilalictus) clelandi	Australia: VIC, Cobboboonee State Forest	JQ266546	JQ266631	JQ266500	JQ266388
Lasioglossum (Chilalictus) cognatum	Australia: VIC, Cobboboonee State Forest	JQ266544	JQ266629	AF264788	AF103953
Lasioglossum (Chilalictus) conspicuum	Australia: VIC, Cobboboonee State Forest	JQ266542	JQ266627	AF264789	AF103952
Lasioglossum (Chilalictus) convexum	Australia: VIC, Cobboboonee State Forest	JQ266540	JQ266625	AF264790	AF103951
Lasioglossum (Chilalictus) erythrurum	Australia: SA, 6 km E. SA/WA border	JQ266548	JQ266633	AF264791	AF103954
Lasioglossum (Chilalictus) fasciatum	Australia: SA: Port Pirie	na	JQ266634	JQ266502	JQ266391

(*continued on next page*)

Table 2 (*continued*)

Taxon	Locality data	LW *rh*	*wnt*-1	*ef*1-α	*co1*
Lasioglossum (Chilalictus) florale	Australia: SA	AY227966	AY222602	AF264792	AF103955
Lasioglossum (Chilalictus) gilesi	Australia: NSW Rugby, 10 km W.	JQ266549	JQ266635	JQ266503	JQ266392
Lasioglossum (Chilalictus) lanarium	Australia: Victoria	AY227967	AY222603	AF264793	AF103956
Lasioglossum (Chilalictus) mediopolitum	Australia: SA, 6 km E. SA/WA border	JQ266550	JQ266636	AF264794	AF103957
Lasioglossum (Chilalictus) mirandum	Australia: WA, Bluff Knoll, Stirling Range NP	JQ266551	JQ266637	AF264795	AF103958
Lasioglossum (Chilalictus) opacicolle	Australia: VIC: Flowerdale	JQ266553	JQ266639	JQ266505	JQ266394
Lasioglossum (Chilalictus) orobatum	Australia: NSW: Armidale, 51 km E.	JQ266552	JQ266638	JQ266504	JQ266393
Lasioglossum (Chilalictus) parasphecodum	Australia: SA, 6 km E. SA/WA border	JQ266554	JQ266640	AF264796	AF103959
Lasioglossum (Chilalictus) representans	Australia: VIC: Cobboboonee State Forest	JQ266555	JQ266641	JQ266506	JQ266395
Lasioglossum (Chilalictus) seductum	Australia: VIC: Mt. Hotham (1740 m)	JQ266556	JQ266642	JQ266507	JQ266397
Lasioglossum (Chilalictus) speculatum	Australia: NSW: Mt. Tomah	JQ266557	JQ266643	JQ266508	JQ266399
Lasioglossum (Chilalictus) supralucens	Australia: WA, Bluff Knoll, Stirling Range NP	JQ266558	JQ266644	AF264797	JQ266402
Lasioglossum (Chilalictus) tamburenei	Australia: VIC: Flowerdale	JQ266559	JQ266645	JQ266510	JQ266403
Lasioglossum (Chilalictus) willsi	Australia: NSW: Rugby, 10 km W.	JQ266560	JQ266646	JQ266511	na
Lasioglossum (Ctenonomia) aethiopicum	South Africa: WCP: Kunje Farm, Citrusdal	JQ342159	JQ266647	JQ266512	JQ266407
Lasioglossum (Ctenonomia) nicolli	Madagascar: Parc National Isalo	EU203307	EU203248	EU203279	JQ266408
Lasioglossum (Ctenonomia) pachyacanthum	South Africa: NCP: Richtersveld National Park	JQ266561	JQ266648	na	JQ266409
Lasioglossum (Dialictus) anomalum	No locality data	JQ266564	JQ266651	JQ266513	JQ266410
Lasioglossum (Dialictus) brevicorne	Spain: Almeria Prov.	AF448876	JQ266666	AF435365	AF435358
Lasioglossum (Dialictus) cressonii	Canada: Ontario	AF448908	JQ266652	AF264801	AF103963
Lasioglossum (Dialictus) ellisiae	USA: New York, Seneca Co.	AF448915	JQ266659	AF264809	AF103971
Lasioglossum (Dialictus) figueresi	Costa Rica: San Jose Province	AF448903	AY455901	AF264802	AF435357
Lasioglossum (Dialictus) gattaca	Republic of Panama, Chiriqui Prov.	AF448898	JQ266673	AF264834	AF104639
Lasioglossum (Dialictus) gundlachii	Puerto Rico	AF448909	JQ266653	AF264803	AF103965
Lasioglossum (Dialictus) near hyalinum	USA: Arizona, Pima Co.	AF448910	JQ266654	AF264804	AF103966
Lasioglossum (Dialictus) imitatum	USA: New York, Tompkins Co.	AF448911	JQ266655	AF264805	AF103967
Lasioglossum (Dialictus) inconditum	USA: Arizona, Santa Cruz Co	AF448883	JQ266675	AF435366	AF435359
Lasioglossum (Dialictus) limbellum	Spain: Almeria Prov.	AF448886	JQ266678	AF435368	AF435361
Lasioglossum (Dialictus) lionotum	USA: New York, Tompkins Co.	AF448919	JQ266732	AF264856	AF104659
Lasioglossum (Dialictus) lucidulum	Spain: Almeria Prov.	AF448887	JQ266679	AF435369	AF435362
Lasioglossum (Dialictus) morio	France: Dordogne	AF448891	JQ266681	AF264827	AF103989
Lasioglossum (Dialictus) parvum	Puerto Rico	AF448912	JQ266656	AF264806	AF103968
Lasioglossum (Dialictus) pectorale	USA, Florida, Polk Co.	AF448894	JQ266684	AF264830	AF104635
Lasioglossum (Dialictus) pilosum	USA: New York, Seneca Co.	AF448913	JQ266657	AF264807	AF103969
Lasioglossum (Dialictus) platyparium	USA	JQ266565	na	JQ266514	JQ266411
Lasioglossum (Dialictus) puncticolle	France: Dordogne	AF448896	JQ266685	AF264832	AF104637
Lasioglossum (Dialictus) rufulipes	Canada: Yukon Terri	JQ266566	na	JQ266515	JQ266412
Lasioglossum (Dialictus) umbripenne	Republic of Panama: Panama	AF448916	JQ266660	AF264810	AF103975
Lasioglossum (Dialictus) versatum	USA: New York, Seneca Co.	AF448914	JQ266658	AF264808	AF103970
Lasioglossum (Dialictus) vierecki	USA: New York, Seneca Co.	AF448917	JQ266661	AF264811	AF103972
Lasioglossum (Dialictus) villosulum	France: Dordogne	AF448901	JQ266691	AF264837	AF104642
Lasioglossum (Dialictus) villosulum	Spain: Almeria Prov.	AF448902	JQ266692	AF435370	AF435364
Lasioglossum (Dialictus) zephyrum	USA: New York, Seneca Co.	AF448918*	AY222607	AF435379	AF103973
Lasioglossum (Evylaeus) albipes (social)	France: Dordogne	AF448873	JQ266663	AF264814	AF103976
Lasioglossum (Evylaeus) albipes (solitary)	France: Vosges	AF448872	JQ266662	AF264813	AF103977
Lasioglossum (Evylaeus) apristum	Japan: Shimane Prefecture	AF448874	JQ266664	AF264815	AF103978
Lasioglossum (Evylaeus) boreale	Canada: Northwest Territories	AF448875	JQ266665	AF264816	AF103979
Lasioglossum (Evylaeus) calceatum	France: Dordogne	AF448877*	AY222608	AF435385	AF103980
Lasioglossum (Evylaeus) cinctipes	USA: New York, Tompkins Co.	AF448878	JQ266667	AF264818	AF103981
Lasioglossum (Evylaeus) comagenense	Canada: Nova Scotia	AF448879	JQ266668	AF264819	AF103982
Lasioglossum (Evylaeus) duplex	Japan: Miyagi Prefecture	AF448880	JQ266670	AF264820	AF103983
Lasioglossum (Evylaeus) fulvicorne	France: Vaucluse	AF448881	JQ266672	AF264821	AF103984
Lasioglossum (Evylaeus) interruptum	Spain: Almeria Prov.	AF448882	JQ266674	AF435367	AF435360
Lasioglossum (Evylaeus) laticeps	France: Dordogne	AF448884	JQ266676	AF264822	AF103985
Lasioglossum (Evylaeus) lineare	France: Meurthe et Moselle	AF448893	JQ266677	AF264823	AF103986
Lasioglossum (Evylaeus) malachurum	France: Dordogne	AF448890	AY455903	AF264826	AF103988
Lasioglossum (Evylaeus) marginatum	France: Dordogne	AF448889	AY455902	AF264825	AF103987
Lasioglossum (Evylaeus) mediterraneum	France: Dordogne	AF448888	JQ266680	AF264824	AF435363
Lasioglossum (Evylaeus) nigripes	France: Vaucluse	AF448892	JQ266682	AF264828	AF103990
Lasioglossum (Evylaeus) pauxillum	Austria: Vienna	AF448885	JQ266683	AF264829	AF104634
Lasioglossum (Evylaeus) politum	France: Dordogne	AF448895	AY455904	AF264831	AF103636
Lasioglossum (Evylaeus) quebecense	No locality data	AF448897	JQ266686	AF264833	AF104638
Lasioglossum ("Evylaeus") sp. (black, impunctate)	South Africa: WCP: 21 km N. Hermanus	JQ266569	JQ266687	JQ266518	na
Lasioglossum ("Evylaeus") mosselinum	South Africa: WCP: Cape Agulhas	JQ266570	JQ266688	JQ266519	na
Lasioglossum (Evylaeus) subtropicum	Japan: Okinawa Prefecture	AF448899	JQ266689	AF264835	AF104640
Lasioglossum (Evylaeus) truncatum	USA: New York, Tompkins Co.	AF448900	JQ266690	AF264836	AF104641
Lasioglossum (Hemihalictus) lustrans	USA: Texas, Bastrop Co.	AF448904*	AY222609	AF435388	AF104643
Lasioglossum (Homalictus) holochlorum	Australia: VIC: Mildura, 56 km S.	JQ266592	JQ266714	JQ266520	JQ266442
Lasioglossum (Homalictus) megastigmum	Australia: W, Bluff Knoll	AY227964	AY222600	AF264839	JQ266443
Lasioglossum (Homalictus) exlautum	Australia: SA, Adelaide	AY227965	AY222601	AF435389	JQ266444
Lasioglossum (Homalictus) sphecodoides	South Africa: Adelaide Univ. Botanical Garden	JQ266593	JQ266715	JQ266521	JQ266445
Lasioglossum (Ipomalictus) sp.	South Africa: KZN: Kosi Bay	JQ266594	JQ266716	JQ266522	na
Lasioglossum (Lasioglossum) athabascense	USA: New York, Tompkins Co.	AF448867	AY222604	AF435390	AF104645
Lasioglossum (Leuchalictus) callizonium	Spain: Almeria Province, Berja to Alcolea	JQ266595	JQ266717	AF264841	AF104644
Lasioglossum (Lasioglossum) coriaceum	No locality data	JQ266596	JQ266718	AF264842	AF104645

Table 2 (*continued*)

Taxon	Locality data	LW *rh*	*wnt*-1	*ef*1-α	*co1*
Lasioglossum (Lasioglossum) desertum	USA: Rose Canyon Lake, AZ	JQ266597	JQ266719	AF264843	AF104646
Lasioglossum (Leuchalictus) discum	France	JQ266598	JQ266720	AF264850	AF104647
Lasioglossum (Lasioglossum) fuscipenne	USA: Michigan	AF448868	JQ266721	AF264844	AF104648
Lasioglossum (Lasioglossum) laevigatum	No locality data	JQ266599	JQ266722	AF264845	AF104949
Lasioglossum (Leuchalictus) leucozonium	Austria: Vienna	JQ266600	JQ266723	AF264846	AF104651
Lasioglossum (Leuchalictus) leucozonium	USA: NY, Tompkins Co., Ithaca vicinity	JQ266601	JQ266724	AF264847	AF104652
Lasioglossum (Leuchalictus) majus	France	JQ266602	JQ266725	AF264849	AF104653
Lasioglossum (Lasioglossum) pavonotum	USA: California, Marin Co.	AF448869	JQ266726	AF264851	AF104654
Lasioglossum (Leuchalictus) scitulum	Japan: Shimane Prefecture	AY227968	AY222605	AF435391	JQ266446
Lasioglossum (Lasioglossum) sexnotatum	France: Morigny-Champigny, Essonne	JQ266603	JQ266728	AF264853	AF104655
Lasioglossum (Lasioglossum) sisymbrii	USA: AZ, Cochise Co.	AF448870	JQ266727	AF264852	AF104656
Lasioglossum (Lasioglossum) titusi	USA: California, San Bernardino Co.	AF448871	JQ266729	AF264854	AF104657
Lasioglossum (Leuchalictus) zonulum	USA: New York, Tompkins Co.	AY227969	AY222606	AF264855	JQ266447
Lasioglossum (Oxyhalictus) sp. 1	South Africa: WCP: Kunje Farm, Citrusdal	JQ266604	JQ266730	JQ266523	JQ266455
Lasioglossum (Parasphecodes) altichum	Australia: VIC: Mt. Hotham (1740 m)	JQ266605	JQ266731	JQ266524	JQ266456
Lasioglossum (Parasphecodes) excultum	Australia: NSW: Oberon, 53 km S.	JQ266606	JQ266733	JQ266525	JQ266458
Lasioglossum (Parasphecodes) hiltacum	Australia: NSW: Rugby, 10 km W.	JQ266607	JQ266734	JQ266526	JQ266459
Lasioglossum (Parasphecodes) hybodinum	Australia: SA	AY227963	AY222599	AF264857	AF104660
Lasioglossum (Parasphecodes) lacthium	Australia: VIC: Mt. Hotham (1740 m)	JQ266608	JQ266735	JQ266527	JQ266460
Lasioglossum (Parasphecodes) olgae	Australia	JQ266563	JQ266650	AF264800	AF103962
Lasioglossum (Parasphecodes) olgae	Australia: VIC: Cobboboonee	JQ266609	JQ266736	JQ266528	JQ266462
Lasioglossum (Parasphecodes) sp.	Australia: SA, Cobbooboonee SP, Victoria	JQ266611	JQ266738	AF264858	na
Lasioglossum (Parasphecodes) subrussatum	Australia: VIC: Mt. Hotham (1740 m)	JQ266610	JQ266737	JQ266529	JQ266463
Lasioglossum (Parasphecodes) sulthicum	South Africa: Burra	JQ266612	JQ266739	JQ266530	JQ266466
Lasioglossum (Pseudochilalictus) imitator	Australia: NSW: Rugby, 14 km. W	JQ266613	JQ266740	JQ266531	JQ266469
Lasioglossum (Pseudochilalictus) nr. imitator	Australia: NSW: Mt. Tomah	JQ266614	JQ266741	JQ266532	na
Lasioglossum (Rubrihalictus) sp.	South Africa: NCP: Richtersveld National Park	JQ266615	JQ266742	JQ266533	JQ266475
Lasioglossum (Rubrihalictus) whiteanum	South Africa: WCP: 21 km N. Hermanus	JQ266616	JQ266743	JQ266534	JQ266476
Lasioglossum (Sellalictus) deceptum	South Africa: WCP: Kunje Farm, Citrusdal	JQ266567	JQ266669	JQ266516	JQ266416
Lasioglossum (Sellalictus) ferinum	South Africa: WCP: 5 km W. Grabouw	JQ266568	JQ266671	JQ266517	JQ266417
Lasioglossum (Sphecodogastra) noctivagum	USA: Texas, Ward Co.	AF448905	JQ266744	AF264859	AF104661
Lasioglossum (Sphecodogastra) oenotherae	USA: New York, Tompkins Co.	AF448906	AY455905	AF264860	AF104662
Lasioglossum (Subgenus nov.) NDA1-(a)	Australia	JQ266562	JQ266649	AF264799	AF103960
Lasioglossum (Sudila) alphenum	Sri Lanka: NE District	AF448907	JQ266745	AF264861	AF104663
Mexalictus arizonensis	USA: Arizona, Santa Cruz Co.	AY227959	AY222595	AF140322	AF102843
Patellapis (Archihalictus) joffrei	Madagascar: Parc National Ranomafana	EU203301	EU203242	EU203273	JQ266377
Patellapis (Chaetalictus) coccinea	Madagascar: Parc National Ranomafana	EU203302	EU203243	EU203274	JQ266389
Patellapis (Chaetalictus) minima	South Africa: Western Cape Prov., Citrusdal	EU203289	EU203231	EU203261	JQ266400
Patellapis (Chaetalictus) sp. 2	South Africa: Kwazulu-Natal	AY227952	AY222588	AF435374	JQ266398
Patellapis (Chaetalictus) rozeni	South Africa: Mpumalanga, Graskop	EU203290	EU203230	EU203262	JQ266401
Patellapis (Lomatalictus) malachurinus	South Africa: Mpumalanga, Graskop	EU203291	EU203232	EU203263	JQ266448
Patellapis (Lomatalictus) sp.	South Africa: Gauteng	AY227953	AY222589	AF435393	JQ266449
Patellapis (Archihalictus) castaneus	Madagascar: Parc National Ranomafana	EU203303	EU203244	EU203275	JQ266450
Patellapis (Archihalictus) inelegans	Madagascar: Parc National Ranomafana	EU203304	EU203245	EU203276	JQ266451
Patellapis (Chaetalictus) fisheri	Madagascar: Parc National Ranomafana	EU203305	EU203246	EU203277	JQ266452
Patellapis (Pachyhalictus) sp.	Vietnam: Ban Don	AY227954	AY222590	AF435400	JQ266467
Patellapis (Patellapis) braunsella	South Africa: WCP., Nieuwoudtville	EU203306	EU203247	EU203278	JQ266457
Patellapis (Patellapis) cf. minutior	South Africa: WCP, Citrusdal	EU203293	EU203234	EU203265	JQ266464
Patellapis (Patellapis) montagui	South Africa: WCP., Clanwilliam	EU203292	EU203233	EU203264	JQ266461
Patellapis (Patellapis) karooensis	South Africa: WCP., Vanrhynsdorp	EU203294	EU203235	EU203266	JQ266465
Patellapis (Patellapis) cf. gessorum	South Africa: NCP., Port Nolloth	EU203288	EU203229	EU203260	JQ266396
Patellapis (Zonalictus) abessinicus	South Africa: Free State, Harrismith	EU203295	EU203236	EU203267	JQ266487
Patellapis (Zonalictus) albofasciatus	South Africa: Free State, Koppies	EU203297	EU203238	EU203269	JQ266491
Patellapis (Zonalictus) andreniformis	South Africa: WCP., Citrusdal	EU203296	EU203237	EU203268	JQ266488
Patellapis (Zonalictus) high elevation sp.	South Africa: Mpumalanga, Sabie	EU203300	EU203241	EU203272	JQ266490
Patellapis (Zonalictus) vittatus	South Africa: Western Cape Prov., Citrusdal	EU203298	EU203239	EU203270	JQ266492
Patellapis (Zonalictus) zacephalum	South Africa: Mpumalanga: Graskop	EU203299	EU203240	EU203271	JQ266489
Thrincohalictus prognathus	Israel: Golan Heights	AY227955	AY222591	AF140326	JQ266484

ar)/codon position/intron, and, finally, (iii) by gene/codon position/intron. Our preferred partitioning scheme was that of genome/codon/intron, but we examined alternative schemes to determine their effects on tree topology. Previous studies based on single copy nuclear gene data sets have suggested that combining nucleotide positions (i.e., nt1, nt2, nt3) across genes, especially when all the genes are evolving at roughly the same rate, is preferred over treating the genes separately (Cardinal et al., 2010). We analysed the data both with and without introns. Ambiguously aligned regions of introns were excluded from all analyses. We used jModelTest (Posada, 2009) to select nucleotide substitution models for each partition of our data sets (Table S2). We ignored any models that could not be implemented using MrBayes v. 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003).

2.4.2. Phylogenetic analyses

We analysed our data set using MrBayes v.3.1.2. Each analysis comprised of eight simultaneous chains run for 50,000,000 generations. Trees were sampled every 5000 generations for a total of 10,000 trees per run. Analyses were performed with all parameters unlinked and branch lengths unconstrained. We used an unconstrained branch lengths prior with an exponential distribution with parameter 100. TRACER v. 1.5 (Rambaut and Drummond, 2009) was used to determine if analyses had run sufficiently long for likelihoods and parameters to stabilize. The online tool AWTY (are we there yet?; Nylander et al., 2007) was used to test for convergence of tree topologies between runs. We examined all pairwise comparisons of post-burnin trees between runs and plotted split posterior probabilities within runs to determine if tree topologies had converged and analyses had reached stationarity. We discarded 2000 trees (equivalent to 10 million generations) from each run to remove all trees generated in the "burn-in" region before parameters had stabilized. Trees were rooted using the Augochlorini based on previous higher level phylogenies (Brady et al., 2006b; Danforth et al., 2004; Pesenko, 1999). Trees from two runs were combined (16,002 trees total) and expressed as a 50% majority rule consensus using Mesquite v. 2.75.

2.4.3. Constrained analyses

We reanalysed our data sets, partitioned by genome/codon/intron, and applying the model selected by the Akaike Information Criterio (AIC) in jModelTest, while constraining *Lasioglossum* to be sister to *Halictus* + *Thrincohalictus*, the topology most often obtained in previous studies (Brady et al., 2006b; Danforth et al., 2004; Fig. 2A). We then tested for significant differences between alternate topologies using a Bayes Factor test. TRACER v. 1.5 was used to estimate marginal likelihoods in order to calculate Bayes Factors.

2.5. Ancestral state reconstruction

We used the results from our MrBayes analyses of the Halictini data set, using the mixed model selected by AIC using jModeltest and the data partitioned by genome/codon/introns, to reconstruct the character state of the most recent common ancestor of *Halictus* and *Lasioglossum*. MrBayes runs were combined into a single tree file using LogCombiner (Rambaut and Drummond, 2010), using a burnin of 2000 trees for each run (equivalent to 10 million generations), and subsampling across the post-burnin region to produce 1000 total trees. This sample of trees allowed us to reconstruct ancestral states while allowing for uncertainty in topology, branch lengths, and relative rates of state transitions. Trees were rooted with the Augochlorini using BayesTrees v. 1.0 (Meade and Pagel, 2009) and then entered into BayesTraits v. 1.0 (Pagel et al., 2004) for ancestral state reconstructions.

Behavioural states of terminal nodes were based on previous behavioural studies of the Halictini (see Appendix 1). Solitary and communal behaviour were treated as a single state, as were eusocial and semisocial colonies. We treated cleptoparasitism and social parasitism in the Sphecodini, *Parathrinchostoma* and *Lasioglossum (Dialictus)* as identical states. In most analyses, the rate parameters for reversal from either cleptoparasitism or social parasitism to nest-building were restricted to zero. This restriction on the rate parameters is justified by the obligate nature of brood parasitism in these taxa due to significant morphological changes that preclude reversals to pollen collection and nest construction. We also ran analyses wherein we fixed the ancestral state of nodes antecedent to Halictini *s. s.* to prevent the probability of a eusocial ancestor being spread over multiple internal nodes. Solitary and communal behavioural states of basal lineages of halictid bees (Rophitinae, Nomiinae, Nomioidinae, Caenohalictini, and Thrinchostomini) strongly support this reconstruction (Eickwort, 1981; Michener, 1968, 1974; Roberts and Brooks, 1987).

We used the multistate model in BayesTraits with maximum likelihood to reconstruct nodes of interest and estimate parameters for each tree. For each maximum likelihood analysis, 100 attempts were made per tree to optimize likelihoods. Maximum likelihood parameter estimates were then used to choose priors for subsequent Markov chain Monte Carlo (MCMC) analyses. We used the multistate model with MCMC for 5×10^8 generations, sampling every 3×10^5 generations, with a burn-in of 2×10^8 generations. We repeated this analysis 4 times to ensure that the analyses converged properly. We examined the acceptance rate in preliminary analyses to determine a suitable value for the ratedev parameter that would allow for effective exploration of tree space while limiting autocorrelation between successive iterations. We tested multiple values until settling upon a ratedev which gave acceptance values between 20% and 40%. We performed analyses using uniform priors and also a hyperprior seeding either an exponential or gamma distribution from a uniform distribution over an interval selected based on the ML results (0–10 with a variance of 0–10 for the gamma). We examined different alternative reconstructions using the fossil command to fix the state at particular nodes. We also performed ancestral state reconstructions based on two alternative topologies (Fig. 2A and C). These topologies were recovered in previous studies (Brady et al., 2006b; Danforth et al., 2004) or using alternative partitions of our data set.

2.6. Divergence dating

We inferred divergence times under a Bayesian framework using BEAST v.1.7.0 (Drummond et al., in press) by employing an uncorrelated lognormal relaxed clock model (Drummond and Rambaut, 2007; Drummond et al., 2006). For most analyses we used the genome/codon/intron partitioning scheme with the AIC selected substitution models (Table S2). Substitution models were unlinked and clock and tree models linked among partitions. A Yule speciation process with a random starting tree was used for the tree prior. We assigned *a priori* age distributions to eight stem group nodes based on evidence from the fossil record (Table 3). In cases where morphological evidence suggests that the fossil likely falls within the crown group we applied a lognormal distribution

Table 3
Fossils used for *a priori* calibration of nodes in divergence dating analyses. All calibrated nodes refer to stem groups. Numbers in parentheses indicate mean and standard deviation values for normal distributions and log(mean), log(standard deviation), and zero offset values for lognormal distributions.

Calibration	Species	Epoch	Site	Node	References	*a priori* distribution
CAL1	*Electrolictus antiquus*	Mid-eocene	Baltic	Halictini *s. s.* or Thrinchostomini	Engel (2001) and De Meulemeester et al. (2012)	Normal (45, 10)
CAL2	*Lasioglossum celinae*	Late oligocene	Bois d'Asson	*Lasioglossum* s.l.	Nel and Petrulevičius (2003)	Lognormal (2, 1, 23)
CAL3	*Nesagapostemon moronei*	Early-mid miocene	Dominican	*Agapostemon*	Engel (2009)	Normal (25, 10)
CAL4	*Eickwortapis dominicana*	Early-mid miocene	Dominican	*Caenohalictus* + *Habralictus*	Michener and Poinar (1996)	Normal (25, 10)
CAL5	*Augochlora leptoloba*	Early-mid miocene	Dominican	*Augochlora*	Engel (2000)	Lognormal (2, 1, 15)
CAL6	*Neocorynura electra*	Early-mid miocene	Dominican	*Neocorynura*	Engel (1995)	Lognormal (2, 1, 15)
CAL7	*Oligochlora* spp.	Early-mid miocene	Dominican	Augochlorini minus *Corynura*	Engel (1996, 1997, 2000, 2009) and Engel and Rightmyer (2000)	Normal (25, 10)
CAL8	*Halictus petrefactus*	Early miocene	Rubielos de Mora	*Halictus*	Engel and Peñalver (2006)	Normal (30, 10)

with a hard lower age bound, whereas if such evidence was lacking then we instead used a normal distribution. We explored the impact of individual *a priori* nodal calibrations on inferred divergence dates by performing jackknifing experiments in which one of the eight calibrations was removed and dates re-estimated under the same conditions. We also tested the effect of our selected partitioning scheme and models by conducting alternative analyses using GTR + I + Γ models under gene/codon/intron and codon (with introns excluded) treatments.

MCMC searches were conducted for 1×10^8 generations with the first 2×10^7 generations discarded as burn-in. Trees and parameter values were saved every 10,000 generations. Convergence and stationarity of inferred age estimates were assessed with TRACER v.1.5 using high ESS scores (>200) and the consistency of results between multiple runs. The results from two independent runs were combined using LogCombiner 1.6.1 (distributed with the BEAST package) and visualised using FigTree v.1.3.1 (Rambaut, 2009).

3. Results

3.1. Data set

Our data set of the Halictinae included 206 taxa, most of which were sequenced for all four genes (see Table 2). After removal of ambiguously aligned portions of the *ef*1-α and LW *rh* introns our data set consisted of 3919 aligned nucleotide sites, including 1707 bp of *ef*1-α (43.6% of data set), 409 bp of *wnt*-1 (10.4%), 1242 bp of *co1* (31.7%), and 561 bp of LW *rh* (14.3%). We also analysed our data set with all introns removed which resulted in 3185 bp, including 1127 bp of *ef*1-α (35.6%), 409 bp of *wnt*-1 (12.9%), 1242 bp of *co1* (39.2%), and 387 bp of LW *rh* (12.2%).

3.2. Bayesian analyses

For the analyses partitioned by codon/intron, AIC and BIC selected GTR + I + Γ as the best model for all codon positions, whereas GTR + Γ was selected for intron regions (Table S2). The genome/codon/intron partitioned data set, was similar but HKY + Γ or GTR + Γ was chosen for 3rd codon positions and the BIC selected SYM + I + Γ for the nuclear 2nd codon position. The AIC and BIC selected five to seven different models for the data set when partitioned by gene/codon/intron (Table S2).

Trees were largely congruent across analyses, but differences were seen in the relationships of the outgroup taxon Sphecodini. The BEAST analyses recovered Sphecodini as sister to Halictini *s. s.* In most MrBayes results Sphecodini + Thrinchostomini together was sister taxon to Halictini *s. s.* but the node uniting Sphecodini + Thrinchostomini was always poorly supported (Fig. 3, S2–S6).

Within the Halictini *s. s.*, the position of *Mexalictus* was not strongly supported. In our preferred partitioned analyses (genome/codon/intron), *Mexalictus* was recovered as sister to the remaining Halictini (Figs. S1–S3). The simplest partitioning scheme (codon/intron), recovered a basal trichotomy with *Mexalictus* (Fig. S4), *Halictus* + *Thrincohalictus* and *Patellapis* + *Lasioglossum* (Fig. 2B and 3). In the most finely partitioned analyses (gene/codon/intron) *Mexalictus* was resolved as the sister to *Halictus* + *Thrincohalictus* (Figs. S5–S6). These analyses were the only ones not to recover *Lasioglossum* and *Patellapis* as sister taxa, but support values for the relationships among Halictini genera were lower than the results from the other analyses. The topology obtained in previous studies (Danforth et al., 2004, 2008; Brady et al., 2006b), with *Mexalictus* sister to *Patellapis*, was never found in our analyses. In our preferred analyses we find strong support for two clades: *Halictus* + *Thrinchohalictus* (PP 100) and *Lasioglossum* + *Patellapis* (PP 98–100; Figs. 2B and 3).

3.3. Constrained analyses

When *Halictus* + *Thrincohalictus* was constrained as sister group to *Lasioglossum* (as obtained in previous studies; Fig. 2A), *Mexalictus* emerged as sister taxon to *Patellapis*; a topology never resolved in the unconstrained data but consistent with earlier published studies (e.g. Brady et al., 2006b; Fig. 2A). A Bayes Factor test indicates positive to strong support (4.7 < ln Bayes Factor < 7.8) for the unconstrained topology (see Kass and Raftery, 1995).

3.4. Ancestral reconstruction

We used BayesTraits to reconstruct the evolutionary history of eusociality in halictid bees with a focus on the common ancestor of *Lasioglossum* and *Halictus*. The maximum likelihood analyses found a much higher transition rate of eusocial to solitary (5.46 SD ± 0.14) behaviour than the reverse (1.46 SD ± 0.23), but this result was not significant when compared to an analysis in which these two transition rates were constrained to be equal (assuming a difference in lnL of 2.0 as significant; Pagel, 1999). The transition rate of cleptoparasitism to nest-building (either solitary or eusocial) was nearly always zero, which supports our decision to restrict this transition in subsequent ML and MCMC analyses.

We found the ancestral state of the node uniting *Halictus* and *Lasioglossum* to be eusocial with greater probability using both ML (89.5%) and MCMC using a variety of priors (72.9–79.1%) than the alternative of a shared solitary state (Table 5). MCMC with uniform priors on the interval 0–100 failed to constrain the parameters so a narrower interval of 0 to 10 was selected based on the ML results. When ancestral nodes antecedent to Halictini *s. s.* were *a priori* constrained to be solitary, eusociality was still found to be more probable for the common ancestor of *Halictus* + *Lasioglossum* (Fig. 4B and F). The two alternative topologies explored (Fig. 2A and C) also found similarly high probabilities for eusocial ancestry (Fig. 4G and H; Table 5).

3.5. Divergence dating

The most recent common ancestor of *Halictus* and *Lasioglossum* is estimated to be 35 Ma (95% Highest Posterior Density: 28, 43 Ma) (Fig. 3). Divergence dating estimates for the three main eusocial clades are: Augochlorini, 17 (15–21) Ma; *Halictus*, 19 (14, 24) Ma; the *Hemihalictus* series of *Lasioglossum* 24 (19, 31) Ma, respectively (Table 4). A eusocial common ancestor of *Halictus* and *Lasioglossum* would shift the estimate for the earliest origin of eusociality in the Halictinae back by >13 million years compared to a scenario of two independent eusocial origins within these genera (Brady et al., 2006b).

The removal of individual fossil calibrations can substantially alter inferred divergence dates in some cases (Buffington et al., 2012). In our data set, however, these jackknifing experiments revealed that no single calibration point had a large impact on estimated divergence dates (Table 4). Furthermore, our inferred dates were robust to alternative partitioning schemes and the inclusion or exclusion of intron data, changing by 0–1 my under these different data treatments.

4. Discussion

4.1. Social evolution

Reconstructing the ancestral state of *Halictus* and *Lasioglossum* is important for understanding how eusociality has arisen and is maintained in the Halictinae. Earlier studies have suggested there may be three nearly synchronous origins of eusociality in Halicti-

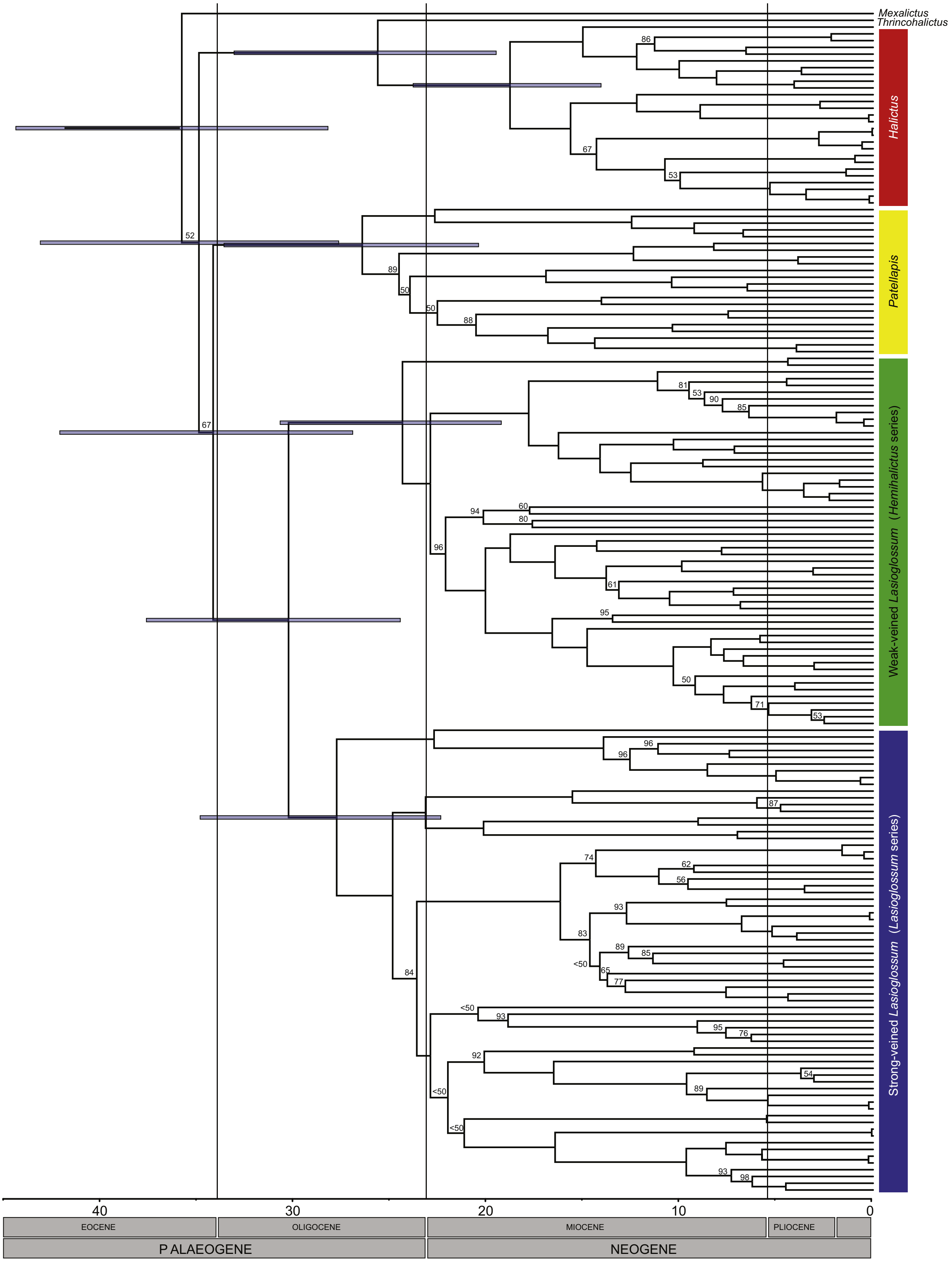

Fig. 3. Fossil-calibrated maximum clade credibility tree of the Bayesian phylogenetic analysis of the Halictini using an uncorrelated lognormal relaxed clock model with the data set partitioned by genome and codon with introns included using a mixed nucleotide substitution model selected using AIC. Posterior probabilities are shown above nodes; values of 99 and 100 have been removed for clarity. Blue bars indicate the 95% Highest Posterior Density on the estimated ages of selected clades. Outgroups not shown (see Fig. S1).

Table 4
Divergence dating estimates for crown group lineages of halictid bees. The first column includes all eight *a priori* calibrations based on the fossil record (Table 3). Subsequent columns give results from experiments excluding one fossil calibration. The final column provides Bayesian dating estimates for the same taxa from a previous study that included substantially fewer species, molecular characters, and fossils (Brady et al., 2006b). Ages are in millions of years with 95% credibility intervals in parentheses.

Taxon	All Calibrations	CAL1 removed	CAL2 removed	CAL3 removed	CAL4 removed	CAL5 removed	CAL6 removed	CAL7 removed	CAL8 removed	Brady et al. (2006a,b)
Halictus	19 (14,24)	18 (14,24)	20 (14,26)	19 (14,25)	19 (14,25)	18 (14,23)	19 (14,25)	19 (14,25)	18 (14,24)	21 (15,28)
Hemihalictus series	24 (19,31)	23 (19,30)	25 (19,33)	25 (19,31)	25 (19,32)	23 (18,29)	25 (19,31)	25 (19,31)	24 (19,31)	22 (15,29)
Halictus + *Lasioglossum* MRCA	35 (28,43)	34 (26,43)	37 (28,47)	35 (28,44)	36 (29,46)	33 (27,41)	36 (28,46)	35 (27,46)	35 (28,44)	40 (35,47)
Augochlora + *Augochlorella*	17 (15,21)	17 (15,20)	17 (15,21)	17 (15,21)	17 (15,21)	14 (9,19)	18 (15,21)	17 (15,21)	17 (15,21)	20 (12,29)

Table 5
Probability (in percentage) of a eusocial common ancestor for several halictid clades of interest under different methods of analysis (ML: maximum likelihood; MCMC: Markov Chain Monte Carlo), alternative historical scenarios, and application of different priors to MCMC analyses. Unless otherwise stated all analyses below disallow reversals from cleptoparasitism to nest-building behaviour (solitary or eusocial). (1) ML with no restrictions; (2) ML with Halictinae most recent common ancestor (MRCA) fixed as solitary; (3) ML with Halictini *s.l.* MRCA fixed as solitary; (4) ML with MRCAs of Halictinae, Augochlorini, Halictini s.l., Caenohalictini and Thrinchostomini fixed as solitary; (5) ML using tree topology from alternative partitioning scheme; (6) ML using constrained tree topology; (7) MCMC with uniform priors on interval from 0 to 100; (8) MCMC with uniform priors on interval from 0 to 10 (based on ML results); (9) MCMC with hyperprior used to seed exponential prior from uniform on interval from 0 to 10; (10) MCMC with hyperprior used to seed gamma prior with mean and variance from uniform interval from 0 to 10; (11) MCMC as in (9) with Halictini s.l. MRCA fixed as solitary; and (12) MCMC as in (9) with MRCAs of Halictinae, Augochlorini, Halictini s.l., Caenohalictini and Thrinchostomini fixed as solitary. Numbers in brackets were fixed *a priori*.

Clade	ML (1)	ML (2)	ML (3)	ML (4)	ML (5)	ML (6)	MCMC (7)	MCMC (8)	MCMC (9)	MCMC (10)	MCMC (11)	MCMC (12)
Halictinae	67.83	[0]	18.72	8.43	77.25	66.88	51.26	52.90	57.05	54.63	19.40	9.20
Augochlorini	40.87	37.07	37.57	[0]	42.00	40.97	43.90	42.54	42.75	42.79	36.71	57.08
Halictini *s. s.*	89.06	80.19	80.88	81.97	92.59	88.34	65.32	72.51	78.69	73.31	69.96	71.57
Halictus	99.85	99.63	99.71	99.75	99.89	99.81	85.24	97.13	98.64	95.39	98.11	98.79
Lasioglossum series	19.07	15.49	15.47	14.89	19.51	19.24	29.60	23.51	22.60	24.05	16.69	15.97
Hemihalictus series	98.68	98.08	98.12	98.32	99.13	98.73	81.77	93.44	96.36	91.93	94.76	96.23
Halictus + *Lasioglossum* MRCA	89.55	80.07	81.41	82.80	92.63	93.45	65.58	72.91	79.06	73.67	70.27	71.93

nae (Brady et al., 2006b; Danforth, 2002) and some authors have speculated the number may be much higher (see Michener, 1990). Not included in previous phylogenetic reconstructions were additional cases of eusociality in the *Lasioglossum* series (Michener, 1968; Miyanaga et al., 1998, 2000; Packer, 1997, 1998). In Brady et al. (2006b), a parsimony-based reconstruction of ancestral states supported dual origins of eusociality in *Halictus* and *Lasioglossum.* A weakness of using equal weights parsimony is that the loss and gain of eusociality are considered equally probable. This is unlikely to be the case given the frequent reversals to solitary behaviour in *Halictus* and *Lasioglossum* (Danforth, 2002).

Model-based ancestral state reconstruction, which takes differences in transition rates into account, provides a different picture. Our analyses with BayesTraits suggest that the common ancestor of *Halictus* and *Lasioglossum* was more likely to have been eusocial than solitary based both on our observed tree topologies (Fig. 2B and C) and a topology obtained in previous studies (Fig. 2A). This implies that the origin of eusociality in these taxa is much more ancient than previously supposed. Based on our fossil-calibrated phylogeny the shared origin of eusociality for *Halictus* and *Lasioglossum* would be pushed back from the Miocene to the early Oligocene or late Eocene; nearly 14 million years earlier than previous estimates. The results of our fossil calibrated divergence dating are largely congruent with earlier estimates (Brady et al., 2006b) for the equivalent nodes (Table 4), even though the earlier study was based on far fewer taxa and calibration points, did not include *co1*, and used a different method of Bayesian dating inference (MULTIDIVTIME; Thorne and Kishino, 2002).

If eusociality originated prior to the origin of *Halictus* and *Lasioglossum s.l.* then subsequent origins of eusociality in the *Lasioglossum* series (*e.g. L. aegyptiellum*; Packer, 1997, 1998) occurred in a lineage that was secondarily solitary. This might help explain why so many origins of eusociality have seemingly occurred in *Lasioglossum* as opposed to other insect lineages. It is reasonable to assume that some of the behavioural and physiological apparatus necessary for developing eusociality were already in place in the *Lasioglossum* series lineages that re-evolved this trait. The alternative hypothesis is that eusociality evolved *de novo* a minimum of four, or perhaps five, times in the Halictini. In either case, halictine bees present an ideal "model" for the study of social evolution.

The basal lineage of Halictidae, the Rophitinae, is mostly solitary (Rozen, 1993), although some communal nests have been reported (Rozen and McGinley, 1976). Communal behaviour is more widespread in the Nomiinae, Nomioidinae, and in the Caeonhalictini (Eickwort et al., 1986; Rozen, 2008; Wcislo, 1993, 1997). This suggests that mutual tolerance is an ancestral characteristic of halictine bees and may be a partial explanation for the subsequent evolution of eusociality. It should be noted, however, that communal behaviour is also observed in other bee lineages that are not known to have evolved eusociality (e.g., Andrenidae). Additional study is needed to elucidate the combination of traits and environmental conditions that allow eusociality to evolve.

For future studies seeking to understand the underlying genetic and ecological mechanisms behind social evolution it is important to account for phylogenetic context (e.g. Schwarz et al., 2011). Unlike other eusocial bees, there have been numerous apparent reversals to solitary behaviour in halictid bees (Danforth, 2002). The tendency for halictid bees to revert to solitary nesting may have a fairly simple biological basis. Because all eusocial halictid bees pass through a temporary solitary phase in the life cycle (the foundress phase), reversals to solitary nesting could arise simply by reversion from a multivoltine life cycle (with one or more worker broods followed by a reproductive brood) to a univoltine life cycle (with just the reproductive brood). In polymorphic species, wherein the solitary phenotype is most often displayed in high altitudes or latitudes, females may encounter environmental signals (temperature, day length, etc.) similar to those in late-season eusocial colonies that cause a switch from worker production to reproductive brood (see Hirata and Higashi, 2008; Kamm, 1974; Yanega, 1993). Reversals to solitary nesting appear to become much less

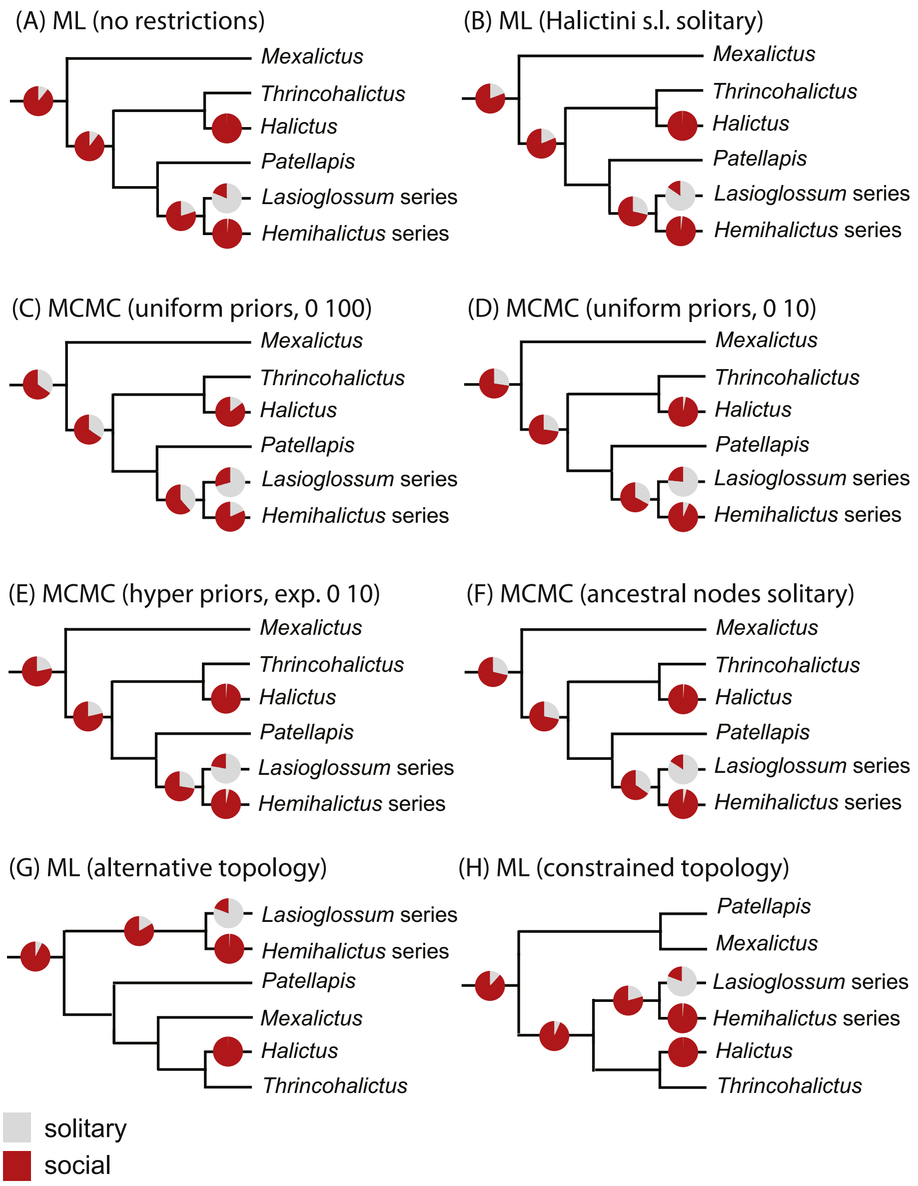
(A) ML (no restrictions)
(B) ML (Halictini s.l. solitary)
(C) MCMC (uniform priors, 0 100)
(D) MCMC (uniform priors, 0 10)
(E) MCMC (hyper priors, exp. 0 10)
(F) MCMC (ancestral nodes solitary)
(G) ML (alternative topology)
(H) ML (constrained topology)

Fig. 4. Summary of ancestral state reconstruction results for the tribe Halictini based on maximum likelihood and Markov Chain Monte Carlo. Unless otherwise stated topologies are based on analyses with data partitioned by genome/codon/intron and reversals from cleptoparasitism are prohibited. (A) ML with no restrictions. (B) ML with common ancestor of Halictini s.l. fixed as solitary. (C) MCMC using uniform, unconstrained priors on the interval 0–100 (D) MCMC using uniform prior on the interval 0–10 based on ML results. (E) MCMC using hyper prior exponential seeded from uniform on interval 0–10. (F) MCMC as in (F) with Halictinae, Augochlorini, Halictini s.l., Caenohalictini and Thrinchostomini fixed as solitary. (G) ML using topology recovered from gene/codon/intron partitioned data set. (H) ML using constrained topology matching previous studies. Red indicates eusociality, black solitary behaviour.

likely in advanced eusocial groups of Apidae (Cardinal and Danforth, 2011), where queens cannot survive without workers and new nests are founded by a reproductive queen and a group of workers (swarm founders). Allodapine bees are totipotent but have apparently never undergone a reversal from eusocial to solitary nesting, perhaps due to their progressive provision of larva (Chenoweth et al., 2007). A recent study using model-based ancestral state reconstruction supports a single origin of social behaviour in xylocopine bees rather than multiple origins (Rehan et al., 2012), a result that mirrors our own and suggests origins of eusociality in bees are less frequent than previously believed.

4.2. Incongruence of phylogenetic topologies

Generic-level relationships within Halictini were found to differ among analyses of our own data set (as a consequence of differing partitioning schemes; Fig. 2B and C) as well as based on previous studies (Brady et al., 2006b; Danforth et al., 2004). These two previous studies resolved *Lasioglossum* and *Halictus* + *Thrincohalictus* as sister clades (Fig. 2A). A subsequent study based on three genes analysed with parsimony resolved *Lasioglossum* as sister taxon to the remaining Halictini (Danforth et al., 2008). Our phylogeny, based on improved taxon sampling and more genes, found *Lasioglossum* to be sister to *Patellapis.* The relationship of *Mexalictus* with the remaining Halictini was unstable in our study, varying depending on nucleotide substitution model and partitioning scheme. Given this uncertainty in tree topology, we performed our BayesTraits analysis on three alternative topologies. In all cases, the probability that the most recent common ancestor of *Lasioglossum* and *Halictus* was eusocial was greater than the probability that their most recent common ancestor was solitary. Since model-based methods favour a single origin of eusociality for *Lasioglossum* and *Halictus* + *Thrincohalictus* even when these taxa are not resolved as monophyletic, our conclusions would remain largely unaffected even if the topology of the Halictini *s. s.* were to change. Behavioural data from *Patellapis* is extremely limited; only a single communal species has been identified (Timmermann and Kuhlmann, 2008) and the nesting biology of *Mexalictus* is unknown. Our results would be further supported should future behavioural studies of *Patellapis* or *Mexalictus* find evidence of eusociality. The genus *Mexalictus* was particularly unstable in our analyses, possibly due to the long-branch created by the limited species-level sampling for this genus. We hope a new revision of *Mexalictus* (Dumesh, in press) will encourage additional sampling efforts that can provide material for future phylogenetic studies. The various topologies recovered for the Halictini *s. s.* and the large amount of missing behavioural data for the tribe and other halictid bees contribute to the remaining uncertainty in ancestral state reconstructions. Our hypothesis testing using BayesTraits failed to reject the possibility of independent origins of eusociality with the Halictini *s. s.* so additional phylogenetic and behavioural study is needed.

4.3. Subgeneric classification of Lasioglossum

The subgeneric classification of the massive genus *Lasioglossum s.l.* is problematic in part due to insufficient understanding of the phylogenetic limits of the constituent subgenera. Our study provides new understanding of *Lasioglossum* phylogeny, particularly in the *Lasioglossum* series. We found strong support for polyphyly of *Lasioglossum s. s.* and *Ctenonomia* (*sensu* Michener, 2007). We therefore recognise the additional subgenera *Leuchalictus* (formerly a synonym of *Lasioglossum s. s.*) and *Ipomalictus* and *Rubrihalictus* (formerly synonyms of *Ctenonomia*). We also found that *Pseudochilalictus* renders *Parasphecodes* paraphyletic, demonstrating that the former name should be synonymised.

Many outstanding difficulties remain in the *Hemihalictus* series of *Lasioglossum*, including the limits of the common subgenera *Evylaeus* and *Dialictus* and whether these names should even be applied (Gibbs et al., 2009; ICZN, 2011). Our results support placing *Sellalictus* within *Dialictus s.l.* Of particular interest are two South African species which form the sister-group to all other members of the *Hemihalictus* series. Additional taxon sampling to include more subgenera and species-groups is required to further clarify the relationships within *Lasioglossum*.

4.4. Summary and future work

Ancestral state reconstruction of social behaviour using model-based methods supports the possibility of a single shared origin for *Halictus* and *Lasioglossum*. This would imply that the initial origin of eusociality in the Halictini is older than previously believed (Brady et al., 2006b). Social behaviour in the *Lasioglossum* series would therefore represent a reacquisition of eusociality rather than a completely *de novo* origin, placing these bees in a unique position in the study of this major evolutionary transition. Understanding the polarity of evolutionary changes in crucial for interpreting more detailed studies of social evolution, whether behavioural, physiological, or genomic.

Our results support the monophyly of five tribes within the Halictinae. The phylogenetic placement of the Thrinchostomini is found to be closer to Sphecodini and Halictini *s. s.* than previous studies suggested. This places two largely Neotropical taxa, Augochlorini and Caenohalictini, as the basal most lineages of the sweat bees. The potential impact of this topology on interpretation of halictid biogeography requires additional study. Some uncertainty remains if the sister taxon of Halictini *s. s.* is Sphecodini or Thrinchostomini; although, based on host-parasite relationships between Halictini and Sphecodini, we expect the former. Our results clearly suggest changes to the subgeneric classification of *Lasioglossum* are necessary. Additional taxon sampling in the taxonomically complex *Hemihalictus* series is needed to resolve long-standing difficulties in the classification of this group.

Additional behavioural studies of particular taxa where data are missing would be beneficial for improved understanding of social evolution in halictid bees. In particular, *Patellapis*, additional members of the *Lasioglossum* series (e.g. *Rubrihalictus*), and basal clades of the *Hemihalictus* series. Comparative genomic studies focused on social evolution in Hymenoptera should be careful to take the patterns of social transitions among halictid bees found above, and the remaining uncertainty, into account during experimental design and the interpretation of results. A multivariate analysis to determine which traits underlie the development of eusociality in these bees is a goal of future research.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2012.08.013.

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