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Phylogeny of the bee genus *Lasioglossum* (Hymenoptera: Halictidae) based on mitochondrial COI sequence data

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Abstract. The bee genus *Lasioglossum* Curtis is a model taxon for studying the evolutionary origins of and reversals in eusociality. This paper presents a phylogenetic analysis of *Lasioglossum* species and subgenera based on a data set consisting of 1240 bp of the mitochondrial cytochrome oxidase I (COI) gene for seventy-seven taxa (sixty-six ingroup and eleven outgroup taxa). Maximum parsimony was used to analyse the data set (using PAUP*4.0) by a variety of weighting methods, including equal weights, *a priori* weighting and *a posteriori* weighting. All methods yielded roughly congruent results. Michener's *Hemihalictus* series was found to be monophyletic in all analyses but one, while his *Lasioglossum* series formed a basal, paraphyletic assemblage in all analyses but one. *Chilalictus* was consistently found to be a basal taxon of *Lasioglossum sensu lato* and *Lasioglossum sensu stricto* was found to be monophyletic. Within the *Hemihalictus* series, major lineages included *Dialictus* + *Paralictus*, the acarinate *Evylaeus* + *Hemihalictus* + *Sudila* and the carinate *Evylaeus* + *Sphecodogastra*. Relationships within the *Hemihalictus* series were highly stable to altered weighting schemes, while relationships among the basal subgenera in the *Lasioglossum* series (*Lasioglossum s.s.*, *Chilalictus*, *Parasphecodes* and *Ctenonomia*) were unclear. The social parasite of *Dialictus*, *Paralictus*, is consistently and unambiguously placed well within *Dialictus*, thus rendering *Dialictus* paraphyletic. The implications of this for understanding the origins of social parasitism are discussed.

Introduction

Genus *Lasioglossum* Curtis includes at least 1000 described species from all continents of the world except Antarctica (Table 1). The monophyly of *Lasioglossum sensu lato* is supported by the weakened 2nd r-m and 2nd m-cu cross veins in females of all species (Fig. 1A,B). This character, while present also in the related Indoaustralian genus *Homalictus* Cockerell (Fig. 1D), appears to be an unreversed synapomorphy of *Lasioglossum*, and monophyly of the genus has not been seriously questioned. All other halictine bees, excluding *Lasioglossum* and *Homalictus*, have well sclerotized 2nd r-m and 2nd m-cu veins in both sexes (Fig. 1C).

The subgeneric groupings within *Lasioglossum* are numerous and are treated by some authors as separate genera (see Krombein *et al*. 1979; Moure & Hurd, 1987) because they comprise such speciose and behaviourally diverse taxa. However, in Europe *Lasioglossum* is treated as a genus consisting of many subgenera (Ebmer, 1987). For the purposes of this paper, I will refer to genus *Lasioglossum* and its numerous subgenera, primarily because, while *Lasioglossum s.l.* is most likely monophyletic, the groupings within *Lasioglossum* (e.g. *Evylaeus*, *Lasioglossum s.s.*, *Ctenonomia*) are potentially paraphyletic and even possibly polyphyletic (*Paralictus*). Michener (1998) divided the subgenera of *Lasioglossum* into two groups: the *Hemihalictus* series, which includes all subgenera with a weakened 1st r-m cross vein (Fig. 1B) in females, and the *Lasioglossum* series, which includes all subgenera with a completely sclerotized 1st r-m cross vein (Fig. 1A). The *Hemihalictus* series includes *Acanthalictus* Cockerell, *Austrevylaeus* Michener, *Evylaeus* Robertson, *Hemihalictus* Cockerell, *Paradialictus* Pauly, *Dialictus* Robertson (including *Paralictus* Robertson), *Sellalictus* Pauly, *Sphecodogastra* Ashmead and *Sudila* Cameron

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Table 1. Classification of the subgenera of *Lasioglossum*.

Subgenus (no. species)	Distribution
Acanthalictus (1)	Siberia
Australictus (11)	Australia
Austrevylaeus (9)	Australia & New Zealand
Callalictus (8)	Australia (Victoria to N. Queensland)
Chilalictus (134)	Australia
Ctenonomia (>100)	Palaeotropical
Dialictus (>300)*	Nearctic/Neotropical
Evylaeus (>100)*	Holarctic/Tropical
Glossalictus (1)	Western Australia
Hemihalictus (1)	Nearctic
Lasioglossum s.s. (>150)*	Holarctic and Mesoamerican
Paradialictus (1)	Africa (Zaire)
Paralictus (3) †	Nearctic
Parasphecodes (92)	Australia & New Guinea
Pseudochilalictus (1)	Australia (New South Wales, Queensland)
Sellalictus (11)	Africa (Zaire to Cape Prov.)
Sphecodogastra (8)	Nearctic
Sudila (6)	Sri Lanka and Malaysia

*Indicates subgenera with both solitary and eusocial species.
† Indicates cleptoparasitic subgenera.

(Table 1). The *Lasioglossum* series includes *Australictus* Michener, *Callalictus* Michener, *Chilalictus* Michener, *Ctenonomia* Cameron, *Glossalictus* Michener, *Lasioglossum* Curtis *s.s.*, *Parasphecodes* Smith and *Pseudochilalictus* Michener. I use Michener's terminology and refer to the *Hemihalictus* and *Lasioglossum* series below. These informal groupings will be evaluated in light of the results of the present study.

Lasioglossum has historically been considered one of the model taxa for studying the evolutionary origins of eusocial behaviour (Michener, 1974) because at least two subgenera contain both solitary and eusocial members: *Dialictus*, *Evylaeus* and perhaps *Lasioglossum s.s.* The social behaviour of *Dialictus* and *Evylaeus* is reviewed in Michener (1990) and Packer (1993). Other subgenera, *Chilalictus* and *Parasphecodes* in Australia (Knerer & Schwarz, 1976, 1978; Kukuk & Schwarz, 1987, 1988; Kukuk & Crozier, 1990; Kukuk, 1992; Kukuk & Sage, 1994; M. P. Schwarz, personal communication) and *Sudila* in Sri Lanka and southern Asia (Sakagami *et al*., 1996), contain mostly communal species. Subgenera containing primarily solitary members include *Hemihalictus* (Daly, 1961), *Sphecodogastra* (Kerfoot, 1967a,b; Knerer & MacKay, 1969; Bohart & Youssef, 1976; McGinley, 1999), and most species of *Lasioglossum s.s.* (Yanega, 1997; Wcislo, 1997a), as

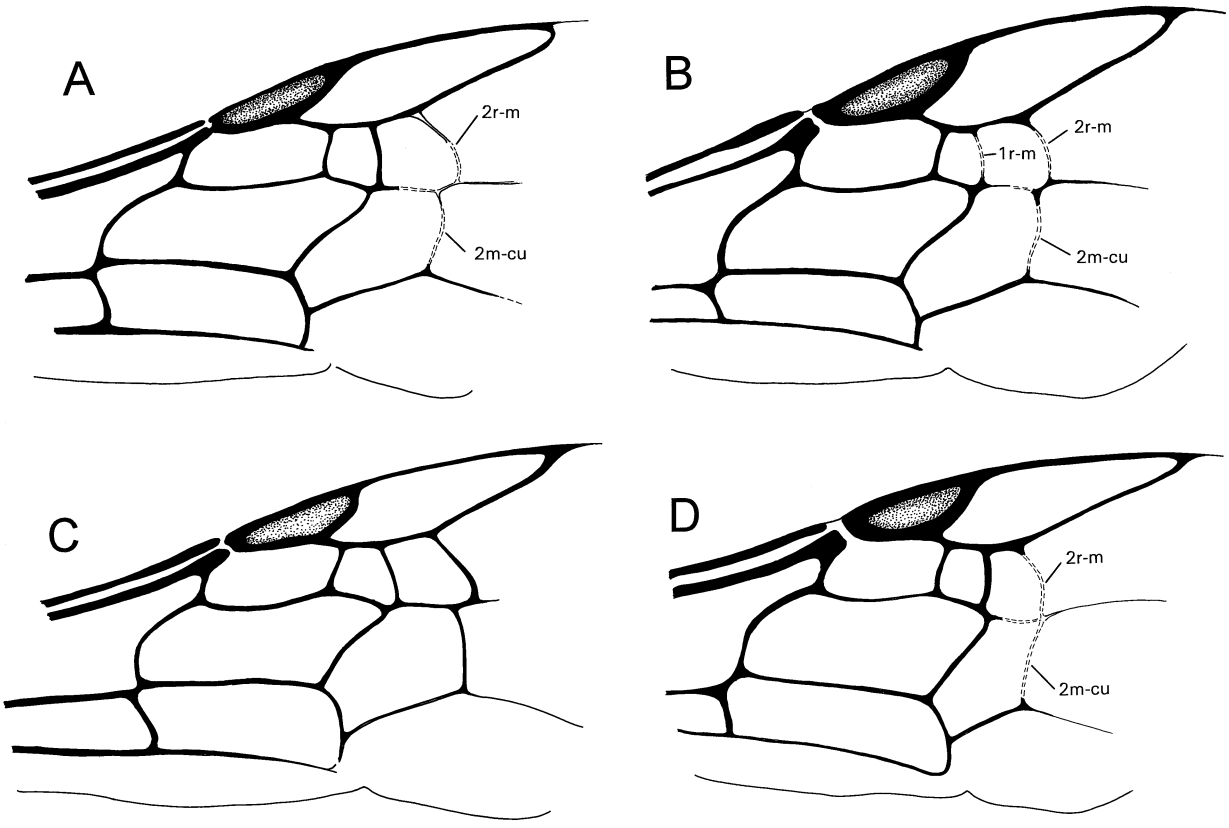

Fig. 1. Wing venation in female halictine bees. A, *L.* (*Lasioglossum*) *leucozonium*; B, *L.* (*Dialictus*) *zephyrum*; C, *Ruizantheda nigrocaerulea*; D, *Homalictus fijiensis*.

well as some *Ctenonomia* and *Chilalictus* (McConnell-Garner & Kukuk, 1997). In addition, one subgenus, *Paralictus*, contains socially parasitic species that enter the nests of eusocial *Dialictus* and lay eggs (Wcislo, 1997b). Such a diversity of social systems makes *Lasioglossum* an ideal group for investigating the evolutionary origins and losses of social behaviour.

As pointed out by McConnell-Garner & Kukuk (1997), to put the behavioural data into historical perspective we will need 'strongly supported phylogenetic hypotheses concerning the relationships among the subgenera of *Lasioglossum*'. However, attempts to trace the evolutionary history of social behaviours within *Lasioglossum s.l.* have been hampered because of a poor understanding of subgeneric and species-level phylogenetic relationships within the group. Many subgenera are potentially paraphyletic and at present we have no phylogenetic analysis at the subgeneric level. One of the major reasons for the inability of previous workers to reconstruct evolutionary relationships within *Lasioglossum* has been the limited number of discretely variable morphological traits that are amenable to cladistic analysis.

The goal of the present study is to examine whether DNA sequence data from the mitochondrial cytochrome oxidase I (COI) gene can provide phylogenetic resolution at the level of the subgenera and species. COI is a slowly evolving mitochondrial protein-coding gene that has been used in a wide variety of insects at the generic and subgeneric levels (Simon *et al*., 1994). The rate of sequence substitution in third positions is roughly equivalent to other mitochondrial protein-coding genes, but the rate of non-synonymous substitution is considerably slower (Simon *et al*., 1994). While it was not possible to obtain representatives of all subgenera, this study includes specimens from all the major subgenera. Of the eighteen widely recognized subgeneric groupings (five of which are monotypic), I included at least one member of nine of these groups and have sampled extensively within the three major North American and European subgenera *Dialictus*, *Evylaeus* and *Lasioglossum s.s*., as well as the major Australian subgenus, *Chilalictus* (Walker, 1995).

Materials and methods

Bees for this study were obtained by the author or generously provided by collaborators in the U.S.A. and other countries (listed in Acknowledgements). Bees were preserved primarily in 95% EtOH but recently collected pinned specimens and frozen specimens were also used. Pinned specimens older than 3–5 years were not suitable for DNA extractions, but those collected more recently provided good quality, high molecular weight DNA for PCR. Outgroup and ingroup taxa included in this study, locality data, specimen voucher numbers and GenBank Accession numbers are listed in Table 2. Species names and author affiliations were checked against the following sources: Moure & Hurd (1987) for the Western Hemisphere, Rasmont *et al*. (1995) and Westrich (1989) for Europe, Cardale (1993) and Walker (1995) for Australia.

Two previously unnamed species were used in this study: *Lasioglossum* (*Ctenonomia*) B4 and *L.* (*Ctenonomia*) NDA1-(A) (both kindly provided by Penelope Kukuk). *Ctenonomia* is not a clearly defined subgenus and some species resemble species of *Lasioglossum s.s.* While these two species are referred to here as *Ctenonomia*, in fact only *L.* (*Ctenonomia*) NDA1-(A) is, in the opinion of this author, a true *Ctenonomia*. Both species are currently being described by Kenneth Walker. Voucher specimens were deposited in the Cornell University Insect Collection.

DNA extractions followed standard protocols. Specimens were briefly frozen in liquid nitrogen and ground in individual 1.5-ml Eppendorf tubes in the presence of 2x CTAB extraction buffer (Saghai-Maroof *et al*., 1984; Doyle & Doyle, 1987, 1990) and 100 µg of proteinase K. Tubes were incubated for 2 h at 55°C, homogenates were extracted with chloroform-isoamylalcohol, digested for 30 min in the presence of 10 µg RNase, and then extracted again with phenol-chloroform-isoamylalcohol and chloroform-isoamylalcohol, in that order. The DNA was precipitated with 2.5 volumes of ice-cold ethanol and 1/10 volume 3 M sodium acetate, washed once in 80% ethanol and resuspended in 50 µl Tris-EDTA (pH 7.6) buffer.

Double-stranded PCR products for sequencing were generated using Promega (Madison, Wisconsin) Taq DNA polymerase and a series of insect mitochondrial primers located within the COI gene or in the flanking tRNA gene (tRNA-leucine; Crozier & Crozier, 1993; see Table 3 for a list of primers used). Two sets of PCR products were used to generate the data set presented below. First, Jerry-Pat produced a 900-bp PCR product that amplified consistently and could be sequenced accurately by automated sequencing. For the upstream (5') end of COI, I used either Ron-Pat or Ron-Madeline. This region amplified less well and not all taxa could be amplified using these primers. The region flanked by Ron-Madeline was sequenced by manual sequencing as described below. *Lasioglossum* (*Hemihalictus*) *lustrans* could not be amplified with either Ron-Madeline or Ron-Pat and a new primer was developed based on comparisons of four taxa that appeared closely related to *L.* (*H.*) *lustrans* based on Jerry-Pat sequences: *L.* (*Evylaeus*) *pectoralis*, *L.* (*Evylaeus*) *morio*, *L.* (*Evylaeus*) *puncticolle* and *L.* (*Evylaeus*) *villosulum*. The new primer (HemiFor; Table 3) in combination with Pat produced a bright band of the expected size and was used in manual sequencing. All PCR amplifications were carried out following standard PCR protocols (Palumbi, 1996), with the following cycle conditions: 94°C, 1 min denaturation; 50–52°C, 1 min annealing; 72°C, 1 min 15 s extension.

Considerable effort was made to avoid contamination of PCR reactions: (1) all glassware and pipettors were cleaned with a dilute solution of sodium hypochlorite on a regular basis (Prince & Andrus, 1992); (2) separate areas of the lab and separate pipettors were used for DNA extractions, DNA amplification, PCR product purification and manual sequencing; (3) negative controls were included in all sets of reactions and, when positive (a rare occurrence), all PCR reactions were discarded (Austin *et al*., 1997).

Prior to sequencing, PCR products were gel-purified in low-melting point agarose gels (FMC, Rockland, Maine) overnight at 4°C. DNA was recovered from ≈ 400 mg gel slices using the Promega Wizard PCR Preps DNA Purification kit. PCR

Table 2. Taxa included in this study, collecting localities, specimen voucher codes and GenBank accession numbers.

Species	Locality	Voucher code	GenBank accession
Outgroup taxa			
Agapostemon kohliellus (Vachal)	Dominican Republic	Agko12	AF102833
Agapostemon sericeus (Forster)	Ithaca vicinity, New York, U.S.A.	Agse162	AF102834
Agapostemon tyleri (Cockerell)	Portal, Arizona, U.S.A.	Agty230	AF102835
Agapostemon viequescensis Cockerell	Puerto Rico	Agvi4	AF102836
Halictus (*Seladonia*) *confusus* Smith	Junius Ponds, New York, U.S.A.	Haco301	AF102837
Halictus (*Halictus*) *farinosus* Smith	Logan, Utah, U.S.A.	Hafa25	AF102838
Halictus (*Halictus*) *ligatus* Say	Rock Hill, South Carolina, U.S.A.	Hali(c)	AF102840
Halictus (*Halictus*) *poeyi* Lepeletier	Marathon, Florida, U.S.A.	Hapo1	AF102839
Halictus (*Halictus*) *poeyi* Lepeletier	Rock Hill, South Carolina, U.S.A.	Hapo(d)	AF102841
Halictus (*Halictus*) *rubicundus* (Christ)	Missoula, Montana, U.S.A.	Haru32	AF102842
Mexalictus arizonensis Eickwort	Miller canyon, Arizona, U.S.A.	Mxaz97	AF102843
Sphecodes minor Robertson	Sydney, Nova Scotia, Canada	Spmi21	AF102844
Ingroup taxa			
L. (*Chi.*) *convexum* (Smith)	Cobboboonee S.F., Victoria, Australia	Chcv156	AF103951
L. (*Chi.*) *conspicuum* (Smith)	Cobboboonee S.F., Victoria, Australia	Chcs155	AF103952
L. (*Chi.*) *cognatum* (Smith)	Cobboboonee S.F., Victoria, Australia	Chcg317	AF103953
L. (*Chi.*) *erythrurum* (Cockerell)	6 km E. SA/WA border, S. Australia	Chey308	AF103954
L. (*Chi.*) *florale* (Smith)	6 km E. SA/WA border, S. Australia	Chfl320	AF103955
L. (*Chi.*) *lanarium* (Smith)	Cobboboonee S.F., Victoria, Australia	Chla316	AF103956
L. (*Chi.*) *mediopolitum* (Cockerell)	6 km E. SA/WA border, S. Australia	Chmd291	AF103957
L. (*Chi.*) *mirandum* (Cockerell)	Bluff Knoll, Stirling Range NP, W. Australia, Australia	Chmi319	AF103958
L. (*Chi.*) *parasphecodum* (Walker)	6 km E. SA/WA border, S. Australia	Chps318	AF103959
L. (*Ctenonomia*) NDA(1)-A	Cobboboonee S.F., Victoria, Australia	Ctsp297	AF103960
L. ('*Ctenonomia*') B4	Cobboboonee S.F., Victoria, Australia	Ctsp153	AF103961
L. ('*Ctenonomia*') B4	S. Australia, Australia	Ctsp397	AF103962
L. (*Dialictus*) *cressonii* (Robertson)	Ontario, Canada	Dicr66	AF103963
L. (*Dialictus*) *cupreicolle* (Friese)	Republic of Panama	Dicu321	AF103964
L. (*Dialictus*) *gundlachii* (Baker)	Puerto Rico	Digu48	AF103965
L. (*Dialictus*) *hyalinum* (Crawford)	Mt. Lemmon, Arizona, U.S.A.	Diha277	AF103966
L. (*Dialictus*) *imitatum* (Smith)	Ithaca, New York, U.S.A.	Diim27	AF103967
L. (*Dialictus*) *parvum* (Cresson)	Puerto Rico	Dipa7	AF103968
L. (*Dialictus*) *pilosum* (Smith)	Junius Ponds, New York, U.S.A.	Dipi71	AF103969
L. (*Dialictus*) *rohweri* (Ellis)	Junius Ponds, New York, U.S.A.	Dirh79	AF103970
L. (*Dialictus*) *tegulare* (Robertson)	Junius Ponds, New York, U.S.A.	Ditg81	AF103971
L. (*Dialictus*) *umbripenne* (Ellis)	Republic of Panama	Dium322	AF103975
L. (*Dialictus*) *vierecki* (Crawford)	Junius Ponds, New York, U.S.A.	Divi67	AF103972
L. (*Dialictus*) *zephyrum* (Smith)	Junius Ponds, New York, U.S.A.	Dizp74	AF103973
L. (*Dialictus*) *zephyrum* (Smith)	Auburn, Alabama, U.S.A.	Ditr329	AF103974
L. (*Evylaeus*) *albipes* (Fabricius)	Les Eyzies, Dordogne, France (social)	Eval99	AF103976
L. (*Evylaeus*) *albipes* (Fabricius)	Longemer & Col de la Schlucht, Vosges, France (solitary)	Eval104	AF103977
L. (*Evylaeus*) *apristum* (Vachal)	Mt.Sanbe, Shimane Prefecture, Japan	Evap145	AF103978
L. (*Evylaeus*) *boreale* Svensson	Inuvik, NWT, Canada	Evbo262	AF103979
L. (*Evylaeus*) *calceatum* (Scopoli)	Les Eyzies, Dordogne, France	Evca105	AF103980
L. (*Evylaeus*) *cinctipes* (Provancher)	Ithaca, New York, U.S.A.	Evci311	AF103981
L. (*Evylaeus*) *comagenense* Knerer & Atwood	Sydney, Nova Scotia, Canada	Evco255	AF103982
L. (*Evylaeus*) *duplex* (Dalla Torre)	Sendai, Miyagi Prefecture, Japan	Evdu142	AF103983
L. (*Evylaeus*) *fulvicorne* (Kirby)	Ventoux, Vaucluse, France	Evfu310	AF103984
L. (*Evylaeus*) *gattaca* Danforth & Wcislo	Chiriquí Province, Republic of Panama	Evsp234	AF104639
L. (*Evylaeus*) *laticeps* (Schenck)	Les Eyzies, Dordogne, France	Evla117	AF103985
L. (*Evylaeus*) *lineare* (Schenck)	Pont-Saint-Vincent, Meurthe et Moselle, France	Evli137	AF103986
L. (*Evylaeus*) *marginatum* (Brullé)	Les Eyzies, Dordogne, France	Evmg108	AF103987
L. (*Evylaeus*) *malachurum* (Kirby)	Les Eyzies, Dordogne, France	Evml111	AF103988
L. (*Evylaeus*) *morio* (Fabricius)	Les Eyzies, Dordogne, France	Evmo148	AF103989
L. (*Evylaeus*) *nigripes* (Lepeletier)	Beaumont du Ventoux, Vaucluse, France	Evng129	AF103990

Table 2. Continued.

Species	Locality	Voucher code	GenBank accession
L. (*Evylaeus*) *pauxillum* (Schenck)	Vienna, Austria	Evpa131	AF104634
L. (*Evylaeus*) *pectorale* (Smith)	Florida, U.S.A.	Evpe10	AF104635
L. (*Evylaeus*) *politum* (Schenck)	Les Eyzies, Dordogne, France	Evpo122	AF104636
L. (*Evylaeus*) *puncticolle* (Morawitz)	Les Eyzies, Dordogne, France	Evpu128	AF104637
L. (*Evylaeus*) *quebecense* (Crawford)	no locality data	Evqu325	AF104638
L. (*Evylaeus*) *subtropicum* Sakagami	Iriomote Is., Okinawa Prefecture, Japan	Evsu139	AF104640
L. (*Evylaeus*) *truncatum* (Robertson)	Ithaca, New York, U.S.A.	Evtr312	AF104641
L. (*Evylaeus*) *villosulum* (Kirby)	Les Eyzies, Dordogne, France	Evvi125	AF104642
L. (*Hemihalictus*) *lustrans* (Cockerell)	Bastrop, Texas, U.S.A.	Helu186	AF104643
L. (*Lasioglossum*) *callizonium* (Pérez)	Berja, Almeria Prov., Spain	Laca380	AF104644
L. (*Lasioglossum*) *coriaceum* (Smith)	no locality data	Laco15	AF104645
L. (*Lasioglossum*) *desertum* (Smith)	Rose Canyon Lake, Arizona, U.S.A.	Lade251	AF104646
L. (*Lasioglossum*) *discum* (Smith)	France	Ladi313	AF104647
L. (*Lasioglossum*) *fuscipenne* (Smith)	Michigan, U.S.A.	Lafu65	AF104648
L. (*Lasioglossum*) *laevigatum* (Kirby)	Les Eyzies, Dordogne, France	Lala23	AF104649
L. (*Lasioglossum*) *lativentre* (Schenck)	Les Eyzies, Dordogne, France	Lalt120	AF104650
L. (*Lasioglossum*) *leucozonium* (Schrank)	Les Eyzies, Dordogne, France	Lale133	AF104651
L. (*Lasioglossum*) *leucozonium* (Schrank)	Ithaca vicinity, New York, U.S.A.	Lale170	AF104652
L. (*Lasioglossum*) *majus* (Nylander)	France	Lamj314	AF104653
L. (*Lasioglossum*) *pavonotum* (Cockerell)	Point Reyes Natl. Sea Shore, California, U.S.A.	Lapa339	AF104654
L. (*Lasioglossum*) *sexnotatum* (Kirby)	Morigny-Champigny, Essonne, France	Lasx136	AF104655
L. (*Lasioglossum*) *sisymbrii* (Cockerell)	Chiricahua Mts., Arizona, U.S.A.	Lasi253	AF104656
L. (*Lasioglossum*) *titusi* (Crawford)	Twentynine Palms, California, U.S.A.	Lati167	AF104657
L. (*Lasioglossum*) *zonulum* (Smith)	Ithaca, New York, U.S.A.	Lazo284	AF104658
L. (*Paralictus*) *asteris* Mitchell	Ithaca, New York, U.S.A.	Paas30	AF104659
L. (*Parasphecodes*) *hybodinum* (Cockl.)	6 km E. SA/WA border, S. Australia, Australia	Pahy299	AF104660
L. (*Sphecodogastra*) *noctivagum* Linsley & MacSwain	Monahans Sand Hills, Texas, U.S.A.	Stno258	AF104661
L. (*Sphecodogastra*) *oenotherae* (Stevens)	Ithaca, New York, U.S.A.	Stoe54	AF104662
L. (*Sudila*) *alphenum* (Cameron)	Hakgala Botanical Garden, NE District, Sri Lanka	Sual390	AF104663

Table 3. Primers used in PCR amplification of mitochondrial cytochrome oxidase I and II genes in *Lasioglossum* and related genera.

Primer name	Sequence	Position*
Sense primers		
Ron	5'-GGATCACCTGATATAGCATTCCC-3'	2049
HemiFor	5'-CGAATAAA[T/C]AATATAAGATTTTG-3'	2073
Jerry	5'-CAACATTTATTTTGATTTTTTGG-3'	2481
Antisense primers		
Madeline	5'TTCTTTTTTT[T/A/C]CC[T/A]CTTTC[A/G]TT[A/G]AA-3'	2585
Pat	5'-TCCAATGCACTAATCTGCCATATTA-3'	3380

*Positions based on the 5′ end of the primer in the honey bee, *Apis mellifera* (Crozier & Crozier, 1993).

products purified in this way provided consistently good sequencing results either by manual or by automated sequencing.

For manual sequencing I used ^{33}P-labelled dideoxy chain termination reactions (Thermo Sequenase radiolabelled terminator cycle sequencing kit; Amersham Inc, Cleveland, Ohio) and standard 8% polyacrylamide gel electrophoresis, as indicated in the Amersham product manual. Labelled products were run out on both a short (2 h) and a long (4 h) gel. Glass plates were cleaned regularly with cerium oxide to remove imperfections (Millard & de Couet, 1995; Rhodite 90, Universal Photonics, Inc., Hicksville, New York) and one plate was treated with RainX (Unelko, Scottsdale, Arizona). The short and long runs were broadly overlapping and allowed 450 bp to be read in a single direction. All Ron-Madeline sequencing was done in both directions. Automated sequen-

Table 4. Base composition in COI (1240 total nucleotide positions; eighty-two sequences total).

	A	C	G	T	*P*
First position	32.9	14.1	19.1	33.9	1.0
Second position	20.6	22.3	14.1	43.0	1.0
Third position	47.4	9.1	0.6	42.9	0.000
Overall	33.6	15.2	11.2	40.0	0.999

cing of Jerry-Pat PCR products was performed on an ABI 377 automated sequencer available through the Cornell Automated Sequencing Facility. Results from automated sequencing routinely exceeded 700 bp and Jerry-Pat PCR products were sequenced in both directions, such that ≈ 95% of the sequence data was confirmed in both directions. Alignment of DNA sequences was unambiguous, with no insertion/deletion mutations detected within the COI gene. One species, *L.* (*Evylaeus*) *lineare*, had a large A/T-rich insert located between the 3′ end of COI and the tRNA-leucine. This region was excised for the purposes of alignment with other species included in this study. Overall I sequenced a total of 1240 bp for seventy-seven species, five of which were represented by more than one locality (giving a total of eighty-two OTUs). The region sequenced includes positions 2072–3311 in *Apis mellifera* (Crozier & Crozier, 1993), or 79.5% of the COI gene (1560 total nucleotide positions in *Apis*).

Phylogenetic analyses of nucleotide and amino acid sequences were performed using test versions of PAUP*4 (PAUP versions 4.0d59, 4.0d60, 4.0d61 and 4.0d64; D. Swofford, personal communication; see Swofford, 1993, for details on earlier versions of the program). For parsimony analyses I used heuristic search with TBR branch swapping, random addition sequence for taxa, ten cladograms retained per search and fifty replicates per search. In order to evaluate the extent to which the data set as a whole and specific partitions of the data set were hierarchically structured, I used the permutation tail probability (PTP) test as implemented in PAUP*4 with 100 replicates (Archie, 1989a,b; Faith, 1990, 1991; Faith & Cranston, 1991). I experimented with a variety of weighting methods, including *a posteriori* weighting, *a priori* weighting by codon position and transition/transversion weighting. These weighting methods are described in detail below. Boostrap analysis (Felsenstein, 1985) was used to evaluate nodal support. Bootstrap values were calculated based on 100 replicates with ten random sequence additions per replicate. MACCLADE version 3.07 (Maddison & Maddison, 1992) was used to map characters on cladograms and to investigate alternative cladogram topologies.

Results

Alignment

The eighty-two sequences were aligned using MEGALIGN in the Lasergene software package (DNASTAR Inc., Madison, Wisconsin). *Apis mellifera* (Crozier & Crozier, 1993) was included as a reference to determine the reading frame of the sequences. Alignments were unambiguous and no insertion/deletions were observed (alignments are available from the author).

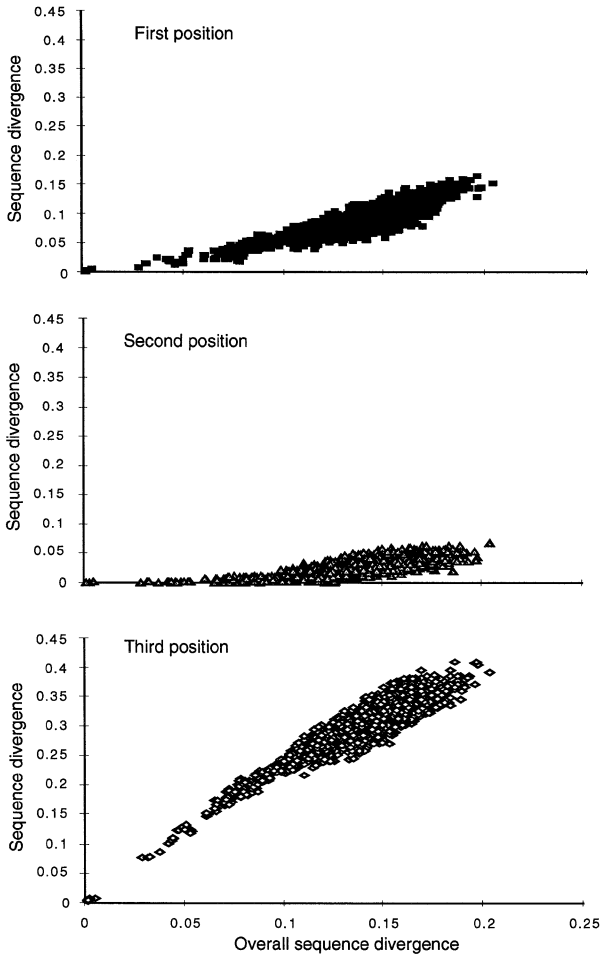

Fig. 2. Divergence in first, second and third positions as a function of overall sequence divergence. Divergence values are uncorrected *P*-values.

Base composition

The base composition for the eighty-two sequences included in this study is given in Table 4. Base composition was overall A/T-biased, as in most insect (especially holometabolan) mitochondrial sequences (Simon *et al*., 1994; Frati *et al*., 1997). This was particularly true of third positions, which showed significant heterogeneity among taxa in base composition.

Sequence divergence

Prior to cladogram construction I investigated the relative sequence divergence in each of the three codon positions. For

first positions, sequence divergence ranged from 0.00 to 0.1646; for second positions, sequence divergence ranged from 0.00 to 0.0678; and for third positions, sequence divergence ranged from 0.0024 to 0.4106. Overall sequence divergence ranged from 0.0016 to 0.2042. Figure 2 shows the divergence in each position with respect to overall (uncorrected) sequence divergence.

Phylogenetic analysis

In all analyses presented below I included twelve outgroup taxa in the following genera: *Halictus* Latreille, *Agapostemon* Guérin-Méneville, *Sphecodes* Latreille and *Mexalictus* Eickwort (Table 2). These are reasonable choices for outgroup taxa, as subfamily Halictinae is clearly monophyletic (Danforth, unpublished data) and these are all genera that are common or present (*Mexalictus*) in the north-temperate regions. Of the 1240 nucleotide positions included in the alignment, 524 were parsimony informative. Of the 524 parsimony informative characters, 114 were first positions (21.8%), forty-five were second positions (8.6%) and 365 were third positions (69.6%).

In order to evaluate classes of positions, I initially analysed the data set partitioned into first, second and third positions alone. This provided a rough idea of the levels of homoplasy in the three data partitions. In each case the data sets were analysed by unweighted parsimony. In an analysis of the 413 first positions 4355 equally parsimonious cladograms were obtained (CI = 0.1738; 1056 steps). A similar analysis was performed on the 413 second positions (> 17 000 cladograms; CI = 0.3722; 201 steps) and on the 414 third positions (4572 cladograms; CI = 0.1403; 4572 steps). It is clear from comparisons of data partitions that first, second and third positions differ in their overall levels of homoplasy, with second positions considerably less homoplasious than either first or third, and third positions the most homoplasious of all. These differential levels of homoplasy are used below in an exploratory analysis of character weighting.

In order to evaluate character congruence of the data set as a whole and individual codon positions, I used the PTP (Archie, 1989a,b; Faith, 1990, 1991; Faith & Cranston, 1991) to test for significant hierarchical structure. For the overall data set and each codon position the results were highly significant ($P < 0.01$ in all cases). The differences between the shortest cladogram obtained in the unpermuted data and the shortest cladogram obtained in the 100 permuted replicates was 1963 steps for the overall data set, 446 steps for first positions, 154 steps for second positions and 1108 steps for third positions. The data set shows significantly more character congruence overall and for each codon position than would be expected by chance for a data set of this nucleotide composition.

In an initial analysis of the complete data set I applied equal weights to all characters and obtained six equally parsimonious cladograms after fifty replicates of random sequence addition (Fig. 3). Cladogram statistics are summarized in Table 5. While some cladograms supported monophyly of the *Hemihalictus* series, in others strong-veined subgenera (*Parasphecode* + *Ctenonomia*) fell within the weak-veined group (*Hemihalictus* series). Other aspects of this cladogram are highly congruent with current views of *Lasioglossum* relationships: *Chilalictus* forms a monophyletic basal lineage, *Dialictus* forms a monophyletic group from which *Paralictus* arises and *Evylaeus* is paraphyletic with respect to *Sphecodogastra*, *Hemihalictus*, *Sudila* and *Dialictus*+*Paralictus*.

Table 5. Descriptive cladogram statistics for parsimony analyses.

Nucleotide data	CI[1]	RI[2]	No. steps	No. cladograms
Unweighted	0.1509	0.4345	5932	6
Successive approx.	0.2732	0.6163	425.4	1
2:2:1 weighting	0.1585	0.4618	7249	7
2:5:1 weighting	0.1718	0.4919	7932	7
5:5:1 weighting	0.1712	0.5030	11170	33
10:10:1 weighting	0.1806	0.5305	17651	12
ts:tv (1:2)	0.1420	0.4735	8781	1

[1] Consistency index excluding uninformative characters.
[2] Retention index.

In order to resolve ambiguous relationships and to impose weights that are based on character congruence, characters were reweighted by the rescaled consistency index via successive approximations character weighting (Farris, 1969; Carpenter, 1988). The single cladogram obtained (Fig. 4) supports monophyly of the *Hemihalictus* series, *Chilalictus* monophyly, *Lasioglossum s.s.* monophyly (including '*Ctenonomia*' B4) and indicates a sister-group relationship between *Parasphecodes*+*Ctenonomia* and the *Hemihalictus* series.

In order to accomodate for variation in the levels of homoplasy among first, second and third positions various weighting schemes were applied based on codon position. The graphs relating sequence divergence for each codon position to overall divergence (Fig. 2) indicate that third positions show much higher levels of divergence than first and second positions. Likewise, the parsimony analyses of each codon position indicated that the levels of homoplasy were considerably lower for second positions as compared to either first or third positions. The following weighting schemes were employed in order to span a range of possible weights: 2:2:1 (first:second:third position), 2:5:1, 5:5:1 and 10:10:1. The goal of *a priori* weighting in this case was to evaluate the robustness of specific aspects of the cladogram.

Relationships similar to those shown in Fig. 4 for the *Hemihalictus* series were obtained with 2:2:1, 2:5:1, 5:5:1 and 10:10:1 weights (cladogram statistics shown in Table 5). However, altered weighting schemes had a marked effect on the relationships among the basal subgenera, such as *Chilalictus*, *Lasioglossum s.s.*, *Parasphecodes* and *Ctenonomia*. Relationships obtained under 2:5:1 weights (Fig. 5B) were the same as those obtained under successive approximations, while under 2:2:1 weights subgeneric relationships were unresolved (Fig. 5C), and under 5:5:1 and 10:10:1 weights (Fig. 5D) the data supported monophyly of the *Lasioglossum* series (contrary to all other analyses, Fig. 5A–C).

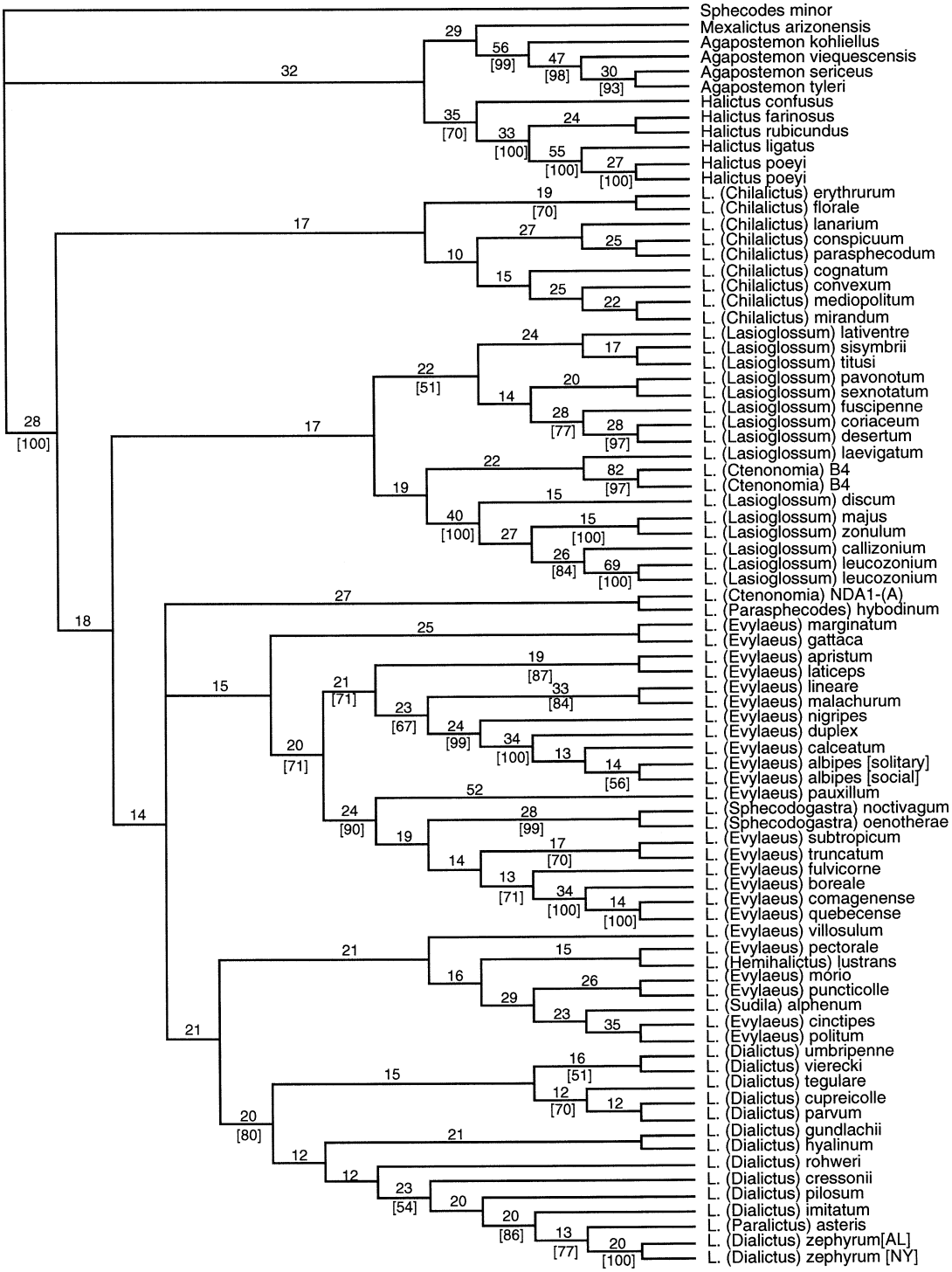

Fig. 3. Strict consensus cladogram based on analysis of unweighted nucleotide data (1240 nucleotide positions; 524 parsimony informative characters). Outgroup taxa included *Agapostemon kohliellus*, *A. viequescensis*, *A. sericeus*, *A. tyleri*, *Mexalictus arizonensis*, *Sphecodes minor*, *Halictus* (*Seladonia*) *confusus*, *Halictus* (*Halictus*) *farinosus*, *H.* (*H.*) *rubicundus*, *H.* (*H.*) *ligatus* and *H.* (*H.*) *poeyi*. Ingroup monophyly was constrained in all analyses. The analysis was run fifty times with random sequence addition. Six equally parsimonious cladograms were obtained (see Table 5 for cladogram statistics). Numbers above branches indicate branch lengths (based on tree no. 1) and numbers in brackets below the branches indicate bootstrap support based on 100 bootstrap replicates.

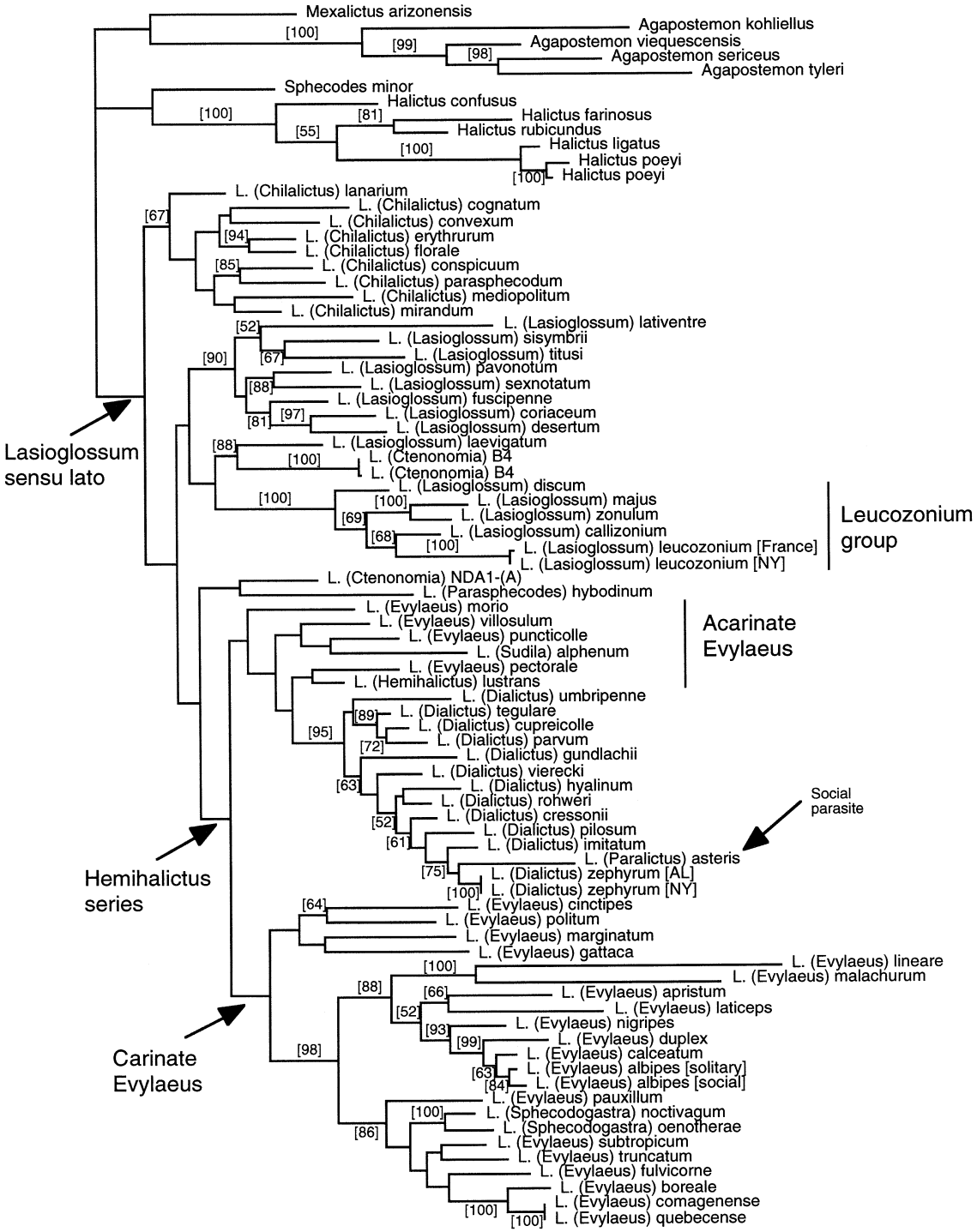

Fig. 4. Cladogram obtained after successive approximations character weighting (1240 nucleotide positions; 524 parsimony informative characters). Outgroup taxa as in Fig. 3. The analysis was run fifty times with random sequence addition after the initial character weights were determined (see Table 5 for cladogram statistics). Numbers in brackets indicate bootstrap support based on 100 bootstrap replicates.

Transition/transversion ratios of 1.0 or less generally indicate saturation of transitional changes and thus justify downweighting of transitions. Transition/transversion (ts:tv) ratios were estimated using a maximum likelihood approach in which the observed ts:tv ratio for the overall data and for each position was calculated independently using a Hasegawa–

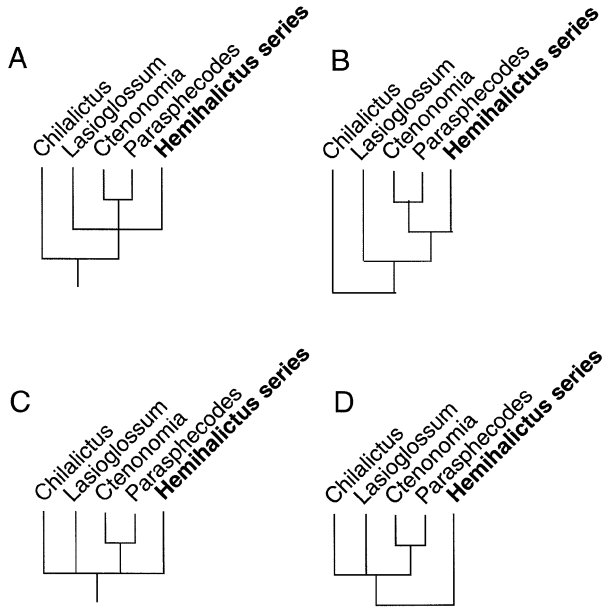

Fig. 5. Alternative cladogram topologies for the *Lasioglossum* series of subgenera included in this analysis (see Table 5 for cladogram statistics). A, Based on unweighted nucleotide data; B, based on successive approximations character weighting, 2:5:1 weights, and downweighting of transitions; C, based on 2:2:1 weights; D, based on 5:5:1 and 10:10:1 weights.

Kishino–Yano model (Hasegawa *et al*., 1985) of sequence evolution, empirically determined base frequencies and the cladograms obtained under unweighted parsimony. This approach indicated that the ts:tv ratio was 1.077 for all the data, while it differed among positions: first position = 2.127; second position = 0.8059; third position = 1.887. These results justified downweighting of transitions relative to transversions (Holmquist, 1983; DeSalle *et al*., 1987; Larson, 1994; Sullivan *et al*., 1995). The cladogram obtained under two-fold downweighting of transitions (Fig. 6) is highly congruent with the cladograms obtained under successive approximations (Fig. 5B): relationships among the basal subgenera are identical to those obtained under successive approximations and relationships within the *Hemihalictus* series are highly congruent with those shown in Fig. 4.

In summary, cladograms obtained under various weighting schemes are highly congruent within the *Hemihalictus* series but basal relationships among members of the *Lasioglossum* series are unstable in the current data set (alternative topologies are summarized in Fig. 5). Within the *Hemihalictus* series major lineages include *Dialictus* (+ *Paralictus*) and carinate *Evylaeus* + *Sphecodogastra*. The acarinate *Evylaeus* either formed the sister group to *Dialictus* (Fig. 3) or a paraphyletic grade from which *Dialictus* arose (Figs 4, 6). The cladogram obtained under successive approximations is considered to be the most accurate representation of relationships based on the current data set, both because the weights applied to the data were obtained based on observed levels of homoplasy among positions, and because this cladogram recovers monophyly of lineages that are considered strongly supported by morphology. This cladogram is also highly congruent with the cladograms obtained under 2:5:1 weights and downweighting of transitions relative to transversions (Fig. 6).

One of the strongest conclusions of this study is that *Paralictus*, a social parasite of *Dialictus*, is nested well within the host clade. In all analyses *L.* (*Paralictus*) *asteris* was placed within *Dialictus*, as closely related to the clade including *L.* (*D.*) *zephyrum*, *L.* (*D.*) *imitatum*, *L.* (*D.*) *pilosus* and *L.* (*D.*) *cressonii*. *Paralictus* therefore makes *Dialictus* paraphyletic (in fact *Paralictus* is the older name for this group; see Michener, 1995). The implications of this for understanding the origins of social parasitism are discussed below.

Discussion

Phylogenetic results

The best way to evaluate the results presented above is to consider them in light of adult morphology and previous phylogenetic studies of the subgenera. There is, in general, a great deal of congruence between the cladograms presented here and morphological characters. First, in all analyses except unweighted parsimony, there was unambiguous support for the monophyly of the 'weak veined' *Lasioglossum*. These are all the species with a weakened 1st r-m cross vein in females (Fig. 1A,B). Michener (1999) refers to this group as the *Hemihalictus* series and it includes *Dialictus* (including *Paralictus*), *Evylaeus*, *Hemihalictus*, *Sphecodogastra* and *Sudila*, as well as several subgenera not included in the present study, such as *Austrevylaeus*, *Acanthalictus* and *Sellalictus* (Table 1).

The group of subgenera not included within the *Hemihalictus* series, *Lasioglossum s.s*., *Chilalictus*, *Parasphecodes* and *Ctenonomia*, was found to be paraphyletic in all analyses except when third positions were heavily downweighted (Fig. 5). The mtCOI data therefore fail to support the monophyly of the *Lasioglossum* series. This is not surprising given that there are no obvious morphological synapomorphies for the group. The *Lasioglossum* series is an informal grouping of subgenera, rather than a well defined higher taxon within *Lasioglossum*.

Within the *Lasioglossum* series the relationships implied by the mtCOI data set are reasonably congruent with morphology. Subgenus *Chilalictus*, which is clearly monophyletic (Walker, 1995), is united by a highly distinctive labral shape with stout marginal setae in females. In all analyses presented here I obtained a monophyletic *Chilalictus*, and found some evidence that *Chilalictus* may be a basal lineage of *Lasioglossum* (Fig. 5A,B).

Subgenus *Lasioglossum* in all cladograms appears to consist of two major lineages. First, a primarily Old World lineage including *L.* (*L.*) *leucozonium* and *L.* (*L.*) *zonulum* (both of which occur in North America and Europe) and the exclusively Palaearctic species, *L.* (*L.*) *discum*, *L.* (*L.*) *majus* and *L.* (*L.*)

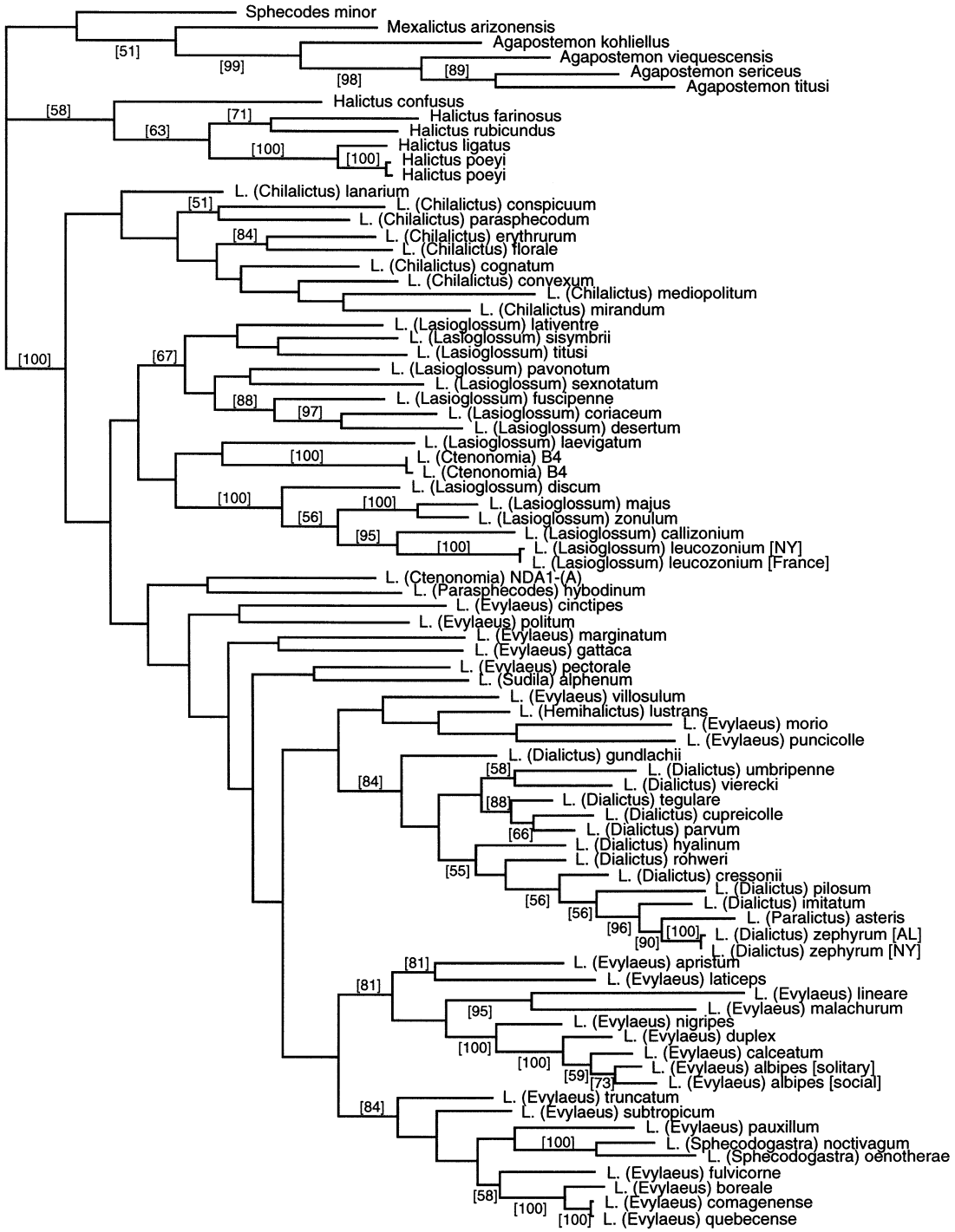

Fig. 6. Single most parsimonious cladogram obtained with transversions weighted twice transitions (1240 nucleotide positions; 524 parsimony informative characters). Outgroup taxa as in Fig. 3. The analysis was run fifty times with random sequence addition (see Table 5 for cladogram statistics). Numbers in brackets indicate bootstrap support based on 100 bootstrap replicates.

callizonium. Packer (1998) refers to these species [plus *L.* (*L.*) *albocinctum*, *L.* (*L.*) *aegyptiellum* and *L.* (*L.*) *subopacum*] as the *L. leucozonium* species group. Based on a study of adult morphology he found the group to be united by three characters: a patch of erect setae on the male S6 (his character 63), a flattened apical gonostylus (his character 76) and ventral retrorse lobes of the gonostylus lacking (his character 78). Females of the *L. leucozonium* group can be recognized by the

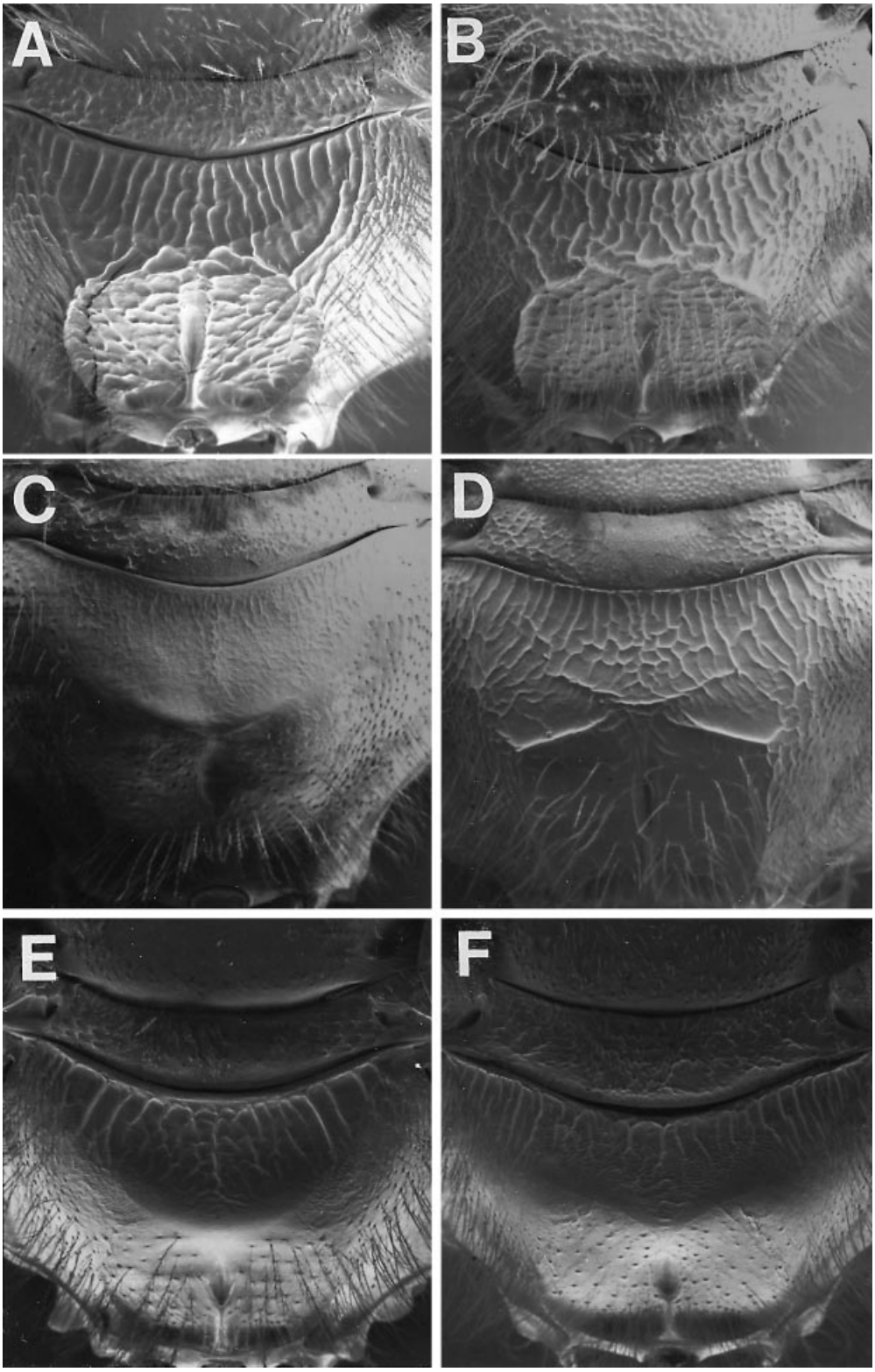

Fig. 7. Scanning electron micrographs of the metanotum and proprodeum in selected species of genus *Lasioglossum*. A, *L.* (*Lasioglossum*) *leucozonium*; B, *L.* (*Lasioglossum*) *zonulum*; C, *L.* (*Lasioglossum*) *coriaceum*; D, *L.* (*Evylaeus*) *albipes*; E, *L.* (*Hemihalictus*) *lustrans*; F, *L.* (*Dialictus*) *versatum*.

relatively short and coarsely sculptured propodeal dorsal area (Fig. 7A,B). In all analyses presented here, this group was monophyletic and formed the sister group to *L.* (*Lasioglossum*) *laevigatum* and *L.* ('*Ctenonomia*') B4.

The second lineage of *Lasioglossum s.s.* is a mixed European and North American lineage that includes *L.* (*L.*) *sexnotatum*, *L.* (*L.*) *lativentre* (European species) plus *L.* (*L.*) *titusi*, *L.* (*L.*) *sisymbrii*, *L.* (*L.*) *coriaceum*, *L.* (*L.*) *pavonotum*, *L.* (*L.*) *fuscipenne* and *L.* (*L.*) *desertum* (all North American species). Most of the species included in this group have a weakly sculptured propodeal dorsal area that is long in relation to the metanotum (Fig. 7C). The relationships within *Lasioglossum s.s.* are roughly congruent with McGinley's (1986) conclusions. For example, the mtCOI data recovered a monophyletic group, including the species *L.* (*L.*) *desertum* and *L.* (*L.*) *coriaceum*, with an acarinarium on the first metasomal tergum of females (McGinley's node 8; Figs 67, 68).

Within the *Hemihalictus* series of subgenera (supported by the weak 1st r-m cross vein in females; Fig. 1B) relationships are most stable to various weighting schemes. In fact, nearly the same cladogram topology is obtained irrespective of the weighting scheme employed (Figs 3, 4, 6). In all analyses the North and Central American *Dialictus* were found to be monophyletic (with the inclusion of *Paralictus*; see below). While there is limited morphological evidence for *Dialictus* monophyly (all North American species possess weak metallic colouration over the head and thorax and most species have a weakly developed propodeal carina; Fig. 7E), the molecular data strongly support the monophyly of *Dialictus* + *Paralictus*. The placement of *Paralictus*, a socially parasitic subgenus, well within *Dialictus* (its host) makes good biological and morphological sense. *Paralictus* are very similar in overall appearance to *Dialictus* but lack all the structures associated with pollen collecting. Some authors have treated *Paralictus* as a genus, equal in status to *Lasioglossum s.l.* (Michener *et al*., 1994), which clearly does not reflect its status as a derivative of *Dialictus*. Surprisingly, the one species of *Paralictus* included in this study, *L.* (*Paralictus*) *asteris*, did not come out as sister to its host, *L.* (*Dialictus*) *imitatum* (Wcislo, 1997b), but as sister to *L.* (*D.*) *zephyrum* (although this relationship was not supported by high bootstrap values; Fig. 4).

According to all the cladograms obtained in this study, subgenus *Evylaeus* is massively paraphyletic. Some species of *Evylaeus*, *L.* (*E.*) *morio*, *L.* (*E.*) *puncticolle*, *L.* (*E.*) *villosulum* and *L.* (*E.*) *pectoralis*, are more closely related to North American *Dialictus* than they are to the remaining species of *Evylaeus*, the 'carinate' *Evylaeus* of Packer (1991) and Ebmer (1987, 1995). [I follow the European workers in not recognizing *L.* (*E.*) *morio* as a *Dialictus*, in spite of the fact that it has metallic colouration; Rasmont *et al*. (1995).] These species, as well as numerous other species, have been variously referred to as the 'black *Dialictus*' (Michener, 1990) and the 'acarinate *Evylaeus*' (Packer, 1991). They are typically small, all black, but some have weak metallic coloration, like *L.* (*E.*) *morio*, with a weak propodeal carina, such that the dorsal and posterior surfaces are not distinctly separated (Fig. 7E). In all analyses the acarinate *Evylaeus* form a paraphyletic grade from which *Dialictus* arises. Because of the morphological similarities between acarinate *Evylaeus* and *Dialictus*, Michener (1998) transfers the acarinate *Evylaeus* to *Dialictus*.

The placement of *Hemihalictus* (an apomorphic, oligolectic species with two submarginal cells) and *Sudila* (a subgenus endemic to Sri Lanka and southern Asia) within the acarinate *Evylaeus* is reasonable based on morphological considerations such as the lack of a propodeal carina (Fig. 7F) and their black (*Hemihalictus*) and weakly metallic (*Sudila*) integument. In none of the cladograms obtained here did *L.* (*E.*) *morio* form the sister group to North American *Dialictus*. This supports Ebmer's (1987) assertion that metallic coloration has arisen multiple times within the *Hemihalictus* series. While no previous work has suggested affinities between the acarinate *Evylaeus*, *Hemihalictus* and *Sudila*, these results are reasonable given the morphology of adults. More species of acarinate *Evylaeus* will be needed to fully resolve relationships within this complex and speciose group.

The remaining lineage of the *Hemihalictus* series includes the carinate *Evylaeus* and *Sphecodogastra* (Fig. 4). This group was supported in the analysis based on successive approximations character weighting and 2:5:1 weights. Most species of *Evylaeus* in this group are medium- to large-sized halictine bees with a complete propodeal carina and a relatively coarsely sculptured dorsal surface to the propodeum (Fig. 7D; Ebmer, 1995). Subgenus *Sphecodogastra* includes species that are all oligolectic on Onagraceae and have a modified femoral scopa. Some species, e.g. *L.* (*S.*) *noctivagum* and *L.* (*S.*) *texanum*, are large, nocturnal or crepuscular bees with huge ocelli (referred to as the Texana group by McGinley, 1999). Other members of *Sphecodogastra*, e.g. *L.* (*S.*) *oenotherae*, are smaller, all black and more like true *Evylaeus* in overall appearance (referred to as the 'Lusoria' group by McGinley, 1999). Linsley & MacSwain (1962) and Knerer & MacKay (1969), for example, considered *L.* (*S.*) *oenotherae* a true *Evylaeus*. McGinley (1999) recently revised the eight species of *Sphecodogastra* and concluded that, even with the inclusion of species like *L.* (*S.*) *oenotherae*, the subgenus is monophyletic, which is in agreement with the results presented here. Morphological synapomorphies of the group include the highly modified femoral scopa (in females), the long sternal vestiture (in males) and the central depression of sternum VI (in males; McGinley, 1999).

The *L. calceatum* species group (*sensu* Ebmer, 1995; including *L.* (*E.*) *albipes*, *L.* (*E.*) *nigripes*, *L.* (*E.*) *calceatum* and *L.* (*E.*) *duplex*) are all very large *Evylaeus* with a distinctly sculptured propodeum (Fig. 7D). The relationships within the carinate *Evylaeus* were investigated previously by Packer (1991, 1997). Based on allozyme data he reconstructed the relationships among eight species, all of which were included in the present study. His results are congruent in many ways with the cladograms obtained here. Both studies found support for the following monophyletic groups: *L.* (*E.*) *lineare* + *L.* (*E.*) *malachurum*; *L.* (*E.*) *albipes* + *L.* (*E.*) *calceatum*; monophyly of *L.* (*E.*) *lineare*, *L.* (*E.*) *malachurum*, *L.* (*E.*) *albipes*, *L.* (*E.*) *calceatum* and *L.* (*E.*) *laticeps*. The only major discrepancy between the two studies is in the relationships among *L.* (*E.*) *marginatum*, *L.* (*E.*) *pauxillum* and *L.* (*E.*) *fulvicorne*.

According to Packer's cladogram *L.* (*E.*) *marginatum* is nested well within the carinate *Evylaeus*, while in my results, *L.* (*E.*) *marginatum* is either a basal member of the carinate *Evylaeus* (Fig. 4) or a basal branch of the *Hemihalictus* series (Fig. 6).

For some species I sequenced individuals from more than one locality. Specimens of *L.* (*L.*) *leucozonium*, a holarctic species, were collected in New York State (U.S.A.) and in central France. COI sequences from these two widely separated localities were virtually identical (0.161% sequence divergence), supporting the hypothesis that *L.* (*L.*) *leucozonium* is indeed a single widely distributed species. Likewise, specimens of *L.* (*D.*) *zephyrum* were obtained and sequenced from New York state and Alabama (U.S.A.). These sequences were virtually identical as well (0.00 to 0.162% sequence divergence). Finally, specimens of *L.* (*E.*) *albipes* were obtained from two populations in France; one of which was eusocial and the other solitary (Plateaux-Quénu, 1989, 1992, 1993; specimens generously provided by Cecile Plateaux-Quénu). The divergence between these two populations was fairly high (2.825% sequence divergence) and could reflect distinct species status (Vogler *et al*., 1993; Packer & Taylor, 1997). However, more extensive geographical sampling will be needed to confirm this. Widely distributed halictine bees can potentially represent multiple, distinct sibling species, in spite of the fact that they appear identical morphologically (Blanchetot & Packer, 1992; Carman & Packer, 1996; Packer & Taylor, 1997; Danforth *et al*., 1998).

The origin of social parasitism

The behaviour of socially parasitic *Paralictus* is very similar to a form of social parasitism that occurs in other bees (see below), polistine wasps (Carpenter, 1997; Carpenter *et al*., 1993) and ants (Buschinger, 1986; Hölldobler & Wilson, 1990; Bourke & Franks, 1991), termed inquilinism. In bees, social parasites are known from the genus *Psithyrus* (Apidae: Bombini), as well as the allodapine bees (Michener, 1970, 1974). In *Psithyrus*, females enter the nests of their hosts and co-exist with the host queen, or, in some cases, kill her (Fisher, 1987, 1988; Franks, 1987; Fisher & Sampson, 1992; Küpper & Schwammberger, 1995). Both hosts and parasites reproduce, but host eggs produced after infiltration of the parasite do not reach adulthood (Küpper & Schwammberger, 1995), and levels of aggression between host and parasite appear related to the extent of host offspring production (Fisher, 1987). In allodapine bees, host and parasite coexist as well (Reyes & Michener, 1990), and parasites functionally replace the host queen in egg production (Batra *et al*., 1993), as in *Psithyrus*. In inquiline ants, a parasitic female enters the nest of the host and coexists with the host queen without producing workers herself. The host workers continue to forage and to rear reproductive offspring of the inquiline, who later disperse and mate (Bourke & Franks, 1991).

The origins of social parasitism in all these groups are similar: social parasites arise from within the host clade, either as sister to their hosts, e.g. in some allodapine bees (Michener, 1974) and some ants (Bourke & Franks, 1991), or as a distinct, monophyletic lineage that renders the host group paraphyletic (bees and social wasps). Distinguishing between these alternative hypotheses requires a well resolved phylogeny for the parasites as well as their hosts. Recent evidence, based both on morphological (Williams, 1994) and molecular (Pedersen, 1996) studies, indicates that *Psithyrus* is monophyletic and has arisen from within the genus *Bombus*, thus rendering *Bombus* paraphyletic. Such a pattern also applies to parasitic allodapine bees (Reyes & Schwarz, 1998) in that *Inquilina* arises from within *Exoneura*, its host. However, socially parasitic species have also arisen multiple times within *Braunsapis*, *Allodape*, *Allodapula* and *Macrogalea*, from closely related non-parasitic forms (Michener, 1974; Reyes & Michener, 1990; Batra *et al*., 1993). In polistine wasps, social parasites (*Sulcopolistes*) form a monophyletic group that arises from within the host lineage (Carpenter *et al*., 1993; Choudhary *et al*., 1994; Carpenter, 1997). In ants, social parasitism has arisen multiple times and inquilinism in particular seems to follow Emery's rule in that inquilines attack closely related species (Bourke & Franks, 1991).

The phylogenetic placement of *Paralictus* conforms to the loose form of Emery's rule rather than the strict form (as distinguished by Bourke & Franks, 1991), because *L.* (*Paralictus*) *asteris* is not sister to its host, *L.* (*D.*) *imitatum*, but is included within the same clade. Indeed, the origin of *Paralictus* from within the eusocial *Dialictus* emphasizes the similarity between social parasitic life cycles and eusocial ones. At present, without additional species of *Paralictus*, one cannot assess the likelihood of either the intra- or the interspecific models of speciation (outlined in Bourke & Franks, 1991; Choudhary *et al*., 1994).

Future work

The mtCOI data set analysed here shows a great deal of promise for resolving relationships within the subgenera of *Lasioglossum* and within and among the subgenera included in the *Hemihalictus* series. Relationships at these levels are stable to altered weighting schemes and the results are congruent with previous phylogenetic studies. However, as far as resolving the relationships among the basal subgenera of *Lasioglossum*, the current data set is weak. Altered weighting schemes yielded different cladogram topologies, particularly among the basal subgenera. One intriguing possibility suggested by the current study is that the Australian subgenus *Chilalictus* might represent a basal branch of genus *Lasioglossum*. Data on elongation factor-1α gene sequences (Danforth & Ji, 1998) are currently being collected for species included in this study as well as species of genus *Homalictus*, which may belong within *Lasioglossum*.

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