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A comprehensive phylogeny of the bumble bees (*Bombus*)

S. A. CAMERON[1]*, H. M. HINES[1] and P. H. WILLIAMS[2]

[1]*Department of Entomology, 320 Morrill Hall, 505 S. Goodwin Avenue, University of Illinois, Urbana, IL 61801, USA*
[2]*Department of Entomology, Natural History Museum, South Kensington, London SW7 5BD, UK*

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Bumble bees (*Bombus* Latreille) occupy a wide diversity of habitats, from alpine meadows to lowland tropical forest, yet they appear to be similar in morphology throughout their range, suggesting that behavioural adaptations play a more important role in colonizing diverse habitats. Notwithstanding their structural homogeneity, bumble bees exhibit striking inter- and intraspecific variation in colour pattern, purportedly the outcome of mimetic evolution. A robust phylogeny of *Bombus* would provide the framework for elucidating the history of their wide biogeographical distribution and the evolution of behavioural and morphological adaptations, including colour pattern. However, morphological studies of bumble bees have discovered too few phylogenetically informative characters to reconstruct a robust phylogeny. Using DNA sequence data, we report the first nearly complete species phylogeny of bumble bees, including most of the 250 known species from the 38 currently recognized subgenera. Bayesian analysis of nuclear (opsin, EF-1α, arginine kinase, PEPCK) and mitochondrial (16S) sequences results in a highly resolved and strongly supported phylogeny from base to tips, with clear-cut support for monophyly of most of the conventional morphology-based subgenera. Most subgenera fall into two distinct clades (*short-faced* and *long-faced*) associated broadly with differences in head morphology. Within the *short-faced* clade is a diverse *New World* clade, which includes nearly one-quarter of the currently recognized subgenera, many of which are restricted to higher elevations of Central and South America. The comprehensive phylogeny provides a firm foundation for reclassification and for evaluating character evolution in the bumble bees. © 2007 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2007, **91**, 161–188.

ADDITIONAL KEYWORDS: classification – corbiculate bees – nuclear genes.

INTRODUCTION

Bumble bees (*Bombus* Latreille) are among the more familiar creatures inhabiting meadows, gardens and grasslands of the temperate world (Darwin, 1859: notes 1854–1861, translated by Freeman, 1968; Sladen, 1912). Robust and vividly coloured, they emerge from hibernation in early Spring in far northerly latitudes, feeding from the earliest willow blossoms before the snows have fully melted. Thermoregulatory mechanisms maintain their body temperature high above ambient in cold to freezing weather (Heinrich, 2004), allowing them full activity under conditions too extreme for other bees. Although they are most abundant in alpine and high-elevation grassland habitats of the northern temperate zone, they range widely from Greenland to the Amazon Basin, from sea level to altitudes of 5800 m in the Himalayas (Williams, 1985). Thus, bumble bees exploit a wide diversity of habitats, from alpine meadows to lowland tropical forest (Sakagami, 1976). Nonetheless, they appear similar in morphology throughout their range (Michener, 1990; Williams, 1998), suggesting that ecological opportunity and behavioural adaptations play an important role in colonizing diverse habitats (Sakagami, 1976; Cameron & Whitfield, 1996; Dornhaus & Cameron, 2003; Dornhaus & Chittka, 2004). For example, temperate species initiate colonies annually from a single queen, whereas the tropical species appear at least facultatively perennial (Sakagami, Akahira & Zucchi, 1967; Olesen, 1989) and even exhibit polygyny (Zucchi, 1973; Cameron & Jost, 1998).

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*Corresponding author. E-mail: scameron@life.uiuc.edu

Despite the apparent constraint on structural diversity, bumble bees display striking inter- and intraspecific variation in colour pattern across their range (Sladen, 1912; Skorikov, 1931; Reinig, 1935, 1939; Tkalců, 1968, 1974, 1989; Williams, 1991, 1998), purportedly the outcome of mimetic evolution (Plowright & Owen, 1980). Not only are *Bombus* predisposed to converge sympatrically on similar colour patterns (Williams, 2007), but other insects mimic the *Bombus* patterns (Evans & Waldbauer, 1982), thus providing excellent opportunities to examine the influence of natural selection on the generation of variation among Müllerian (Plowright & Owen, 1980) and Batesian (Waldbauer, Sternberg & Maier, 1977; Evans & Waldbauer, 1982) mimics. Elucidating the evolution of diversity requires knowledge of the historical pattern of variation, which, in turn, provides unique insights into evolutionary processes underlying the pattern of variation. A robust phylogeny of *Bombus*, the goal of our research, is essential for inferring the history of their wide geographical distribution and for testing hypotheses of behavioural and mimetic adaptations.

CLASSIFICATION AND CURRENT STATUS OF *BOMBUS* PHYLOGENY

Bombus comprises the monotypic tribe Bombini, which together with Apini (honey bees), Meliponini (stingless bees), and Euglossini (orchid bees), constitutes the corbiculate bees, distinguished by the pollen-carrying structure (corbicula) on the hindleg. Their closest relatives, estimated from DNA sequences of multiple genes, are the stingless bees (Cameron, 1993, 2003; Mardulyn & Cameron, 1999; Cameron & Mardulyn, 2001, 2003; Lockhart & Cameron, 2001; Thompson & Oldroyd, 2004), although some morphological data (Roig-Alsina & Michener, 1993; Schultz, Engel & Ascher, 2001) place them as sister to honey bees + stingless bees.

Subsequent to Linnaeus (1758), approximately 2800 formal names, including synonyms, have been assigned to *Bombus* species and lower-ranking taxa (Williams, 1998). Today, we recognize approximately 250 species (Williams, 1998) assigned to 38 subgenera, primarily on the basis of male genitalia (Radoszkowski, 1884; Krüger, 1917; Skorikov, 1922; Richards, 1968). Many of the subgenera are monotypic (Richards, 1968; Williams, 1998) as a result of the distinctness of the male genitalia. The inflated number of species names was often the result of distinguishing species (and later, subspecies) by colour pattern. Numerous alternative classifications have been proposed (Milliron, 1961, 1971, 1973a, b; Tkalců, 1972) but largely disregarded.

Previous research on bumble bee phylogeny was restricted in scope and utility by either: (1) the application of phenetic methods to infer phylogenetic relationships; (2) relatively sparse species sampling; or (3) insufficient numbers of informative characters. Examples of phenetic applications include the investigation by Ito (1985) of male genitalia and analyses of allozymes in regional taxa (Pekkarinen, 1979; Pekkarinen, Varvio-Aho & Pamilo, 1979; Obrecht & Scholl, 1981; Pamilo, Pekkarinen & Varvio, 1987). Williams (1985, 1994) applied parsimony to morphological data to address the higher-level relationships among subgenera, but the available characters led to considerable lack of subgeneric resolution. DNA analyses of relationships have been restricted to regional fauna (analyses of European taxa: Pedersen, 1996, 2002) or have included few species (assessment of *Pyrobombus* monophyly, 10–12 species overall: Koulianos, 1999; analysis of subgenera based on 19 species: Koulianos & Schmid-Hempel, 2000). Kawakita *et al*. (2003, 2004) estimated relationships among many of the subgenera using sequence data, but their sampling of only 76 species limits their ability to draw robust conclusions about the monophyly of groups and the evolution of traits.

Comprehensive molecular studies include examination of relationships within the large New World subgenus *Fervidobombus*, including DNA sequences and morphology, by Cameron & Williams (2003) and sequence analysis of 37 of the 43 recognized *Pyrobombus* species by Hines, Cameron & Williams (2006). In the present study, we report on the full hierarchy of *Bombus* relationships for most of the world fauna, including rare and potentially endangered species such as *Bombus franklini* and *Bombus occidentalis* (Thorp, 2005), exploiting the large number of characters available from DNA sequences collected from multiple genes.

MATERIAL AND METHODS

TAXA EXAMINED

A total of 218 *Bombus* taxa, representing most of the species from all 38 subgenera listed by Williams (1998), was sampled for analysis and their locality, collector, and voucher numbers recorded (Table 1). Intraspecific variants and taxa of controversial species status (Williams, 1998) were included when possible (Table 1). Outgroups were represented by exemplars selected from each of the other tribes of corbiculate bees (Table 1). Identification of taxa received from other collectors was verified by the authors before sequencing. Voucher specimens for all taxa used in the molecular investigation are deposited at the Illinois Natural History Survey, Urbana, Illinois. Additional vouchers from the morphological character examination are maintained by PHW at the Natural History Museum, London.

Table 1. *Bombus* and outgroup taxa examined, their collector, collection localities, voucher numbers, and GenBank accession numbers

Subgenus	Species	Collection locality	Collector	V.#	16S	EF-1α	Opsin	ArgK	PEPCK
Ag *Alpigenobombus* Skorikov (6/5)	*breviceps* Smith	Chiang Mai, Thailand	N. Warrit	190	DQ787983	DQ788165	DQ788320	DQ788408	EF050915
	grahami (Frison)	Meigu, Sichuan, China	T. Ya	273	EF032347	EF03237	EF032389	EF032408	EF050916
	kashmirensis Friese	Hongyuan, Sichuan, China	SC *et al.*	121	DQ788040	DQ788215	DQ788342	DQ788447	EF050913
	nobilis Friese	Qionglai Sh, Sichuan, China	SC *et al.*	098	DQ788071	DQ788245	AY739485^{H}	DQ788473	EF050912
	wurflenii Radoszkowski	Obergurgl, Austria	SC, JW	001	DQ788137	DQ788305	AF493007^{K}	AF492873^{K}	EF050914
Al *Alpinobombus* Skorikov (5/5)	*alpinus* (Linnaeus)	Gurgltal, Austria	SC, JW	029	DQ787963	DQ788146	AY739452^{H}	DQ788393	EF050871
	balteatus Dahlbom	Lappland Co., Sweden	B. Cederberg	039	DQ787974	DQ788157	AY739455^{H}	DQ788402	EF050870
	hyperboreus Schönherr	Dalarna Co., Sweden	B. Cederberg	070	DQ788028	DQ788204	AY739470^{H}	DQ788440	EF050868
	neoboreus Sladen	Alaska, USA	A. Scholl	188	DQ788068	DQ788242	AY739484^{H}	DQ788470	EF050869
	polaris Curtis	Lappland Co., Sweden	H. Elmquist	223	DQ788083	AF492970^{K}	AF493037^{K}	AF492903^{K}	EF050872
Bi *Bombias* Robertson (2/2)	*auricomus* (Robertson)	Illinois, USA	H. Hines	062	DQ787972	DQ788154	AY739454^{H}	AF492892^{K}	EF051014
	nevadensis Cresson	Alberta, Canada	S. Cameron	139	DQ788069	DQ788243	DQ788352	DQ788471	EF051015
Bo *Bombus* Latreille (10/10)	*affinis* Cresson	Illinois, USA	H. Hines	167	DQ787961	DQ788144	AY739451^{H}	DQ788391	EF050860
	cryptarum (Fabricius)*	Erzincan Prov., Turkey	PR *et al.*	127	DQ787995	DQ788175	AY739461^{H}	DQ788416	EF050855
	franklini (Frison)	Oregon, USA	R. Thorp	256	EF032345	EF032366	EF032385	EF032403	EF050861
	hypocrita Pérez	Kyushu, Japan	S. Huang	123	DQ788030	DQ788206	AF493023^{K}	AF492889^{K}	EF050864
	ignitus Smith	Beijing, China	SC *et al.*	096	DQ788031	DQ788207	AF493032^{K}	AF492898^{K}	EF050866
	lucorum (Linnaeus) ♂	E. Pyrenees, France	HH, PR	217	DQ788051	DQ788225	AF493021^{K}	AF492887^{K}	EF050862
	♂	Qionglai Sh, Sichuan, China	SC *et al.*	184	DQ788050	DQ788224	AY739479^{H}	DQ788456	EF050863
	moderatus Cresson*	Alberta, Canada	S. Cameron	163	DQ788062	DQ788236	AY739481^{H}	DQ788464	EF050856
	occidentalis Greene*	New Mexico, USA	SC, JW	025				DQ788476	EF050858
		Montana, USA	S. Garner	026	DQ788074	DQ788248	AY739486^{H}		
	patagiatus Nylander	Hongyuan, Sichuan, China	SC *et al.*	111	DQ788078	DQ788252	AF493020^{K}	AF492886^{K}	EF050857
	sporadicus Nylander	Lappland Co., Sweden	B. Cederberg	193	DQ788108	DQ788279	AY739491^{H}	DQ788501	EF050867
	terrestris (Linnaeus) ♂	San Quírico, Italy	SC, JW	003	DQ788118	DQ788288	AF493022^{K}	AF492888^{K}	EF050865
	terricola Kirby ♂	Ontario, Canada	J. Whitfield	205	DQ788119	DQ788289	AF493019^{K}	AF492885^{K}	EF050859
	tunicatus Smith ♂	Uttaranchal, India	M.Saini	248	DQ788124				
Br *Brachycephalibombus* Williams (2/1)	*brachycephalus* Handlirsch	Jalisco, Mexico	F. Noguera	030	DQ787982				
		Guerrero, Mexico	H. Hines	230		DQ788163	DQ788319	DQ788407	EF050889
Cc *Coccineobombus* Skorikov (2/2)	*baeri* Vachal ♂	Puno, Peru	J. Arcas	174	DQ787973	DQ788156	DQ788317	DQ788401	EF050886
	coccineus Friese	Huarochiri, Peru	C. Rasmussen	137	DQ787991	DQ788172	DQ788323	DQ788413	EF050885
Cf *Confusibombus* Ball (1/1)	*confusus* Schenck ♂	Dorres, France	HH, PR	083	DQ787992	DQ788173	DQ788324	DQ788414	EF051016
Cr *Crotchiibombus* Franklin (1/1)	*crotchii* Cresson	California, USA	D. Zungri	071	DQ787994	AF492973	AF493040^{K}	AF492906^{K}	EF050882

Table 1. *Continued*

Subgenus	Species	Collection locality	Collector	V.#	16S	EF-1α	Opsin	ArgK	PEPCK
Cu *Cullumanobombus* Vogt (4/3)	*apollineus* Skorikov*	Erzurum Prov., Turkey	PR *et al.*	084	DQ787964	DQ788147	DQ788313	DQ788394	EF050890
	rufocinctus Cresson	Alberta, Canada	A. Scholl	186	DQ788097	DQ788268	AF493034^{K}	AF492900^{K}	EF050892
	semenoviellus Skorikov	Brandenburg, Germany	B. Cederberg	236	DQ788101	DQ788271	DQ788369	DQ788494	EF050891
Ds *Dasybombus* Labougle & Ayala (2/2)	*macgregori* Labougle & Ayala	Guerrero, Mexico	R. Ayala	231	DQ788053	DQ788226	DQ788347	DQ788457	EF050881
	handlirschi Friese	Pasco, Peru	D. Takiya	132	DQ788021	DQ788197	DQ788336	DQ788435	EF050887
Dv *Diversobombus* Skorikov (4/4)	*diversus* Smith	Kyushu, Japan	S. Huang	120	DQ788000	AF492961^{K}	AF493028^{K}	AF492894^{K}	EF051010
	longipes Friese	Qionglai Sh, Sichuan, China	SC *et al.*	194	DQ788049	DQ788223	DQ788346	DQ788455	EF051009
	trifasciatus Smith	Himachal Pradesh, India	D. Johnson	015	DQ788122	AF492918^{K}	AF492985^{K}	AF492851^{K}	EF051007
	ussurensis Radoszkowski	S. Korea, Mulan Valley	P. Tripotin	130	AF364829^{B}	AF492919^{K}	AF492986^{K}	AF492852^{K}	EF051008
	wilemani Cockerell*	Meifang, Taiwan	H. Townes	182	DQ788135				
Ev *Eversmannibombus* Skorikov (1/1)	*persicus* Radoszkowski	Kars Prov., Turkey	PR *et al.*	054	DQ788080	DQ788255	DQ788358	DQ788480	EF050935
Ex *Exilobombus* Skorikov (1/1)	*exil* (Skorikov)	Hövsgöl Nuur, Mongolia	NHM	232	DQ788004	DQ788185	DQ788330	DQ788425	EF050962
Fv *Fervidobombus* Skorikov (19/18)	*atratus* Franklin	Ribeirão Preto, Brazil	R. Zucchi	305	AY268398^{C}	DQ788152	AY268376^{C}	DQ788398	EF050943
	bellicosus Smith ♂	Buenos Aires, Argentina	A. Roig	221	AY268399^{C}	DQ788159	AY268377^{C}	DQ788404	EF050956
	brasiliensis Lepeletier	Paraná, Brazil	La Roca	219	AY268400^{C}	DQ788164	AY268378^{C}		EF050950
	californicus Smith*	Alberta, Canada	R. Owen	306	AY268401^{C}	DQ788167	AY268379^{C}	DQ788410	EF050957
	dahlbomii Guérin-Méneville	Arauco Prov., Chile	C. Carlton	016	DQ787996	AF492931^{K}	AF492998^{K}	AF492864^{K}	EF050940
	digressus (Milliron)	R. Grande de Orosí, Mexico	R. Delgado	307	AY268403^{C}	DQ788178	AY268381^{C}	DQ788418	EF050959
	diligens Smith	Jalisco, Mexico	F. Noguera	171	AY268404^{C}	DQ788179	AY268382^{C}	DQ788419	EF050955
	excellens Smith	Aragua, Venezuela	J. Garc'a	308	AY268405^{C}	DQ788184	AY268383^{C}	DQ788424	EF050942
	fervidus (Fabricius)	Missouri, USA	S. Cameron	309	AY268406^{C}	AF492930^{K}	AF492997^{K}	AF492863^{K}	EF050958
	medius Cresson	Chiapas, Mexico	RA, RB	222	AY268407^{C}	DQ788228	AY268385^{C}	DQ788459	EF050951
	mexicanus Cresson	Chiapas, Mexico	RA, RB	220	AY268408^{C}	DQ788232	AY268386^{C}	DQ788461	EF050952
	morio (Swederus)	Porto Alegre, Argentina	D. Wittman	310	AY268409^{C}	DQ788239	AY268387^{C}	DQ788467	EF050941
	opifex Smith	Puno, Peru	J. Arcas	175	DQ788075	DQ788249	DQ788356	DQ788477	EF050954
	pensylvanicus (DeGeer)	Missouri, USA	S. Cameron	317	EF032352	EF032374	EF032392	EF032411	EF050948
		Missouri, USA	S. Cameron	311	EF032351	EF032375	EF032393	EF032412	EF050949
	pullatus Franklin	Pitilla, Costa Rica	S. Cameron	312	AY268411^{C}	DQ788261	AY268389^{C}	DQ788486	EF050945
	sonorus Say*	Arizona, USA	T. Griswold	051	DQ822475	DQ788277	EF032394	DQ788500	EF050946
		Mexico	S. Cameron	318	AY268412^{C}	EF032376	AY268390^{C}	EF032410	EF050947
	steindachneri Handlirsch	Morelos, Mexico	R. Ayala	313	AY268413^{C}	DQ788280	AY268391^{C}	DQ788502	EF050953
	transversalis (Olivier)	Madre de Dios, Peru	S. Cameron	314	AY268414^{C}	DQ788291	AY268392^{C}	DQ788510	EF050944
	trinominatus Dalla Torre	Oaxaca, Mexico	H. Hines	229	DQ788123	DQ788292	DQ788379	DQ788511	EF050960
	weisi Friese	Jalisco, Mexico	F. Noguera	315	AY268415^{C}	DQ788303	AY268393^{C}	DQ788520	EF050961
Fs *Festivobombus* Tkalců (1/1)	*festivus* Smith	Guizhou Prov., China	M. Yun	032	DQ788007	DQ788187			
		Qionglai Sh, Sichuan, China	SC *et al.*	104			AY739465^{H}	DQ788427	EF050910

Fr *Fraternobombus* Skorikov (1/1)	*fraternus* (Smith)	Illinois, USA	H. Hines	183	DQ788013	AF492974^{K}	AF493041^{K}	AF492907^{K}	EF050884
Fn *Funebribombus* Skorikov (1/1)	*funebris* Smith	Abancay, Peru	C. Rasmussen	128	DQ788016	DQ788194	DQ788334	DQ788433	EF050883
Kl *Kallobombus* Dalla Torre (1/1)	*soroeensis* (Fabricius)	E. Pyrenees, France	HH, PR	136	DQ788107	DQ788278	AF493008^{K}	AF492874^{K}	EF051013
Ls *Laesobombus* Krüger (1/1)	*laesus* Morawitz	Kars Prov., Turkey	PR *et al.*	052	DQ788044	DQ788218	DQ788344	DQ788451	EF050936
Mg *Megabombus* Dalla Torre (14/12)	*argillaceus* (Scopoli)	Kayseri Prov., Turkey	PR *et al.*	058	DQ787967	DQ788150	AY739453^{H}	DQ788396	EF051001
	consobrinus Dahlbom ♂	Altai Mts, Kazakhstan	P. Mardulyn	261	EF032354	EF032379	AY267150^{K}	AY267166^{K}	EF050994
	gerstaeckeri Morawitz	E. Pyrenees, France	HH, PR	065	DQ788017	DQ788195	DQ788335	DQ788434	EF051003
	hortorum (Linnaeus)	Toscana, Italy	PR *et al.*	005	DQ788024	DQ788200	AF492987^{K}	AF492853^{K}	EF050999
	koreanus (Skorikov)	Kangwondo, S. Korea	P. Tripotin	277	EF032355	AF492969^{K}	AF493036^{K}	AF492902^{K}	EF050995
	portchinsky Radoszkowski	Artvin Prov., Turkey	PR *et al.*	072	DQ788085	DQ788259	DQ788361	DQ788484	EF051000
	religiosus (Frison)	Qionglai Sh, Sichuan, China	SC *et al.*	141	DQ788091	DQ788264	DQ788364	DQ788489	EF050998
	ruderatus (Fabricius)	Valdivia Prov., Chile	C. Carlton	018	DQ788096	AF492977^{K}	AF493044^{K}	AF492910^{K}	EF051002
	securus (Frison)	Qionglai Sh, Sichuan, China	SC *et al.*	142	DQ788100	DQ788270	DQ788368	DQ788493	EF050997
	supremus Morawitz	Qionglai Sh, Sichuan, China	SC *et al.*	101	DQ788112	DQ788284	DQ788375	DQ788505	EF051004
	sushkini (Skorikov)	Hongyuan, Sichuan, China	SC *et al.*	143	DQ788113	AF492921^{K}	AF492988^{K}	AF492854^{K}	EF050996
	tichenkoi (Skorikov)					AF492922^{K}	AF492989^{K}	AF492855^{K}	
Ml *Melanobombus* Dalla Torre (14/10)	*alagesianus* Reinig*	Artvin Prov., Turkey	PR *et al.*	085	DQ787962	DQ788145	DQ788312	DQ788392	EF050903
	erzurumensis (Özbek)*	Artvin Prov., Turkey	PR *et al.*	126	DQ788003	DQ788183	AY739463^{H}	DQ788423	EF050899
	formosellus (Frison)					AF492939^{K}	AF493006^{K}	AF492872^{K}	
	friseanus Skorikov	Qionglai Sh, Sichuan, China	SC *et al.*	105	DQ788015	DQ788193	AY739467^{H}	DQ788432	EF050906
	incertus Morawitz	Erzurum Prov., Turkey	PR *et al.*	086	DQ788035	DQ788211	DQ788341	DQ788443	EF050901
	keriensis Morawitz	Hongyuan, Sichuan, China	SC *et al.*	114	DQ788041	DQ788216	AY739474^{H}	DQ788448	EF050904
	ladakhensis Richards	Aba, Sichuan, China	SC *et al.*	158	DQ788043	AY739575^{H}	AY739475^{H}	DQ788450	EF050905
	lapidarius (Linnaeus) ♂	San Quírico, Italy	SC, JW	006	DQ788045	DQ788219	AF493005^{K}	AF492871^{K}	EF050902
	miniatus Bingham	Himachal Pradesh, India	M. Saini	244	DQ788059	DQ788233	DQ788348	DQ788462	EF050908
	rufofasciatus Smith	Hongyuan, Sichuan, China	SC *et al.*	133	DQ788098	DQ788269	AY739489^{H}	DQ788492	EF050907
	sichelii Radoszkowski	Obergurgl, Austria	SC, JW	034	DQ788103	DQ788273	DQ788371	DQ788496	EF050900
	simillimus Smith ♂	Himachal Pradesh, India	M. Saini	243	DQ788104	DQ788274	DQ788372	DQ788497	EF050909
Md *Mendacibombus* Skorikov (12/7)	*avinoviellus* (Skorikov) ♂	Himachal Pradesh, India	M. Saini	242	AY268416^{C}	DQ788155	AY268416^{C}	DQ788400	EF051020
	convexus Wang	Qionglai Sh, Sichuan, China	SC *et al.*	109	DQ787993	DQ788174	DQ788325	DQ788415	EF051021
	defector Skorikov					AF492958^{K}	AF493025^{K}	AF492891^{K}	
	handlirschianus Vogt	Artvin Prov., Turkey	PR *et al.*	087	DQ788022	DQ788198	DQ788337	DQ788436	EF051017
	mendax Gerstaecker	Gurgltal, Austria	SC, JW	019	DQ788057	AY739584^{H}	AF493024^{K}	AF492890^{K}	EF051019
	shaposhnikovi Skorikov	Artvin Prov., Turkey	PR *et al.*	099	DQ788102	DQ788272	DQ788370	DQ788495	EF051018
	waltoni Cockerell	Qionglai Sh, Sichuan, China	SC *et al.*	102	DQ788134	DQ788302	DQ788385	DQ788519	EF051022
Mc *Mucidobombus* Krüger (1/1)	*mucidus* Krüger	E. Pyrenees, France	PR *et al.*	059	DQ788066	AF492935^{K}	AF493002^{K}	AF492868^{K}	EF050934

Table 1. *Continued*

Subgenus	Species	Collection locality	Collector	V.#	16S	EF-1α	Opsin	ArgK	PEPCK
Ob *Obertobombus* Reinig (2/1)	*oberti* Morawitz	Mt. Kailas, Tibet	T. Bardell	234	DQ788073	DQ788247	DQ788355	DQ788475	EF050898
Or *Orientalibombus* Richards (3/2)	*funerarius* Smith	Luojishan, Sichuan, China	P. Williams	270	EF032356	EF032378	EF032396	EF032415	EF051012
	haemorrhoidalis Smith	Chiang Mai Prov., Thailand	N. Warrit	191	DQ788020	AF492935^{K}	AF493002^{K}	AF492868^{K}	EF051011
Pe *Pressibombus* Frison (1/1)	*pressus* (Frison)	Uttaranchal, India	M. Saini	239	DQ788088	EF032368			EF050837
Ps *Psithyrus* Lepeletier (29/21)	*ashtoni* (Cresson) ♂	Ottawa, Canada	J. Whitfield	164	DQ787969	AF492983^{K}	AF493050^{K}	AF492916^{K}	EF050978
	barbutellus (Kirby) ♂	Uppland Co., Sweden	B. Cederberg	073	DQ787975	DQ788158	DQ788318	DQ788403	EF050972
	bohemicus (Seidl) ♂	E. Pyrenees, France	PR *et al.*	055	DQ787980	AF492925^{K}	AF492992^{K}	AF492858^{K}	EF050979
	campestris (Panzer)	Dalarna Co., Sweden	B. Cederberg	040	DQ787986	AF492927^{K}	AF492994^{K}	AF492860^{K}	EF050974
	chinensis (Morawitz) ♂	Qionglai Sh, Sichuan, China	SC *et al.*	152	DQ787988	DQ788170	DQ788321	DQ788412	EF050982
	citrinus (Smith) ♂	Virginia, USA	A. Deans	170	DQ787990	DQ788171	DQ788322	AY267169^{K}	EF050975
	coreanus (Yasumatsu)				AF364830^{B}				
	cornutus (Frison) ♂	Ningnan, Sichuan, China	T. Ya	271	EF032353	EF032377	EF032395	EF032413	EF050984
	fernaldae (Franklin) ♂	New Mexico, USA	SC, JW	088	DQ788006	AF492926^{K}	AF492993^{K}	AF492859^{K}	EF050969
	flavidus Eversmann ♂	Lappland Co., Sweden	B. Cederberg	041	DQ788010	DQ788189	DQ788332	DQ788429	EF050970
	insularis (Smith)	California, USA	H. Hines	162	DQ788038	AF492975^{K}	AF493042^{K}	AF492908^{K}	EF050976
	maxillosus Klug ♂	Kayseri Prov., Turkey	PR *et al.*	074	DQ788054	DQ788227	AY739480^{H}	DQ788458	EF050973
	norvegicus (Sparre-Schneider)	Dalarna Co., Sweden	B. Cederberg	089	DQ788072	DQ788246	DQ788354	DQ788474	EF050971
	quadricolor (Lepeletier) ♂	Uppland Co., Sweden	B. Cederberg	090	DQ788090	DQ788263	DQ788363	DQ788488	EF050966
	rupestris (Fabricius)	Uppland Co., Sweden	B. Cederberg	009	DQ788099	AF492928^{K}	AF492995^{K}	AF492861^{K}	EF050983
	skorikovi (Popov) ♂	Aba, Sichuan, China	SC *et al.*	159	DQ788106	DQ788276	DQ788373	DQ788499	EF050968
	suckleyi Greene	Colorado, USA	SC, JW	091	DQ788110	DQ788282	DQ788374	DQ788503	EF050981
	sylvestris (Lepeletier)	Uppland Co., Sweden	B. Cederberg	020	DQ788115	DQ788286	DQ788377	DQ788507	EF050967
	tibetanus (Morawitz) ♂	Min Shan, Sichuan, China	SC *et al.*	134	DQ788120	DQ788290	DQ788378	DQ788509	EF050985
	variabilis (Cresson) ♂	Missouri, USA	S. Cameron	316	AY268419^{C}	DQ788295	AY268397^{C}	DQ788513	EF050977
	vestalis (Geoffroy) ♂	Kent, England	P. Williams	169	DQ788128	DQ788297	AY739495^{H}	DQ788515	EF050980
Pr *Pyrobombus* Dalla Torre (43/40)	*alboanalis* Franklin*	Alaska, USA	SAC, JBW	257	EF032343	EF032360	EF032380	EF032398	EF050813
	ardens Smith ♂	Dae-Dong, S. Korea	D. Kim	131	DQ787966	DQ788149	AF493031^{K}	AF492897^{K}	EF050823
	avanus (Skorikov)*	Luojishan, Sichuan, China	P. Williams	272	EF032344	EF032365	EF032384	EF032402	EF050830
	beaticola (Tkalců)					AF492963^{K}	AF493030^{K}	AF492896^{K}	
	bifarius Cresson	New Mexico, USA	M. Hauser	208	DQ787977	DQ788160	AF493010^{K}	AF492876^{K}	EF050838
	bimaculatus Cresson	Arkansas, USA	S. Cameron	218	DQ787978	DQ788161	AY739456^{H}	DQ788405	EF050847
	biroi Vogt	Ketmen Mts., Kazakhstan	M. Hauser	210	DQ787979	DQ788162	AY739457^{H}	DQ788406	EF050825
	brodmannicus Vogt	Artvin Prov., Turkey	PR *et al.*	077	DQ787984	DQ788166	AY739458^{C}	DQ788409	EF050818
	caliginosus (Frison)	California, USA	H. Hines	150	DQ787985	DQ788168	AF493035^{K}	AF492901^{K}	EF050853
	centralis Cresson	Washington, USA	J. Whitfield	146	DQ787987	DQ788169	AY739459^{C}	DQ788411	EF050852

cingulatus Wahlberg	Lappi, Finland	P. Rasmont	212	DQ787989	AF492948^{K}	AF493015^{K}	AF492881^{K}	EF050812
ephippiatus Say	Oaxaca, Mexico	R. Ayala	042	DQ788002				
	Chiapas, Mexico	M. Guzman	198		DQ788182	AY739462^{H}	DQ788422	EF050844
flavescens (Smith)	Mei-fang, Taiwan	H. Townes	181	DQ788009	AF492950^{K}	AF493017^{K}	AF492883^{K}	EF050824
flavifrons Cresson ♂	California, USA	J. Whitfield	095	DQ788011	DQ788190	AF493016^{K}	AF492882^{K}	EF050850
frigidus Smith	Alaska, USA	A. Scholl	185	DQ788014	DQ788192	AY739466^{H}	DQ788431	EF050811
haematurus Kriechbaumer ♂	Trabzon Prov., Turkey	P. Rasmont	211	DQ788019	EF032364			EF050829
huntii Greene	Washington, USA	J. Whitfield	151	DQ788027	DQ788203	AF493045^{K}	AF492911^{K}	EF050840
hypnorum (Linnaeus)	Klösterle, Austria	SC, JW	078	DQ788029	DQ788205	AF493013^{K}	AF492879^{K}	EF050826
	Qionglai Sh, Sichuan,China	SC *et al.*	207	EF032359	EF032363	EF032383	EF032401	EF050827
impatiens Cresson	Illinois, USA	H. Hines	060	DQ788033	DQ788209	AF493009^{K}	AF492875^{K}	EF050842
infirmus (Tkalců) ♂	Qionglai Sh, Sichuan, China	SC *et al.*	157	DQ788036	DQ788212	AY739471^{H}	DQ788444	EF050836
infrequens (Tkalců)*	Qionglai Sh, Sichuan, China	SC *et al.*	140	DQ788037	DQ788213	AY739472^{H}	DQ788445	EF050831
jonellus (Kirby)	Lappland Co., Sweden	B. Cederberg	079	DQ788039	DQ788214	AY739473^{H}	DQ788446	EF050814
lapponicus (Fabricius)	Lappland Co., Sweden	B. Cederberg	103	DQ788046	DQ788220	DQ788345	DQ788452	EF050845
lemniscatus Skorikov	Qionglai Sh, Sichuan, China	SC *et al.*	161	DQ788047	DQ788221	AY739477^{H}	DQ788453	EF050834
lepidus Skorikov	Qionglai Sh, Sichuan, China	SC *et al.*	155	DQ788048	DQ788222	AY739478^{H}	DQ788454	EF050835
luteipes Richards	Pokhara, Nepal	S. Martin	195	DQ788052				
melanopygus Nylander	California, USA	H. Hines	215	DQ788055	DQ788229	AF493011^{K}	AF492877^{K}	EF050849
mixtus Cresson	Washington, USA	SC, JW	024	DQ788060	DQ788234	AF493014^{K}	AF492880^{K}	EF050816
modestus Eversmann	Aba, Sichuan, China	SC *et al.*	160	DQ788063	DQ788237	AY739482^{H}	DQ788465	EF050820
♂	S. Altai, Kazakhstan	P. Mardulyn	238	EF032358	EF032362	EF032382	EF032400	EF050821
monticola Smith	E. Pyrenees, France	PR *et al.*	176	DQ788064	DQ788238	AY739483^{H}	DQ788466	EF050848
parthenius Richards	Himachal Pradesh, India	M. Saini	241	DQ788076	DQ788250	DQ788357	DQ788478	EF050832
perplexus Cresson	Ottawa, Canada	J. Whitfield	166	DQ788079	DQ788254	AF493012^{K}	AF492878^{K}	EF050828
picipes Richards	Qionglai Sh, Sichuan, China	SC *et al.*	180	DQ788082	DQ788257	AY739487^{H}	DQ788482	EF050833
pratorum (Linnaeus)	Klösterle, Austria	SC, JW	075	DQ788087	AF492966^{K}	AF493033^{K}	AF492899^{K}	EF050819
pyrenaeus Pérez	Gurgltal, Austria	SC, JW	035	DQ788089	DQ788262	AY739488^{H}	DQ788487	EF050822
sandersoni Franklin	Isle au Haut, Maine, USA	SC, JW	255	EF032346	EF032361	EF032381	EF032399	EF050815
sitkensis Nylander	California, USA	H. Hines	144	DQ788105	DQ788275	AY739490^{H}	DQ788498	EF050817
sonani (Frison)*					AF492951^{K}	AF493018^{K}	AF492884^{K}	
sylvicola Kirby ♂*	New Mexico, USA	SC, JW	108	DQ788116	DQ788287	AY739493^{H}	DQ788508	EF050847
ternarius Say	Nova Scotia, Canada	SC, JW	116	DQ788117	AF492979^{K}	AF493046^{K}	AF492912^{K}	EF050839
vagans Smith	Wisconsin, USA	J. Whitfield	044	DQ788125	DQ788293	DQ788380	DQ788512	EF050854
vandykei (Frison)	Washington, USA	J. Whitfield	149	DQ788126	DQ788294	AF493049^{K}	AF492915^{K}	EF050851
vosnesenskii Radoszkowski ♂	Washington, USA	J. Whitfield	112	DQ788133	DQ788301	AF493047^{K}	AF492913^{K}	EF050841
wilmattae Cockerell*	Chiapas, Mexico	R. Vandame	199	DQ788136	DQ788304	AY739496^{H}	DQ788521	EF050843

Table 1. *Continued*

Subgenus	Species	Collection locality	Collector	V.#	16S	EF-1α	Opsin	ArgK	PEPCK
Rh *Rhodobombus* Dalla Torre (3/3)	*armeniacus* Radoszkowski	Kayseri Prov., Turkey	PR *et al.*	080	DQ787968	DQ788151	DQ788315	DQ788397	EF050937
	mesomelas (Gerstaecker)	Switzerland	C. Gerlach	037	DQ788058	DQ788231	EF032390	EF032414	EF050938
	pomorum (Panzer) ♂	Erzincan Prov., Turkey	PR *et al.*	053	DQ788084	DQ788258	DQ788360	DQ788483	EF050939
Rb *Robustobombus* Skorikov (7/7)	*ecuadorius* Meunier ♂	Abancay, Peru	C. Rasmussen	135	DQ788001	DQ788181	DQ788329	DQ788421	EF050874
	hortulanus Friese ♂	Magdalena, Colombia	J. Cantillo	200	DQ788025	DQ788201	AY739468^{H}	DQ788438	EF050875
	melaleucus Handlirsch	Oxapampa, Peru	C. Rasmussen	173	DQ787960	DQ788143	DQ788311	DQ788460	EF050876
	robustus Smith	Boyacá, Colombia	S. Cameron	050	DQ788093	DQ788266	DQ788366	EF032405	EF050877
	tucumanus Vachal	Tucumán, Argentina	M. Lucia	276	EF032349	EF032367	EF032386	EF032404	EF050873
	vogti Friese* ♂	Abancay, Peru	C. Rasmussen	172	DQ788130	DQ788299	DQ788383	DQ788517	EF050878
	volucelloides Gribado*	San Jose, Costa Rica	P. Hanson	122	DQ788131	AY267133^{K}	AY267149^{K}	AY267165^{K}	
Rc *Rubicundobombus* Skorikov (1/1)	*rubicundus* Smith	Cundinamarca, Colombia	A. Perez	021	DQ788094				
		Boyacá, Colombia	S. Cameron	202		DQ788267	DQ788367	DQ788491	EF050888
Rf *Rufipedibombus* Skorikov (2/1)	*eximius* Smith	Alishan, Taiwan	J. de Boer	049	DQ788005	DQ788186	AY739464^{C}	DQ788426	EF050911
Sx *Senexibombus* Frison (4/2)	*bicoloratus* Smith	Nantou, Taiwan	C. Lin	225	DQ787976	AF492971^{K}	AF493038^{K}	AF492904^{K}	EF051005
	kulingensis Cockerell	Zhejiang Prov., China	SC *et al.*	097	DQ788042	DQ788217	DQ788343	DQ788449	EF051006
Sp *Separatobombus* Frison (2/2)	*griseocollis* (DeGeer)	Illinois, USA	H. Hines	082	DQ788018	DQ788196	AF493039^{K}	AF492905^{K}	EF050879
	morrisoni Cresson	Arizona, USA	T. Sillett	014	DQ788065				
		Utah, USA	T. Griswold	196		DQ788240	DQ788350	DQ788468	EF050880
Sb *Sibiricobombus* Vogt (5/4)	*asiaticus* Morawitz ♂	Himachal Pradesh, India	M. Saini	249	DQ787970	EF032369	EF032387	EF032406	EF050896
	niveatus Kriechbaumer	Kayseri Prov., Turkey	PR *et al.*	093	DQ788070	DQ788244	DQ788353	DQ788472	EF050893
	sibiricus (Fabricius)	Khövsgöl Nuur, Mongolia	D Sheppard	274	EF032348	EF032370	EF032388	EF032407	EF050897
	sulfureus Friese	Kayseri Prov., Turkey	PR *et al.*	064	DQ788111	DQ788283	AY739492^{H}	DQ788504	EF050895
	vorticosus Gerstaecker*	Aksaray Prov., Turkey	PR *et al.*	124	DQ788132	DQ788300	DQ788384	DQ788518	EF050894
St *Subterraneobombus* Vogt (9/7)	*appositus* Cresson ♂	Utah, USA	T. Griswold	145	DQ787965	DQ788148	DQ788314	DQ788395	EF050986
	borealis Kirby	Maine, USA	SC, JW	250	DQ787981	AF492976^{K}	AF493043^{K}	AF492909^{K}	EF050987
	difficillimus Skorikov*	Hongyuan, Sichuan, China	SC *et al.*	154	DQ787998	DQ788177	DQ788327	DQ788417	EF050990
	distinguendus Morawitz ♂	Edesbecka, Finland	C. Grinter	197	DQ787999	DQ788180	DQ788328	DQ788420	EF050988
	fragrans (Pallas)	Kayseri Prov., Turkey	PR *et al.*	061	DQ788012	DQ788191	DQ788333	DQ788430	EF050992
	melanurus Lepeletier	Dzungarskij Alatau, Kazakh.	M. Hauser	022	DQ788056	DQ788230	AF492990^{K}	AF492856^{K}	EF050991
	personatus Smith	Hongyuan, Sichuan, China	SC *et al.*	138	DQ788081	DQ788256	DQ788359	DQ788481	EF050993
	subterraneus (Linnaeus)	Uppland Co., Sweden	B. Cederberg	046	DQ788109	DQ788281	AF493027^{K}	AF492893^{K}	EF050989
Th *Thoracobombus* Dalla Torre (19/16)	*deuteronymus* Schulz	Primorskiy Krai, Russia	K. Holston	147	DQ787997	DQ788176	DQ788326	AY267170^{K}	EF050922
	filchnerae Vogt ♂	Hongyuan, Sichuan, China	SC *et al.*	206	DQ788008	DQ788188	DQ788331	DQ788428	EF050933
	hedini Bischoff	Qionglai Sh, Sichuan, China	SC *et al.*	129	DQ788023	DQ788199	DQ788338	DQ788437	EF050930
	honshuensis (Tkalců)					AF493029^{K}	AF492962^{K}	AF492895^{K}	
	humilis Illiger	E. Pyrenees, France	HH, PR	056	DQ788026	DQ788202	AY739469^{H}	DQ788439	EF050924
	impetuosus Smith	Xinmian, Sichuan, China	P. Williams.	284	EF032350	EF032373	EF032391	EF032409	EF050927
	mlokosievitzii Radoszkowski	Artvin Prov., Turkey	PR *et al.*	081	DQ788061	DQ788235	DQ788349	DQ788463	EF050917

	muscorum (Linnaeus)	Öland Co., Sweden	B. Cederberg	033	DQ788067	DQ788241	DQ788351	DQ788469	EF050925
	pascuorum (Scopoli)	Toscana, Italy	SC, JW	023	DQ788077	DQ788251	AF493001^{K}	AY492867^{K}	EF050932
	potanini Morawitz*	Hongyuan, Sichuan, China	SC *et al.*	113	DQ788086	DQ788260	DQ788362	DQ788485	EF050928
	pseudobaicalensis Vogt	Primorskiy Kray, Russia	K. Holston	253	EF032357	EF032372	AY267155^{K}	AY267171^{K}	EF050923
	remotus Tkalců ♂	Qionglai Sh, Sichuan, China	SC *et al.*	192	DQ788092	DQ788265	DQ788365	DQ788490	EF050929
	ruderarius (Müller)	Obergurgl, Austria	SC, JW	047	DQ788095	AF492932^{K}	AF492999^{K}	AF492865^{K}	EF050920
	schrencki Morawitz ♂	W. Khentey, N. Mongolia	M. Mühlenberg	298	AF364828^{B}	AF492933^{K}	AF493000^{K}	AF492866^{K}	EF050931
	sylvarum (Linnaeus)	Uppsala Co., Sweden	SC, JW	110	DQ788114	DQ788285	DQ788376	DQ788506	EF050918
	velox (Skorikov)*	Artvin Prov., Turkey	PR *et al.*	094	DQ788127	DQ788296	DQ788381	DQ788514	EF050921
	veteranus (Fabricius)	Jura, France	A. Scholl	187	DQ788129	DQ788298	DQ788382	DQ788516	EF050919
	zonatus Smith	Aksaray Prov., Turkey	PR *et al.*	063	DQ788138	DQ788306	DQ788386	DQ788522	EF050926
Tr *Tricornibombus* Skorikov (3/3)	*atripes* Smith	Zhejiang Prov., China	P. Meihua	066	DQ787971	DQ788153	DQ788316	DQ788399	EF050964
	imitator Pittioni ♂	Guizhou Prov., China	M. Yun	028	DQ788032	DQ788208	DQ788339	DQ788441	EF050965
	tricornis Radoszkowski	Primorskiy Kray, Russia	K. Holston	148	DQ788121	AF492937^{K}	AF493004^{K}	AF492870^{K}	EF050963
OUTGROUPS									
Tribe	**Species**								
Apini	*Apis dorsata* Fabricius	Bangalore, India	S. Cameron	321	L22893^{C2}	AY267146^{K}	AF091733^{M}	AY267178^{K}	EF051028
	Apis mellifera Linnaeus	Arkansas, USA	S. Cameron	320	L22891	AF015267^{D}	AF091732^{M}	EF032397	EF051030
Euglossini	*Euglossa imperialis* Cockerell	São Paulo, Brazil	S. Cameron	319	AJ581085^{Mi}	AY267144^{K}	AY267160^{K}	AY267176^{K}	
	Eulaema boliviensis (Friese)	La Paz Prov., Bolivia	M. Hauser	213	DQ788139	DQ788307	DQ788387	DQ788523	EF051029
Meliponini	*Geniotrigona thoracica* Smith	Kuala Muda, Malaysia	R. Hepburn	303	DQ788140	DQ788308	DQ788388	DQ788524	EF051023
	Heterotrigona itama Cockerell	Kuala Muda, Malaysia	R. Hepburn	304	DQ788141	DQ788309	DQ788389	DQ788525	EF051024
	Hypotrigona gribodoi (Magretti)	Bwindi, Uganda	R. Kajobe	322	DQ790440^{R}	DQ813121^{R}	DQ813199^{R}	DQ813043^{R}	EF051026
	Liotrigona mahafalya Brooks & Michener	Mahajanga Prov., Madagascar	B. Fisher		DQ790442^{R}	DQ813126^{R}	DQ813204^{R}	DQ813048^{R}	
	Plebeia frontalis Friese	Puebla, Mexico	M. Villamar	323	DQ790459^{R}	DQ813138^{R}	DQ813216^{R}	DQ813059^{R}	EF051027
	Trigona amazonensis Ducke	Madre de Dios, Peru	S. Cameron	178	DQ788142	DQ788310	DQ788390	DQ788526	EF051025

The two-letter acronym (in **bold**) in column 1 denotes the abbreviated subgenus name. Numbers in parentheses following subgeneric names refer to total number of species recognized by Williams (1998) (excluding extinct species) followed by the number represented in the present study. Asterisks indicate taxa that were recognized as conspecific with other species by Williams (1998). Superscript letters following accession numbers indicate sequences obtained from previous studies. ^{B}Bae et al., unpubl. GenBank reference, 2001; ^{C}Cameron & Williams (2003); ^{C2}Cameron (1993); ^{D}Danforth & Ji (2001); ^{H}Hines *et al*. (2006); ^{K}Kawakita *et al*. (2003, 2004); ^{M}Mardulyn & Cameron (1999); ^{Mi}Michel-Salzat, Cameron & Oliveira (2004); ^{R}Rasmussen & Cameron, 2007).

HH, PR = H. Hines, P. Rasmont; SC *et al*. = S. Cameron, P. Williams, J. Whitfield; PR *et al*. = P. Rasmont, M. Aytekin, H. Hines, M. Terzo, I. Barbier; SC, JW = S. Cameron, J. Whitfield; RA, RB = R. Ayala, R. Brooks.

GENES

Five genes were selected for analysis based on prior testing for useful signal: mitochondrial 16S rRNA, and the nuclear protein-encoding genes long-wavelength rhodopsin copy 1 (opsin), elongation factor-1 alpha F2 copy (EF-1α), arginine kinase (ArgK) and phosphoenolpyruvate carboxykinase (PEPCK). 16S was selected for its high evolutionary rate, useful for resolving tip relationships; the nuclear genes provide better phylogenetic signal at deeper hierarchical levels in the phylogeny. A full discussion of the first four genes, primer sequences, and rationale underlying their selection is provided elsewhere (Hines *et al*., 2006). PEPCK was selected because it has recovered relationships within Lepidoptera at similar taxonomic levels to those in *Bombus* (Friedlander *et al*., 1996) and contains both highly variable intron and conserved exon regions. PEPCK primers used for most taxa were FHv4-5′-TGTATRATAATTCGCAAYTTCAC-3′ and RHv4-5′-CTGCTGGRGTYCTAGATCC-3′. Some taxa required an alternative forward primer, either FH2-5′-GTSTCTTATGGGAGSGGTTACGG-3′ or FHv5-5′-AGAACAATTATCTYAAATRCTAARCTTC-3′. The forward primer sequences began within an intron and were unable to amplify some of the outgroup taxa. For these and two problematic *Bombus* taxa (*Bombus haematurus* and *Bombus pressus*), primers were designed to amplify only the exon region: PEPCKexF-5′-GATAYTGGCTATCACARATCC-3′ and 21dNrc from Friedlander *et al*. (1996). Some previously reported sequences were obtained from GenBank (Table 1). Two of these were renamed to conform to current species names, deduced from their collection locality: *Bombus parthenius* from Taiwan is recognized as *Bombus sonani* and *Bombus nevadensis* from the eastern USA is recognized as *Bombus auricomus*.

MORPHOLOGY

We analysed an updated version of the morphological data set of Williams (1994), incorporating 47 bumble bee taxa scored for 53 morphological characters, including character states of the male genitalia (30 characters), male soma (seven characters) and female soma (16 characters) (Appendices 1 and 2). Each subgenus is represented by one or two exemplars, which express the character state for the remainder of the subgenus; variable subgenera were represented by more than one species. Because genitalic characters of Apini and Meliponini used in the molecular analyses were difficult to homologize with Bombini, the next closest outgroup, Euglossini (*Eufriesea*), was selected, along with the slightly more distant Xylocopinae (*Lestis*).

POLYMERASE CHAIN REACTION (PCR) AND DNA SEQUENCING

Detailed extraction, PCR and sequencing protocols are reported in Hines *et al*. (2006). PCR amplification of PEPCK was conducted at annealing temperatures of 49–50 °C and extension at 72 °C. Gene fragments of the following sizes were amplified: (1) ~530 nucleotides (bp) of 16S; (2) ~680 bp of opsin, including two introns comprising 178 bp (Mardulyn & Cameron, 1999); (3) ~725 bp of EF-1α F2 copy, containing an intron of ~200 bp; (4) ~910 bp of ArgK containing an intron of ~325 bp; and (5) ~900 bp of PEPCK containing two introns of ~525 bp. Sequences obtained from these fragments, using the Applied Biosystems BigDye Terminator kit version 3.0 or 3.1, were visualized with an ABI 3730XL automated sequencer at the W. M. Keck Center for Comparative and Functional Genomics, University of Illinois. Both strands were sequenced for all taxa and consensus sequences were deposited in GenBank (accession numbers are shown in Table 1).

ALIGNMENT

Sequences were edited and aligned (default parameters, CLUSTAL W) in BioEdit (version 5.0.9) (Hall, 1999), with some manual adjustment if identical or highly similar regions were aligned differently across taxa. A comparative check on the 16S alignment was made using CLUSTAL X, version 1.81 (Jeanmougin *et al*., 1998), with gap opening of 10 and gap extension of 0.10. Nucleotides of uncertain alignment for 16S (~50 bp from four hypervariable AT-rich regions) were excluded from the data matrices. ArgK and PEPCK contained a few indels that were problematic to align for a few taxa; the ambiguous regions for these were treated as missing data, as in Kawakita *et al*. (2003). Nucleotides of EF-1α that varied between our taxa and those of Kawakita *et al*. (2003) were confirmed by reinspection of chromatograms. Gap regions of unambiguous alignment were coded as separate binary characters following the methods of Simmons & Ochoterena (2000) and Simmons, Ochoterena & Carr (2001), incorporating only the regions that were parsimony informative in collective analyses. With the introduction of gaps in the alignments, individual gene fragments had the following numbers of aligned/gap-coded sites: 16S, 573/8; opsin, 751/5; EF-1α, 906/13; ArgK, 1108/43; and PEPCK, 1425/57. The combined (five-genes) data set included 4763 bp and 126 gap-coded characters.

PHYLOGENETIC ANALYSES

DNA sequences

Inter- and intrasubgeneric relationships of *Bombus* were inferred largely from Bayesian analyses, implemented in MrBayes, version 3.1.2 (Ronquist & Huelsenbeck, 2003) on an IBM p-series 690 supercomputer operated by the National Center for Supercomputing Applications (University of Illinois at Urbana-Champaign, Champaign, IL). Genes were analysed individually and collectively, each partitioned into exon, intron and gap regions (when applicable) to account for variation in evolutionary rates among gene regions. Four taxa sequenced only for the 16S fragment (*Bombus tunicatus*, *Bombus wilemani*, *Bombus coreanus*, and *B. luteipes*) were excluded from collective analyses.

Model selection for each gene was based on Akaike information criteria in Modeltest, version 3.7 (Posada & Crandall, 1998) and MrModeltest (Nylander, 2004). The model parameters used for each gene partition were: 16S (GTR + I + G), 16S gap characters (standard + G); EF-1α intron (GTR + I + G), EF-1α exon (GTR + I + G), EF-1α gap characters (standard + G); opsin intron (GTR + I + G), opsin exon (HKY + G), opsin gap characters (standard + G); ArgK intron (GTR + G), ArgK exon (GTR + I + G), ArgK gap characters (standard + G); PEPCK intron (GTR + G), PEPCK exon (HKY + G) and PEPCK gap characters (standard + G). In combined analyses, gap-coded characters were treated as a single partition (standard + G).

Four to six independent analyses were carried out for each gene fragment and for the combined data (eight million generations for individual genes, 12 million for combined analyses, four chains with mixed-models, flat priors, saving trees every 1000 generations). Consensus trees were estimated from at least three independent analyses, with convergent log-likelihood plots examined in Tracer 1.2 (Rambaut & Drummond, 2003). All trees estimated prior to stationarity were discarded. Trees remaining after convergence from replicate runs were combined to create a single majority rule consensus tree. Posterior probability (*PP*) values represent the proportion of all Markov chain samples that contain a given node, and are interpreted as the probability that a node or monophyletic group is correct given the model and the data.

To assess the influence of the gap regions on tree topology, additional parallel analyses were conducted with gap-coded characters excluded.

Maximum parsimony nonparametric bootstrap analysis of the combined five-genes data [heuristic search, 300 replicates, ten random additions per replicate, retaining a maximum of 300 trees in each replicate, tree-bisection-reconnection (TBR) branch swapping] was implemented in PAUP*4.0 (Swofford, 2001) to compare parsimony bootstrap values with Bayesian posterior probabilities. Correspondingly high values for these two different measures of support provide good confidence for testing the monophyly of the conventional *Bombus* subgenera and accepting other monophyletic groups.

Morphological data

Morphological characters were analysed under parsimony in PAUP* (heuristic search, 100 replicates with a maximum of 500 trees saved per replicate, TBR branch-swapping, all characters of equal weight). Because male genitalic characters have been the focus in prior examinations of *Bombus* phylogeny (Williams, 1985, 1994), we separated the morphological characters into two partitions, one with the full set of characters, the other with only genitalic characters. Replicate analyses established that tree topology and tree statistics were stable. Nonparametric bootstrapping (heuristic search: 100 replicates, ten random additions per replicate, maximum of 500 trees saved per replicate) provided measures of relative support for each node in the parsimony tree.

Morphological characters were also combined with a reduced five-genes data set of the 47 *Bombus* taxa analysed under parsimony, implemented in MrBayes (two independent runs, three million generations, four chains, saving trees every 1000 generations, flat priors, mixed models using the same parameters as those applied to the total sequence data set, standard model applied to the morphological characters). Results were compared with those from a Bayesian analysis of the reduced five-genes data set alone (same model parameters and conditions as applied to genes + morphology). Outgroups for this analysis were selected from the full-scale molecular data set (*Plebeia*, *Trigona*, *Heterotrigona*, *Apis*, and *Eulaema*; Table 1), coded as missing data for the morphological characters.

RESULTS

RESOLUTION AND SUPPORT FOR THE PHYLOGENY

Independent Bayesian analyses of each nuclear gene data set resulted in highly resolved and well supported phylogenies overall (supplementary trees online). On the whole, opsin was less useful near the tips of the phylogeny but resolved many of the deeper clades, whereas EF-1α, ArgK, and PEPCK provided good resolution and support for relationships within and between clades throughout the trees. By contrast, mitochondrial 16S (supplementary tree online) was especially useful in resolving the tip clades within conventional groupings (subgenera) but generally poor at resolving intersubgeneric and deeper relationships (Table 2).

Simultaneous Bayesian analysis of the combined sequence data produced a tree with nearly complete resolution and high branch support at all levels

Table 2. The left half of the table indicates resolution (number of internal nodes resolved) and node support (*PP* = posterior probability) for the conventional *Bombus* subgenera from Bayesian analyses of the combined sequences from five genes. In the right half of the table, posterior probability values are reported for subgeneric monophyly based on individual gene sequences. Abbreviations of subgeneric names are given in Table 1. The 12 monotypic subgenera (Table 1) are not listed, nor are subgenera comprising two species for which we have only one representative (Br, Fn, Ob, Rf). u, unresolved; pa, paraphyletic; po, polyphyletic

Subgenus	Number of taxa in study	Number of internal nodes resolved in combined gene tree	Number of nodes $PP \geq 0.95$ in combined gene tree	*PP* support for monophyly of subgenera				
				16S	Opsin	ArgK	Ef-1α	PEPCK
Pr	46	42	28	u	1.0	1.0	1.0	1.0
Bo	13	10	4	1.0	1.0	1.0	1.0	1.0
Al	5	4	4	u	1.0	1.0	1.0	1.0
Rb	7	6	5	1.0	1.0	0.86	1.0	1.0
Cc	2	1	1	0.87	1.0	1.0	0.98	1.0
Sp	2	0	0	u	u	u	u	u
Ds	2	0	0	u	po	po	po	po
Cu	3	1	1	0.99	u	pa	u	u
Ml	12	11	10	1.0	0.89	1.0	1.0	0.91
Ag	5	4	4	u	0.85	1.0	1.0	1.0
Sb	5	4	3	u	0.99	0.84	1.0	0.98
Fv*	19	17	15	u	1.0	0.97	u	1.0
Tr	3	1	1	po	u	pa	u	po
Rh	3	2	2	1.0	1.0	0.88	1.0	1.0
Th	18	15	11	1.0	u	1.0	1.0	1.0
Ps	20	19	17	1.0	1.0	1.0	1.0	1.0
Mg	12	11	9	u	0.94	1.0	1.0	0.78
Sx	2	1	1	1.0	0.98	1.0	1.0	1.0
Dv	4	2	1	1.0	1.0	1.0	1.0	1.0
St	8	7	5	1.0	0.98	1.0	1.0	1.0
Or	2	1	1	1.0	1.0	1.0	1.0	1.0
Bi	2	1	1	1.0	1.0	1.0	1.0	1.0
Md	7	6	5	1.0	1.0	1.0	1.0	1.0

**Fervidobombus* minus the *dahlbomii* + *morio* + *excellens* group.

(Fig. 1, Table 2). Of the 204 *Bombus* nodes in the tree, all but 12 were resolved and 158 (77%) were supported by posterior probability values > 0.95. Relationships among subgenera indicated by high support (*PP* > 0.95) are summarized in Fig. 2.

SUBGENERIC RELATIONSHIPS

The Bayesian combined (Fig. 1) and individual nuclear gene phylogenies, and to some extent the 16S phylogeny, indicated strong support for all but the following five conventional *Bombus* subgenera (Table 2): *Separatobombus*, *Dasybombus*, *Cullumanobombus*, *Fervidobombus*, and *Tricornibombus*. Of the three larger nonmonophyletic subgenera, *Cullumanobombus* was paraphyletic as *Bombus rufocinctus* fell out as sister group to the remaining *Cullumanobombus* species (*Bombus apollineus* + *Bombus semenoviellus*) + a New World clade (Fig. 1). *Fervidobombus* emerged as polyphyletic as the group (*Bombus morio* + *Bombus excellens* + *Bombus dahlbomii*) separated and attached to the *Thoracobombus*–*Rhodobombus* clade (*PP* = 0.81), leaving the remaining *Fervidobombus* strongly supported (*PP* = 1.0). In turn, a polyphyletic *Tricornibombus*, which excluded *Bombus* (*Tricornibombus*) *imitator*, and included *Bombus* (*Exilobombus*) *exil* (*PP* = 1.0), attached as sister group to *Fervidobombus* (minus the *dahlbomii*-group) with strong support (*PP* = 1.0). Of the remaining two nonmonophyletic subgenera (both ditypic), *Separatobombus* was unresolved due to poor support (Fig. 1), and *Dasybombus* was polyphyletic as *Bombus* (*Dasybombus*) *macgregori* and *Bombus* (*Dasybombus*) *handlirschi* fell into different well supported clades (both with *PP* = 1.0). Two other ditypic subgenera represented in this study by both species (*Coccineobombus* and *Bombias*) were resolved as strongly monophyletic (*PP* = 1.0).

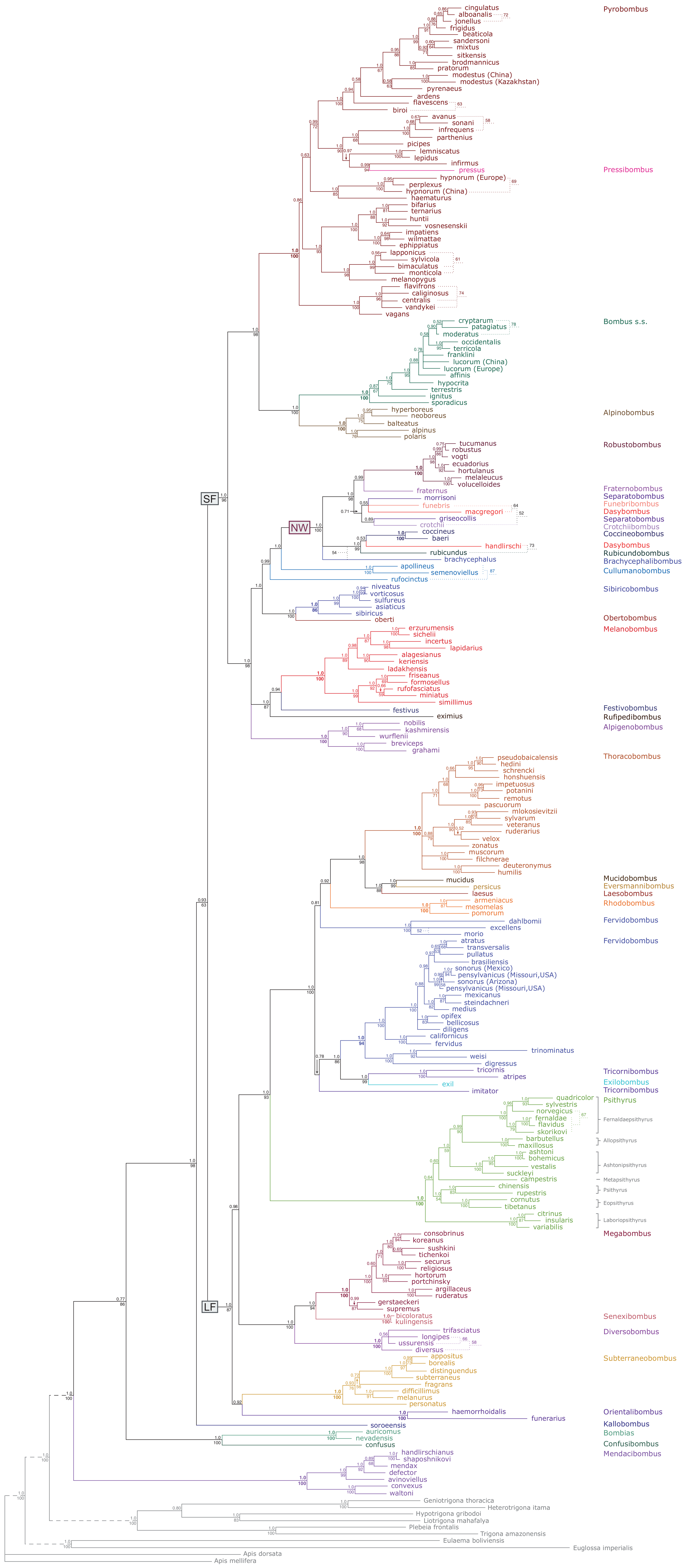

Figure 1. Phylogeny of *Bombus* estimated from Bayesian analysis of combined sequence data from five gene fragments (16S, opsin, ArgK, EF-1α, and PEPCK). Subgeneric clades are individually colour-coded and labelled with the subgeneric name. Values above branches are Bayesian posterior probabilities; values below branches are parsimony bootstrap values. Alternative resolution from parsimony analysis shown as dotted lines. Outgroup branches (in grey), represented by dashed lines, have been shortened for visual purposes. NW, *New World* clade; SF, *short-faced* clade; LF, *long-faced* clade.

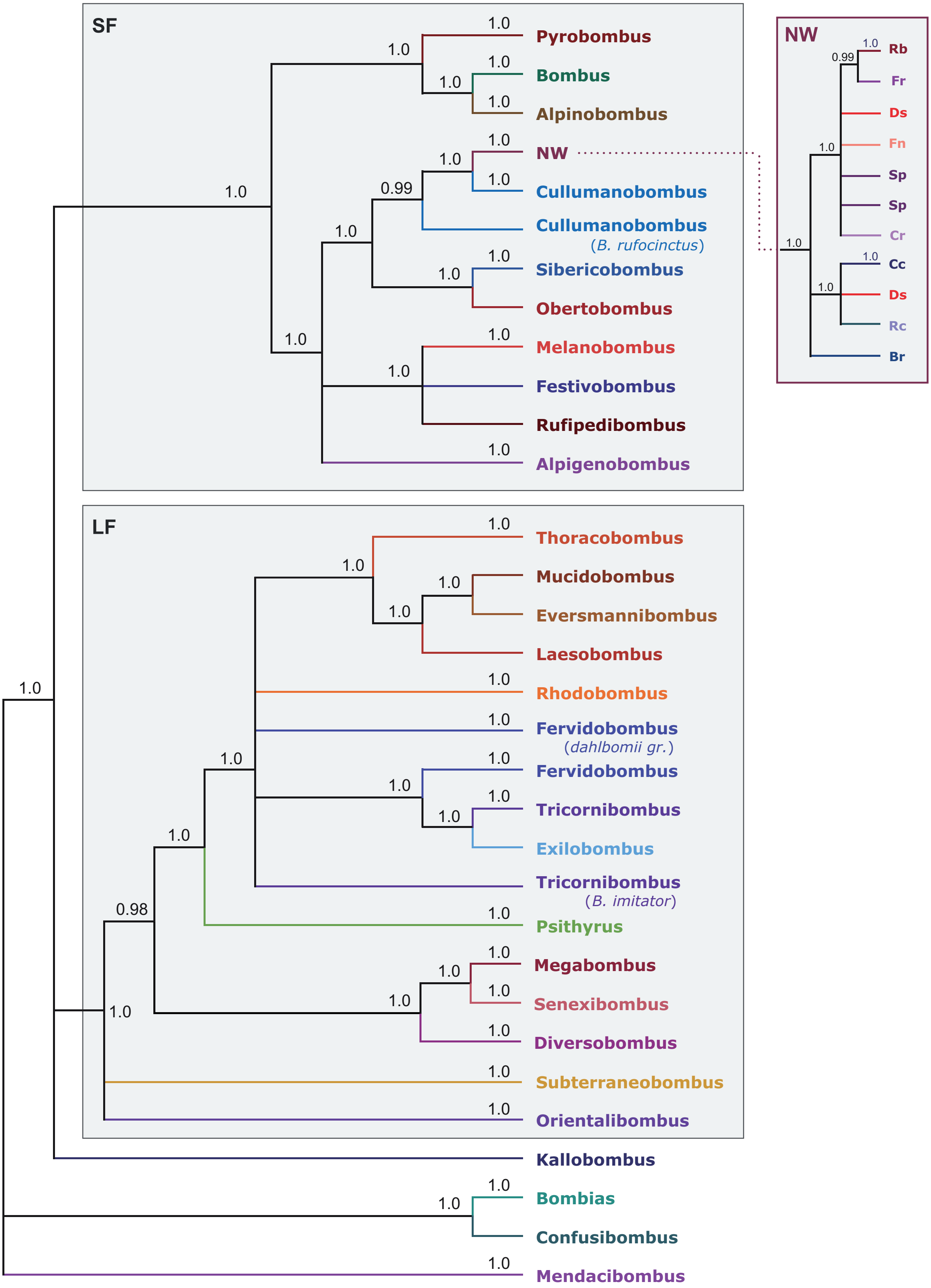

Figure 2. *Bombus* phylogeny representing only the subgeneric relationships with strong support (*PP* = 0.95) from the Bayesian tree in Fig. 1. Branches are colour-coded as in Fig. 1. Values on branches are Bayesian posterior probability values.

Table 3. Synopsis of the closest relatives of each of the 11 conventional monotypic subgenera of *Bombus*

Subgenus	Sister group relationship	*PP* value/BV
Pressibombus	Pe + *Bombus* (Pr) *infirmus*	0.99/94
Fraternobombus	Fr + Rb	0.99/–
Crotchiibombus	Cr + *Bombus* (Sp) *griseocollis*	0.89/–
Rubicundobombus	Rc + Cc + *Bombus* (Ds) *handlirschi*	1.0/99
Festivobombus	Fs + Ml	0.94/–
Mucidobombus/*Eversmannibombus*/	(Mc + Ev) + Ls	1.0/88
Laesobombus	((Mc + Ev) + Ls) + Th	1.0/98
Exilobombus	Ex + (*Bombus* (Tr) *atripes* + *Bombus tricornis*)	1.0/99
Kallobombus	Kl + LF + SF	1.0/98
Confusibombus	Cf + Bi	1.0/100

The sister group relationships are inferred from combined DNA sequences of 16S, EF-1α, opsin, and ArgK.
Abbreviated subgeneric names in column 2 are given in Table 1.
PP, posterior probability; BV, parsimony bootstrap value; –, no support.

Of the 11 recognized monotypic subgenera (Table 1), most of their relationships to other taxa were well resolved with good support (Figs 1, 2). Notably, the attachment of *Exilobombus* (*B. exil*) within *Tricornibombus* was strongly supported (*PP* = 1.0), as was the placement of *Pressibombus* (= *B. pressus*) as sister to *Bombus infirmus* within *Pyrobombus* (*PP* = 0.99; Fig. 1). Sister group relationships of the monotypic subgenera are summarized in Table 3.

The four species sequenced only for the 16S fragment were resolved in the 16S tree within their expected subgenera. In particular, *B. luteipes* fell within the *Pyrobombus* clade (*Bombus avanus* + *B. sonani* + *Bombus infrequens* + *B. parthenius*) (*PP* = 0.90), attaching to *B. infrequens* + *B. parthenius* as an unresolved trichotomy (*PP* = 0.97); *B. tunicatus* attached as sister to *Bombus terricola* (*PP* = 0.74); *B. coreanus* was sister to *Bombus ashtoni* + *Bombus bohemicus* (*PP* = 0.85); and *B. wilemani* was sister to *Bombus trifasciatus* (*PP* = 1.0).

Parsimony nonparametric bootstrap analysis of the combined sequences resulted in a tree nearly identical to but somewhat less resolved than the Bayesian tree [Fig. 1, bootstrap values (BV) shown below branches]. The only substantive contradiction was *Cullumanobombus*, which was monophyletic in the parsimony tree (BV = 87%) but paraphyletic in the Bayesian tree (*PP* = 0.99). Parsimony resulted in decreased resolution within portions of *Pyrobombus*, *Bombus s.s.*, *Psithyrus*, and *Fervidobombus*, principally for relationships that were poorly supported with Bayesian analysis. Inconsistencies between parsimony and Bayesian support values (Fig. 1) also occurred near a few of the tip taxa within several subgenera, where posterior probability values were generally low (*PP* = 0.65). Parsimony gave stronger support for the hypothesis that *Mendacibombus* falls nearest the root (BV = 86%).

Deep relationships

The individual nuclear- and combined-genes phylogenies resolved nearly all subgenera into two large and strongly supported sister clades (Figs 1, 2), *short-faced* (SF) and *long-faced* (LF), which broadly correspond to differences in facial morphology and ecologically important differences in tongue-length, with some exceptions. ArgK (Fig. 3D) gave somewhat weaker support for SF (*PP* = 0.83) and opsin (Fig. 3C) did not fully resolve it, whereas PEPCK did not fully resolve the LF clade (Fig. 3E). However, the sister group relationship between SF + LF, with or without *Kallobombus*, was supported in all nuclear gene analyses (Fig. 3B–E).

Nested within the *short-faced* clade was a large, well supported group consisting of taxa found exclusively in the New World, including *Robustobombus* and numerous mono- and ditypic subgenera (Figs 1, 2). Eight of the 19 mono- and ditypic subgenera fell into this *New World* (NW) clade, which was also strongly supported in the individual gene trees, except 16S. The subgeneric tree (Fig. 2, inset) shows clearly that the *New World* clade has poorer resolution among subgenera than any other part of the tree.

Although the nuclear gene trees were generally compatible, uncertainty surrounded both the root and the placement of the monotypic *Kallobombus* (Kl) with respect to the *short-* and *long-faced* clades. A consensus of the basal relationships for the five genes (Fig. 3A) summarizes the uncertainty. *Kallobombus* in the EF-1α tree (Fig. 3B) formed a trichotomy with the *short-* and *long-faced* clades whereas, in the opsin tree,

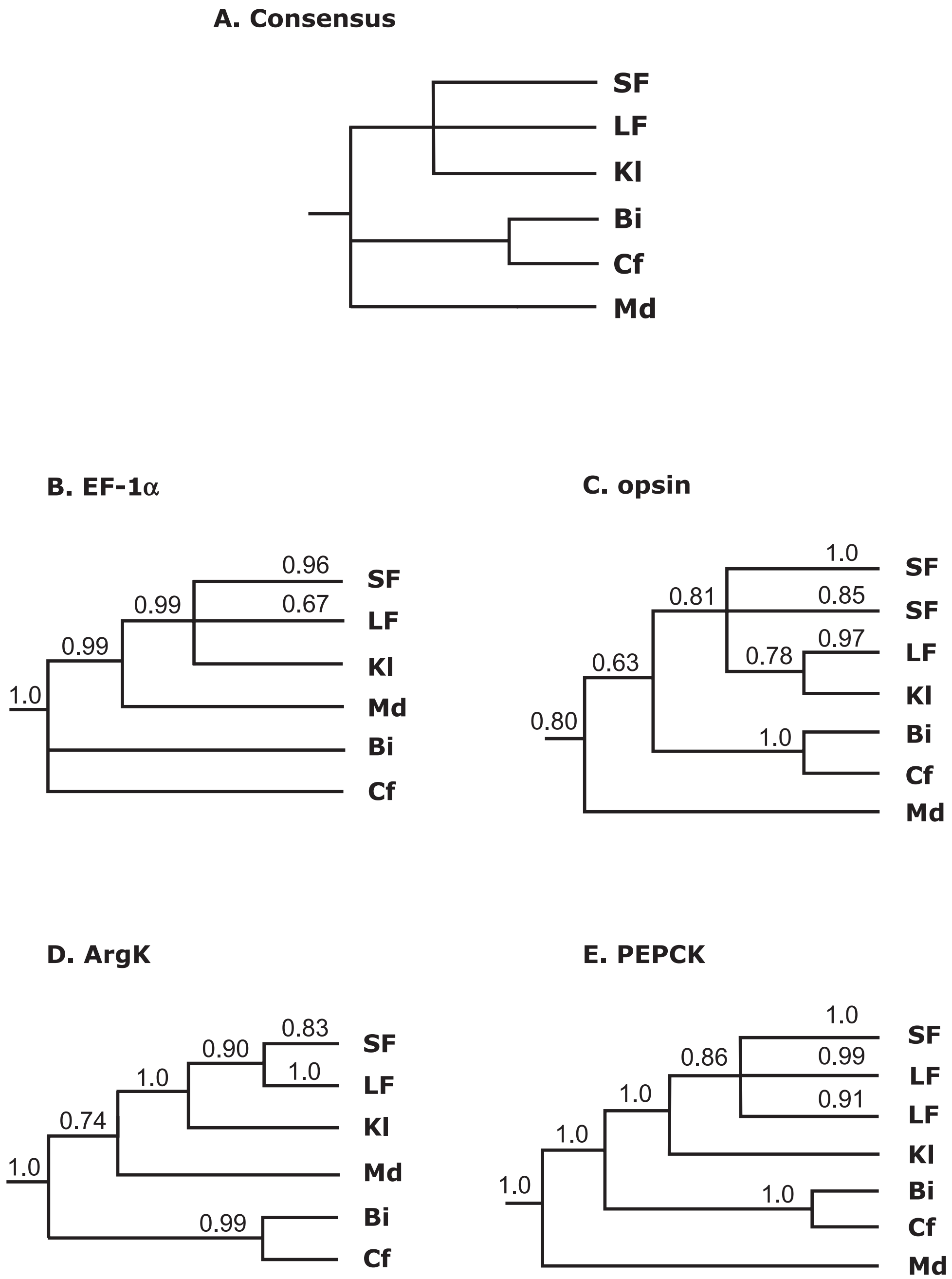

Figure 3. *Bombus* trees summarizing the basal relationships for each of four nuclear genes. A, summary consensus of the four individual gene trees: EF-1α (B), opsin (C), ArgK (D), and PEPCK (E). Kl, *Kallobombus*; Bi, *Bombias*; Cf, *Confusibombus*; Md, *Mendacibombus*. SF and LF refer to the *short-faced* and *long-faced* clades, respectively. Proportions shown on the branches of individual gene trees are Bayesian posterior probabilities.

it was more closely related to the *long-faced* clade (Fig. 3C) and, with ArgK and PEPCK, it was sister to SF + LF (Fig. 3D, E). In the combined-genes analysis, *Kallobombus* was resolved as sister to SF + LF with moderate support ($PP = 0.93$, BV = 63%). With respect to rooting the *Bombus* tree, opsin and PEPCK suggested a rooting along the *Mendacibombus* branch (Fig. 3C, E), although support from opsin was poor ($PP = 0.63$), and EF-1α and ArgK indicated a rooting with *Confusibombus* + *Bombias* ($PP = 0.99$ and 0.74, respectively). EF-1α did not resolve the sister group relationship between *Bombias* + *Confusibombus*, which was strongly supported by all the other genes ($PP = 0.98$–1.0). The combined analysis (Fig. 1) suggested *Mendacibombus* as sister group to the remaining bumble bees, with weak to moderate support (*PP* = 0.77, BV = 86%).

EFFECTS OF GAP-CODED CHARACTERS

Excluding the gap-coded characters from the five-genes data set had minor influences on tree topology overall and support values were highly similar to the complete data set. In a few cases, gap characters substantially increased support for a clade, including (1) the sister group relationship between *B. rufocinctus* and members of the *New World* clade + (*B. semenoviellus* + *B. apollineus*) (*PP* = 0.65 without gaps, 0.99 with gaps); (2) the sister group relationship between *Bombus citrinus* and *Bombus insularis* within *Psithyrus* (from 0.90 to 1.0); and (3) the sister group relationship between (*Megabombus* + *Senexibombus* + *Diversobombus*) and *Thoracobombus–Psithyrus* (from 0.91 to 0.98). By contrast, gap characters substantially decreased support for the relationship between *Bombus velox* and *Bombus ruderarius* (within *Thoracobombus*) from 0.99 to 0.52. Regarding topological changes, excluding the gap characters caused the *Bombus hypnorum–B. haematurus* group within *Pyrobombus* (Fig. 1) to attach as sister to the clade *Bombus cingulatus–B. biroi* (*PP* = 0.62), and within *Alpinobombus* (Fig. 1), *Bombus balteatus* attached as sister to *Bombus hyperboreus* in place of *Bombus neoboreus* (*PP* = 0.94). Most importantly, excluding gap characters shifted the position of the root from *Mendacibombus* to *Bombias* + *Confusibombus* (*PP* = 0.61).

MORPHOLOGY

Figure 4 illustrates comparative results of parsimony bootstrap analyses based on the complete set of morphological characters (Fig. 4A) and on the male genitalia alone (Fig. 4B), with bootstrap values indicating support for nodes > 50%. Genitalic characters alone resulted in higher tree resolution than when somatic characters were included, although support for the additional resolution was weak. Both data sets recovered a *New World* clade + *Sibiricobombus* but bootstrap support was < 50% with the inclusion of somatic characters. The genitalic data resolved a *short-faced* clade, with weak support (BV = 59%), and there was no resolution of a *long-faced* clade in either tree. The trichotomy *Senexibombus* + *Diversobombus* + *Megabombus* was well supported in both trees (BV = 83% and 78%). Five of the nine subgenera represented by two exemplars in the complete data set were recovered with good support (BV > 70%), only three were supported by genitalia alone. The basal divergences resembled the comprehensive DNA tree (Figs 1, 2), except that *Mendacibombus* was paraphyletic and *Bombias* and *Confusibombus* did not form a clade.

Bayesian analysis of the sequence data for these 47 taxa recovered a tree (not shown) entirely consistent with the subgeneric relationships from the all-taxon Bayesian analysis (Fig. 2), with nearly identical high support values (all but a few *PP* = 0.98). Adding the morphological characters to the 47-taxon sequence data resulted in decreased support for *Subterraneobombus* + *Orientalibombus* (from *PP* = 0.92 to 0.85) and for the placement *Kallobombus* as sister to the *short-faced* + *long-faced* clades (from *PP* = 0.94 to 0.68). Also, *Alpigenobombus* moved from its position as an unresolved polytomy with the clades circumscribing the *New World* taxa–*Sibiricobombus* and *Melanobombus–Rufipedibombus* to that of sister to these two clades, although support was weak (*PP* = 0.85).

DISCUSSION

MONOPHYLY OF CONVENTIONAL SUBGENERA

An unequivocal result of this molecular phylogenetic examination is the strong support that it provides for groups of species represented by the conventional subgeneric system, as developed over many years from morphological taxonomy (Radoszkowski, 1884; Vogt, 1911; Krüger, 1917; Skorikov, 1922; Frison, 1927; Richards, 1968; summarized in Williams, 1998). This is consistent with the more limited molecular analyses of Pedersen (2002) and Kawakita *et al*. (2004). In retrospect, the long stability of the *Bombus* subgenera is notable given the intense controversies over their higher-level classification (for a review, see Ito, 1985). Clearly, characters of the male genitalia (Radoszkowski, 1884; Vogt, 1911; Richards, 1968; Williams, 1985, 1994) have proven reliable indicators of species-group membership (see also Hines *et al*., 2006).

The problems generally arise when these same characters are used to assess relationships among the subgenera. On the whole, morphological characters, whether analysed phenetically (Plowright & Stephen, 1973; Ito, 1985) or cladistically (Williams, 1985, 1994; Ito & Sakagami, 1985), have been unable to fully resolve the intersubgeneric relationships or to reconstruct higher-level clades that are congruent with the DNA-based phylogeny. Although finding morphological characters that can serve as synapomorphies for higher-level groupings of *Bombus* could be useful, another approach to the study of morphology would make use of the well-resolved DNA phylogeny to elucidate the evolution of functionally interesting morphological traits, such as features of the male genitalia, the tongue, and colour pattern.

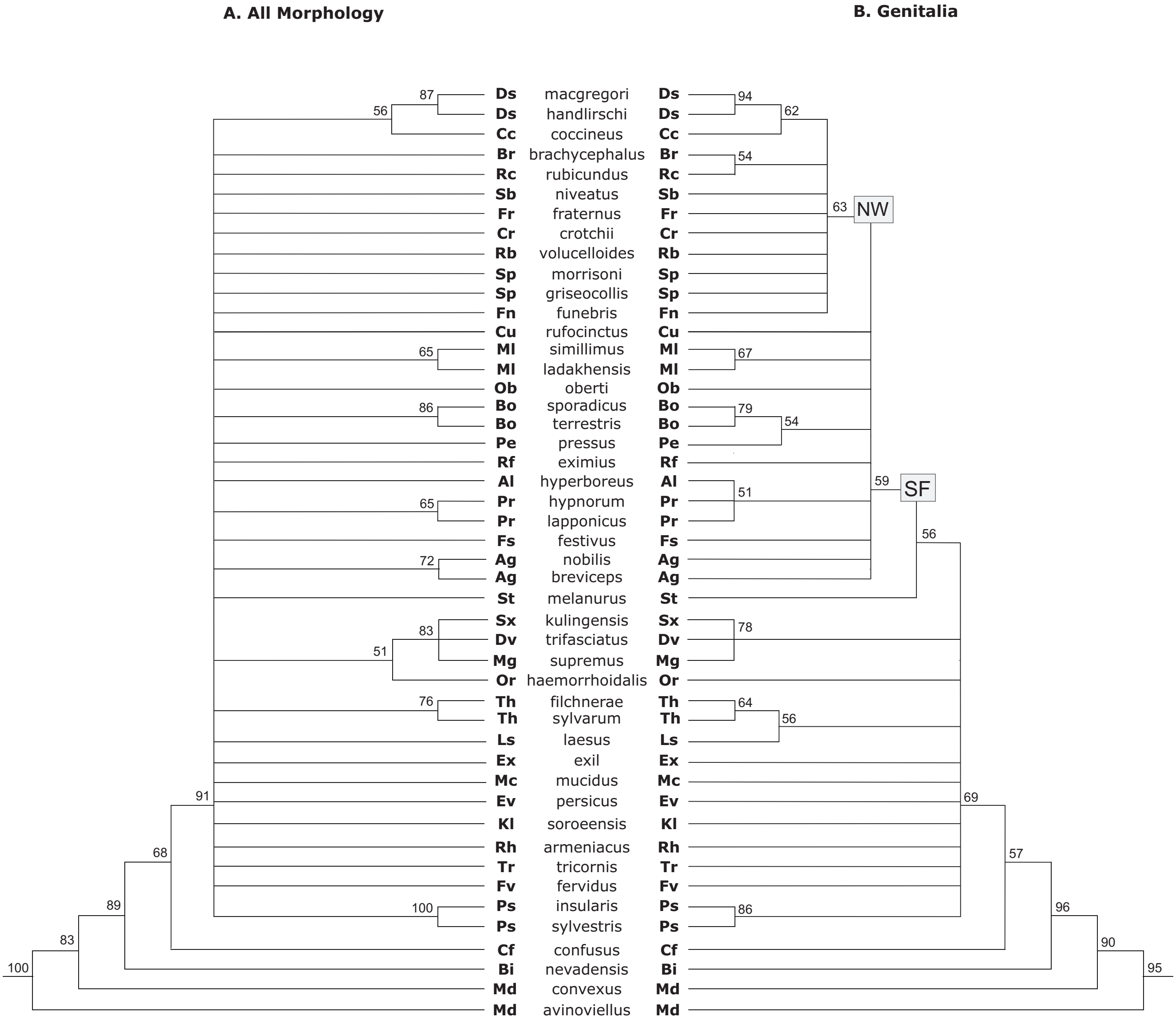

Figure 4. Nonparametric parsimony bootstrap trees estimated from morphological characters for 47 *Bombus* subgeneric exemplars. A, analysis of the total morphology data set. B, analysis of genitalic characters alone. Values above branches indicate bootstrap support (BV) > 50%. Subgeneric taxon labels are given in Table 1.

In a few cases, our results lead to the splitting of conventional subgenera. The large subgenus *Fervidobombus* is a conspicuous example, as the *dahlbomii*-group (Fig. 1) falls outside this clade, leaving behind a strongly supported clade attached to a polyphyletic *Tricornibombus*. The polyphyly of *Fervidobombus* was suggested in earlier phenetic (Ito, 1985) and cladistic analyses of subgeneric relationships (Koulianos & Schmid-Hempel, 2000; Kawakita *et al*., 2003). The report by Cameron & Williams (2003), rooting *Fervidobombus* with *Thoracobombus* (thought to be among its closest relatives), produced an apparently monophyletic clade because other subgenera were not represented in the analysis. More complete taxon sampling outside *Fervidobombus* updates this.

Williams (1998) assigned *B. handlirschi* to the monotypic *Dasybombus* (Labougle & Ayala, 1985) based on similarities of the male genitalia, but the DNA data show that *B. handlirschi* is closely related to *Rubicundobombus* (*sensu* Franklin, 1912, 1913) and *Coccineobombus* (Fig. 1). The two remaining occurrences of nonmonophyly of subgenera (*Cullumanobombus* and *Separatobombus*) are less clear cut in terms of support, and would benefit from targeted studies of those groups.

In one of the more surprising results, it appears that *Pressibombus* is a part of *Pyrobombus*. Morphologically, *B. pressus* is characterized by distinctive male genitalia, particularly by a strong reduction of the gonostylus, which appears to a lesser extent in other members of the *parthenius*-group, and by a unique ventrolateral production of the head of the penis valve. Apart from this new addition to the group, *Pyrobombus*, the largest subgenus, is decisively monophyletic (for additional discussion, see Hines *et al*., 2006). In contrast to earlier reports by Williams (1991, 1994, 1998) suggesting that *Mendacibombus* is paraphyletic and *Sibiricobombus sensu* Richards (1968) (which included *Obertobombus*) is polyphyletic, both groups are monophyletic and, from the single species studied here, *Obertobombus* indeed forms a monophyletic group with *Sibiricobombus*. The monotypic *Festivobombus* and *Rufipedibombus* form a strongly supported group with the larger *Melanobombus*.

The socially parasitic subgenus *Psithyrus* has provoked a great of deal of taxonomic controversy. Historically, *Psithyrus* was considered a separate genus of bumble bees (Lepeletier, 1832) because of the divergent biology and morphology associated with its inquiline mode of life. Our results strongly support *Psithyrus* as a monophyletic group within *Bombus*, which is consistent with the majority of findings over the last few decades (Plowright & Stephen, 1973; Pekkarinen *et al*., 1979; Ito, 1985; Williams, 1985, 1991, 1994; Pamilo *et al*., 1987; Pedersen, 2002; Kawakita *et al*., 2004). Furthermore, *Psithyrus* divides into the same clades (Fig. 1) established as subgenera in earlier taxonomic studies (Franklin, 1912, 1913; Frison, 1927; Popov, 1931; Pittioni, 1949). Williams (1991, 1998) treated these as species groups, synonymizing the subgeneric names; Rasmont *et al*. (1995) considered them as separate subgenera within *Bombus*. The relationships among these species groups receive weaker support, highlighting the consistency in relative degree of support between the molecular and morphological data.

Of potential interest to *Psithyrus* evolution is the finding by Bromham & Leys (2005) that rates of molecular evolution in social parasites are faster than those of their social relatives. If this conclusion is accepted, and if substitution rates are expected to evolve in a lineage-dependent fashion, it follows that the socially parasitic *Psithyrus* should exhibit faster rates than most of their social relatives. There may be a hint of this in the greater combined branch lengths of *Psithyrus* relative to the other subgenera, but testing this requires sister-group comparison of substitution rates in their non-parasitic sister taxa.

DEEP DIVISIONS WITHIN *BOMBUS*

Krüger (1917) recognized a division of the bumble bees into two larger sections, *Odontobombus* ('with spine') and *Anodontobombus* ('without spine'), based on several diagnostic morphological characters, including head shape and presence/absence of a mid-basitarsal spine. His *Odontobombus* (*Thoracobombus*, *Laesobombus*, *Mucidobombus*, *Rhodobombus*, *Subterraneobombus*, and *Megabombus*) and *Anodontobombus* (*Pyrobombus*, *Bombus s.s.*, *Alpinobombus*, *Cullumanobombus*, *Melanobombus*, *Kallobombus*, *Confusibombus*, and *Mendacibombus*) correspond approximately to our *long-faced* and *short-faced* clades. In detail, the *long-faced* clade includes an additional eight subgenera (Fig. 1) not included in Krüger's more restricted investigation of Central European fauna, and the *short-faced* clade includes 13 subgenera omitted by Krüger. Krüger's characters led him to group *Kallobombus*, *Confusibombus*, and *Mendacibombus* within the equivalent of the *short-faced* clade, whereas our results reveal that these taxa fall outside both divisions, representing more basal divergences in the phylogeny. Kawakita *et al*. (2004) discussed two higher-level *Bombus* groupings (clades A and B), which are consistent with our *long-faced* and *short-faced* divisions, but with many fewer taxa and weak support for their A clade. Interestingly, with Bayesian analysis of sequences from only 47 (~20%) of the 218 taxa collected for this study, we recovered the identical structure to that of the comprehensive tree, with well supported intersubgeneric relationships, including the *short-faced*, *long-faced*, and *New World* subdivisions. This indicates that taxon sampling is less important in recovering higher level phylogenetic structure of *Bombus* than is the availability of a large number of informative characters.

RELATIONSHIPS NEAR THE RANK OF SPECIES

In several cases, sister taxa share such a remarkable degree of sequence similarity that we might ask whether they are conspecific. Given the 1–2 bp differences we commonly see within a gene for many taxon samples, for example, within nuclear genes of heterozygous females or from comparisons of conspecific taxa sequenced both by us and by Kawakita *et al*. (2003), we consider it likely that two nominal taxa are conspecific if they differ by only 1–2 bp across all gene sequences (equivalent to only 0.03–0.05% sequence divergence). Of course, final decisions about species status will require examining the patterns of variation among a broad sampling of individuals.

Several sympatric species pairs exhibit discordant colour patterns yet have virtually identical sequences. For example, *Bombus* (*Sibiricobombus*) *niveatus* (Fig. 1), which is white-banded, and *Bombus vorticosus*, which is yellow-banded, differed by a single nucleotide (ArgK) across all five genes, supporting their status as dimorphic colour forms of the same species.

Williams (1991, 1998) concluded this from morphology and Rasmont *et al*. (2005) from mandibular gland secretions. *Bombus* (*Mendacibombus*) *handlirshianus* (white-band) and *Bombus shaposhnikovi* (yellow-banded) differ by two bases (1 bp 16S, 1 bp PEPCK), and individuals of these two colour forms were even observed to share a common nest entrance at a site in north-east Turkey (H. M. Hines, pers. observ.). Some of these colour dimorphisms may be the result of intraspecific diallelic differences (Owen & Plowright, 1980). The lack of correspondence between colour pattern variation and degree of nucleotide differentiation shows that much remains to be learned about colour pattern evolution, and sets the stage for examining possible adaptive causes, including mimicry.

Some putative allopatric species pairs, also described from distinct colour morphs, may be conspecific. One example involves the two *Senexibombus* species, *Bombus bicoloratus* (Taiwan) and *Bombus kulingensis* (mainland China), which differ by only a single base in both the 16S and PEPCK sequences. Another example includes *Bombus pensylvanicus* from eastern North America and *Bombus sonorus* from western North America: both taxa, which are similar in morphology but variable in colour pattern (Williams, 1998), exhibit signs of introgression as *B. sonorus* from San Luis Potosí, Mexico (an area of sympatry with *B. pensylvanicus*) and *B. sonorus* from Arizona are not monophyletic relative to two *B. pensylvanicus* sequences from Missouri (Fig. 1). Other likely conspecific taxa, questioned by Williams (1998), include *Bombus erzurumensis*/*Bombus sichelii* (3 bp difference for 16S, 3 bp for EF1α, 1 bp for PEPCK) and *Bombus volucelloides*/*Bombus melaleucus* (2 bp 16S, 3 bp ArgK).

In two cases, allopatric pairs of *Psithyrus* taxa exhibit nearly identical sequences and could be conspecific: *Bombus fernaldae*/*Bombus flavidus* (1 bp difference EF1a, 1 bp ArgK) and *B. ashtoni*/*B. bohemicus* (1 bp ArgK). It is unusual that one member of each pair occurs only in the New World and the other only in the Old World, respectively. If conspecific, each pair would encompass a nearly Holarctic temperate distribution, thus far found only in the *lucorum* complex and some *Alpinobombus* species. The possible conspecificity of *B. fernaldae* and *B. flavidus* has not been considered until now, but Williams (1991) suggested that *B. ashtoni* and *B. bohemicus* could be parts of the same species. Both taxa specialize on hosts of the subgenus *Bombus s.s.*: *B. ashtoni* on *Bombus affinis* and *B. terricola* (Plath, 1934; Fisher, 1984), *B. bohemicus* on *Bombus lucorum* (Alford, 1975)

Other species pairs considered potentially conspecific (Williams, 1998) are somewhat genetically divergent based on 16S, including *B. occidentalis*/*B. terricola* (7 bp, 1.3%); *Bombus fervidus*/*Bombus californicus* (8 bp, 1.6%); *B. velox*/*Bombus deuteronymus* and *Bombus sylvicola*/*Bombus lapponicus* (10 bp, 1.9%); *B. moderatus* + *B. cryptarum*/*B. lucorum* (11 bp, 2.1%, 14 bp, 2.6%; neither taxon is monophyletic with *B. lucorum*); *Bombus alagesianus*/*Bombus keriensis* (14 bp, 2.6%) and *Bombus difficillimus*/*Bombus melanurus* (20 bp, 3.8%). The separation of *B. auricomus* and *B. nevadensis* on the basis of morphology, colour pattern (Williams, 1998), and allozymes (Scholl *et al*., 1992) is also supported by our sequence data (22 bp, 4.2%). Interestingly, *Bombus modestus* from Kazakhstan is genetically distinct from *B. modestus* from Sichuan, China for 16S, exhibiting greater genetic distance (24 bp, 4.5%) than many pairs of sister species.

IMPLICATIONS FOR *BOMBUS* CLASSIFICATION

Several authors have suggested that the subgeneric system for bumble bees would benefit from simplification (Menke & Carpenter, 1984; Williams, 1998). The challenge is to recognize monophyletic groups that are morphologically and behaviourally meaningful, while reducing the number of small subgenera. Our *Bombus* phylogeny raises the possibility of revising the classification with more confidence by providing a framework to ensure monophyletic subgenera and reduce the proliferation of formal group names. Here we intend no nomenclatural action as specific recommendations concerning recognition of groups will be made elsewhere, based in part on some of the following relevant details.

Concerning the *New World* clade, rather than recognizing even more monotypic subgenera, it might be more practical to recognize the entire *New World* clade as a single subgenus consisting of several species groups, including *Robustobombus*. Further reductions in numbers of subgenera could be achieved by placing *Obertobombus* (*Bombus oberti*) into *Sibiricobombus*, and by including *Festivobobombus* (*Bombus festivus*) and *Rufipedibombus* in *Melanobombus*. Whether to consolidate these taxa into one group is complicated by the fact that many of the monotypic subgenera are sister to clades subtended by conspicuously long branches, suggesting long separation/distinctness (Fig. 1). In view of these apparently large intersubgeneric genetic distances, it might be prudent to consider additional biological characters in decisions as to whether to combine the taxa.

Given the strong support for *Pressibombus* falling within *Pyrobombus*, it is reasonable to synonymize *Pressibombus* under *Pyrobombus*. The clade comprising the monotypic subgenera *Mucidobombus*, *Eversmannibombus*, and *Laesobombus* could be considered as members of *Thoracobombus* or as a separate three-species subgenus. *Exilobombus* attaches to *Tricornibombus* (excluding *B. imitator*) and should be considered a part of *Tricornibombus*. *Megabombus* could include *Senexibombus*. *Bombias* and *Confusibombus* might be combined into a single subgenus, although the branches appear long and the respective taxa distinct.

New names might be required to accommodate the splitting of a few subgenera. The obvious division of *Fervidobombus* into two monophyletic groups could require a new species group or subgenus designation for *B. dahlbomii* + *B. morio* + *B. excellens*. Other classification decisions may involve *Cullumanobombus*, *Separatobombus*, *Dasybombus*, and *Tricornibombus*.

FUTURE RESEARCH

Phylogeny

Within some clades (e.g., *Bombus s.s.* and *Fervidobombus*), a few of the internodes are short but have high posterior probabilities (Fig. 1). We interpret these relationships with caution because Bayesian inference is highly sensitive to phylogenetic signal (indicating high posterior probability values with relatively few characters) and has been shown (with simulated data) to occasionally attach high support values to incorrect short internodes (Alfaro, Zoller & Lutzoni, 2003). Gathering additional characters should resolve the uncertainties associated with these short branches. Importantly, new characters from other genes should increase resolution and support for relationships within the *New World* clade and strengthen the placement of *Kallobombus* and assignment of the root.

The missing putative species (Williams, 1998) from this study are most notably from *Psithyrus* (eight species), *Mendacibombus* (five species) and *Melanobombus* (four species). Examination of the positions of these and remaining species from small subgenera (e.g., *Cullumanobombus*, *Rufipedibombus*, *Senexibombus*, and *Orientalibombus*) would secure our knowledge of *Bombus* relationships and subgeneric monophyly.

Diversification in the New World

The pattern of diversification of the *New World* clade, with its numerous distinctive taxa that span North America, Mexico, and the Andes, suggests there may have been at least two episodes of relatively rapid divergence after colonization of the New World. The first of these is implied by the very short branches that subtend relatively long branches of the morphologically distinct monotypic subgenera, which are difficult to resolve with good support (Fig. 1). This pattern poses interesting questions concerning the interplay between colonization of the New World and trends in bumble bee diversification. Comparative studies of sister taxa outside this group, which are all Old World (except *B. rufocinctus*), could reveal whether any morphological or physiological innovations occurred in association with episodes of continental colonization and diversification (Kay *et al.*, 2005). The second episode occurred with *Robustobombus*, the only speciose monophyletic subgenus within the *New World* clade. This group seems to have diverged more recently in upland areas of the northern Andes, perhaps in association with new ecological opportunities provided after the northern Andean uplift (3–5 Mya) that involved the colonization by new plant genera (Hughes & Eastwood, 2006). Our robust species phylogeny affords the opportunity to test this hypothesis with estimates of divergence times.

The only other large endemic New World subgenus, *Fervidobombus*, suggests a similar pattern of diversification after colonization from the Old World. However, in contrast to taxa of the *New World* clade, many of which inhabit cooler, mountainous habitats, *Fervidobombus* inhabit warmer lowland habitats, including tropical rain forest (Williams, 1985; Cameron & Williams, 2003). Their predisposition to nest above ground, a characteristic of many of their closest Old World relatives, may underlie their mostly lowland distribution, and at least partially explain the innovation in nest architecture associated with the atypical rain forest species, *Bombus transversalis* (Taylor & Cameron, 2003) and *Bombus pullatus* (Hines, Cameron, & Deans, 2007). Additional research on the biology of Old World relatives should clarify influences of ecological shifts and phylogeny on life history of the tropical bumble bees.

Colour pattern evolution

Colour pattern mimicry in bumble bees has been suggested by several authors (Plowright & Owen, 1980; Williams, 1991) to explain many geographical trends in colour pattern variation. However, conclusions that colour patterns do not also evolve along phylogenetic lineages have been limited by incomplete species sampling and coding of the full range of patterns and intraspecific polymorphisms. Our comprehensive phylogenetic results permit the first comparative examination of convergence and mimicry on a worldwide scale.

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APPENDIX 1

The morphological character states represented in the matrix are listed. Multistate characters analysed as ordered are shown with asterisks. Characters of the penis refer to the penis valves unless otherwise specified.

MALE PENIS

1. Spatha broader than long (0); spatha longer than broad (1).
2. Spatha basally broadly rounded (0); spatha basally acutely pointed (1).
3. Spatha laterally continuous with valves (0); spatha laterally overhanging valves (1).
4. Dorsal lightly sclerotized channel narrow (0); dorsal lightly sclerotized channel broad (1).
5. Shaft without a distinct ventro-lateral angle (0); shaft with an acute ventro-lateral angle near the midpoint of its length (1); shaft with only a weak trace of a ventro-lateral angle (2); shaft with ventro-lateral angle broadly rounded as a shallow convexity (3); shaft with ventro-lateral angle pronounced as a very broadly rounded right angle (4).
6. Shaft dorso-ventrally narrow or irregular in breadth (0); shaft uniformly dorso-ventrally expanded (1).
7. Apex straight or curved outwards (0); apex curved in towards body midline (1).

8.* Head strongly laterally compressed (0); head nearly tubular (1); head strongly dorso-ventrally flattened (2).

(9) Inner basal shelves broad (0); inner basal shelves narrow (1).

10.* Inner shelves of head absent or weakly defined (0); inner shelves of head strongly marked basally by a pronounced right angle, then running parallel to shaft axis as far as recurved head (1); inner shelves expanded basally (2).

11.* Head with outer shelf narrow (0); head with outer shelf extended laterally by more than the same breadth as head (1); head with outer shelf curved ventrally and then twisted towards apex, to form half of a funnel (2).

12. Head with outer shelf narrow or board (0); head with outer margin of shaft with straight section narrow subapically (1).

13. Head with inner (median) margin of recurved section convex (0); head with inner margin of recurved section concave.

14. Head with or without inwardly recurved hook (0); head straightened with secondary loss of inwardly recurved hook (1).

15. Head without basal ventral projection (0); head with basal ventral projection (1).

16. Shaft nearly straight in lateral aspect (0); shaft with a strong ‘S’-shaped dogleg in lateral aspect (1).

MALE VOLSELLA

17.* Small irregular sclerite, not extending apically further than gonostylus (0); large clasping organ, extending apically further that gonostylus (1); entire volsella elongated and narrowed (2).

18. Outer margin with long setae (0); outer margin without long setae (1).

19. Lateral margins subapically converging (0); lateral margins broadened immediately subapically and then truncated apically (1).

20. Inner margin without a distinct subapical process (0); inner margin with a subapical process, which is usually toothed, arising just before inner margin and projecting in towards midline of body (1); inner subapical process produced distinctly beyond inner margin, often in the form of a broad curved tongue (2); inner subapical process reduced to an indistinct curve in margin, always lacking teeth (3).

21. Inner subapical process distinctly separated from apex, often nearer midpoint of length (0); inner subapical process narrowly subapical, at least on long axis of volsella.

22.* Inner ventral ridge not swollen or swelling not curved back proximally towards outer margin (0); inner ventral ridge, near mid-point of volsella length, pronounced at the inner edge of coarsely sculptured area in the apical half and curved back proximally towards the outer margin (1); coarsely sculptured ventral area broadened basally before an inner constriction to a narrower subapical neck, and pear-shaped (2).

23.* Coarsely sculptured ventral area weakly defined or proximal half reaching outer margin adjacent to gonocoxite (0); proximal half of coarsely sculptured ventral area separated from outer margin by a concave, weakly sculptured area, forming a narrow shining submarginal groove (1); proximal half of coarsely sculptured ventral area separated from outer margin by a concave, weakly sculptured area, forming a submarginal groove as broad as coarsely sculptured area (2); proximal half of coarsely sculptured ventral area separated from margin by broad submarginl groove with long setae (3).

24.* Inner apical margin without stout setae (0); inner apical margin with stout setae (1); inner apical margin with stout setae very long and dense (2).

MALE GONOSTYLUS

25. Inner basal corner without a distinct process (0); inner basal corner with a distinct rounded process projecting in towards midline of body (1); inner basal corner with a distinct process; distally narrowed in the form of a single sharp spine (2); inner basal process with teeth (3).
26. Basal inner margin associated with setae (0); basal inner margin without associated setae (1).
27. Inner basal process produced (0); inner basal process reduced to a stub or membranous or both (1).
28. Inner apical margin simple (0); inner apical margin double with a submarginal groove (1).
29. Apical process present (0); apical process absent (1).
30. Apical process rounded or narrowing (0); apical process broadened (1).

MALE HEAD

31. Mandible with sparse long setae from posterior margin (0); mandible with dense setae from posterior margin, forming a 'beard' (1).
32. Antenna short, not reaching back beyond wing bases (0); antenna long, reaching back beyond wing bases (1).
33. Flagellar segments nearly straight and cylindrical (0); flagellar segments curved (1).
34. Compound eye similar in relative size to female eye (0); compound eye distinctly enlarged relative to female eye (1).

MALE THORAX

35. Hind tibia with outer surface uniformly convex (0); hind tibia with outer surface partially concave medially in distal third (1).
36. Hind tibia with short or long hairs over entire outer surface (0); hind tibia without even short hairs medially in distal third (1).

MALE ABDOMEN

37. Gastral sternum VII with posterior margin medially convex or irregular, but not broadly concave (0); gastral sternum VII with posterior margin medially broadly concave (1).

FEMALE HEAD

38.* Labrum with median longitudinal ridge (0); labrum with complete transverse ridge between two grooves (1); labrum with transverse ridge broadly interrupted medially (2); labrum with median part of transverse ridge displaced towards apex of labrum to form a projecting lamella, which reaches towards the anterior margin of the labrum (3).
39. Labrum broadly rectangular (0); labrum broadly triangular (1).
40. Mandible distally broadly rounded (0); mandible distally pointed (1).
41. Mandible with two to four teeth (0); mandible with six teeth (1).
42. Mandible with basal keel not reaching distal margin (0); mandible with basal keel reaching distal margin (1).
43. Oculo-malar distance less than the basal breadth of mandible (0); oculo-malar distance equal to or greater than the basal breadth of mandible (1).
44. Oculo-malar area broadly rounded into the face anteriorly, the area below the eye uniformly convex (0); oculo-malar area separated from the face anteriorly by a narrowly rounded angle, the area immediately below the eye partially concave (1).

FEMALE THORAX

45.* Mid-basitarsus with disto-posterior corner broadly rounded or forming a right angle (0); mid-basitarsus with acute disto-posterior corner (1); mid-basitarsus with pronounced disto-posterior spine (2).
46. Hind tibia without corbicula (0); hind tibia with corbicula (1).
47. Hind tibia with disto-posterior corner forming right angle (0); hind tibia with disto-posterior corner acute or spinosely produced (1).
48. Hind basitarsus with proximo-posterior process no longer broad (0); hind basitarsus with proximo-posterior process longer than broad (1).
49. Hind basitarsus with proximal process with few scattered hairs on outer surface (0); hind basitarsus with proximal process densely hairy on outer surface (1).

FEMALE ABDOMEN

50.* Gastral sternum II without transverse ridge (0); gastral sternum II with weakly rounded traverse ridge (1); gastral sternum II with strongly raised transverse ridge (2).
51. Gastral sternum VI without subapical swellings, curving gradually dorsally (0); gastral sternum VI with paired subapical swellings, lateral areas abruptly turned dorsally (1).
52. Gastral sternum VI without lateral keels (0); gastral sternum VI with lateral keels (1).
53. Gastral segments V–VI nearly coaxial with segments I-IV (0); gastral segments V–VI curled ventrally and back towards anterior (1).

APPENDIX 2

Morphological character matrix. Morphological character states are explained in Appendix 1.

	11111111112222222222333333333344444444445555 12345678901234567890123456789012345678901234567890123
Lestis	00000-000000-00000-----000000000110000010100001000000
Eufriesea	00100-000000-00001-----000000000000100010100010000000
avinoviellus	10000-000000-0001000-000010000100110010000100100000000
convexus	10010-001000-00010010000010000100110010000100100000000
nevadensis	10010-001000-00011021001110000100110120000101110000000
confusus	10010-011000-00011020001110000100110030000101110000000
soroeensis	111120011000-0001102100101000011100013000100111101000
haemorrhoidalis	110120011000-0001112000201100111101103000110011101000
kulingensis	110120011000-0001102100231000111101103000100211100000
trifasciatus	110120011000-0001102100231000111101103000110211100000
supremus	110120011000-0001102100231000111101103000110211100000
melanurus	11112011100000001102100111000011101003000110211101000
insularis	110120011000-0001103000110100011100003110001200002111
sylvestris	110120011000-0001103000110100010000003110001200002111
exil	110120011000-0001102000211000011100003000100211101000
tricornis	110120011000-0001102100111000011100003000110211101000
fervidus	110120011000-0001102000111000011100003000110211101000
armeniacus	111120011000-0001102100111000011100003000110211101000
persicus	110120011000-0001103000211000011100003000110211101100
mucidus	110120011000-0001102000211000011100003000110211101000
laesus	110120011000-0011112000211000011101003000100211101000
filchnerae	110120011000-0011102000221000011100003000110211101000
sylvarum	110120011000-0011102000221000011100003000110211101000
hyperboreus	111120121000-1101102100111110010001103000110111101000
sporadicus	11114012103001101102100111100010001103000100011100000
terrestris	11114012103001101102100111100010001103000100011100000
hypnorum	11112012100000001102100111110010001003000100011100000
lapponicus	11112012100000001102100111110010001003000100011100000
pressus	11114012102000101102100111001010000103000110011100000
breviceps	11112012100000001102100111000010000003001100111101000
nobilis	11112012100000001102100111000010000103001100111101000
eximius	11013012101000001102100111001011001103000110011100000
festivus	11111012100000001102100111100010000003000100011100000
simillimus	11111012100110001102100111100010011013000110011100000
ladakhensis	11011012100110001102100111100010000013000110010100000
oberti	11111012100010001102100111000010010003000110011110000
niveatus	11111012110000001102110111000011011013000110011110000
rufocinctus	11111012100000001102100111000010010113000100011100000
brachycephalus	11111012120000001102123111100010010013000100011100000
rubicundus	111110121200-0001102123111000010010013000100011100000
handlirschi	11111112110010002102123101100010010013000100011100000
coccineus	11111112110010001102123111000010010013000100011100000
fraternus	11111012100000001102120111000001010013000100011100000
crotchii	11111012110000001102121111000011011103000100011100000
griseocollis	11111012100000001102122111100010010013000100011100000
morrisoni	11111012100000001102122111000010011013000100011100000
funebris	111110121000-0001102122111000010010013000100011100000
volucelloides	11111012110010001102122111000011010013000100011100000
macgregori	11111112100010002102123101100010010013000100011100000

SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article:

Figure S1. 16S. Phylogeny of *Bombus* estimated from Bayesian analysis of mitochondrial 16S rRNA nucleotide data. Values above branches are Bayesian posterior probabilities. The left hand portion of the figure connects via the dashed lines at the bottom to the dotted lines at the top of the right hand portion of the figure.

Figure S2. EF-1∝. Phylogeny of *Bombus* estimated from Bayesian analysis of elongation factor-1alpha F2 copy nucleotide sequences. Values above branches are Bayesian posterior probabilities. The left hand portion of the figure connects via the dashed lines at the bottom to the dotted lines at the top of the right hand portion of the figure.

Figure S3. Opsin. Phylogeny of *Bombus* estimated from Bayesian analysis of long-wavelength rhodopsin copy 1 nucleotide sequences. Values above branches are Bayesian posterior probabilities. The left hand portion of the figure connects via the dashed lines at the bottom to the dotted lines at the top of the right hand portion of the figure.

Figure S4. ArgK. Phylogeny of *Bombus* estimated from Bayesian analysis of arginine kinase nucleotide sequences. Values above branches are Bayesian posterior probabilities. The left hand portion of the figure connects via the dashed lines at the bottom to the dotted lines at the top of the right hand portion of the figure.

Figure S5. PEPCK. Phylogeny of *Bombus* estimated from Bayesian analysis of phosphoenolpyruvate carboxykinase nucleotide sequences. Values above branches are Bayesian posterior probabilities. The left hand portion of the figure connects via the dashed lines at the bottom to the dotted lines at the top of the right hand portion of the figure.

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