

Short Communication

Phylogeny, historical biogeography, and character evolution in bumble bees (*Bombus*: Apidae) based on simultaneous analysis of three nuclear gene sequences[☆]

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1. Introduction

Most of the nearly 250 bumble bee species are primitively eusocial and live as colonies (Michener, 1974, 2000). All bumble bee species are now placed in genus *Bombus* (Apidae: Bombini), including a lineage of obligate social parasites of bumble bees formally placed in a separate genus, *Psithyrus*. For over a century, *Bombus* has been a renowned model taxon for illuminating topics of general biological interest (Darwin, 1859; Heinrich, 1979; Michener, 2000; Sakagami, 1976), but the overall morphological similarity among bumble bee species has hindered full resolution of bumble bee phylogeny and interpretation of their evolution (Michener, 2000; Williams, 1994). Previous studies employing DNA sequence data sets, mostly consisting of mitochondrial sequences, provided some phylogenetic insight (Cameron and Williams, 2003; Koulianos, 1999; Koulianos and Schmid-Hempel, 2000; Pedersen, 1996; Pedersen, 2002; Tanaka, 2001). However, the taxonomic sampling in these studies was limited for a robust assessment of *Bombus* phylogeny. Also, high substitution rates and biased base composition of bee mitochondrial DNA impede sufficient resolution of higher-level phylogeny.

Our recent work (Kawakita et al., 2003) has recovered robust phylogenetic relationships among most major *Bombus* lineages based on three nuclear genes, long-wavelength rhodopsin (LW Rh), arginine kinase (ArgK), and elongation factor-1 α F2 copy (EF-1 α). This study also showed that these genes contain gaps within introns that can reinforce results obtained from base substitutions. In this study, we incorporate the methodological advance in our previous study and analyse an expanded collection of samples (Table 1) to interpret historical biogeography and evolution of behavioral and morphological attributes in bumble bees. We show that early diversification of bumble bees took place primarily in the Palearctic, followed by repeated intercontinental faunal interchanges. We also describe major evolutionary trends in brood-rearing behavior and proboscis length, characters of potential importance for shaping the diversification of bumble bees.

2. Materials and methods

A list of bumble bee and outgroup exemplar species is given as Supplementary material. Methods of DNA sequencing and phylogenetic analyses are described in Kawakita et al. (2003). As in our previous assessment (Kawakita et al., 2003), we conducted simultaneous parsimony analysis of exon + intron data for three nuclear gene fragments (LW Rh, ArgK, and EF-1 α) including appropriately coded indel nucleotides and gap

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Table 1

A list of *Bombus* subgenera with information on distribution and species number

Subgenera	Distribution	No. species	No. sampled
<i>Mendacibombus</i>	Pa	12	2
<i>Bombias</i>	Ne	1	1
<i>Confusibombus</i>	Pa	1	—
<i>Mucidobombus</i>	Pa	1	1
<i>Fervidobombus</i>	Ne, Nt	20	3
<i>Exilobombus</i>	Pa	1	—
<i>Psithyrus</i>	Ho	29	7
<i>Eversmannibombus</i>	Pa	1	—
<i>Tricornibombus</i>	Pa	3	1
<i>Thoracobombus</i>	Pa	19	7
<i>Orientalibombus</i>	Pa	3	1
<i>Laesobombus</i>	Pa	1	—
<i>Megabombus</i>	Pa	14	6
<i>Diversobombus</i>	Pa	4	3
<i>Senexibombus</i>	Pa	4	1
<i>Rhodobombus</i>	Pa	3	1
<i>Alpinobombus</i>	Ho	5	1
<i>Kallobombus</i>	Pa	1	1
<i>Subterraneobombus</i>	Ho	9	3
<i>Alpigenobombus</i>	Pa	6	1
<i>Pyrobombus</i>	Ho	43	21
<i>Festivobombus</i>	Pa	1	—
<i>Rufipedibombus</i>	Pa	2	—
<i>Bombus</i> s. str.	Ho	10	7
<i>Pressibombus</i>	Pa	1	—
<i>Cullumanobombus</i>	Ho	4	1
<i>Melanobombus</i>	Pa	14	2
<i>Obertobombus</i>	Pa	2	—
<i>Sibiricobombus</i>	Pa	5	—
<i>Fraternobombus</i>	Ne	1	1
<i>Crotchiibombus</i>	Ne	1	1
<i>Robustobombus</i>	Nt	5	1
<i>Funebribombus</i>	Nt	2	—
<i>Separatobombus</i>	Ne	2	2
<i>Dasybombus</i>	Nt	2	—
<i>Coccineobombus</i>	Nt	2	—
<i>Rubicundobombus</i>	Nt	1	—
<i>Brachycephalobombus</i>	Nt	2	—

The order of arrangement generally reflects the cladistic results of Williams (1994), with basal subgenera listed first. *Mendacibombus* was inferred to be paraphyletic in Williams (1994). Ho, Holarctic; Ne, Nearctic; Nt, Neotropical; Pa, Palearctic.

characters; this approach allows all relevant data from our nuclear sequences to contribute to the resolution of *Bombus* phylogeny. We performed heuristic searches with 100 random addition analyses and TBR branch swapping using PAUP*4.0b10 (Swofford, 2002). Nodal

support was assessed using bootstrap analysis and Bremer support (Bremer, 1994).

To evaluate the historical biogeography of bumble bees, we optimized the known distribution of each species onto the shortest trees using DIVA 1.1 (Ronquist, 1996, 1997). DIVA infers ancestral distributions using a cost matrix derived from a simple biogeographic model not requiring a general hypothesis of area relationships. Each species was coded as present/absent on each of the three biogeographic areas: the Palearctic, Nearctic, and Neotropics (Fig. 1). Distributional data were based on the Natural History Museum *Bombus* database (Williams, P. H., <http://www.nhm.ac.uk/entomology/bombus/index.html>). The obtained 72 shortest trees were each evaluated using DIVA to assess sensitivity of the reconstruction to tree selection.

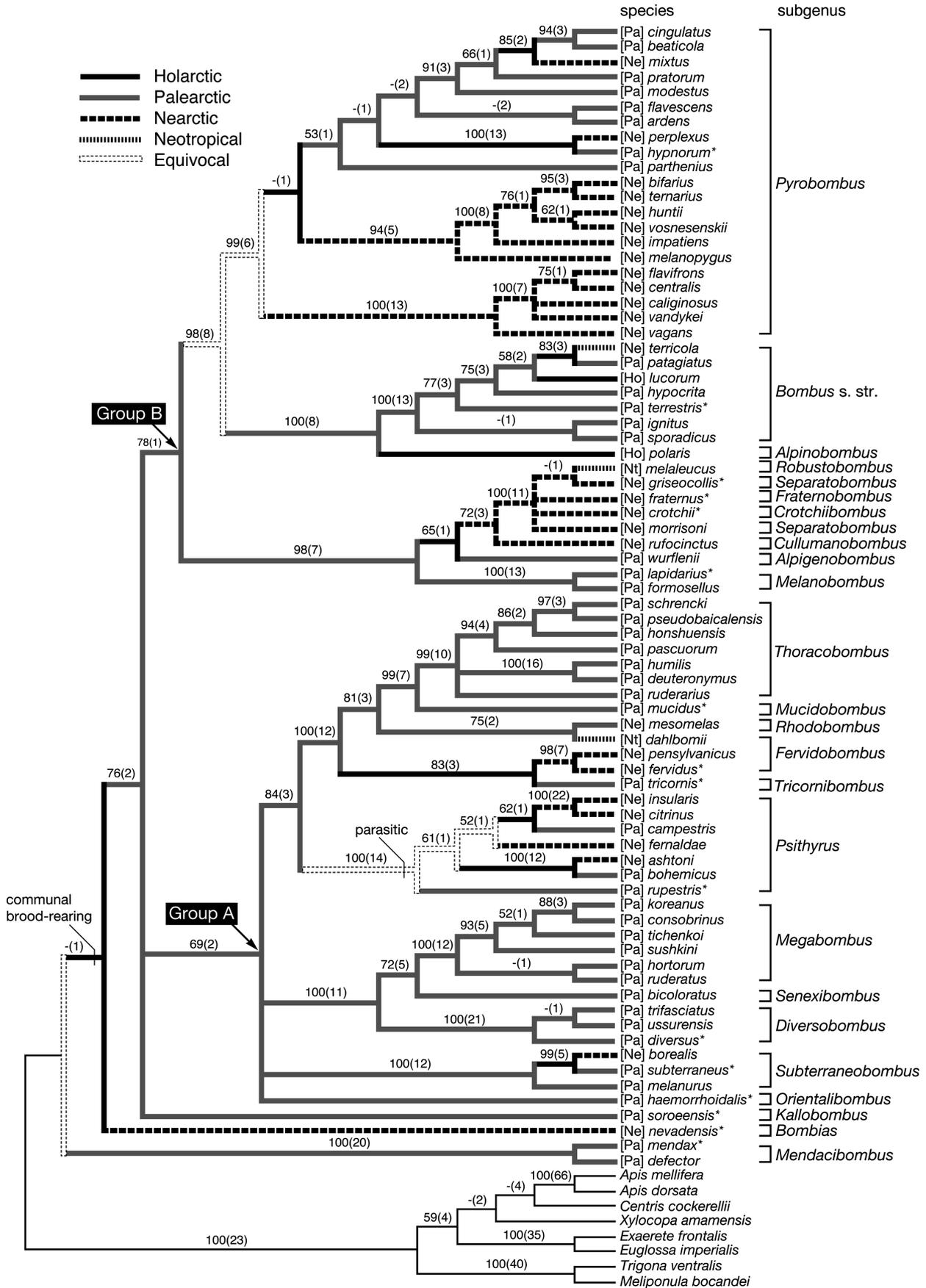
We also used our phylogenetic hypothesis to evaluate the evolution of proboscis length in bumble bees. To trace the evolution of this character on the shortest phylogenetic trees, we reconstructed ancestral states of proboscis lengths using published estimates of mean proboscis length, which were available for 32 species sequenced in the present study (see Fig. 2 for data sources). Criteria for measuring proboscis length differs among studies; we only considered studies that measured proboscis length as prementum + glossa. The proboscis lengths were log₁₀-transformed, and ancestral states were estimated using CAIC software version 2.6.9 (Purvis and Rambout, 1995).

3. Results and discussion

3.1. Phylogeny

Simultaneous parsimony analysis of the three gene fragments produced a well-resolved phylogeny (Fig. 1; strict consensus of 72 trees of 2240 steps; Consistency Index excluding uninformative characters = 0.49; Retention Index = 0.76). Monophyly of *Fervidobombus* and *Separatobombus* were not verified, as anticipated by morphological (Ito, 1985; Williams, 1994) and molecular data (Kawakita et al., 2003; Koulianos and Schmid-Hempel, 2000). The basal placement of *Mendacibombus*, *Bombias*, and *Kallobombus* is consistent with previous phylogenetic studies (Kawakita et al., 2003; Pedersen, 2002; Williams, 1994). Apart from these three basal

Fig. 1. The strict consensus of 72 shortest trees (length = 2240) inferred from the simultaneous parsimony analysis of three nuclear genes. Numbers above branches are bootstrap values based on 1000 replicates (“-” when <50%) followed by Bremer supports (Branch support indices) in parentheses. Type species of each subgenus sequenced are indicated with asterisks. “Group A” and “Group B” indicate two major clades identified in the present study. Ancestral distributions reconstructed using DIVA is shown, which was not affected by selection of different shortest trees. Letters in parentheses indicate distribution area of each species: Pa, Palearctic; Ne, Nearctic; Nt, Neotropical. The ancestral distribution of *B. dahlbomii* and *B. mesomelas* was Palearctic–Neotropical according to DIVA. However, this distribution pattern could also result from migration of a Palearctic ancestor to the Nearctic and subsequently to the Neotropics, followed by extinction of Nearctic populations. At present, we prefer to interpret the ancestral distribution of *B. dahlbomii* and *B. mesomelas* as Palearctic.



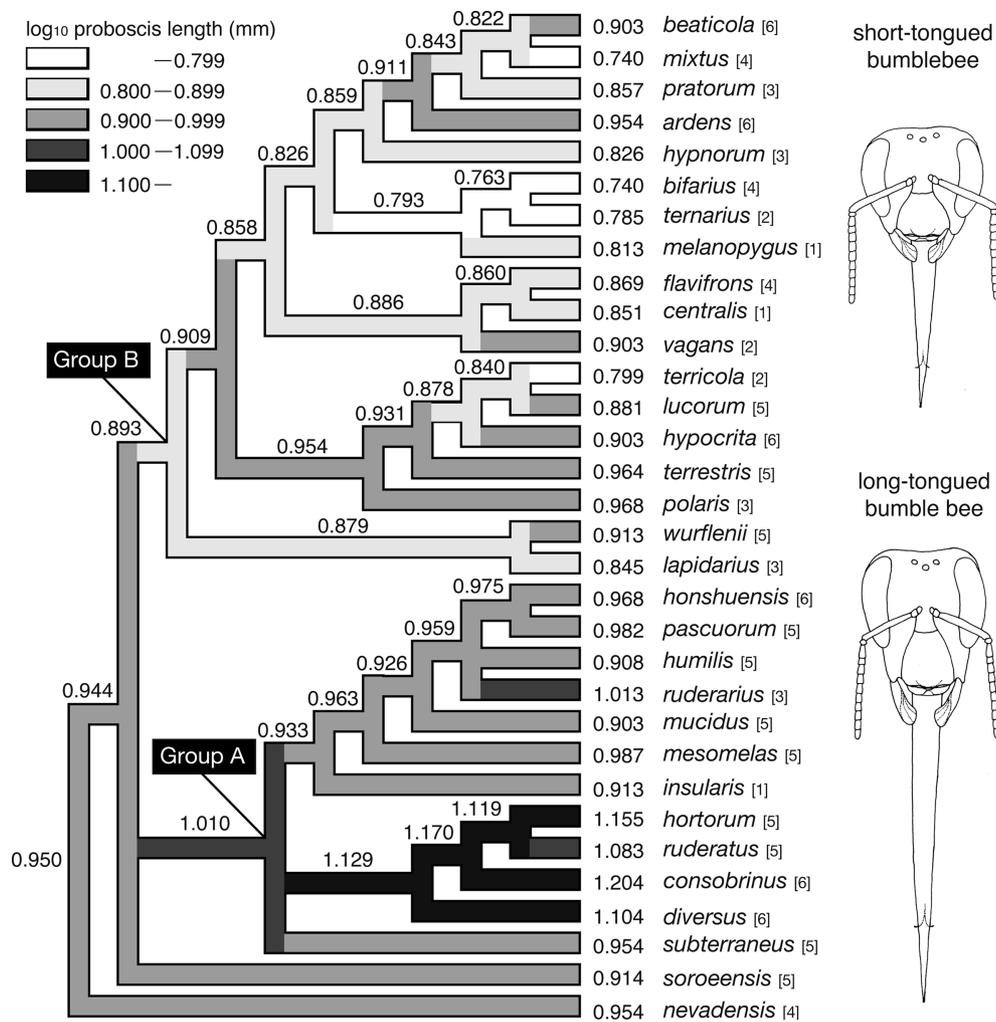


Fig. 2. Ancestral states of mean proboscis length estimated using CAIC. Terminal and ancestral values are log₁₀-transformed length in mm. Data on proboscis lengths were based on published information; the source is indicated in parenthesis following each species name: 1, Macior (1974); 2, Heinrich (1976); 3, Ranta and Vepsäläinen (1981); 4, Bauer (1983); 5, Obesco (1992); 6, Sota et al. (1997). “Group A” and “Group B” indicate two major clades identified in the present study. Typical head morphologies of short-tongued and long-tongued bumble bees are illustrated.

subgenera, there are two major clades, *Orientalibombus*–*Thoracobombus* clade (“Group A” in Fig. 1) and *Melanobombus*–*Pyrobombus* clade (“Group B” in Fig. 1). Group A corresponds with the section *Odontobombus* proposed by Krüger (1917) and more or less similar supraspecific groupings proposed by later researchers (reviewed in Ito, 1985), except for the addition of *Orientalibombus* and *Psithyrus* (cuckoo bumble bees) as members of this group (Fig. 1). Inclusion of *Psithyrus* in Group A is consistent with morphological studies indicating that some *Odontobombus* subgenera are related to *Psithyrus* (Ito, 1985; Williams, 1994). Group B corresponds with one of the clades also recovered by Williams (1994), except that *Alpinobombus* is indicated, with strong support, as the sister group to *Bombus* s. str. (Fig. 1). The sister relationship of *Alpinobombus* and *Bombus* s. str. is also suggested by Pedersen (2002) based on a molecular phylogeny. Group B includes most

subgenera restricted to the New World and a few subgenera with Holarctic distributions and many boreal and/or arctic species.

3.2. Biogeography

Fig. 1 shows the optimal inferred ancestral distributions obtained by DIVA analysis. There were 12 optimal reconstructions that require 16 dispersal/extinction events. These reconstructions were not affected by selection of different shortest trees, because all taxa forming polytomies shared the same distributions (Fig. 1).

The two basal bumble bee subgenera, *Mendacibombus* and *Bombias*, occur in the Palearctic and the Nearctic, respectively, suggesting a rather ancient Holarctic distribution followed by Palearctic/Nearctic vicariance. Early diversification of bumble bees was suggested to have occurred primarily in the Palearctic (Fig. 1), a

result consistent with the highest diversity of extant bumble bee species in this region (Williams, 1998). Subsequent migrations to the Nearctic regions and less frequent re-colonizations of the Palearctic have occurred repeatedly during the evolution of bumble bees (Fig. 1). Both ancient and more recent faunal interchanges can be inferred. Older dispersals include immigration to the Nearctic by ancestors of *Fervidobombus*, *Pyrobombus*, and the clade consisting of several exclusively New World subgenera, and re-colonization of the Palearctic within *Pyrobombus*. More recent interchanges are characteristic of *Pyrobombus*, *Bombus* s. str., and *Psithyrus*, which include many boreal, arctic, and high montane species. Those relatively recent dispersals are responsible for Nearctic/Palearctic pairs of closely related species such as *B. perplexus* and *B. hypnorum*, *B. terricola* and *B. patagiatus*, and *B. ashtoni* and *B. bohemicus* (Fig. 1). The most recent dispersals involve the few boreal/arctic species with natural Holarctic distributions (e.g., *B. polaris*, *B. lucorum*). Both recent and more ancient faunal interchanges between the Palearctic and the Nearctic are paralleled by several other naturally Holarctic bee groups such as *Colletes*, *Hylaeus*, *Andrena*, *Lasioglossum*, *Halictus*, *Osmiini*, and *Anthophora* (Michener, 1979, 2000).

Colonization of Central/South America involves species of *Fervidobombus* and *Robustobombus* (Fig. 1), although one species of *Pyrobombus* also occurs south to Panama (Williams, 1998). The potentially non-monophyletic subgenus *Fervidobombus* has ca. 20 species distributed from southern Canada through the Neotropics to southern South America (Williams, 1998). Most *Fervidobombus* occur at low to moderate elevations, while a single atypical species, *Bombus dahlbomii*, occurs south to southern Chile and Argentina. The remaining five exclusively Neotropical subgenera not included in our analysis are closely related to *Robustobombus* (Williams, 1994) and have radiated in Andean South America and montane Central America, as have a few other primarily Holarctic bee groups such as *Anthophora*, *Anthidium*, and weak-veined *Lasioglossum* (Michener, 1979, 2000).

3.3. Evolution of behavioral and morphological characters

A distinctive behavioral characteristic of most bumble bees is the communal rearing of multiple larvae in a single larval brood chamber (Michener, 1974). *Mendacibombus* and *Bombias* are exceptions, since these make individual brood cells (Haas, 1976; Hobbs, 1965), representing the inferred ancestral state for bumble bees (Fig. 1) (Williams, 1994). In communal brood cells, an individual larva receives more or less nutrition depending on its position within a chamber, and this has been regarded as the principal cause of extensive size polymorphism among workers in bumble bees (Inoue and

Kato, 1992; Michener, 1974), as compared to honey bees and stingless bees in which larvae are reared in individual cells and worker size variation is negligible. Such size-variation within colonies facilitates specialization of a foraging worker to flowers of different sizes thus maximizing the resource gathering ability of a colony (Heinrich, 1979). Communal rearing behavior therefore increases the reproductive success of bumble bee colonies, which may explain the marked difference in the number of extant species between basal lineages (13 species in *Mendacibombus* plus *Bombias*) and the almost 200 communally rearing species.

Species of the subgenus *Psithyrus* are obligate social parasites. Queens invade and usurp eusocial nests of other bumble bee species and rely on host workers to provision their brood. Our phylogeny places *Psithyrus* sister to a clade including *Fervidobombus* and four exclusively Palearctic subgenera, but the single Palearctic species of *Eversmannibombus*, which shares some morphological synapomorphies with *Psithyrus* (Williams, 1994), was not included in our analysis. Hosts of *Psithyrus* include *Bombias* and diverse subgenera of Groups A and B (Hobbs, 1965; Pekkarinen and Teräs, 1993; Thorp et al., 1983), so the host range is evidently not constrained by phylogeny. Socially parasitic behavior (perhaps facultative) has arisen independently in at least two species of *Alpinobombus* (Richards, 1973) and *Thoracobombus* (Yarrow, 1970), which are distantly related to *Psithyrus* and to each other as shown by our phylogeny (Fig. 1).

Bumble bee species reduce overlap in resource utilization within local species assemblages primarily by differences in proboscis length, thereby partitioning flower resources with different corolla depths (Heinrich, 1976; Inouye, 1977; Pyke, 1982). Fig. 2 indicates that proboscis length of bumble bees is more or less conserved across the phylogenetic tree; the ancestors of species belonging to Group A had relatively high values between 0.9 and 1.2 (log₁₀-transformed mean proboscis length in mm), whereas most ancestral values within Group B are less than 0.9 (Fig. 2). These results are correlated with a long malar area that characterizes species of Group A (Krüger, 1917; Williams, 1994), which is adapted to foraging on long, tubular flowers (Inoue and Kato, 1992; Sakagami, 1976), and a generally short head in species of Group B. The general phylogenetic conservatism of proboscis length is perhaps unexpected, because ecologically important characters may exhibit great evolutionary lability mediated by strong natural selection. It is worth noting the diversity of relatively long-tongued bumble bees (Group A) in the Palearctic, which contrasts to the prevalence of short-tongued bumble bees (Group B) in the Nearctic (Figs. 1, 2). This pattern is possibly attributable to historical factors (e.g., primary absence of Group A from the Nearctic), floral resource specialization, and/or

competition between other major nectarivores. The original absence of the short-tongued honey bees (genus *Apis*) in the Nearctic may explain why short-tongued *Bombus* predominate in the New World, whereas many Old World *Bombus* have relatively longer tongues.

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