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BIOGEOGRAFIA HISTÓRICA DAS ESPÉCIES DE MAMANGAVAS COM ÊNFASE NAS ESPÉCIES NEOTROPICAIS: IMPLICAÇÕES SISTEMÁTICAS E PARA A CONSERVAÇÃO

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Pós-Graduação

"BIOGEOGRAFIA HISTÓRICA DAS ESPÉCIES DE MAMANGAVAS COM ÊNFASE NAS ESPECIES NEOTROPICAIS: IMPLICAÇÕES SISTEMÁTICAS E PARA A CONSERVAÇÃO"

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LISTA DE ABREVIATURAS

+j	a free parameter accounting for the possibility of a founder-event speciation;
12S	small subunit of the mitochondrial ribosome;
16S	subunit of the mitochondrial ribosome;
AD	Average interspecific distance ± standard deviation;
AIC	Akaike's information criterion;
AICc	corrected Akaike information criterion;
AICM	Akaike's information criterion (AIC) through Markov chain Monte Carlo (MCMC);
Argk	arginine kinase;
B.	*Bombus;*
BA	Bahia;
BAMM	used Bayesian Analysis of Macroevolutionary Mixtures;
BayArea	Bayesian Analysis of Biogeography when the Number of Areas is Large;
BI	Bayesian Inference methods;
BioGeoBEARS	BioGeography with Bayesian and likelihood Evolutionary;
BOLD	The Barcode of Life Data Systems is designed to support the generation and application of DNA barcode data;
bp	pares de base;
BSP	Bayesian Skyline Plot;
CLOCK	Strict clock;
COI	Cytochrome oxidase I;
CytB	Cytochrome B;
DEC	Dispersal-extinction-cladogenesis;
DF	Distrito Federal;
DIVA	Dispersal-vicariance analysis;
dNTP	Bases nucleotídicas;
EF1α F2copy	elongation factor 1-alpha gene - 2copy;
ES	Espírito Santo;
ESS	estimated sample size;
Fig.	Figure;
FLONA	National Forest;
GenBank	sequence database is an open access of the International Nucleotide Sequence Database Collaboration;
GO	Goiás;
gr.	group;
H	high haplotype diversity;
HPD	highest posterior density;
I	I statistic;
LF	long-faced
LF1	long-faced clade 1;

LF2	long-faced clade 2;
LTT	lineage through-time;
MaD	maximum intraspecific distance ± standard deviation;
MCMC	Markov chain Monte Carlo;
MG	Minas Gerais;
MgCl2	Cloreto de Magnésio;
MiD	minimum interspecific distance ± standard deviation;
ML	maximum likelihood
mM	mile molar;
MP	maximum parsimony;
MS	Mato Grosso do Sul;
MT	Mato Grosso;
mtDNA	mitochondrial DNA;
mya	milhion years ago;
N.	Number;
NAF	northern Atlantic Forest;
ng	Nano Gramas;
Opsin	long-wavelength rhodopsin gene;
PARNA	National Park;
PCA	Principal Components Analysis;
PCR	Reação em Cadeia de Polimerase;
PEG20%	purified using polyethylene glycol 20%;
PEPCK	phosphoenolpyruvate carboxykinase;
pp	posterior probability values
PR	Paraná;
REBIO	Biological Reserve;
RJ	Rio de Janeiro;
RR	relative rank;
RS	Rio Grande do Sul;
S.E.	standard errors;
SAF	southern Atlantic Forest;
SAR	Simultaneous autoregressive model;
SC	Santa Catarina;
SF	short-faced
SP	São Paulo;
sp. n	new species
TBR	Tree Bisection Reconnection;
UCED	Uncorrelated Exponential Relaxed Clock;
UCLN	Unncorrelated Lognormal Relaxed Clock model;
UENF	Universidade Federal do Norte Fluminense;
UESC	Universidade Estadual de Santa Cruz;
UFMG	Universidade Federal de Minas Gerais

UFMG IHY	Universidade Federal de Minas Gerais;
UFOP	Universidade Federal de Ouro Preto;
UFSC	Universidade Federal de Santa Catarina;
USP/SP	Universidade de São Paulo;
USP: FFCLRP	Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto – Universidade de São Paulo;
μL	microlitro;
μM	micro molar;
π	nucleotide diversity;

RESUMO

A biogeografia é uma ciência que usa dados biológicos e geográficos para compreender os padrões de distribuição dos táxons em um contexto histórico-geográfico. Atualmente, são reconhecidas seis regiões biogeográficas no mundo, sendo o território brasileiro parte da região Neotropical. Esta região possui uma complexa história geológica, sendo constituída por um mosaico de ambientes com composições diferentes de espécies. A busca pelo entendimento dos processos geográficos e biológicos que resultaram nos padrões de distribuição dos táxons da região neotropical é dificultada por ela apresentar registro fóssil escasso e poucos estudos filogenéticos e filogeográficos. Dentro do contexto das subregiões biogeográficas que compõem a região Neotropical, estão as montanhas do leste do Brasil, que possuem uma rica variedade de solos, microrregiões climáticas e uma complexa cobertura vegetal ao longo dos gradientes de altitude, o que confere a estas áreas um alto endemismo. Através de métodos biogeográficos, análises filogenéticas e por técnicas utilizadas para inferir modelos de distribuição de espécies ("*species distribution models*" – SDM), o aumento do conhecimento das mamangavas (*Bombus* Latreille, 1802) Neotropicais contribuirá para a elucidação dos processos que moldaram a distribuição das abelhas na Região Neotropical. Neste trabalho, foram empregadas análises filogenéticas e biogeográficas, com base em dados moleculares, modelos de nicho ecológico e análises de nicho para a compreensão dos processos que moldaram a distribuição das espécies neotropicais do gênero *Bombus* Latreille, 1802. O primeiro capítulo intitulado, *Hitting an Unintended Target: Phylogeography of Bombus brasiliensis Lepeletier, 1836 and the First New Brazilian Bumblebee Species in a Century (Hymenoptera: Apidae,)* foca nos estudos fitogeográficos de *B. brasiliensis* e na descrição de uma nova espécie com distribuição restrita entre o norte do Espírito Santo e o sul da Bahia. O Segundo capítulo intitulado *Peripatric speciation followed by niche differentiation between sister bumblebee species from the Atlantic Tropical Rain Forest, Brazil,* testa os fatores relacionados ao evento de especiação entre *B. brasiliensis* e *B. bahiensis* Santos Júnior & Silveira, 2015 e chama a atenção para o status de conservação de *B. bahiensis*. O terceiro capítulo intitulado, *A new perspective on historical biogeography, divergence times and diversification patterns of Neotropical bumblebees (Hymenoptera: Apidae),* compara o ritmo de diversificação entre as regiões biogeográficas e busca

compreender os principais processos que moldaram a atual distribuição das mamangavas, com ênfase na região Neotropical.

ABSTRACT

Biogeography is a science that uses biological and geographical data to understand the distribution patterns of taxa in a historical and geographical context. Six biogeographical regions are currently recognized in the world with the Brazilian territory integrating the Neotropics. This region presents a complex geological history and constitutes a mosaic of environments with different species assemblages. The search for understanding the geographical and biological processes that resulted in the distribution patterns of Neotropical taxa is hindered by the scarcity of its fossil record and of phylogenetic and phylogeographic studies. Within the context of the biogeographical subregions composing the Neotropics are the eastern Brazilian mountains, which contains a rich variety of soils, climatic microregions and a complex vegetation cover along the altitudinal gradients, which gives these areas a high rate of endemism.Biogeographical methods, phylogenetic analyses and species distribution modelling techniques, the increase in the knowledge on Neotropical bumblebees will contribute to the elucidation of the processes that shaped the distribution of bees in the Neotropics. In this work we use phylogenetic and Biogeographic analyses, based on molecular data, ecological niche models and niche analysis, to understand the processes that shaped the distribution of the Neotropical species of the genus *Bombus* Latreille, 1802. The first chapter, entitled Hitting an Unintended Target: Phylogeography of *Bombus brasiliensis* Lepeletier, 1836 and the First New Brazilian Bumblebee Species in a Century (Hymenoptera: Apidae), focuses in the phylogeographic studies of *B. brasiliensis* and the description of a new bumblebee species with a restricted range between the north of Espírito Santo and south of Bahia. The second chapter, entitled Peripatric speciation followed by niche differentiation between sister bumblebee species from the Atlantic Tropical Rain Forest, Brazil, assess the factors related to the speciation event between *B. brasiliensis* e *B. bahiensis* Santos Júnior & Silveira, 2015 and draws attention to the conservation status of *B. bahiensis*. The third chapter, entitled A new perspective on historical biogeography, divergence times and diversification patterns of Neotropical bumblebees (Hymenoptera: Apidae), compares the rhythm of diversification between the biogeographical regions and aims to comprehend the main processes that shaped the current distribution of bumblebees, with emphasis on the Neotropical region.

INTRODUÇÃO GERAL

A biogeografia é uma ciência multidisciplinar que através de dados biológicos, climáticos e geográficos busca compreender os padrões de distribuição dos táxons em um contexto histórico e geográfico. De um ponto de vista mais amplo, ela fornece a matéria prima necessária para elucidação dos processos geológicos e biológicos que moldaram a distribuição das biotas no planeta e de como se deu a evolução dos táxons que compõem estas biotas (Carvalho & Almeida 2011).

O território brasileiro, juntamente com os territórios dos outros países que compõem a América do Sul, está inserido na região Neotropical, da qual também fazem parte as áreas que abrangem a América Central, o Caribe, as Antilhas e Bahamas e as áreas tropicais do México. Essa região corresponde a uma das seis regiões zoogeográficas, de um sistema de divisão do globo em regiões baseadas na similaridade da fauna (Rafael *et al.* 2012).

A complexa história geológica desse continente, composto por distintas áreas biogeográficas (Morrone 2004), com diferentes latitudes e altitudes, o que garante a ele um mosaico de ambientes com composições diferentes de espécies (Rafael *et al.* 2012), induziu a busca pelo entendimento dos padrões de distribuição dos táxons dessa região. No entanto, estas análises, biogeográficas, tornam-se mais robustas e eficientes se forem incluídos táxons pertencentes a diferentes grupos taxonômicos (Sigrist & Carvalho 2008).

A dificuldade na obtenção de uma reconstrução da história biogeográfica completa para a região Neotropical é agravada por apresentar um registro histórico escasso, por exemplo causada pela dificuldade de fossilização (Burnham & Johnson 2004). Mesmo tendo aumentado o número de dados e métodos disponíveis e o conhecimento sobre os processos, ainda falta muito para compreender melhor a evolução das áreas de endemismo(ver regiões biogeográficas Morrone 2004, 2013) dessa região. Para isso, é necessário que mais e mais processos que moldaram os padrões de distribuição sejam inferidos para que a comparação entre eles possa ajudar no melhor entendimento do cenário evolutivo espacial da América do Sul, utilizando assim as congruências das áreas de distribuição e os padrões biogeográficos dos táxons que ocorrem nessas áreas (Carvalho & Almeida 2011).

As montanhas têm um papel importante nos processos de diversificação da biota Neotropical (ex.Silveira & Cure (1993); Hines (2008); Silveira *et al.* (2016); Dias *et al.* (2017)). Entre elas estão a cordilheira dos Andes, as montanhas imersas no domínio da Amazonia e as montanhas do leste do Brasil (Steinbauer *et al.* 2016).Dentro do contexto das subregiões biogeográficas neotropicais as montanhas do leste do Brasil possuem uma rica variedade de solos, micro-regiões climáticas e uma complexa cobertura vegetacional ao longo dos gradientes de altitude, o que confere a essas áreas alto endemismo (Stannard *et al.* 1995; Rapini *et al.* 2008). Relativamente pouco se sabe sobre a ecologia, biogeografia e história natural da biota das regiões montanhosas do leste do Brasil (Behling 1998; Rapini *et al.* 2008; Silveira *et al.* 2016).

Áreas montanhosas funcionam como ilhas, pois separam por longas distâncias populações.Quando a dispersão entre uma população e outra é interrompida, por um longo período de tempo, pode resutar na divergência entre duas populações isoladas dando origem a duas novas espécies (Futuyma 2009; Frankham *et al.* 2010). As taxas de especição causadas pelo isolamento com a elevação sugerem que o padrão global de endemismo nas áreas elevadas são uma explicação para os processo de especiação em áreas montanhosas. O isolamento baseado na topografia, como são chamados, aumenta as taxas de especiação global em todas as áreas montanhosas, principalmente quando aproximam-se da linha do Equador (Steinbauer *et al.* 2016).

Dos padrões de distribuição geográficos propostos por Silveira e Cure (1993) para a distribuição das espécies de abelhas existentes nas áreas montanhosas do leste do Brasil, três merecem destaque, por serem testáveis através de métodos biogeográficos e por análises de distribuição potencial das espécies (*Environmental niche modelling* – ENM). Os três padrões são: i) espécies restritas às cadeias de montanhas do sudeste; ii) espécies comuns nas áreas montanhosas e/ou áreas baixas acima da latitude 24^{o} Sul; e iii) espécies comuns às áreas de altitude do sudeste e do Planalto Central.

A espécie brasilieira, *Bombus (Thoracobombus) brasiliensis* Lepeletier, 1836, pertence a um gênero cuja história biogeográfica e explicada em parte por processos de dispersão ao longo das cadeias de montanhas, Monhanhas Rochosas e cordilheira dos Andes (Hines 2008). Por pussuir uma distribuição disjunta, segundo padrão (ii) proposto por Silveira e Cure (1993), levantou-se a hipótese de *B. brasiliensis* tratar na verdade de um complexo de espécies.

O gênero *Bombus* Latrille, 1802 possui atualmente 270 espécies, conhecidas popularmente como mamangavas (ver catalogo em http://www.nhm.ac.uk/). A região Neotropical é a única área de clima quente e úmido que possui representantes desse gênero. Ao todo 32 espécies de *Bombus* estão presentes na região Neotropical, pertencentes a quatro subgêneros *Cullumanobombus* Vogt, 1911(13 espécies), *Pyrobombus* Dalla Torre, 1880 (uma espécie), *Psithyrus* Lepeletier, 1832 (uma espécie) e *Thoracobombus* Dalla Torre, 1880 (17 espécies) (http://www.nhm.ac.uk/; Williams (1998); Williams *et al.* (2008)). Neste trabalho testamos o segundo padrão proposto por Silveira e Cure (1993) – espécies comuns nas áreas montanhosas e/ou áreas baixas acima da latitude 24^{o} Sul. No primeiro capítulo foi testado se as populações de *B. brasiliensis* das áreas montanhosas do leste do Brasil pertenceriam a uma espécie diferente das polunações existentes nas áreas baixas (orla marítima e Bacia do Rio Paraná). E uma nova espécie do gênero *Bombus* e descrita com distribuição restrita entre o sul da Bahia e o norte do Espírito Santo. No segundo capítulo os processos que causaram a separação entre as espécies irmãs, *B. brasiliensis* e *B. bahiensis* foi testado e no terceiro capítulo foi comparado o ritmo de diversificação do gênero *Bombus* entre as regiões biogeográficas com os resultados anteriormente encontrados pela Hines (2008). Novas análises foram realizadas (Análises do ritmo de diversificação no programa BAMM – *Bayesian Analysis of Macroevolutionary Mixtures*, estimativas dos tempos de divergência BEAST – *Bayesianv Evolutionary Analysis* e inferências biogeográficas BioGeoBEARS – *BioGeography with Bayesian and likelihood Evolutionary*), com um aumento no número de terminais (espécies do gênero) e o número de fosseis disponíveis para a datação. Além disso, essas foram realizadas através de novos métodos (Matzke 2013; Bouckaert *et al.* 2014; Mitchell & Rabosky 2017) que possibilitaram com maior precisão a busca pela compreensão dos principais processos que moldaram a atual distribuição das mamangavas (do gênero *Bombus*) da região Neotropical (ver Hines (2008)), para a comparação entre os métodos novos e antigos).

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Capítulo 1 – Hitting an Unintended Target: Phylogeography of *Bombus brasiliensis* Lepeletier, 1836 and the First New Brazilian Bumblebee Species in a Century (Hymenoptera: Apidae)

RESEARCH ARTICLE

Hitting an Unintended Target: Phylogeography of *Bombus brasiliensis* Lepeletier, 1836 and the First New Brazilian Bumblebee Species in a Century (Hymenoptera: Apidae)

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Abstract

This work tested whether or not populations of *Bombus brasiliensis* isolated on mountain tops of southeastern Brazil belonged to the same species as populations widespread in lowland areas in the Atlantic coast and westward along the Paraná-river valley. Phylogeographic and population genetic analyses showed that those populations were all conspecific. However, they revealed a previously unrecognized, apparently rare, and potentially endangered species in one of the most threatened biodiversity hotspots of the World, the Brazilian Atlantic Forest. This species is described here as *Bombus bahiensis* sp. n., and included in a revised key for the identification of the bumblebee species known to occur in Brazil. Phylogenetic analyses based on two *mt*DNA markers suggest this new species to be sister to *B*. *brasiliensis*, from which its workers and queens can be easily distinguished by the lack of a yellow hair-band on the first metasomal tergum. The results presented here are consistent with the hypothesis that *B*. *bahiensis* sp. n. may have originated from an ancestral population isolated in an evergreen-forest refuge (the so-called Bahia refuge) during cold, dry periods of the Pleistocene. This refuge is also known as an important area of endemism for several animal taxa, including other bees. Secondary contact between *B*. *bahiensis* and *B*. *brasiliensis* may be presently prevented by a strip of semi-deciduous forest in a climate zone characterized by relatively long dry seasons. Considering the relatively limited range of this new species and the current anthropic pressure on its environment, attention should be given to its conservation status.

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Introduction

Despite the fact that, taxonomically, bees are among the best known insects in the world (for example [1]), there are taxonomic problems to be solved in the group, including sex association in dimorphic species and distinction of cryptic species, even in relatively well-known taxa. In these cases, molecular data, including the so called DNA barcode, alone or integrated to other types of characters, have proven to be much useful (for example [2, 3]).

The bumblebees (genus *Bombus* Latreille, 1802) comprise approximately 250 species widely distributed in the world, but occurring mainly in the cool subtropical and temperate areas of the Nearctic and, especially, Palearctic regions [4]. Defining species limits in *Bombus* is frequently difficult, because of the lack of useful structural characters and the great variability in hair-color patterns. This has led to an array of recent studies employing DNA markers to elucidate bumblebee-species boundaries (for example [5–12]). Molecular tools have also been used in the genus to investigate intraspecific genetic structure, phylogeography and phylogenetic relationships (for example [13–22]).

In South America, most of the species of *Bombus* are distributed along the Andes and in temperate regions, with only a few species recorded in the warm lowlands—the later, actually, the only bumblebees to occur in such environments in the world [1]. Only six species of the genus are generally referred to occur in Brazil [23], all belonging to the same subgenus—*Fervidobombus* Skorikov, 1922 or *Thoracobombus* Dalla Torre, 1880, depending on which classification one adopts (for example [1, 24] or [25]). Five of these species (*B. bellicosus* Smith, 1879; *B. brasiliensis* Lepeletier, 1836; *B. brevivillus* Franklin, 1913; *B. pauloensis* Friese, 1913; and *B. transversalis* (Olivier, 1789)) seem to be very closely related, while the sixth one, *B. morio* (Swederus, 1787) belongs to a distinctive clade in the same subgenus [19, 21]. *Bombus pauloensis* has been widely treated as *B. atratus* Franklin, 1913 in the literature (for an explanation for the adoption of *B. pauloensis* as a valid name, see [24]). The occurrence of a seventh species in western Brazil, *B. pullatus* Franklin, 1913, was reported by Milliron [26], but considered with suspicion by Abrahamovich & Díaz [27], and ignored by Moure & Melo [24], and needs confirmation. An eighth species, *B. rubriventris* Lepeletier, 1836, was recorded with doubts by Milliron [26] as possibly occurring in the state of Goiás. According to him, this species, known only from its female holotype, would be very rare or extinct. One cannot rule out the possibility that the assignment of *B. rubriventris* to Brazil resulted from mere labeling mistake or from wrong interpretation of the type locality (see [26] for details).

In their thorough revision of the Brazilian bumblebees, Moure & Sakagami [23]) noted that *B. brasiliensis* Lepeletier, 1836 was the only species to be more common on mountaintops (mainly in the ranges along the southern and eastern Brazilian coasts) than in lowlands, and that it was the only one recorded in elevations above 1,800 m. Later, Silveira & Cure [28] noted that *B. brasiliensis* is absent in the lowlands surrounding the mountain ranges where it is relatively common further inland in southeastern Brazil. The occurrence of these isolated mountaintop populations of *B. brasiliensis* raised the suspicion that they might in fact belong to a species different from the one present in the lowlands along the coast and in the Paraná River valley. Here, this hypothesis is tested, mainly by means of phylogenetic and population-genetic analyses of two *mt*DNA genes. Since the question of how to delimit species boundaries is still in debate (see, for instance, the revision by Wiley & Lieberman [29]), different character sources (molecular and morphological) and analytical methods (tree and non-tree based) were complementarily employed to decide whether or not a set of populations should be considered as a new taxon.

Materials and Methods

Morphological and Molecular Procedures

The description of the new species presented below was based on six specimens—three workers and a queen from the municipality of Ilhéus, in the Brazilian state of Bahia, and two workers collected in the municipality of Conceição da Barra, state of Espírito Santo. These specimens are deposited at the Taxonomic Collections of the 'Universidade Federal de Minas Gerais'—UFMG—and in the 'Padre Jesus Santiago Moure' entomological collection, of the 'Universidade Federal do Paraná'—DZUP—, as detailed in the section "Taxonomic treatment", below. Two collecting expeditions were conducted in southern Bahia and northern Espírito Santo states (coordinates of sampling sites are, in Bahia: Eunápolis—16°25'8"S, 39°34'55"W, 162m; Itamaraju—16°58'39"S, 39°33'16"W, 81m; Porto Seguro—16°27'3"S, 39°17'16"W, 114m; and, in Espírito Santo: Conceição da Barra—18°20'54"S,39°51'06"W, 47m; São Mateus—18°45'13"S, 39°51'39"W, 39 m; Sooretama—19°03'01"S, 40°08'02"W, 94m), under the collecting permit number 23784 (granted by "Instituto Chico Mendes de Conservação da Biodiversidade" to JESJ), in an attempt to increase molecular sample size and geographic representation. No additional specimens were found, however, during these expeditions.

The specimens were examined under a dissection microscope (Leica M125) and compared to the descriptions in Moure & Sakagami [23]. Comparisons were also made with photographs of the lectotype of *B. brasiliensis* Lepeletier, 1836, reproduced here in Fig 1A and 1B. Direct observation of this type was not done because, after examining hundreds of specimens of *B. brasiliensis* from all its geographic range, an easily-observable diagnostic character was found to distinguish the two concerned species, which could be readily checked in the photographs obtained from the type. Morphological terminology employed here is mostly that of Moure & Sakagami [23]. Flagellomeres are designated as F1, F2, F3 etc. Accordingly, metasomal terga and sterna are designated as T1, T2, T3 etc and S1, S2, S3 etc. The apical width of the malar area is the shortest width of the malar area, measured along the mandible base.

Two mitochondrial markers, Cytochrome oxidase I (COI) and Cytochrome B (CytB) were employed in addition to the morphological characters. DNA was extracted from one of the hind legs of each of 168 *Bombus* specimens, 142 of them belonging to *B. brasiliensis*, three to the species described as new below, and the rest belonging to the other species of the genus known to occur in Brazil (S1 Table). This was done through the phenol–chloroform method [30]. The extracted DNA was re-suspended in 40μL of TE buffer. The 5'region of the COI gene was amplified using the primers LepF1 and LepR1 [31], mtd_6 and mtd_9 [32], and that of the CytB was amplified using the primers mtd_26 [32] and CytB_R1, 5'TTCAATTATTTGACTT CCTAATCAAG3' (designed for this analysis). Amplification of both genes was done in a 30μL polymerase chain reaction (PCR) mix, including 0.5 units of Taq DNA polymerase, 2 mM $MgCl_2$, in 1x PCR buffer, 0.5 μM of each primer, 2.5 mMdNTPs and about 20 ng of genomic DNA. Amplification was carried out in a thermocycler using a program consisting of 5 min of denaturation at 94°C, followed by 35 30-second cycles at 94°C, 40 s at 48–51°C, 1 min at 72°C, and a final extension for 10 min at 72°C. PCR products were visualized in a 0.8% agarose gel. All PCRs that generated a single product were purified using polyethylene glycol 20% (PEG) precipitation (modified of Sambrook & Russel [30]—S2 Table). This purified PCR product was sequenced in both directions using a MegaBACE 1000 sequencer (GE Healthcare) with the same amplification primers following the manufacturer's recommendations. The raw sequences were obtained using the software Phred v. 0.20425 [33]. The final sequences were assembled with the software Phrap v. 0.990319 (http//www.phrap.org). Consed 19.0 [34] was used to view and edit the high quality consensus sequences. Alignments of the consensus

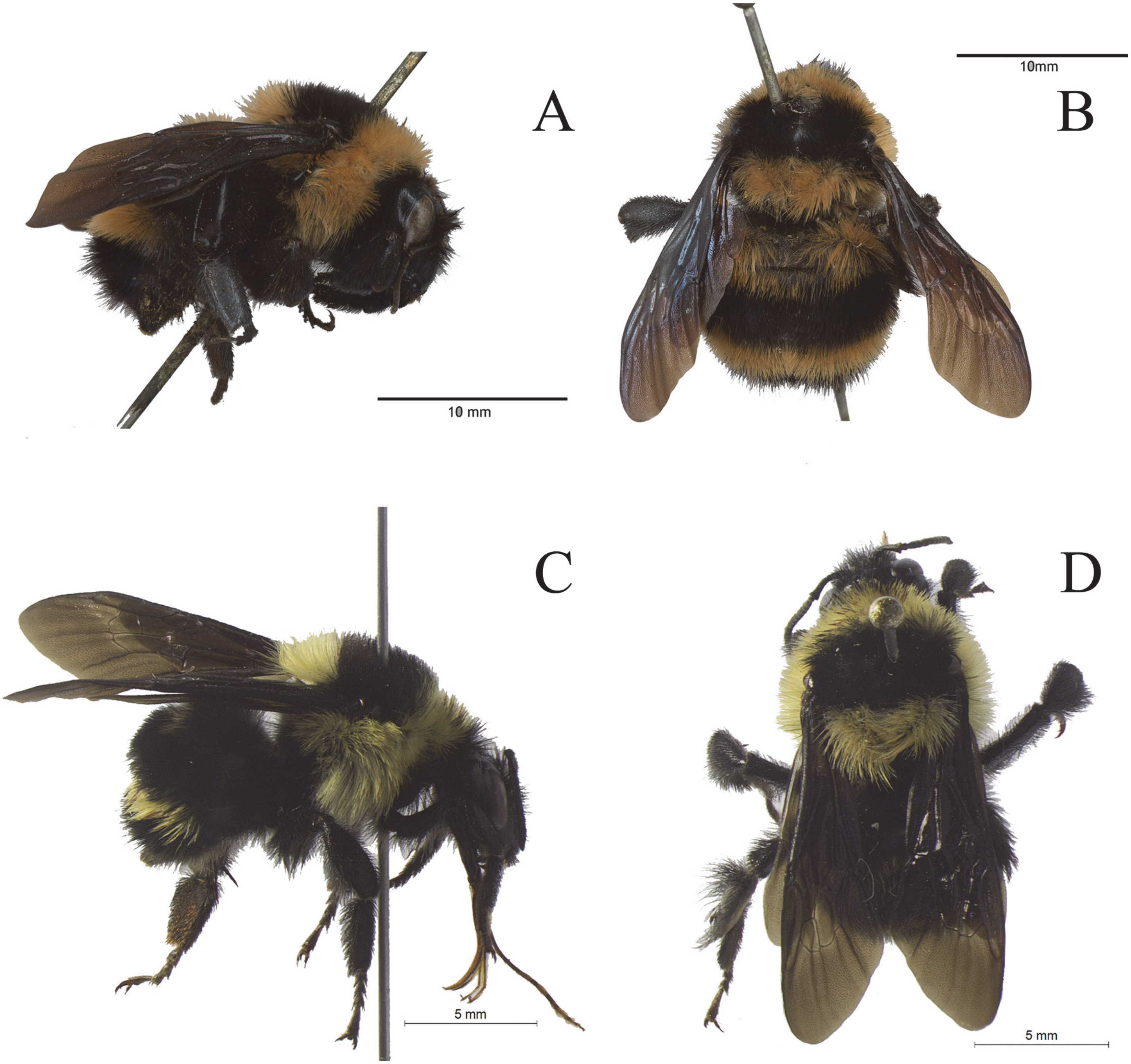

Fig 1. Types of *Bombus brasiliensis* Lepeletier, 1836 and *Bombus bahiensis* sp. n. Lectotype of *B*. *brasiliensis*—A: lateral view of head, mesosoma and metasoma; B: dorsal view of mesosoma and metasoma. Holotype of *B*. *bahiensis* sp. n.—C: lateral view of head, mesosoma and metasoma; D: dorsal view of mesosoma and metasoma.

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sequences for all individuals were generated using MUSCLE [35] implemented in the program MEGA 5.01 [36].

Molecular extraction and sequencing were done in the Biodiversity and Molecular Evolution Lab (LBEM), at the Universidade Federal de Minas Gerais, Belo Horizonte, Brazil. In addition to the 306 sequences generated here (168 COI, and 138 CytB sequences) for five species,

Table 1. Specimens belonging to GenBank and BOLD Systems databases, which were used in analyzes with the COI gene.

Accession number	Species	Database
KC853321.1	*Bombus excellens* Smith, 1879	GenBank
KC853363.1	*Bombus medius* Cresson, 1863	GenBank
KC853356.1	*Bombus pauloensis* Friese, 1913	GenBank
KC853357.1	*Bombus pauloensis* Friese, 1913	GenBank
KC853358.1	*Bombus pauloensis* Friese, 1913	GenBank
KC853359.1	*Bombus pauloensis* Friese, 1913	GenBank
KC853360.1	*Bombus pauloensis* Friese, 1913	GenBank
KC853361.1	*Bombus pensylvanicus* (De Geer, 1773)	GenBank
ARG-06832-75	*Bombus bellicosus* Smith, 1879	BOLDSYSTEMS
ARG-06832-76	*Bombus bellicosus* Smith, 1879	BOLDSYSTEMS
ARG-7205-37	*Bombus bellicosus* Smith, 1879	BOLDSYSTEMS
AF385820.1	*Bombus deuteronymus* Schulz, 1906	GenBank
KC853366_1	*Bombus diligens* Smith, 1861	GenBank
FJ582118.1	*Bombus fervidus* (Fabricius, 1798)	GenBank
FJ582119.1	*Bombus fervidus* (Fabricius, 1798)	GenBank
FJ582120.1	*Bombus fervidus* (Fabricius, 1798)	GenBank
FJ582122.1	*Bombus fervidus* (Fabricius, 1798)	GenBank
AY181106.1	*Bombus humilis* Illiger, 1806	GenBank
AY181127.1	*Bombus mesomelas* Gerstäcker, 1869	GenBank
AY181128.1	*Bombus mesomelas* Gerstäcker, 1869	GenBank
DQ225325.1	*Bombus morio* (Swederus, 1787)	GenBank
KC853367.1	*Bombus morio* (Swederus, 1787)	GenBank
KC853368.1	*Bombus morio* (Swederus, 1787)	GenBank
KC853369.1	*Bombus morio* (Swederus, 1787)	GenBank
KC853370.1	*Bombus morio* (Swederus, 1787)	GenBank
KC853371.1	*Bombus morio* (Swederus, 1787)	GenBank
AY181133.1	*Bombus muscorum* (Linnaeus, 1758)	GenBank
AY181134.1	*Bombus muscorum* (Linnaeus, 1758)	GenBank
AY181135.1	*Bombus muscorum* (Linnaeus, 1758)	GenBank
KC853365.1	*Bombus opifex* Smith, 1879	GenBank
AY181136.1	*Bombus pascuorum* (Scopoli, 1763)	GenBank
AY181137.1	*Bombus pascuorum* (Scopoli, 1763)	GenBank
AY181138.1	*Bombus pascuorum* (Scopoli, 1763)	GenBank
AY181139.1	*Bombus pascuorum* (Scopoli, 1763)	GenBank
AY181140.1	*Bombus pascuorum* (Scopoli, 1763)	GenBank
AY181141.1	*Bombus pascuorum* (Scopoli, 1763)	GenBank
AY181142.1	*Bombus pascuorum* (Scopoli, 1763)	GenBank
AY181143.1	*Bombus pascuorum* (Scopoli, 1763)	GenBank
JQ909709.1	*Bombus pascuorum* (Scopoli, 1763)	GenBank
JQ909710.1	*Bombus pascuorum* (Scopoli, 1763)	GenBank
AY181152.1	*Bombus ruderarius* (Müller, 1776)	GenBank
AY181153.1	*Bombus ruderarius* (Müller, 1776)	GenBank
AY181154.1	*Bombus ruderarius* (Müller, 1776)	GenBank
AY181155.1	*Bombus ruderarius* (Müller, 1776)	GenBank
AF385821.1	*Bombus schrencki* Morawitz, 1881	GenBank
GU674500.1	*Bombus schrencki* Morawitz, 1881	GenBank
AY181166.1	*Bombus sylvarum* Linnaeus, 1761	GenBank

(*Continued*)

Table 1. (*Continued*)

Accession number	Species	Database
AY181167.1	*Bombus sylvarum* Linnaeus, 1761	GenBank
AY181168.1	*Bombus sylvarum* Linnaeus, 1761	GenBank

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49 COI sequences available in the GenBank were employed for 16 species belonging to the subgenus *Thoracobombus* (sensu Williams et al. [25]) (Table 1). Additionally, sequences from three others specimens of *B. bellicosus*, stored in the Barcode of Life Database, and kindly made available by Dr. P. Tubaro, from the Museo Argentino de Ciencias Naturales, were also employed (Table 1).

The computer programs Arlequin version 3.5.1.2 [37], DnaSP version 5.10.01 [38] and MEGA 5.01 were used to estimate the following intra and interpopulational parameters: 1) haplotype diversity (H); 2) average number of nucleotide differences (k); 3) mean number of pairwise differences (π); 4) number of polymorphic sites (S); and 5) Tajima's D and Fu's F_S test of selective neutrality. DnaSP was also used to verify non-synonymous and synonymous substitutions. Haplotype networks constructed using the median-joining algorithm (MJ) [39], available in the NETWORK 4.5 software, were used for inferences about phylogenetic relationships among haplotypes and their possible geographical correlation. Population analyses were performed separately for *B. brasiliensis* (124 specimens) and *B. bahiensis* sp. n. (3 specimens) using a CytB and COI concatenated matrix (127 specimens).

Average intra and interspecific genetic distances were obtained with Mega 5.01, using the parameters of the Kimura 2 model—K2P [40]. Two analyses were performed with different data sets, one using only the COI gene and the other employing a concatenated data set of the COI and CytB genes. This was done because: 1) There were more COI than CytB sequences available in GenBank and BOLD for species of *Thoracobombus* (sensu Williams et al.[25]); and 2) There were no CytB sequences available for many of the specimens from which DNA was extracted for this work.

Since primers used here were not the same as those employed for obtaining the sequences in GenBank and BOLD, only 402 bp were present in all sequences used in the COI-only analyses. Intraspecific distances were estimated only for species represented by three or more specimens, with at least two different haplotypes.

Phylogenetic analyses were performed using a concatenated matrix with CytB and COI-gene data, including a total of 138 specimens, with *B. morio* as the outgroup. The best fit substitution model estimated with Modeltest 3.7 [41] for this analysis was the GTR+G model.

Phylogenies were generated through Bayesian Inference (BI) in MrBayes 3.1 [42], and through Maximum Parsimony (MP) and Maximum Likelihood (ML) algorithms using the program PAUP* 4.0b10 [43]. Inferences of trees with MP and ML methods were performed using heuristic search, with the following parameters: stepwise addition (random) starting from a single initial tree and branch swapping (tree bisection and reconnection—TBR). MP analysis was set to retain up to 1000 most parsimonious trees. The bootstrap method was used as a measure of branch support for the recovered phylogenies, using a total of 10,000 and 100 replications, respectively, for MP and ML. Phylogenetic analyses using BI used two sets of Markov chains, each containing three hot chains and one cold, with 20 million generations with a 25% burn-in, to seek for convergence to the same subset of best trees.

Nomenclatural Acts

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the new names contained herein are available under that Code from the electronic edition of this article. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix "http://zoobank.org/". The LSID for this publication is: urn:lsid:zoobank.org:act: D4CB4F65-BA90-4E9B-BFCF-F420A9718C25. The electronic edition of this work was published in a journal with an ISSN, and has been archived and is available from the following digital repositories: PubMed Central, LOCKSS.

Results

Concatenated population analyses

The phylogenetic relationships among haplotypes and their geographical correlation, using the concatenated CytB (683 bp) and COI (471 bp) sequences (totaling 1154 bp), showed that populations from almost all the geographic range of *B. brasiliensis* share haplotypes, as shown by the haplotype network, which suggests lack of population structure (Fig 2). In contradiction to the initial hypothesis, thus, this result suggests that populations of *B. brasiliensis* isolated in mountain tops away from the coast belong in the same species as those living in coastal forests from the state of Rio de Janeiro southward to the state of Santa Catarina and westward to Paraguay in the Paraná-river valley.

On the other hand, these results indicate that the three specimens collected in the coastal forest of southern Bahia state (municipality of Ilhéus, all with the same haplotype) diverged from *B. brasiliensis* by 38 mutation steps (Fig 2), suggesting that this population belongs to a different species (described below as *B. bahiensis* sp. n.)

The population analysis of *B. brasiliensis* included a total of 124 specimens, from 30 localities (Fig 3). It resulted in 27 haplotypes, 17 polymorphic sites (11 synonymous and six nonsynonymous mutations), 10 of which were parsimoniously informative and seven were autapomorphies (singleton sites). The total haplotype diversity (Hd), nucleotide diversity (π), and average number of nucleotide differences (K) were 0.886, 0.003 and 3.057, respectively. The statistics for the neutrality tests were $D = -0.083$ ($p = 0.5$) for Tajima's, and $FS = -12.28$ ($p = 0.001$) for Fu's. Although the Tajima's D was not statistically significant, the significant value of Fu's FS, associated with the large number of unique haplotypes and the star-shaped haplotype network, suggests recent population expansion.

The genetic distance between *B. bahiensis* sp. n. and *B. brasiliensis* was 3.7 ± 0.6%, while the average intraspecific distance within *B. brasiliensis* was 0.26 ± 0.09%. The population parameters above were not calculated for *B. bahiensis* sp. n., since all three specimens had the same haplotype.

COI-only analyses

Of the 402 bp used in the COI-only analyses, 241 were constant and, among the variable characters, 28 were parsimony-uninformative, and 133 parsimony-informative. The mean genetic distance between *B. bahiensis* sp. n. and *B. brasiliensis* estimated with the COI gene was 2.89 ± 0.8%. Among the comparisons made for the 21 *Thoracobombus* species employed in this analysis, distances smaller than that between *B. brasiliensis* and *B. bahiensis* were obtained for seven other species pairs (S3 Table). Moreover, while the largest distance between any two

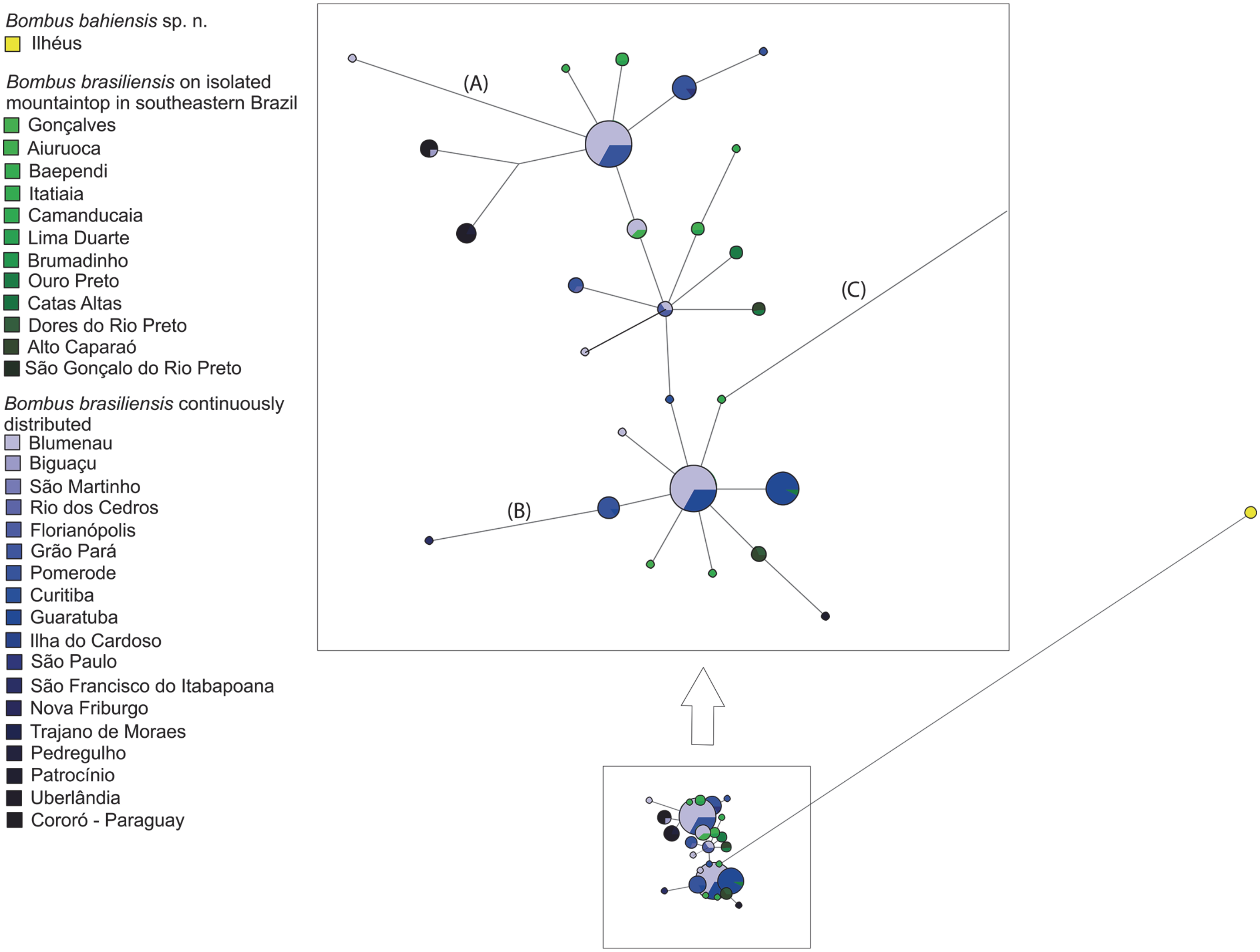

Fig 2. Network of haplotypes for *Bombus brasiliensis* Lepeletier, 1836 and *Bombus bahiensis* sp. n. Median-joining network of haplotypes for *B. brasiliensis* and *B.bahiensis* sp. n. The *B. brasiliensis* on isolated moutaintops in southeastern Brazil are coloured of different shades of green and the *B. brasiliensis* continuously distributed are coloured of different shades of blue. *B.bahiensis* sp. n. is coloured yellow. All lines joining haplotypes are one mutation step long, except for three of them, marked with "a", "b" and "c", which are three, two and 38 steps long, respectively.

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specimens of *B. brasiliensis* was of 1 ± 0.5%, the smallest distance between a specimen of *B. brasiliensis* and a specimen of *B. bahiensis* was of 2.5± 0.8%.

The intraspecific distances among the *Thoracobombus* species analyzed here, estimated with the COI sequences, were all equal or smaller than 0.5% (Table 2). One exception was the mean intraspecific distance for *B. brevivillus*, which was very high when all specimens originally attributed to this species where considered together (Table 2). Moreover, the average distance between the two "*B. brevivillus*" clades obtained in the phylogenetic results described below is 3.92 ± 0.93%. This suggests that the samples considered here as belonging to *B. brevivillus* include specimens of two distinct species (*B. brevivillus* (1) and (2) in S3 Table). This finding is being examined in detail and results will be published elsewhere.

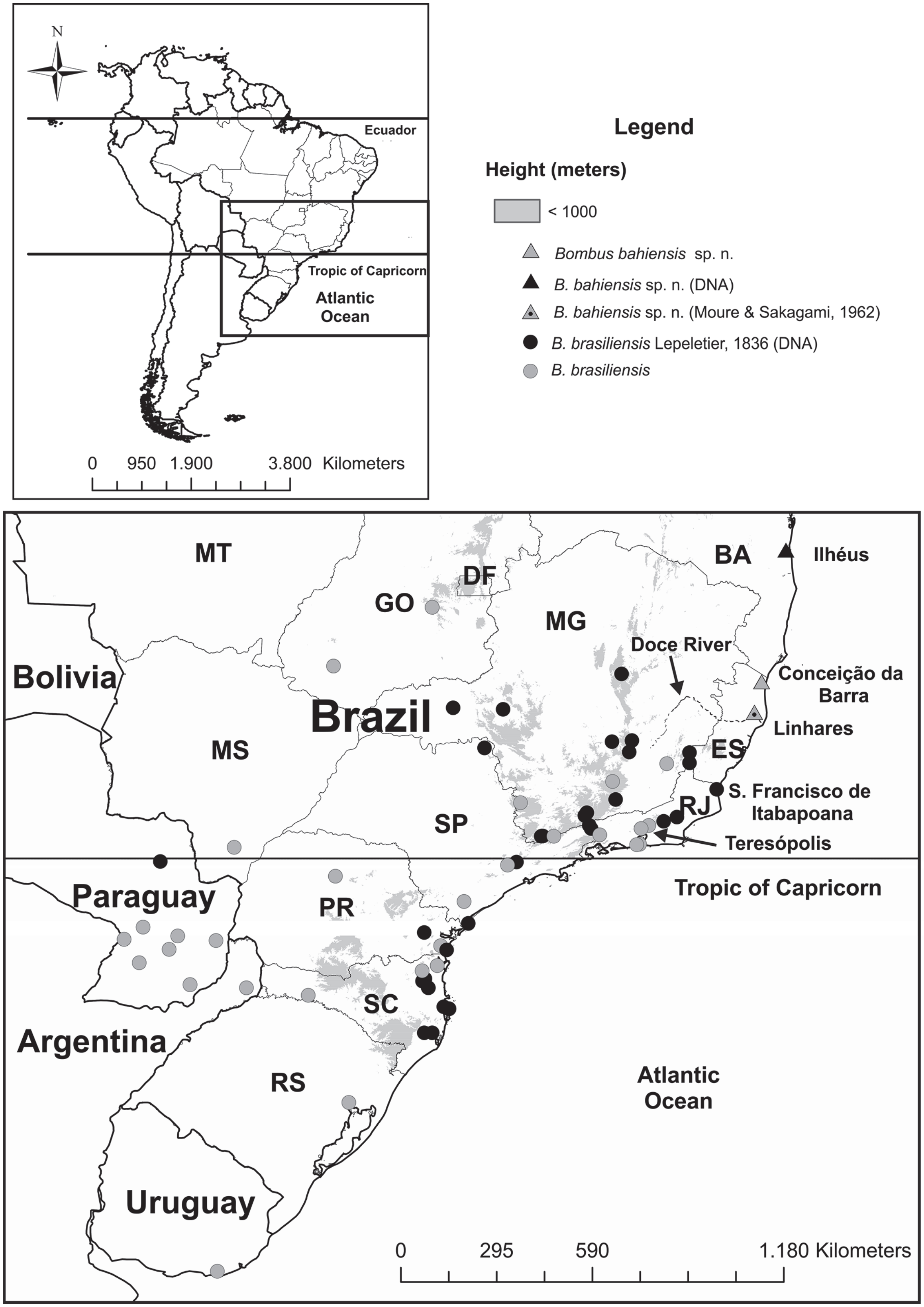
N
Ecuador
Tropic of Capricorn
Atlantic Ocean
0 950 1.900 3.800 Kilometers
Legend
Height (meters)
▨ < 1000
△ Bombus bahiensis sp. n.
▲ B. bahiensis sp. n. (DNA)
△ B. bahiensis sp. n. (Moure & Sakagami, 1962)
● B. brasiliensis Lepeletier, 1836 (DNA)
● B. brasiliensis
MT
DF
GO
BA
Ilhéus
MG
Doce River
Bolivia
Brazil
Conceição da Barra
Linhares
MS
ES
S. Francisco de Itabapoana
RJ
SP
Teresópolis
Tropic of Capricorn
Paraguay
PR
SC
Argentina
RS
Atlantic Ocean
Uruguay
0 295 590 1.180 Kilometers

Fig 3. Distribution of *Bombus brasiliensis* Lepeletier, 1836 and *Bombus bahiensis* sp. n. Records for *B*. *bahiensis* are represented by triangles (the black triangle, the gray triangle and the gray triangle with black point represents a population used in mtDNA analyzed, a population used in morphologic analyzed and a population inferred to belong to the species based on descriptions in Moure &Sakagami [23], respectively); records for *B*. *brasiliensis* are represented by circles (the black circle denote sites represented by mtDNA). Records were compiled from the literature [23, 60, 73] and from specimens deposited at UFMG and/or listed in S1 Table. Acronyms represent Brazilian states, as follows: BA = Bahia; ES = Espírito Santo; MG = Minas Gerais; RJ = Rio de Janeiro; SP = São Paulo; PR = Paraná; SC = Santa Catarina; RS = Rio Grande do Sul; MT = Mato Grosso; GO = Goiás; MS = Mato Grosso do Sul; DF = Distrito Federal. Localities mentioned in the text are indicated. The stippled line indicated by black arrow represents the Doce river.

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Phylogenetic analyses

Of the 1157 bp employed in the phylogenetic analyses (686 bp for CytB and 471 for COI), 916 were constant, three were gaps; eight variable characters were parsimony-uninformative, and 233 were parsimony-informative. Those three gaps are due to one CytB codon appearing exclusively in *B*. *morio* (included as the outgroup) and were the cause for the difference in pair-base numbers between the data employed in the phylogenetic and the population analyses.

The phylogenetic analyses yielded the same single topology for Parsimony, Maximum Likelihood and Bayesian analysis (Fig 4). This tree was yielded after 1,102,282 rearrangements in the MP analyses and was 302-steps long. The average standard deviation of split frequencies after 20 million generations in the Bayesian analysis was of 0.002869, indicating convergence to a single subset of trees.

The phylogenetic analyses are congruent with the population analyses in suggesting that populations of *B*. *brasiliensis* isolated on the top of mountain ranges in southeastern Brazil belong to the same species as those in the Atlantic coast and in the lowlands of the Paraná-river valley (S1 Fig). They also show that specimens originally attributed to *B*. *brasiliensis* in the coastal forest of southern Bahia constitute a clade *(B*. *bahiensis* sp. n.), sister to the rest of the populations of *B*. *brasiliensis*.

Table 2. Genetic distances for COI sequences (%) within species of bumblebees.

Species	Average distance	MaD
B. *pauloensis*	0.46±0.17	1.51±0.60
B. *brasiliensis*	0.39±0.21	1.01±0.50
B. *morio*	0.35±0.14	1.77±0.67
B. *bahiensis* sp. n.	0.00±0.00	0.00±0.00
B. *transversalis*	0.10±0.10	0.25±0.24
B. *bellicosus*	0.50±0.29	0.75±0.43
B. *brevivillus* (2)	0.50±0.28	0.75±0.43
B. gr. *Brevivillus*	2.50±0.60	4.09±0.99
B. *muscorum*	0.00±0.00	0.00±0.00
B. *fervidus*	0.00±0.00	0.00±0.00
B. *pascuorum*	0.14±0.10	0.50±0.34
B. *ruderarius*	0.00±0.00	0.00±0.00

Analysis was done in species with three or more specimens. The model used was the Kimura 2-parameter. *B*. *brevivillus* was considered as belonging in two distinct species (*B*. *brevivillus* cluster 1 and cluster 2 see S3 Table) and *B*. gr. *brevivillus* (all specimens). AD = average intraspecific distance ± standard deviation; MaD = maximum intraspecific distance ± standard deviation.

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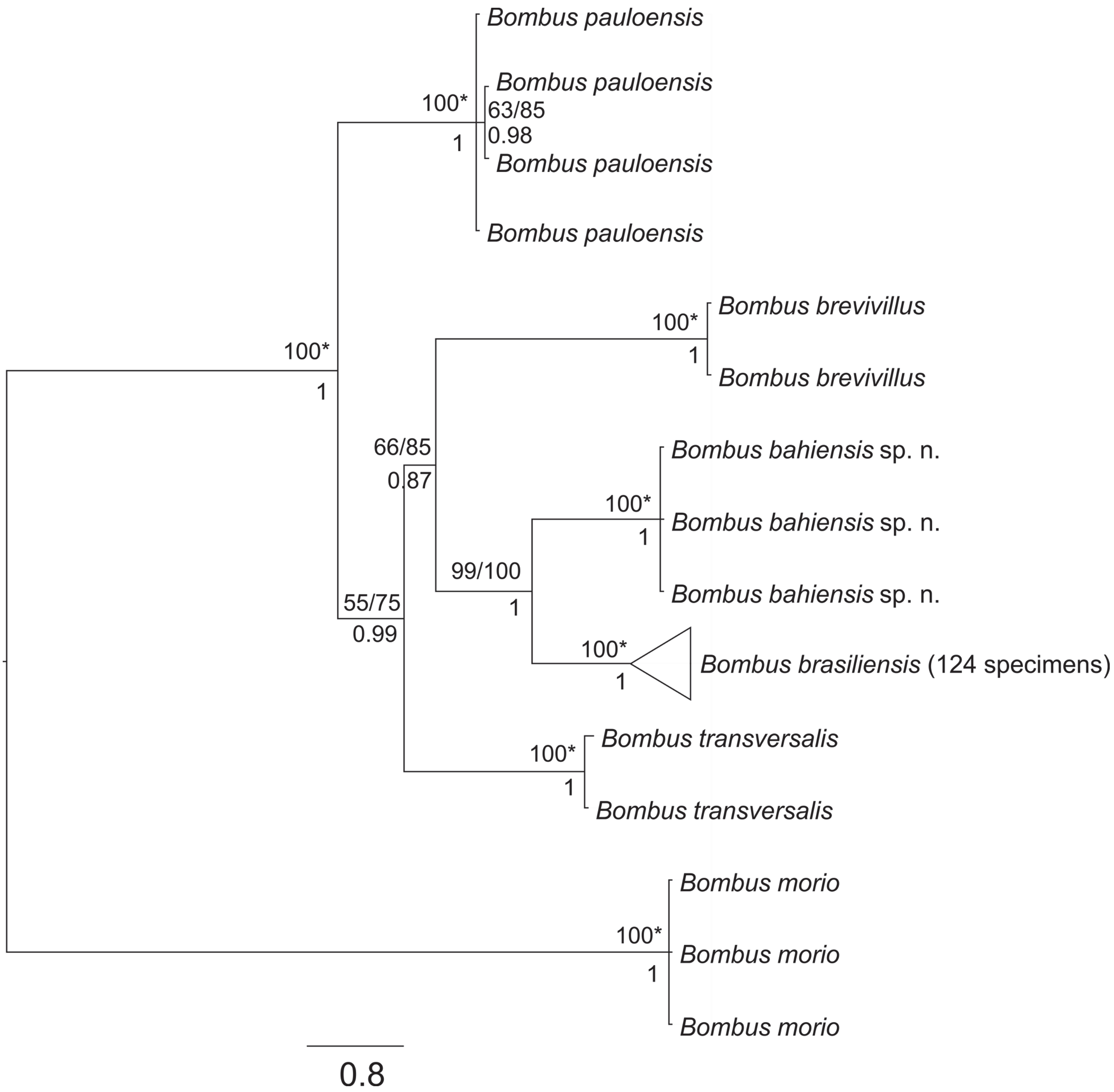

Fig 4. Phylogenetic relationships among Brazilian bumblebees obtained from concatenated phylogenetic analyses performed with CytB and COI sequences, using Bayesian inference and all specimens available for each species. Values shown under each branch are posterior probabilities. The values shown above each branch are bootstrap values represented here by Maximum Parsimony (MP)/ Maximum Likelihood (ML), because an identical topology was obtained with MP and ML. An analysis employing only unique haplotypes yields the same topology. "*" Represents the same values for MP and ML. *Bombus morio* (Swederus, 1787) was used as outgroup. The collapsed branch includes all *Bombus brasiliensis* haplotypes found on the haplotype network from Fig 2 and S1 Fig.

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Morphology and taxonomic decisions

Examination of the external morphology of specimens attributed by the molecular analyses to *B. brasiliensis* and to *B. bahiensis* sp. n. revealed a character also allowing for their distinction: The pilosity of T1, which is entirely yellow in *B. brasiliensis* and mostly black in *B. bahiensis* (see key below). Furthermore, this character led to the recognition that two additional specimens from Conceição da Barra (northern Espírito Santo state) also belong in *B. Bahiensis* sp.n. (these latter specimens were too old and DNA extraction from them was not attempted). Considering the molecular and morphological evidence, the specimens from southern Bahia attributed to *B. brasiliensis* in our samples are considered here to be a species distinct from that containing the remaining populations of *B. brasiliensis*.

Two pieces of evidence indicate that the name *B. brasiliensis* should be applied to the species occurring along the coast of Rio de Janeiro and southwards: *a)* the holotype of *B. brasiliensis* has its first metasomal tergum covered with yellow pilosity (Fig 1B), as opposed to black, as in specimens from southern Bahia (Fig 1C and 1D); and *b)* the fact that this type specimen was probably collected in the vicinities of the city of Rio de Janeiro [23].

Moreover, two synonyms are recognized for *B. brasiliensis*: *B. venustus* Smith, 1861 and *B. brasiliensis* var. *palliventris* Friese, 1931 [23, 24], the former described from Teresópolis (on the top of the Serra do Mar mountain range, in the state of Rio de Janeiro—Fig 3) and the latter from Paraguay. The fact that populations sampled in Paraguay and on the mountaintops in southeastern Brazil showed to be conspecific with specimens from the southern Atlantic coast in the phylogenetic and population-genetic analyses, indicate that those names should continue to be considered as synonyms of *B. brasiliensis*. Taking all above in account, the populations occurring to the north of the Doce river, in the coastal forests of northern Espírito Santo and southern Bahia, are described below as a new species.

Taxonomic treatment

***Bombus bahiensis* Santos Júnior & Silveira sp. n.** urn:lsid:zoobank.org:act:D4CB4F65-BA90-4E9B-BFCF-F420A9718C25

Fig 1C and 1D

Diagnosis. No single morphological character is known to be exclusive of *Bombus bahiensis* sp. n., which can be distinguished from most other Brazilian bumblebee species, except *B. brasiliensis*, for the mesosoma dorsally yellow, traversed by a black intertegular band, which extends laterally over the lower half of the mesepisternum (and generally reaching its ventral area), and the predominantly yellow metasoma traversed by a black band in T3; from *B. brasiliensis* it can be distinguished by the mostly black-haired T1, with yellow and partially-yellow hairs restricted to the mid-apical margin of the tergum. Only two species are potentially sympatric with *B. bahiensis* sp. n., *B. pauloensis* (melanic form) and *B. brevivillus*, which can be readily distinguished from *B. bahiensis* for their entirely black pilosity.

Description (worker holotype). *Measurements* (mm): approximate body length—16.2; head (length:width)– 4.5: 4.0; labrum (length: width)– 0.4:1.7; malar area (length × width)– 0.9:1.3; clypeocellar distance—1.9; interantennal distance—0.7; antennocular distance—0.6; ocellocular distance—0.6; interocellar distance—0.7; ocellar diameter—0.2; ocelloccipital distance—0.8; scape (length:diameter)– 2.1:0.3; length of F1 and F2–0.5, 0.3; forewing length—14.3; length of 2nd and 3rd submarginal cells (measured on their posterior margins)– 1.7, 1.3.

Structure: labrum biconvex; disc of clypeus depressed; malar area slightly shorter than wide; interocellar distance larger than ocellorbital.

Body color: black, except reddish-brown on posterior tibia; wings dark brown, with light cupreous hue.

Body surface: clypeus coarsely punctate, except on disc, irregularly punctate with shiny interspaces; area between lateral ocelli and eye impunctate, smooth and shiny, except for a micro-punctate region on upper paraocular area, near upper inner-margin of compound eyes, which is narrower than mid-ocellar diameter, generally occupying one third of ocellorbital distance; glabrous area on disc of mesoscutum ill-defined, mostly restricted to region posterior to median mesoscutal line, lightly micro-reticulate but shiny; glabrous shiny area on inner surface of hind femur relatively narrow (less than half the width of the surface) occupying the apical three-fourths of the femoral length.

Pubescence: on head, black bristles intermixed with greyish plumose hairs, most abundant between antennal sockets; on mesosoma, long, fine hairs with long branches, light yellow, except for black intertegular band; on inner surface of hind tibia, fine, spatulate, the flat apical portion triangular, restricted to the very tip of each seta; on T1- T5 long and fine, with long branches, on T3 light yellow, on other terga, black; on T6 black and short; on sterna and legs, black and short.

Variation: the integument varies from black to reddish brown, especially on legs and metasoma; black hairs with light-yellow tips may occur on T1.

Queen. Similar to worker, except for the vertex much elevated above level of ocelli, as normally occurs in *Bombus* queens.

Male. Unknown.

Holotype. “Ilhéus BA; Brasil 18/11/2009; A. Nemésio”, “Euglossina da Hiléia Baiana, Campus UESC; 16724–47065”, “*Bombus bahiensis*; Santos Júnior & Silveira, sp. n.; HOLOTYPUS”. Collected in flight. Deposited at UFMG (accession number 16724–47065). The holotype lacks the right hind-leg, removed for DNA extraction.

Paratypes. “Euglossina da Hiléia Baiana, Campus UESC; 18339–52893”, “Ilhéus BA; Brasil 20/02/2010; A. Nemésio”, “*Bombus bahiensis*; Santos Júnior & Silveira sp. n.; PARATYPUS”; “Euglossina da Hiléia Baiana, Campus UESC; 18339–52894”, “Ilhéus BA; Brasil 20/02/2010; A. Nemésio”, “*Bombus bahiensis*; Santos Júnior & Silveira, sp. n.; PARATYPUS” (both workers, deposited at UFMG). “Conceição da Barra ES; Brasil 31/01/1969; C. Elias & T. Elias”, “*Bombus bahiensis*; Santos Júnior & Silveira, sp. n.; PARATYPUS” (two workers, deposited at DZUP).“Ilhéus BA; Brasil 2003; M. A. Costa”, “*Bombus bahiensis;* Santos Júnior & Silveira, sp. n.; PARATYPUS” (queen, deposited at DZUP).

Etymology. The name *bahiensis* refers both to the Brazilian state of Bahia, where the holotype and some of the paratypes were collected, and to its habitat, the Bahia Forest (see explanation below, under “distribution”).

Distribution. The species seems to be restricted to the so-called Bahia Forest, which is the especially luxuriant Atlantic Forest formation that covers southern Bahia and northern Espírito Santo states, in Brazil (Fig 3).

Key to females of Brazilian species of *Bombus* Latreille, 1802. (Modified from [23])

1. - Pilosity entirely black, except for apically-pale hairs on ventral parts of body **2**
   - Pilosity at least partially yellow on mesosoma, metasoma or both, forming bands or entirely covering one or more terga **4**

2. - Malar area longer than its apical width; posterior glabrous area on disc of mesoscutum well delimited, micro-reticulate and dull ....***B. morio* (Swederus, 1787)**
   - Malar area shorter than its apical width; posterior glabrous area on disc of mesoscutum poorly or well delimited but never micro-reticulate and shiny **3**

3. - Micro-punctate region on upper paraocular area, near upper inner-margin of compound eyes wide, as wide as or wider than mid-ocellar diameter, occupying half of ocellorbital

distance; pilosity relatively dense, velvety, especially dorsally on mesoscutum of queens .... ***B. brevivillus*** **Franklin, 1913***
- Micro-punctate region on upper paraocular area, near upper inner-margin of compound eyes narrow, narrower than mid-ocellar diameter, generally occupying one third of ocellorbital distance; pilosity looser, not velvety .... ***pauloensis*** **Friese, 1913** (black form)

4. - Pilosity of pronotum, mesoscutum and scutellum light-yellow to yellowish-brown, without black intertegular hair-band; pilosity on T4-T6 ferruginous .... ***B. bellicosus*** **Smith, 1879**
   - Yellow pilosity on pronotum, mesoscutum and scutellum relatively well developed, but always with an intertegular black band; T3 generally with yellow hair band; T4-T6 black-haired **5**
5. - Yellow pilosity on mesosoma never totally covering meso and metepisterna, which are black haired on lower half, including venter **6**
   - Yellow pilosity entirely covering meso and metepisterna, frequently reaching venter of mesosoma **7**
6. - Posterior glabrous area on disc of mesoscutum ill-defined, with sparse punctures reaching its middle; pilosity on pronotum, scutellum and T3 deep yellow, black intertegular band narrower than pronotal band .... ***B. transversalis*** **(Olivier, 1789)**
   - Posterior glabrous area on disc of mesoscutum well defined, with no punctures on its middle; yellow pilosity entirely light; intertegular hair band wider than pronotal band .... ***B. pauloensis*** **Friese, 1913** (yellow-banded form)
7. - T1 covered with yellow pilosity ....***B. brasiliensis*** **Lepeletier, 1836**
   - T1 covered with black pilosity, frequently intermingled with yellow or partially yellow hairs mid-apically .... ***B. bahiensis*** **Santos Júnior & Silveira sp. n.**

* Probably a compound species (see text).

Discussion

Variation discontinuity, Phylogeny and Species Status

The genetic distance between *B. bahiensis* sp. n. and *B. brasiliensis* was relatively small, and this may raise the suspicion that they might in fact be a single species. However, comparable small divergences have been commonly estimated for several species-pairs believed to have diverged recently, in other bee taxa. For example, divergences found for COI-sequences by Dick et al. [44] and Nemésio et al. [45] between species pairs of orchid-bee (Euglossina) in South America were all below 2%, while Gibbs [2] found only a 3.06% average COI-sequence divergence among five species of *Lasioglossum* previously misinterpreted as a single species (with the lowest recorded value of 1.7%*)*. In *Bombus*, such small genetic distances were found, also, among cryptic species in northern Europe ([5, 10], see also Table 5 in [12]). Moreover, although the average genetic distance between *B. brasiliensis* and *B. bahiensis* sp. n. was the smallest recorded between pairs of Brazilian species of *Bombus* in this study, it was higher, for example, than those obtained for seven other pairs of undisputed species in Asia, Europe, and South America (S3 Table). The fact that the smallest distance between a specimen of *B. brasiliensis* and one of *B. bahiensis* is 2.5 times larger than the largest distance between any two specimens of *B. brasiliensis* is an additional support for a genetic gap between *B. bahiensis* and *B. brasiliensis*.

Traditionally, discontinuity in variation among populations has been considered, both in morphological and molecular-based studies, as evidence that independently-evolving lineages are involved (for example [29]). In the barcoding context, average intra-population divergences much smaller than the inter-population divergences (the so called barcode gap—[46]) is considered evidence that they are cohesive sets of populations, isolated from each other. Much has been discussed in the literature about the reality and convenient thresholds of such a gap (for example [46]). However, there is some agreement that one is safe in recognizing different species when the barcode gap between them reaches the order of one magnitude, as in the case of *B. brasiliensis* and *B. bahiensis* sp. n. A problem here is that large intra-specific divergence could not be expected for *B. bahiensis* sp. n., considering that it is represented by a small sample from a single site. However, the situation could be looked at from the other way around: *B. brasiliensis* from sites more than 1500 km apart along the latitudinal gradient or from isolated populations in areas differing more than 1500 m in elevation did not show divergences as large as that found between populations of *B. bahiensis* sp. n. and those of *B. brasiliensis* about 240 Km apart in lowland evergreen forests.

The fact that *B. brasiliensis* and *B. bahiensis* sp. n. are reciprocally monophyletic also supports their recognition as separate species (for example [47]), as it suggests that each of them is a mutually-independent evolutionary lineage. It could be argued that the phylogenetic hypothesis produced here, being based on two linked genes (maternally-inherited mtDNA), may merely indicate a phylogeny for those genes, which may be different from the correct phylogeny of the species involved (for example [48]). Nevertheless, as pointed out by Williams et al. [49], this problem has not been detected for the use of COI in bumblebees and, moreover, *mt*DNA may actually be more suitable for species delimitation than other molecular and morphological data [50].

Biogeographic considerations

Before the arrival of the Portuguese settlers, the presumed geographic range of *B. bahiensis* sp. n. was covered by an especially luxurious evergreen forest (from now on, the Bahia forest), coinciding with an important area of endemism for many plant and animal taxa, including insects [51], and including several recently-described endemic species of orchid bees (Apidae: Euglossina) (for example [52–56]). Intra- and interspecific disjunctions of populations or sister-species involving this area and other areas in the Amazonian Forest or to the south in the Atlantic Forest also have been recorded for several taxa (for example [57–59]).

A closer look to the distributions of *B. brasiliensis* and *B. bahiensis* sp. n. shows a 240-km gap between the northernmost known record of the species in the coastal lowlands of Rio de Janeiro state (in the municipality of São Francisco de Itabapoana, see Fig 3 and S1 Table), and its southernmost record in the lowlands of Espírito Santo state (municipality of Linhares; [23]—see comments on this population below) (Fig 3). *Bombus brasiliensis* does occur in southeastern Brazil, in latitudes between those of Linhares and São Francisco de Itabapoana [23, 28, 60], but all these records (and additional localities represented in the UFMG collection) refer to cloud forests (or their immediate vicinities) on the top of mountain chains, further inland. These mountaintop records represent populations which are isolated from those near the coast (Fig 3) by semi-deciduous forests in areas under climates with relatively long dry seasons (4–6 months)—the same kind of environment found in the gap between the humid northern area occupied by *B. bahiensis* sp. n. and the humid southern area occupied by *B. brasiliensis* (compare, for example, the map in Fig 3 with that of the Atlantic Forest vegetation types presented by Carnaval et al. [61] in their supplementary material' Figure S1).

Close relationship between elements of the Bahia forest and the Amazonian Forest biotas has been pointed out for organisms such as birds (for example [62]) and also for orchid bees, with several pairs of presumed sister species of the latter occurring disjunctly in the two phytogeographic domains (for example [54, 55]). This may suggest that *B. bahiensis* sp. n. could be sister to the only species in the genus restricted to Amazonia, *B. transversalis* [23]. However, this hypothesis is not supported by any of the results obtained here.

The presumed range of *B. bahiensis* sp. n. also falls within a forest refuge that existed in the Atlantic Forest domain during cold, dry periods of the Pleistocene, the so-called Bahia refuge, which was predicted by climatic models and validated by paleopalynological data (for example [61, 63,64]). In the Bahia refuge, limited in the south by the Doce river, in Espírito Santo, and extending northward into southern Bahia, evergreen forests persisted even in the driest periods of the Pleistocene. Carnaval et al. [64] predicted that surveys in this area would still reveal undescribed species and cryptic lineages. If Hines [65] is right in her estimate that the closest common ancestor of *B. brasiliensis*, *B. transversalis* and *B. pauloensis* existed at about 2 Mya, then the most recent common ancestor of *B. brasiliensis* and *B. bahiensis* sp. n. lived in the Pleistocene, and this would be consistent with the hypothesis that the isolation of part of its populations in the Bahia refuge during that period could be the vicariance event responsible for its genetic divergence. The close association of *B. brasiliensis* and *B. bahiensis* sp. n. with evergreen forests in southeastern Brazil suggests that interbreeding of their populations may have been prevented by their inability to settle in lowland semi-deciduous forests, which occur under climates with relatively long dry seasons and that reaches the coast between southern Espírito Santo and northern Rio de Janeiro states. It should be noted, however, that this hypothesis is weakened by the occurrence of *B. brasiliensis* in areas dominated by climates with relatively long dry seasons and covered with semideciduous forests along the Paraná River valley, westward into Paraguay and central Brazil (Fig 3). A closer look at the habitats used by this species in that region is necessary for a better understanding of this question.

Morphological distinction and geographic range

Bombus is a relatively monotonous genus, as far as morphological characters are concerned (for example [1]), and this makes them frequently difficult to distinguish, especially among closely-related species (for example [5–11, 49]. Thus, it is not surprising that not a single exclusive autoapomorphy of *B. bahiensis* sp. n. could be found, and that the only morphological character distinguishing it from its presumed sister species, the black-haired T1, is a plesiomorphic trait or, at most, a homoplastic apomorphy, also found in all other Brazilian species but *B. brasiliensis* (for example [23]).

Moure & Sakagami [23] noticed that specimens identified by them as *B. brasiliensis* from Linhares, by the northern margin of the Doce river, in Espírito Santo state (shown by a grey triangle with a central black dot in Fig 3) had their first metasomal tergum completely covered by black pilosity. They interpreted this population as a melanic variety of *B. brasiliensis*. These specimens quite probably belong to *B. bahiensis* sp. n. and may represent the southernmost population of the species, since the Doce river is the southern limit for the evergreen forests of the Bahia refuge and the Bahia forest (although some authors, as Silva & Casteleti [51] and Ribeiro et al. [66] consider the Bahia forest to extend further south in Espírito Santo).

Conservation Status

Local or regional decline or extirpation of bumblebee populations has been reported in the northern hemisphere (for example [49, 67–70]) and in Brazil [71]. It seems that the small available sample of *B. bahiensis* sp. n. reflects a low abundance in nature and not a small sampling effort in its habitat. The bee fauna of northern Espírito Santo was intensively sampled by C. Elias, a collector for the DZOL collection in Curitiba in the 1960's and early 1970's, and the only specimens of the species collected among thousands of bee specimens, apparently are those few listed in the "Taxonomic treatment". Moreover, two expeditions were set to southern Bahia and northern Espírito Santo to search for the species, during the development of this project (January and June/July, 2014), but resulted unsuccessful. With this in mind, two facts should be considered concerning the conservation of this new species: 1) its presumed geographic range is the smallest among all Brazilian *Bombus* species; and 2) its natural habitat is under heavy anthropic impact—the rain forest that originally covered about 86% of the region, now covers only between 12% and 17% of it [51, 66]—and continues to be fragmented. Moreover, bees in general and bumblebees specifically may be more susceptible to fragmentation than other organisms, due to issues related to effective population size, social behavior, nesting, sex determination mechanisms etc. (see discussions in [72, 68]). Thus, *B. bahiensis* sp. n. may actually be an endangered species and efforts should be made to map the remaining populations of the species and their abundances, better defining its current geographic range and conservation status.

Supporting Information

S1 Fig. Phylogenetic relationships among *Bombus brasiliensis* obtained from concatenated phylogenetic analyses performed with CytB and COI sequences, using Bayesian inference. Values shown under each branch are posterior probabilities.
(TIF)

S1 Table. Specimens sequenced for the genetic analyses with their geographic origins. Universidade Federal de Minas Gerais—UFMG IHY; Universidade Federal de Santa Catarina—UFSC; Universidade Federal do Norte Fluminense—UENF; Universidade Federal de Ouro Preto—UFOP; Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto—Universidade de São Paulo—USP: FFCLRP; Universidade de São Paulo—USP/SP.
(DOCX)

S2 Table. Protocol of the purification of Polyethylene Glycol 20% (PEG 20%) for elimination of bands <300–400 bp.
(DOCX)

S3 Table. Genetic distances for COI sequences (%) between bumblebee-species. The model used was the Kimura 2-parameter. Bees currently considered as *B. brevivillus* belong in two distinct species, identified below as *B. brevivillus* (1) and *B. brevivillus* (2). AD = Average interspecific distance ± standard deviation; MiD = minimum interespecific distance ± standard deviation.
(DOCX)

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Author Contributions

Conceived and designed the experiments: JESJ FAS. Performed the experiments: JESJ. Analyzed the data: JESJ FRS FAS. Contributed reagents/materials/analysis tools: FRS FAS. Wrote the paper: JESJ FAS.

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Capítulo 2 – Peripatric speciation followed by niche differentiation between sister bumblebee species from the Atlantic Tropical Rain Forest

Peripatric speciation followed by niche differentiation between sister bumblebee species from the Atlantic Tropical Rain Forest

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Abstract

In this study, we analyzed the processes resulting in the speciation event leading to the origin of two endemic sister species of bumblebees in a Brazilian rain forest. We studied the phylogeographic pattern and historical demography of *Bombus bahiensis*, which is restricted to small fragments in eastern Brazil, and *B. brasiliensis,* which is widely distributed in southern and southeastern Brazil and neighboring regions of Uruguay, Paraguay and Argentina. We used ecological niche models, niche analyses, and genetic data (1) to test the role of niche differentiation on the divergence between the two species, and (2) to find potential distribution areas for the most restricted *B. bahiensis*, where likely to be found. Our results showed that *B. brasiliensis* populations are able to disperse across mosaics of anthropogenic and preserved areas and exhibit low levels of spatial genetic structure. Otherwise, *B. bahiensis* presented low genetic diversity restricted to a small distribution

range, where it is suffering with continuous habitat loss. In addition, being restricted by many environmental constraints, which suggests that this species may be at an elevated risk of extinction. The climatic oscillations of the Pleistocene influenced the population structure of both species in different ways, probably due to changes in their effective population sizes, physiological differences and demographic expansion. Specifically, while *B. brasiliensis* expanded its distribution range in the last 500,000 years throughout most of the Atlantic Forest, *B. bahiensis* has been restricted to a small area between southern Bahia and northern Espírito Santo states in Brazil.

Introduction

Speciation is the process responsible for species diversification, and most new species appear in allopatry (Endler 1978; White 1978). Two main mechanisms are associated with allopatric speciation: vicariance and dispersal (Endler 1978; White 1978). In addition, different environmental factors may lead to allopatric speciation processes (Wiens & Graham 2005), for instance, those factors promoting extinction, spatial displacement – dispersion – and population isolation – vicariance (Jakob *et al.* 2007). This is because different species display distinct tolerance levels to certain environmental conditions and may have different dispersal abilities related to hereditary characteristics associated to particular niches (Soberón 2007). Niche-associated traits such as this can present evolutionary conservatism among related species, which may be due to their phylogenetic proximity, or be similar in unrelated species due to convergent responses to environmental changes, even over vast temporal and spatial scales (Wiens & Graham 2005; Moen *et al.* 2013; Tanentzap & Lee 2017). Thus, climatic oscillations over time may have directly affected the speciation process and the patterns of current species distribution (Carnaval & Moritz 2008). Climatic fluctuations during the Plio-Pleistocene helped shaping the present diversity in temperate and boreal systems (Hewitt 2000). In the tropics, many models of diversification have been used to understand historical processes that resulted in the separation of species during those epochs (Williams & Pearson 1997; Perez *et al.* 2016; Menezes *et al.* 2016). Understanding processes related to speciation events is not a simple or straightforward task. There are few studies assessing biogeographical scenarios to explain them (Brooks & McLennan 2002) and different evolutionary models were proposed to understand the high biodiversity of Tropical rainforests (Gentry 1992; Rull 2008, 2011).

Some environments in the tropics are especially interesting, as they serve as a rich natural history laboratory for testing biogeographical and evolutionary questions. The Atlantic Forest hotspot is one such environment in South America, presenting a great species diversity and areas with different environmental conditions (Myers *et al.* 2000; Carnaval *et al.* 2014). In addition, this forest environment suffered large variation in vegetation cover throughout the Pleistocene due to climate oscillations (Carnaval & Moritz 2008). These factors directly contributed to the formation of the current biota in this ecosystem (Carnaval *et al.* 2014). However, insights on these biogeographic features were mainly based on the study of the diversification of Atlantic Forest vertebrates (Cabanne *et al.* 2007; Batalha-Filho *et al.* 2012; Valdez & D’Elía 2013; Menezes *et al.* 2016).

Phylogeographic studies can provide relevant biogeographic evidence on the speciation process (Avise *et al.* 1987; Hewitt 1996; Edwards & Beerli 2000), and may serve as well as the basis for conservation genetics approaches (Frankham *et al.* 2010). For instance, conservation biologists usually classify populations as evolutionarily significant units – ESUs (lineages evolving independently) – for management purposes, which are mostly based on phylogeographic data (De Queiroz 2007). The ESUs are populations that need a greater protection due their unique status based on genetic and ecological data (Moritz 1994; Crandall *et al.* 2000). Other tools, such as species distribution models (SDM) and spatially explicit models, can provide powerful tests of evolutionary hypotheses (Elith & Leathwick 2009) explaining speciation events, which can be applied particularly on studies of sister species or small clades (Silva *et al.* 2014).

The process of allopatric speciation is closely linked to the patterns of population and species distribution. In this way, the understanding of the speciation processes can help to recognize some of the causes of a species distribution. For example, endemism is a particular type of distribution pattern of several species, which is an important characteristic for conservation assessment (Kerr 1997). The understanding of the processes related to the geographic restriction of species distributions is fundamental for the creation of effective conservation strategies and for the identification of endemism areas. However, few studies focused on processes that have caused endemism patterns and niche differentiation (e.g. (Meyer *et al.* 2005; Kozak & Wiens 2006; Johnson 2010; Hanson *et al.* 2012; Nieto Feliner 2014).

The sister bumblebee species, *Bombus bahiensis* Santos Júnior & Silveira, 2015 and *B. brasiliensis* Lepeletier, 1836, of the Brazilian Atlantic Rainforest domain, are a good model for this kind of study (Santos Júnior *et al.* 2015). A recent study on phylogeographic and population genetic structure (Santos Júnior *et al.* 2015) resulted in the description of *B. bahiensis*, with a very restrict range, inhabiting small remnant fragments in the northern portion of that ecosystem, in the states of Bahia (BA) and Espirito Santo (ES). On the other hand, *B. brasiliensis*, is distributed throughout the Atlantic Forest of Brazil (southern ES towards to the southernmost states) and neighboring regions of Uruguay, Paraguay and Argentina (Santos Júnior *et al.* 2015).

In this study, we searched for factors related to the current distributions and speciation event that originated *B. brasiliensis* and *B. bahiensis*. For this, we used spatially explicit models associated with the evolution of the lineages of populations of both species. We tested the hypothesis of niche conservatism and the possible abiotic events associated with the divergence between species. In addition, we reevaluated through species distribution models (SDM) the distribution of *B. bahiensis* related to different environmental factors and its conservation status according to IUCN (International Union for Conservation of Nature) criteria.

Material and Methods

To assess the distribution of the genetic variability of *B. brasiliensis* and *B. bahiensis* in a geographical and temporal context, we used the database available (Cyt-b and COI concatenated matrix, *B. brasiliensis* – 124 specimens and *B. bahiensis* – 3 specimens) and the information, of neutrality tests (Tajima’s D and Fu’s FS), median-joining network of haplotypes, haplotype diversity (Hd), nucleotide diversity (π), and average number of nucleotide differences (K), published by Santos Júnior *et al.* (2015).

Barrier analyses and population structure of *B. brasiliensis*

To identify the most likely barriers to gene flow among populations of *B. brasiliensis* we used the Monmonier algorithm with the software Barrier 2.2 (Manni *et al.* 2004). This algorithm tests the most significant differences between genetic distance and geographic distribution among sampling locations. To generate genetic distance matrices, we used software Mega 7.0.14 (Kumar *et al.* 2016) (Kimura 2-parameter model) that we imported into Barrier 2.2 (Manni *et al.* 2004). We estimated the population structure indices using information on the allelic variation between haplotypes, as well as their frequencies through Analysis of Molecular Variance – AMOVA (Excoffier *et al.* 1992) in Arlequin 3.5.2.2 software (Excoffier & Lischer 2010).

Population Demography

We generated Bayesian Skyline Plots (BSP) using Beast 2.3.1 (Bouckaert *et al.* 2014) to visualize the changes in effective population sizes (Ne) through time for the *B. brasiliensis* data set. The analyses were run under HKY + I model of substitution. Three independent runs of 50 million generations were performed, with the Markov chain sampled every 1000 generations and the first 10% of trees were discarded as burn-in. Tracer 1.6 (Rambaut *et al.* 2013) was used to assess convergence of posterior distributions (determined by monitoring effective sample size, ESS > 200, and trace plots), mixing and stationary of the MCMC process and generated Bayesian Skyline Plots. Based on analyzes to be published elsewhere (Santos Júnior *et al.* unpublished data), we assumed 4.5mya (normal distribution; mean 4.5; standard deviation 0.9 Ma) as the age of the most recent common ancestor of *B. brasiliensis* and *B. bahiensis*. Three different clock models were tested: a Strict clock (CLOCK), Unncorrelated Lognormal Relaxed Clock model (UCLN) and Uncorrelated Exponential Relaxed Clock (UCED). We used Bayes factors and Akaike's information criterion (AIC) through Markov chain Monte Carlo (MCMC) (AICM) (Baele *et al.* 2012) to evaluate the evidence in favor of each model and select the one that best explained our data in Tracer version 1.5 and 1.6 respectively (Appendix S1). This analysis was not done for *B. bahiensis*, due to the insufficient number of samples.

Modeling Approach and tests of niche overlap

To test the niche overlap between the studied species, we used species distribution models (SDM) to estimate *B. brasiliensis* and *B. bahiensis* ranges. For this, we used four algorithms with different logics: Bioclim (Booth *et al.* 2014), GARP (Stockwell 1999), Maxent 3.3.3 (Phillips *et al.* 2006) and Support Vector Machine – SVM (Joachims 1999). We transformed altitude and bioclimatic variables of Wordclim (http://www.worldclim.org/) into axes of Principal Components Analysis (PCA) with correlation matrix in ArcGIS 10.3. This procedure was performed to avoid the multicollinearity of the variables. To evaluate the models, we used 30% of the presence data (obtained from well sampled areas) and absence data (inferred from areas without records of the studied species and with high sampling effort). To estimate the sampling effort, we created a kernel density map of bee collections for South America with records obtained from GBIF – Global Biodiversity Information Facility (http://www.gbif.org/), CRIA (http://splink.cria.org.br/) and literature (Appendix S2). To interpolate the sampling effort, we used the value calculated by the spatial variant of Silverman’s Rule of Thumb, as implemented in ArcGIS, as the influence area for each distribution record. This procedure enables an approximation of the Gaussian distribution of the distances of interpolated points. Bee records were verified for spatial accuracy, with all data checked for accuracy and geographic-coordinate validity through data crossing with databases of Brazilian political units in a geographic information system – GIS. Records that lacked geographic coordinates or presented georeferencing errors were georeferenced based on IBGE databases of localities and municipalities (http://mapas.ibge.gov.br). To transform the results of the models into binary maps the threshold of the minimum presence was applied. We chose this threshold considering that it is as a conservative approach, since it assumes that the minimum suitability is present in the samples. Since few records were available for *B. bahiensis*, the validation for this species could not be carried out. Thus, we assumed that the validation results for the *B. brasiliensis* model should satisfactorily reflect the predictive capacity of the *B. bahiensis* models. To evaluate the models we used the Area under Curve metric (AUC). To evaluate the niche overlap between species, we used three tests: I statistic (Warren *et al.* 2008) and relative rank – RR (Warren & Seifert 2011) in ENMTools (http://enmtools.blogspot.com.br/). To test the overlap, we used the distribution models as test variables.

Explanatory factors of evolution of lineages.

In order to assess which variables might be more related to the cladogenesis process generating the species, we used two models: SAR (Simultaneous autoregressive model) and Lagged model, in SAM (Spatial Analysis in Macroecology) (Rangel *et al.* 2010). We chose these models because they incorporate spatial autocorrelation in the analysis. As a connectivity matrix, we used a Minimum Spanning Tree. As the variable response, we used the phylogenetic tree. It is necessary to use vector values for the analysis in models, thus, phylogenetic data were converted into a phylogenetic distance matrix, which was converted to vector values by a Non-metric multidimensional scaling – NMDS. As it is not possible to analyze the relations of the predictor variables with the distance matrix, the matrix was converted into one axis values through NMDS analysis (analysis performed with 10,000 replications). These analyzes were carried out in the R software packages ape, picante, phylotools and phytools.

Two sets of variables were used as predictors: in the first, climatic variables and altitude, which were converted into axes of a PCA, due to the great correlation between them. Besides these variables, climatic stability (temperature and rainfall variation during the last 140,000 years) was also used (Oliveira & Santos unpublished data). In a second round of analyses, the following variables were used: soil moisture; soil carbon; soil pH; aridity; orientation of the terrain; slope; tree density; presence of evergreen forests; presence of herbaceous vegetation; potential evapotranspiration; potential vegetation; primary productivity, besides the previously mentioned variables. For the visualization of the evolution of the characters presenting higher coefficients of explanation in the models, we optimized these characters in the trees by Brownian model in Mesquite 3.2 software (Maddison & Maddison 2017).

Results

Barrier analyses and population structure of B. brasiliensis

The results identified a putative barrier to gene flow in *B. brasiliensis* between northern and southern Atlantic Forest populations. The break occurred between the Brazilian states of São Paulo and Minas Gerais, except for populations of the so called "Triângulo Mineiro" region (westernmost Minas Gerais), which remained as part of the southern Atlantic Forest populations (Figure 1).

The results of the AMOVA test and their corresponding F-statistics of genetic differentiation are presented next. The three sources of variation were: Φ_{CT} was low and marginally significant ($\Phi_{CT} = 0.06177$, p-value = 0.03519), showing that there was a low level of genetic differentiation between northern Atlantic Forest (NAF) and southern Atlantic Forest (SAF); Φ_{SC} was moderate and significant ($\Phi_{SC} = 0.28373$, p-value = 0) indicating a moderate differentiation among populations within groups; and Φ_{ST} value was also slightly high and significant ($\Phi_{ST} = 0.32798$, p-value = 0), indicating that there is moderate to high genetic differentiation among individuals within populations. Only a small fraction of the variation (6.18%) is explained by differences between groups NAF and SAF, while the greatest amount of variation is explained by differences among individuals (67.20%), which is expected when populations are not significantly differentiated.

Population Demography

The best model for the BSP analysis was UCED (Supplementary 1). BSP revealed a demographic expansion in *B. brasiliensis* since ca. 500,000mya (Figure 2) This is in accordance with the negative and significant neutrality test result (Fu's FS = -12.28, p-value = 0.001), all genetic diversity estimates (high haplotype diversity, H = 0.886 and low nucleotide diversity, $\pi = 0.003$), and a star-shaped haplotype network presented by Santos-Junior *et al.* (2015).

Species distribution modeling and tests of niche overlap

The models that presented better performance were GARP and SVM (AUC = 0.97 for both models in *B. brasiliensis* and 0.99 for both models in *B. bahiensis*). These models showed a continuous distribution of *B. brasiliensis* in southeastern South America in regions predominantly covered by seasonal semideciduous forests. On the other hand, *B. bahiensis*, presented an extremely restricted distribution in the eastern Brazilian state of Bahia, a region characterized by an evergreen tropical rainforest (Instituto Brasileiro de Geografia e Estatística 2012; Nemesio 2012; Rolim *et al.* 2016) named as the "*Hiléia Bahiana*" by

Andrade-Lima (1966). All the methods used for niche divergence test indicated niche divergence between *B. brasiliensis* and *B. bahiensis* (I = 0.07497; RR = 0.5249 in GARP analysis and I = 0.40762; RR = 0.5082 in SVM analysis) (Figure 3).

The results of the NMDS analysis satisfactorily represented the phylogenetic distances (non-metric fit R2 = 0.99, linear fit R2 = 0.99). The models with high spatial autocorrelation in errors were discarded. Among the remaining models, those better controlling the spatial autocorrelation in the errors, we chose the one presenting the smallest AIC, SAR (Figure 4). Most of the explanation of phylogenetic variation in the populations of *B. brasiliensis* and *B. bahiensis* was given by the predictor variables (86%). About 12% of the phylogenetic variation was explained by the spatial distance between samples. A small portion of the variation (about 2%) was unexplained (Figure 4). The main predictors of phylogenetic variation were climatic variables, corresponding to the first two axes of the PCA (Appendix S3); climatic stability was the second-best predictor of phylogenetic variation; and soil-related variables, such as soil moisture and soil pH, were also significant explaining phylogenetic variation among species populations (Appendix S4). The optimization of the variables of the species niche, using a Brownian model, showed the divergence of characters among the population clades of *B. brasiliensis* and *B. bahiensis* (Figure 4).

Discussion

The geographic range of *B. bahiensis*, the "*Hiléia Bahiana*", is an important area of endemism for different taxa (Peixoto & Silva 1997; Thomas *et al.* 1998; Peixoto *et al.* 2008; Rolim *et al.* 2016), where several other endemic bee species have also been recorded (*e.g.* Pedro & Camargo 2003; Graf & Urban 2008; Faria & Melo 2012; Hinojosa-Díaz *et al.* 2012; Nemesio & Engel 2012). The available evidence suggests that this ecosystem has been a long-lasting, stable forest environment at least along the Pleistocene and has functioned as a forest refuge along that period (Carnaval & Moritz 2008; Carnaval *et al.* 2009). In such a scenario, causes of speciation are especially relevant, since they may help to explain the origin and evolution of a whole set of endemic species (Whitaker 2003; Haffer 2008; Boucher *et al.* 2011). Thus, evidence on species divergence due to niche specialization, as those brought up by our niche-modeling analysis, is an interesting explanation, alternative to general vicariance explanations.

In addition to niche-modeling data, there are other pieces of evidence supporting the events resulting in the niche divergence between *B. brasiliensis* and *B. bahiensis*. Populations of the former species share haplotypes in almost all its geographic range (some haplotypes are shared among populations some 1,200 km apart (Figure 1), which indicates the occurrence of gene flow between distant populations—see figure 2 in Santos Júnior *et al.* (2015) and Figure S1), and suggesting the lack of population structure. On the other hand, the minimum distance between populations of *B. bahiensis* and *B. brasiliensis* is around 600 km (Figure 1). This leads to the conclusion that genetic differentiation between *B. brasiliensis* and *B. bahiensis* cannot be attributed only to geographic isolation. This is suggested also by the low explanation given by the variable "distance" in the SAR model (see above) (Figure 4). Moreover, being powerful fliers, bumblebees should be able to disperse across such distances, if environmental conditions were favorable to their establishment along the way and in the newly colonized area (Santos Júnior *et al.* 2015; Francisco *et al.* 2016). Therefore, our results suggest that environmental conditions influenced the origin and evolution, and the geographic range of these species.

The geographic disjunction between *B. brasiliensis* and *B. bahiensis* is also interesting and could be alternatively explained as due to 1) the division of a wide range of their ancestor species, due to climatic changes along the land stripe between their current ranges (vicariance); 2) the dispersion of individuals of the ancestor species across a pre-existing environmental barrier; or 3) the emergence of an environmentally unsuitable stripe of land between the parapatric ranges of two previously-diverged sister species.

Genetically testing if the speciation process leading to the origin of *B. bahiensis* and *B. brasiliensis* was due to dispersion or vicariance is difficult since both processes may have similar effects on genetic diversity (Frankham *et al.* 2010). Moreover, making inferences based on the population genetics of *B. bahiensis* is somewhat speculative, due to the very small sample available for genetic studies (four specimens) and, especially considering that all specimens for which DNA was available were collected virtually in the same spot and could belong to a same colony. Nevertheless, irrespective of which of the scenarios proposed above is more probable, we expect them to have the same effect on *B. bahiensis* ancestor population size and genetic diversity. This allows us to assume that *B. bahiensis* had, in its origin, a small population size and low genetic diversity as consequence of a process of peripatric speciation.

The usual model of peripatric speciation proposes one of the diverging populations to be much smaller and located in the periphery of its sister species range (Mayr 1940; Templeton 1980). In this case, the range of *B. bahiensis* is much smaller than that of *B. brasiliensis* and the former has a peripheral distribution in relation to the latter. This may occur by dispersion followed by a founding effect, which occurs when a new population is established by a very small number of specimens from a larger population or by factors that promoted extinction of intermediate populations (Frankham *et al.* 2010).

The lack of variability reduces the capacity of a species to evolve in response to environmental changes in medium to long term (Wiens & Graham 2005; Frankham *et al.* 2010). This inability to respond to different environmental conditions may be an additional explanation for the restrict geographical range of *B. bahiensis*. Stochastic effects have a much greater impact on the genetic variability of small populations than on large populations. Thus, a species with small population and low genetic diversity, well adapted to specific environmental conditions found in a long standing refuge (Stockwell *et al.* 2003; Frankham *et al.* 2010), as is the case of *B. bahiensis,* may find it difficult to expand its range and adapt itself to other environmental conditions, especially during the abrupt climatic variations of the Pleistocene. If, in one hand, tests of the role of demographic history on *B. bahiensis* population cannot be performed, due to the low number of specimens available, this could be done for *B. brasiliensis,* which apparently was able to expand its range during Pleistocene climatic fluctuations, as reported by Santos Júnior *et al.* (2015). These authors indicated that populations of *B. brasiliensis* located in low altitude areas served as sources for the colonization of high altitude areas during interglacial periods.

In addition to environmental conditions, intrinsic phenotypic traits such as nesting behavior, foraging strategy and dispersion ability may be important factors shaping species distribution and structure. *Bombus* species, for example, are known to be able to disperse over several kilometers and to exhibit low levels of spatial genetic structure (e.g. Lepais *et al.* 2010; Dreier *et al.* 2014; Santos Júnior *et al.* 2015; Duennes *et al.* 2016). Differences in dispersion capacity and genetic structure among sympatric Brazilian bumblebees have been reported in the literature though (Françoso *et al.* 2016), indicating that barriers to gene flow may be species-specific.

In regard to nesting behavior of bumblebees, studies are scant and usually comprise occasional reports. The nesting behavior of *B. bahiensis,* for instance, is unknown, and the little we know about *B. brasiliensis* is based on just the study of four nests (Table 1). We believe that disparity in this specific trait may also account for differences in species distribution and, since Brazilian bumblebee nests are usually found on the ground surface for the importance of soil moisture in explaining this difference (as predicted by SAR model). Besides that, differences in nest architecture may influence defense strategies against predators and parasitoids (Whitfield *et al.* 2001; Ramírez & Cameron 2003) the permeability of the nest in wet rainforests, which could interfere in the health of the colony (Taylor & Cameron 2003).

Conservation Status

Only seven specimens of *B. bahiensis* are known: four workers from Ilhéus, BA, deposited at UFMG, and one queen from Ilhéus and two workers from Conceição da Barra, ES, deposited in the DZUP collection (Santos Júnior *et al.* 2015). This small number of specimens collected in such a long time span (Ilhéus two specimens collected in 2010, one in 2009 and one in 2003; Conceição da Barra two specimens collected in 1969) suggests that *B. bahiensis* is probably a rare species.

After *B. bahiensis* was described, 702 hours of sampling were spent in and around the areas where it was previously recorded. In its type locality, after 45 sampling hours, a single individual was collected (the seventh specimen collected in Ilhéus, BA and Conceição da Barra ES, in 48 years). However, in the southern portion of its historical distribution range (Figure 1), no specimen was collected since 1969, despite the large sampling effort deployed there recently (up to 225 hours in some locations, table 2). This data suggests a retraction in its distribution range and/or local extinction along the last 48 years. The population size criterion for a species to be considered as threatened by IUCN considers the total number of mature individuals of the species (http://www.iucnredlist.org/). *Bombus bahiensis* is a social bee, with colonies potentially housing tens or hundreds of individuals (Laroca 1972, 1976;

Varela 1992b; Gonzalez *et al.* 2004; Goulson 2010; de Oliveira *et al.* 2015). Except for *B. bellicosus,* which suffered a steep abundance decline in the northern portion of its range (Martins & Melo 2010; Martins *et al.* 2013, 2015), the other Brazilian bumblebee species are rather abundant bees in nature (Santos *et al.* 2004; Araújo *et al.* 2006; Silva-Pereira & Santos 2006; Luz *et al.* 2010; Hakim & Laroca 2010; Martins *et al.* 2013). Thus, considering the increased collection effort in its range in recent years, and the expectation that workers should be relatively abundant nearby their nests, the fact that only a small number of B. *bahiensis* are still known suggests an elevated extinction risk.

Another important criterion to define a species conservation status is its habitat size and quality. According to the IUCN criteria, populations ranging in fragmented areas under 100 km^2 are considered endangered (http://www.iucnredlist.org/). The approximately 37.000 km^2 predicted by the ENM (considering only southern Bahia, where the species still exists) as the occurrence for *B. bahiensis* is of (area an area under heavy anthropic impact – its rainforests now cover only between 12% and 17% of the area, when it originally covered around 86% of the region (Ribeiro *et al.* 2009) and continues to be deforested. This means that the species actually occupies at most about 6,300 km^2 of forests, as isolated populations in small forest fragments.

Bombus brasiliensis may adapt to changing climatic conditions, because of its genetic diversity and ability to occupy a larger distribution area. Thus it should be of little concern. On the other hand, *B. bahiensis* given the low genetic diversity and small population size may not be able to adapt to climate changes in its range, especially if environmental changes occur at an accelerated rate (Frankham 2005; Araujo & Rahbek 2006). The species’ restrict geographic range is area high urbanization, habitat destruction and geographic constraints, which together with its putative low genetic diversity, puts it at risk in a critical extinction risk. These facts raise a big concern over the conservation status of *B. bahiensis,* and following the IUCN criteria, we propose that this species should be considered Critically Endangered (CR).

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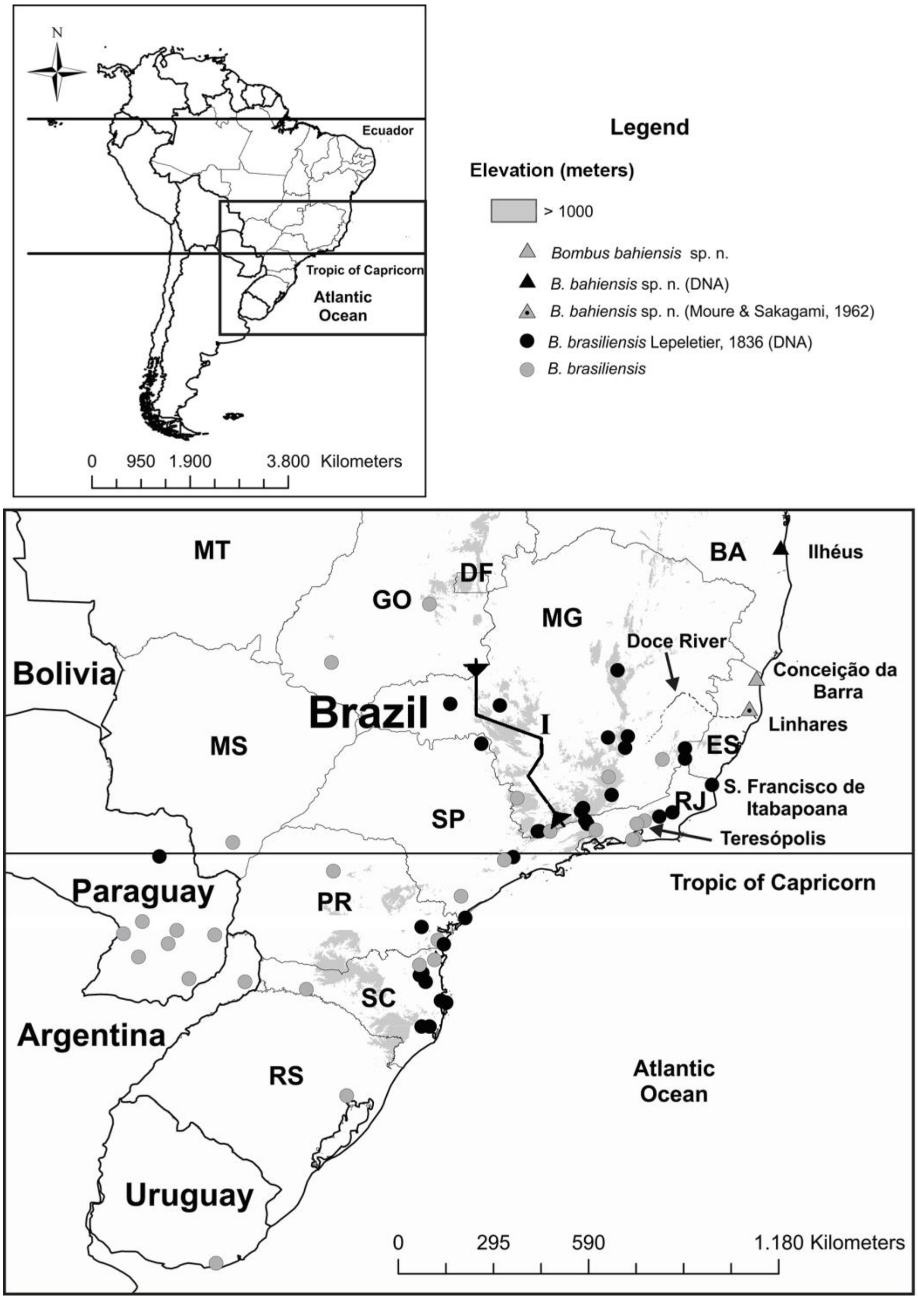
Figures



Figurte1. Map modified from Santos Júnior et al. (2015) depicting a putative barrier to gene flow suggested by the Monmier algorithm ("I"), Distribution records for *B. bahiensis* are represented by triangles (the black triangle, the gray triangle and the gray triangle with black point represents samples used in mtDNA analyzes, samples used in morphological analyzes and samples inferred to belong to the species based on descriptions in Moure & Sakagami (1962), respectively); records for *B. brasiliensis* are represented by circles (the black circle denote sites represented by mtDNA). Acronyms represent Brazilian states, as follows: BA = Bahia; ES = Espírito Santo; MG = Minas Gerais; RJ = Rio de Janeiro; SP = São Paulo; PR =

Paraná; SC = Santa Catarina; RS = Rio Grande do Sul; MT = Mato Grosso; GO = Goiás; MS = Mato Grosso do Sul; DF = Distrito Federal. Localities mentioned in the text are indicated. The stippled line indicated by black arrow represents the Doce river.

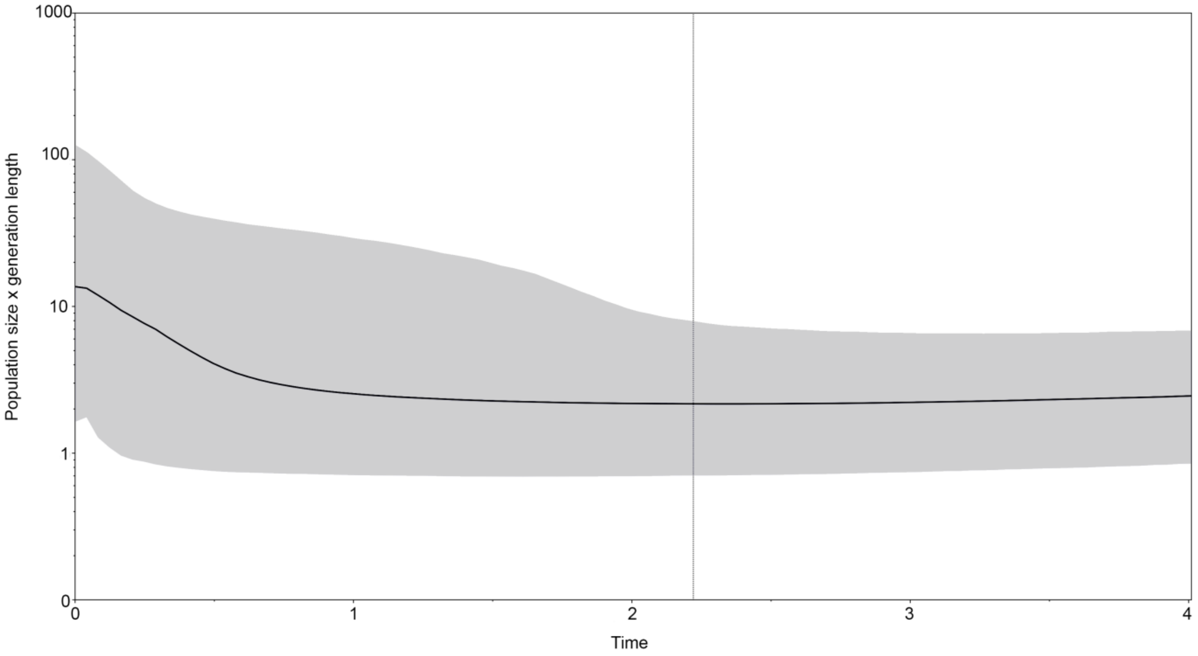


Figure 2. Bayesian Skyline Plot (BSP) depicting changes in effective population size of *B. brasiliensis* through time. The black horizontal line shows the median estimate of population size and the blue area shows the upper and lower 95% highest posterior density limits.

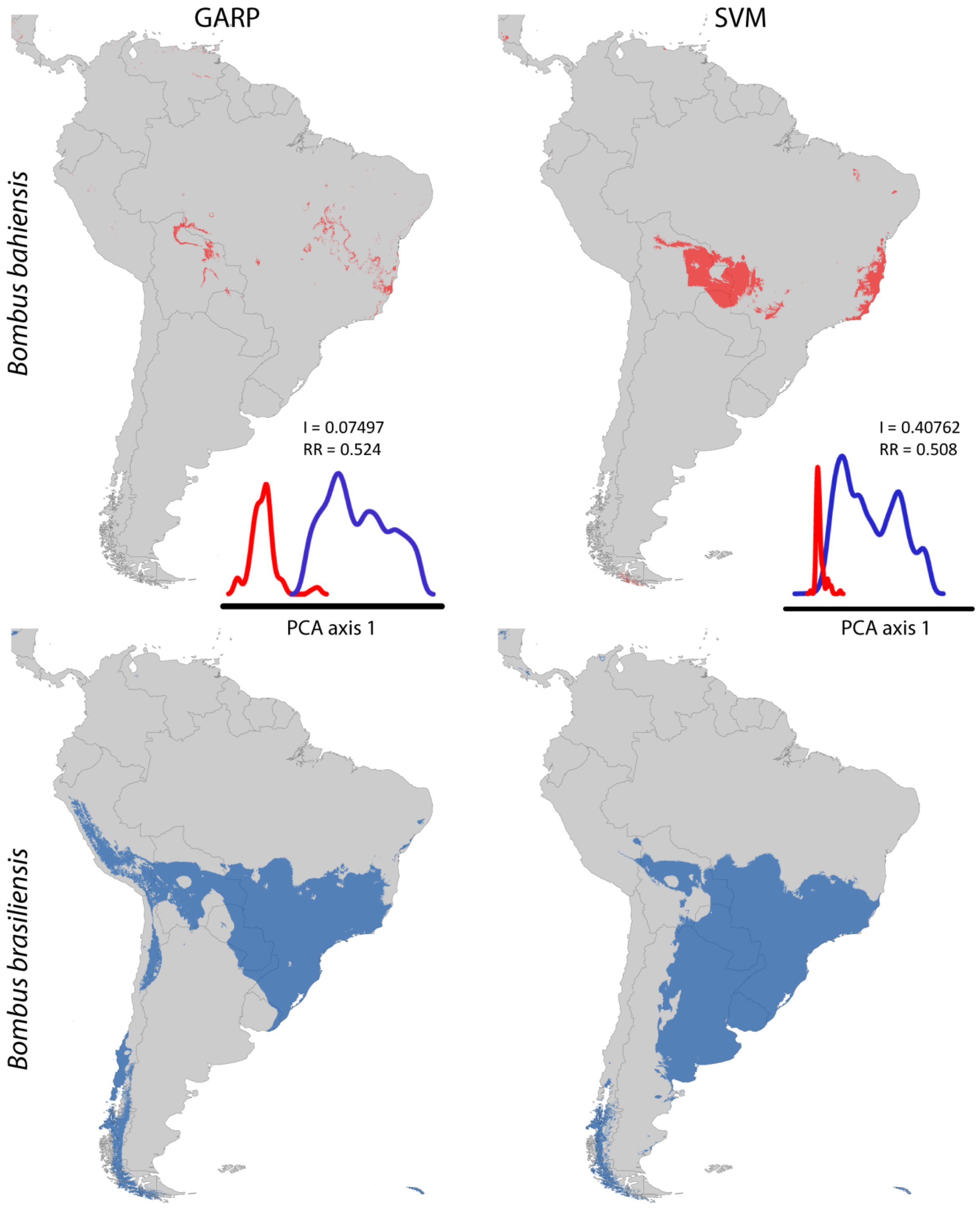
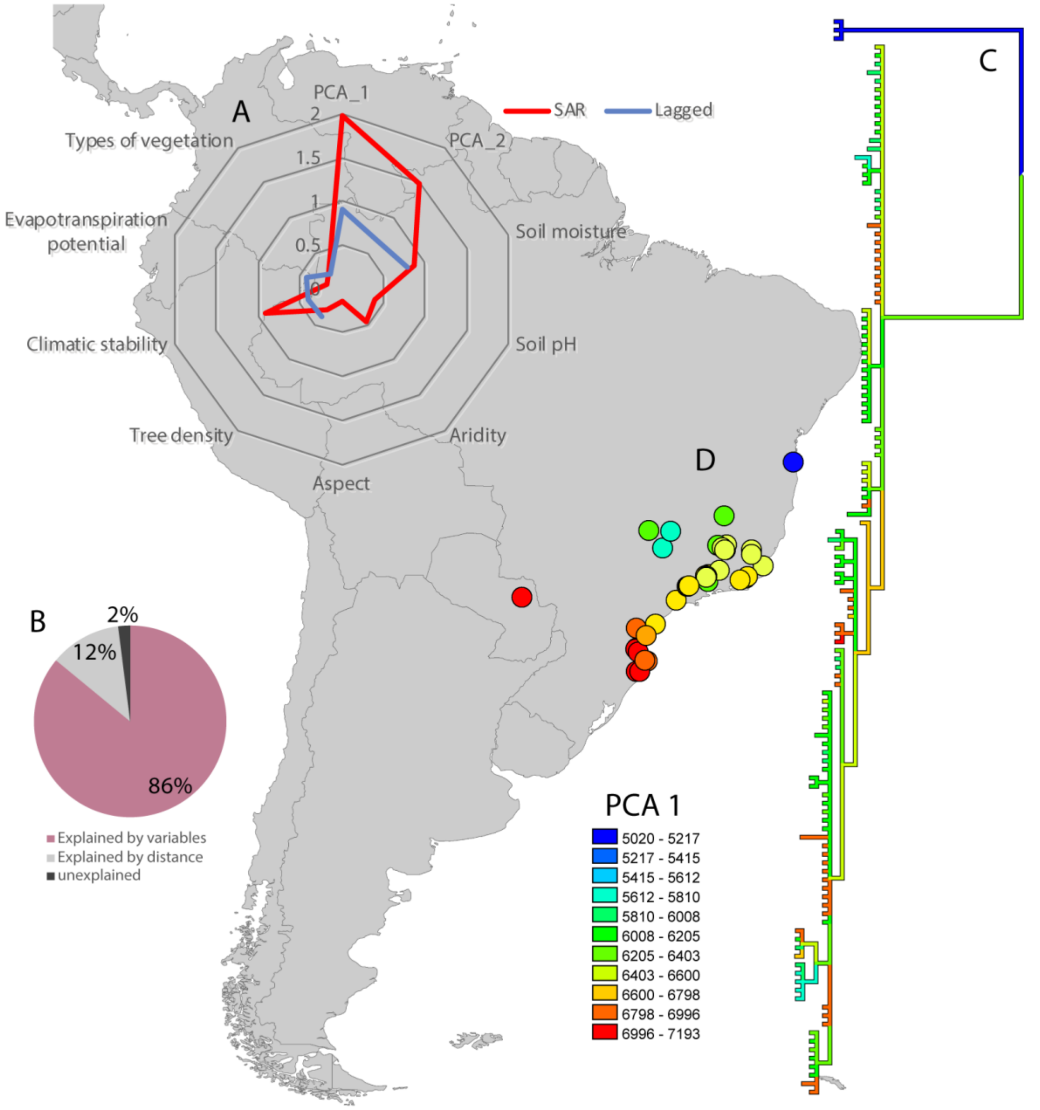


Figure 3. Best distribution models of *B. brasiliensis* (blue) and *B. bahiensis* (red) species. Graphs indicate the frequencies of environmental *conditions* represented by axis 1 of the PCA (estimated niche). Values indicate the two tests: I statistic (Warren *et al.* 2008) and relative rank – RR (Warren & Seifert 2011) for niche overlap.



Fufure 4. Niche evolution and explanatory factors of evolution of lineages. A: standardized coefficients for each variable in SAR model (red) and Lagged model (Blue). B: Pie chart indicates proportion of SAR model explanation by variables, distance and unexplained. C: Phylogenetic tree of populations of the *B. brasiliensis* and *B. bahiensis* species with the optimization by Brownian motion of the PCA axis 1, representing the niche of the species. D: Points of the populations represented in C, colors represent the values on axis 1 of the PCA.

Tables

Table 1. Nesting sites described for Brazilian species of *Bombus*. Nesting site preferences are shown between parentheses.

Species and nesting site	Description of nest site	Locality	Bibliography
B. bellicosus (Surface)	In a meadow located on a level curve. In this pasture area had been cultivated *Festuca* sp., *Trifolium repens* Linnaeus and *Lotus* sp.	La Estanzuela, Colonia, Uruguay	(Varela 1992a; b)
B. brasiliensis (Surface and semi-subterranean)	The border of a coffee plantation and within dense bunches of grass in a pasture area.	Pedregulho, São Paulo, Brazil.	(Mateus *et al*. 2006)
	Secondary forest next to the border.	Antonina, Paraná, Brazil.	(Laroca 1972)
	Cavity in an old wall next to the floor.	Brazil.	(Moure & Sakagami 1962)
B. brevivillus (Surface and subterranean)	Next to a banana commercial plantation.	Itatira, Ceará, Brazil.	(de Oliveira *et al*. 2015)
B. morio (Surface and subterranean)	Mammal burrow next to São Marcos River.	Catalão, Goiás, Brazil.	In this paper
	In the middle of the pasture in a grassland.	Serra do Salitre, Minas Gerais, Brazil.	In this paper
	Secondary vegetation, in the middle of a grassland near a road and in the middle of the secondary shrubby vegetation in a cavity in the ground.	Paranaguá, Paraná, Brazil.	(Laroca 1976)
B. pauloensis (Surface, arboreal and subterranean)	In a cane plantation near the forest in a cavity in the ground.	Derrubadas, Rio Grande do Sul, Brazil.	In this paper
	Pre-existing cavity in an area of Canga.	Nova Lima, Minas Gerais	In this paper
	In the forest.	Rio Claro, São Paulo, Brazil.	(Sakagami *et al.* 1967)
	Highly disturbed areas such as grazing pastures for cows and horses with scattered exotic trees such as *Eucalyptus* and *Pinus*.	Facatativa, Cundinamarca, Colombia; Loja, Ecuador.	(Gonzalez *et al*. 2004)
	Hidden under grassy vegetation on a well-drained slope.	Ribeirão Preto, São Paulo, Brazil.	(Cameron & Jost 1998)
	In boards in the garden of a house in urban area	La Plata, Argentina.	(Telleria 1998)
B. transversalis (Surface)	In the forest in a small cavity just below ground.	Rio Napo, Añangu, Ecuador.	(Olesen 1989)
	The forest floor at each nest site.	Amacayacu National Park, along the Amazon river, Colombia; Palmarí Reserve along the Javarí River, Brazil; Tiputini Biodiversity Station, Quito along the Tiputini River, Ecuador; Yasuní Scientific Research Station, near the Tiputini River, Orellana, Ecuador; Tambopata-Candamo Reserve in the state of Madre de Dios, Peru.	(Taylor & Cameron 2003)
	Along marked trails on the Tambopata Nature Reserve.	Malinowski, Tambopata, and Madre de Dios Rivers in southeastern Peru.	(Cameron *et al*. 1999)

Table 2. Number of collectors, hours, days and specimens collected in *B. bahiensis* distribution range. UESC – Universidade Estadual de Santa Cruz; BA – Bahia state; PARNA – National Park; FLONA – National Forest; ES – Espírito Santo state; REBIO – Biological Reserve; * – sampling before formal description of

species. After Santos Júnior *et al.* (2015) only one specimen of *B. bahiensis* was collected in Ilhéus, Cabruca/UESC, Bahia state from Brazil. All other specimens collected belongs to different species.

Locality	**Date**	**N. of collectors**	**N. of days**	**N. of hours**	**N. of specimens**	***B. bahiensis***	***B. morio***
Ilhéus, Cabruca/UESC, BA	May 2015	5	1	45	368	1	0
PARNA do Monte Pascoal, Porto Seguro, BA	December 2015	2	3	54	690	0	0
PARNA do Descobrimento, Prado, BA	December 2015	2	3	54	358	0	0
FLONA do Rio Preto, Conceição da Barra, ES	*July 2014	4	2	72	867	0	0
	May 2015	5	2	90	426	0	12
REBIO de Sooretama, Linhares, ES	May 2015	5	2	90	266	0	0
REBIO de Sooretama, Sooretama, ES	*July 2014	4	3	108	115	0	0
	May 2015	5	5	225	487	0	0
	December 2015	2	8	144	1150	0	0
Total before Santos junior et al. 2015			**5**	**180**	**982**	**0**	**0**
Total after Santos junior et al. 2015			**24**	**702**	**3745**	**1**	**12**

Supplementary

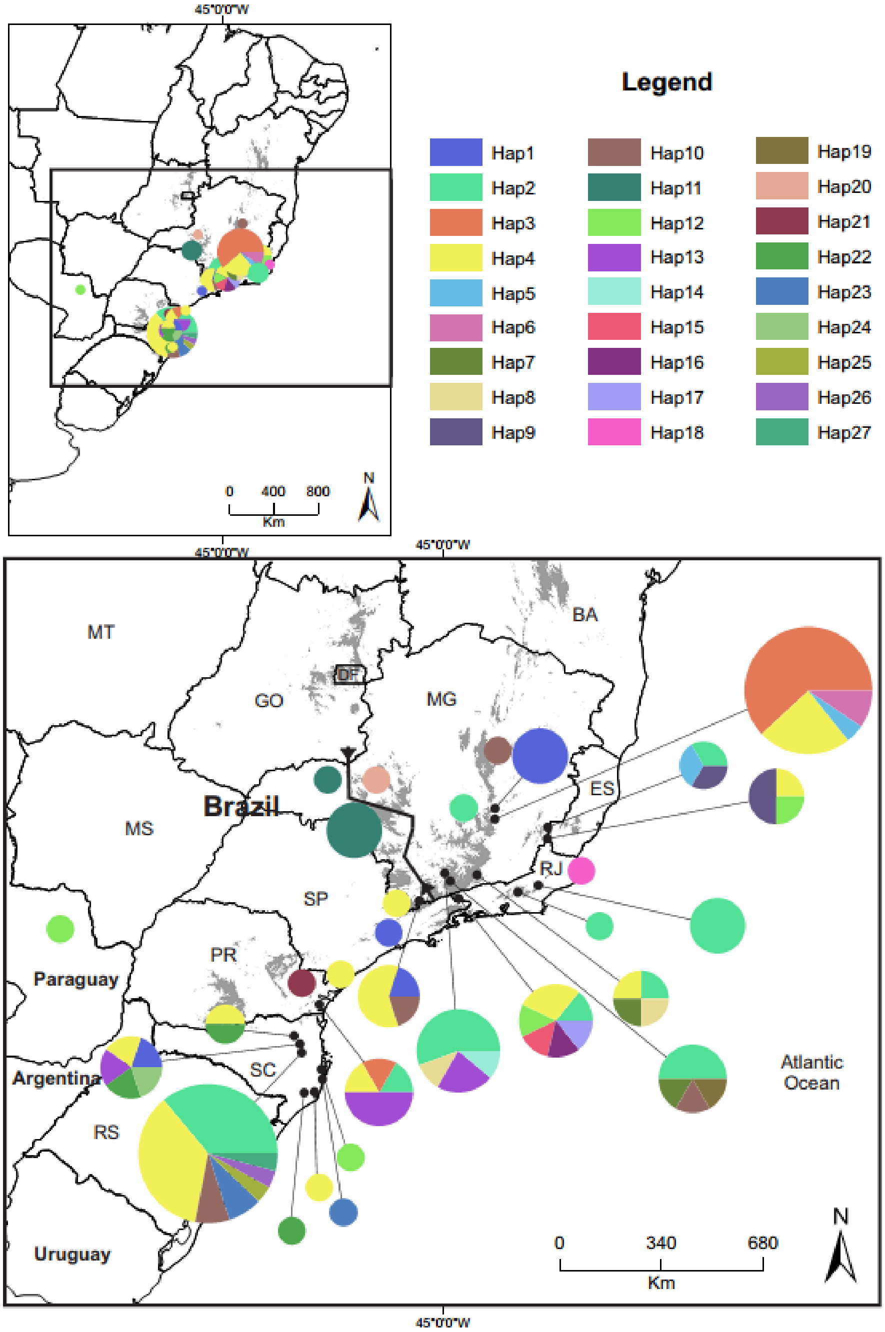


Figure S1: Distribution of *B. brasiliensis* haplotypes along sampled areas. Diameter of circles indicates the relative sample size in each area. For the relationship among these haplotypes see figure 2 in Santos Júnior *et al.* (2015). The gray area in of the map represents the areas above 1000 meters. The barrier labelled with "I" depicts a putative geographic barrier to gene flow in B. brasiliensis. Acronyms represent Brazilian states, as follows: BA = Bahia; ES = Espírito Santo; MG = Minas Gerais; RJ = Rio de Janeiro; SP = São Paulo; PR =

Paraná; SC = Santa Catarina; RS = Rio Grande do Sul; MT = Mato Grosso; GO = Goiás; MS = Mato Grosso do Sul; DF = Distrito Federal.

Appendix S1. The best model indicated by bayes factors statistics and AICM – Akaike's information criterion (AIC) through Markov chain Monte Carlo (MCMC).

Table 1. Statistics used bayes factors for bayesian model comparison. The run marked gray indicated the best model. S.E. standard errors; CLOCK – Strict clock; UCLN – Unncorrelated Lognormal Relaxed Clock model; and UCED – Uncorrelated Exponential Relaxed Clock.

Trace 1.5	ln P(model | data)	S.E.	UCLN	CLOCK	UCED
UCLN	-1666,045	+/- 0,207	-	1,8	-2,95
UCED	-1659,254	+/- 0,18	2,95	4,749	-
CLOCK	-1670,189	+/- 0,23	-1,8	-	-4,749

Table 2. Statistics used AICM – Akaike's information criterion (AIC) through Markov chain Monte Carlo (MCMC), for bayesian model comparison. The run marked gray indicated the best model. S.E. standard errors; CLOCK – Strict clock; UCLN – Unncorrelated Lognormal Relaxed Clock model; and UCED – Uncorrelated Exponential Relaxed Clock.

Trace 1.6	AICM	S.E.	CLOCK	UCED	UCLN
CLOCK	3.549.238	+/- 0.415	-	-111.713	-98.322
UCED	3.437.524	+/- 0.273	111.713	-	13.391
UCLN	3.450.915	+/- 0.124	98.322	-13.391	-

Appendix S2 Distribution data of bumblebees in Atlantic forest, highlighting for *B. brasiliensis* and *B. bahiensis*. A – altitude; NM – Number of months; N – Number of sampling hours; A – Abundance; R – Richness; Acronyms represent Brazilian states, as follows: BA = Bahia; MG = Minas Gerais; RJ = Rio de Janeiro; PB – Paraíba; PE – Pernambuco; PR = Paraná; RS = Rio Grande do Sul; SP = São Paulo; SC = Santa Catarina.

State	**City**	**Locality**	**Latitude**	**Longitude**	**Altitude**	**NM**	**N**	**A**	**R**	***B. brasiliensis***	***B. bahiensis***	**Others**	**Reference**
PR	Lapa	Reserva Passa Dois	25°45'S	49°47'30"W	910	13	216	2361	158	0	0	2	(Barbola & Laroca 1993)
PR	Ponta Grossa	Parque Estadual de Vila Velha	25°13’47,9"S 25°13’55,8"S	49°59’26,9"W 49°59’42,6"W		10		1552	181	0	0	2	(Gonçalves & Melo 2005)
PE	Chã-Grande	Fazenda Água Fria	8°11’19.0”S	35°28’13.6”W	600	12	288	1004	79	0	0	1	(Milet-Pinheiro & Schlindwein 2008)
MG	Paraopeba	Floresta Nacional de Paraopeba	19°20'S	44°20'W	740	13	233	1408	182	0	0	2	(Silveira & Campos 1995)
SP	Corumbataí	Reserva de Cerrado da FAPESP	22°15'S	47°00'W	800	36	872	696	124	0	0	2	
MG	Ponte Nova	Estação Experimental da EPAMIG	20°30'S 20°24'S	43°00'W 42°48'W	500 400	18	82	1132	150	0	0	2	(Silveira *et al.* 1993)
MG	Viçosa	Mata do Paraíso	20°48’07”S	42°51’31”W		7		712	98	0	0	2	(Cure *et al.* 1992).
PB	Mamanguape	Reserva Biológica de Guaribas	6°40'S 6°45’S	35°07'W 35°12’W		12	402	3022	114	0	0	1	(Aguiar & Martins 2003)

State	City	Locality	Latitude	Longitude	Altitude	NM	N	A	R	***B. brasiliensis***	***B. bahiensis***	Others	Reference
BA	Itatim	Morro do Agenor	12°42’S	39°46’W		15	180	1189	60	0	0	2	(Aguiar & Zanella 2005)
MG	Belo Horizonte	Estação Ecológica da UFMG	19°52’S	43°58’W		12		350	98	0	0	2	(Antonini & Martins 2003).
MG	Ouro Preto		20°23’S 20°24’S	43°30’W 43°31’W	1250	13	260	325	46	2	0	1	(Araújo *et al.* 2006)
MG	Ouro Branco	Serra de Ouro Branco	20°26’S 20°30’S	43°46’W 43°33’W	1573	10	160	352	66	0	0	1	
MG	Uberlândia	Reserva Ecológica do Panga	19°11'10"S	48°23'30"W 48°24'30"W	800	13		1226	128	0	0	1	(Carvalho & Bego 1996)
SC	Porto União		26°19′S	50°55′W	794	13		1339	130	0	0	2	(Krug & Alves-dos-Santos 2008)
SC	Indaial	PN Serra do Itajaí - Rancho do Mono	27°03'00"S	49°08'57W	700	11		1616	89	71	0	1	(Luz *et al.* 2010)
SC	Blumenau	PN Serra do Itajaí - Terceira Vargem	27°03'37"S	49°06'43"W	390	11							
RS	Viamão	Parque Estadual de Itapuã	30°20’W	51°05’W	5 - 263	12		3306	95	0	0		(Truylio & Harter-Marques 2007)
RJ	Campos dos Goytacazes	Campus da UENF	21°45’42,4”S	41°17’28,5”W		12	192	827	50	0	0	1	(Silva Neto 2008)

State	**City**	**Locality**	**Latitude**	**Longitude**	**Altitude**	**NM**	**N**	**A**	**R**	***B. brasiliensis***	***B. bahiensis***	**Others**	**Reference**
RJ	São João da Barra	Restinga Iquipari/Grussaí	21°44’S	41°02’W	5	12	288	748	37	0	0	0	Gaglianone MC (inf.pes.)
RJ	São Francisco do Itabapoana	Estação Ecológica Estadual de Guaxindiba (Floresta estacional semidecidual sobre tabuleiro)	21°24’45”	41°05’10”	40	12	96	436	58	0	0	1	Gaglianone MC (inf.pes.)
RJ	Trajano de Moraes	Mata da Cabecinha e arredores (floresta ombrófila densa montana)	22°05’S	42°05’W	750-1000	3	90	526	63	6	0	14	Gaglianone MC (inf.pes.)
RJ	São José de Ubá	Mata da Prosperidade e arredores (floresta estacional semidecidual)	21°24’S	42°02’W	350-500	7	72	133	27	0	0	1	Gaglianone MC (inf.pes.)
SP	Salesópolis	Estação Biológica da Boracéia	23°37'51"S	5°52'11"W				8101	259	103	0	2	(Wilms 1995)

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Appendix S3. Principal components (PC) calculated from climatic variables. The first spreadsheet corresponds to PCs and the fraction of variation explained by them. The second spreadsheet corresponds to variables' coefficients for each PC.

Simultaneous autoregressive model (SAR)

Results for Non-metric multidimensional scaling – NMDS 2 as a response variable, and 16 predictor variable(s). Number of samples is (n) 127, 124 *B. brasiliensis* and three *B. bahiensis;* F: 41.37 and P: 0. See Figures 4 and S1 in Santos Júnior *et al.* (2015) for the relationship between *B. brasiliensis* and *B. bahiensis* and of the data that were converted in a distance matrix by NMDS.

OLS Result: r: 0.955; r^2: 0.911; AICc: -164.54

Explained by Predictor Variables: r: 0.926; r^2: 0.857; AICc: -104.523

Total Explained (Predictor + Space): r: 0.989, r^2: 0.977, AICc: -337.597

Spatial autoregressive parameter (rho): 0.895

Connectivity Matrix: Minimum Spanning Tree

Table 1. Results of SAR model (Simultaneous autoregressive model). The red rows showed were not significant values.

Variable	OLS Coeff	SAR Coeff	Std Coeff	Std Error	t	P Value
Constant	-0.456	1.525	0	0.668	2.284	0.024
pcac1	-0.001	-0.002	-1.987	<.001-19.953	0	
pcac2	<.001	<.001	1.494	<.001	10.682	0
pcac3	<.001	<.001	0.124	<.001	1.811	0.073
soilmoistu	0.013	0.011	0.859	<.001	12.305	0
soilcarbon	-0.037	-0.004	-0.014	0.014	-0.261	0.795
soil_ph	0.305	0.419	0.391	0.076	5.48	<.001
aridity	-0.329	-0.412	-0.47	0.067	-6.191	<.001
asp	<.001	<.001	0.14	<.001	2.957	0.004
densitree2	<.001	<.001	0.296	<.001	4.964	<.001
estabilid	48.528	48.729	0.909	6.435	7.573	<.001
evergreen	-0.001	-0.001	-0.107	<.001	-1.457	0.148
herb	0.006	0.001	0.014	0.003	0.483	0.63
p_evapotra	0.004	-0.006	-0.165	0.002	-2.47	0.015
potveg	-0.019	-0.028	-0.24	0.006	-5.049	<.001
produt_pri	0.277	0.016	0.023	0.083	0.194	0.847
slope	0.009	<.001	0.008	0.004	0.148	0.883

Descriptive Statistics:

	NMDS_2	Estimated	Error
Min	-0.055	-0.464	-0.161
Max	2.278	2.234	0.409
Mean	<.001	-0.004	0.004
Std.Dev.	0.356	0.352	0.054
Skewness	6.349	6.112	3.304
Kurtosis	38.92	37.074	26.853
Moran's I	-0.02	0.017	0.403

Table 2. Results of analysis of Moran'I spatial autocorrelation for 12 distances classes in a SAR analysis. The results are presented in the figure below. The blue rows showed the categories of distance in which the spatial autocorrelation were not controlled by model.

D.Class	Count	DistCntr	Moran's I	P	I (max)	I/I(max)
1	1450	12.707	0.086	0.005	0.387	0.221
2	1238	71.128	-0.005	0.839	0.591	-0.009
3	1316	148.644	-0.031	0.286	0.598	-0.052
4	1546	216.609	-0.042	0.141	0.273	-0.155
5	1118	304.681	0.016	0.528	0.584	0.028
6	1334	431.389	-0.042	0.121	0.636	-0.065
7	1546	589.308	-0.049	0.095	0.654	-0.075
8	1154	691.606	0.048	0.121	0.32	0.15
9	1304	768.173	0.048	0.146	0.46	0.105
10	1388	872.689	-0.064	0.07	0.791	-0.081
11	1302	959.354	-0.035	0.241	0.491	-0.072
12	1306	1527.74	-0.006	0.749	1.265	-0.004

Expected: -0.008

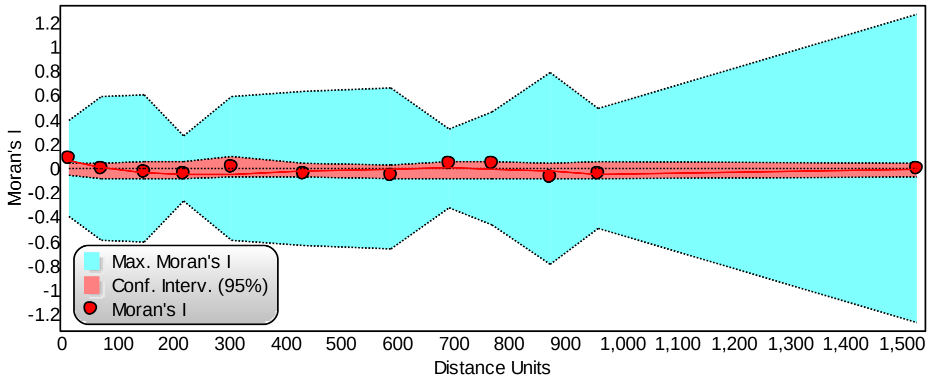

Figure 1. Relation between Moran's I and 12 distances classes in a SAR analysis presented in table 2.

Lagged Model

Results for NMDS_2 as a response variable, and 16 predictor variable(s).

Number of samples is (n) 127; r: 0.862; r^2: 0.742

Response Variable Spatial Autoregressive Coefficient (rho): 0.895

Standard error of rho: 3.344

Akaike's Information Criterion (AICc): 44.648

Table 3. Results of Lagged Model. The red rows showed were not significant values.

Variable	**Coefficient**	**Std Coeff.**	**Std Error**	**t**	**P Value**
Constant	2.737	0	4.108	0.666	0.507
pcac1	-0.003	-0.912	<.001	-5.478	<.001
pcac2	0.001	0.688	<.001	2.681	0.008
pcac3	<.001	-0.087	<.001	-0.746	0.457
soilmoistu	0.04	0.791	0.007	6.041	<.001
soilcarbon	-0.233	-0.229	0.119	-1.962	0.052
soil_ph	-0.345	-0.084	0.456	-0.757	0.451
aridity	-0.241	-0.072	0.359	-0.67	0.504
asp	0.002	0.153	0.001	1.624	0.107
densitree2	<.001	0.381	<.001	3.654	<.001
estabilid	79.795	0.39	34.363	2.322	0.022
evergreen	-0.017	-0.347	0.006	-2.871	0.005
herb	-0.136	-0.379	0.019	-7.141	<.001
p_evapotra	0.053	0.414	0.014	3.778	<.001
potveg	-0.093	-0.21	0.032	-2.892	0.005
produt_pri	0.524	0.193	0.525	0.998	0.321
slope	0.048	0.154	0.032	1.477	0.142

Descriptive Statistics:

	NMDS_2	**Estimated**	**Residuals**
Min	-0.055	-6.006	-3.886
Max	2.278	5.756	3.408
Mean	<.001	<.001	<.001
Std.Dev.	0.356	1.17	0.689
Skewness	6.349	1.533	0.285
Kurtosis	38.92	18.035	13.47
Moran's I	-0.02	0.758	-0.448

Table 4. Results of analysis of Moran'I spatial autocorrelation for 12 distances classes in a Lagged model analysis. The results are presented in the figure below. The blue rows showed the categories of distance in which the spatial autocorrelation were not controlled by model.

D.Class	Count	DistCntr	Moran's I	P	I (max)	I/I(max)
1	1450	12.707	0.037	0.286	0.303	0.121
2	1238	71.128	-0.028	0.317	0.407	-0.07
3	1316	148.644	-0.029	0.357	0.407	-0.071
4	1546	216.609	-0.012	0.578	0.316	-0.039
5	1118	304.681	-0.096	0.03	0.629	-0.152
6	1334	431.389	0.062	0.045	0.353	0.176
7	1546	589.308	-0.035	0.211	0.275	-0.127
8	1154	691.606	-0.053	0.111	0.702	-0.075
9	1304	768.173	0.088	0.03	0.695	0.127
10	1388	872.689	-0.063	0.09	0.542	-0.116
11	1302	959.354	0.007	0.724	0.283	0.026
12	1306	1527.74	0.011	0.593	0.379	0.028

Expected: -0.008

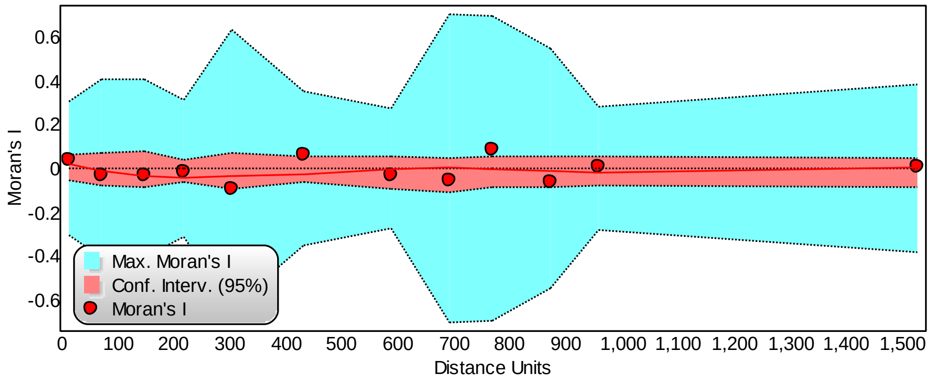

Figure 2. Relation between Moran's I and 12 distances classes in a Lagged model analysis presented in table 4.

Reference

Santos Júnior JE, Santos FR, Silveira FA (2015) Hitting an Unintended Target: Phylogeography of Bombus brasiliensis Lepeletier, 1836 and the First New Brazilian Bumblebee Species in a Century (Hymenoptera: Apidae) (S Brady, Ed,). *PLOS ONE*, **10**, e0125847.

Appendix 4 Detailed results of the SAR (Simultaneous autoregressive model) and Lagged model. Table with values of standardized coefficients and spatial autocorrelation values of the model errors.

PC	Layer	EigenValue Percent	Accumulative
1	8,74E+05	70,0581	70,0581
2	2,43E+05	19,4653	89,5234
3	1,02E+05	8,159	97,6824
4	1,69E+04	1,3545	99,0369
5	6,74E+03	0,5402	99,5771
6	2,69E+03	0,2159	99,793
7	1,69E+03	0,1353	99,9283
8	4,50E+02	0,0361	99,9644
9	1,71E+02	0,0137	99,9781
10	1,41E+02	0,0113	99,9894
11	6,00E+01	0,0048	99,9942
12	3,56E+01	0,0029	99,9971
13	1,69E+01	0,0014	99,9984
14	7,66E+00	0,0006	99,999
15	6,37E+00	0,0005	99,9995
16	4,27E+00	0,0003	99,9999
17	9,90E-01	0,0001	100
18	3,05E-01	0	100
19	1,09E-01	0	100
20	-3,12E-09	0	100

Eigenvalues and eigenvectors

PCA layer	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Bio1	-2,53E-02	2,84E-02	-3,39E-02	1,80E-02	6,64E-02	1,39E-01	3,36E-01	2,94E-02	5,24E-02	-2,52E-02	-1,13E-02	-1,34E-02	1,27E-01	-8,91E-02	3,62E-02	-5,30E-01	-1,09E-01	6,89E-01	-2,52E-01	1,56E-09
Bio2	6,89E-03	-8,98E-03	8,60E-04	1,92E-02	5,07E-02	3,40E-02	8,19E-02	-5,20E-02	-9,91E-02	4,79E-01	-4,43E-02	9,05E-02	-1,57E-02	5,46E-01	1,44E-01	-1,74E-01	-5,94E-01	-1,87E-01	-5,75E-03	-1,04E-09
Bio3	-6,26E-03	-3,07E-03	-7,07E-04	-4,42E-03	-4,77E-03	1,20E-02	3,08E-02	2,20E-02	7,71E-03	2,43E-02	-2,38E-02	3,56E-02	8,19E-02	7,25E-01	4,64E-02	-2,41E-02	6,59E-01	1,59E-01	3,69E-02	1,01E-09
Bio4	8,83E-01	3,14E-01	3,33E-01	6,52E-03	8,09E-02	8,61E-03	4,18E-02	1,50E-02	2,80E-02	-3,85E-02	7,02E-03	-5,54E-03	1,78E-03	1,27E-02	1,07E-03	-6,61E-03	-6,36E-03	3,13E-03	1,75E-02	1,98E-10
Bio5	-1,01E-02	3,15E-02	-2,94E-02	1,76E-02	1,06E-01	1,23E-01	3,70E-01	-3,06E-02	1,39E-02	2,85E-01	-1,14E-01	7,82E-02	3,17E-01	-9,31E-02	-5,10E-02	5,22E-01	2,05E-02	1,22E-01	-1,53E-02	5,77E-01
Bio6	-3,89E-02	3,27E-02	-3,62E-02	-1,36E-02	2,06E-02	1,01E-01	2,81E-01	8,89E-02	1,83E-01	-3,58E-01	-1,08E-02	1,34E-03	3,67E-01	1,87E-01	-2,46E-03	4,12E-01	-2,51E-01	3,39E-02	-1,43E-02	-5,77E-01
Bio7	2,88E-02	-1,19E-03	6,78E-03	3,12E-02	8,51E-02	2,27E-02	8,89E-02	-1,20E-01	-1,69E-01	6,43E-01	-1,04E-01	7,68E-02	-5,04E-02	-2,80E-01	-4,86E-02	1,10E-01	2,71E-01	8,85E-02	-1,04E-03	-5,77E-01
Bio8	-2,05E-02	2,95E-02	-3,23E-02	4,79E-02	7,46E-02	2,08E-01	4,34E-01	2,75E-03	-5,73E-01	-2,55E-01	-4,35E-03	-8,56E-02	-5,74E-01	7,39E-02	-7,84E-03	1,51E-01	-6,36E-03	-2,46E-03	3,51E-03	1,03E-10
Bio9	-3,09E-02	2,62E-02	-3,52E-02	-2,09E-02	5,12E-02	6,07E-02	2,36E-01	7,68E-02	7,51E-01	1,39E-01	1,02E-01	-4,18E-02	-5,68E-01	3,04E-02	-1,18E-02	9,21E-02	2,02E-02	-6,57E-03	1,91E-03	-2,52E-11
Bio10	-1,42E-02	3,33E-02	-2,95E-02	1,39E-02	6,72E-02	1,31E-01	3,36E-01	2,69E-02	5,25E-02	-2,87E-02	-2,08E-02	-4,65E-03	1,89E-01	-8,90E-02	4,25E-03	-2,82E-01	2,27E-01	-6,30E-01	-5,35E-01	-9,86E-09
Bio11	-3,68E-02	2,52E-02	-3,82E-02	1,50E-02	6,53E-02	1,34E-01	3,36E-01	2,77E-02	5,43E-02	-2,42E-02	-2,92E-02	-1,99E-03	1,76E-01	-1,15E-01	3,56E-03	-3,38E-01	7,75E-02	-2,09E-01	8,05E-01	6,62E-09
Bio12	-3,93E-01	3,39E-01	7,09E-01	1,76E-01	1,86E-02	-3,01E-01	1,39E-01	-2,82E-01	1,86E-02	-4,11E-02	6,34E-02	1,64E-02	1,13E-04	1,31E-02	-1,47E-02	-7,45E-03	-2,69E-03	3,33E-05	8,12E-04	2,54E-12
Bio13	-5,73E-02	3,35E-02	6,31E-02	2,04E-03	2,43E-01	5,73E-02	-7,69E-02	2,70E-01	-1,84E-02	-1,07E-01	2,29E-02	9,10E-01	-8,51E-02	-4,01E-02	-3,19E-02	-2,34E-02	6,98E-03	1,59E-04	-3,44E-03	-9,29E-11
Bio14	-1,02E-02	1,79E-02	4,76E-02	1,10E-02	-1,82E-01	-4,39E-02	3,97E-02	2,54E-01	-3,92E-02	8,17E-02	-8,31E-03	-4,46E-02	8,37E-03	1,10E-01	-9,29E-01	-7,56E-02	-6,81E-02	-7,86E-03	-6,78E-03	-1,84E-10
Bio15	-2,74E-04	-1,44E-02	-1,40E-02	-2,45E-03	9,10E-02	5,74E-02	2,33E-02	9,01E-02	-1,06E-01	1,23E-01	9,67E-01	-5,18E-02	1,11E-01	-6,54E-03	8,35E-03	4,16E-02	2,16E-02	8,40E-03	8,13E-03	1,71E-10
Bio16	-1,60E-01	9,70E-02	1,82E-01	1,63E-02	6,52E-01	1,40E-01	-2,35E-01	5,19E-01	-3,04E-02	6,00E-02	-1,43E-01	-3,69E-01	2,54E-02	3,66E-03	3,59E-02	1,35E-02	-9,90E-04	-8,58E-04	3,34E-05	2,24E-11
Bio17	-3,68E-02	6,09E-02	1,60E-01	2,95E-02	-5,73E-01	-1,45E-01	1,42E-01	6,78E-01	-7,91E-02	1,37E-01	-3,30E-02	-1,88E-02	-2,79E-03	-7,46E-02	3,24E-01	3,40E-02	2,50E-02	3,97E-03	9,93E-04	6,85E-11
Bio18	-5,28E-02	4,95E-02	1,60E-01	5,22E-01	-2,63E-01	7,44E-01	-2,52E-01	-7,35E-02	6,68E-02	1,68E-02	1,33E-03	1,12E-02	1,24E-02	-2,89E-03	-2,42E-03	1,84E-02	2,99E-03	-9,71E-04	-5,69E-04	-2,49E-12
Bio19	-1,15E-01	1,16E-01	2,58E-01	-8,31E-01	-1,58E-01	4,22E-01	-8,19E-02	-7,58E-02	-1,80E-02	3,55E-02	-3,49E-03	6,52E-03	-3,89E-03	-8,92E-03	-3,84E-03	-1,18E-03	6,20E-04	1,81E-04	-2,72E-04	-3,21E-12
Altitude	1,19E-01	-8,66E-01	4,70E-01	-3,79E-03	5,20E-02	2,05E-02	1,04E-01	8,76E-03	2,73E-02	-2,87E-02	-9,67E-03	-4,73E-03	3,74E-03	-6,06E-03	-2,53E-03	2,48E-03	-4,63E-04	2,49E-05	-1,43E-04	-6,10E-14

Capítulo 3 – A new perspective on historical biogeography, divergence times and diversification patterns of Neotropical bumblebees (Hymenoptera: Apidae)

A new perspective on historical biogeography, divergence times and diversification patterns of Neotropical bumblebees (Hymenoptera: Apidae)

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Abstract

Aim: This study tries to refine the knowledge about bumblebees by (1) comparing the diversification rates in different biogeographic regions and (2) investigating which are the main processes underlying their diversification in the Neotropical Region.

Location: Neotropical Region.

Methods: We construct a dated phylogeny to infer on the biogeography and time of diversification, estimate divergence times using fossil data derived from the literature and reconstruct the historical distribution of bumblebees in a temporal framework.

Results: These analyses reveal dispersal of *Bombus* concordant with geographic and climatic events of the late Cenozoic in the Neotropical region. The initial diversification of bumblebee lineages in South America was estimated at around 36.25 and 16.11 mya.

Main conclusions: The Old World *Bombus* ancestor occurred in the Holarctic (60.5 to 28.2 mya), with its early diversification events largely restricted to the eastern Old World. The ancestor of the Neotropical bumblebee occurred in the Nearctic region. Radiations were observed from the Neotropical region to South America between 36.25 and 16.11 mya.

Keywords

Bombus, BioGeoBears, BAMM, LTT

Introduction

Ecological services provided by pollinating insects have an immeasurable value, as they contribute to one-third of global crop production and improve quality, quantity and market value of fruits (Tepedino, 1979; Klatt et al., 2013). They also play a critical role in maintaining wild plant communities by ensuring seed production and avoiding self-pollination (Kearns & Inouye, 1997; Michener, 2007). This interaction has shaped the evolution of both angiosperms and their pollinators since the rise of the flowering plants in the early Cretaceous (Soltis et al., 2005). Bees are one of the major groups of angiosperm-pollinating insects (Michener, 2007) and though they are widely studied in both basic and applied researches, there is still no clear understanding of the phylogeny and evolutionary history of their sufamilial lineages (Danforth et al., 2013).

Corbiculate bees (Apidae, Apini sensu Roig-Alsina in Roig-Alsina & Michener, 1993(Roig-Alsina & Michener, 1993)) are characterized by presenting a spoon-shaped hind tibiae with reduced scopae forming pollen baskets known as corbiculae. Four monophyletic extant groups (subtribes Euglossina, Bombina, Meliponina, Apina sensu Silveira et al. (2002) and three extinct groups (subtribes Melikertina, Electrapina and Electrobombina) compose the corbiculate bees (Michener, 2007).

Among the extant corbiculate bees, the Bombina, consisting solely by *Bombus* Latreille, 1802, include 270 living species (http://www.nhm.ac.uk/), and is the only subtribe with a high extant diversity in temperate and cold environments (125 species in Palaearctic Region, 118 species in Oriental Region, 57 species in Nearctic Region, 20 species in the Arctic Region and 15 species in the Japanese Region). In the Neotropics the genus is represented by 32 species, 20 of wich endemic of that region (Williams, 1998) (http://www.nhm.ac.uk/). The only bumblebee species known to inhabit tropical lowlands are the eight species occurring in Brazil and nearby areas in neighbouring countries (Michener, 2007). *Bombus* species were distributed into four areas of the Neotropical Region: the Northern (nine species), Eastern (13 species), Southern (four species), and Western (20 species) (Williams, 1996; http://www.nhm.ac.uk/). Seven species of *Bombus* are reported in Brazil (*B. applanatus* Oliveira, Françoso & Arias, 2016; *B. bahiensis* Santos Júnior & Silveira, 2015, *B. bellicosus* Smith, 1879; *B. brasiliensis* Lepeletier, 1836*; B. brevivillus* Franklin, 1913; *B. pauloensis* Friese, 1913;

and *B. transversalis* (Olivier, 1789)). An eigth species, *B. rubriventris* Lepeletier, 1836, was reported as possibly occurring in the state of Goiás, Brazil. This species, which is known only through its female holotype, would be very rare or extinct (Milliron, 1973). According to Santos Júnior et al. (2015) it is possible that the assignment of *B. rubriventris* to Brazil resulted from labeling mistake or from wrong interpretation of the type locality (see Milliron, (1973) for details).

The earliest, and still doubtful, fossils attributed to *Bombus* are from the Oligocene (Goulson, 2010), and twelve fossil species of Bombina are known from the Oligocene through the Miocene (Wappler et al., 2012). According to Dehon et al. (2014).Among *B. cerdanyensis* and *B. randeckensis* are the most thoroughly examined species, with the latter being the only one with a reliable subgeneric classification, providing an accurate calibration point (Wappler et al., 2012; Dehon et al., 2014). Anyway, the addition of fossil information to phylogenetic and biogeographic analyses may improve our understanding of the relationships between extant taxa (Engel, 2001).

Divergence times estimates reported by the first bumblebee biogeographical study suggest that they arose in Asia, with an initial divergence of bumblebee lineages estimated to have occurred about 40–25 mya (Hines, 2008). This period matches a dramatic global cooling at the Eocene–Oligocene boundary which may have favoured the bumblebees due to their adaptation to cold climates. According to Hines (2008) bumblebees probably dispersed westwards from Asia through Europe in this time interval. Their initial dispersal towards North America (Palearctic-Nearctic) probably occurred about 20 mya, given that few taxa have dispersed in the opposite direction (Nearctic-Palearctic) about 4 mya. They eventually reached South America about 7.5

mya, with early regional diversification events taking place between 6 and 7.5 mya. However, most of the speciation events seem to have taken place 3.5 mya in the last (Hines, 2008).

Hines (2008) presented some lineage through-time (LTT) plots with an increased rate of diversification in recent times. This phenomenon observed in LTT plots can result from the fact that lineages arising in the recent past have more chances of being represented in the phylogeny than lineages arising in the more distant past. This is called "*pull of the present"* and is a property of the birth-death model (Nee et al., 1994; Jablonski et al., 2003; Nee, 2006; Etienne & Rosindell, 2012).

Here we used new analytical tools to improve our knowledge of the biogeographic history and diversification of neotropical bumblebees , and in the processes that resulted in their current distribution. For this, we employed a more comprehensive taxon sampling, and calibration points based on recently described corbiculate fossils species: *Eulaema zigrasi* Engel, 2014, *Oligobombus cuspidatus* Antropov 2014 and *B. randeckensis* Wappler & Engel, 2012. We also include the reinterpretation of the fossil *Paleoeuglossa melissiflora* Poinar, 1998, which belongs to the *Eufriesea* gourp according to Engel et al. (2012). This study tried to refine the knowledge about bumblebees following these main objectives: 1) to test the monophyly of the 15 subgenera of Bmonus; 2) to compare the diversification rates in different biogeographic regions; 3) to understand which are the main processes underlying bumblebee diversification in the Neotropical Region.

Material & methods

Taxon and character sampling

DNA samples of the nine Brazilian specimens of bumblebees were extracted from one hind leg of each specimen (dry pinned specimen) or thoracic musculature (fresh specimen) using the phenol chloroform method (Sambrook & Russel, 2001), Table S1**.** The extracted genomic DNA was re-suspended in 50 µL of TE buffer. Three mitochondrial genes, Cytochrome oxidase I (COI) Cytochrome B (CytB) and 16S, and four nuclear genes, arginine kinase (Argk), elongation factor 1-alpha gene (EF1α F2copy), long-wavelength rhodopsin gene (Opsin) and phosphoenolpyruvate carboxykinase (PEPCK) were sequenced using the primers listed in Table S2 . DNA amplification was performed in a total volume of 50 µL 0.3 unit/tube with Platinum® Taq polymerase, $MgCl_2$ 2 mM, PCR buffer 1X, 0.5µM of each primer, dNTPs 200 µM and approximately 20 ng of genomic DNA.

Amplification was carried out in a thermocycler using a program consisting of 5 min of denaturation at 94°C, followed by 37 30-second cycles at 94°C, 40s at 50-57°C (Table S3), 1.30 min at 72°C, and a final extension for 10 min at 72°C. PCR products were visualized in a 2% agarose gel. All PCRs that generated a single product were purified using polyethylene glycol 20% (PEG) precipitation (Santos Júnior et al., 2015), and further sequenced using BigDye Terminator v3.1 in both directions using an ABI 3130xl Genetic Analyzer sequencer with the same amplification primers following the manufacturer's recommendations (Thermo Fisher Scientific). The consensus sequences were obtained using SeqScape® Software v2.6. In addition to sequences generated here, we included sequences available in the Genbank and BOLD Systems v3 255 bumblebee species, three Apina species, six Euglossina species and six Meliponina species (Table S1). Alignments of consensus sequences for all individuals were generated using MAFFT v7.017 (Kazutaka et al., 2002) implemented in the program Geneious 8.1.8 (Kearse et al., 2012). The introns of nuclear genes Argk, EF1α, Opsin and PEPCK were identified and separated from exons using Mega 7.0.14 (Kumar et al., 2016). Regions of ambiguous alignment of genes 12S and 16S were removed using GBlocks 0.91b (Castresana, 2000; Talavera & Castresana, 2007). We used the default setting, except gap option that was set to'with half'. After that, the "12 genes" alignments were concatenated using the software SequenceMatrix v1.8 (Vaidya et al., 2011). We explored best partitioning schemes and substitution models simultaneously using PartitionFinder v.1.1.1 (Lanfear et al., 2012), under a Bayesian Information Criterion for the entire matrix.

Phylogenetic inference

Here we followed the sytem of subgenera proposed by Williams et al. (Williams et al., 2008) to test the monophyly of the 15 subgenera and to use in our following phylogenetic and divergence times estimation analyses.

We used TNT (Goloboff et al., 2008) for maximum parsimony (MP) analyses. The inference was performed using a heuristic search, with 1000 replications (saving at most 100 trees in each replication) of random (stepwise) addition of taxa followed by Tree Bisection Reconnection – TBR branch swapping. Two additional runs using trees in memory were performed to refine searches. For the support of branches we made 10,000 replicates of Standard Bootstrap.

For the maximum likelihood analysis (ML), we used RAxML version 8 (Stamatakis, 2014). The model GTR+G was used for all 16 partition obtained with PartitionFinder v.1.1.1 (Lanfear et al., 2012) and 1,000 bootstrap replicates search were used to calculate branch support (ML optimizations were performed every five bootstrap replicates and were followed by a final search for the best-scoring ML tree once bootstrap search over). The RaxML also was used on the full-length concatenated alignment data (–J MR_DROP command) in order to identify rogue taxa (Pattengale et al., 2011).

Bayesian inference (BI) was done using MRBAYES v 3.2.2 (Huelsenbeck & Ronquist, 2001). Six independent analyses were made all using the PartitionFinder results:

1) with 150 million generations, two runs, four chains and temp set to 0.05;
2) same as 1) but on 250 million generations;
3) same as 1) but with 300 million generations;
4) with 300 million generations, two runs, eight chains and temp set to 0.05;

For all four analyses, each pair of run and chain we used the Markov chain Monte Carlo – MCMC method. The convergence of the runs was assessed using the following statistics: standard deviation of split frequencies, potential scale reduction factor (PSRF) and estimated sample size (ESS) for each parameter. According to the manual of the program values between 0.01 and 0.05 may be adequate depending on the purpose of the analysis.

Divergence times estimation

Divergence time analyses based on our "full-length concatenated alignment data", including partitioning scheme and models used in our previous analyses, were performed using BEAST v.2.4.4 (Bouckaert et al., 2014) implemented on the workstations and the Linux cluster of the Sagarana – high-performance computer, at the bioinformatics department of the Universidade Federal de Minas Gerais (UFMG). In order to allow for clock rate variation among branches, an Unncorrelated Lognormal Relaxed Clock model (UCLN) was used. In order to apply fossil ages as node calibration points and to optimize the search for optimal ages by starting at high likelihood in topology space, we constrained the monophyly of *Bombus* subgenera according to our BI topology (see Appendix 1 for priors, Fig. S1.2 and Fig.S2.2 for BI and BEAST analyses, respectively). Analyses were carried for 500 million generations with samples every 1000 generations. Convergence of Markov Chains was assessed in Tracer 1.5 or Tracer 1.6 (Rambaut & Drummond, 2009; Rambaut et al., 2013) by inspecting the trace and ESS of the parameters. Different burn-in (20 – 90 %) were applied after checking the log-likelihood curves and a maximum credibility tree with median ages and their 95% highest posterior density (HPD) were subsequently generated using TreeAnnotator 2.4.3 (Bouckaert et al., 2014).

Biogeographic Analyses

In order to infer the biogeographic history of bumblebees we used the R package BioGeoBEARS – BioGeography with Bayesian and likelihood Evolutionary Analysis in R Scripts (Matzke, 2013). The maximum clade credibility tree obtained from divergence time analyses was used to map the ancestral areas probability (see Table S5). Based on this tree and the areas defined in Fig. 1, we carried out unconstrained analyses employing the following models: DEC (Ree & Smith, 2008), DIVA (Ronquist, 1997), BayArea (Landis et al., 2013) and a “+j” (a free parameter accounting for the possibility of a founder-event speciation) version of each of them. All six models were compared and the best fit model was chosen using the corrected Akaike information criterion (AICc) (Landis et al., 2013; Matzke, 2014).

Diversification Patterns

In order to understand diversification of bumblebee lineages through time and the variation in rates of speciation and extinction we used Bayesian Analysis of Macroevolutionary Mixtures (BAMM).This software employs reversible jump MCMC to explore the space of lineage diversification models, allowing us to test hypothesis about the number of process of diversification responsible for a given phylogenetic pattern (Rabosky et al., 2014). It is a flexible Bayesian framework for inferring the number and location of shifts in macroevolutionary rate across a dated phylogenetic tree. We used the maximum credibility tree as the input phylogeny (Fig. S2.2). Rates were inferred across the phylogeny and variation in rates of speciation and extinction of bumblebee species through time plotted in graphs. The analyses were made with 10 million generations, with a burn-in fraction of 0.20. Priors were set by BAMMtools package in R and all output from BAMM was also processed BAMMtools (Rabosky et al., 2014).

Results

Full-length concatenated alignment data

Our full-length concatenated alignment data had a total length of 6,188 bp (412 – 4,935 bp), which was used for phylogenetic inferences (see Table S4 for specimens used and amount of missing data). Best partitioning schemes and substitution models are presented in table 1.

Missing data and the choice for Bayesian Inference methods

Although we used MP, ML and BI analysis, we chose to focus our discussion on BI results because many studies demonstraded that ML and BI are less sensitive to the missing data, whereas MP has been shown to present decreased accuracy (Dunn et al., 2003; Simmons, 2012). Additionally, Bayesian Inference methods (BI) outperforms the other two inference methods cited above (Wiens & Morrill, 2011; Guillerme & Cooper, 2016). The BI can accurately reconstruct the position of taxa with until 95 % of missing data provided that the overall number of characters in the analysis is large (Wiens & Moen, 2008; Wiens & Morrill, 2011).

Phylogenetic inference

The best score of MP was 13,176 (total rearrangements examined was 21,224,652,872, see Fig. S3.2). The subgenera *Alpigenobombus, Melanobombus, Sibiricobombus* and *Cullumanobombus* formed a polyphyletic group, where *B. festivus* and *B. eximius* (*Melanobombus*) grouped with *Sibiricobombus* making *Melanobombus* paraphyletic. The ML tree recovered *B. rufocinctus* as part of a polytomy leaving the subgenera

Cullumanobombus paraphyletic. The polytomy was composed of *Cullumanobombus* + *Sibiricobombus* + *B. (Cullumanobombus) rufocinctus* Fig. S4.2. All subgenera were monophyletic in BI analyses, although some of them were supported by low posterior probability values (pp). The relationships among species within some subgenera were not well resolved or were weaklly supported, Fig. S1.2.

As the focus of this paper is on Neotropical species, a summary of the results for the four subgenera occurring in that region is presented here. *Psithyrus* (one species in the northern Neotropical borders), *Thoracobombus* (17 species in the Neotropics) and *Cullumanobombus* (13 species) were all well supported subgenera, but the relationships among their species not fully resolved. *Pyrobombus* (one species in the Neotropics) was found as a highly supported clade (pp = 1) and the relationships among the species of this subgenera were resolved, with few presenting low posterior probability values.

Divergence time estimation

Dates for diversification of the main *Bombus* lineages are summarized (Table 2). Neotropical species from mainly Nearctic subgenera, *B. (Psithyrus) variabilis* and *B. (Pyrobombus) ephippiatus*, have diverged at the same period, between 1.02 and 4.7 million years ago (mya). The subgenus *Cullumanobombus* has 13 Neotropical species, among which, *B. baeri*, *B. coccineus, B. handlirschi* and *B. rubicundus* form a monophyletic group highly supported in BI analyses, which diverged during the Miocene (between 9.6 to 19.21 mya, Fig. S1.2). In the BI analysis *B. (Cullumanobombus) brachycephalus* is the sister group to the former clade, although weakly supported (pp <95). However, in the BEAST analysis it groups with *B. brachycephalus,* a non-Neotropical species in *B. (Cullumanobombus)* (Fig. S2.2). The clade formed by the species *B. ecuadorius, B. hortulanus, B. robustus, B. vogti, B.tucumanus, B. melaleucus* and *B. volucelloides* was well-supported clade both in BI and BEAST analyses and had its divergence estimated to have occurred between 3.31 to 8.52 Ma. The subgenus *Thoracobombus* has 17 Neotropical species, which were not a single clade nor in the BEAST and neither in the BI analyses. Neotropical species were scattered into two larger clades: 1) LF1 – long-faced clade 1 (including *B. morio, B excellens* and *B. dahlbomii,* with divergence estimated to have occurred between 11.78 to 24.95 Ma). 2) LF2 – long-faced clade 2 (incluinding *B. applanatus, B. brevivillus, B. bahiensis, B. brasiliensis, B. pauloensis, B. pullatus* and *B. transversalis,* which forms a monophyletic group in BI - 7.98 to 14.2 Ma). *Bombus bellicosus* and *B. opifex* (4.35 to 12.02 Ma) also formed a monophyletic Neotropical LF2 subgroup. Other Neotropical species grouped with non-Neotropical species (see Fig. S1.2). The diversification of most Neotropical bumblebee species occurred during the Middle Miocene and the Pliocene (See Fig. S2.2). *Bombus applanatus* and *B. brevivillus* are the only pair of sister species diverging within the Plio-Pleistocene period (between 1.51 to 4.2 Ma).

Biogeographic Analyses

The best-fitting model for BioGeoBEARS analysis was BAYAREALIKE+J (Table 3 and Fig. 1 for area codes), wich suggests dispersion and extinction to be important factors on the biogeographic history of bumblebees. According to this model the most likely biogeographic distribution of the most recent common ancestor (MRCA) of all bumblebees was in the Holarctic region. The most likely biogeographic distributions estimated for the MRCAs of the different clades containing Neotropical bumblebees were: 1) Nearctic Region (*B. (Psithyrus) variabilis, B. (Pyrobombus) ephippiatus*); 2) Nearctic Region and north-western regions of the Andes area (LF2); 3) north-western region of the Andes and the Chaco areas (*B. morio, B. excelles* and *B. dahbomii* in LF1 group); north-western regions of the Andes-Amazonian areas and Nearctic region (*Cullumanobombus*). The most probable ancestral distributions of the Brazilian species were: 1) north-western regions of the Andes and the Chaco areas (*B. bellicossus* and *B. morio*); and 2) Amazonian area (*B. transversalis, B. pauloensis, B. brasiliensis, B. bahiensis, B brevivillus* and *B. appanatus*, Fig. 2 and Fig. S5.2).

Five independent events of dispersion from Nearctic to Neotropical region may have occurred: one in *Cullumanobombus*, one in *Pyrobombus*, one in *Psithyrus* and two in *Thoracobombus*. No dispersion event on the opposite direction (from Neotropics to Nearctics) has been detected for this groups and extinction events are the sole factor accounting for exclusively nearctic species in those clades.

Diversification Patterns

Despite sampling models with up to eight different evolutionary regimes, schemes with zero-shift models were consistently selected in the BAMM analysis – for example a single, constantly varying net diversification rate throughout the history of the bumblebee species (genus *Bombus* – pp = 0.83, Fig. S6.2, Fig. S7.2 and Fig. S8.2). BAMM analysis results showed an overall decreasing pattern (Fig. 3) of marginal Bayesian posterior densities of macroevolutionary rates of diversification.

Discussion

Full-length concatenated alignment data, effects of the missing data in BIM analysis and Phylogenetic inference

In addition to phylogenetic questions within the bumblebee species, we also tested for the presence of rogue taxa. Although, two terminals have been shown to behave as rogue taxa (*B. (Psithyrus) coreanus* and *B. (Thoracobombus) anachoreta*) we decided to keep them in our analyses, since their presence did not affect the relationships among the subgenra and other taxa of interest. Some papers evaluating the analytical problems concerning the topologies and measures of branch support have showed that missing data can not be strictly correlated with the degree of ambiguity in the results (Kearney, 2002; Fulton & Strobeck, 2006). According to Kearney (Kearney, 2002; Wiens, 2003, 2006; Wiens & Morrill, 2011), it is entirely possible to find a suitable result despite an extensive amount of missing data, depending on the distribution of congruent characters and homoplasy in a specific matrix. Moreover, many studies demonstrated that adding incomplete taxa do not necessarily increases overall ambiguity (Crepet & Nixon, 1989a, 1989b; Norell & De Queiroz, 1991; Novacek, 1992; Grande & Bemis, 1998; Fulton & Strobeck, 2006; Guillerme & Cooper, 2016; Streicher et al., 2016).

Our results suggested that a monophyletic clade comprised of *Mendacibombus* + *Bombias* is a sister group of the other *Bombus* subgenera (pp = 1), differing from previous studies in which *Mendacibombus* was a sister group of *Bombus* (Cameron et al., 2007; Hines, 2008). *Kallobombus* was found to be the sister group of the LF and SF, in congruence with other studies (Cameron et al., 2007; Hines, 2008). The relationships among the subgerera comprising SF were significantly different from previous works and are summarized as follows. *Alpinobombus, Bombus* st.r. and *Pyrobombus* subgenera formed a monophyletic group (Fig. S3.2 and Fig. S4.2) corroborating with previous analyses (Cameron et al., 2007; Hines, 2008). *Melanobombus* subgenus was sister group of *Alpigenobombus, Sibiricobombus* and *Cullumanobombus,* and the relationships among these subgenera were all well resolved (pp > 0.95), with the exception of *Cullumanobombus*. In previous studies these groups constituted a polytomy (Cameron et al., 2007). The relationships among LF bumblebees were similar to those found in previous works (Cameron et al., 2007; Hines, 2008).

Divergence time estimation and biogeographic analyses

The divergence time for the *Bombus* root estimated by Hines (2008) with missing data was similar to ours (60.5 to 28.2mya, see Table 2). Studies have shown that during the last 65 millions years the Earth's climate has experienced continuous changes (from extreme warmth until extreme cold throughout the world and occurring in different ways over distinctive latitudinal distributions Zachos et al., 2001).

Differently from Hines (2008), who has reported a single event of dispersion from South to North America, we detected none of such events. We propose, based on our biogeographic analyses, that the restricted range observed in Nearctic species (from *Cullumanobombus* and *Thoracobombus*) results from range contraction during the evolution of those taxa (Fig. 4 and Fig. 5). In other words, they result from extinction of Neotropical ancestor populations.

Results from BAYAREALIKE+J and BEAST place the first South American bumblebees ancestral nodes between 36.25 and 16.11 mya (HPD 95% between groups 1 and 3 see Table 2; Fig. S5.2). Early South American diversification events were estimated between 24.95 and 8.6 mya (HPD 95% between *B. rubicundus* and *B. dahlbomii* see Table 2) and most remaining speciation events appear to have taken place after 7.39 mya.These resuts are supported by evidences suggesting that the Panama Isthmus was at least partialy uplifted between 13 and 15 mya (Montes et al., 2015). Other studies also indicated initial dispersal pulses of the Great American Biotic Interchange (GABI) since the Oligocene-Miocene boundary (Bacon et al., 2015; ODea et al., 2016). However, the evidence for older Isthmus uplift and GABI pulses must be taken with caution (ODea et al., 2016). Despite that, bumblebees are known to have great dispersal abilities being capable of mantaining gene flow over several kilometers (Lepais et al., 2010; Dreier et al., 2014; Santos Júnior et al., 2015; Francisco et al., 2016; Duennes et al., 2016). Thus, even if landmasses connecting North and South America were not thoroughly present at the time of the early bumblebee divergences, some species could have crossed the gap between continents and colonized South America.

Of the three waves of dispersal towards South America suggested by Hines (2008), only one was recovered here between 15 and 7 mya (groups 2, 4 and 5 see Table 2; Fig. S5.2). *Bombus (Cullumanobombus) funebris* may represents on other wave (< 7 mya) of dispersal proposed by the author, but its interval of diversification (between 14.78 and 6.16 mya see Table 2; Fig. S5.2) is closer to the one cited above.

Two species of the *Cullumanobombus* subgenus, *B. handlirschi* and *B. rubicundus* (HPD 95% between 19.21 and 7.21 see Table 2), based on our biogeographic analyses have its ancestral range between north and western regions of the Andes and Amazonian areas (see map Fig. 1 and Fig. 5). The *Thoracobombus* subgenus, *B. excellens, B. morio, B. dahlbomii, B. opifex* (likely ancestral area North and western regions of the Andes and Chaco) and *B. transversalis* (likely ancestral range Nearctic region and North and western regions of the Andes area), Table 2.The diversification process of these species may be releted to climatic changes that occurred during Middle Miocene, mainly its climatic optimum, between 17 and 15 mya and/or geologic changes, as reconfiguration of drainage patterns during the onset Amazon river (Hoorn et al., 2010). After that, global temperature started to decrease until the reestablishment the Antarctic ice sheet around 10 mya (Zachos et al., 2001). The speciation events may have been influenced by the uplift of the Eastern Cordilleras of the Central and Northern Andes between 13 and 11 mya (Taylor, 1991; Hoorn et al., 1995; Hungerbühler et al., 2002). The process of Plio-Plestocene glaciations also played a role in the divergences within the groups 1 and 2 of the *Cullumanobombus* and group 4 of the *Thoracobombus* subgenera between 5.99 and 0.22 mya, table 2 (Hoorn et al., 1995; Hewitt, 2000; Carnaval & Moritz, 2008; Carnaval et al., 2009, 2014; Françoso et al., 2016).

Sometimes the difficulty to infer the processes and time intervals in which the speciation events occurred forces researchers to use differents methods for finding the events. For example, *B. brasiliensis* and *B. bahiensis* are sister species identified with the use of different approaches including BI, niche modelling and biogeographic analyses, all of which suggested speciation by niche divergence (Santos Júnior et al.

unplublished data). The concordance with geographic and climatic events is very important and may be used to assess hypotheses: i) as in the case of *B. pauloensis* and *B. pullatus* (7.17 to 2.63 mya), which likely became separated during de last period of intense mountain building in the Northern Andes (Lundberg et al., 1998); and ii) in the case of the sister species, *B. (Thoracobombus) applanatus* and *B. (Thoracobombus) brevivillus* (4.2 to 1.51 mya), which likely became separated during the climatic fluctuations of the Plio-Pleistocene (Werneck et al., 2012b, 2012a).

Diversification Patterns

The previous study by Hines (2008) presented some LTT plots with an increased rate of diversification in recent times. We found contrasting results when compared to the diversification history found by Hines (2008), who observed a lineage accumulation between 34 and 24 Ma, followed by a constant rate of diversification towards the present. Our results showed a downcurving plot suggesting a decline on the rate of diversification towards the present (Fig. 3). The previous results found by Hines (2008), were likely caused by a phenomenon called the "*pull of the present"* (Nee et al., 1994). This bias can be avoided by adding a more complete sampling of recent species (Raup et al., 1973; Jablonski et al., 2003; Nee, 2006).

Other two explanations for the LTT plots observed by Hines (2008), besides the sampling artifact (Nee et al., 1994), can be: 1) slowdown observed if there is age dependency in whether nodes are deemed to be speciation events (Purvis et al., 2009) and 2) diversity dependence (species speciation/extinction rates are not constant (Phillimore & Price, 2008; Ricklefs, 2010)). Our results are supported by the observation of the diversification rates (Fig. 3) and can be explained by the assumption that speciation takes time (Etienne & Rosindell, 2012; Etienne et al., 2014; Rosindell et al., 2015). But we do not exclude the possibility of diversity-dependent diversification (see Ricklefs, 2010; Rosindell et al., 2015). Here we only regarded speciation as a more parsimonious alternative than that proposed by Hines (2008). According to Etienne and Rosindell (Etienne & Rosindell, 2012) this is an explanation that cannot be ignored in future models of diversification.

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Figures

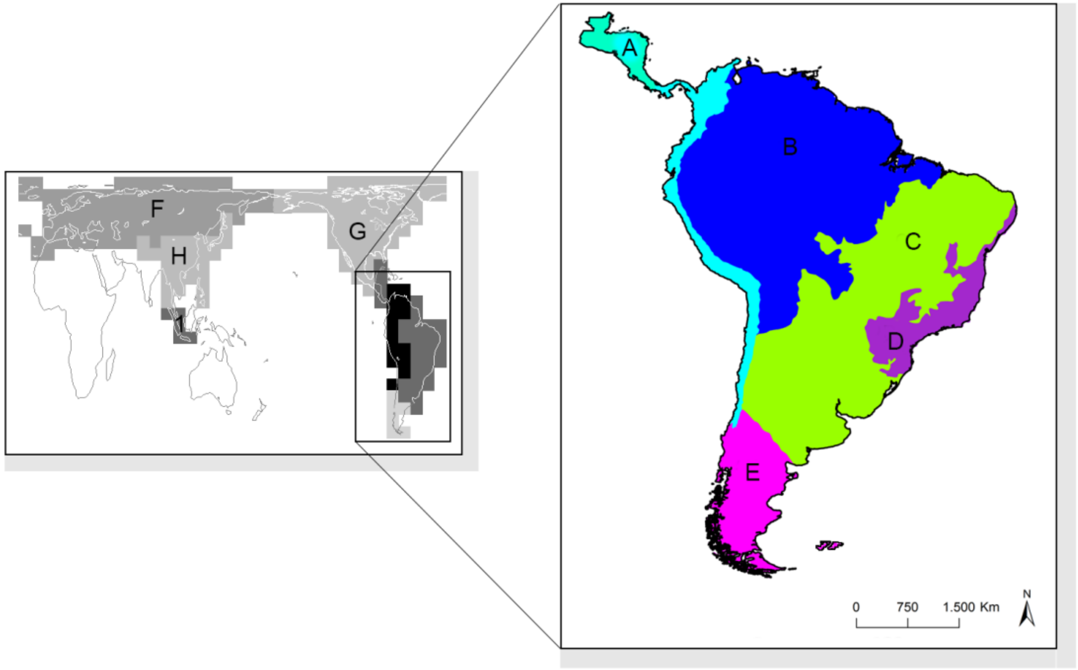

Fig. 1. Map of world bumblebee species by region. Biogeographic regions of the world proposed by Willians *et al.* (Williams, 1996) were modified. The Sumatran Region was not represented by any species on the analyses (Represented by number “1” in the map – *B. senex* Vollenhoven, 1873, *B. melanopoda* Cockerell, 1910 and *B. rufipes*). Neotropical Region modified from Morrone (Morrone, 2013) with the biogeographic areas analyzed by BioGeoBEARS. A – North and western regions of the Andes; B – Amazonian; C – Chaco; D – Parana; E – Andean region; F – Palaearctic region; G – Nearctic region; H – Oriental region.

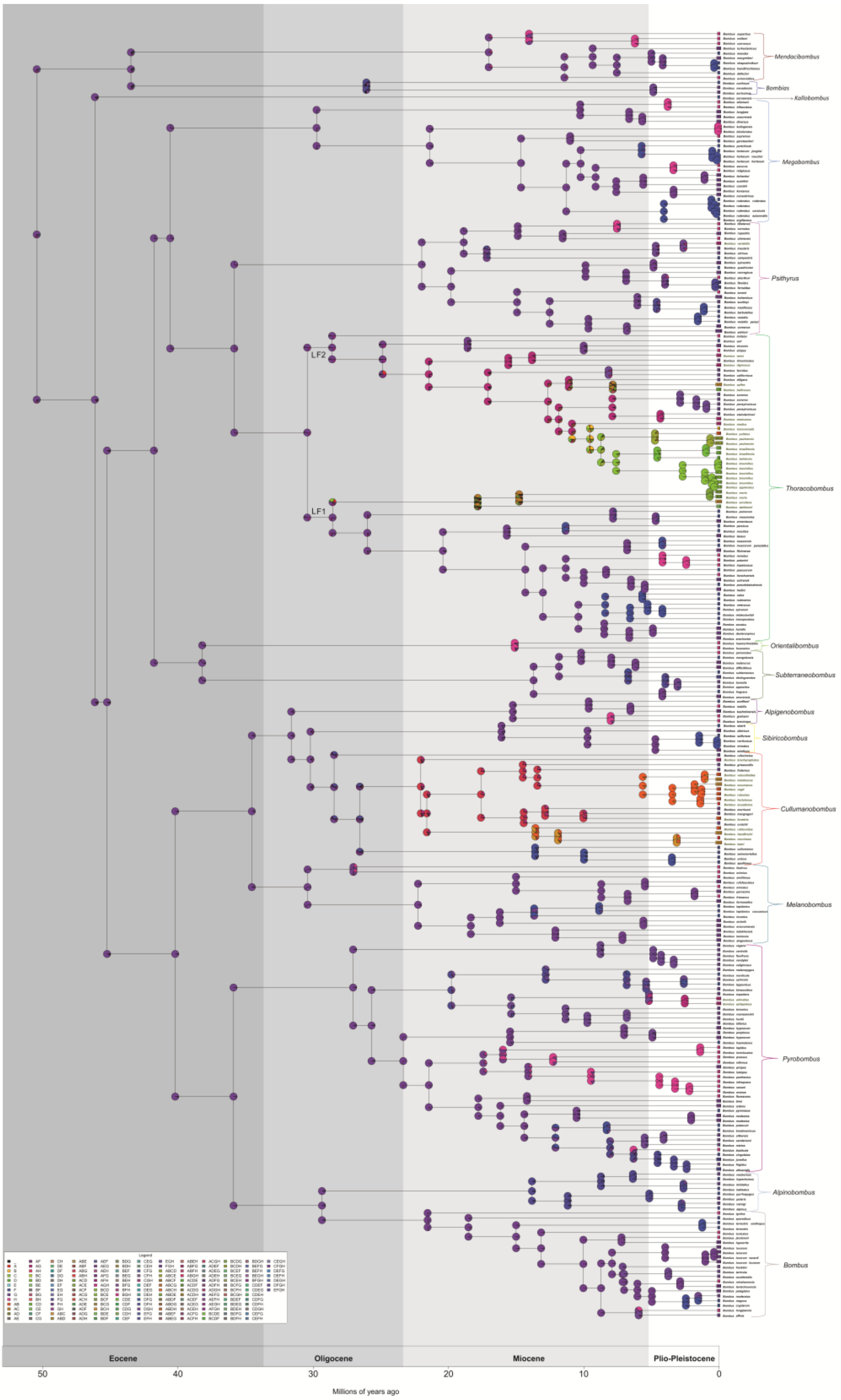
Mendacibombus
Bombias
Kallobombus
Megabombus
Psithyrus
LF2
Thoracobombus
LF1
Orientalibombus
Subterraneobombus
Alpigenobombus
Sibiricobombus
Cullumanobombus
Melanobombus
Pyrobombus
Alpinobombus
Bombus
Legend
Eocene
Oligocene
Miocene
Plio-Pleistocene
50
40
30
20
10
0
Millions of years ago

Fig. 2. Distribution of occurrence of data for ancestry inference of Bumblebee species. In map, Fig. 1, are letters and number showed the areas. Node panels show the probability of each geographic state from BioGeoBEARS analysis. Time tree for Bumblebees obtained under an Unncorrelated Lognormal Relaxed Clock model (UCLN) calibrated with eight fossils (Appendix 1 and Fig. S2.2). LF1 – long-faced clade 1 and LF2 – long-faced clade 2.

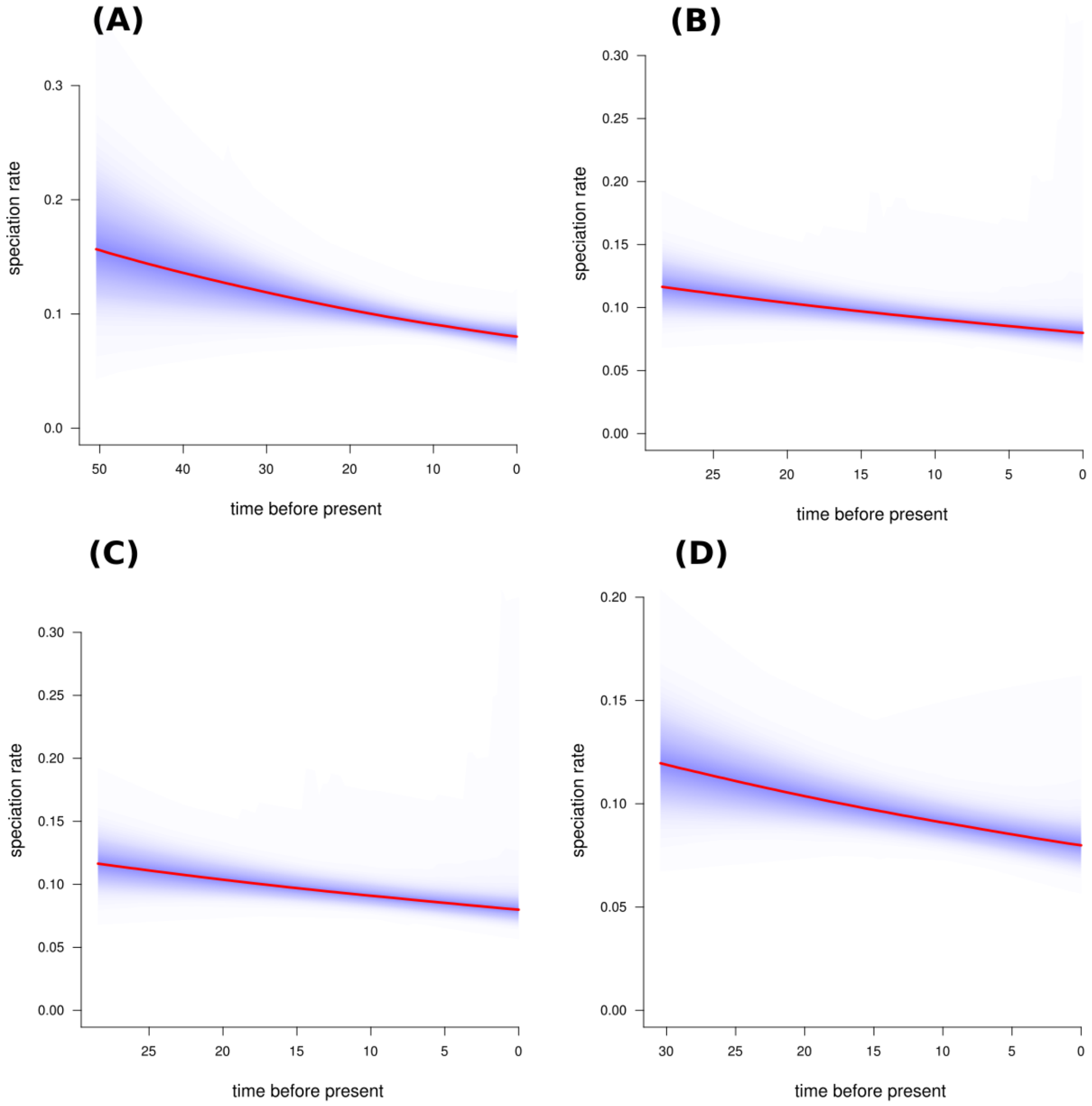

Fig. 3. Estimates of speciation rate variability across the Bumblebees (red line), with average global climate overlain (Zachos et al., 2001); purple line). A – All species; B – Brazilian species; C – *Cullumanobombus* and D – *Thoracobombus* subgenus.

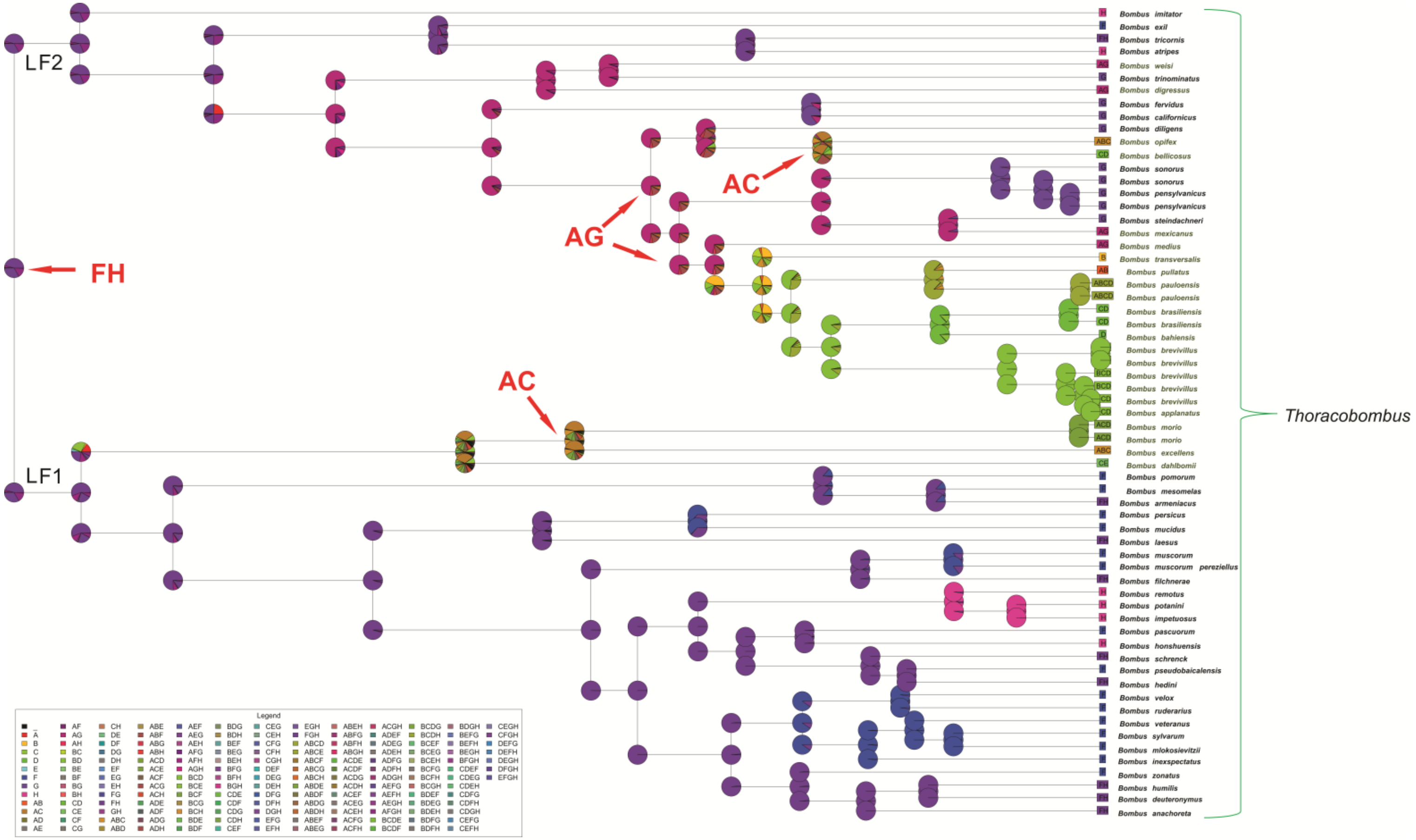

Fig. 4. Global ancestral state estimates under the unconstrained model for the BAYAREALIKE+J. Time tree for *Thoracobombus*. In map, Fig. 1, are letters and number showed the areas. LF1 – long-faced clade 1 and LF2 – long-faced clade 2.

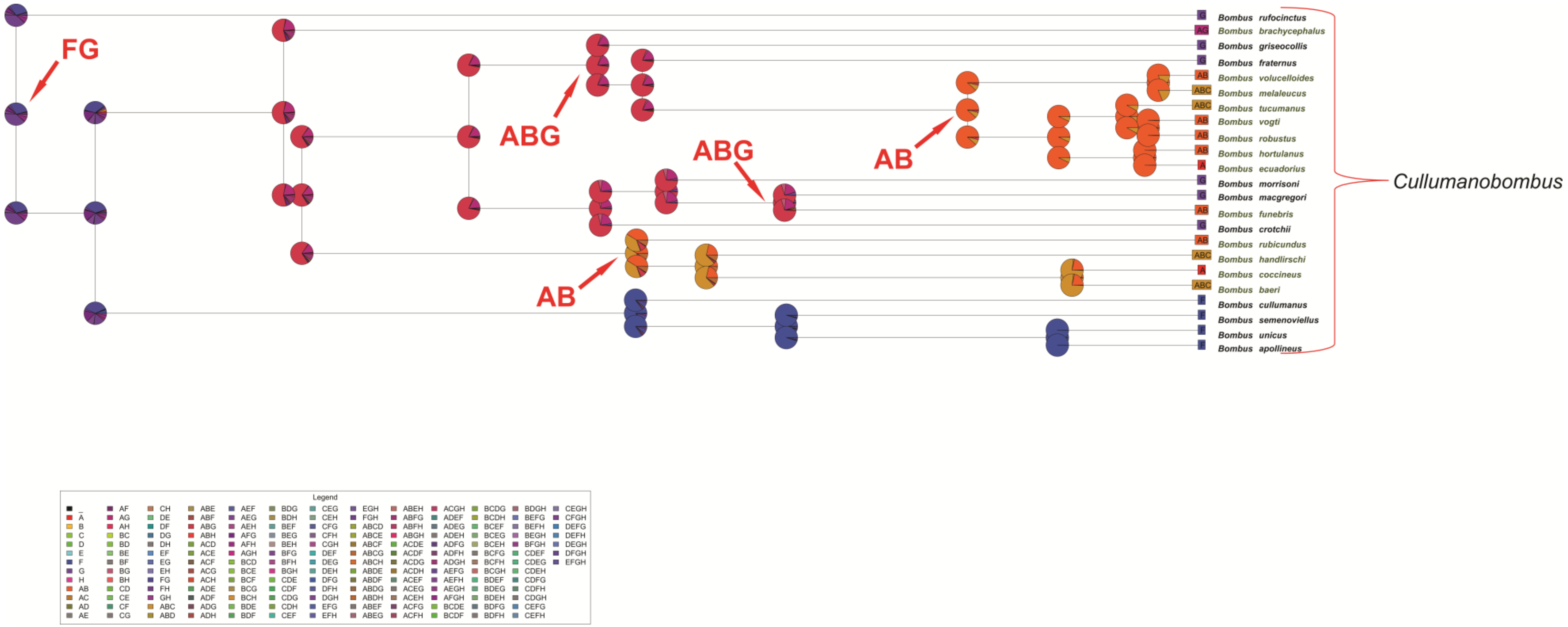

Fig. 5. Global ancestral state estimates under the unconstrained model for the BAYAREALIKE+J. Time tree for *Cullumanobombus*. In map, Fig. 1, are letters and number showed the areas.

Tables

Table 1. Best partitioning schemes and substitution models

Subset	Best Model	Subset Partitions	Subset Sites
1	GTR+I+G	12S and 16S	1-389, 390-830
2	GTR+I+G	Argk exon1	831-1429\3
3	GTR+I+G	Argk exon2, EF1α exon2 and Opsin exon2	832-1429\3, 3811-4628\3, 4715-5247\3
4	SYM+G	Argk exon3, Opsin exon3 and PEPCK exon3	833-1429\3, 4716-5247\3, 5351-5748\3
5	GTR+G	Argk intron and PEPCK intron	1430-1677, 5749-6188
6	GTR+I+G	COI1 and Cytb1	1678-3221\3, 3222-3809\3
7	GTR+I+G	COI2	1679-3221\3
8	GTR+I+G	COI3	1680-3221\3
9	GTR+I+G	Cytb2	3223-3809\3
10	GTR+I+G	Cytb3	3224-3809\3
11	GTR+I	EF1α exon1	3810-4628\3
12	GTR+G	EF1α exon3	3812-4628\3
13	HKY+I+G	EF1α intron	4629-4713
14	HKY+I+G	Opsin exon1 and PEPCK exon1	4714-5247\3, 5349-5748\3
15	GTR+G	Opsin intron	5248-5348
16	SYM+I+G	PEPCK exon2	5350-5748\3

Table 2. Age estimates of the main bumblebee clades. Dates for diversification of main *Bombus* subgenus and *Bombus* are summarized. Tree with the maximum clade credibilities and branch lengths equal to the median ages as calculated from 75,001 post burn-in chronograms (85%). Calibration points are show by the numbers in parentheses indicated around red dots, these refer to the fossils of appendix 1.mya– million years ago. group[1] – monophyletic group in BI, (species)[1]; group[2] – monophyletic group in BI, (species)[2]; group[3] – monophyletic group in BI, (species)[3]; group[4] – monophyletic group in BI, (species)[4]; . group[5] – monophyletic group in BI, (species)[5]; group[6] – monophyletic group in BI, (species)[6]; group[7] – monophyletic group in BI, (species)[7]; * artifact summarizing method (BEAST); OBS – observation.

Taxonomic	Taxa	HPD 95% interval	Median	Area	Locality	OBS
Cullumanobombus	*(B. baeri)*[1]	5.99 to 1.09 mya	3.35 mya	A,B,C	S. America	Andes
Cullumanobombus	*(B. brachycephalus)**					
Cullumanobombus	*(B. coccineus)*[1]	5.99 to 1.09 mya	3.35 mya	A	S. America	Andes
Cullumanobombus	*(B. ecuadorius)*[2]	2.72 to 0.42 mya	1.49 mya	A	S. America	Andes
Cullumanobombus	*(B. funebris)*	14.78 to 6.16 mya	10.23 mya	A,B	S. America	
Cullumanobombus	*(B. handlirschi)*[1]	17.27 to 7.21 mya	12.15 mya	A,B,C	S. America	
Cullumanobombus	*(B. hortulanus)*[2]	2.72 to 0.42 mya	1.49 mya	A,B	S. America	Andes
Cullumanobombus	*(B. melaleucus)*[2]	2.38 to 0.22 mya	1.18 mya	A,B,C	S. America	Andes
Cullumanobombus	*(B. robustus)*[2]	2.46 to 0.44 mya	1.38 mya	A,B	S. America	Andes
Cullumanobombus	*(B. rubicundus)*[1]	19.21 to 8.6 mya	13.82 mya	A,B	S. America	Early Neotropical bumblebees diversification
Cullumanobombus	*(B. tucumanus)*[2]	3.14 to 0.75 mya	1.89 mya	A,B,C	S. America	Andes
Cullumanobombus	*(B. vogti)*[2]	2.46 to 0.44 mya	1.38 mya	A,B	S. America	Andes
Cullumanobombus	*(B. volucelloides)*[2]	2.38 to 0.22 mya	1.18 mya	A,B	Neotropical	Andes
Cullumanobombus	group[1]	28.17 to 16.11 mya	21.85 mya		S. America	The first Neotropical bumblebees ancestral
Cullumanobombus	group[2]	18.82 to 8.59 mya	14.74mya		S. America	

Taxonomic	Taxa	HPD 95% interval	Median	Area	Locality	OBS
Psithyrus	*(B. variabilis)*	4.74 to 1.02 mya	2.77 mya	A,G	Neotropical	
Pyrobombus	*(B. ephippiatus)*	8.61 to 2.75 mya	5.39 mya	A,G	Neotropical	
Thoracobombus	*(B. applanatus)*[4]	4.2 to 1.51 mya	2.78 mya	B,C,D	S. America	
Thoracobombus	*(B. bahiensis)*[4]	6.77 to 2.72 mya	4.67 mya	D	S. America	
Thoracobombus	*(B. bellicosus)*[5]	12.02 to 4.35 mya	7.96 mya	C,D	S. America	
Thoracobombus	*(B. brasiliensis)*[4]	6.77 to 2.72 mya	4.67 mya	C,D	S. America	
Thoracobombus	*(B. brevivillus)*[4]	4.2 to 1.51	2.78 mya	B,C,D	S. America	
Thoracobombus	*(B. dahlbomii)*[3]	24.95 to 11.78 mya	18.05 mya	C,E	S. America	Early Neotropical bumblebees diversification
Thoracobombus	*(B. digressus)*[7]	21.28mya to 10.15	15.77 mya	A,G	Neotropical	
Thoracobombus	*(B. excellens)*[3]	21.55 to 8.87 mya	14.98 mya	A,B,C	S. America	
Thoracobombus	*(B. medius)*[*]					
Thoracobombus	*(B. mexicanus)*[6]	7.39 to 1.94 mya	4.5 mya	A,G	Neotropical	
Thoracobombus	*(B. morio)*[3]	21.55 to 8.87 mya	14.98 mya	A,C,D	S. America	
Thoracobombus	*(B. opifex)*[5]	12.02 to 4.35 mya	7.96 mya	A,B,C	S. America	
Thoracobombus	*(B. pauloensis)*[4]	7.17 to 2.63 mya	4.84 mya	A,B,C,D	S. America	
Thoracobombus	*(B. pullatus)*[4]	7.17 to 2.63 mya	4.84 mya	A,B	S. America	
Thoracobombus	*(B. transversalis)*[4]	12.59 to 6.88 mya	9.66 mya	B	S. America	
Thoracobombus	*(B. weisi)*[7]	19.61 to 8.59 mya	13.99 mya	A,G	Neotropical	
Thoracobombus	group[3]	36.25 to 21.77 mya	28.78 mya		S. America	The first Neotropical bumblebees ancestral
Thoracobombus	group[4]	14.2 to 7.98 mya	10.97 mya		S. America	

Taxonomic	Taxa	HPD 95% interval	Median	Area	Locality	OBS
Thoracobombus	group[5]	14.2 to 7.98 mya	11.25 mya	A,G	S. America	
Thoracobombus	group[6]	11.16 to 4.88 mya	8.01 mya		Neotropical	
Thoracobombus	group[7]	27.74 to 15.86 mya	21.66 mya		Neotropical	

Table 3. BioGeoBEARS results for each model implemented in the analysis: d – dispersal, e – extinction; j – founder, LnL – values of log-likelihood, and AICc – Corrected Akaike Information Criterion. Best-fitting model in bold .

Model	LnL	d	e	j	AICc	AIC
BAYAREALIKE+J	**-537.24**	**0.00096**	**0.03175**	**0.00332**	**1080.58**	**1080.49**
BAYAREALIKE	-542.34	0.00092	0.03888	0	1088.72	1088.67
DEC+J	-570.01	0.02251	1.00E-12	0.00303	1146.11	1146.01
DEC	-578.19	0.02461	0.00221	0	1160.43	1160.38
DIVALIKE	-608.43	0.02902	0.00185	0	1220.9	1220.85
DIVALIKE+J	-795.78	0.00651	1.00E-12	0.01013	1597.65	1597.56

Supplementary material

Table S1. Specimens sequenced for the genetic analyses with their geographic origins and specimens belonging to GenBank and BOLD Systems, which were used in phylogenetic and biogeographic analyzes with their geographic origins.

Species	Collection locality	Voucher	16S (245)	EF-1 (254)	Opsin (240)	ArgK (238)	PEPCK (247)	CytB (80)	COI (185)	12S (18)	OBS
B. (Alpigenobombus) breviceps Smith, 1852	Chiang Mai, Thailand	190	DQ787983	DQ788165	DQ788320	DQ788408	EF050915		FJ175357		
B. (Alpigenobombus) grahami Frison, 1933	Meigu, Sichuan, China	273	EF032347	EF032371	EF032389	EF032408	EF050916		FJ175354		
B. (Alpigenobombus) kashmirensis Friese, 1909	Hongyuan, Sichuan, China	121	DQ788040	DQ788215	DQ788342	DQ788447	EF050913				
B. (Alpigenobombus) nobilis Friese, 1905	Qionglai Sh, Sichuan, China	98	DQ788071	DQ788245	AY739485	DQ788473	EF050912				
B. (Alpigenobombus) wurflenii Radoszkowski, 1860	Obergurgl, Austria	1	DQ788137	DQ788305	AF493007	AF492873	EF050914	AF066975	FJ175355		
B. (Alpinobombus) alpinus Linnaeus, 1758	Gurgltal, Austria	29	DQ787963	DQ788146	AY739452	DQ788393	EF050871		HQ948121		
B. (Alpinobombus) balteatus Dahlbom, 1832	Lappland Co., Sweden	39	DQ787974	DQ788157	AY739455	DQ788402	EF050870	AF066967	AY181097		
B. (Alpinobombus) hyperboreus Schönherr, 1809	Dalarna Co., Sweden	70	DQ788028	DQ788204	AY739470	DQ788440	EF050868	AF066968	AY181108		
B. (Alpinobombus) kirbiellus Curtis, 1835		3534					KT954154				
B. (Alpinobombus) natvigi Richards, 1931		B06					KP901106				
B. (Alpinobombus) neoboreus Sladen, 1919	Alaska, USA	188	DQ788068	DQ788242	AY739484	DQ788470	EF050869				
B. (Alpinobombus) polaris Curtis, 1835	Lappland Co., Sweden	223	DQ788083	AF492970	AF493037	AF492903	EF050872		AY181144		
B. (Alpinobombus) pyrrhopygus Friese, 1902		3832					KT954150				
B. (Bombias) auricomus Robertson, 1903	Illinois, USA	62	DQ787972	DQ788154	AY739454	AF492892	EF051014		KM585615		It is renamed to *B. nevadensis*
B. (Bombias) confusus Schenk, 1859	Dorres, France	83	DQ787992	DQ788173	DQ788324	DQ788414	EF051016		KJ838797		
B. (Bombias) nevadensis Cresson, 1874	Alberta, Canada	139	DQ788069	DQ788243	DQ788352	DQ788471	EF051015	AF077920	AF066990		
B. (Bombus) affinis Cresson, 1863	Illinois, USA	167	DQ787961	DQ788144	AY739451	DQ788391	EF050860		GU707738		
B. (Bombus) cryptarum (Fabricius, 1775)	Erzincan Prov., Turkey	127	DQ787995	DQ788175	AY739461	DQ788416	EF050855		JQ843418		
B. (Bombus) franklini (Frison, 1921)	Oregon, USA	256	EF032345	EF032366	EF032385	EF032403	EF050861		AY694097		
B. (Bombus) hypocrita Pérez, 1905	Kyushu, Japan	123	DQ788030	DQ788206	AF493023	AF492889	EF050864	JQ820710	JQ843426		
B. (Bombus) ignitus Smith, 1869	Beijing, China	96	DQ788031	DQ788207	AF493032	AF492898	EF050866	EU110100	JQ843438		
B. (Bombus) jacobsoni Skorikov, 1912		COI-5P5							BBBO397-11		It's the same the voucher JQ843439
B. (Bombus) lantschouensis Vogt, 1908		6873A01							JQ692953		
B. (Bombus) longipennis Friese, 1918		1551							JQ843472		
B. (Bombus) lucorum (Linnaeus, 1761)	E. Pyrenees, France	217	DQ788051	DQ788225	AF493021	AF492887	EF050862	JQ820751	LN714024		
B. (Bombus) lucorum (Linnaeus, 1761)	Qionglai Sh, Sichuan, China	184	DQ788050	DQ788224	AY739479	DQ788456	EF050863	EF469487	AF279497		
B. (Bombus) lucorum lucorum (Linnaeus, 1761)		LucL24		JQ820938			JQ821143	JQ820735	JQ820532		
B. *(Bombus) lucorum renardi* (Linnaeus, 1761)		LucR18		JQ820956			JQ821161	JQ820753	JQ820550		
B. (Bombus) magnus Vogt, 1911		B08							AY181124		
B. (Bombus) minshanensis Bischoff, 1936		6873B05							JQ692961		
B. (Bombus) moderatus Cresson, 1863	Alberta, Canada	163	DQ788062	DQ788236	AY739481	DQ788464	EF050856		KR901669		Synonymous of B. cryptarum

Species	Collection locality	Voucher	16S (245)	EF-1 (254)	Opsin (240)	ArgK (238)	PEPCK (247)	CytB (80)	COI (185)	12S (18)	OBS
B. (Bombus) occidentalis Greene, 1858	Montana, USA;New Mexico, USA	26	DQ788074	DQ788248	AY739486	DQ788476	EF050858		JQ692962		
B. (Bombus) patagiatus Nylander, 1848	Hongyuan, Sichuan, China	111	DQ788078	DQ788252	AF493020	AF492886	EF050857	EF469488	JQ843590		
B. (Bombus) sporadicus Nylander, 1848	Lappland Co., Sweden	193	DQ788108	DQ788279	AY739491	DQ788501	EF050867	JQ820819	JQ843602		
B. (Bombus) terrestris (Linnaeus, 1758)	San Quírico, Italy	3	DQ788118	DQ788288	AF493022	AF492888	EF050865	KP670308	JQ843670		
B. (Bombus) terrestris xanthopus Kriechbaumer, 1870		TerX19		JQ821081			JQ821284	JQ820876	JQ820674		
B. (Bombus) terricola Kirby, 1837	Ontario, Canada	205	DQ788119	DQ788289	AF493019	AF492885	EF050859		GU707716		
B. (Bombus) tunicatus Smith, 1852	Uttaranchal, India	248	DQ788124						JQ843676		
B. (Cullumanobombus) apollineus Skorikov, 1910	Erzurum Prov., Turkey	84	DQ787964	DQ788147	DQ788313	DQ788394	EF050890				Synonymous of B. cullumanus
B. (Cullumanobombus) baeri Vachal, 1904	Puno, Peru	174	DQ787973	DQ788156	DQ788317	DQ788401	EF050886				
B. (Cullumanobombus) brachycephalus Handlirsch, 1888	Guerrero, Mexico	230	DQ787982	DQ788163	DQ788319	DQ788407	EF050889				
B. (Cullumanobombus) coccineus Friese, 1903	Huarochiri, Peru	137	DQ787991	DQ788172	DQ788323	DQ788413	EF050885				
B. (Cullumanobombus) crotchii Cresson, 1878	California, USA	71	DQ787994	AF492973	AF493040	AF492906	EF050882				
B. (Cullumanobombus) cullumanus (Kirby, 1802)		COI-5P4							BBWP490-10		Voucher 84
B. (Cullumanobombus) ecuadorius Meunier, 1890	Abancay, Peru	135	DQ788001	DQ788181	DQ788329	DQ788421	EF050874				
B. *(Cullumanobombus) fraternus* (Smith, 1854)	Illinois, USA	183	DQ788013	AF492974	AF493041	AF492907	EF050884		KM585682		
B. (Cullumanobombus) funebris Smith, 1854	Abancay, Peru	128	DQ788016	DQ788194	DQ788334	DQ788433	EF050883	AF066974	KC853374		
B. (Cullumanobombus) griseocollis (DeGeer, 1773)	Illinois, USA	82	DQ788018	DQ788196	AF493039	AF492905	EF050879				
*B. (Cullumanobombus) handlirsch*i Friese, 1903	Pasco, Peru	132	DQ788021	DQ788197	DQ788336	DQ788435	EF050887				
B. (Cullumanobombus) hortulanus Smith, 1904	Magdalena, Colombia	200	DQ788025	DQ788201	AY739468	DQ788438	EF050875				
B. (Cullumanobombus) macgregori Labougle & Ayala 1985	Guerrero, Mexico	231	DQ788053	DQ788226	DQ788347	DQ788457	EF050881				
B. (Cullumanobombus) melaleucus Handlirsch, 1888	Oxapampa, Peru	173	DQ787960	DQ788143	DQ788311	DQ788460	EF050876				
B. (Cullumanobombus) morrisoni Cresson, 1878	Utah, USA	196	DQ788065	DQ788240	DQ788350	DQ788468	EF050880				
B. (Cullumanobombus) robustus Smith, 1854	Boyacá, Colombia	50	DQ788093	DQ788266	DQ788366	EF032405	EF050877	AF066973	AF066986		
B. (Cullumanobombus) rubicundus Smith, 1854	Boyacá, Colombia	202	DQ788094	DQ788267	DQ788367	DQ788491	EF050888				
B. (Cullumanobombus) rufocinctus Cresson, 1863	Alberta, Canada	186	DQ788097	DQ788268	AF493034	AF492900	EF050892		KT706869		
B. (Cullumanobombus) semenoviellus Skorikov, 1910	Brandenburg, Germany	236	DQ788101	DQ788271	DQ788369	DQ788494	EF050891		KJ839366		
B. (Cullumanobombus) tucumanus Vachal, 1904	Tucumán, Argentina	276	EF032349	EF032367	EF032386	EF032404	EF050873				
B. (Cullumanobombus) unicus Morawitz, 1883		COI-5P3							BBBO198-10		
B. (Cullumanobombus) vogti Friese, 1903	Abancay, Peru	172	DQ788130	DQ788299	DQ788383	DQ788517	EF050878				
B. (Cullumanobombus) volucelloides Gribodo, 1892	San Jose, Costa Rica	122	DQ788131	AY267133	AY267149	AY267165					Synonymous of *B. melaleucus*
B. (Kallobombus) soroeensis (Fabricius, 1777)	E. Pyrenees, France	136	DQ788107	DQ788278	AF493008	AF492874	EF051013		KJ839741		
B. (Megabombus) argillaceus (Scopoli, 1763)	Kayseri Prov., Turkey	58	DQ787967	DQ788150	AY739453	DQ788396	EF051001	AF066965	KP671609		
B. (Megabombus) bicoloratus Smith, 1879	Nantou, Taiwan	225	DQ787976	AF492971	AF493038	AF492904	EF051005		KP671641		
B. (Megabombus) consobrinus Dahlbom, 1832	Altai Mts, Kazakhstan	261	EF032354	EF032379	AY267150	AY267166	EF050994	EF469489	KT334303		
B. (Megabombus) czerskii Skorikov, 1910		CZ00024						EF469490	KJ734241		
B. (Megabombus) diversus Smith, 1869	Kyushu, Japan	120	DQ788000	AF492961	AF493028	AF492894	EF051010		KT334306		
B. (Megabombus) gerstaeckeri Morawitz, 1881	E. Pyrenees, France	65	DQ788017	DQ788195	DQ788335	DQ788434	EF051003		KT334300		
B. (Megabombus) hortorum (Linnaeus, 1761)	Toscana, Italy	5	DQ788024	DQ788200	AF492987	AF492853	EF050999	JQ820706	KP671630		
B. (Megabombus) hortorum jonghei (Linnaeus, 1761)		HorJ01		JQ820911			JQ821116	JQ820708	KF468689		
B. (Megabombus) hortorum hortorum (Linnaeus, 1761)		HorH01		JQ820909			JQ821114	JQ820706	JQ820505		
B. (Megabombus) koreanus (Skorikov, 1933)	Kangwondo, S. Korea	277	EF032355	AF492969	AF493036	AF492902	EF050995		KJ734250		
B. (Megabombus) kulingensis Cockerell, 1917	Zhejiang Prov., China	97	DQ788042	DQ788217	DQ788343	DQ788449	EF051006		FJ175232		Synonymous of B. bicoloratus
B. (Megabombus) longipes Friese, 1905	Qionglai Sh, Sichuan, China	194	DQ788049	DQ788223	DQ788346	DQ788455	EF051009		KP671681		

Species	Collection locality	Voucher	16S (245)	EF-1 (254)	Opsin (240)	ArgK (238)	PEPCK (247)	CytB (80)	COI (185)	12S (18)	OBS
B. (Megabombus) portchinsky Radoszkowski, 1883	Artvin Prov., Turkey	72	DQ788085	DQ788259	DQ788361	DQ788484	EF051000		KJ734252		
B. (Megabombus) religiosus (Frison, 1935)	Qionglai Sh, Sichuan, China	141	DQ788091	DQ788264	DQ788364	DQ788489	EF050998		KP671608		
B. (Megabombus) ruderatus (Fabricius, 1775)	Valdivia Prov., Chile	18	DQ788096	AF492977	AF493044	AF492910	EF051002	JQ820816	KP671610		
B. (Megabombus) ruderatus ruderatus (Fabricius, 1775)		RudR20		JQ821021			JQ821226	JQ820816	JQ820615		
B. (Megabombus) ruderatus autumnalis (Fabricius, 1775)		RudA05		JQ820982			JQ821187	JQ820777	JQ820576		
B. (Megabombus) ruderatus corsicola (Fabricius, 1775)		RudC19		JQ821001			JQ821206	JQ820796	JQ820595		
B. (Megabombus) securus (Frison, 1935)	Qionglai Sh, Sichuan, China	142	DQ788100	DQ788270	DQ788368	DQ788493	EF050997		KJ734256		
B. (Megabombus) supremus Morawitz, 1887	Qionglai Sh, Sichuan, China	101	DQ788112	DQ788284	DQ788375	DQ788505	EF051004		KP671604		
B. (Megabombus) sushkini (Skorikov, 1931)	Hongyuan, Sichuan, China	143	DQ788113	AF492921	AF492988	AF492854	EF050996		KT334302		Synonymous of *B. tichenkoi*
B. (Megabombus) tichenkoi (Skorikov, 1923)		J1		AF492922	AF492989	AF492855			AF279518		
B. (Megabombus) trifasciatus Smith, 1852	Himachal Pradesh, India	15	DQ788122	AF492918	AF492985	AF492851	EF051007		FJ175236		
B. (Megabombus) ussurensis Radoszkowski, 1877	S. Korea, Mulan Valley	130	AF364829	AF492919	AF492986	AF492852	EF051008		KP671675		
B. (Megabombus) wilemani Cockerell, 1911	Meifang, Taiwan	182	DQ788135								Synonymous of *B. trifasciatus*
B. (Melanobombus) alagesianus (Skorikov, 1923)	Artvin Prov., Turkey	85	DQ787962	DQ788145	DQ788312	DQ788392	EF050903	KC915396	KM458064		Synonymous of B. keriensis
B. (Melanobombus) caucasicus Radoszkowski, 1860		L084TR		KC916483	KC915984		KC916232	KC915481	KC915732		*B. lapidarius caucasicus*
B. (Melanobombus) erzurumensis Özbek, 1990	Artvin Prov., Turkey	126	DQ788003	DQ788183	AY739463	DQ788423	EF050899		KM458066		Synonymous of B. sichelii
B. (Melanobombus) eximius Smith, 1852	Alishan, Taiwan	49	DQ788005	DQ788186	AY739464	DQ788426	EF050911		GU085206		
B. (Melanobombus) festivus Smith, 1861	Qionglai Sh, Sichuan, China	104	DQ788007	DQ788187	AY739465	DQ788427	EF050910				
B. (Melanobombus) formosellus (Frison, 1934)		J2		AF492939	AF493006	AF492872			AF279522		Synonymous of B. pyrosoma
B. (Melanobombus) friseanus Skorikov, 1933	Qionglai Sh, Sichuan, China	105	DQ788015	DQ788193	AY739467	DQ788432	EF050906				
B. (Melanobombus) incertus Morawitz, 1881	Erzurum Prov., Turkey	86	DQ788035	DQ788211	DQ788341	DQ788443	EF050901	KC915397	KC915649		
B. (Melanobombus) keriensis Morawitz, 1887	Hongyuan, Sichuan, China	114	DQ788041	DQ788216	AY739474	DQ788448	EF050904				
B. (Melanobombus) ladakhensis Richards, 1928	Aba, Sichuan, China	158	DQ788043	AY739575	AY739475	DQ788450	EF050905				
B. (Melanobombus) lapidarius (Linnaeus, 1758)	San Quírico, Italy	6	DQ788045	DQ788219	AF493005	AF492871	EF050902	KC915637	KF936492		
B. (Melanobombus) miniatus Bingham, 1897	Himachal Pradesh, India	244	DQ788059	DQ788233	DQ788348	DQ788462	EF050908				
B. (Melanobombus) pyrosoma Morawitz, 1890		B04	FJ159114					EF424427	DQ835585		
B. (Melanobombus) rufofasciatus Smith, 1852	Hongyuan, Sichuan, China	133	DQ788098	DQ788269	AY739489	DQ788492	EF050907				
B. (Melanobombus) sichelii Radoszkowski, 1860	Obergurgl, Austria	34	DQ788103	DQ788273	DQ788371	DQ788496	EF050900	KC915640	KJ839131		
B. (Melanobombus) simillimus Smith, 1852	Himachal Pradesh, India	243	DQ788104	DQ788274	DQ788372	DQ788497	EF050909				
B. (Mendacibombus) avinoviellus (Skorikov, 1914)	Himachal Pradesh, India	242	AY268416	DQ788155	AY268394	DQ788400	EF051020	AF181610			Mistake in table of Cameron et al. 2007
B. (Mendacibombus) convexus Wang, 1979	Qionglai Sh, Sichuan, China	109	DQ787993	DQ788174	DQ788325	DQ788415	EF051021				
B. (Mendacibombus) defector Skorikov, 1910		J3	KX452099	AF492958	AF493025	AF492891	KX452106		AF385807		
B. (Mendacibombus) handlirschianus Vogt, 1909	Artvin Prov., Turkey	87	DQ788022	DQ788198	DQ788337	DQ788436	EF051017				
B. (Mendacibombus) margreiteri Vogt, 1910		B01	KX452100				KX452107				
B. (Mendacibombus) mendax Gerstaecker, 1869	Gurgltal, Austria	19	DQ788057	AY739584	AF493024	AF492890	EF051019		HQ563801		
B. (Mendacibombus) shaposhnikovi Skorikov, 1910	Artvin Prov., Turkey	99	DQ788102	DQ788272	DQ788370	DQ788495	EF051018	JQ820818	JQ820617		Synonymous of *B. handlirschianus*
B. (Mendacibombus) superbus (Tkalcu, 1968)		B02	KX452097		KX452101		KX452104				
B. (Mendacibombus) turkestanicus Skorikov, 1910		B03	KX452098		KX452102		KX452105				
B. (Mendacibombus) waltoni Cockerell, 1910	Qionglai Sh, Sichuan, China	102	DQ788134	DQ788302	DQ788385	DQ788519	EF051022		GU085207		
B. (Orientalibombus) funerarius Smith, 1852	Luojishan, Sichuan, Chin	270	EF032356	EF032378	EF032396	EF032415	EF051012		FJ175342		
B. (Orientalibombus) haemorrhoidalis Smith, 1852	Chiang Mai Prov., Thailand	191	DQ788020	AF492983	AF493050	AF492916	EF051011		KT334307		Mistake in table of Cameron et al. 2007
B. (Psithyrus) ashtoni (Cresson, 1864)	Ottawa, Canada	164	DQ787969	AF492924	AF492991	AF492857	EF050978		FJ582103		Synonymous of B. bohemicus and mistake in table of Cameron et al. 2007

Species	Collection locality	Voucher	16S (245)	EF-1 (254)	Opsin (240)	ArgK (238)	PEPCK (247)	CytB (80)	COI (185)	12S (18)	OBS
B. (Psithyrus) barbutellus (Kirby, 1802)	Uppland Co., Sweden	73	DQ787975	DQ788158	DQ788318	DQ788403	EF050972		KJ839426		
B. (Psithyrus) bohemicus Seidl, 1937	E. Pyrenees, France	55	DQ787980	AF492925	AF492992	AF492858	EF050979	JQ820705	KJ839694		
B. (Psithyrus) campestris (Panzer, 1801)	Dalarna Co., Sweden	40	DQ787986	AF492927	AF492994	AF492860	EF050974		HM401453		
B. (Psithyrus) chinensis (Morawitz, 1890)	Qionglai Sh, Sichuan, China	152	DQ787988	DQ788170	DQ788321	DQ788412	EF050982				
B. (Psithyrus) citrinus (Smith, 1854)	Virginia, USA	170	DQ787990	DQ788171	DQ788322	AY267169	EF050975		FJ582113		
B. (Psithyrus) coreanus (Yasumatsu, 1934)		J4	AF364830								
B. (Psithyrus) cornutus (Frison, 1933)	Ningnan, Sichuan, China	271	EF032353	EF032377	EF032395	EF032413	EF050984				
B. (Psithyrus) fernaldae (Franklin, 1911)	New Mexico, USA	88	DQ788006	AF492926	AF492993	AF492859	EF050969		JX832855		Synonymous of B. flavidus
B. (Psithyrus) flavidus Eversmann, 1852	Lappland Co., Sweden	41	DQ788010	DQ788189	DQ788332	DQ788429	EF050970		AY181184		
B. (Psithyrus) insularis (Smith, 1861)	California, USA	162	DQ788038	AF492975	AF493042	AF492908	EF050976		FJ582127		
B. (Psithyrus) maxillosus Klug, 1817	Kayseri Prov., Turkey	74	DQ788054	DQ788227	AY739480	DQ788458	EF050973				Synonymous of *B. barbutellus*
B. (Psithyrus) norvegicus (Sparre-Schneider, 1918)	Dalarna Co., Sweden	89	DQ788072	DQ788246	DQ788354	DQ788474	EF050971	AF066964	KJ838809		
B. (Psithyrus) quadricolor (Lepeletier, 1832)	Uppland Co., Sweden	90	DQ788090	DQ788263	DQ788363	DQ788488	EF050966		HQ563809		
B. (Psithyrus) rupestris (Fabricius, 1793)	Uppland Co., Sweden	9	DQ788099	AF492928	AF492995	AF492861	EF050983		KJ839543		
B. (Psithyrus) skorikovi (Popov, 1927)	Aba, Sichuan, China	159	DQ788106	DQ788276	DQ788373	DQ788499	EF050968				
B. (Psithyrus) suckleyi Greene, 1860	Colorado, USA	91	DQ788110	DQ788282	DQ788374	DQ788503	EF050981				
B. (Psithyrus) sylvestris (Lepeletier, 1832)	Uppland Co., Sweden	20	DQ788115	DQ788286	DQ788377	DQ788507	EF050967	KT164644	KJ839213		
B. (Psithyrus) tibetanus (Morawitz, 1887)	Min Shan, Sichuan, China	134	DQ788120	DQ788290	DQ788378	DQ788509	EF050985				
B. (Psithyrus) turneri (Richards, 1929)		B09							GU085208		
B. (Psithyrus) variabilis (Cresson, 1872)	Missouri, USA	316	AY268419	DQ788295	AY268397	DQ788513	EF050977				Synonymous of *B. intrudens*
B. (Psithyrus) vestalis (Geoffroy, 1785)	Kent, England	169	DQ788128	DQ788297	AY739495	DQ788515	EF050980	JQ820905	KJ839596		
B. (Psithyrus) vestalis perezi (Schulthess-Rechberg, 1886)		Pere19		JQ820977			JQ821182	JQ820772	JQ820571		
B. (Pyrobombus) alboanalis Franklin, 1913	Alaska, USA	257	EF032343	EF032360	EF032380	EF032398	EF050813				Synonymous of B. jonellus
B. (Pyrobombus) ardens Smith, 1879	Dae-Dong, S. Korea	131	DQ787966	DQ788149	AF493031	AF492897	EF050823		FJ593628		
B. (Pyrobombus) avanus (Skorikov, 1938)	Luojishan, Sichuan, China	272	EF032344	EF032365	EF032384	EF032402	EF050830				
B. (Pyrobombus) beaticola (Tkalcu, 1968)		J5		AF492963	AF493030	AF492896			AF279532		
B. (Pyrobombus) bifarius Cresson, 1878	New Mexico, USA	208	DQ787977	DQ788160	AF493010	AF492876	EF050838	AF084910	AF084915		
B. (Pyrobombus) bimaculatus Cresson, 1863	Arkansas, USA	218	DQ787978	DQ788161	AY739456	DQ788405	EF050847		KM585629		
B. (Pyrobombus) biroi Vogt, 1911	Ketmen Mts., Kazakhstan	210	DQ787979	DQ788162	AY739457	DQ788406	EF050825				
B. (Pyrobombus) brodmannicus Vogt, 1909	Artvin Prov., Turkey	77	DQ787984	DQ788166	AY739458	DQ788409	EF050818		KP033346		
B. (Pyrobombus) caliginosus (Frison, 1927)	California, USA	150	DQ787985	DQ788168	AF493035	AF492901	EF050853				
B. (Pyrobombus) centralis Cresson, 1864	Washington, USA	146	DQ787987	DQ788169	AY739459	DQ788411	EF050852		KR875595		
B. (Pyrobombus) cingulatus Wahlberg, 1854	Lappi, Finland	212	DQ787989	AF492948	AF493015	AF492881	EF050812		AF385814		
B. (Pyrobombus) ephippiatus Say, 1837	Chiapas, Mexico	198	DQ788002	DQ788182	AY739462	DQ788422	EF050844		JF799015		
B. (Pyrobombus) flavescens Smith, 1852	Mei-fang, Taiwan	181	DQ788009	AF492950	AF493017	AF492883	EF050824		GU085209		
B. (Pyrobombus) flavifrons Cresson, 1863	California, USA	95	DQ788011	DQ788190	AF493016	AF492882	EF050850	AF084912	JX832113		
B. (Pyrobombus) frigidus Smith, 1854	Alaska, USA	185	DQ788014	DQ788192	AY739466	DQ788431	EF050811	AF084909	JX833457		
B. (Pyrobombus) haematurus Kriechbaumer, 1870	Trabzon Prov., Turkey	211	DQ788019	EF032364			EF050829				
B. (Pyrobombus) huntii Greene, 1860	Washington, USA	151	DQ788027	DQ788203	AF493045	AF492911	EF050840		JN400357		
B. (Pyrobombus) hypnorum (Linnaeus, 1758)	Klösterle, Austria	78	DQ788029	DQ788205	AF493013	AF492879	EF050826	AF066971	AY181110		
B. (Pyrobombus) hypnorum (Linnaeus, 1758)	Qionglai Sh, Sichuan, China	207	EF032359	EF032363	EF032383	EF032401	EF050827	EF424423	AF385815		
B. (Pyrobombus) impatiens Cresson, 1863	Illinois, USA	60	DQ788033	DQ788209	AF493009	AF492875	EF050842		HQ978604		
B. (Pyrobombus) infirmus (Tkalcu, 19368)	Qionglai Sh, Sichuan, China	157	DQ788036	DQ788212	AY739471	DQ788444	EF050836				

Species	Collection locality	Voucher	16S (245)	EF-1 (254)	Opsin (240)	ArgK (238)	PEPCK (247)	CytB (80)	COI (185)	12S (18)	OBS
B. (Pyrobombus) infrequens (Tkalcu, 1989)	Qionglai Sh, Sichuan, China	140	DQ788037	DQ788213	AY739472	DQ788445	EF050831				
B. (Pyrobombus) jonellus (Kirby, 1802)	Lappland Co., Sweden	79	DQ788039	DQ788214	AY739473	DQ788446	EF050814	F066970	KJ839707		
B. (Pyrobombus) lapponicus (Fabricius, 1793)	Lappland Co., Sweden	103	DQ788046	DQ788220	DQ788345	DQ788452	EF050845	AF066969	KF434333		
B. (Pyrobombus) lemniscatus Skorikov, 1912	Qionglai Sh, Sichuan, China	161	DQ788047	DQ788221	AY739477	DQ788453	EF050834				
B. (Pyrobombus) lepidus Skorikov, 1912	Qionglai Sh, Sichuan, China	155	DQ788048	DQ788222	AY739478	DQ788454	EF050835				
B. (Pyrobombus) luteipes Richards, 1934	Pokhara, Nepal	195	DQ788052								
B. (Pyrobombus) melanopygus Nylander, 1848	California, USA	215	DQ788055	DQ788229	AF493011	AF492877	EF050849		KR876897		
B. (Pyrobombus) mixtus Cresson, 1878	Washington, USA	24	DQ788060	DQ788234	AF493014	AF492880	EF050816		JX832459		
B. (Pyrobombus) modestus Eversmann, 1852	Aba, Sichuan, China	160	DQ788063	DQ788237	AY739482	DQ788465	EF050820		AF279547		
B. (Pyrobombus) modestus Eversmann, 1852	S. Altai, Kazakhstan	238	EF032358	EF032362	EF032382	EF032400	EF050821	EF406355	AF279545		
B. (Pyrobombus) monticola Smith, 1844	E. Pyrenees, France	176	DQ788064	DQ788238	AY739483	DQ788466	EF050848		KF434341		
B. (Pyrobombus) parthenius Richards, 1934	Himachal Pradesh, India	241	DQ788076	DQ788250	DQ788357	DQ788478	EF050832				
B. (Pyrobombus) perplexus Cresson, 1863	Ottawa, Canada	166	DQ788079	DQ788254	AF493012	AF492878	EF050828		FJ582129		
B. (Pyrobombus) picipes Richards, 1934	Qionglai Sh, Sichuan, China	180	DQ788082	DQ788257	AY739487	DQ788482	EF050833	EF424426			
B. (Pyrobombus) pratorum (Linnaeus, 1761)	Klösterle, Austria	75	DQ788087	AF492966	AF493033	AF492899	EF050819		GU705924		
B. (Pyrobombus) pressus (Frison, 1935)	Uttaranchal, India	239	DQ788088	EF032368			EF050837				
B. (Pyrobombus) pyrenaeus Pérez, 1880	Gurgltal, Aust	35	DQ788089	DQ788262	AY739488	DQ788487	EF050822		KJ837876		
B. (Pyrobombus) sandersoni Franklin, 1913	Isle au Haut, Maine, USA	255	EF032346	EF032361	EF032381	EF032399	EF050815		FJ582137		
B. (Pyrobombus) sitkensis Nylander, 1848	California, USA	144	DQ788105	DQ788275	AY739490	DQ788498	EF050817				
B. (Pyrobombus) sonani (Frison, 1934)		J6		AF492951	AF493018	AF492884			AF385816		Changed for the *B. parthenius species*
B. (Pyrobombus) sylvicola Kirby, 1837	New Mexico, USA	108	DQ788116	DQ788287	AY739493	DQ788508	EF050846		JX833556		Mistake in PEPCK
B. (Pyrobombus) ternarius Say, 1837	Nova Scotia, Canada	116	DQ788117	AF492979	AF493046	AF492912	EF050839	AF084911	FJ582149		
B. (Pyrobombus) vagans Smith, 1854	Wisconsin, US	44	DQ788125	DQ788293	DQ788380	DQ788512	EF050854		FJ582159		
B. (Pyrobombus) vandykei (Frison, 1927)	Washington, USA	149	DQ788126	DQ788294	AF493049	AF492915	EF050851		KJ845649		
B. (Pyrobombus) vosnesenskii Radoszkowski, 1862	Washington, USA	112	DQ788133	DQ788301	AF493047	AF492913	EF050841		JN400358		
B. (Pyrobombus) wilmattae Cockerell, 1912	Chiapas, Mexi	199	DQ788136	DQ788304	AY739496	DQ788521	EF050843		JF799021		Synonymous of *B. ephippiatus*
B. (Sibiricobombus) asiaticus Morawitz, 1875	Himachal Pradesh, Indi	249	DQ787970	EF032369	EF032387	EF032406	EF050896				
B. (Sibiricobombus) niveatus Kriechbaumer, 1870	Kayseri Prov., Turkey	93	DQ788070	DQ788244	DQ788353	DQ788472	EF050893				
B. (Sibiricobombus) oberti Morawitz, 1883	Mt. Kailas, Tibet	234	DQ788073	DQ788247	DQ788355	DQ788475	EF050898				
B. (Sibiricobombus) sibiricus (Fabricius, 1781)	Khövsgöl Nuur, Mongolia	274	EF032348	EF032370	EF032388	EF032407	EF050897	EF424424	GU085210		
B. (Sibiricobombus) sulfureus Friese, 1905	Kayseri Prov., Turkey	64	DQ788111	DQ788283	AY739492	DQ788504	EF050895				
B. (Sibiricobombus) vorticosus Gerstaecker, 1872	Aksaray Prov., Turkey	124	DQ788132	DQ788300	DQ788384	DQ788518	EF050894				Synonymous of *B. niveatus*
B. (Subterraneobombus) amurensis Radoszkowski, 1862		COI_5P2							ASBEE035-08		
B. (Subterraneobombus) appositus Cresson, 1878	Utah, USA	145	DQ787965	DQ788148	DQ788314	DQ788395	EF050986		KR873657		
B. (Subterraneobombus) borealis Kirby, 1837	Maine, USA	250	DQ787981	AF492976	AF493043	AF492909	EF050987		FJ582105		
B. (Subterraneobombus) difficillimus Skorikov, 1912	Hongyuan, Sichuan, China	154	DQ787998	DQ788177	DQ788327	DQ788417	EF050990				
B. (Subterraneobombus) distinguendus Morawitz, 1869	Edesbecka, Finland	197	DQ787999	DQ788180	DQ788328	DQ788420	EF050988		KJ837828		
B. (Subterraneobombus) fragrans (Pallas, 1771)	Kayseri Prov., Turkey	61	DQ788012	DQ788191	DQ788333	DQ788430	EF050992	EF424425			
B. (Subterraneobombus) melanurus Lepeletier, 1835	Dzungarskij Alatau, Kazakh	22	DQ788056	DQ788230	AF492990	AF492856	EF050991	EF434859	KT334308		
B. (Subterraneobombus) mongolensis Williams, 2011		COI-5P							ASBEE039-08		
B. (Subterraneobombus) personatus Smith, 1879	Hongyuan, Sichuan, China	138	DQ788081	DQ788256	DQ788359	DQ788481	EF050993				
B. (Subterraneobombus) subterraneus (Linnaeus, 1758)	Uppland Co., Sweden	46	DQ788109	DQ788281	AF493027	AF492893	EF050989		HQ446456		
B. (Thoracobombus) anachoreta (Skorikov, 1914)		AN00001							KP671683		

Species	Collection locality	Voucher	16S (245)	EF-1 (254)	Opsin (240)	ArgK (238)	PEPCK (247)	CytB (80)	COI (185)	12S (18)	OBS
B. (Thoracobombus) applanatus Francoso, *et al.* 2015	Bahia, Brazil	EF209		KT187912		KT187900			KT187877		
B. (Thoracobombus) armeniacus Radoszkowski, 1877	Kayseri Prov., Turkey	80	DQ787968	DQ788151	DQ788315	DQ788397	EF050937				
B. (Thoracobombus) atripes Smith, 1852	Zhejiang Prov., China	66	DQ787971	DQ788153	DQ788316	DQ788399	EF050964				
B. (Thoracobombus) bahiensis Santos-Júnior & Silveira, 2015	Ilhéus, Bahia, Brazil	b85 52893	in this paper	in this paper	in this paper	in this paper	in this paper	KJ849088	KJ848944		
B. (Thoracobombus) bellicosus Smith, 1879	Buenos Aires, Argentina	221	AY268399	DQ788159	AY268377	DQ788404	EF050956		KT187864	AF529427	
B. (Thoracobombus) brasiliensis Lepeletier, 1835	Paraná, Brazil	219	AY268400	DQ788164	AY268378		EF050950	KJ849033	KJ848884	AF529428	
B. (Thoracobombus) brasiliensis Lepeletier, 1835	Pedregulho, São Paulo, Brazil	1218400	in this paper	in this paper	in this paper	in this paper	in this paper	KJ848974	KJ848808		
B. (Thoracobombus) brevivillus gr Franklin, 1913	Prata, Paraíba, Brazil	1200916	in this paper	in this paper	in this paper	in this paper	in this paper	KJ849027	KJ848874		
B. (Thoracobombus) brevivillus gr Franklin, 1913	Remígio, Paraíba, Brazil	1202754	in this paper	in this paper	in this paper	in this paper	in this paper	KJ849028	KJ848876		
B. (Thoracobombus) brevivillus gr Franklin, 1913	Ubajara, Ceará, Brazil	1207320	in this paper	in this paper	in this paper	in this paper	in this paper	in this paper	KJ848951		
B. (Thoracobombus) brevivillus gr Franklin, 1913	Ubajara, Ceará, Brazil	1207321	in this paper	in this paper	in this paper	in this paper	in this paper	in this paper	KJ848952		
B. (Thoracobombus) brevivillus gr Franklin, 1913	Ubajara, Ceará, Brazil	1207210	in this paper	in this paper	in this paper	in this paper	in this paper	in this paper	KJ848953		
B. (Thoracobombus) californicus (Fabricius, 1798)	Alberta, Canada	306	AY268401	DQ788167	AY268379	DQ788410	EF050957			AF529429	Synonymous of B. fervidus
B. (Thoracobombus) dahlbomii Guérin-Méneville, 1835	Arauco Prov., Chile	16	DQ787996	AF492931	AF492998	AF492864	EF050940	AF066962	AF066979	AF529430	
B. (Thoracobombus) deuteronymus Schulz, 1906	Primorskiy Krai, Russia	147	DQ787997	DQ788176	DQ788326	AY267170	EF050922		AF385820		
B. (Thoracobombus) digressus (Milliron, 1962)	R. Grande de Orosí, Mexico	307	AY268403	DQ788178	AY268381	DQ788418	EF050959			AF529431	
B. (Thoracobombus) diligens Smith, 1861	Jalisco, Mexico	171	AY268404	DQ788179	AY268382	DQ788419	EF050955		KC853366	AF529432	
B. (Thoracobombus) excellens Smith, 1879	Aragua, Venezuela	308	AY268405	DQ788184	AY268383	DQ788424	EF050942		KC853321	AF529433	
B. (Thoracobombus) exil (Skorikov, 1823)	Hövsgöl Nuur, Mongolia	232	DQ788004	DQ788185	DQ788330	DQ788425	EF050962				
B. (Thoracobombus) fervidus (Fabricius, 1798)	Missouri, USA	309	AY268406	AF492930	AF492997	AF492863	EF050958		FJ582122	AF529434	
B. (Thoracobombus) filchnerae Vogt, 1908	Hongyuan, Sichuan, China	206	DQ788008	DQ788188	DQ788331	DQ788428	EF050933	EF406353	KF468688		
B. (Thoracobombus) hedini Bischoff, 1936	Qionglai Sh, Sichuan, China	129	DQ788023	DQ788199	DQ788338	DQ788437	EF050930	EF406354			
B. (Thoracobombus) honshuensis (Tkalcu, 1968)		J7		AF492962	AF493029	AF492895			AF279559		
B. (Thoracobombus) humilis Illiger, 1806	E. Pyrenees, France	56	DQ788026	DQ788202	AY739469	DQ788439	EF050924	AF017517	KJ839745		
B. (Thoracobombus) imitator Pittioni, 1949	Guizhou Prov., China	28	DQ788032	DQ788208	DQ788339	DQ788441	EF050965				
B. (Thoracobombus) impetuosus Smith, 1871	Xinmian, Sichuan, China	284	EF032350	EF032373	EF032391	EF032409	EF050927				
B. (Thoracobombus) inexspectatus (Tkalcu, 1963)		B05	FJ159110	FJ159108	FJ159109	FJ159107	FJ159106				
B. (Thoracobombus) laesus Morawitz, 1875	Kars Prov., Turkey	52	DQ788044	DQ788218	DQ788344	DQ788451	EF050936		KP671682		
B. (Thoracobombus) medius Cresson, 1863	Chiapas, Mexico	222	AY268407	DQ788228	AY268385	DQ788459	EF050951		KC853364		
B. (Thoracobombus) mesomelas Gerstaecker, 1869	Switzerland	37	DQ788058	DQ788231	EF032390	EF032414	EF050938	AF066963	KJ839160		
B. (Thoracobombus) mexicanus Cresson, 1878	Chiapas, Mexico	220	AY268408	DQ788232	AY268386	DQ788461	EF050952		KC853372		
B. (Thoracobombus) mlokosievitzii Radoskowski, 1877	Artvin Prov., Turkey	81	DQ788061	DQ788235	DQ788349	DQ788463	EF050917				
B. (Thoracobombus) morio (Swederus, 1787)	Porto Alegre, Brazil	310	AY268409	DQ788239	AY268387	DQ788467	EF050941	KJ848968	KJ848802	AF529435	
B. (Thoracobombus) morio (Swederus, 1787)	Catalão, Goiáis, Brazil	b13	in this paper	in this paper	in this paper	in this paper	in this paper	KJ848979	KJ848813		
B. (Thoracobombus) mucidus Gerstaecker, 1869	E. Pyrenees, France	59	DQ788066	AF492935	AF493002	AF492868	EF050934		KJ839017		
B. (Thoracobombus) muscorum (Linnaeus, 1758)	Öland Co., Sweden	33	DQ788067	DQ788241	DQ788351	DQ788469	EF050925		AY181133	AF529442	
B. (Thoracobombus) muscorum pereziellus (Skorikov, 1923)		Perl01		KF468702					KF468695		
B. (Thoracobombus) opifex Smith, 1879	Puno, Peru	175	DQ788075	DQ788249	DQ788356	DQ788477	EF050954		KC853365		
B. (Thoracobombus) pascuorum (Scopoli, 1763)	Toscana, Italy	23	DQ788077	DQ788251	AF493001	AF492867	EF050932	EU122104	GU705929	AF529443	Mistake in table of Cameron et al. 2007 (Argk)
B. (Thoracobombus) pauloensis Friese, 1913	Ribeirão Preto, Brazil	305	AY268398	DQ788152	AY268376	DQ788398	EF050943	KJ849067	KJ848921	AF529426	Is like B. atratus Cameron et al. 2007
B. (Thoracobombus) pauloensis Friese, 1913	Salinas, Minas Gerais, Brazil	77202	in this paper	in this paper	in this paper		in this paper	in this paper	KJ848786		
B. (Thoracobombus) pensylvanicus (DeGeer, 1773)	Missouri, USA	317	EF032352	EF032374	EF032392	EF032411	EF050948	AF181611	KC853361	AF529436	

Species	Collection locality	Voucher	16S (245)	EF-1 (254)	Opsin (240)	ArgK (238)	PEPCK (247)	CytB (80)	COI (185)	12S (18)	OBS
B. (Thoracobombus) pensylvanicus (DeGeer, 1773)	Missouri, USA	311	EF032351	EF032375	EF032393	EF032412	EF050949				
B. (Thoracobombus) persicus Radoszkowski, 1881	Kars Prov., Turkey	54	DQ788080	DQ788255	DQ788358	DQ788480	EF050935				
B. (Thoracobombus) pomorum (Panzer, 1805)	Erzincan Prov., Turkey	53	DQ788084	DQ788258	DQ788360	DQ788483	EF050939				
B. (Thoracobombus) potanini Morawitz, 1890	Hongyuan, Sichuan, China	113	DQ788086	DQ788260	DQ788362	DQ788485	EF050928				Synonymous of *B. impetuosus*
B. (Thoracobombus) pseudobaicalensis Vogt, 1911	Primorskiy Kray, Russia	253	EF032357	EF032372	AY267155	AY267171	EF050923	EU110101	AF279564		
B. (Thoracobombus) pullatus Franklin, 1913	Pitilla, Costa Rica	312	AY268411	DQ788261	AY268389	DQ788486	EF050945	AF066961	AF066978	AF529437	
B. (Thoracobombus) remotus (Tkalcu, 1968)	Qionglai Sh, Sichuan, China	192	DQ788092	DQ788265	DQ788365	DQ788490	EF050929				
B. (Thoracobombus) ruderarius (Müller, 1776)	Obergurgl, Austria	47	DQ788095	AF492932	AF492999	AF492865	EF050920	AF002723	KJ838913		
B. (Thoracobombus) schrencki Morawitz, 1881	W. Khentey, N. Mongolia	298	AF364828	AF492933	AF493000	AF492866	EF050931		GU674500		
B. (Thoracobombus) sonorus Say, 1837	Arizona, USA	51	DQ822475	DQ788277	EF032394	DQ788500	EF050946				Synonymous of *B. pensylvanicus*
B. (Thoracobombus) sonorus Say, 1837	Mexico	318	AY268412	EF032376	AY268390	EF032410	EF050947			AF529438	Synonymous of *B. pensylvanicus*
B. (Thoracobombus) steindachneri Handlirsch, 1888	Morelos, Mexico	313	AY268413	DQ788280	AY268391	DQ788502	EF050953			AF529439	
B. (Thoracobombus) sylvarum (Linnaeus, 1761)	Uppsala Co., Sweden	110	DQ788114	DQ788285	DQ788376	DQ788506	EF050918		KJ839388		
B. (Thoracobombus) transversalis (Olivier, 1789)	Madre de Dios, Peru	314	AY268414	DQ788291	AY268392	DQ788510	EF050944	KJ849091	KJ848950	AF529440	
B. (Thoracobombus) tricornis Radoszkowski, 1888	Primorskiy Kray, Russia	148	DQ788121	AF492937	AF493004	AF492870	EF050963		AF279573		
B. (Thoracobombus) trinominatus Dalla Torre, 1890	Oaxaca, Mexico	229	DQ788123	DQ788292	DQ788379	DQ788511	EF050960				
B. (Thoracobombus) velox (Skorikov, 1914)	Artvin Prov., Turkey	94	DQ788127	DQ788296	DQ788381	DQ788514	EF050921				
B. (Thoracobombus) veteranus (Fabricius, 1793)	Jura, France	187	DQ788129	DQ788298	DQ788382	DQ788516	EF050919		HQ563800		
B. (Thoracobombus) weisi Friese, 1903	Jalisco, Mexico	315	AY268415	DQ788303	AY268393	DQ788520	EF050961		KC853373	AF529441	
B. (Thoracobombus) zonatus Smith, 1854	Aksaray Prov., Turkey	63	DQ788138	DQ788306	DQ788386	DQ788522	EF050926				
Apis dorsata Fabricius, 1793	Bangalore, India	321	L22893	AY267146	AF091733	AY267178	EF051028	KP259252	KJ513470	KC294229	
Apis mellifera Linnaeus, 1758	Arkansas, USA	320	L22891	AF015267	AF091732	EF032397	EF051030	EF184045	KP844947	KM458618	
Apis cerana Fabricius, 1793		isolate_63	HQ318940	EU184774	EU184839	EU184832		AP017314	KJ755628	NC_014295	
Euglossa (Glossura) imperialis Cockerell, 1922	São Paulo, Brazil	319	AJ581085	AY267144	AY267160	AY267176		AY916117	AJ581106		
Euglossa (Euglossa) championi Cheesman, 1929		EU16	AJ581089	EU421368	AJ581740	EU421629			EU421497		
Eulaema (Apeulaema) boliviensis Friese, 1898	La Paz Prov., Bolivia	213	DQ788139	DQ788307	DQ788387	DQ788523	EF051029		EU421493		
Eulaema (Apeulaema) nigrita Lepeletier, 1841		EU7	AJ581097	EU421427	AJ581732	EU421685		AY916095	EU421555		
Eufriesea pulchra (Smith, 1854)		DWR-F24		EU184769	EU184834	EU184828			AY506359		
Eufriesea surinamensis (Linnaeus, 1758)		EU25		EU421379	GU245272	EU421639			EU421508		
Geniotrigona thoracica (Smith, 1857)	Kuala Muda, Malaysia	303	DQ788140	DQ788308	DQ788388	DQ788524	EF051023				
Heterotrigona itama (Cockerell, 1918)	Kuala Muda, Malaysia	304	DQ788141	DQ788309	DQ788389	DQ788525	EF051024				
*Hypotrigona gribodoi (*Magretti, 1884)	Bwindi, Uganda	322	DQ790440	DQ813121	DQ813199	DQ813043	EF051026				
Liotrigona mahafalya Brooks & Michener, 1988	Mahajanga Prov., Madagascar	J8	DQ790442	DQ813126	DQ813204	DQ813048			HQ012811		
Plebeia frontalis (Friese, 1911)	Puebla, Mexico	323	DQ790459	DQ813138	DQ813216	DQ813059	EF051027				
Trigona amazonensis (Ducke, 1916)	Madre de Dios, Peru	178	DQ788142	DQ788310	DQ788390	DQ788526	EF051025				

Table S2. List of primers used to amplify mitochondrial and nuclear genes.

Gene	Primer	Primer sequence	Reference
COI	LepF1	5’–ATTCAACCAATCATAAAGATATTGG–3’	(Hebert et al., 2004)
COI	LepR1	5’–TAAACTTCTGGATGTCCAAAAAATCA–3’	(Hebert et al., 2004)
COI	mtd_6	5’–GGAGGATTTGGAAATTGATTAGTTCC–3’	(Simon C et al., 1994)
COI	mtd_9	5’–CCCGGTAAAATTAAAATATAAACTTC–3’	(Simon C et al., 1994)
COI	COI_R_PR3	5’–GCTCGTGTGTCAACATCTAA–3’	designed here
CytB	MBI_30	5’–TCGAGTTCCATTTGATTT–3’	(Silvestre et al., 2008)
CytB	mtd_26	5’–TATGTACTACCATGAGGACAAATATC–3’	(Simon C et al., 1994)
16S	LR13943F	5’–CACCTGTTTATCAAAAACAT–3’	(Costa *et al.* 2003)
16S	874	5’–ATAGATAGAAACCAATCTG–3’	(Cameron S A et al., 1992)
Opsin	LWRhF	5’–AATTGCTATTAYGARACNTGGGT–3’	(Mardulyn & Cameron, 1999)
Opsin	LWRhR	5’–ATATGGAGTCCANGCCATRAACCA–3’	(Mardulyn & Cameron, 1999)
EF1α	EF1αF	5’–GGACACAGAGATTTCATCAARAA–3’	(Kawakita et al., 2003)
EF1α	EF1αR	5’–TTGCAAAGCTTCRTGRTGCATTT–3’	(Kawakita et al., 2003)
Argk	ArgkF1	5’–GTTGACCAAGCYGTYTTGGA–3’	(Kawakita et al., 2003)
Argk	ArgkR1	5’–CATGGAAATAATACGRAGRTG–3’	(Kawakita et al., 2003)
PEPCK	FHv4	5′-TGTATRATAATTCGCAAYTTCAC-3″	(Cameron et al., 2007)
PEPCK	RHv4	5′-CTGCTGGRGTYCTAGATCC-3′	(Cameron et al., 2007)

Table S3. PCR primer pairs and annealing temperatures used for analysis. See table S2 for primer sequences used.

Gene	Primers	Temperature	Product size
COI	LepF1 + COI_R_PR3	50°C	~888pb
	LepF1 + mtd_9	50°C	~729pb
	LepF1 + LepR1	50°C	~711pb
	mtd_6 + COI_R_PR3	50°C	~686pb
	mtd_6 + mtd_9	50°C	~527pb
CytB	mtd_26 + MBI_30	50°C	~1100pb
16S	LR13943F+874	50°C	~500pb
Opsin	LWRhF + LWRhR	57°C	~700pb
EF1α	EF1αF + EF1αR	53°C	~1200pb
Argk	ArgkF1 + ArgkR1	50°C	~700pb
PEPCK	FHv4 + RHv4	50°C	~900pb

Table S4. Data of full-length concatenated alignment data used on phylogenetic analysis. Introns of the genes Argk, Opsin and PECK belonging to outgroup taxa were removed of analyses. TL – Total length; N – Number of charset; Numbers between parenthesis refer to indels and when followed by 'N' the nucleotide base is not known.

Taxon	**TL (bp)**	**N**	**12S**	**16S**	**COI**	**CytB**	**Argk exon**	**Argk intron**	**EF1a exon**	**EF1a intron**	**Opsin exon**	**Opsin intron**	**PEPCK exon**	**PEPCK intron**
Apis cerana	4225	8	389	412 (3)	972	587	530	0	759	85	491	0	0	0
Apis dorsata	4059	9	389 (1)	384 (6)	621	503	468	0	759	85	502	0	348	0
Apis mellifera	4605	9	389	384 (4)	1044	469	599	0	819	85	462	0	354	0
B. affinis SC167	3984	10	0	441	658	0	599	248	529	85	502	101	381	440 (40)
B. alagesianus SC085	4674	11	0	441	943	465	539	248	529	85	502	101	381	440
B. alboanalis SC257	3326	9	0	441	0	0	599	248	529	85	502	101	381	440
B. alpinus SC029	3942	10	0	441	616	0	599	248	529	85	502	101	381	440
B. amurensis COI 5P2	602	1	0	0	602	0	0	0	0	0	0	0	0	0
B. anachoreta AN00001	657	1	0	0	657	0	0	0	0	0	0	0	0	0
B. apollineus SC084	3286	9	0	441	0	0	599	248 (32)	529	85	502	101	357	424
B. applanatus EF209	1987	5	0	0	631	0	488	248 (1)	535	85	0	0	0	0
B. appositus SC145	3887	10	0	441	561	0	599	248 (1)	529	85	502	101	381	440 (11)
B. ardens SC131	3947	10	0	441	658	0	530	248	529	85	534	101	381	440
B. argillaceus SC058	4536	11	0	441	657	553	599	248 (1)	529	85	502	101	381	440
B. armeniacus SC080	3326	9	0	441	0	0	599	248 (1)	529	85	502	101	381	440
B. ashtoni SC164	3928	10	0	441	639	0	530	248 (2)	529	85	534	101	381	440 (37)
B. asiaticus SC249	3168	9	0	441	0	0	539	248	529	85	502	92	381	351
B. atripes SC066	3312	9	0	441	0	0	588	248 (57)	529	85	502	101	378	440
B. auricomus SC062	3829	10	0	441	658	0	444	248 (49)	529	85	502	101 (11)	381	440 (3)

Taxon	**TL (bp)**	**N**	**12S**	**16S**	**COI**	**CytB**	**Argk exon**	**Argk intron**	**EF1a exon**	**EF1a intron**	**Opsin exon**	**Opsin intron**	**PEPCK exon**	**PEPCK intron**
B. avanus SC272	3265	9	0	441	0	0	599	248	529	85	502	101	357	403 (4)
B. avinoviellus SC242	3809	10	0	441	0	572 (3 'N')	539	248 (12)	529	85	473	101 (1 'N', 2)	381	440
B. baeri SC174	3266	9	0	441	0	0	539	248 (17)	529	85	502	101	381	440
B. bahiensis 52893	4162	11	0	441	656	414	539	248 (1)	513	85	473	101	348	344 (1)
B. balteatus SC039	4935	11	0	441	1056	553	599	248	529	85	502	101	381	440
B. barbutellus SC073	3897	10	0	441	571 (4 'N')	0	599	248 (1)	529	85	502	101	381	440 (37)
B. beaticola J5	2907	7	0	0	891	0	530	248	518	85	534	101	0	0
B. bellicosus SC221	4056	11	389 (10 'N')	441	430	0	539	248 (1)	529	85	473	101 (1 'N', 2)	381	440
B. bicoloratus SC225	3849	10	0	441	657	0	530	248 (1)	529	85	534	101	348	376
B. bifarius SC208	4175	11	0	441	333	553 (2 'N')	530	248 (78)	529	85	534	101	381	440
B. bimaculatus SC218	3984	10	0	441	658	0	599	248 (78)	529	85	502	101	381	440
B. biroi SC210	3266	9	0	441	0	0	539	248	529	85	502	101	381	440
B. bohemicus SC055	4319	11	0	441	571 (4 'N')	459	530	248 (2)	529	85	534	101	381	440 (37)
B. borealis SC250	3863	10	0	441	622	0	530	248 (1)	529	85	534	101	351	422 (11)
B. brachycephalus SC230	3326	9	0	441	0	0	599	248 (17)	529	85	502	101	381	440
B. brasiliensis 1218400	4177	11	0	441	658	414	539	248 (1)	529	85	473	101	348	341 (1)
B. brasiliensis SC219	3635	10	389	441	471	414	0	0	529	85	473 (3 'N')	101 (7 'N', 2)	381	351
B. breviceps SC190	4286	10	0	441	960	0	599	248	529	85	502	101	381	440
B. brevivillus 1200916	3862	11	0	441	471	414	414	248 (1)	521	85	473	101	348	346 (1)
B. brevivillus 1202754	3952	11	0	441	471	414	508	248 (1)	521 (1)	85	473	101	348	342 (1)
B. brevivillus 1207210	3916	11	0	441	608	362	414	248 (1)	497	85	473	101	348	339 (1)
B. brevivillus 1207320	3764	11	0	441	601	305	414	248 (1)	529	85	473	101	348	219
B. brevivillus 1207321	3941	11	0	441	601	362	414	248 (1)	529	85	473	101	348	339 (1)
B. brodmannicus SC077	4174	10	0	441	848	0	599	248	529	85	502	101	381	440
B. californicus SC306	3686	10	389 (1)	441	0	0	599	248 (1)	529	85	473	101 (2)	381	440

Taxon	**TL (bp)**	**N**	**12S**	**16S**	**COI**	**CytB**	**Argk exon**	**Argk intron**	**EF1a exon**	**EF1a intron**	**Opsin exon**	**Opsin intron**	**PEPCK exon**	**PEPCK intron**
B. caliginosus SC150	3289	9	0	441	0	0	530	248	529	85	534	101	381	440
B. campestris SC040	3914	10	0	441	634	0	530	248 (2)	520	85	534	101	381	440 (37)
B. centralis SC146	3869	10	0	441	543 (1 'N')	0	599	248	529	85	502	101	381	440
B. chinensis SC152	3326	9	0	441	0	0	599	248 (1)	529	85	502	101	381	440 (37)
B. cingulatus SC212	4091	10	0	441	891	0	530	248	529	85	534	101	381	351
B. citrinus SC170	3896	10	0	441	639	0	530	248 (53)	529	85	502	101	381	440 (37)
B. coccineus SC137	3326	9	0	441	0	0	599	248 (17)	529	85	502	101	381	440
B. confusus SC083	3984	10	0	441	658	0	599	248 (173)	529	85	502	101	381	440
B. consobrinus SC261	4534	11	0	441	658	587	530	248 (1)	529	85	534	101	381	440
B. convexus SC109	3190	9	0	441	0	0	539	248 (12)	529	85	502	101	348	397
B. coreanus J4	412	1	0	412	0	0	0	0	0	0	0	0	0	0
B. cornutus SC271	2930	9	0	441	0	0	539	248 (1)	529	85	234	33	381	440 (37)
B. crotchii SC071	3289	9	0	441	0	0	530	248 (17)	529	85	534	101	381	440
B. cryptarum SC127	3984	10	0	441	658	0	599	248	529	85	502	101	381	440 (40)
B. cullumanus COI 5P4	568	1	0	0	568	0	0	0	0	0	0	0	0	0
B. czerskii CZ00024	1245	2	0	0	658	587	0	0	0	0	0	0	0	0
B. dahlbomii SC016	4564	12	389 (1)	441	333	553	530	248 (1)	529	85	534	101	381	440
B. defector J3	4173	10	0	441	891	0	530	248 (12)	529	85	534	101	381	433
B. deuteronymus SC147	4067	10	0	441	891	0	530	248 (3)	529	83	502	101	381	361 (4)
B. difficillimus SC154	3326	9	0	441	0	0	599	248 (1)	529	85	502	101	381	440
B. digressus SC307	3537	10	389 (1)	441	0	0	539	248 (26)	529	85	473	101 (4 'N', 2)	381	351 (1)
B. diligens SC171	4317	11	389	441	631	0	599	248 (24)	529	85	473	101 (1 'N', 2)	381	440
B. distinguendus SC197	3927	10	0	441	601	0	599	248 (11)	529	85	502	101	381	440 (11)
B. diversus SC120	4177	10	0	441 (2)	658	0	530	248 (1)	759	85	534	101	381	440
B. ecuadorius SC135	3326	9	0	441	0	0	599	248 (17)	529	85	502	101	381	440 (5)

Taxon	TL (bp)	N	12S	16S	COI	CytB	Argk exon	Argk intron	EF1a exon	EF1a intron	Opsin exon	Opsin intron	PEPCK exon	PEPCK intron
B. ephippiatus SC198	4137	10	0	441 (1)	811	0	599	248 (78)	529	85	502	101	381	440
B. erzurumensis SC126	4269	10	0	441	943	0	599	248	529	85	502	101	381	440
B. excellens SC308	4257	11	389 (1)	441	631	0	539	248 (1)	529	85	473	101 (2)	381	440
B. exil SC232	3246	9	0	441	0	0	539	248 (49)	529	85	502	101	361	440
B. eximius SC049	4035	10	0	441	709	0	599	248	529	85	502	101	381	440
B. fernaldae SC088	3937	10	0	441	648	0	530	248 (2)	529	85	534	101	381	440 (37)
B. fervidus SC309	4316	11	388 (1)	441	639	0	530	248 (1 'N', 1)	529	85	534	101	381	440
B. festivus SC104	3326	9	0	441 (1)	0	0	599	248	529	85	502	101	381	440
B. filchnerae SC206	4390	11	0	441	477	587	599	248 (1)	529	85	502	101	381	440 (4)
B. flavescens SC181	3998	10	0	441	709	0	530	248	529	85	534	101	381	440
B. flavidus SC041	4382	10	0	441	1056	0	599	248 (2)	529	85	502	101	381	440 (37)
B. flavifrons SC095	4500	11	0	441	658	553	530	248	529	85	534	101	381	440
B. formosellus J2	2918	7	0	0	891	0	530	248	529	85	534	101	0	0
B. fragrans SC061	3913	10	0	441	0	587 (1)	599	248 (1)	529	85	502	101	381	440
B. franklini SC256	4193	10	0	417	891	0	599	248	529	85	502	101	381	440 (40)
B. fraternus SC183	3947	10	0	441 (1)	658	0	530	248 (17)	529	85	534	101	381	440
B. frigidus SC185	4535	11	0	441	658	553	597 (2 'N')	248	529	85	502	101	381	440
B. friseanus SC105	3326	9	0	441	0	0	599	248	529	85	502	101	381	440
B. funebris SC128	4500	11	0	431	631 (30 'N')	553 (1 'N')	599	248 (17)	529	85	502	101	381	440 (1)
B. funerarius SC270	4291	10	0	441	965	0	599	248 (1)	529	85	502	101	381	440 (13)
B. gerstaeckeri SC065	3981	10	0	441	655	0	599	248 (1)	529	85	502	101	381	440
B. grahami SC273	4088	10	0	441	960 (152 'N')	0	519	248	529	85	502	101	348	355 (4 'N')
B. griseocollis SC082	3289	9	0	441	0	0	530	248 (17)	529	85	534	101	381	440
B. haematurus SC211	1373	5	0	441	0	0	0	0	529	85	0	0	201	117
B. haemorrhoidalis SC191	3908	10	0	441	658	0	491	248 (1 'N', 1)	529	85	534	101	381	440

Taxon	**TL (bp)**	**N**	**12S**	**16S**	**COI**	**CytB**	**Argk exon**	**Argk intron**	**EF1a exon**	**EF1a intron**	**Opsin exon**	**Opsin intron**	**PEPCK exon**	**PEPCK intron**
B. handlirschi SC132	3212	9	0	441	0	0	539	248 (17)	529	85	502	101	351	416 (4)
B. handlirschianus SC087	3266	9	0	441	0	0	539	248 (16)	529	85	502	101	381	440
B. hedini SC129	3913	10	0	441	0	587	599	248 (6)	529	85	502	101	381	440 (4)
B. honshuensis J7	2918	7	0	0	891	0	530	248 (6)	529	85	534	101	0	0
B. h hortorum HorH01	2788	6	0	0	849	459	0	0	574	85	0	0	381	440
B. h jonghei HorJ01	2788	6	0	0	849	459	0	0	574	85	0	0	381	440
B. hortorum SC005	4405	11	0	441	657	459	530	248 (1)	529	85	534	101	381	440
B. hortulanus SC200	3326	9	0	441	0	0	599	248 (17)	529	85	502	101	381	440 (5)
B. humilis SC056	4303	11	0	441	658	400	599	248 (3)	529	83	502	101	381	361 (4)
B. huntii SC151	4100	10	0	441	811	0	530	248 (78)	529	85	534	101	381	440
B. hyperboreus SC070	4935	11	0	441	1056	553	599	248 (6)	529	85	502	101	381	440
B. hypnorum SC078	4872	11	0	441	1056	553	530	248	529	85	534	101	355	440
B. hypnorum SC207	4795	11	0	432	891	587	599	248	529	85	502	101	381	440
B. hypocrita SC123	4387	11	0	441	639	459	530	248	529	85	534	101	381	440 (40)
B. ignitus SC096	4534	11	0	441	658	587	530	248	529	85	534	101	381	440 (49)
B. imitator SC028	3326	9	0	441	0	0	599	248 (1)	529	85	502	101	381	440 (30)
B. impatiens SC060	3925	10	0	441	658	0	530	248 (78)	529	85	534	101	359	440
B. impetuosus SC284	3305	9	0	441	0	0	578	248 (6)	529	85	502	101	381	440 (46)
B. incertus SC086	4675	11	0	441	944	465	539	248	529	85	502	101	381	440
B. inexspectatus B05	3042	9	0	411	0	0	509	248 (1)	529	85	459	101	348	352 (46)
B. infirmus SC157	3316	9	0	441	0	0	599	248 (26)	529	85	502	101	381	430
B. infrequens SC140	3326	9	0	441	0	0	599	248	529	85	502	101	381	440
B. insularis SC162	3897	10	0	441	608 (1 'N')	0	530	248 (53)	529	85	534	101	381	440 (37)
B. jacobsoni COI 5P5	425	1	0	0	425 (1 'N')	0	0	0	0	0	0	0	0	0
B. jonellus SC079	4537	11	0	441	658	553	599	248	529	85	502	101	381	440

Taxon	TL (bp)	N	12S	16S	COI	CytB	Argk exon	Argk intron	EF1a exon	EF1a intron	Opsin exon	Opsin intron	PEPCK exon	PEPCK intron
B. kashmirensis SC121	3325	9	0	441	0	0	598	248	529	85	502	101	381	440
B. keriensis SC114	3326	9	0	441	0	0	599	248	529	85	502	101	381	440
B. kirbiellus 3534	821	2	0	0	0	0	0	0	0	0	0	0	381	440
B. koreanus SC277	3947	10	0	441	658	0	530	248 (1)	529	85	534	101	381	440
B. kulingensis SC097	4238	10	0	441	980	0	539	248 (1)	521	85	502	101	381	440
B. ladakhensis SC158	3320	9	0	441	0	0	599	248	523	85	502	101	381	440
B. laesus SC052	3970	10	0	441	657	0	599	248 (1)	529	85	502	101	369	439 (77)
B. lantschouensis 6873A01	658	1	0	0	658	0	0	0	0	0	0	0	0	0
B. l caucasicus L084TR	3651	8	0	0	1056	465	0	0	589	85	534	101	381	440
B. lapidarius SC006	4804	11	0	441	1050	465	530	248	529	85	534	101	381	440
B. lapponicus SC103	4536	11	0	441	658	553	599	248 (78)	528	85	502	101	381	440
B. lemniscatus SC161	3289	9	0	441	0	0	599	248 (22)	529	85	502	101	358	426
B. lepidus SC155	3326	9	0	441	0	0	599	248 (22)	529	85	502	101	381	440
B. longipennis 1551	658	1	0	0	658	0	0	0	0	0	0	0	0	0
B. longipes SC194	3983	10	0	441 (2)	657	0	599	248 (1)	529	85	502	101	381	440 (6)
B. l lucorum LucL24	2788	6	0	0	849	459	0	0	574	85	0	0	381	440 (40)
B. l renardi LucR18	2788	6	0	0	849	459	0	0	574	85	0	0	381	440 (40)
B. lucorum SC184	4804	11	0	441	891	587	599	248	529	85	502	101	381	440 (40)
B. lucorum SC217	4357	11	0	441	609	459	530	248	529	85	534	101	381	440 (40)
B. luteipes SC195	441	1	0	441	0	0	0	0	0	0	0	0	0	0
B. macgregori SC231	3326	9	0	441	0	0	599	248 (17)	529	85	502	101	381	440
B. magnus B08	1056	1	0	0	1056	0	0	0	0	0	0	0	0	0
B. margreiteri B01	1262	3	0	441	0	0	0	0	0	0	0	0	381	440
B. maxillosus SC074	3326	9	0	441	0	0	599	248 (1)	529	85	502	101	381	440 (37)
B. medius SC222	3868	10	0	441	631 (24 'N')	0	539	248 (6)	529	85	473	101 (3 'N', 2)	381	440

Taxon	TL (bp)	N	12S	16S	COI	CytB	Argk exon	Argk intron	EF1a exon	EF1a intron	Opsin exon	Opsin intron	PEPCK exon	PEPCK intron
B. melaleucus SC173	3266	9	0	441	0	0	539	248 (18)	529	85	502	101	381	440 (5)
B. melanopygus SC215	3947	10	0	441	658	0	530	248 (78)	529	85	534	101	381	440
B. melanurus SC022	4445	11	0	441	658	587	530	248 (1)	529	85	534	101	381	351
B. mendax SC019	3941	10	0	441	658	0	530	248 (12)	523	85	534	101	381	440
B. mesomelas SC037	4537	11	0	441	658	553	599	248 (1)	529	85	502	101	381	440
B. mexicanus SC220	3560	10	0	441	335	0	527	248 (6)	529	85	473	101 (3 'N', 2)	381	440
B. miniatus SC244	3266	9	0	441	0	0	539	248	529	85	502	101	381	440
B. minshanensis 6873B05	658	1	0	0	658	0	0	0	0	0	0	0	0	0
B. mixtus SC024	3912	10	0	441	623 (1 'N')	0	530	248	529	85	534	101	381	440
B. mlokosievitzii SC081	3326	9	0	441	0	0	599	248 (1)	529	85	502	101	381	440 (46)
B. moderatus SC163	3984	10	0	441	658 (1 'N')	0	599	248	529	85	502	101	381	440 (40)
B. modestus SC160	4217	10	0	441	891	0	599	248	529	85	502	101	381	440
B. modestus SC238	4804	11	0	441 (1)	891	587	599	248	529	85	502	101	381	440
B. mongolensis COI 5P	600	1	0	0	600	0	0	0	0	0	0	0	0	0
B. monticola SC176	3984	10	0	441	658	0	599	248 (78)	529	85	502	101	381	440
B. morio B13	3998	11	0	441	471	414	537	248 (1)	529	85	473	101	348	351 (1)
B. morio SC310	4571	12	389 (3)	441	471	414	599	248 (1)	529	85	473 (1 'N')	101 (2)	381	440
B. morrisoni SC196	3326	9	0	441	0	0	599	248 (17)	529	85	502	101	381	440
B. mucidus SC059	3852	10	0	441	573	0	530	248 (1)	519	85	534	101	381	440 (4)
B. m pereziellus Perl01	1508	3	0	0	849	0	0	0	574	85	0	0	0	0
B. muscorum SC033	4771	11	389 (2)	441	1056	0	599	248 (1)	529	85	502	101	381	440 (4)
B. natvigi B06	840	2	0	0	0	0	0	0	0	0	0	0	400	440
B. neoboreus SC188	3326	9	0	441	0	0	599	248 (6)	529	85	502	101	381	440
B. nevadensis SC139	4007	11	0	441	333	553	428	248	529	85	502	101 (11)	357	430 (6)
B. niveatus SC093	3225	9	0	441	0	0	599	248	529	84	502	90	381	351

Taxon	**TL (bp)**	**N**	**12S**	**16S**	**COI**	**CytB**	**Argk exon**	**Argk intron**	**EF1a exon**	**EF1a intron**	**Opsin exon**	**Opsin intron**	**PEPCK exon**	**PEPCK intron**
B. nobilis SC098	3326	9	0	441	0	0	599	248	529	85	502	101	381	440
B. norvegicus SC089	4510	11	0	441	631	553 (1 'N')	599	248 (2)	529	85	502	101	381	440 (37)
B. oberti SC234	3317	9	0	441	0	0	599	248	529	85	502	92	381	440 (2)
B. occidentalis SC026	3918	10	0	441	592 (1 'N')	0	599	248	529	85	502	101	381	440 (40)
B. opifex SC175	3957	10	0	441	631	0	599	248 (1)	529	85	502	101	381	440
B. parthenius SC241	3266	9	0	441	0	0	539	248	529	85	502	101	381	440
B. pascuorum SC023	4797	12	389 (1)	441	658	461	530	248 (5)	529	85	534	101	381	440 (4)
B. patagiatus SC111	4534	11	0	441	658	587	530	248	529	85	534	101	381	440 (40)
B. pauloensis 77202	3302	9	0	441	568	415	0	0	529	85	473	101	348	342 (1)
B. pauloensis SC305	4509	12	389 (1)	441	471	414	539	248 (6)	527	85	473	101 (1 'N', 2)	381	440
B. pennsylvanicus SC311	3323	9	0	441	0	0	599	248 (1)	529	85	502	101	381	437
B. pennsylvanicus SC317	4917	12	388	441	631	572	599	248 (1)	529	85	502	101	381	440
B. perplexus SC166	3928	10	0	441	639	0	530	248	529	85	534	101	381	440
B. persicus SC054	3274	9	0	441	0	0	573	248 (15)	527	85	502	101	359	438
B. personatus SC138	3326	9	0	441	0	0	599	248 (1)	529	85	502	101	381	440
B. picipes SC180	3913	10	0	441	0	587	599	248	529	85	502	101	381	440
B. polaris SC223	4256	10	0	441	1056	0	530	248	529	85	534	101	381	351
B. pomorum SC053	3326	9	0	441	0	0	599	248 (1)	529	85	502	101	381	440
B. portchinsky SC072	3946	10	0	441	658	0	599	248 (1)	529	85	502	101	353	430 (12 'N')
B. potanini SC113	3318	9	0	441	0	0	599	248 (6)	521	85	502	101	381	440 (46)
B. pratorum SC075	3897	10	0	441	658	0	530	248	529	85	534	101	351	420
B. pressus SC239	1365	5	0	441	0	0	0	0	529	85	0	0	201	109
B. pseudobaicalensis SC253	4767	11	0	441	891	587	530 (2 'N')	248 (6)	529	85	534	101	381	440 (4)
B. pullatus SC312	4572	12	389	441	333	553	599	248 (1)	529	85	473 (1 'N')	101 (3 'N', 2)	381	440
B. pyrenaeus SC035	3984	10	0	441	658	0	599	248	529	85	502	101	381	440

Taxon	**TL (bp)**	**N**	**12S**	**16S**	**COI**	**CytB**	**Argk exon**	**Argk intron**	**EF1a exon**	**EF1a intron**	**Opsin exon**	**Opsin intron**	**PEPCK exon**	**PEPCK intron**
B. pyrosoma B04	1764	3	0	394	783	587	0	0	0	0	0	0	0	0
B. pyrrhopygus 3832	821	2	0	0	0	0	0	0	0	0	0	0	381	440
B. quadricolor SC090	3984	10	0	441	658	0	599	248 (2)	529	85	502	101	381	440 (37)
B. religiosus SC141	3777	10	0	441	657	0	599	248 (1)	529	85	296	101	381	440
B. remotus SC192	3326	9	0	441	0	0	599	248 (6)	529	85	502	101	381	440 (46)
B. robustus SC050	4152	11	0	441	333	553	539	248 (17)	529	85	502	101	381	440 (5)
B. rubicundus SC202	3326	9	0	441	0	0	599	248 (17)	529	85	502	101 (2)	381	440
B. ruderarius SC047	4443	11	0	441	658	506	530	248 (1)	519	85	534	101	381	440 (46)
B. r autumnalis RudA05	2788	6	0	0	849	459	0	0	574	85	0	0	381	440
B. r corsicola RudC19	2788	6	0	0	849	459	0	0	574	85	0	0	381	440
B. r ruderatus RudR20	2788	6	0	0	849	459	0	0	574	85	0	0	381	440
B. ruderatus SC018	4405	11	0	441	657	459	530	248 (1)	529	85	534	101	381	440
B. rufocinctus SC186	3916	10	0	441	627	0	530	248 (8)	529	85	534	101 (1)	381	440
B. rufofasciatus SC133	3297	9	0	441	0	0	599	248	529	85	502	101	352	440
B. rupestris SC009	3947	10	0	441	658	0	530	248 (1)	529	85	534	101	381	440 (37)
B. sandersoni SC255	3942	10	0	418	639 (4 'N')	0	599	248	529	85	502	101	381	440
B. schrencki SC298	3902	10	0	412	658	0	530	248 (6)	513	85	534	101	381	440 (4)
B. securus SC142	3984	10	0	441	658	0	599	248 (1)	529	85	502	101	381	440
B. semenoviellus SC236	3984	10	0	441	658	0	599	248 (32)	529	85	502	101	381	440
B. shaposhnikovi SC099	4574	11	0	441	849	459	539	248 (16)	529	85	502	101	381	440
B. sibiricus SC274	4522	11	0	441	709	587 (2 'N')	599	248	529	85	502	90	381	351
B. sichelii SC034	4063	11	0	441	658	465	599	248	529	85	196	21	381	440
B. simillimus SC243	3325	9	0	441	0	0	599	248	529	85	502	101	380	440
B. sitkensis SC144	3326	9	0	441	0	0	599	248 (7)	529	85	502	101	381	440
B. skorikovi SC159	3326	9	0	441	0	0	599	248 (2)	529	85	502	101	381	440 (37)

Taxon	TL (bp)	N	12S	16S	COI	CytB	Argk exon	Argk intron	EF1a exon	EF1a intron	Opsin exon	Opsin intron	PEPCK exon	PEPCK intron
B. sonani J6	2918	7	0	0	891	0	530	248	529	85	534	101	0	0
B. sonorus SC051	3316	9	0	431	0	0	599	248 (1)	529	85	502	101	381	440
B. sonorus SC318	3686	10	389 (1 'N')	441 (1)	0	0	599	248 (1)	529	85	473	101 (1 'N', 2)	381	440
B. soroeensis SC136	3947	10	0	441	658	0	530	248	529	85	534	101 (3)	381	440 (17)
B. sporadicus SC193	4405	11	0	441	620 (2 'N')	459	599	248 (61)	529	85	502	101	381	440 (40)
B. steindachneri SC313	3686	10	389 (1)	441	0	0	599	248 (1 'N', 1)	529	85	473	101 (2)	381	440
B. subterraneus SC046	4281	10	0	441	992	0	530	248 (1)	529	85	534	101	381	440
B. suckleyi SC091	3326	9	0	441	0	0	599	248 (1)	529	85	502	101	381	440 (37)
B. sulfureus SC064	3220	9	0	441	0	0	594	248	529	84	502	90	381	351
B. superbus B02	1858	5	0	441	0	0	0	0	0	0	502	101	381	433
B. supremus SC101	3983	10	0	441	657	0	599	248 (1)	529	85	502	101	381	440
B. sushkini SC143	3947	10	0	441	658	0	530	248 (1)	529	85	534	101	381	440
B. sylvarum SC110	3948	10	0	441	658	0	599	248 (1)	529	85	502	101	348	437 (44)
B. sylvestris SC020	4522	11	0	441 (1 'N')	658	587	599	248 (2)	516	85	466	101	381	440 (37)
B. sylvicola SC108	3949	10	0	441	650	0	599	248 (78)	529	85	502	101	354	440
B. ternarius SC116	4481	11	0	441	639 (2 'N')	553	530	248 (78)	529	85	534	101	381	440
B. terrestris SC003	4478	11	0	441	658	531	530	248	529	85	534	101	381	440 (47)
B. t xanthopus TerX19	2788	6	0	0	849	459	0	0	574	85	0	0	381	440 (47)
B. terricola SC205	3947	10	0	441	658	0	530	248	529	85	534	101	381	440 (40)
B. tibetanus SC134	3326	9	0	441	0	0	599	248 (1)	529	85	502	101	381	440 (37)
B. tichenkoi J1	2918	7	0	0	891	0	530	248 (1)	529	85	534	101	0	0
B. transversalis SC314	4758	12	389	441	658	414	599	248 (6)	529	85	473	101 (3 'N', 2)	381	440
B. tricornis SC148	4180	10	0	441 (1)	891	0	530	248	529	85	534	101	381	440
B. trifasciatus SC015	4265	10	0	441 (2)	980	0	530	248 (1)	525	85	534	101	381	440
B. trinominatus SC229	3326	9	0	441	0	0	599	248 (60)	529	85	502	101	381	440

Taxon	**TL (bp)**	**N**	**12S**	**16S**	**COI**	**CytB**	**Argk exon**	**Argk intron**	**EF1a exon**	**EF1a intron**	**Opsin exon**	**Opsin intron**	**PEPCK exon**	**PEPCK intron**
B. tucumanus SC276	3326	9	0	441	0	0	599	248 (17)	529	85	502	101	381	440 (5)
B. tunicatus SC248	1099	2	0	441	658	0	0	0	0	0	0	0	0	0
B. turkestanicus B03	1858	5	0	441	0	0	0	0	0	0	502	101	381	433
B. turneri B09	709	1	0	0	709	0	0	0	0	0	0	0	0	0
B. unicus COI 5P3	658	1	0	0	658	0	0	0	0	0	0	0	0	0
B. ussurensis SC130	3866	10	0	412 (2)	657	0	530	248 (1)	529	85	534	101	350	420 (6)
B. vagans SC044	3948	10	0	424	639	0	599	248	529	85	502	101	381	440
B. vandykei SC149	4226	10	0	441	937	0	530	248	529	85	534	101 (1 'N')	381	440
B. variabilis SC316	3297	9	0	441	0	0	599	248 (1)	529	85	473	101 (3 'N', 2)	381	440 (37)
B. velox SC094	3253	9	0	441	0	0	526	248 (1)	529	85	502	101	381	440 (46)
B. vestalis perezi Pere19	2788	6	0	0	849	459	0	0	574	85	0	0	381	440 (37)
B. vestalis SC169	4443	11	0	441	658	459	599	248 (1)	529	85	502	101	381	440 (37)
B. veteranus SC187	3984	10	0	441	658	0	599	248 (6)	529	85	502	101	381	440 (46)
B. vogti SC172	3326	9	0	441	0	0	599	248 (17)	529	85	502	101	381	440 (5)
B. volucelloides SC122	2468	7	0	441	0	0	530	248 (18)	529	85	534	101	0	0
B. vorticosus SC124	3159	9	0	441	0	0	533	248	529	84	502	90	381	351
B. vosnesenskii SC112	4100	10	0	441	811	0	530	248 (78)	529	85	534	101	381	440
B. waltoni SC102	3946	10	0	441	709	0	539	248 (12)	529	85	502	101	352	440
B. weisi SC315	3889	11	389 (1)	441	203	0	599	248 (89)	529	85	473 (1 'N')	101 (3 'N', 2)	381	440
B. wilemani SC182	441	1	0	441 (2)	0	0	0	0	0	0	0	0	0	0
B. wilmattae SC199	4137	10	0	441	811	0	599	248 (78)	529	85	502	101	381	440
B. wurflenii SC001	4802	11	0	441	960	553	530	248	529	85 (1 'N')	534	101	381	440
B. zonatus SC063	3326	9	0	441	0	0	599	248 (17)	529	85	502	101	381	440 (46)
Eufriesea pulchra	2414	5	0	0	550	0	530	0	759	85	490	0	0	0
Eufriesea surinamensis	2875	5	0	0	1069	0	583	0	636 (3 'N')	85	502	0	0	0

Taxon	TL (bp)	N	12S	16S	COI	CytB	Argk exon	Argk intron	EF1a exon	EF1a intron	Opsin exon	Opsin intron	PEPCK exon	PEPCK intron
Euglossa championi	3415	6	0	416 (10)	1050	0	582	0	795 (5 'N')	85	487	0	0	0
Euglossa imperialis	3624	7	0	383 (1)	871	534	467	0	759	85	525	0	0	0
Eulaema boliviensis	3586	7	0	441 (2)	1082	0	599	0	529	85	502	0	348	0
Eulaema nigrita	3236	7	0	416 (3)	641 (4 'N')	536	474 (2 'N')	0	597	85	487	0	0	0
Geniotrigona thoracica	2423	6	0	441 (1)	0	0	556	0	494 (5 'N')	85	475	0	372	0
Heterotrigona itama	2408	6	0	441 (2)	0	0	539	0	506	85	486	0	351	0
Hypotrigona gribodoi	2411	6	0	441	0	0	539	0	542 (1 'N', 3)	85 (1)	443	0	361	0
Liotrigona mahafalya	2739	6	0	441	665	0	539	0	566 (1 'N')	85 (1)	443 (1 'N')	0	0	0
Plebeia frontalis	2451	6	0	441	0	0	539	0	566	85 (1)	443	0	377	0
Trigona amazonensis	2464	6	0	441 (2)	0	0	530	0	529	85 (1)	502	0	377 (3)	0

Table S5. Table with distribution for each bumblebee species. The column distribution contains the data set used in BioGeoBEARS (BioGeography with Bayesian – and likelihood – Evolutionary Analysis in R Scripts). The biogeographic regions followed (Williams, 1996) and http://www.nhm.ac.uk/research-curation/research/projects/bombus/regions.html.

Species	BioGeoBEARS	Distribution	Collection locality	Voucher
B. affinis	G	Nearctic Region	Illinois, USA	Voucher 167
B. alagesianus	F,H	Oriental, Palaearctic Regions	Artvin Prov., Turkey	Voucher 85
B. alboanalis	F,G	Palaearctic, Nearctic Regions	Alaska, USA	Voucher 257
B. alpinus	F	Palaearctic Region	Gurgltal, Austria	Voucher 29
B. amurensis	F,H	Oriental, Palaearctic Regions		COI 5P2
B. anachoreta	F,H	Palaearctic, Oriental Regions		AN00001
B. apollineus	F	Palaearctic Region	Erzurum Prov., Turkey	Voucher 84
B. applanatus	B,C,D	Neotropical Region	Bahia, Brazil	EF209
B. appositus	G	Nearctic Region	Utah, USA	Voucher 145
B. ardens	F,H	Oriental, Palaearctic Regions	Dae-Dong, S. Korea	Voucher 131
B. argillaceus	F	Palaearctic Region	Kayseri Prov., Turkey	Voucher 58
B. armeniacus	F,H	Oriental, Palaearctic Regions	Kayseri Prov., Turkey	Voucher 80
B. ashtoni	F,G,H	Palaearctic, Oriental, Nearctic Regions	Ottawa, Canada	Voucher 164
B. asiaticus	F,H	Oriental, Palaearctic Regions	Himachal Pradesh, Indi	Voucher 249
B. atripes	H	Oriental Region	Zhejiang Prov., China	Voucher 66
B. auricomus	G	Nearctic Region	Illinois, USA	Voucher 62
B. avanus	H	Oriental Region	Luojishan, Sichuan, China	Voucher 272
B. avinoviellus	H	Oriental Region	Himachal Pradesh, India	Voucher 242
B. baeri	A,B,C	Neotropical Region	Puno, Peru	Voucher 174
B. bahiensis	D	Neotropical Region	Ilhéus, Bahia, Brazil	52893

Species	**BioGeoBEARS**	**Distribution**	**Collection locality**	**Voucher**
B. balteatus	F	Palaearctic Region	Lappland Co., Sweden	Voucher 39
B. barbutellus	F	Palaearctic Region	Uppland Co., Sweden	Voucher 73
B. beaticola	H	Oriental Region		J5
B. bellicosus	C,D	Neotropical Region	Buenos Aires, Argentina	Voucher 221
B. bicoloratus	H	Oriental Region	Nantou, Taiwan	Voucher 225
B. bifarius	G	Nearctic Region	New Mexico, USA	Voucher 208
B. bimaculatus	G	Nearctic Region	Arkansas, USA	Voucher 218
B. biroi	F,H	Palaearctic, Oriental Regions	Ketmen Mts., Kazakhstan	Voucher 210
B. bohemicus	F,G,H	Palaearctic, Oriental, Nearctic Regions	E. Pyrenees, France	Voucher 55
B. borealis	G	Nearctic Region	Maine, USA	Voucher 250
B. brachycephalus	A,G	Nearctic, Neotropical Regions	Guerrero, Mexico	Voucher 230
B. brasiliensis	C,D	Neotropical Region	Paraná, Brazil	Voucher 219
B. brasiliensis	C,D	Neotropical Region	Pedregulho, São Paulo, Brazil	1218400
B. breviceps	H	Oriental Region	Chiang Mai, Thailand	Voucher 190
B. brevivillus gr	B,C,D	Neotropical Region	Prata, Paraíba, Brazil	1200916
B. brevivillus gr	B,C,D	Neotropical Region	Remígio, Paraíba, Brazil	1202754
B. brevivillus gr	B,C,D	Neotropical Region	Ubajara, Ceará, Brazil	1207320
B. brevivillus gr	B,C,D	Neotropical Region	Ubajara, Ceará, Brazil	1207321
B. brevivillus gr	B,C,D	Neotropical Region	Ubajara, Ceará, Brazil	1207210
B. brodmannicus	F	Palaearctic Region	Artvin Prov., Turkey	Voucher 77
B. californicus	G	Nearctic Region	Alberta, Canada	Voucher 306
B. caliginosus	G	Nearctic Region	California, USA	Voucher 150
B. campestris	F	Palaearctic Region	Dalarna Co., Sweden	Voucher 40
B. caucasicus	F	Palaearctic Region		L084TR
B. centralis	G	Nearctic Region	Washington, USA	Voucher 146
B. chinensis	H	Oriental Region	Qionglai Sh, Sichuan, China	Voucher 152
B. cingulatus	F	Palaearctic Region	Lappi, Finland	Voucher 212

Species	**BioGeoBEARS**	**Distribution**	**Collection locality**	**Voucher**
B. citrinus	G	Nearctic Region	Virginia, USA	Voucher 170
B. coccineus	A	Neotropical Region	Huarochiri, Peru	Voucher 137
B. confusus	F	Palaearctic Region	Dorres, France	Voucher 83
B. consobrinus	F,H	Oriental, Palaearctic Regions	Altai Mts, Kazakhstan	Voucher 261
B. convexus	H	Oriental Region	Qionglai Sh, Sichuan, China	Voucher 109
B. coreanus	F,H	Oriental, Palaearctic Regions		J4
B. cornutus	H	Oriental Region	Ningnan, Sichuan, China	Voucher 271
B. crotchii	G	Nearctic Region	California, USA	Voucher 71
B. cryptarum	H	Palaearctic Region	Erzincan Prov., Turkey	Voucher 127
B. cullumanus	F	Palaearctic Region		COI-5P4
B. czerskii	F,H	Oriental, Palaearctic Regions		CZ00024
B. dahlbomii	C,E	Neotropical Region	Arauco Prov., Chile	Voucher 16
B. defector	F	Palaearctic Region		J3
B. deuteronymus	F,H	Palaearctic, Oriental Regions	Primorskiy Krai, Russia	Voucher 147
B. difficillimus	F,H	Oriental, Palaearctic Regions	Hongyuan, Sichuan, China	Voucher 154
B. digressus	A,G	Nearctic, Neotropical Regions	R. Grande de Orosí, Mexico	Voucher 307
B. diligens	G	Nearctic Region	Jalisco, Mexico	Voucher 171
B. distinguendus	G	Palaearctic, Nearctic Regions	Edesbecka, Finland	Voucher 197
B. diversus	F,H	Oriental, Palaearctic Regions	Kyushu, Japan	Voucher 120
B. ecuadorius	A	Neotropical Region	Abancay, Peru	Voucher 135
B. ephippiatus	A,G	Nearctic, Neotropical Regions	Chiapas, Mexico	Voucher 198
B. erzurumensis	F,H	Oriental, Palaearctic Regions	Artvin Prov., Turkey	Voucher 126
B. excellens	A,B,C	Neotropical Region	Aragua, Venezuela	Voucher 308
B. exil	F	Palaearctic Region	Hövsgöl Nuur, Mongolia	Voucher 232
B. eximius	H	Oriental Region	Alishan, Taiwan	Voucher 49
B. fernaldae	F,G	Palaearctic, Nearctic Regions	New Mexico, USA	Voucher 88
B. fervidus	G	Nearctic Region	Missouri, USA	Voucher 309

Species	**BioGeoBEARS**	**Distribution**	**Collection locality**	**Voucher**
B. festivus	H	Oriental Region	Qionglai Sh, Sichuan, China	Voucher 104
B. filchnerae	F,H	Palaearctic, Oriental Regions	Hongyuan, Sichuan, China	Voucher 206
B. flavescens	H	Oriental Region	Mei-fang, Taiwan	Voucher 181
B. flavidus	F,G	Palaearctic, Nearctic Regions	Lappland Co., Sweden	Voucher 41
B. flavifrons	G	Nearctic Region	California, USA	Voucher 95
B. formosellus	F,H	Oriental, Palaearctic Regions		J2
B. fragrans	F	Palaearctic Region	Kayseri Prov., Turkey	Voucher 61
B. franklini	G	Nearctic Region	Oregon, USA	Voucher 256
B. fraternus	G	Nearctic Region	Illinois, USA	Voucher 183
B. frigidus	G	Nearctic Region	Alaska, USA	Voucher 185
B. friseanus	H	Oriental Region	Qionglai Sh, Sichuan, China	Voucher 105
B. funebris	A,B	Neotropical Region	Abancay, Peru	Voucher 128
B. funerarius	H	Oriental Region	Luojishan, Sichuan, Chin	Voucher 270
B. gerstaeckeri	F	Palaearctic Region	E. Pyrenees, France	Voucher 65
B. grahami	H	Oriental Region	Meigu, Sichuan, China	Voucher 273
B. griseocollis	G	Nearctic Region	Illinois, USA	Voucher 82
B. haematurus	F	Palaearctic Region	Trabzon Prov., Turkey	Voucher 211
B. haemorrhoidalis	H	Oriental Region	Chiang Mai Prov., Thailand	Voucher 191
B. handlirschi	A,B,C	Neotropical Region	Pasco, Peru	Voucher 132
B. handlirschianus	F	Palaearctic Region	Artvin Prov., Turkey	Voucher 87
B. hedini	F,H	Palaearctic, Oriental Regions	Qionglai Sh, Sichuan, China	Voucher 129
B. honshuensis	H	Oriental Region		J7
B. hortorum	F	Palaearctic Region	Toscana, Italy	Voucher 5
B. hortorum jonghei	F	Palaearctic Region		HorJ01
B. hortorum hortorum	F	Palaearctic Region		HorH01
B. hortulanus	A,B	Neotropical Region	Magdalena, Colombia	Voucher 200
B. humilis	F,H	Palaearctic, Oriental Regions	E. Pyrenees, France	Voucher 56

Species	BioGeoBEARS	Distribution	Collection locality	Voucher
B. huntii	G	Nearctic Region	Washington, USA	Voucher 151
B. hyperboreus	F	Palaearctic Region	Dalarna Co., Sweden	Voucher 70
B. hypnorum	F,H	Palaearctic, Oriental Regions	Klösterle, Austria	Voucher 78
B. hypnorum	F,H	Palaearctic, Oriental Regions	Qionglai Sh, Sichuan, China	Voucher 207
B. hypocrita	F,H	Oriental, Palaearctic Regions	Kyushu, Japan	Voucher 123
B. ignitus	H	Oriental Region	Beijing, China	Voucher 96
B. imitator	H	Oriental Region	Guizhou Prov., China	Voucher 28
B. impatiens	G	Nearctic Region	Illinois, USA	Voucher 60
B. impetuosus	H	Oriental Region	Xinmian, Sichuan, China	Voucher 284
B. incertus	F	Palaearctic Region	Erzurum Prov., Turkey	Voucher 86
B. inexspectatus	F	Palaearctic Region		B05
B. infirmus	H	Oriental Region	Qionglai Sh, Sichuan, China	Voucher 157
B. infrequens	H	Oriental Region	Qionglai Sh, Sichuan, China	Voucher 140
B. insularis	G	Nearctic Region	California, USA	Voucher 162
B. jacobsoni	H	Oriental Region		COI-5P5
B. jonellus	F,G	Palaearctic, Nearctic Regions	Lappland Co., Sweden	Voucher 79
B. kashmirensis	F,H	Oriental, Palaearctic Regions	Hongyuan, Sichuan, China	Voucher 121
B. keriensis	F,H	Oriental, Palaearctic Regions	Hongyuan, Sichuan, China	Voucher 114
B. kirbiellus	G	Nearctic Region		Voucher 3534
B. koreanus	F,H	Oriental, Palaearctic Regions	Kangwondo, S. Korea	Voucher 277
B. kulingensis	H	Oriental Region	Zhejiang Prov., China	Voucher 97
B. ladakhensis	F,H	Oriental, Palaearctic Regions	Aba, Sichuan, China	Voucher 158
B. laesus	F,H	Palaearctic, Oriental Regions	Kars Prov., Turkey	Voucher 52
B. lantschouensis	F,H	Palaearctic, Oriental Regions		6873A01
B. lapidarius	F	Palaearctic Region	San Quírico, Italy	Voucher 6
B. lapponicus	F	Palaearctic Region	Lappland Co., Sweden	Voucher 103
B. lemniscatus	H	Oriental Region	Qionglai Sh, Sichuan, China	Voucher 161

Species	**BioGeoBEARS**	**Distribution**	**Collection locality**	**Voucher**
B. lepidus	H	Oriental Region	Qionglai Sh, Sichuan, China	Voucher 155
B. longipennis	H	Oriental Region		Voucher 1551
B. longipes	F,H	Oriental, Palaearctic Regions	Qionglai Sh, Sichuan, China	Voucher 194
B. lucorum	F,H	Palaearctic, Oriental Regions	E. Pyrenees, France	Voucher 217
B. lucorum	F,H	Palaearctic, Oriental Regions	Qionglai Sh, Sichuan, China	Voucher 184
B. lucorum lucorum	F,H	Palaearctic, Oriental Regions		LucL24
B. lucorum renardi	F,H	Palaearctic, Oriental Regions		LucR18
B. luteipes	H	Oriental Region	Pokhara, Nepal	Voucher 195
B. macgregori	G	Nearctic Region	Guerrero, Mexico	Voucher 231
B. magnus	F	Palaearctic Region		B08
B. margreiteri	F,H	Palaearctic, Oriental Regions		B01
B. maxillosus	F	Palaearctic Region	Kayseri Prov., Turkey	Voucher 74
B. medius	A,G	Nearctic, Neotropical Regions	Chiapas, Mexico	Voucher 222
B. melaleucus	A,B,C	Neotropical Region	Oxapampa, Peru	Voucher 173
B. melanopygus	G	Nearctic Region	California, USA	Voucher 215
B. melanurus	F,H	Oriental, Palaearctic Regions	Dzungarskij Alatau, Kazakh	Voucher 22
B. mendax	F	Palaearctic Region	Gurgltal, Austria	Voucher 19
B. mesomelas	F	Palaearctic Region	Switzerland	Voucher 37
B. mexicanus	A,G	Nearctic, Neotropical Regions	Chiapas, Mexico	Voucher 220
B. miniatus	H	Oriental Region	Himachal Pradesh, India	Voucher 244
B. minshanensis	H	Oriental Region		6873B05
B. mixtus	G	Nearctic Region	Washington, USA	Voucher 24
B. mlokosievitzii	F	Palaearctic Region	Artvin Prov., Turkey	Voucher 81
B. moderatus	H	Nearctic Regions	Alberta, Canada	Voucher 163
B. modestus	F,H	Oriental, Palaearctic Regions	Aba, Sichuan, China	Voucher 160
B. modestus	F,H	Oriental, Palaearctic Regions	S. Altai, Kazakhstan	Voucher 238
B. mongolensis	F	Palaearctic Region		COI-5P

Species	**BioGeoBEARS**	**Distribution**	**Collection locality**	**Voucher**
B. monticola	F	Palaearctic Region	E. Pyrenees, France	Voucher 176
B. morio	A,C,D	Neotropical Region	Porto Alegre, Brazil	Voucher 310
B. morio	A,C,D	Neotropical Region	Catalão, Goiáis, Brazil	b13
B. morrisoni	G	Nearctic Region	Utah, USA	Voucher 196
B. mucidus	F	Palaearctic Region	E. Pyrenees, France	Voucher 59
B. muscorum	F	Palaearctic Region	Öland Co., Sweden	Voucher 33
B. muscorum pereziellus	F	Palaearctic Region		Perl01
B. natvigi	G	Nearctic Region		B06
B. neoboreus	G	Nearctic Region	Alaska, USA	Voucher 188
B. nevadensis	G	Nearctic Region	Alberta, Canada	Voucher 139
B. niveatus	F	Palaearctic Region	Kayseri Prov., Turkey	Voucher 93
B. nobilis	H	Oriental Region	Qionglai Sh, Sichuan, China	Voucher 98
B. norvegicus	F,H	Palaearctic, Oriental Regions	Dalarna Co., Sweden	Voucher 89
B. oberti	F	Palaearctic Region	Mt. Kailas, Tibet	Voucher 234
B. occidentalis	G	Nearctic Region	Montana, USA;New Mexico, USA	Voucher 26
B. opifex	A,B,C	Neotropical Region	Puno, Peru	Voucher 175
B. parthenius	H	Oriental Region	Himachal Pradesh, India	Voucher 241
B. pascuorum	F	Palaearctic Region	Toscana, Italy	Voucher 23
B. patagiatus	F,H	Oriental, Palaearctic Regions	Hongyuan, Sichuan, China	Voucher 111
B. pauloensis	A,B,C,D	Neotropical Region	Ribeirão Preto, Brazil	Voucher 305
B. pauloensis	A,B,C,D	Neotropical Region	Salinas, Minas Gerais, Brazil	77202
B. pensylvanicus	G	Nearctic Region	Missouri, USA	Voucher 317
B. pensylvanicus	G	Nearctic Region	Missouri, USA	Voucher 311
B. perplexus	G	Nearctic Region	Ottawa, Canada	Voucher 166
B. persicus	F	Palaearctic Region	Kars Prov., Turkey	Voucher 54
B. personatus	H	Oriental Region	Hongyuan, Sichuan, China	Voucher 138
B. picipes	F,H	Oriental, Palaearctic Regions	Qionglai Sh, Sichuan, China	Voucher 180

Species	**BioGeoBEARS**	**Distribution**	**Collection locality**	**Voucher**
B. polaris	G	Nearctic Region	Lappland Co., Sweden	Voucher 223
B. pomorum	F	Palaearctic Region	Erzincan Prov., Turkey	Voucher 53
B. portchinsky	F	Palaearctic Region	Artvin Prov., Turkey	Voucher 72
B. potanini	H	Oriental Region	Hongyuan, Sichuan, China	Voucher 113
B. pratorum	F	Palaearctic Region	Klösterle, Austria	Voucher 75
B. pressus	H	Oriental Region	Uttaranchal, India	Voucher 239
B. pseudobaicalensis	F	Palaearctic Region	Primorskiy Kray, Russia	Voucher 253
B. pullatus	A,B	Neotropical Region	Pitilla, Costa Rica	Voucher 312
B. pyrenaeus	F	Palaearctic Region	Gurgltal, Aust	Voucher 35
B. pyrosoma	F,H	Oriental, Palaearctic Regions		B04
B. pyrrhopygus	F	Palaearctic Region		Voucher 3832
B. quadricolor	F	Palaearctic Region	Uppland Co., Sweden	Voucher 90
B. religiosus	H	Oriental Region	Qionglai Sh, Sichuan, China	Voucher 141
B. remotus	H	Oriental Region	Qionglai Sh, Sichuan, China	Voucher 192
B. robustus	A,B	Neotropical Region	Boyacá, Colombia	Voucher 50
B. rubicundus	A,B	Neotropical Region	Boyacá, Colombia	Voucher 202
B. ruderarius	F	Palaearctic Region	Obergurgl, Austria	Voucher 47
B. ruderatus	F	Palaearctic Region	Valdivia Prov., Chile	Voucher 18
B. ruderatus ruderatus	F	Palaearctic Region		RudR20
B. ruderatus autumnalis	F	Palaearctic Region		RudA05
B. ruderatus corsicola	F	Palaearctic Region		RudC19
B. rufocinctus	G	Nearctic Region	Alberta, Canada	Voucher 186
B. rufofasciatus	F,H	Oriental, Palaearctic Regions	Hongyuan, Sichuan, China	Voucher 133
B. rupestris	F,H	Palaearctic, Oriental Regions	Uppland Co., Sweden	Voucher 9
B. sandersoni	G	Nearctic Region	Isle au Haut, Maine, USA	Voucher 255
B. schrencki	F,H	Palaearctic, Oriental Regions	W. Khentey, N. Mongolia	Voucher 298
B. securus	H	Oriental Region	Qionglai Sh, Sichuan, China	Voucher 142

Species	**BioGeoBEARS**	**Distribution**	**Collection locality**	**Voucher**
B. semenoviellus	F	Palaearctic Region	Brandenburg, Germany	Voucher 236
B. shaposhnikovi	F	Palaearctic Region	Artvin Prov., Turkey	Voucher 99
B. sibiricus	F,H	Oriental, Palaearctic Regions	Khövsgöl Nuur, Mongolia	Voucher 274
B. sichelii	F,H	Oriental, Palaearctic Regions	Obergurgl, Austria	Voucher 34
B. simillimus	H	Oriental Region	Himachal Pradesh, India	Voucher 243
B. sitkensis	G	Nearctic Region	California, USA	Voucher 144
B. skorikovi	H	Oriental Region	Aba, Sichuan, China	Voucher 159
B. sonani	H	Oriental Region		J6
B. sonorus	G	Nearctic Region	Arizona, USA	Voucher 51
B. sonorus	G	Nearctic Region	Mexico	Voucher 318
B. soroeensis	F	Palaearctic Region	E. Pyrenees, France	Voucher 136
B. sporadicus	F	Palaearctic Region	Lappland Co., Sweden	Voucher 193
B. steindachneri	G	Nearctic Region	Morelos, Mexico	Voucher 313
B. subterraneus	F	Palaearctic Region	Uppland Co., Sweden	Voucher 46
B. suckleyi	G	Nearctic Region	Colorado, USA	Voucher 91
B. sulfureus	F	Palaearctic Region	Kayseri Prov., Turkey	Voucher 64
B. superbus	H	Oriental Region		B02
B. supremus	H	Oriental Region	Qionglai Sh, Sichuan, China	Voucher 101
B. sushkini	F,H	Palaearctic, Oriental Regions	Hongyuan, Sichuan, China	Voucher 143
B. sylvarum	F	Palaearctic Region	Uppsala Co., Sweden	Voucher 110
B. sylvestris	F,H	Oriental, Palaearctic Regions	Uppland Co., Sweden	Voucher 20
B. sylvicola	G	Nearctic Region	New Mexico, USA	Voucher 108
B. ternarius	G	Nearctic Region	Nova Scotia, Canada	Voucher 116
B. terrestris	F	Palaearctic Region	San Quírico, Italy	Voucher 3
B. terrestris xanthopus	F	Palaearctic Region		TerX19
B. terricola	G	Nearctic Region	Ontario, Canada	Voucher 205
B. tibetanus	H	Oriental Region	Min Shan, Sichuan, China	Voucher 134

Species	**BioGeoBEARS**	**Distribution**	**Collection locality**	**Voucher**
B. tichenkoi	F,H	Palaearctic, Oriental Regions		J1
B. transversalis	B	Neotropical Region	Madre de Dios, Peru	Voucher 314
B. tricornis	F,H	Oriental, Palaearctic Regions	Primorskiy Kray, Russia	Voucher 148
B. trifasciatus	H	Oriental Region	Himachal Pradesh, India	Voucher 15
B. trinominatus	G	Nearctic Region	Oaxaca, Mexico	Voucher 229
B. tucumanus	A,B,C	Neotropical Region	Tucumán, Argentina	Voucher 276
B. tunicatus	H	Oriental Region	Uttaranchal, India	Voucher 248
B. turkestanicus	F,H	Oriental, Palaearctic Regions		B03
B. turneri	H	Oriental Region		B09
B. unicus	F	Palaearctic Region		COI-5P3
B. ussurensis	F,H	Oriental, Palaearctic Regions	S. Korea, Mulan Valley	Voucher 130
B. vagans	G	Nearctic Region	Wisconsin, US	Voucher 44
B. vandykei	G	Nearctic Region	Washington, USA	Voucher 149
B. variabilis	A,G	Nearctic, Neotropical Regions	Missouri, USA	Voucher 316
B. velox	F	Palaearctic Region	Artvin Prov., Turkey	Voucher 94
B. vestalis	F	Palaearctic Region	Kent, England	Voucher 169
B. vestalis perezi	F	Palaearctic Region		Pere19
B. veteranus	F	Palaearctic Region	Jura, France	Voucher 187
B. vogti	A,B	Neotropical Region	Abancay, Peru	Voucher 172
B. volucelloides	A,B	Neotropical Region	San Jose, Costa Rica	Voucher 122
B. vorticosus	F	Palaearctic Region	Aksaray Prov., Turkey	Voucher 124
B. vosnesenskii	G	Nearctic Region	Washington, USA	Voucher 112
B. waltoni	H	Oriental Region	Qionglai Sh, Sichuan, China	Voucher 102
B. weisi	A,G	Nearctic, Neotropical Regions	Jalisco, Mexico	Voucher 315
B. wilemani	H	Oriental Region	Meifang, Taiwan	Voucher 182
B. wilmattae	A,G	Nearctic, Neotropical Regions	Chiapas, Mexi	Voucher 199
B. wurflenii	F	Palaearctic Region	Obergurgl, Austria	Voucher 1

Species	**BioGeoBEARS**	**Distribution**	**Collection locality**	**Voucher**
B. zonatus	F	Palaearctic Region	Aksaray Prov., Turkey	Voucher 63

Appendix 1

Divergence time estimates. We describe here below the paleontological evidence upon which our eight calibration points are based.

Calibration 1 –The fossil bee *Euglossa moronei* Engel, 1999, which is from Miocene Dominican Republic amber (Engel, 1999). Based on biostratigraphic and paleogeographic data, Dominican amber has been dated from of late Early Miocene through early Middle Miocene (20.43-13.65 Mya) age (Iturralde-Vinent & MacPhee, 1996). For this calibration point, we applied a lognormal distribution with mean of 1.0, Stdev of 0.8 and offset of 15 Mya (95% HPD: 15.6-28 Mya) to the node uniting *Euglossa* subgenus.

Calibration 2– *Apis lithohermaea* Engel, 2006 is the oldest fossil record for crown group *Apis* (15.97-13.65 Mya). The fossil is from the Chôjabaru Formation of Iki Island, Japan which has been estimated to be from the middle Miocene. Based on some key morphological characters, it was assigned to the *dorsata* species group and was described as being quite similar to modern *Apis dorsata* (Engel, 2006). This fossil therefore provides a minimum age for the diversification of *Apis dorsata* from *Apis cerana*, a member of the *mellifera* species group. A lognormal distribution with mean of 1.0, Stdev of 0.8 and offset of 10 Mya (95% HPD: 10.6-23) was applied as a prior for most recent common ancestor (MRCA) between *A. dorsata* and *A. cerana.*

Calibration 3 –The fossil *bee Eulaema (Apeulaema) zigrasi* Engel, 2014 (23.03-15.97 Mya), which is from Early Miocene Mexican amber (Engel, 2014). For this calibration point, we applied a lognormal distribution with mean of 0.8 and Sigma of 0.9 and offset of 16 Mya (95% HPD: 16.4-29 Mya) to the node uniting *Apeulaema* species.

Calibration 4 –The fossil bee *Paleoeuglossa melissiflora* Poinar, 1998 (20.43-13.65 Mya), which is from Dominican Republic (Poinar Jr, 1998). Belongs to *Eufriesea* according to Engel et al. (2012). For this calibration point, we applied a lognormal distribution with mean of 0.8 and Sigma of 0.9 and offset of 14 Mya (95% HPD: 14.4-27 Mya) to the node uniting *Eufriesea* species.

Calibration 5 –The fossil bee *Bombus (Bombus) randeckensis* Wappler & Engel, 2012 (18.97-13.65 Mya), which is from the Miocene Randeck Maar of southwestern Germany (Wappler et al. 2012). For this calibration point, we applied lognormal distribution with a mean of 0.8 and Sigma of 1 and offset of 14 Mya (95% HPD: 16.5-22.5 Mya) to the node uniting *Bombus* s.str.

Calibration 6 – *Oligobombus cuspidatus* Antropov 2014 (37.2-33.9 Mya), which if from the Late Eocene Bembridge Marls (A'Court Smith Collection) (Antropov et al., 2014). For this calibration point, we applied a lognormal distribution with mean of 1.2 and Sigma of 0.7 and offset of 33 Mya (95% HPD: 33.8-46.1 Mya) to the node uniting *Bombus* species.

Calibration 7 –*Cretotrigona prisca* (Michener and Grimaldi 1988) (70.6-66.043 Mya) is the oldest fossil bee and the oldest record of eusocial behavior among the Apoidea (Michener & Grimaldi, 1988). This fossil is from Late Cretaceous New Jersey amber, Delaware River, Kinkora (Engel, 2000). For this calibration point, we applied a lognormal distribution with mean of 1 and Sigma of 0.9 and offset of 66 Mya (95% HPD: 66.5-81.9 Mya) to the node uniting Meliponina.

Calibration 8. The fossil bees *Liotrigonopsis rozeni* Engel 2001 and *Kelneriapis eocenica* (Kelner-Pillault, 1969) these fossils are from eocenic Baltic amber (AMNH collection) (Engel, 2001). For this calibration point, we applied a lognormal distribution with mean of 1 and Sigma of 0.8 and offset of 40 Mya (95% HPD: 40.6-53 Mya) to the node uniting *Liotrigona* and *Hypotrigona.*

Appendix 2

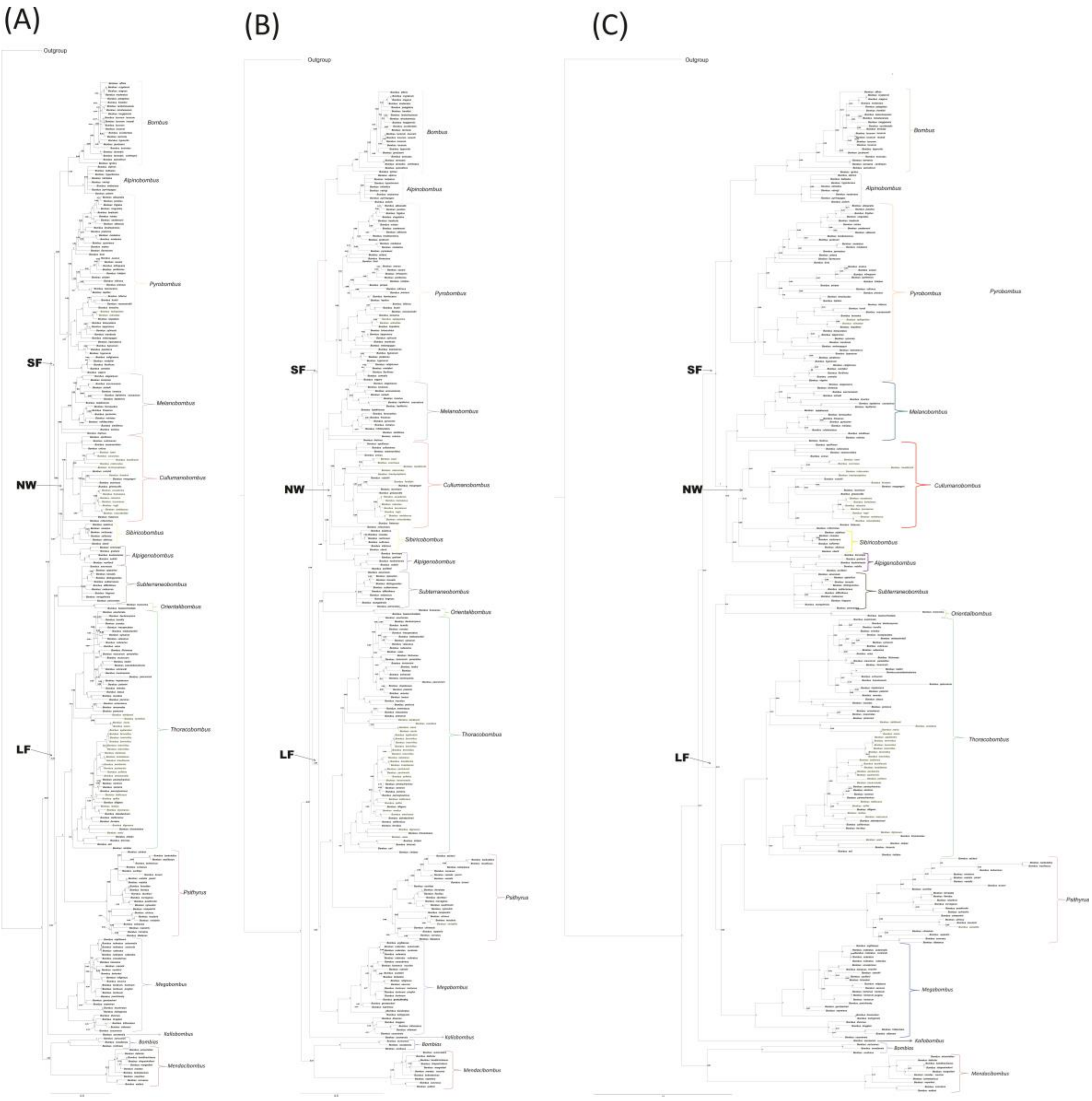

Fig. S1.2. Phylogenetic relationships among bumblebees using Bayesian inference - BI and all species available for each subgenus. Topologies recovered with the data of matrix presented in Table S4. Values shown under each branch are posterior probabilities. The letters shown between parentheses refer to the 150 (A), 250 (B) and 300 (C) millions of generations. SF LF1 – long-faced clade 1 and LF2 – long-faced clade 2. The standard deviations were 0.033707, 0.011348 and 0.011991.

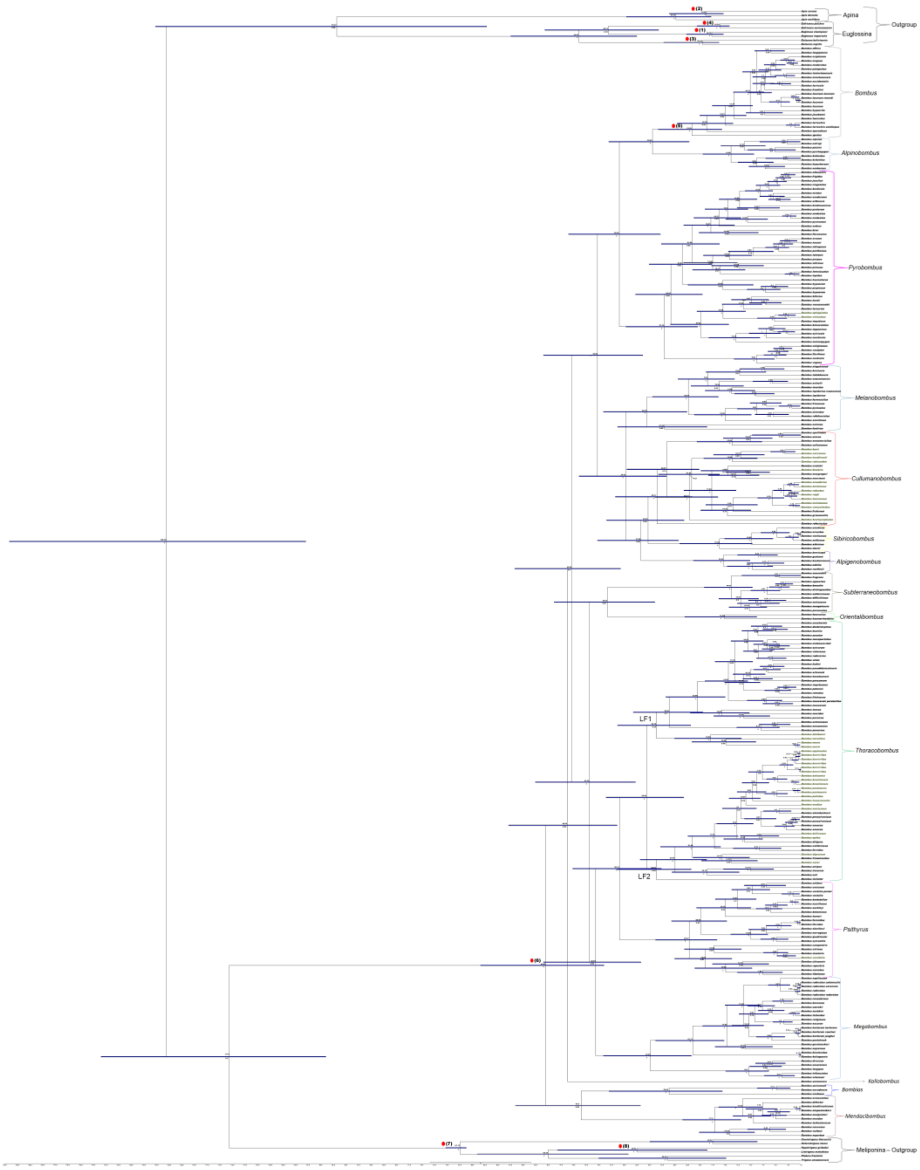

Fig. S2.2. Estimated divergence times using Unncorrelated Lognormal Relaxed Clock model (BEAST v2.4.4). Maximum clade credibilities tree depicting branch lengths equal to the median ages as calculated from 75,001 post burn-in chronograms (85%). Bars show 95% Highest Posterior Density intervals of age nodes. Calibration points are shown by the numbers in parentheses indicated around red dots, these refer to the fossils of appendix 1. The values shown above each branch are posterior probabilities. The red circles – calibration points; LF1 – long-faced clade 1 and LF2 – long-faced clade 2.

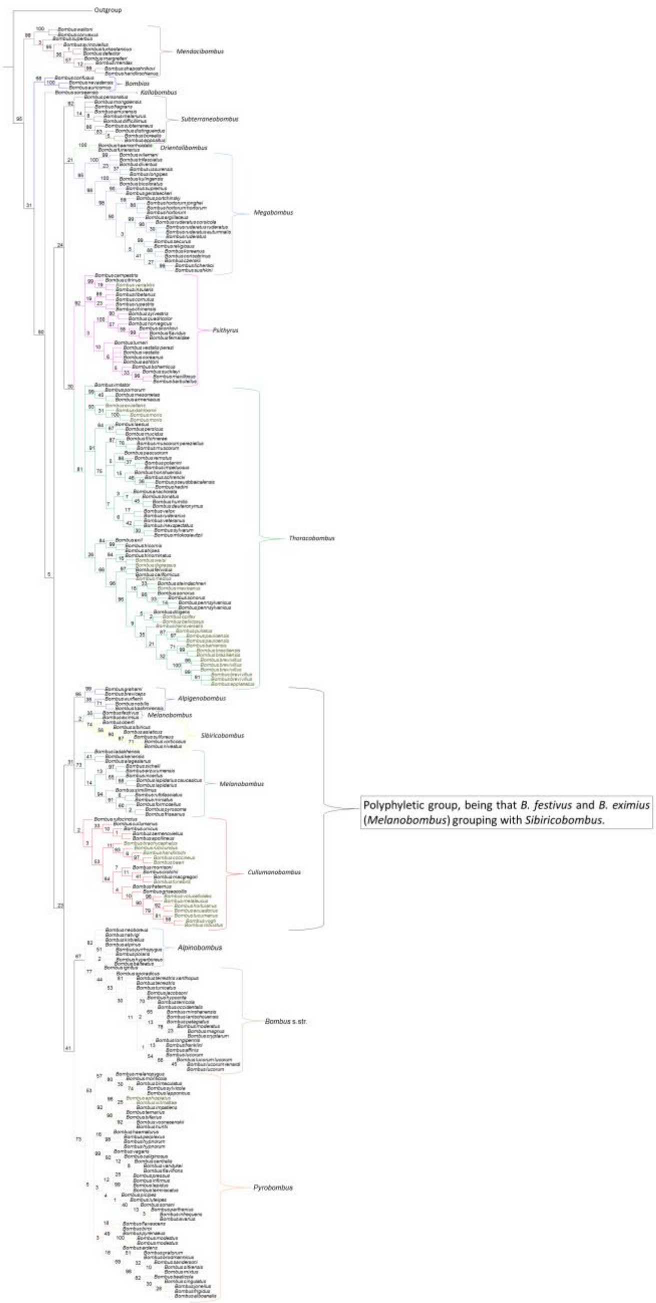

Fig. S3.2. Strict consensus tree of Maximum Parsimony – MP analysis of bumblebees' species. Topologies recovered with the data of matrix presented in Table S4. The values shown above each branch are bootstrap values.

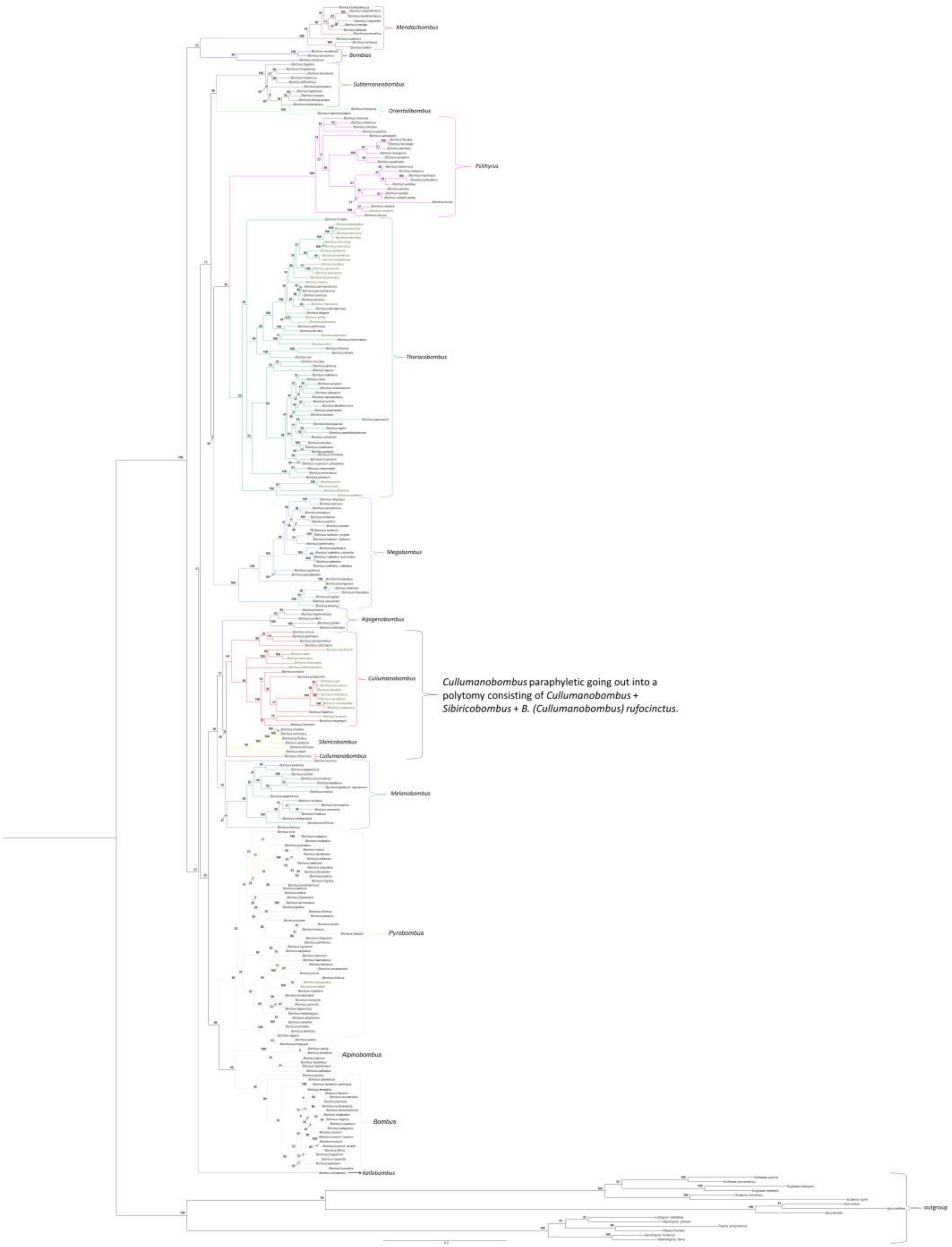

Fig. S4.2. Maximum Likelihood tree – ML search obtained with RAxML. Topologies recovered with the data of matrix presented in Table S4. Bootstrap support values are indicated above each branch.

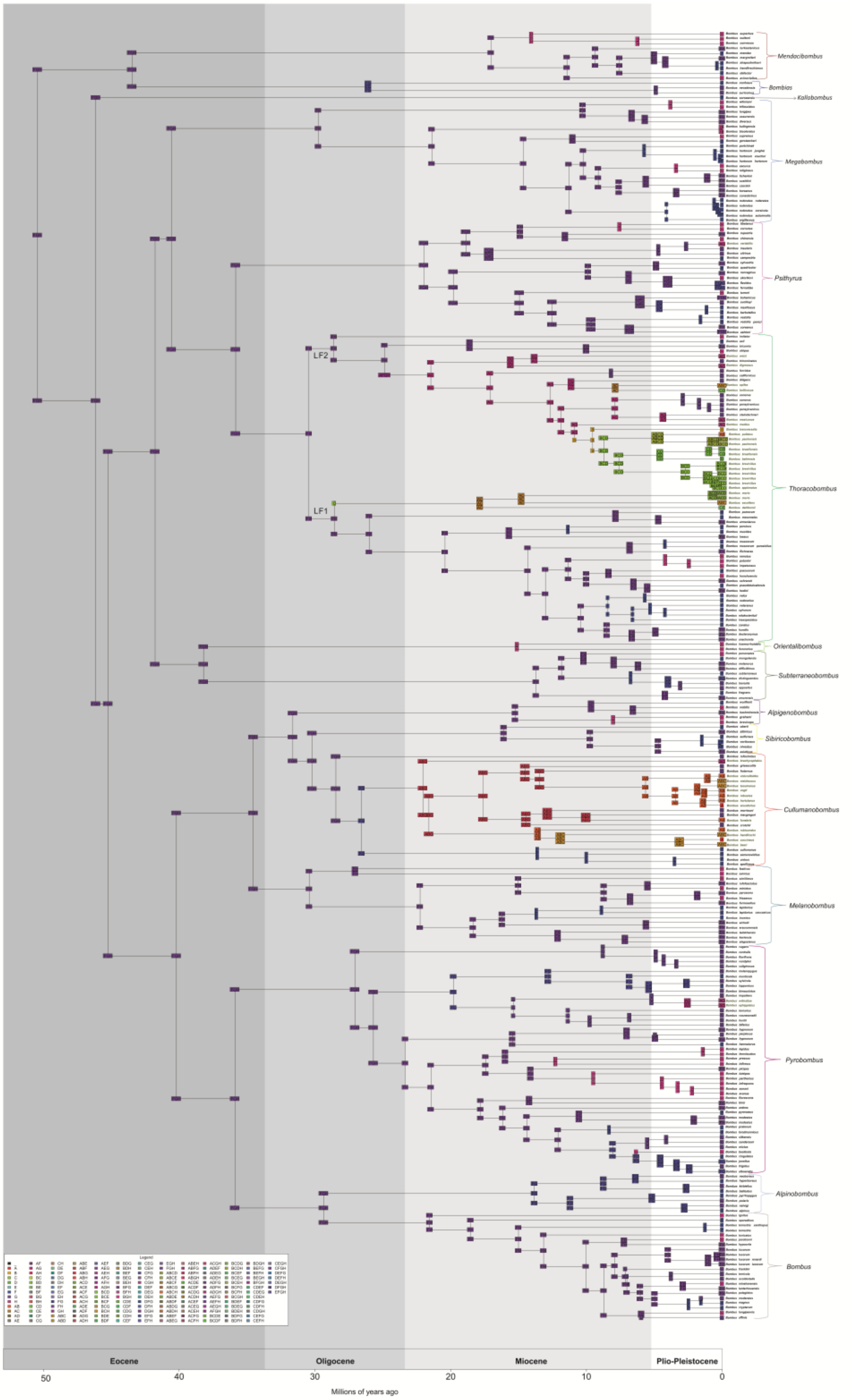

Fig. S5.2. Global ancestral state estimates under the unconstrained model for the BAYAREALIKE+J. Time tree for Bumblebees obtained under an Unncorrelated Lognormal Relaxed Clock model (UCLN) calibrated with eight fossils (Appendix, S2.2 Fig.). In map, Fig. 1, are letters and number showed the areas. LF1 – long-faced clade 1 and LF2 – long-faced clade 2.

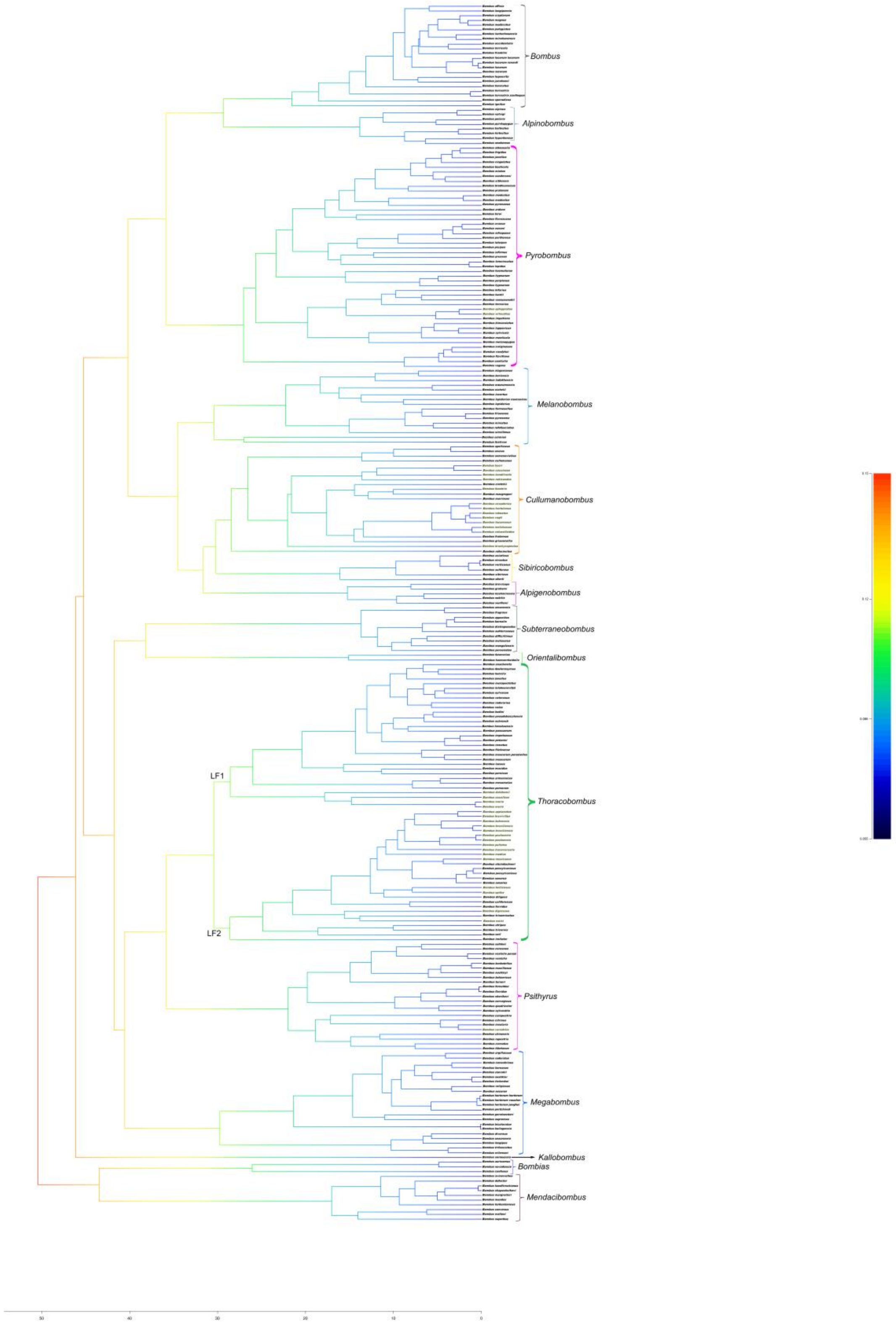

Fig. S6.2. Bumblebee species chronogram with shading of branches reflective of estimated diversification rates (see scale at right) estimated in BAMM. Diversifications are the means of the marginal densities of the rates.

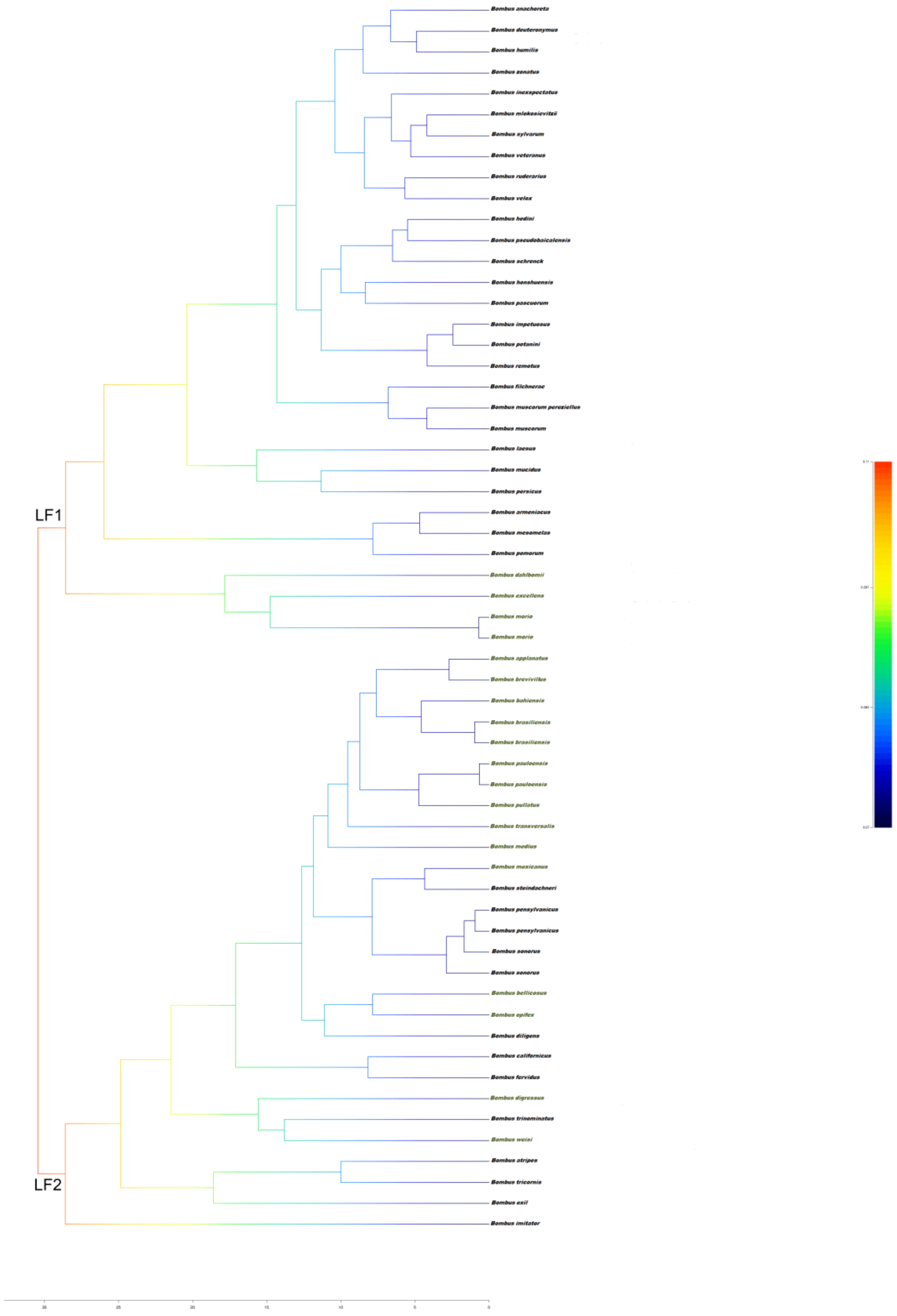

Fig. S7.2. *Thoracobombus* species chronogram with shading of branches reflective of estimated diversification rates (see scale at right) estimated in BAMM. Diversifications are the means of the marginal densities of the rates.

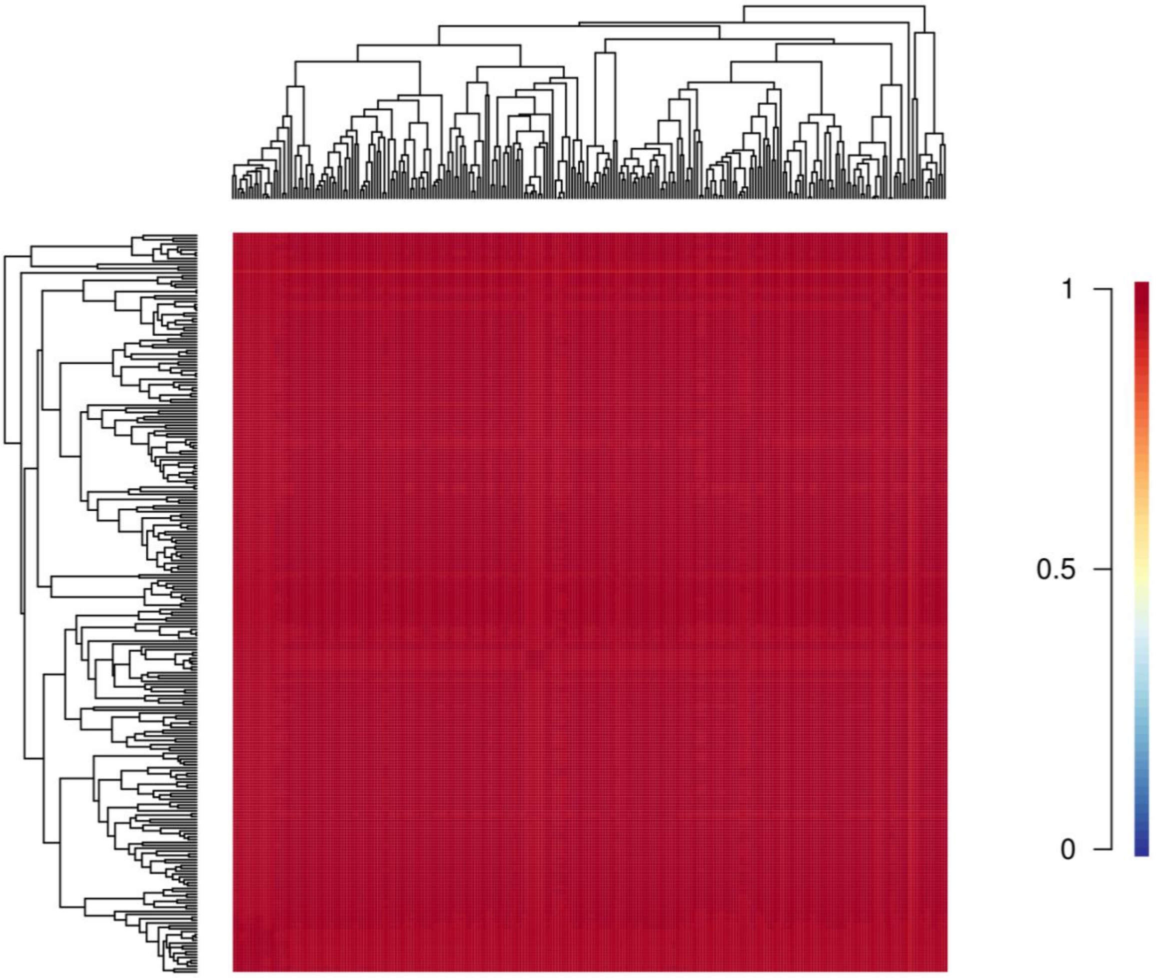

Fig. S8.2. Macroevolutionary cohort matrix for diversification in bumblebees. Pairs of taxa with highly similar rates are depicted in hotter colors (red) and dissimilar rates are shown as colder colors (blue).

Fig. S9.2. Global ancestral state estimates under the unconstrained model for the BAYAREALIKE+J. Time tree for *Pyrobombus*. In map, Fig. 1, are letters and number showed the areas.

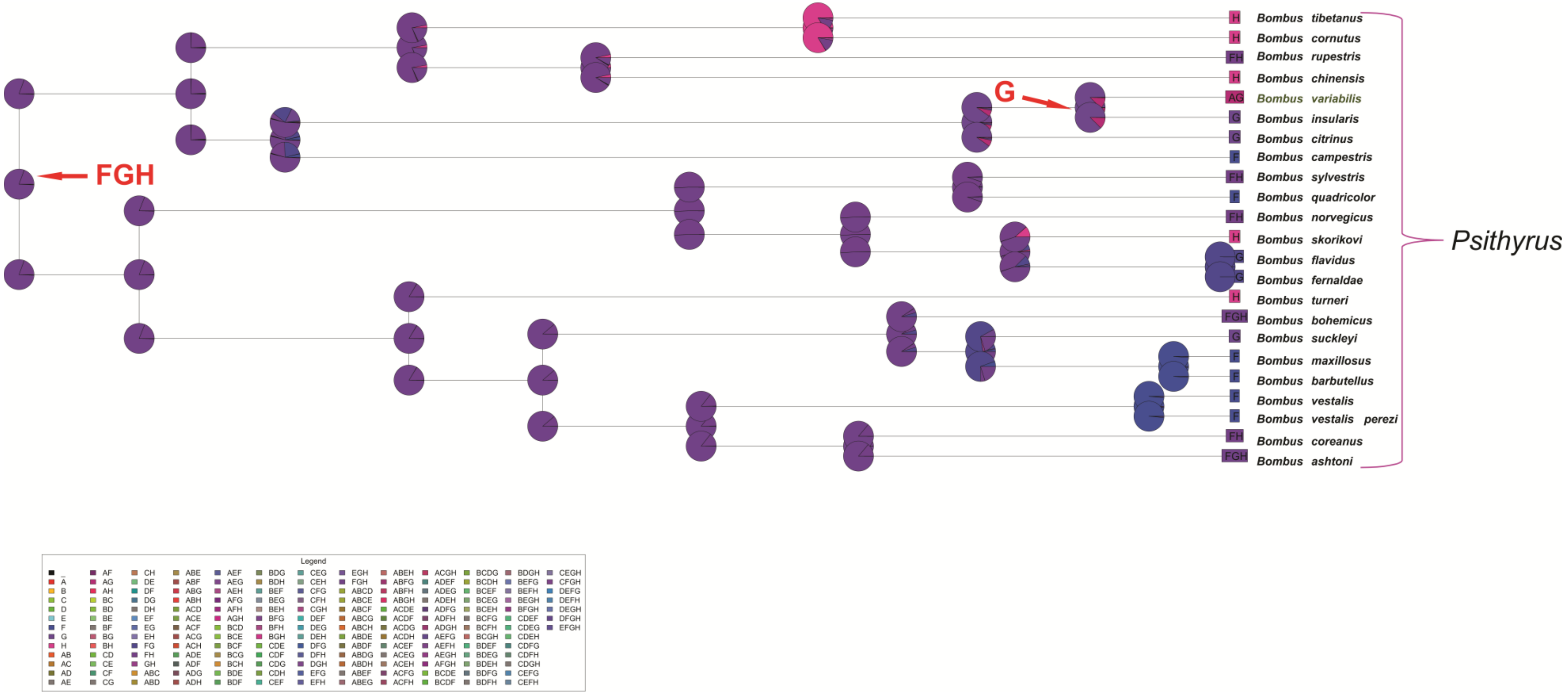

Fig. S10.2. Global ancestral state estimates under the unconstrained model for the BAYAREALIKE+J. Time tree for *Psithyrus*. In map, Fig. 1, are letters and number showed the areas.

CONCLUSÕES GERAIS

O presente trabalho fornece, uma varredura dos principais processos que moldaram a distribuição biogeográfica das espécimes de mamangavas neotropicas. Além da descrição de uma nova espécie e e dos possíveis eventos que levaram a diversificação dessas espécioes. O trabalho também fornece novas hipóteses a serem testadas em trabalhos futuros.

Todas as análises foram realizados pela primeira vez no presente grupo de pesquisa. No entanto, essas análises permitiram traçar estratégias para a conservação de algumas mamangavas brasileiras. Assim, o presente trabalho fornece material para várias outras pesquisas, além de ter realizado formação de recursos humanos em análise bioinformacional.

ANEXOS

Capítulo 1

Supporting Information

(http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0125847)

S1 Figure. Phylogenetic relationships among *Bombus brasiliensis* obtained from concatenated phylogenetic analyses performed with CytB and COI sequences, using Bayesian inference. Values shown under each branch are posterior probabilities. (TIF)

S1 Table. Specimens sequenced for the genetic analyses with their geographic origins. Universidade Federal de Minas Gerais—UFMG IHY; Universidade Federal de Santa Catarina— UFSC; Universidade Federal do Norte Fluminense—UENF; Universidade Federal de Ouro Preto—UFOP; Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto—Universidade de São Paulo—USP: FFCLRP; Universidade de São Paulo—USP/SP. (DOCX)

S2 Table. Protocol of the purification of Polyethylene Glycol 20% (PEG 20%) for elimination of bands <300–400 bp. (DOCX)

S3 Table. Genetic distances for COI sequences (%) between bumblebee-species. The model used was the Kimura 2-parameter. Bees currently considered as *B. brevivillus* belong in two distinct species, identified below as *B. brevivillus* (1) and *B. brevivillus* (2). AD = Average interspecific distance ± standard deviation; MiD = minimum interspecific distance ± standard deviation. (DOCX).