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Research Article

New insight into the phylogeny of the orchid bees (Apidae: Euglossini)

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Abstract

Orchid bees occur across the American continent, from the southern United States to Paraguay and northern Argentina. There are 240 described species of orchid bees. The phylogeny of these bees has been studied by several researchers. In most cases, phylogenetic trees with different topologies have been introduced, because the tree topology of the orchid bees is very unstable. In this work, using 244 gene sequences consisting of three mitochondrial genes (cytochrome b, cytochrome c oxidase I, and 16S ribosomal RNA) and a single nuclear gene (RNA polymerase II), the phylogenetic relationships within the tribe Euglossini were re-evaluated. Although we cannot describe the phylogenetic tree of the tribe Euglossini with confidence yet; I found that there are probably two distinct evolutionary pathways or two distinct evolutionary lineages in this tribe. Moreover, I found that the evolutionary pathway of the genus *Euglossa* is probably different from other genera belonging to the tribe Euglossini. Nevertheless, definite viewpoints on this matter need more studies.

Keywords: Euglossini, *Euglossa*, phylogeny, new insight, distinct lineages.

Introduction

The orchid bees are classified as under phylum: Arthropods, Class: Insects, Order: Hymenoptera, Sub-order: Apocrita, Infera-order: Acuelata, Super-family: Apoidea, Family: Apidae, Subfamily: Apinae, and Tribe: Euglossini (Michener 2007; Ghassemi-Khademi 2016). The orchid bees are only corbiculate apines (pollen basket bees) that do not exhibit large colonies and may rather be solitary, communal, semisocial, or social (Ramírez *et al.* 2002). These honeybees occur across the American continent, from the southern United States of America to Paraguay and northern Argentina, where they can make up to 25% of bee communities in Neotropical wet forests (Ferronato *et al*. 2017). Female orchid bees collect nectar, pollen, and resin fornest construction and brood-cell provisioning, while male bees collect perfume compounds from floral and nonfloral sources and use them during courtship display for attracting conspecific females (Brand *et al.* 2017).

The highest diversity of the orchid bees belongs to Amazon basin, in South America (Ferronato *et al*. 2017). There are 240 described species of orchid bees (Moure *et al.* 2012; McCravy *et al.* 2016) with medium to large sizes (8.5-29.0 mm length) distributed in five current genera: *Eufriesea*, *Euglossa*, *Eulaema*, and two cuckoo genera, *Aglae* (in nests of the genus *Eulaema*) and *Exaerete* (in nests of the genera: *Eulaema* and *Eufriesea*)(O'Toole and Raw 2004; Ramírez *et al.* 2002; Michener 2007; Carvalho-Filho and Oliveira 2017).

Except for the genus *Aglae*, which is known from South America and eastern Panama, each genus in this tribe ranges from Mexico to

Argentina, mostly in the moist forests (Michener 2007). Recently, the bees of this tribe have received serious attention in particular because they are very effective pollinators of nearly 700 orchid species, which is the main reason for calling them "orchid bees" (Fernandes *et al.* 2013). In addition, this tribe contains some of the most colorful and gaudy bees, many of which are metallic green, blue, and bronze or purple (O'Toole and Raw 2004). Probably, the tribe Euglossini has a common ancestry with the tribe Bombini (the bumblebees) (O'Toole and Raw 2004).

Meanwhile, phylogeny of corbiculate bees, especially the orchid bees (as a member of the corbiculate bees), have been studied by several researchers (Bembé 2007; Darveau *et al.* 2005; Roig-Alsina and Michener 1993; Schultz *et al.* 1999, 2001; Noll 2002; Michel-Salzat *et al.* 2004; Brand *et al.* 2017; Fernandes *et al.* 2013; Kawakita *et al.* 2008; Engel 1999; Ramírez *et al.* 2010; Penha *et al.* 2014). Each of these studies has introduced phylogenetic trees with different topologies because the tree topology of the orchid bees is very unstable. In this work, using the nucleotide sequences of three mitochondrial genes (cytochrome b, cytochrome c oxidase I, and 16S ribosomal RNA) and a single nuclear gene (RNA polymerase II), specially the submitted gene sequences in gen bank recently, I re-evaluated the phylogenetic relationships within the tribe Euglossini and provided a theory about the phylogeny of the orchid bees. Although this theory is probably close to reality, definite viewpoints need more investigations on this matter.

Material and methods

All gene sequences including cytochrome b (n=87), cytochrome c oxidase I (n=55), RNA polymerase II (n= 78), and 16S ribosomal RNA (or 16S rRNA) (n=24) genes (A total of 244 gene sequences) belonging to the tribe Euglossini were downloaded from NCBI (Tabs: 1, 2, 3, and 4). The sequences were aligned with Mega6 (Tamura *et al.* 2013) using the Clustral W alignment method.

The evolutionary history was inferred using the Maximum Likelihood method for each of the studied genes separately. The trees were calculated with the highest log-likelihood. In all of the phylogenetic trees, the percentage of replicate trees, in which the associated taxa are clustered together in the bootstrap test (1000 replicates), were shown next to the branches (Felsenstein 1985).

In all above analyses, all positions containing gaps and missing data were eliminated. All the evolutionary analyses were computed using the Kimura 2-parameter method (Kimura 1980) and were conducted in MEGA6 (Tamura *et al.* 2013). Also, the robustness of clades was calculated by the bootstrap method. In this study, 50-60% was considered as a weak support (as bootstrap values), 61-75% as a moderate support, 76-88% as a good support, and ≥89% as a strong support as values (Retrieved from Win *et al.* (2017), with minor modification).

In addition, Bayesian analyses of studied gene sequences were run with the parallel version of MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) on a Linux cluster with one processor assigned to each Markov chain under the most generalizing model (GTR+G+I) because overparametrization apparently does not negatively affect Bayesian analyses (Huelsenbeck and Ranala, 2004). Each Bayesian analysis comprised two simultaneous runs of four Metropolis-coupled Markov-chains at the default temperature (0.2). Analyses were terminated after the chains converged significantly, as indicated by the average standard deviation of split frequencies<0.01. Bayesian inference of phylogeny was conducted for 6,000,000 generations. In this study, 700 bootstrap replicates were used as ML branch support values. The posterior probabilities equal/higher than 0.95 and bootstrap supports equal/higher than 70% were considered as strong support values (Sayyadzadeh *et al.* 2016).

Table 1. The accession numbers of cytochrome b genes and scientific names of studied species from the tribe Euglossini (n=87) received from GenBank (www.ncbi.nlm.nih.gov)(n=89).

Species	Accession Numbers	Scientific Names	Accession Numbers
Eufriesea schmidtiana	AY916094	*Euglossa flammea*	AY916116
Eugloss aiopoecila	KF443107	*Euglossa asarophora*	AY916118
	KX249839	*Euglossa allosticta*	AY916119
	KX249852	*Euglossa crassipunctata*	AY916120
	KX249853	*Euglossa dodsoni*	AY916121
	KF443124	*Euglossa gorgonensis*	AY916122
Euglossa townsendi	KF443108	*Euglossa allosticta*	AY916119
	KF443109	*Euglossa crassipunctata*	AY916120
	KF443110	*Euglossa dodsoni*	AY916121
	KF443111	*Euglossa gorgonensis*	AY916122
	KF443112	*Euglossa allosticta*	AY916119
	KF443113	*Eulaema bombiformis*	KF888747
	KF443114		KF888751
	KF443115		KF888755
Euglossa stellfeldi	KF443116		KF888756
	KF443117		KF888753
	KF443118		KF888815
	KF443119		KF888816
	KF443120		
	KF443121		
	KF443122	*Eulaema meriana*	KF888818
	KF443123		KF888819
Euglossa imperialis	AF002730		KF888820
	AY916117		KF888821
Euglossa dissimula	AY916100		KF888822
Euglossa hansoni	AY916101		KF888863
			AF181614
Euglossa heterosticta	AY916102	*Eulaema cingulata*	KF895451
Euglossa analis	AY916103		KF895491
Euglossa cognata	AY916104		KF895492
Euglossa tridentata	AY916105		KF895493
Euglossa mixta	AY916106		KF895494
Euglossasa pphirina	AY916107		KF895495
Euglossa despecta	AY916108		KF895511
Euglossa dressleri	AY916109		AY916096
Euglossa cybelia	AY916110	*Eulaema nigrita*	AY916095
Euglossa maculilabris	AY916111	*Eulaema speciosa*	AY916097
Euglossa decorata	AY916112	*Exaerete smaragdina*	AY916099
Euglossa bursigera	AY916113	*Exaerete frontalis*	AY916098
Euglossa ignita	AY916114		AF002729
Euglossa chalybeata	AY916115	*Eufriesea ornata*	AY916093
Eulaema atleticana	KU135609	*Eufriesea venusta*	AY916092
	KU135608	*Eufriesea rufocauda*	AY916091
	KU135607	*Eufriesea chrysopyga*	AY916090
	KU135606	*Eufriesea caerulescens*	AF181613
	KU135604	*Apis dorsata*	KP259252
	KU135629	*Apis mellifera*	EF184045
	KU135628		
	KU135627		
	KU136206		

Table 2. The accession numbers of cytochrome c oxidase I (COX1) genes and scientific names of studied species from the tribe Euglossini (n=55) received from GenBank (www.ncbi.nlm.nih.gov)(n=59).

Species	Accession Numbers	Scientific Names	Accession Numbers
Aglae caerulea	EU163091	*Euglossa iopoecila*	KX250213
	EU421542		KX250207
	AY506458		KX250206
	AY506457		KX250205
Eufriesea ornata	KC313077		KX250203
Eufriesea atlantica	KC313076		KX250200
Eufriesea superba	KC313075		KX250198
Eufriesea nordestina	KC313074		KX250196
	KC313073		KX250193
	KC313072		KX250192
	KC313071		KX250175
	KC313070		KX250176
	KC313069		KX250173
	KC313068		KX250171
	KC313067		KX250165
Eufriesea nigrohirta	KC313065		KX250163
Eufriesea auriceps	KC313064	*Eulaema peruviana*	AJ581111
	KC313063	*Eulaema polyzona*	AJ581112
Eufriesea xantha	AJ581110	*Eulaema polychroma*	AJ581113
Eufriesea flaviventris	AJ581109	*Eulaema meriana*	AJ581114
Exaerete smaragdina	KC313078	*Eulaema speciosa*	AJ581115
	AJ582625	*Eulaema nigrita*	AJ581116
Aglae caerulea	EU421542	*Eulaema atleticana*	KU134960
	AY506458		KU134955
	AY506457		KU134954
Apis dorsata	KU752355		KU135003
	KT960840		KU135002
Apis mellifera	KU874168		KU135000
	KU874167		KU134999
			KU134991
			KU134984
			KU134985
			KU134986

Table 3. The accession numbers of RNA polymerase II genes and scientific names of studied species from the tribe Euglossini (n=78) received from GenBank (www.ncbi.nlm.nih.gov)(n=79).

Species	Accession Numbers	Scientific Names	Accession Numbers
Eufriesea magrettii	EU421303	*Euglossa macrorhyncha*	EU421321
Eufriesea pulchra	EU184723	*Euglossa cordata*	EU421297
Eufriesea anisochlora	EU421325	*Euglossavariabilis*	EU421232
Eufriesea macroglossa	EU421311	*Euglossa tridentata*	EU421320
Eufriesea corusca	EU421296	*Euglossa bursigera*	EU421319
Eufriesea mussitans	EU421294	*Euglossa retroviridis*	EU421317
Eufriesea violascens	EU421286	*Euglossa villosiventris*	EU421316
Eufriesea chalybaea	EU421285	*Euglossa laevicincta*	EU421274
Eufriesea tucumana	EU421283	*Euglossa tridentata*	EU421275
	EU421282	*Euglossa hemichlora*	EU421306
Eufriesea pulchra	EU421257	*Euglossa obtusa*	EU421304
Eufriesea violacea	EU421228	*Euglossa dressleri*	EU421298
Eufriesea lucida	EU421223	*Euglossa erythrochlora*	EU421295
Eufriesea concava	EU421216	*Euglossa deceptrix*	EU421293
Eufrieseac hrysopyga	EU421258	*Euglossa analis*	EU421292
Eufriesea rufocauda	EU421212	*Euglossa rufipes*	EU421291
Euglossa ioprosopa	EU421270	*Euglossa azureoviridis*	EU421288
Euglossa augaspis	EU421267	*Euglossa imperialis*	EU421284
Euglossa orellana	EU421263	*Euglossa igniventris*	EU421281
Euglossa cognata	EU421261	*Euglossa fuscifrons*	EU421279
Euglossa heterosticta	EU421252	*Eugloss aturbinifex*	EU421273
Euglossa sapphirina	EU421251	*Euglossa chlorina*	EU421272
Euglossa hansoni	EU421250	*Euglossa flammea*	EU421231
Euglossa championi	EU421249	*Euglossa dodsoni*	EU421234
Euglossa asarophora	EU421247	*Euglossa truncata*	EU421238
Euglossa crassipunctata	EU421246	*Euglossa amazonica*	EU421218
Euglossa piliventris	EU421244	*Euglossa rugilabris*	EU421217
Euglossa villosiventris	EU421242	*Euglossa paisa*	EU421213
Euglossa ignita	EU421241	*Euglossa purpurea*	EU421220
Euglossa flammea	EU421240	*Eulaema mocsaryi*	EU421254
Euglossa securigera	EU421239	*Eulaema polychroma*	EU421324
Euglossa cordata	EU421221	*Eulaema chocoana*	EU421323
Euglossa jamaicensis	EU421224	*Eulaema sororia*	EU421322
Euglossa parvula	EU421227	*Eulaema nigrita*	EU421300
Exaerete throcanterica	EU421313	*Eulaema cingulata*	EU421253
Exaerete dentata	EU421229	*Eulaema meriana*	GU245394
Exaerete smaragdina	EU421211	*Eulaema bombiformis*	EU421271
Bombus lapidarius	KF936151		
Aglae caerulea	EU162842		
	EU421289		
	GU245396		

Table 4. The accession numbers of 16S ribosomal RNA (or 16S rRNA) genes and scientific names of studied species from the tribeEuglossini (n=24) received from GenBank (www.ncbi.nlm.nih.gov) (n=25).

Scientific Names	Accession Numbers	Scientific Names	Accession Numbers
Aglae caerulea	EU162926	*Eulaema meriana*	AF181585
	AJ581103		AJ581095
Eufriesea xantha	AJ581091	*Eulaema boliviensis*	DQ788139
Eufriesea flaviventris	AJ581090.1	*Eulaema bombiformis*	AJ581100
Euglossa iopoecila	KX155770	*Eulaema mocsaryi*	AJ581099
	KX155767	*Eulaema cingulata*	AJ581098
	KX155764	*Eulaema speciosa*	AJ581096
	KX155763	*Eulaema polychroma*	AJ581094
	KX155734	*Eulaema polyzona*	AJ581093
	KX155725	*Eulaema peruviana*	AJ581092
	KX155715	*Exaerete azteca*	EU162927
	KX155713	*Exaerete smaragdina*	AJ581101
		Aglae caerulea	AJ581103
		Bombus hypocrita	AF364824

Results

As the results indicated, outgroups were separated from the tribe members in all of the phylogenetic trees (Figs. 1-8), implying the presence of relatively close genetic distances among tribe members. Also, in all of the phylogenetic trees, all species belonging to a single genus were clustered together. In addition, to show the accuracy of the phylogenetic analyzes, different genera of this tribe were demarcated precisely.

Based on the topology of Maximum Likelihood phylogenetic tree of Cytochrome b sequences, the relationship of different genera belonging to the tribe Euglossiniis is as follows:

[{*Euglossa*}+{*Exaerete*+(*Eufriesea*+*Eulaema*)}]. So, the genus *Euglossa* is sistered to the other genera and we can distinguish two distinct major clusters in this tree. The results showed that all species belonging to the genus *Euglossa* have the high supported ML bootstrap (=82) and BI posterior probability (=78.64) values. In another cluster, three genera including *Exaerete*, *Eufriesea*, and *Eulaema* constructed a monophyletic group with very high BI posterior probability (=87.7) and weak (but acceptable) supported ML bootstrap (=51) values.

Moreover, based on the topology of Maximum Likelihood phylogenetic tree of 16S ribosomal RNA (16S rRNA) sequences, the relationship of different genera belonging to the tribe Euglossini is as follows:

[{(*Eulaema*)+*Exaerete*+*Eufriesea*+*Aglae*}+{*Euglossa*}]. So, the genus *Euglossa* is sistered to the other genera in this tree and we can distinguish two distinct major clusters; where all of the sequences belonging to the genus *Euglossa* showed good supported ML bootstrap (=77). In another cluster, four genera including *Exaerete*, *Eufriesea*, *Eulaema*, and *Aglae* built a monophyletic group with moderate (but acceptable) supported ML bootstrap (=62) and very strong BI posterior probability (=98.57) values.

Furthermore, based on the topology of Maximum Likelihood phylogenetic tree of Cytochrome c oxidase I (COX1) sequences, the relationship of different genera belonging to the tribe Euglossini is as follows:

[{(*Eufriesea*+*Exaerete*)+*Eulaema*}+{*Aglae*+*Euglossa*}]. Hence, two genera of *Euglossa* and *Aglae* are sistered to the other genera and thus we can distinguish two distinct major clusters; where all of the species belonging to the two

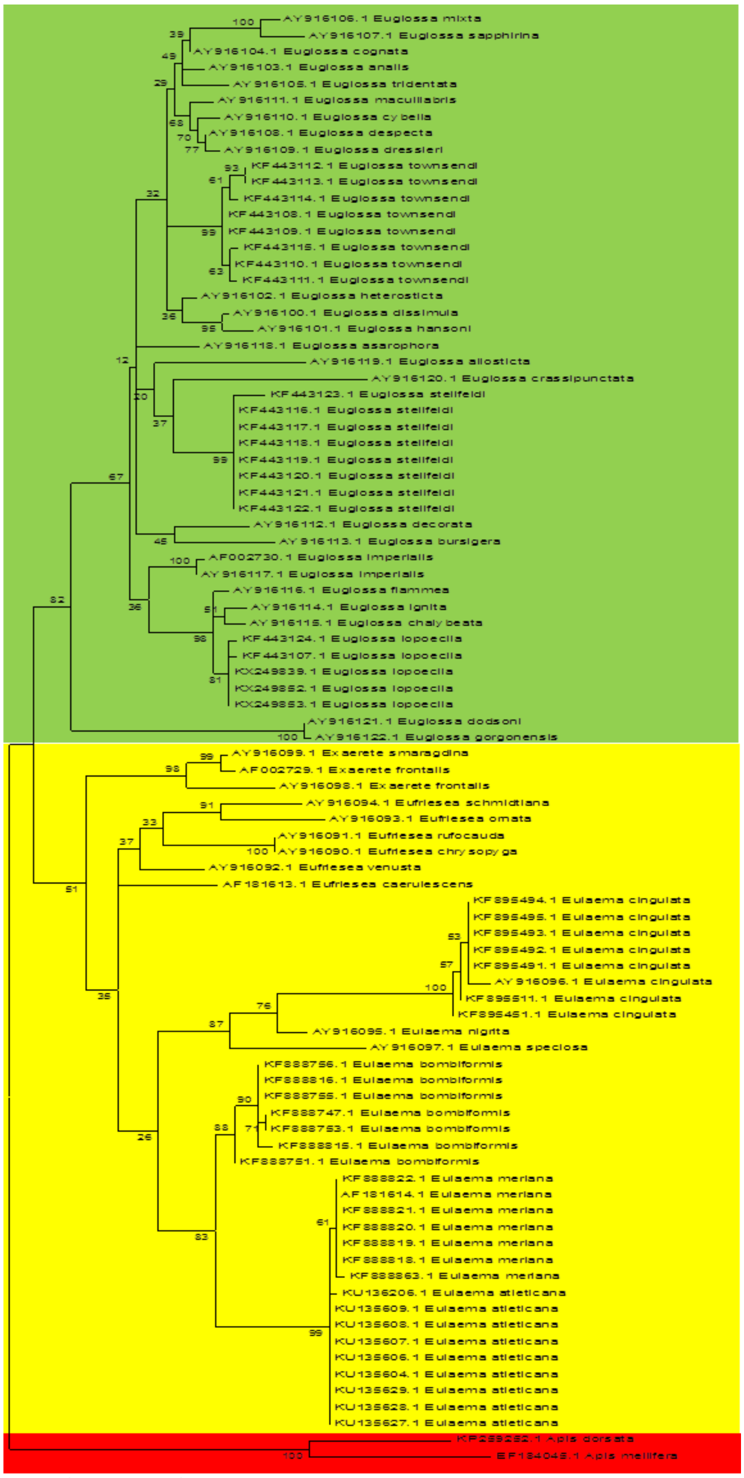

Figure 1. Maximum Likelihood tree based on Kimura 2- parameter distance using Cytochrome b sequences; the numbers on each branch correspond to bootstrap support values. The tree was rooted with two *Apis* spp. sequences.

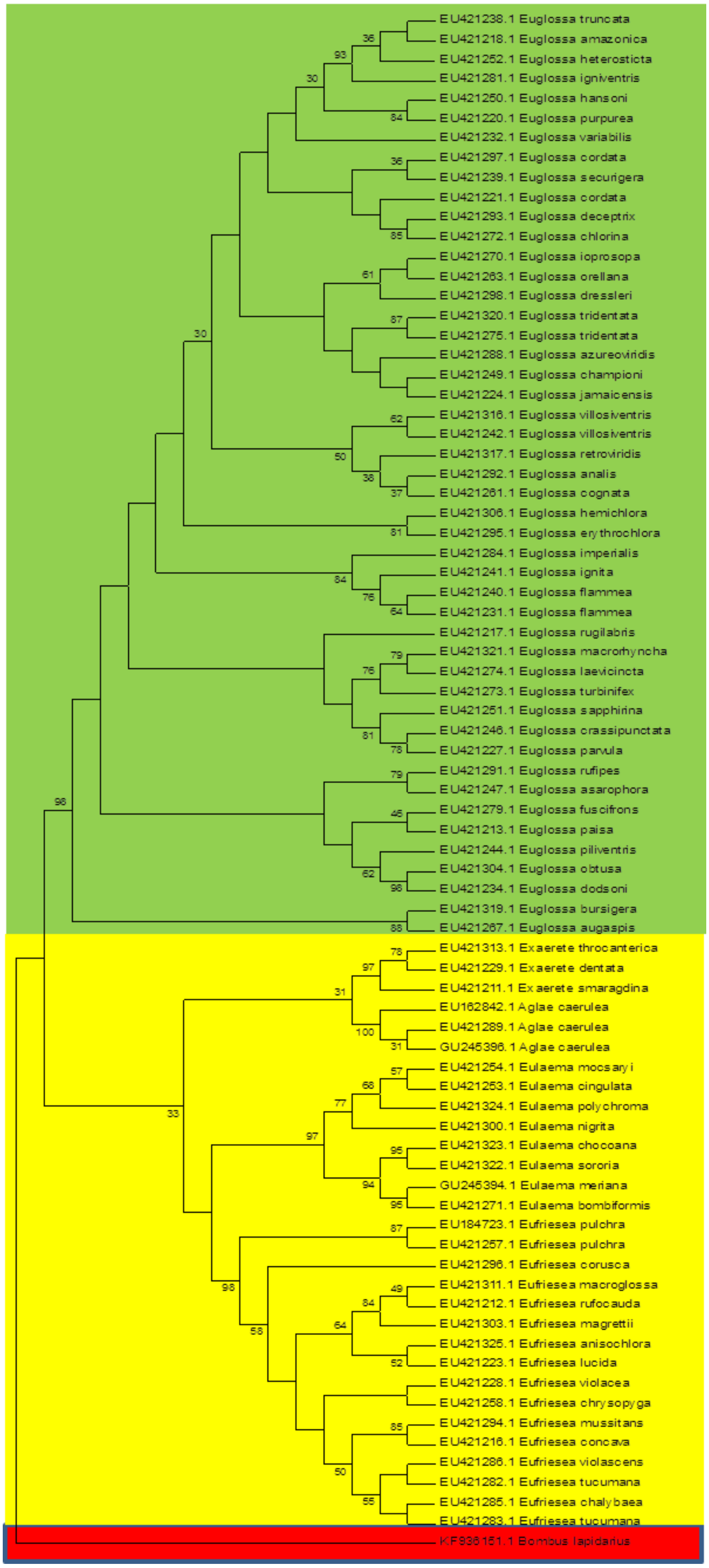

Figure 2. Maximum Likelihood tree based on Kimura 2- parameter distance using RNA polymerase II sequences; the numbers on each branch correspond to bootstrap support values. The tree was rooted with a single *Bombus lapidarius* sequences (percentages lower than 30 are not shown).

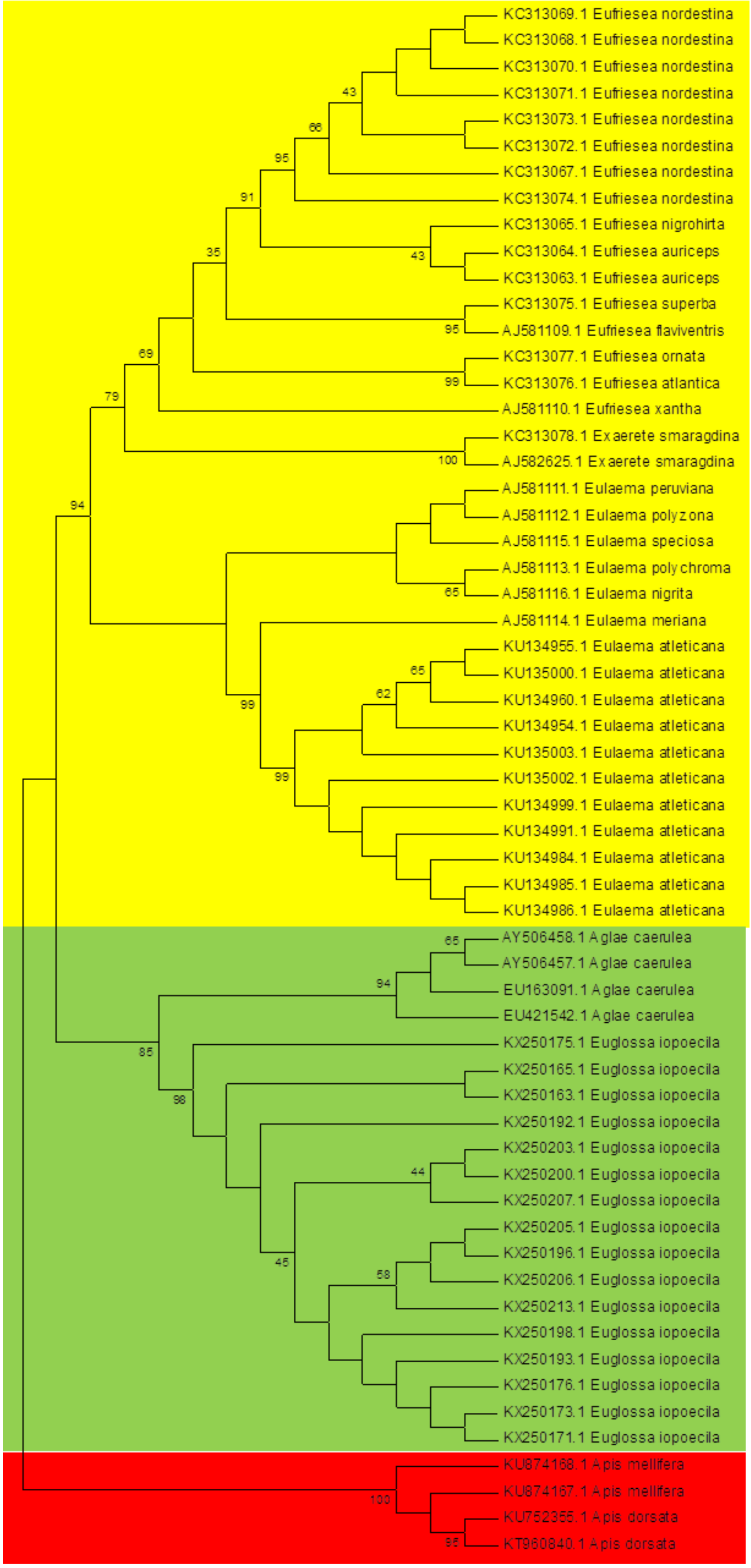

Figure 3. Maximum Likelihood tree based on Kimura 2- parameter distance using Cytochrome c oxidase I (COX1) sequences; the numbers on each branch correspond to bootstrap support values. The tree was rooted with four *Apis* spp. sequences (percentages lower than 30 are not shown).

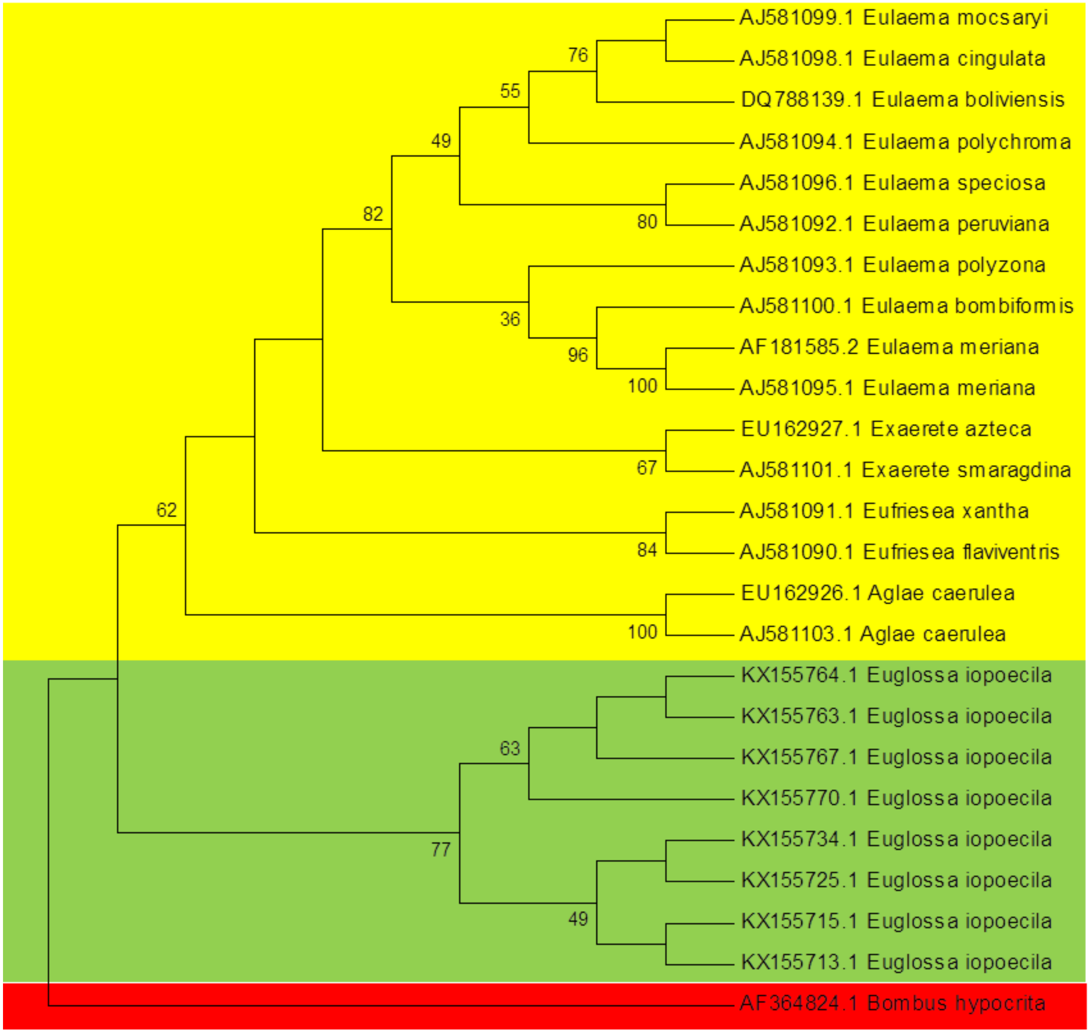

Figure 4. Maximum Likelihood tree based on Kimura 2- parameter distance using 16S ribosomal RNA (16S rRNA) sequences; the numbers on each branch correspond to bootstrap support values. The tree was rooted with a single *Bombus hypocrita* sequences (percentages lower than 30 are not shown).

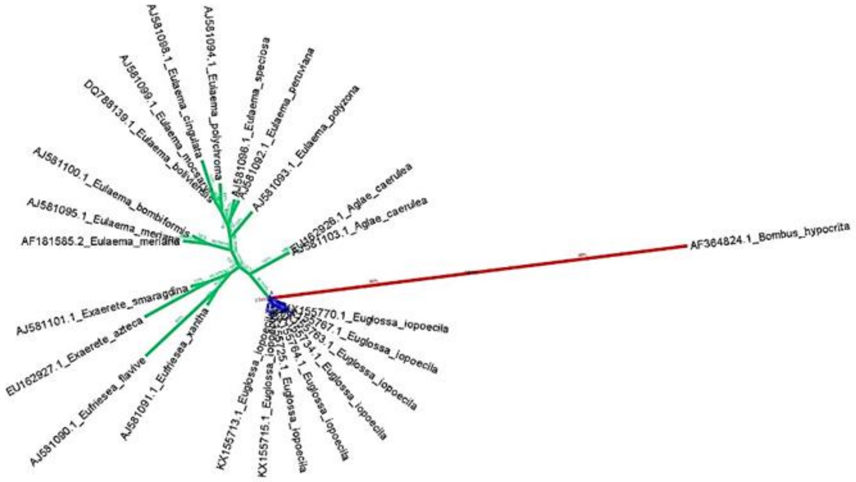

Figure 5. Bayesian phylogeny reconstructed based onbased on16S ribosomal RNA (16S rRNA) sequences. The values beside the branches are BI posterior probability values. The tree was rooted with a single *Bombus hypocrita* sequences.

0.07

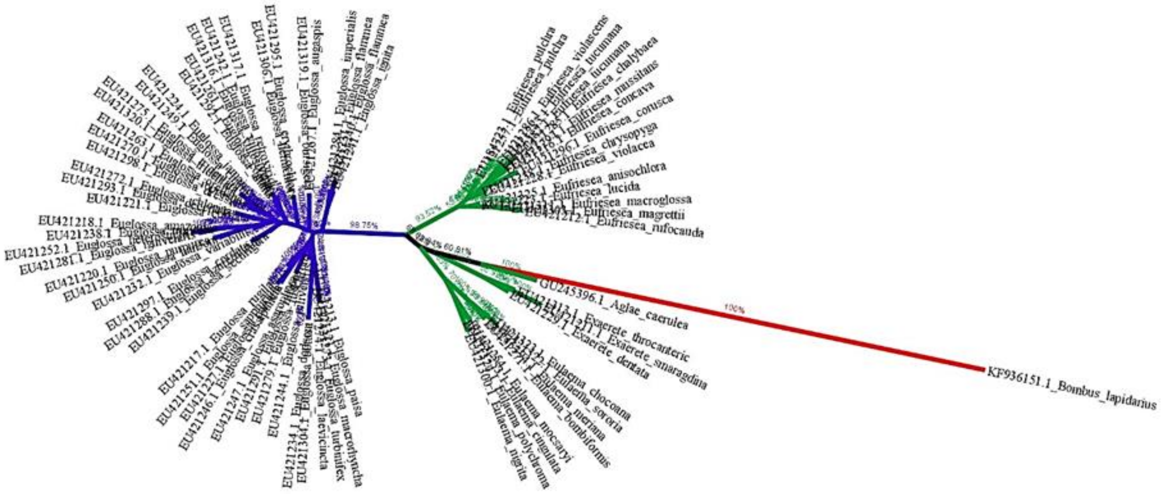

Figure 6. Bayesian phylogeny reconstructed based on RNA polymerase II sequences. The values beside the branches are BI posterior probability values. The tree was rooted with a single *Bombus lapidarius* sequences.

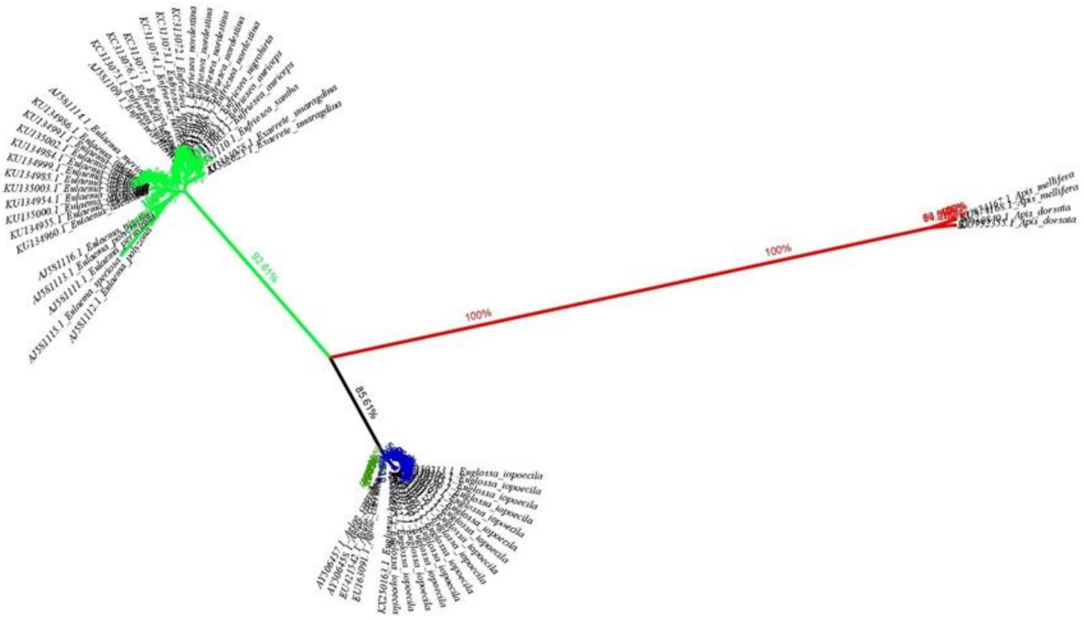

Figure 7. Bayesian phylogeny reconstructed based onCytochrome c oxidase I (COX1) sequences. The values beside the branches are BI posterior probability values. The tree was rooted with four *Apis* spp. sequences.

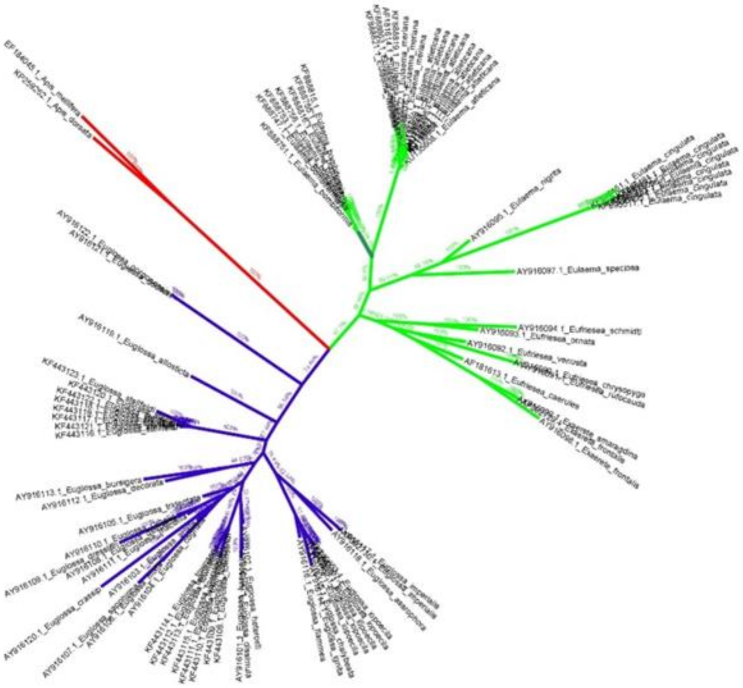

Figure 8. Bayesian phylogeny reconstructed based on Cytochrome b sequences. The values beside the branches are BI posterior probability values. The tree was rooted with two *Apis* spp. sequences.

genera of *Euglossa* and *Aglae* showed a high supported ML bootstrap (=85) and BI posterior probability (=85.61) values. In another cluster, three genera including *Exaerete*, *Eufriesea*, and *Eulaema* constructed a monophyletic group with a high supported ML bootstrap (=94) and BI posterior probability (=92.61) values. The BI posterior probability values of two mentioned clusters were equal to 100.

Eventually, based on the topology of Maximum Likelihood phylogenetic tree of RNA polymerase II sequences, the relationship of different genera belonging to the tribe Euglossini is as follows:

[(*Euglossa*)+{(*Exaerete*+*Aglae*)+(*Eulaema*+*Eufriesea*)}]. In this tree, similar to the previous phylogenetic trees, the genus *Euglossa* is In all of the phylogenetic trees, the BI posterior probability value of two mentioned clusters was equal to 100; thus, it can be inferred that the tribe Euglussini is a monophyletic group with the highest BI posterior probability value.

sistered to the other genera and we can distinguish two distinct major clusters; where all of the sequences belonging to the genus *Euglossa* showed very strong supported ML bootstrap (=98) and very strong BI posterior probability (=98.75) values. In another cluster, four genera including of *Exaerete*, *Eufriesea*, *Eulaema*, and *Aglae* constructed a monophyletic group with a very weak supported ML bootstrap (=33). Definitely, in this case, the topology of the Bayesian phylogenetic tree was slightly different from the topology of ML phylogenetic tree. However, as can be seen from Fig. 6, similar to the other trees, we can detect two different groups consisting of the genus *Euglossa* and other genera, clearly separated from each other.

Discussion

In this study, four gene fragments were used to evaluate the phylogenetic relationships among five genera of the orchid bees.

As one of the first comprehensive studies on this subject, Kimsey (1987) evaluated generic relationships among the tribe Euglossini based on morphological characters. The relationships of different genera based on the topology of Kimsey's phylogenetic tree were as:

[{(*Euglossa*+*Exaerete*)}+{*Eufriesea* +(*Aglae*+*Eulaema*)}](Kimsey 1987)(Fig. 9).

Michener's tree based on morphological characters (1990) had the following phylogenetic relationships:

[*Exaerete*+*Euglossa*+{(*Eufriesea*+*Aglae*+*Eulaema*)}](Michener 1990)(Fig. 9)

In another study, Engel (1999) depicted the phylogenetic tree of the tribe Euglossini based on cladistic analysis of 15 morphological characters. In this study, the generic relationships among orchid bees were as under:

[{(*Exaerete*+*Euglossa*)+*Eufriesea*}+{*Eulaema* +*Aglae*}](Engel 1999)(Fig. 9)

Elsewhere, Michel-Salzat et al. (2004), using 37 morphological characters and sequencing of two widely used mitochondrial genes (16S rDNA and COI) and two protein-encoding nuclear genes (long-wavelength rhodopsin, LWRh (also known as opsin), and the F2 copy of elongation factor-1a, EF-1a), presented the generic phylogenetic relationships among orchid bees as:

[((*Eulaema*+*Eufriesea*)+*Euglossa*+*Exaerete*)+*Aglae*]

(Michel-Salzat*et al.* 2004) (Fig.9)

Also, Oliveira's phylogenetic tree based on morphological characters was exactly similar to the Michel-Salzat's tree (Oliveira 2006).

Darveau et al. (2005) used a single mitochondrial marker to infer the relationships within orchid bees. The topology of the phylogenetic tree in their study was as:

[(*Euglossa*)+{(*Eufriesea*+*Eulaema*)+*Exaerete*}

(Darveau *et al.* 2005)

Recently, Ramírez et al. (2010), used four loci of cytochrome oxidase (CO1), elongation factor 1-a (EF1-a), arginine kinase (ArgK), and RNA polymerase II (Pol-II) for evaluating the phylogenetic relationships within the tribe Euglossini (Fig. 10). In this study, the phylogenetic relationships of orchid bees based on the topology of combined Bayesian and combined parsimony phylogenetic trees were as:

[((*Euglossa*+*Aglae*)+*Eulaema*+*Eufriesea*)+*Exaerete*](Ramírez *et al.* 2010)(Fig. 10)

Among the five genera of Euglossini, the genus *Euglossa* is composed of six subgenera with about 122 species based on external morphological characters (Nemésio 2009; Ramírez *et al.* 2010), so this genus is the most diverse genus into the tribe Euglossini (Fernandes *et al.* 2013). This number may be much higher than the current number. Moreover, the taxonomy of this group is extremely complicated due to a large number of morphological similarities (Fernandes *et al.* 2013). The phylogeny of the genus *Euglossa* is still to be investigated (Michel-Salzat *et al.* 2004).

In a study, Fernandes et al. (2013) evaluated cytogenetically two species of the genus *Euglossa* including *Eu. Carolina* and *Eu. Townsend*. They reported that in the genus *Euglossa* other mechanisms might have caused karyotype evolution as individuals have a high number of chromosomes, higher than other bees species (Fernandes *et al.* 2013). In this genus, the chromosomes are large and present submetacentric morphology, which is contrary to the characteristics predicted in the theory of Minimal Interaction (Fernandes *et al.* 2013).

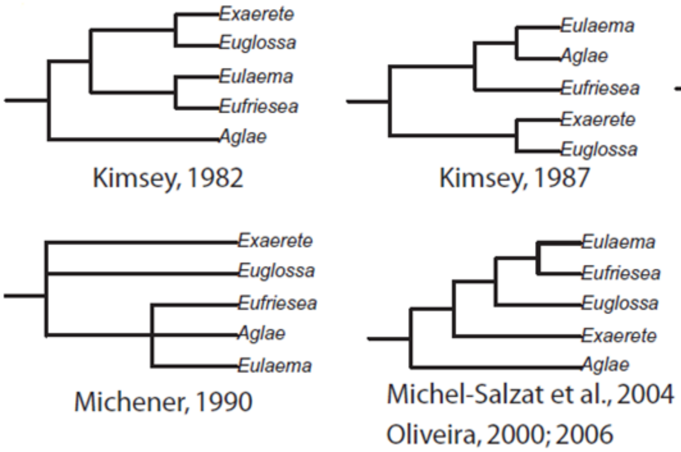

Figure 9. The generic relationships among orchid bees recovered in previous studies since 1982 to 2006 (retrieved from Ramírez *et al.* 2010).

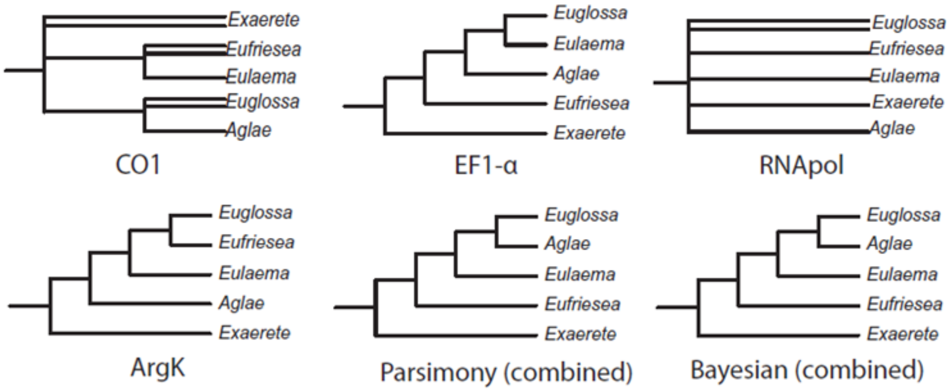

Figure 10. The phylogenetic relationships among orchid bees using four different genes (Ramírez *et al.* 2010).

In another study, Darveau et al. (2005) sequenced a single mitochondrial gene (cytb) in four genera belonging to the tribe Euglossini (GenBank accession number AY916090-AY916122) (Darveau *et al.* 2005). They reported the cytb information places the *Euglossa* genus as the sister of the other genera (Darveau *et al.* 2005). Also, they emphasized, the alternative methods of phylogenetic inference and genetic distance methods yielded similar topologies with nodes with bootstrap values greater than 50% generally conserved (Darveau *et al.* 2005).

Overall, the tree topology of the orchid bees is unstable (Darveau *et al.* 2005), which make the introduction of phylogenetic trees with different topologies. Thus, in this scientific report, the results are presented with caution. Although we cannot describe the phylogenetic tree of the tribe Euglossini with confidence yet, I found that there are probably two distinct evolutionary pathways or two distinct evolutionary lineages in this tribe. Moreover, I found that the evolutionary pathway of the genus Euglossa is probably different from other genera belonging to the tribe Euglossini.

As mentioned, the genus *Euglossa* is the most diverse genus in the tribe Euglossini and the karyotypic evolution of this genus has a particular state different from other bees (Fernandes *et al.* 2013). Although, to make a definite conclusion about karyotype evolutionary pathways in these bees we must perform karyotype analyses in all the genera belonging to the tribe Euglossini, based on the results of present study, the results of Fernandes et al. (2013), and considering that the genus *Euglossa* is the most diverse genus into the tribe Euglossini (Nemésio 2009; Ramírez *et al.* 2010), the theory presented in this paper is likely to be close to reality. However, a definite conclusion needs more studies on this matter.

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