Comparative Genital Morphology, Phylogeny, and Classification of the Orchid Bee Genus *Euglossa* Latreille (Hymenoptera: Apidae), with Distributional Modeling of Adventive Euglossines

BY

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ABSTRACT

Orchid bees (tribe Euglossini) are conspicuous members of the corbiculate bees owing to their metallic coloration, long labiomaxillary complex, and the fragrance-collecting behavior of the males, more prominently (but not restricted) from orchid flowers (hence the name of the group). They are the only corbiculate tribe that is exclusively Neotropical and without eusocial members. Of the five genera in the tribe, *Euglossa* Latreille is the most diverse with around 120 species. Taxonomic work on this genus has been linked historically to the noteworthy secondary sexual characters of the males, which combined with the other notable external features, served as a basis for the subgeneric classification commonly employed. The six subgenera *Dasystilbe* Dressler, *Euglossa sensu stricto, Euglossella* Moure, *Glossura* Cockerell, *Glossurella* Dressler and *Glossuropoda* Moure, although functional for the most part, showed some intergradations (especially the last three), and no phylogenetic evaluation of their validity has been produced. A general paucity in the use of male genitalic morphology has also been characteristic for the lineage. Here a comparative study of the male genitalic morphology for *Euglossa* is presented, and in turn, along with other external morphological features, used as a source of characters included in a phylogenetic analysis. A total of 41 *Euglossa* species representing the taxonomic diversity within the genus (all subgenera and species groups) plus five taxa as outgroups, and 79 characters are included in a comprehensive cladistic treatment. The resulting 64 shortest trees combined in a strict consensus tree recover as natural groups the subgenera *Dasystilbe* (monotypic), *Euglossa s. str.,* and *Euglossella* (sister to all other *Euglossa s. lat.*). *Glossura* appears as paraphyletic in a derived clade that also includes *Glossuropoda* (*sensu* Moure) and some *Glossurella*; *Glossura* is proposed to be expanded to include all the species groups in this derived clade. Most *Glossurella* form a heterogeneous paraphyletic assemblage, and the subgenus is proposed to be narrowed to include the monophyletic group formed by *E. bursigera* Dressler and allies. A morphologically distinctive group in the *Glossurella* grade is also proposed as a new subgenus with *E. oleolucens* Dressler as type species, while the remaining species in the grade are classified as *Euglossa incertae sedis*. Recent molecular phylogenetic work largely agrees with the results here presented. The presence of two adventives orchid bee species in the USA is analyzed using occurrence data across their natural ranges to produce Species Distribution Models, so that the recently discovered naturalized populations of *Euglossa viridissima* Friese in southeastern Florida are projected not to expand beyond the Florida Peninsula, and the isolated records of *Eulaema polychroma* (Mocsáry) in the southwestern USA are most likely due to individual dispersion events or human introduction.

To the memory of my mother;
you have always been with me

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CHAPTER 1

A Briefing on Orchid Bees with emphasis on the genus *Euglossa* Latreille (Hymenoptera: Apidae)

The bee genus *Euglossa* Latreille, is one of five genera in the tribe Euglossini of the subfamily Apinae. Two of the other four genera are cleptoparasitic – *Aglae* Lepeletier and Serville and *Exaerete* Hoffmannsegg – while the other two – *Eulaema* Lepeletier and *Eufriesea* Cockerell – are pollen-collecting lineages as is *Euglossa* (Dressler, 1982a; Michener, 2007). Collectively these genera are known as orchid bees, owing to the collection of fragrant compounds by males of euglossine species. As a result, euglossines are the most significant and in many instances sole pollinators of various Neotropical Orchidaceae (van der Pijl and Dodson, 1966; Ackerman, 1983a). The tribe is confined to the Neotropical region, while *Euglossa* itself ranges from northern Mexico to Paraguay and has the only extant euglossine species that occur in the West Indies (e.g., Michener, 1979, 2007; Dressler, 1982a). *Euglossa* embraces a little over 100 described species (Michener, 2007; Ramírez *et al.*, 2002; Roubik and Hanson, 2004; Nemésio and Silveira, 2007), but new taxa are regularly discovered (e.g., Roubik 2004, Ramírez, 2005, 2006), and the total number of valid species might rise as high as 120 (Roubik and Hanson, 2004).

Euglossini, is one of four tribes of the corbiculate Apinae; i.e., those apine bees with a corbicula on the hind tibia and associated structures (e.g., Schultz *et al*., 1999; Michener, 2009). While the corbiculates are widely supported as monophyletic (e.g., Roig-Alsina and Michener, 1993; Schultz *et al*., 1999, 2001; Engel, 2001a, b; Michener, 2007), relationships among the tribes – Euglossini, Bombini (bumble bees), Meliponini (stingless bees), and Apini (honey bees) – are quite controversial. Euglossini are strongly placed as basal among the corbiculates by morphological (Roig-Alsina and Michener, 1993; Schultz *et al.*, 1999, 2001; Engel, 2001a, b), paleontological (Engel, 2001a, b), and behavioral data (Noll, 2002), as well as simultaneous analyses of all of these along with DNA sequence data (e.g., Schultz *et al*., 1999, 2001). Alternatively a set of DNA sequences has suggested that Euglossini are sister to Apini (Cameron and Mardulyn, 2001). Monophyly of the five genera is well supported (e.g., Kimsey, 1987; Oliveira, 2006; Ramírez *et al*., 2010), although again relationships have been contentious. Six different phylogenetic hypotheses have been proposed, four based exclusively on morphology (Kimsey, 1982, 1987; Michener, 1990; Engel, 1999), one supported by morphological data (Oliveira, 2006) and DNA sequences (Michel-Salzat *et al.,* 2004), and a recent one supported by molecular data (Ramírez *et al*., 2010). From the five topologies proposed prior to 2010, *Euglossa* appears three times as sister to *Exaerete* (Kimsey, 1982, 1987; Engel, 1999), once as sister to *Eufriesea* + *Eulaema* (Oliveira, 2006; Michel-Salzat *et al.,* 2004), and in one falls into a basal trichotomy (Michener, 1990). The recent molecular analysis of Ramírez *et al*. (2010) adds yet another possible scenario for the putative sister group of *Euglossa*; their combined, four-loci analysis supported a *Euglossa* + *Aglae* clade. In the same study, Ramírez *et al*. (2010) present a robust phylogenetic hypothesis for *Euglossa* at the infrageneric level. A small section of the subgenus *Euglossa* (i.e., *Euglossa s.str*.), has also been recently analyzed phylogenetically using morphological data (Bembé, 2007). For the other genera in the tribe, there is a species-level phylogeny for *Eulaema*

(Oliveira, 2006), and a preliminary one has been produced for *Exaerete* (Engel, 1999).

Euglossa species are noticeable by their striking metallic coloration and their conspicuously elongate mouthparts (*Euglossa* meaning “true tongue”) (Fig. 1.1), features also displayed by the other euglossine genera, as well as the comb of bristles in place of the jugal lobe in the hind wing, and the secondary sexual modifications of the males involved in the collection and manipulation of the aromatic compounds (Michener, 2007; Dressler, 1978b). The characters that differentiate *Euglossa* from the other orchid bees include the presence of two dark oval spots on the labrum, males with a tibial slit not reaching the apical margin of the hind tibia, generally with two small felty patches on the basal end of the large middle tibial patch, as well as females usually with a median, black scutellar tuft (Michener, 2007). A significant body of taxonomic work has been done for *Euglossa*, particularly in the last four decades after the discovery of the chemical compounds to which males are attracted, and their use as collecting baits (Dodson *et al*., 1969), numerous new species (more than 50% of the total, as can be seen in Ramírez *et al*., 2002), as well as previously unknown males, have been described. It is remarkable that among the available descriptions of species in the genus, the genital structures of both sexes have received almost no attention, being considered only in a few classic works (e.g., Dressler, 1978a), but mainly in recent descriptions (i.e., Ramírez, 2005, 2006; Parra-H *et al*., 2006; Rasmussen and Skov, 2006; Hinojosa-Díaz and Engel, 2007). Most of the taxonomic work focusses on the striking secondary sexual features of the males (i.e., Roubik, 2004). The taxonomic work of Cockerell (1917), Moure (1967, 1989), and Dressler (1978b, 1982b, c, d) produced the subgeneric classification so far in use for *Euglossa*, consisting of six subgenera (*Dasystilbe*, *Glossura*, *Glossurella*, *Glossuropoda*, *Euglossa s. str*., and *Euglossella*). Moreover, Dressler (1978b, 1982c), recognized 17 species groups. The six subgenera have been in common use in new descriptions (e.g., Rebêlo and Moure, 1995; Roubik, 2004; Ramírez, 2005, 2006; Parra-H *et al*., 2006; Nemésio, 2007; Hinojosa- Díaz and Engel, 2007), as well as in synoptic and local lists (Moure, 1967; Kimsey and Dressler, 1986; Bonilla-Gómez and Nates-Parra, 1992; Ramírez *et al*., 2002; Faria and Melo, 2007; Moure *et al*., 2008). However, Michener (2007) synonymized all the subgenera based on the seeming intergradation among them, situation that was somewhat addressed by Dressler (1978b) while delimiting subgenera and species groups.

The biology of several individual species of *Euglossa*, and euglossine bees in general, has been studied in different aspects, among others, bee-orchid association (e.g., Janzen, 1981; Ackerman, 1983a), nesting behavior (e.g., Garófalo, 1992; Ramírez-Arriaga *et al.*, 1996), and seasonality (e. g., Janzen *et al*., 1982; Ackerman, 1983b, 1989). In a general approach, euglossine biology was first discussed by Zucchi *et al.* (1969) and Dressler (1982a), and has been recently summarized and updated by Ramírez *et al*. (2002), Cameron (2004), and Roubik and Hanson (2004). All of these authors allude to the fragrance collecting behavior of the males from orchid flowers as the most distinctive biological feature of these bees. About 10% of the Neotropical orchids are exclusively pollinated by male euglossines (Ackerman, 1983; Ramírez *et al*., 2002), but in their quest for fragrances, they also visit other plant families, noticeably Araceae (Dressler, 1982a; Ramírez *et al*., 2002), as well as non floral sources such as plant sap (Dressler, 1979), fungi growing on rotting wood (Whitten *et al*.,1993), terrestrial mushrooms (Capellari and Harter-Marques, 2010) and even insecticides (Roberts *et al*., 1982) and cadavers of other euglossine males (Roubik, 1998). The role that the fragrances play in the biology of the euglossine bees, although not completely understood, seems to have some function in mating (Eltz *et al.*, 1999; Eltz *et al*., 2005). The pollinating activities of the females, not restricted to visit orchids, are perhaps more important than those of the males, as they seem to promote outcrossing among tropical plant species with low population density (Janzen, 1971). Both, male and female pollination activities are enhanced as they are powerful flyers, have longer lifespans than usual for bees, and perform trap lining in their flower visits (Ackerman, 1982, 1985). Some other biological features of the euglossines make them of special interest with respect to the other corbiculate bees, especially their lack of eusocial behavior (some *Euglossa* species are communal or semisocial (Garófalo, 1985)), which in a phylogenetic context will enlighten the evolution of social behavior in the clade.

Orchid bees in general are highly appreciated among those interested in bees, as noticed by their briefly described notorious external morphological features, and for the appealing orchid-pollinator relationship. In the last decade a new generation of researchers has increased the knowledge of local faunas, described new species, particularly from areas poorly explored in previous years (e.g. Colombia), provided new insights on the role of the fragrances collected by the males and in general created a broader basis to understand aspects of the biology and systematics of this group. A robust phylogenetic approach was lacking until the recent publication of the study by Ramírez *et al*. (2010), in which the authors produced a phylogenetic hypothesis not only for the tribe, but for each genus. That molecular analysis was produced almost in parallel to the present study, such that this work was produced in the absence of any prior cladistic analyses and thereby no preconceived notions of cladistic affinity. Nonetheless the remarkable coincidences in the phylogenetic hypothesis of Ramírez *et al*. (2010), and the hypothesis presented in Chapter 3, only confirm that the systematic knowledge of the group (and particularly of *Euglossa*) is reaching some maturity. So, as a major contribution, here a morphological comparative work on the genitalia of the genus *Euglossa* is presented in the following chapter, in order to apply those characters in an infrageneric phylogenetic analysis (Chapter 3). In addition, as *Euglossa* has adventitiously reached the United States, an ecological model of the potential distribution of *Euglossa viridissima* (the recent adeventive) along with *Eulaema polychroma*, another orchid bee with scarce distributional records in the USA, is presented in the last chapter.

Fig. 1.1. *Euglossa asarophora* male, lateral habitus.

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CHAPTER 2

Male Genitalic Morphology of Orchid Bees of the Genus *Euglossa* Latreille

(Hymenoptera: Apidae)

INTRODUCTION

The tribe Euglossini encompasses one of the most remarkable groups of bees, outstanding for their biology, their morphological peculiarities, and their phylogenetic position. The striking metallic integumental coloration of most species, particularly of those in the genus *Euglossa* Latreille, makes them hard to pass by. Their common name "orchid bees" derives from the association between male euglossines and orchids, in which males visit orchid flowers in their quest for volatile chemicals offered by the plant as reward in exchange for the pollination services provided (Dressler, 1982a). This peculiar behavior is accompanied by a suite of interesting external morphological features, most of them linked to the gathering, handling, and storing of the chemicals. Species of the genus *Euglossa* exhibit, besides the aforementioned male secondary sexual characteristics, variation in other external features in both sexes, notably, besides the metallic coloration, the length of the labiomaxillary complex from which the genus derives its name (*Euglossa*; Gr. *eu* = true, *glossa* = tongue). Not surprisingly, taxonomic work on this genus has relied heavily on these noteworthy external characteristics, giving almost no attention to the genital structures. With few exceptions (Dressler, 1978a; Parra-H *et al*., 2006; Ramírez, 2006; Rasmussen and Skov, 2006; Hinojosa-Díaz and Engel, 2007), the vast majority of the little more than 100 species of *Euglossa* (Ramírez *et al.*, 2002; Roubik and Hanson, 2004, Nemésio and Silveira, 2007) have been described without written or graphic reference to the male genital capsule or its associated hidden sterna, in spite of the known importance of these structures in studies of related groups of bees. Likewise, Cockerell (1917), Moure (1967, 1989), and Dressler (1978b, 1982b, c) established the current subgeneric classification based solely on external morphology. In a recent attempt to draw attention to male genital characters for *Euglossa*, Ospina-Torres *et al*. (2006) presented a short review of the variation of gonostylar morphology within the genus. The systematic and phylogenetic value of characters from the male genital capsule and hidden sterna in the tribe Euglossini has been demonstrated when applied to the whole tribe (Kimsey, 1987; Engel, 1999), as well as in studies of *Eufriesea* Cockerell (Kimsey, 1982), *Eulaema* Lepeletier de Saint Fargaeu (Oliveira, 2006) and *Exaerete* Hoffmannsegg (Kimsey, 1979; Anjos-Silva *et al*., 2007). In *Euglossa*, a recent revision of a section of *Euglossa sensu stricto* (Bembé, 2007), has also demonstrated the utility of this kind of information. The present work presents a detailed review of the male genitalic morphology of the genus *Euglossa* with standardized terminology that will be applied in a phylogenetic study of the genus (Chapter 3) and that could serve as a basis for other studies as well as descriptions of new species.

MATERIALS AND METHODS

Male genital capsules and hidden sterna of several specimens of *Euglossa imperialis* Cockerell were examined by dissecting them from specimens deposited in the Division of Entomology, Natural History Museum and Biodiversity Research Center, University of Kansas. The dissections were performed by severing the metasomal tip consisting of terga 7 to 8, sterna 6 to 8 and the genital capsule. The severed sections were cleared in a solution of potassium hydroxide (KOH) at room temperature for periods of 18 to 36 hours depending on the condition of each specimen, and then transferred to water and alcohol to be finally kept in glycerin. Electronic drawings were generated, using a drawing tablet, for the dissected metasomal hidden sterna (7 and 8) and the genital capsule, as observed on an Olympus SZ60 microscope. For comparative purposes dissections were made of representatives of several other species of *Euglossa* and all other euglossine genera, as well as specimens of *Bombus* Latreille, *Centris* Fabricius, *Epicharis* Klug and *Anthophora* Latreille (Appendix 1). In addition, available literature on the genital structures for the aforementioned groups, as well as Meliponini, was studied (Appendix 1). No comparative statement is made with *Apis* Linnaeus since the male genitalia of this genus is highly derived.

COMPARATIVE MORPHOLOGY

Euglossa imperialis Cockerell, 1922

The morphology of the male genitalia and hidden sterna of *Euglossa* is based on that of *E*. *imperialis*, as this species exhibits one of the widest distributional ranges of all species within the genus, occurring from southern Mexico to Atlantic Southeast Brazil (Ramírez *et al*., 2002; Roubik, 2004; Roubik and Hanson, 2004; Rebêlo, 2001). Moreover it is typically abundant and commonly collected, well represented in collections, thus ideal as a source of specimens for dissection and as a comparative basis for other species.

Terminology applied to the major sclerotic components of bee male genitalia has been rather stable with some variation in terms according to different authors and taxa, especially if compared to the numerous, sometimes confusing, sets of terms for male genitalic structures throughout Hymenoptera as a whole and insects in general. A sample of different terminologies applied to the different parts of bee and Hymenoptera male genitalia is presented in Table 1.

Table 2.1. Diferent sets of terminology applied to some relevant parts of male genitalia of bees and Hymenoptera. Terms in the same row are equivalent. The specific group of organisms to which every study refers is shown below the author.

Michener (1944) Bees (applied in present work)	Ito (1985) *Bombus*	Schulmeister (2001) Lower Hymenoptera	Michener (2007) Bees
Gonobase	Gonobase	Cupula	Gonobase
Gonocoxite	Gonocoxite	Gonostipes	Gonocoxite
Gonostylus	Squama Gonostylus	Harpe	Gonostylus Volsella*
Penis valve	Penis valve	Penisvalva	Penis valve

* The term volsella was applied by Michener (2007) to different structures in different groups of bees, here is shown in the context for Bombini and Euglossini.

The terminology of Michener (1944a) is here preferred, as it is the most commonly used in bee morphology and taxonomy. The terminology used for the hidden sterna associated with the male genitalia has varied depending on the treatment of them as parts of either the abdomen (eighth and ninth abdominal sterna) or the metasoma (seventh and eighth metasomal sterna); the abdominal numbering was initially used as an attempt to present a regular treatment of these segments for comparison with other Hymenoptera (Michener, 1944a, 1956) and other lineages of insects (Michener 1944b), but the metasomal terminology is by far dominant (e.g., Eickwort, 1969; Brooks, 1988; Michener, 2007; Engel, 2007) and will be followed here.

A description of the male hidden metasomal sterna and the different components of the genital capsule of *E*. *imperialis* is presented here with comments on variation seen in other *Euglossa* as well as other euglossine genera and related apine bees. Comparative statements of subgeneric assemblages within *Euglossa* in this work refer to the names as they are currently used in most new species descriptions and synoptic lists; the monotypic *Dasystilbe* Dressler offers no interpretational problem and will be referred to by mentioning its only member *E*. *villosa* Moure. *Euglossa s*. *str*. and *Euglossella* Moure are treated as Dressler stated in his original subgeneric division (1978b). *Glossura* Cockerell and *Glossurella* Dressler are as subsequently diagnosed by Dressler (1982c), while *Glossuropoda* Moure is taken in its original sense. All comparative statements in the following description containing references to “all species” belonging to subgeneric assemblages refer to those species reviewed (Appendix 1). As a general practice, when necessary, each structure will be subdivided into major recognizable “sections” referred to in accordance with their general anatomical position. The structures will be presented in the following sequence: seventh metasomal sternum, eighth metasomal sternum, genitalia, gonobase, gonocoxite, gonostylus, aedeagus, penis valve, and penis.

Seventh metasomal sternum.—This is the first of the pair of hidden metasomal sterna associated with the male genitalia (often referred to as S7) and is invaginated into the metasoma together with the eighth metasomal sternum and the genital capsule (hence the name hidden sterna). In *E. imperialis* the seventh metasomal sternum agrees basically with the general description for *Anthophora* given by Michener (1944a). The whole sternum is curved upwards such that the dorsal (inner) surface is concave and the ventral (outer) surface is convex. It has a rather trapezoidal disc continued laterally by the long anterolateral arms (Fig. 2.1 A). The anterior edge is strongly concave. The posterior edge of the disc is divided by an acute triangular incision whose depth (length) equals its width and forms an interior angle of around 70°. The lobes of the disc, formed by the incision, are slightly wider than long and are oriented posterolaterally. The lateral edges, defined between the posterior discal edge and the posterolateral apices of the slender arms, run evenly concave. The whole sternum is a well sclerotized structure reinforced along its entire anterior edge by the conspicuous antecostal ridge, which projects dorsally on the ventral surface and defines the very brief acrosternite, noticeable mesally. The anterolateral apices of the sternal arms bear the sternal apodemes (Fig. 2.1 A). The lines of insertion of the conjunctiva that connects with the eight metasomal sternum are located on the ventral surface of the disc.

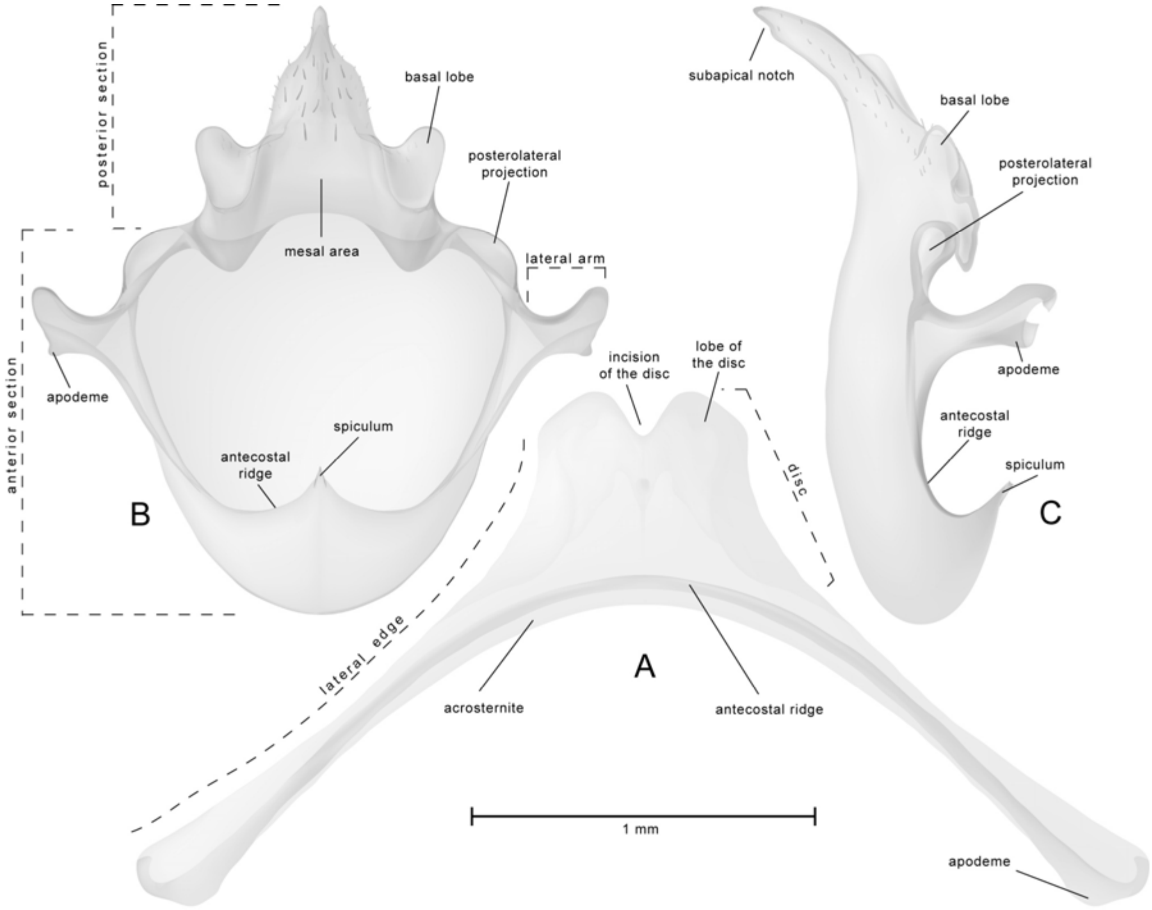

Fig. 2.1. *Euglossa imperialis*, male hidden metasomal sterna. **A.** Seventh metasomal sternum, ventral view. **B.** Eighth metasomal sternum, dorsal view. **C.** Eighth metasomal sternum, lateral view.

This kind of seventh metasomal sternum, with a bulky trapezoidal disc and devoid of setae (see below), is typical of the subgenera *Glossura*, *Glossuropoda*, and some species of *Glossurella*.

There is significant taxonomic and systematic value in the features of the seventh metasomal sternum. The basic construction of the sternum in the species of *Euglossa* as well as in the rest of euglossine genera follows the scheme described for *E*. *imperialis*; however there are variations that give great value to the study of this sclerite. The most significantly variable features of this sternum involve the disc, particularly its posterior edge, which in the majority of the species bears an incision (as described above), with depth and shape in most cases being species specific, although exceptions can be found in groups like *E*. *decorata* and allied species, which show a range of intraspecific variation (Hinojosa-Díaz, in prep.). The posterior edge is entire in some species, especially of *Eufriesea* (e.g., Kimsey, 1982), as well as in *Exaerete*, in which it sometimes has a mesal acute projection instead of an incision (Kimsey, 1979; Anjos-Silva *et al*., 2007). The discal lobes resulting when the edge is notched are also variable in shape and orientation. A feature of most euglossine species, absent in *E*. *imperialis*, is the presence of setae on the posterior edge of the seventh metasomal sternum, sometimes restricted to the disc (notched or not) but often sparsely extended to areas on the lateral edges proximal to the disc. The lateral edges of the sternum can be slightly concave, as in *E*. *imperialis*, or slightly convex, this affected by the length and shape of the disc. Some species of *Euglossa* (e.g., Hinojosa-Díaz and Engel, 2007) as well as of *Eufriesea* (e.g., Kimsey, 1982) and *Eulaema* (e.g., Oliveira, 2006) have either protuberances or notches along this lateral edge. The seventh metasomal sternum of *Bombus* lacks long arms so the lateral edges are defined mainly by the lateral edges of the disc. Otherwise the posterior edge presents features similar to those of Euglossini (Ito, 1985). In Meliponini the sternum is reduced to a small plate (Michener, 2007). Centridini and Anthophorini have seventh metasomal sterna in which the arms are clearly differentiated but never as long as in Euglossini and with an entire posterior edge (e.g., Brooks, 1988; Ayala, 1998).

Eighth metasomal sternum.—The eighth metasomal sternum (usually referred to as S8), rests between the seventh metasomal sternum and the genital capsule, all invaginated in the metasomal apex, as mentioned before. As is the norm for bees and other Hymenoptera (Michener, 1944a, 1956), the eighth metasomal sternum is substantially different from the rest of the metasomal sterna. In *E. imperialis* in particular, and Euglossini in general, these differences are marked. Instead of being a depressed sclerotic plate as are the rest of the sterna, this sternum is strongly three-dimensional. In a simplified view it is formed by two main sections plus the short lateral arms (Fig. 2.1 B). The anterior section, with a depressed globular shape, provides most of the surface and volume of the sternum; in dorsal and ventral views, it looks roughly ovoid, wider posteriorly, with two convex posterolateral sclerotic thickenings projected outwards flanking the posterior section of the sternum. The dorsal surface of the posterior section is completely open, bearing the posteriorly-projected spiculum. The posterior section of the sternum projects from the ovoid anterior section and is hollow and roughly triangular with two lateral basal lobes (Fig 2.1 B). In lateral view the posterior section is projected ventrally, forming an angle of about 140° degrees with the longitudinal axis of the anterior section (Fig. 2.1 C). The arms come from the mesoposterior portions of the anterior section of the sternum and project laterodorsally, bearing the small apodemes at their anterior corners (Fig. 2.1 B).

The extreme morphology of the eighth metasomal sternum can be explained by looking at it in terms of a modified flat sternum. The globular posterior section is the product of the inflection dorsally of the anterior edge that curves posteriorly, and the basalmost section of the disc projects anteriorly forming the rounded anterior apex of the ovoid shape. The antecostal ridge, as a result, is projected ventrally towards the inner cavity of the sternum, while there is no clear evidence of the acrosternite. The mesally-located spiculum is also, as a consequence of the inflection of the anterior edge, directed posteriorly on the now anterodorsal edge; the spiculum is unque to the eighth metasomal sternum and in most bees projects anteriorly rather than posteriorly (Michener, 2007); the posteriorly projected spiculum was called “notospiculum” by Hinojosa-Díaz and Engel (2007). The relatively complex structure of the posterior section of the eighth metasomal sternum likewise involves inflection of edges and projection of the discal surface; however it is not easily explained, since there are no clear clues. Nonetheless, the structure of the posterior section deserves extra consideration. Its dorsally basal (anterior) edge is strongly sclerotic with a series of folds (continuing from the posterolateral sclerotic projections of the anterior section of the sternum), and two projections directed anteriorly. The dorsal surface is elevated posterior to the two projections. Each elevated sector ends in a carina that flanks the basal and lateral sides of the basal lobes, which in turn are a product of the invagination of the lateral edges of the triangular shape. The two elevated sectors define a smooth, depressed mesal area (Fig. 2.1 B). The remainder of the anterior section, beyond the basal lobes, is basically the one deviating from the longitudinal axis of the sternum, as seen in lateral view (Fig. 2.1 C). This area is covered with scattered, short, simple setae on its dorsal and lateral surfaces and bears, dorsally, a mesal ridge that rises evenly, ending midway towards the posterior apex. This ridge seems to be a variable feature within the species, since in some specimens it is just briefly insinuated. The apex of the sternum narrows abruptly in lateral view at around the last third of its length, and bears a ventral subapical notch (Fig. 2.1 C).

The systematic and taxonomic value of the traits seen in the eighth metasomal sternum are relevant within *Euglossa* and Euglossini. The shape of the anterior globular section varies little among *Euglossa* species, for the most part being as described above. In the other euglossines, *Eufriesea* and *Exaerete* have an eighth metasomal sternum similar to that of *Euglossa*, longer in *Eulaema* and *Aglae*. The spiculum is extremely reduced in *Aglae*. The shape and length of the lateral arms present some variation among *Euglossa* species, as well as in the other genera. The posterolateral projections of the anterior section seem to be present in all euglossine species, except in *Aglae*; their shape, evenly roundly convex in *Euglossa*, is otherwise acutely projected in *Eufriesea*, *Eulaema*, and *Exaerete*. Of considerable value is the variation observed in the posterior section of the sternum. The basal lobes and associated invaginations of the lateral integument of the triangular apex, seem to be important in defining infrageneric assemblages, as they are presently understood in *Euglossa*. The species generally classified under *Euglossa sensu stricto* show just slightly projected basal lobes, having an almost perfectly triangular posterior section of the eighth metasomal sternum. Very well developed lobes as seen in *E*. *imperialis*, are found in all species of *Euglossella*, *Glossura*, *Glossuropoda*, and most of *Glossurella*. Of the remainder of euglossine genera, *Eufriesea* and *Eulaema* are the only ones with evident basal lobes in the posterior section, but theirs are acute projections (e.g,. Kimsey, 1982; Oliveira, 2006), while in *Euglossa*, when present, these are rounded convexities, for the most part. The very apex of the posterior section is also variable among groups in *Euglossa*. It is narrower, in dorsal, ventral, or lateral views in almost all species with well-developed basal lobes. The shape of the posterior section in *Eufriesea* is also triangular and narrow, although with different conformation than in *Euglossa* (e.g., Kimsey, 1982). The same can be said for *Eulaema*, but here the apex after the basal lobes may take oval shapes (e.g., Oliveira, 2006). In *Exaerete* the apex of the posterior section is broadly rounded or truncate (Kimsey, 1979), while in *Aglae* it appears like an unmodified rectangular projection with rounded sides. The subapical ventral notch, present in most species of *Euglossa*, is absent in *E. villosa*. The notch is also present in species of *Eufriesea* (although larger [Kimsey, 1982]) and *Eulaema*, and absent in *Exaerete* and *Aglae*. The setae on the posterior section are also good characters to distinguish species and perhaps species groupings in *Euglossa*. Most species have pilosity as described for *E. imperialis*, but relatively long plumose setae on the ventral surface are characteristic of most species of *Euglossella* (e.g., Hinojosa-Díaz and Engel, 2007). In *Bombus*, the eighth metasomal sternum is rather flattened; the spiculum is where most of the variation occurs, and the posterior section is morphologically simpler than in Euglossini (Ito, 1985). In Meliponini the eighth metasomal sternum is reduced or absent (Michener, 2007). In Centridini this sternum is flat, but seemingly not as flat as in *Bombus*; both groups of bees have the spiculum directed anteriorwards, the anterior edge not curved posteriorly (or just slightly), and a posterior section of varied shape (e.g., Ayala, 1998). Some subgenera of *Anthophora* have a posteriorly inflected anterior edge and a spiculum directed dorsally or posteriorly (Brooks, 1988), but the sternal disc is never anteriorly evaginated as in the globular-shaped sternum of Euglossini.

Genitalia.—The male genitalia, or genital capsule, of *E. imperialis* is a heavily sclerotic structure consisting of individual units that articulate or link to form the copulating apparatus. It is hidden at the posterior apex of the metasoma, overlying the hidden sterna. The distinct recognizable parts composing it are: gonobase, gonocoxites, gonostyli, and aedeagus (Figs. 2.2-2.3).

Gonobase.—The most anterior component of the male genitalia in *E. imperialis*, the gonobase, is a sclerotic plate about three times as wide as long, inflected in such a way that its convex surface arches forming an extended bowl-shaped structure, running mainly dorsolaterally, and attached to the gonocoxites via its postelolateral edges (Fig. 2.4). The ventral edge is bentposteriorly and firmly

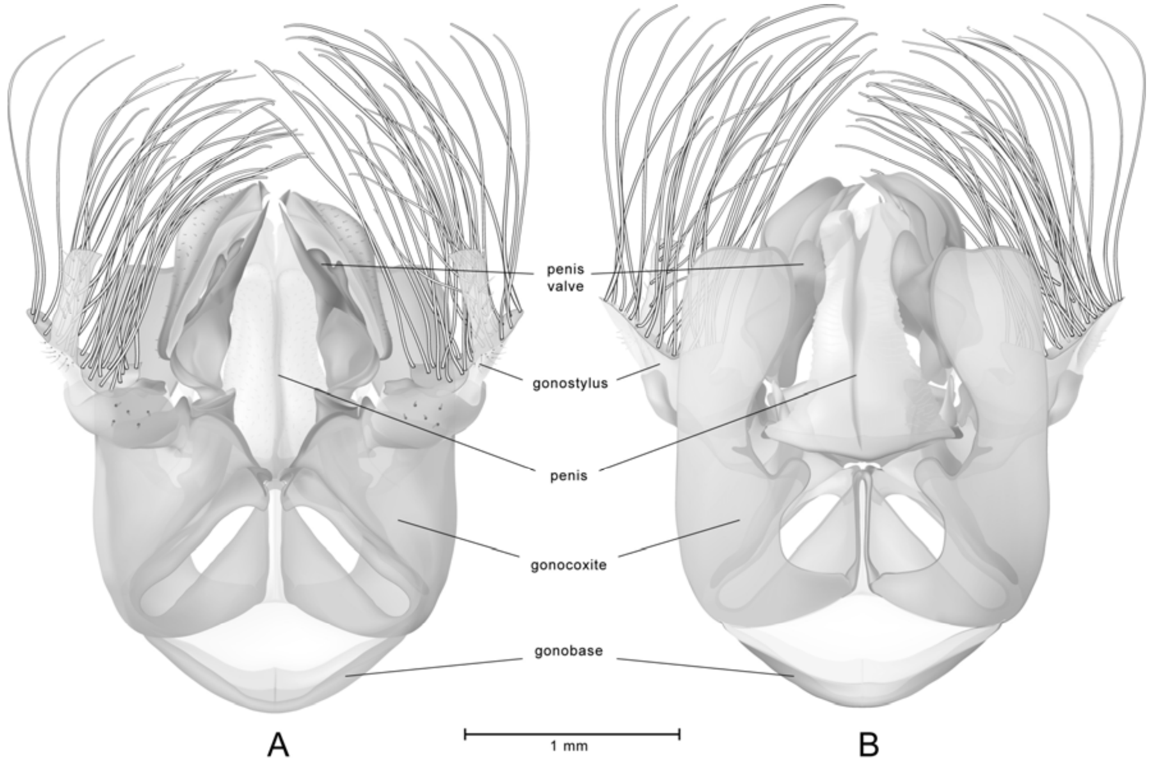

Fig. 2.2. *Euglossa imperialis*, male genital capsule. **A.** Ventral view. **B.** Dorsal view. The setae on gonostylus are here depicted at a lower density than they are on real organisms.

sclerotic. A soft mesal line divides the gonobase into lateral symmetrical halves, while defining a small dorsomesal projection. Dorsally the convex blade weakens as it runs posteriorly to meet the gonocoxites, making it hard to find the limit between the sclerite and the continuing membrane that closes the dorsal section between gonobase and gonocoxites. In lateral view the anteriormost section of the ventral edge projects ventrally (Fig. 2.4 B).

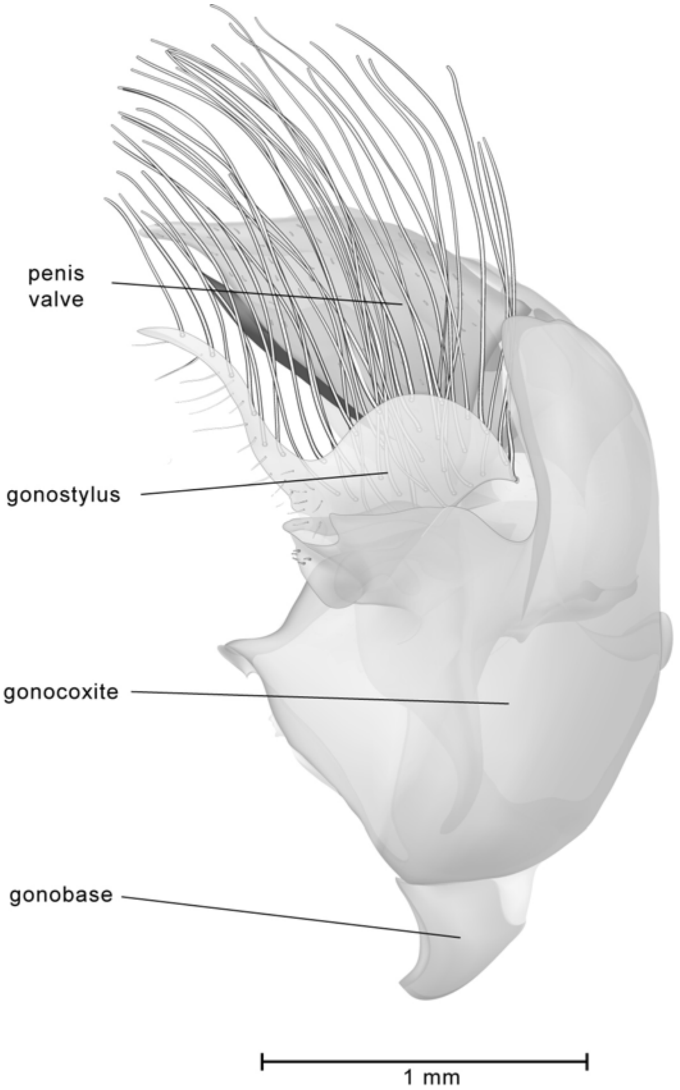

Fig. 2.3. *Euglossa imperialis*, male genital capsule, lateral view. The setae on gonostylus are here depicted at a lower density than they are on real organisms.

The variation observed in the gonobase of all euglossine species involves basically its length and width. An important species-specific character seems to be the way the ventral edge projects on its anterior section, or if it does not project at all (e.g. Hinojosa-Díaz and Engel, 2007).

In *Bombus* the gonobase is a complete sclerotized ring enclosing the genital foramen, although the ventral section is considerably narrower than the dorsum, sometimes appearing as fused to the gonocoxites (Ito, 1985). In Meliponini this structure is reduced as a whole, present as a very narrow dorsal belt (Camargo *et al*., 1967; Michener, 2007). The ventral part of the ring is obviously absent in Euglossini (i.e., membranous), and is generally very narrow in Centridini and Anthophorini, looking almost absent in some species of *Centris* (e.g., Ayala, 1998).

Gonocoxite.—The pair of gonocoxites constitutes the bulkiest part of the genitalia. Viewing the insect male genitalia as homologous to walking appendages, the gonocoxites plus the gonobase would correspond to the basal segments of the outer section of the embryonic claspers (Michener, 1944a, 1956); this is important in terms of the name of the structure. In *E. imperialis* each gonocoxite is a strongly-sclerotic unit with a well defined basal (anterior) section, and the main body of the gonocoxite as a laterally convex structure open towards the inner area where the aedeagus is located (Figs. 2.4 A-B). The basal section corresponds to the anterior surface of the gonocoxite, forming a posterolateral wall to each side of the genital foramen. In a comparative study of the male genitalia of Andrenidae, a likely homologous structure to the basal section is referred to as the gonocoxal apodeme (Rozen, 1951). The basal section is divided by a deep diagonal incision through which musculature and the ejaculatory ducts pass. The triangular, anteromesal subsection defined by the incision, connects on its inner mesal edge (reinforced by a dorsal inflection), with the corresponding subsection of the opposite gonocoxite (Fig. 2.4 A). This longitudinal membranous connection articulates both gonocoxites in a hinge fashion, giving them a forceps like movement. The posterolateral subsection is narrow, broadened on its posteromesal extreme with an acute projection directed anteriorly, and with a strong carina on its apex. The apical projection of the carina forms the second point of articulation with the opposite gonocoxite. Both subsections, are differentially oriented forming an acute separation angle, at the anterior extreme of the incision, that increases or decreases as the gonocoxites move acting as forceps.

The main body of the gonocoxite is strongly concave continuing from the basal section, and with distinctive processes on its dorsal and ventral surfaces. The dorsal surface of the body of the gonocoxite rises convexly from the anterodorsal edge of the basal section. It is dominated by two processes, a dorsal process projected posteromesally, and a larger apical (posterior) process. The dorsal process is a thumblike projection of the dorsomesal edge, slightly longer than wide and evenly rounded apically; the apex of the dorsal process is reinforced by an infold (Fig. 2.4 C). The dorsal process separates two major incisions, one basal and another subapical. The basal incision is evenly concave, while the subapical incision forms an acute angle with the dorsal process. The subapical incision precedes the large apical process, which is a projection of the whole dorsal surface of the gonocoxite. The apical process is a hollow flattened structure with sharp, carinate edges, and a short perpendicular

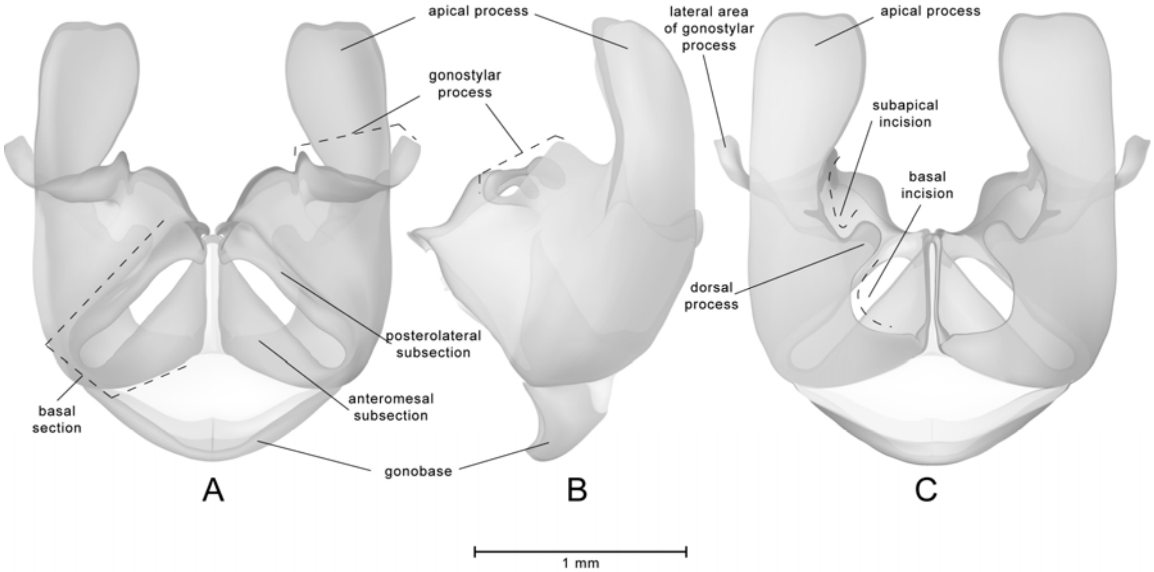

Fig. 2.4. *Euglossa imperialis*, male gonocoxites. **A.** Ventral view. **B.** Lateral view. **C.** Dorsal view.

thickening at the concave edge of the subapical incision (Fig. 2.4). The inner edge of the apical process, immediately after the subapical incision, is convex, making the process wider near its apex. The posterior edge is truncate with a minor emargination proximal to the posteromesal corner. The straight outer edge of the apical process meets basally with the gonostylar process of the ventral surface of the gonocoxite (see below).

The ventral surface of the gonocoxite continues from the posterior edge of the basal section after a sharp bend. It is rather flat ventrally (Fig. 2.4 A), becoming convex laterally (Fig. 2.4 B) to converge with the dorsal surface. The inner edge is posteromesally convex and is hardened by infolds and thickenings; two strong prong-like projections, directed towards the lumen of the genital capsule, are located at the posteromesal angle. The ventral surface bears apically its main feature, the gonostylar process, which is a ventrolateral projection with a basket-like shape where the gonostylus rests (Figs. 2.2-2.3). The gonostylar process extends laterally beyond the outer edge of the gonocoxite and is reinforced by infolds (Fig. 2.4). The whole lateral section of this process is fused to the thin sclerotic blade of the ventral section of the gonostylus (Figs. 2.2-2.3); however the border between both structures can be recognized on the basis of change of thickness of the sclerotic blade.

The inner concavity created by the gonocoxites is occupied by soft tissue and by the apodemes of the penis valves (Figs. 2.2-2.3). The open areas between the inner edges of the dorsal and ventral surfaces are protected by membranes.

The morphology of the gonocoxite is a good source of characters of definite taxonomic and systematic value. Perhaps the most noticeable feature of the gonocoxite of *E. imperialis* is the large apical process. The variation of this structure within *Euglossa* involves the alignment of its posterior edge. As seen in *E. imperialis* this edge is perpendicular to the sagittal plane of the genital capsule (Fig. 2.2 B), a condition found in all species of *Glossura* and seemingly all *Euglossa sensu stricto*, as well as in some *Glossurella* (e.g., Parra-H *et al.*, 2006; Rasmussen and Skov, 2006). In the subgenus *Euglossella* (and some *Glossurella*) this edge is oblique, having the posterolateral corner displaced anteriorly, making the apical process look acute in dorsal view (e.g., Hinojosa-Díaz and Engel, 2007), while in *Glossuropoda* the opposite oblique situation is observed. The carina of the posterior edge is in some species slightly projected especially the corners, making it shallowly concave. In the other euglossine genera the edge is rather oblique the way it is in *Glossuropoda* (the opposite to that of *Euglossella*), although in some of them it is hard to evaluate this situation, especially in *Eufriesea* in which the edge is deeply concave (e.g., Kimsey, 1982), being just slightly concave in *Eulaema* and *Aglae*, and slightly convex in *Exaerete* (e.g., Anjos-Silva and Rebêlo, 2006). The apical process is larger in *Euglossa* than in the rest of the Euglossini. This process seems to be present in *Bombus* as a posterior enlargement; however, it is not a free apical projection since it bears on its apex the structure homologized with a gonostylus by Michener (2007), and called the squama by Ito (1985). In Meliponini the apical process seems to be present in some groups, but reduced and with varied shape. The apical process is absent in Centridini and Anthophorini. The dorsal process is also subject to variation, in *Euglossa* varying in shape and orientation, which in turn alters the shape of the basal and subapical incisions, particularly the latter. The variation in shape of the dorsal process and adjacent incisions seems to be species specific, varying among species of the same subgenus. This process is present in the other euglossine genera except *Aglae*. The basal incision is noticeably concave throughout all *Euglossa* species, while in *Eufriesea*, *Eulaema*, and *Exaerete* it can hardly be considered an incision since the inner edges of the dorsal surface of both gonocoxites run parallel to each other, basal to the dorsal process. *Bombus* has a dorsal process located in a more basal position than in Euglossini; therefore the basal incision is shorter and the subapical incision enlarged (e.g., Ito, 1985). No dorsal process is seen in Meliponini. Several groups of Centridini have a dorsal process, with various shapes as well as a basal incision also varied. In Anthophorini there is no obvious dorsal process although the convexity of the basal inner edge of the dorsum of the gonocoxite could be mistaken for it. In *Euglossa* the gonostylar process also shows substantial variation, which is correlated with the variation of the gonostylus (see below).

In general the larger gonostylar processes, with well differentiated basket surfaces, are found in *Glossura*, *Glossuropoda*, and some *Glossurella*, while in the rest of the species the process is rather small. Only in the aforementioned groups (not in all species of *Glossurella*) is the gonostylar process fused to the gonostylar blade. In the rest of the species of *Euglossa* the lateral edge of the gonostylar process barely exceeds the lateral margin of the gonocoxite, and besides not being fused to the gonostylar sclerotic lamina, it can have acute, truncate, rounded or prong-like shapes. The inner delimitation of the gonostylar process also varies in its separation from the posteromesal prongs of the inner ventral edge, being almost contiguous in most *Glossura*, and some *Glossurella*, and of varied nature in other groups. The gonostylar process of the other euglossine genera is, despite the different opinion of Kimsey (1987), not as developed as the one described here, being more similar to the one seen in species of *Euglossa sensu stricto*; however, in both *Eufriesea* and *Eulaema*, the lateral projection of the process is rather slender and setose on its apex. In the other corbiculate and non-corbiculate apine bees, used here for comparison, there is no strong modification of the ventral surface of the gonocoxite to support the gonostylus.

Gonostylus.—The gonostylus of *E. imperialis* is a very noticeable structure arising from the gonostylar process of the gonocoxite. It is mainly membranous, setose, and composed of two main parts, a ventral section and a lateral section (Fig. 2.5). The ventral section has been treated differently, as can be seen in Table 1, sometimes as part of the gonostylus (Michener, 1944a), as the sole gonostylus (in *Bombus* [Ito, 1985]), as a different structure called the volsella (Kimsey, 1987; Michener, 2007), or ignored as a gonostylar component (Ospina-Torres *et al.*, 2006). Here this section is discussed as part of the gonostylus since it is structurally connected to the lateral section. The term volsella as used by Kimsey (1987) and Michener (2007) in Euglossini (also in Bombini by the second author) is particularly misleading. This gonostylar section is not homologous to the pinching structures found in the genital capsule of other Hymenoptera, including other groups of bees, and as such the term volsella should be avoided in this context. The bulky membranous ventral section of the gonostylus is continuous on its posterolateral area with the lateral section of the gonostylus (Fig. 2.3); it is roughly rod-shaped, running dorso-ventrally, with its ventral half resting entirely on the basket of the gonostylar process of the gonocoxite. The oval, flat ventral surface of the ventral section, exceeds the gonocoxite basket edge. The dorsum of the ventral section is embedded in the lumen of the genital capsule (Fig. 2.3). The overhanging ventral surface, as well as the posterior and lateral surfaces, are covered with a continuous cuticular layer (Fig. 2.5 A). This lamina is connected laterally to the lateral projection of the gonostylar process of the gonocoxite, and is continuous with the inner surface of the lateral section of the gonostylus. As mentioned before, gonostylus and gonocoxite are fused on the lateral projection of the gonostylar process, but the limit of both structures can be traced by the changes in thickness of the sclerotized continuous cuticle. The ventral overhanging surface is flat and centrally covered with sparse, minute, simple setae.

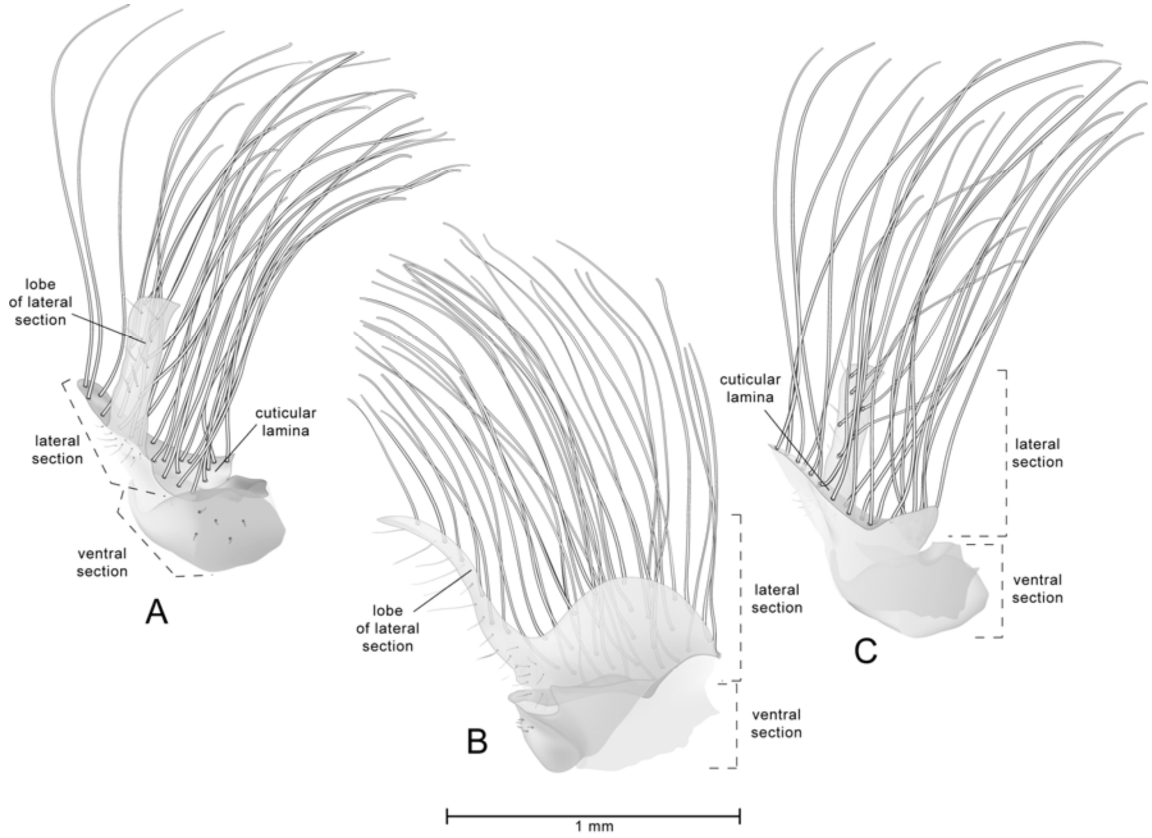

Fig. 2.5. *Euglossa imperialis*, right male gonostylus. **A.** Ventral view. **B.** Lateral view. **C.** Dorsal view. The setae on gonostylus are here depicted at a lower density than they are on real organisms

The conspicuous lateral section of the gonostylus, also membranous, rests posterolaterally to the ventral section, as a free projection. It is somewhat compressed, diagonally oriented, and scoop-shaped if seen from the middle axis of the genital capsule (Fig. 2.5). In lateral view, the posterior edge of the gonostylar lateral section is convex on its dorsal main sector (Fig. 2.5 B), while ventrally it bears a slender membranous lobe that appears rectangular in ventral or dorsal views (Fig. 2.5 A-C). The whole inner surface, including the lobe, is covered by the cuticular lamina that joins it with the posterior surface of the ventral section of the gonostylus, from which it can be differentiated by a shallow channel and by the presence of dense, long, simple setae covering the entire inner surface, including the lobe. The outer (ventral) surface of the lobe is covered by sparse, short, simple setae (Fig. 2.5).

The gonostylus is perhaps the most diversified structure of the genital capsule of *Euglossa*, and is as such of substantial taxonomic and systematic value in terms of infrageneric assemblages. The ventral section is generally overhanging, but this is definitely much more noticeable in species of *Glossura*, *Glossuropoda*, and some *Glossurella*. The rest of the species have a ventral section not as large, just surpassing the basket edge, and the ventral surface is convexly projected, while in the aforementioned groups it is rather flat, truncate (as described for *E*. *imperialis*). A feature that covaries with the overhanging ventral section is the presence of the continuous cuticular lamina connecting the ventral section with the lateral section, as well as with the gonocoxite on its gonostylar process. In those species in which the ventral section is neither bulky, nor strongly overhanging, the gonostylar process of the gonocoxite, the lamina of the posterior surface of the ventral section, and the inner surface of the lateral section, is only connected on its dorsalmost extreme, while there is no continuity between the ventral lamina and the lateral part of the gonostylar process of the gonocoxite, which according to Kimsey (1987), is considered the derived condition in Euglossini. The minute setae on the ventral section are usually on the ventral overhanging surface, but in some species they are also on the inner or posterior surfaces.

The variation of the lateral section of the gonostylus deserves special attention. Ospina-Torres *et al*. (2006), in their brief comparative study of this structure in *Euglossa*, proposed five different arrangements, mainly based on its division into two lobes. Their "ventral lobe" is the slender lobe here described for *E. imperialis*, and their "dorsal lobe" would be equivalent to the dorsal sector of the lateral edge, which is noticeably convex. Although in a lateral view this latter feature can certainly be considered a lobe, it is more accurate to look at the structure over its whole shape, not just laterally. In doing so, it seems more reliable to consider this just as a strongly convex edge of the setose dorsal sclerotic blade of the gonostylus. Ospina-Torres *et al*. (2006), examined the gonostylar lateral sections by mounting them on flat slides, not considering the whole tridimensional arrangement of it as it is situated on the gonocoxite. This arrangement is important, having two alternatives: the dorso-posterior blade (i.e., the cuticular blade bearing the long setae) oriented diagonally (as in *E. imperialis*), in all species of *Euglossa sensu stricto* (e.g., Bembé, 2007), *Glossura* and *Glossuropoda*, and some *Glossurella*, or the dorso-posterior blade almost totally compressed and oriented straight posteriorly, as in most *Glossurella*, and all *Euglossella* species. Nonetheless, the nature of the posterior edge (rather lateral in the diagonally-oriented blades) is also valuable in a taxonomic and systematic context. Clearly convex in several groups (as in *E*. *imperialis*), it appears flat, or even concave in some species; in some cases the basal sector is either enlarged with a minute ventral lobe, or reduced with a dominant ventral lobe. The location, density, and nature of the setae on the lateral section of the gonostylus also vary considerably. The longer setae are always on the dorsal blade, sometimes being plumose as in *Euglossella* species (Hinojosa-Díaz and Engel, 2007), while the ventral, or lateral surfaces of the ventral lobe bear sparse, simple, minute setae. Some species have the gonostylus almost devoid of setae, noticeably *E. nigrosignata* and *E. oleolucens*.

It is important to mention that beyond the usefulness of the gonostylar morphology to define infrageneric assemblages in *Euglossa*, there are differential cases of their application to determine species boundaries. Most of the species in the genus have a particular morphology with little variation as is the case of *E*. *imperialis*, but there are cases like *E*. *decorata* and allied species in which there seems to be a great deal of intraspecific variation in gonostylar morphology (Hinojosa-Díaz, in prep.) although all of it within the overall morphology that characterizes *Euglossella*.

The gonostylar variation in other euglossines occurs mainly in the lateral section (i.e., the one treated as gonostylus). In *Eufriesea* it is laterally compressed, not diagonal, appearing more sclerotized than in *Euglossa*, but most importantly, strongly bilobate. The large dorsal lobe is probably homologous to the dorsal sector of the lateral section of the gonostylus of

Euglossa judging by the disposition of the setae. In *Eulaema* and *Aglae* the also sclerotic laterally-compresed lateral section of the gonostylus consists of a simple lobe, with just a small dorsal bump seemingly homologous to the dorsal sector. In *Exaerete* the membranous lateral section of the gonostylus has a ventral rod-shaped lobe, and a flat and small dorsal sector (Kimsey, 1979). In *Bombus* the ventral section (treated in other studies as volsella [Kimsey, 1987; Michener, 1990, 2007], or as the exclusive gonostylus [Ito, 1985]) is considerably sclerotized and larger than in Euglossini, lying apically on the unmodified ventral area of the gonocoxite, and with apical modifications (Ito, 1985). The lateral section (treated in other studies as the squama [Ito, 1985]), also sclerotic, is rather subapical on the dorsal surface of the gonocoxite. In Meliponini the gonostylus is represented by a single slender lobe coming from the base of the gonocoxite. In Centridini and Anthophorini, all the features of the gonostylus observed in Euglossini are found, with a range of variation entirely their own.

Aedeagus.—This is the structure between the gonocoxites, composed of the strongly sclerotic penis valves articulated at each side of the penis (Fig. 2.6). The aedeagus is joined to the genital capsule by the insertion of the apodemal projection of each penis valve into the lumen of the gonocoxites (Figs. 2.2-2.3).

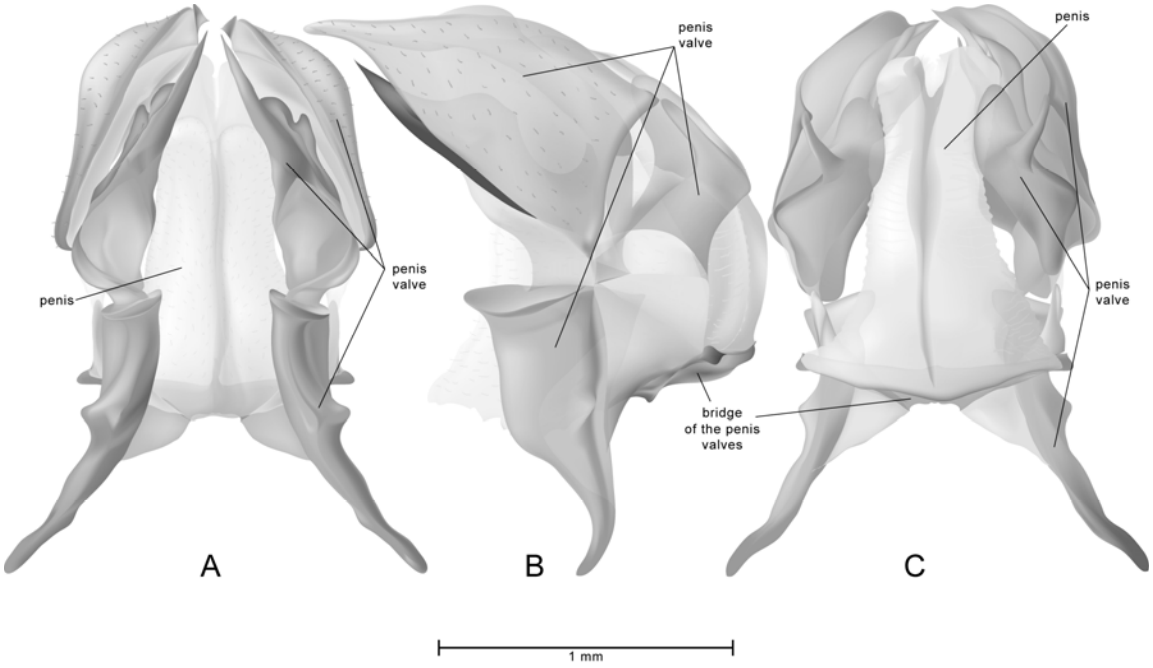

Fig. 2.6. *Euglossa imperialis*, aedeagus. **A.** Ventral view. **B.** Lateral view. **C.** Dorsal view.

Penis valve.—Following the idea that the insect male genitalia is homologous to walking appendages, the penis valves would correspond to the inner section of the embryonic claspers (Michener, 1944a, 1956). Each penis valve is a sclerotic structure with membranous areas connecting three distinguishable sections: apodeme-base, dorsal section, and ventral blades (Figs. 2.7). The apodeme-base is the most anterior section. It is a somewhat compressed, cone-like structure, that takes about half of the total length of the valve, and is the one by which each valve is united to the rest of the genital capsule. The attaching acute apodeme is embedded in the inner matrix of the capsule, at the base of the gonocoxite, is curved ventrolaterally towards the lateral wall of the gonocoxite. Outside the lumen of the gonocoxite this section widens dorsoventrally and an acute prong is projected laterally (Fig. 2.7 A). The dorsal side of the apodeme-base bears a lamella extending in two directions; the anterior part is directed mesally while the posterior part extends posteriorly following the orientation of the whole penis valve. The fold that delimits the two lamellar sections strengthens the anterodorsal edge of the apodeme-base and projects dorsally in an acute angle.

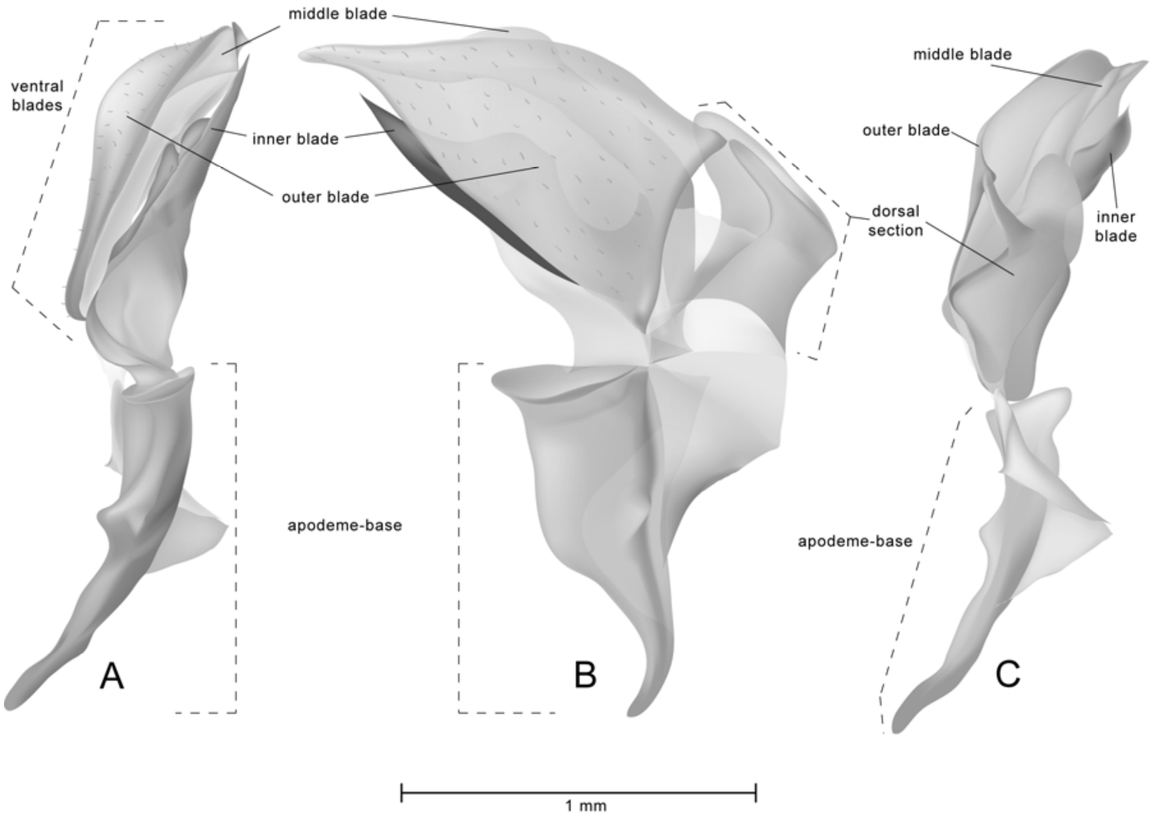

Fig. 2.7. *Euglossa imperialis*, penis valve. **A.** Ventral view. **B.** Lateral view. **C.** Dorsal view.

The anterior part of this lamella articulates with the bridge of the penis valves and the posterior part does the same with the dorsal section of the penis valve (Fig. 2.6 B-C). The ventral side of the apodeme-base takes up most of the posterior widening of the conic shape. The posterior surface takes a scoop-like shape with a carinate edge, and leans slightly towards the gonocoxite. This posterior widened area of the apodeme-base lies close to the pair of strong, prong-like projections of the ventral surface of the gonocoxite (Fig. 2.2 A). The scoop-like posterior surface is articulated to the ventral blades of the penis valve by a sinuate, slightly-sclerotic membranous fold.

The dorsal section of the penis valve consists of a sclerotic unit with a compressed trapezoidal shape, running anteroposteriorly, the posterior part broadened and divided laterally in two prong-like projections, one on each side, forming a posterior concave surface (Figs. 2.7 B-C). The inner prong is larger. The whole dorsal section, as mentioned above, articulates anteriorly with the apodeme-base and ventrally, via weakly sclerotic and membranous segments, with the ventral blades.

The ventral blades section of the penis valve comprises three compressed blade-like structures appressed one against another and oriented dorsoventrally. The outer blade has a sublanceolate shape, with all edges folding towards the meson (Fig. 2.7 C). It articulates with the dorsal section of the penis valve via a membrane attached to its strong, rather truncate dorsal edge, and with the scoop-like posterior surface of the apodeme-base via a sinuate slightly sclerotic membranous fold. The entire outer surface of the outer blade is covered with sparse, minute setae. The middle blade is strongly sclerotic on its dorsal and posterior margins, being attached to the mixture of sclerotic and membranous junctures that come from the dorsoposterior area of the apodeme-base. The rest of the middle blade is rather membranous and united to the outer blade all along its anterior edge, both blades together forming a sort of compressed sheath. The inner blade is the most sclerotized and runs along the anterior edge of the other two blades. It is joined to the rest of the penis valve on the same sclerotic-membranous juncture coming from the dorsoposterior area of the apodeme-base. This inner blade has a knife-like shape, with a very acute ventral apex and a series of thickenings and convolutions on its posterior edge (Fig. 2.7). Each penis valve serves as a support for the penis as it forms a movable articulation with the bridge of the penis valves through the dorsal lamellae that run mesaly from the dorsum of the apodeme-base on each penis valve (Fig. 2.6).

The morphology of the penis valves is conserved in *Euglossa*. There is some slight variation in size and projection of the ventral blades. Moreover, some variation can be found in the shape of the posterior edge of the outer blade, which is more noticeably convex in groups like *Euglossella* and some *Glossurella*. The same edge on its dorsal extreme is conspicuously notched in *Euglossella* (e.g., Hinojosa-Díaz and Engel, 2007).

Eufriesea and *Eulaema* have penis valves composed of the same sections described for *Euglossa* although the whole structure seems to be more sclerotized, with less membranous interconnections. In both genera, the posterior edge of the outer blade is notched, as mentioned for *Euglossella*, but to a greater extent in some cases. The scoop-like modification of the posterior surface of the apodeme-base is exclusive to *Euglossa*, while in *Eufriesea* and *Eulaema* it is truncate, and convex in *Exaerete*. In *Eulaema*, in particular, the ventral blades are smaller and the dorsal section (although distinguishable) is fused to them. In *Exaerete* there is no definite dorsal section and the inner blade is rather small. *Aglae* has a completely different situation: both penis valves and the bridge of the penis valves are fused in a continuous, non-articulated structure with bulgy penis valves covered by dense, moderately-long setae on their outer surfaces (Kimsey, 1987). In *Bombus* the penis valves are less elaborate than in Euglossini, with smaller ventral projections, although with interesting modifications on their own (Ito, 1985). In Meliponini the penis valves are simple, hook-like projections. In Centridini there are several species groups in which the conformation of the penis valves is very similar to the one described for *Aglae*, although the bulgy ventral projections show different arrangements depending on the group, and in several of them also take sheath-like shapes. Similar situations are found in Anthophorini.

Penis.—The penis in *E. imperialis* is a saclike structure occupying a mesoposterior location in the genital capsule, between the penis valves by which it is supported. The base of the penis is formed by the bridge of the penis valves (Fig. 2.8). The bridge of the penis valves is a strong plate articulated to the penis valves through their dorsal lamellar projections. The structure of the bridge is built along three main sclerotic ridges, two of them expanding laterally to form the acute arms and a mesal one that continues dorsally as the main axis of the spatha (Figs. 2.8 B-C). Ventrally this bridge has two small acute projections that form a semicircular invagination. The whole dorsal edge of the bridge bends dorsally to form the convex spatha, which is a weaker sclerotic lamina narrowing apically, with a central axis (continuing from the mesal sclerotic ridge), slightly invaginated all along (Figs. 2.8 B-C). The lateral sections are strengthened by minor ridges perpendicular to the main axis. The apex of the spata is bilobed.

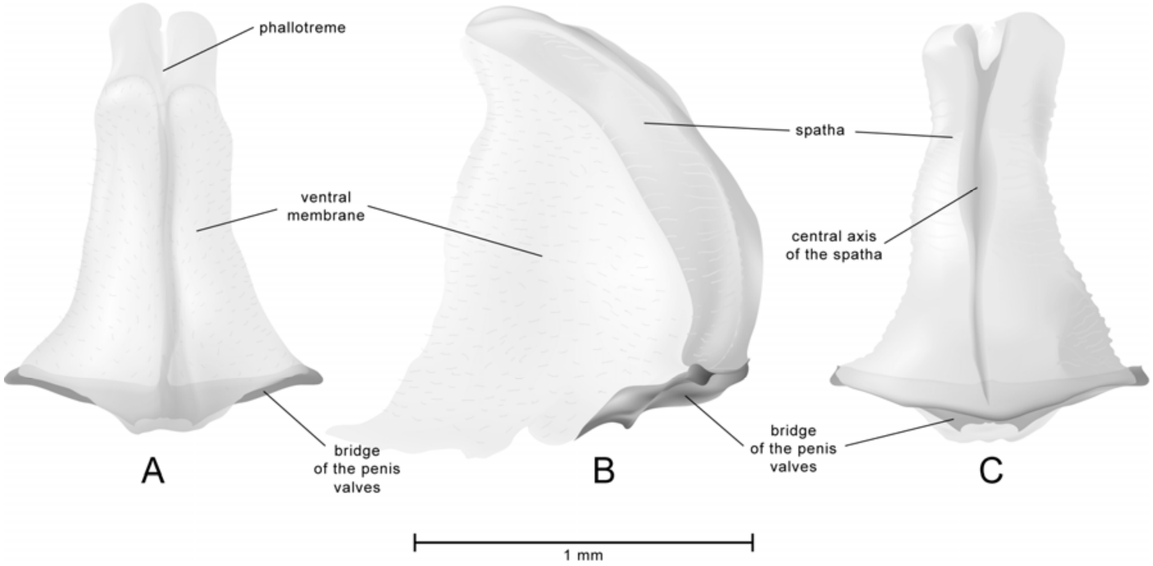

Fig. 2.8. *Euglossa imperialis*, penis. **A.** Ventral view. **B.** Lateral view. **C.** Dorsal view.

Together the bridge of the penis valves and the spatha serve as the anterior and dorsal sclerotic supports of the membranous ventral surface of the penis. This ventral membrane is attached anteriorly to the membranes that close the inner open sections of the gonocoxites and to the posteromesal extremes of the basal section of the gonocoxite. Most of the membrane surface is covered with short setae. The phallotreme is located subapically between the bilobed apex of the spatha. (Fig. 2.8). According to Roig-Alsina (1993) the genus *Euglossa* has a simple, bag-like eversible endophallus with conspicuous spiculation, evidence of which can be seen in dissected genital capsules through the external membranous wall of the penis.

The penis variation in *Euglossa*, in taxonomic and systematic terms, can be seen in the relative length and width of the spatha, as well as in the extent of the central axis of the spatha. The spatha is evidently longer than its basal width in most species of *Euglossa sensu strict*, all *Glossura*, most *Glossuropoda*, and some *Glossurella*, while the rest of the species have a spatha about as long as its basal width. *Euglossa sensu stricto*, *Glossura*, *Glossuropoda*, and some *Glossurella* have a complete central axis, slightly invaginated, as described for *E. imperialis*. Most other species have a non-invaginated central axis running only mid-distance to the apex of the penis. In *Euglossella* it is restricted to the very anterior extreme of the spatha (e.g. Hinojosa-Díaz and Engel, 2007). The axis is absent in *E. villosa*. In *Euglossella* the spatha is reinforced by small longitudinal wrinkles instead of the transverse ones present in most of the species.

In the other Euglossini, the bridge of the penis valves (either differentiated or fused) is oriented dorsally in the same plane as the spatha; this causes the two ventrally-oriented projections seen in *Euglossa* to be oriented anteriorly. The spatha is rather short, although wide in *Eufriesea* and *Eulaema*, short and narrow in *Aglae*, and practically absent in *Exaerete*. In none of these genera is there a noticeable central axis to the spatha. Kimsey (1987) mentioned the setose penis as a derived characer in *Euglossa* and in the other euglossine genera appearing asetose. However, on a closer examination, there seems to be setose areas in all Euglossini, very noticeable in *Aglae* and restricted to small ventral areas in the other genera. Setose penes are also easily identifiable in at least Centridini.

In *Bombus*, the bridge of the penis valves is rather different in shape from the one in Euglossini, while the spatha is narrow (in most species) and long (Ito, 1985). In Meliponini the spatha is wide. In Centridini, several groups have a very weak bridge of the penis valves, and the spatha is generally configured as in *Eufriesea* and *Eulaema*. In Anthophorini the bridge is well sclerotized and the spatha, likewise, similar to the aforementioned euglossines.

DISCUSSION

The immediately captivating external morphology of euglossine bees combined with the interesting suite of external secondary sexual characteristics of the males have served as the foundation for the taxonomy of the tribe. This situation is particularly evident for *Euglossa*, the largest genus of the tribe, in which the dominance of these characters has relegated to obscurity the use of other important sets of morphological features, notably the male genitalia and hidden metasomal sterna. As mentioned before, few species of *Euglossa* were described with references to the male genital structures; those original descriptions that include these features are mainly of new species described in the last two years (Parra-H *et al*., 2006; Ramírez, 2006; Rasmussen and Skov, 2006; Hinojosa-Díaz and Engel, 2007). The traditionally used external characters are obviously useful, but there is no reason to ignore the important information contained in the genital capsule and the hidden metasomal sterna.

As detailed in the present work, the diversity of the different components of the genitalia and the hidden sterna in the genus *Euglossa* provides a considerable source of characters important in terms of recognition of species or species groups, but perhaps more significantly, as valuable added information to be applied in systematic and phylogenetic studies. A good example of this usage is the recently published phylogenetic study and revision of an assemblage within *Euglossa sensu stricto* (Bembé, 2007). The infrageneric categories in use for *Euglossa* (Cockerell, 1917; Moure, 1967, 1989; Dressler,1978b, 1982b, c, d), based exclusively on external morphology, are applicable without much trouble to a good number of species. However, there are instances in which the sole presence or absence of particular characters of the male make it hard to be certain of appropriate subgeneric assignment. The subgenus *Glossurella* is a clear example of a difficult assemblage of species that do not seem to be necessarily closer to one another than to other groups within the genus. For instance, the group of species allied to *E. allosticta*, all included in *Glossurella* (Dressler, 1982b), are externally very similar to bees in the subgenus *Glossura*, from which they differ by the lack of some structures (mesotibial tufts, sternal cowls) that are used for defining this subgenus. Upon reviewing the genital characters, it becomes clear that this assemblage belongs in *Glossura*. This can be seen in the depiction of the genital structures of *E. asarophora* and *E. rufipes* (Rasmussen and Skov, 2006), which are similar to those described here for *E. imperialis*, a member of *Glossura*. This situation is repeated for other groups within *Glossurella*, only varying the characters involved. Another good example of troubled taxonomic status, with the exclusive use of non-genital external morphology, is that of *E. nigrosignata*, originally described in *Glossura* by Moure

(1969), then moved to *Glossurella* by Dressler (1982b), and recently transferred to *Glossuropoda* by Roubik (2004). The male genitalia of *E*. *nigrosignata* reveal few similarities to those of *Glossura* or *Glossuropoda* since it poses a unique gonostylar morphology more similar to that of some species in *Glossurella*. There are more examples like these, which reveal the necessity of a revision of the present subgeneric classification of *Euglossa* (see Chapter 3). Notwithstanding, the external characters used in the subgeneric classification define some well demarcated groupings inside *Euglossa*, such as *Euglossella* whose genital characters only reinforce the distinctivenes of this subgenus. With the current situation, it would be preferred to take a more conservative position, such as the one of Michener (2007) who prefers to place all the subgeneric names as synonyms under the name *Euglossa*. The outstanding secondary sexual characters of the males that heavily influence this classification are useful characters; however, their usefulness is limited if ignoring other sources of information that can help to solve the controversies created when such characters are unable to clearly resolve taxonomic status, and it is in this context where the characters from the genital structures enter to play their best role.

It is clear that a phylogenetic framework is needed to produce a more stable and reliable infrageneric partition of *Euglossa* and the main purpose of this chapter is to present a basis of the male genital morphology, in order to be included in an infrageneric phylogenetic analysis based on morphology (Chapter 3), but also to provide a basis of comparative morphology of the structures here described, and to encourage their use.

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CHAPTER 3

Phylogeny and Classification of the Orchid Bee Genus *Euglossa* Latreille (Hymenoptera: Apidae)

INTRODUCTION

Among the five euglossine genera, *Euglossa* is the one that exhibits the highest diversity, both in number of species, as well as in morphological differentiation. As such, the just under 120 nominal species known for the genus (Ramírez *et al*., 2002, Nemésio and Silveira, 2007) have been assigned to six subgenera in an attempt to reflect the morphological diversification inside the group. The current subgeneric classification is a summary of the significant taxonomic work invested in the genus. Cockerell (1917) produced the first subdivision of the genus while erecting *Glossura* to initially include two species [*E*. *piliventris* (type species) and *E*. *ignita*] with considerably elongated mouthparts and "bigibbous scutellum", consequently leaving the rest of the species in *Euglossa s. str*. Moure (1967) added a third subgenus, *Euglossella* (*E*. *viridis* as type species), aiming to regroup those species with males with tridentate mandibles formerly in *Euglossa s. str.* Dressler (1978b) made an integral approach to the infrageneric classification of the genus, whilst proposing several species groups (see below); he reinterpreted *Glossura* and *Euglossella* and created a fourth subgenus (*Dasystilbe*) by bringing into consideration additional morphological features, mainly from secondary sexual characters of the males. Most of his attention in the redefinition of the subgenera was focused on characters found on the meso and metatibiae of the males. He created *Dasystilbe* as a monotypic subgenus to include *E*. *villosa*, which in his view shared features of both *Glossura* and *Euglossella*, and as such the erection of *Dasystilbe* would bring stability to the classification (Dressler, 1978b). Dressler

(1982c) furthermore segregated one of the species groups that he had originally (Dressler, 1978b) seen as part of *Glossura*, and created *Glossurella* (*E*. *bursigera* as type species) as an assemblage of those *Glossura*-like species with characteristic “pockets” on the second metasomal sternum. Moure (1989) added the last of the six subgenera in use, *Glossuropoda*, to include *E*. *intersecta* (type species; originally in *Glossura*) and allied species (two more described by Moure at the time), with an enlarged and posteriorly sinuate mesobasitarsus.

The seeming usefulness of the secondary sexual features of the *Euglossa* males have for the most part modeled the delimitation of the subgenera, specially as rearranged by Dressler (1978b, 1982b, c, d), who also provided an outline of the species groups, based on the same set of external features, plus characters of the mouthparts (length), punctuation and the dorsolateral angle of the pronotum. In his original infrageneric treatment, Dressler (1978b) proposed 12 species groups (all of them identified by roman numerals and, in most cases, a representative species name) along with the four subgenera there outlined: *Glossura* and *Euglossa s. str.*, each with five species groups, and *Dasystilbe* and *Euglossella* each with one species group. As mentioned above, one of his original *Glossura* species groups was later granted subgeneric status on its own (Dressler, 1982c) as *Glossurella*, and subdivided into six more or less informal species groups (see Table 3.1). Moure’s *Glossuropoda* (Moure, 1989), as stated originally, corresponds to Dressler’s (1978b) *Glossura* species group one (I), and was expanded and redefined by adding *E*. *nigrosignata* (originally in *Glossura*) to it (Roubik, 2004). Dressler’s species groups I and II (i.e. *Glossuropoda*) are better seen as a single group, since synonymies have been proposed back and forth between *E. rugilabris* (group II) and species described by Moure (1989) as being allied to *E. intersecta* as part of *Glossuropoda* (therefore belonging to group I) (see Roubik, 2004; Nemésio, 2009). Additional changes of status (replacement in either subgenus or species group) for some species have resulted from some studies of assemblages within the genus, such as relocation of some *Euglossa s. str*. to different species groups within the subgenus (Bembé, 2007), transferences from *Glossurella* to *Glossuropoda* (Roubik, 2004), or from *Glossura* to *Glossurella* (Faria and Melo, 2007). A current account of the status of species composition for the six subgenera and 15 species groups as proposed by the discussed authors is presented in table 3.1.

Table 3.1. Species composition for the subgenera and species groups of *Euglossa* as presently understood. Species assignation and species group nomenclature is based on Dressler (1978b, 1982b, c, d); species described after Dressler's proposals (i.e. after 1982) are positioned according to the affiliation directly stated by the species' authors or by the authors indirect indication of association with other species.

Dasystilbe Dressler, 1978
One species group [species group VI (Dressler, 1978b)], one species.

Euglossa villosa Moure, 1968

Euglossa s. str. Latreille, 1802
Five species groups [species groups VIII—XII (Dressler, 1978b, 1982b, d)], 54 species.

VIII. *analis* species group
- *E. analis* Westwood, 1840
- *E. bidentata* Dressler, 1982
- *E. cognata* Moure, 1970
- *E. iopyrrha* Dressler, 1982
- *E. mixta* Friese, 1899
- *E. retroviridis* Dressler, 1982
- *E. villosiventris* Moure, 1968

IX. *viridissima* species group
- *E. viridissima* Friese, 1899

X. *cybelia* species group
- *E. aureiventris* Friese, 1899
- *E. championi* Cheesman, 1929

E. cybelia Moure, 1968
E. dressleri Moure, 1968
E. ioprosopa Dressler, 1982
E. laurensi Bembé, 2008
E. maculilabris Moure, 1968
E. nigropilosa Moure, 1965
E. tridentata Moure, 1970

XI. *purpurea* species group

E. alleni Moure, 1968
E. amazonica Dressler, 1982
E. anodorhynchi Nemésio, 2006
E. atroveneta Dressler, 1978
E. crininota Dressler, 1978
E. dissimula Dressler, 1978
E. gibbosa Dressler, 1982
E. hansoni Moure, 1965
E. heterosticta Moure, 1968
E. igniventris Friese, 1925
E. magnipes Dressler, 1982
E. micans Dressler, 1978
E. mourei Dressler, 1982
E. pleosticta Dressler, 1982
E. purpurea Friese, 1899
E. sovietica Nemésio, 2007
E. townsendi Cockerell, 1904
E. truncata Rebêlo & Moure, 1995

XII. *cordata* species group

E. chlorina Dressler, 1982
E. cordata (Linnaeus, 1758)
E. cyanaspis Moure, 1968
E. deceptrix Moure, 1968
E. despecta Moure, 1968
E. erythrochlora Moure, 1968
E. fimbriata Rebêlo & Moure, 1995
E. hemichlora Cockerell, 1917
E. jamaicensis Moure, 1968
E. leucotricha Rebêlo & Moure, 1995
E. liopoda Dressler, 1982
E. melanotricha Moure, 1967
E. milenae Bembé, 2007
E. modestior Dressler, 1982
E. platymera Dressler, 1982
E. securigera Dressler, 1982
E. variabilis Friese, 1899

Euglossa s. str. Incerta sedis

†*E. cotylisca* Hinojosa-Díaz & Engel, 2007 (belongs to either group XI or XII)
E. pictipennis Moure, 1943

Euglossella Moure, 1967

One species group [species group VII (Dressler, 1978b)], 15 species.

E. bigibba Dressler, 1982
E. cosmodora Hinojosa-Díaz & Engel, 2007
E. cyanea Friese, 1899
E. cyanura Cockerell, 1917
E. decorata F. Smith, 1874
E. granti Cheesman, 1929

- *E. jacquelinae* Nemésio, 2007
- *E. mandibularis* Friese, 1899
- *E. perfulgens* Moure, 1967
- *E. perpulchra* Moure & Schlindwein, 2002
- *E. perviridis* Dressler, 1985
- *E. polita* Ducke, 1902
- *E. singularis* Mocsáry, 1899
- *E. urarina* Hinojosa-Díaz & Engel, 2007
- *E. viridis* (Perty, 1833)

Glossura Cockerell, 1917
Two species groups [species groups III and IV (Dressler, 1978b, 1982b)], 12 species.

III. *piliventris* species group
- *E. chalybeata* Friese, 1925
- *E. flammea* Moure, 1969
- *E. ignita* F. Smith, 1874
- *E. imperialis* Cockerell, 1922
- *E. lugubris* Roubik, 2004
- *E. occidentalis* Roubik, 2004
- *E. orellana* Roubik, 2004
- *E. piliventris* Guérin, 1845
- *E. tiputini* Roubik, 2004

IV. *stellfeldi* species group
- *E. annectans* Dressler, 1982
- *E. solangeae* Nemésio, 2007
- *E. stellfeldi* Moure, 1947

Glossurella Dressler, 1982
Dressler (1982c) gave subgeneric status to its original *bursigera* species group [species group V (Dressler, 1978b)] and designated six assemblages within it (species groups here treated as Va, Vb, Vc, etc.). Currently 25 species.

Va. *crassipunctata* species group
- *E. crassipunctata* Moure, 1968
- *E. parvula* Dressler, 1982
- *E. sapphirina* Moure, 1968

Vb. *gorgonensis* species group
- *E. fuscifrons* Dressler, 1982
- *E. gorgonensis* Cheesman, 1929
- *E. hyacinthina* Dressler, 1982
- *E. oleolucens* Dressler, 1978
- *E. paisa* Ramírez, 2005
- *E. samperi* Ramírez, 2006
- *E. stilbonota* Dressler, 1982
- *E. trinotata* Dressler, 1982

Vc. unnamed species group
- *E. dodsoni* Moure, 1965
- *E. obtusa* Dressler, 1978

Vd. unnamed species group
- *E. augaspis* Dressler, 1982
- *E. bursigera* Moure, 1970
- *E. prasina* Dressler, 1982

Ve. unnamed species group
- *E. carinilabris* Dressler, 1982
- *E. laevicincta* Dressler, 1982
- *E. macrorhyncha* Dressler, 1982
- *E. turbinifex* Dressler, 1978

Vf. unnamed species group

E. allosticta Moure, 1969
E. asarophora Moure, 1969
E. natesi Parra-H, Ospina & Ramírez, 2006
E. rufipes Rasmussen & Skov, 2006
E. viridifrons Dressler, 1982

Glossuropoda Moure, 1989
One species group [species group I, (Dressler, 1978b)], six species.

E. cyanochlora Moure, 1995
E. inflata Roubik, 2004
E. intersecta Latreille, 1824
E. juremae Moure, 1989
E. nigrosignata Moure, 1969
E. rugilabris Moure, 1967

Incertae sedis
Three species

E. auriventris Friese, 1925
E. lazulina Friese, 1923
†*E. moronei* Engel, 1999

The use of external morphology, almost exclusively secondary sexual characters of the males, has provided this basic and seemingly coherent arrangement of infrageneric assemblages within the genus; however Dressler (1978b) when initially proposing his subdivision of the genus, and asserting that it was based entirely on these external features, gave room for future modifications of the scheme, specially by adding the then poorly known and scarcely used features of the male genitalia.

The monophyletic nature of *Euglossa* is supported by the presence of numerous exclusive morphological features; however until very recently, no phylogenetic approach existed to prove the monophyletic nature of the otherwise seemingly coherent morphologically based subgenera and species groups described above. It is not a necessary assumption of the original authors of either the subgenera or the species groups that they represent natural phylogenetic entities (i.e. monophyletic groups), but under a phylogenetic approach, assertions of relatedness (in the form of inclusion in a group of taxa) must be tested in terms of their possible monophyly. Since no phylogenetic study existed until the very recent publication of a molecular phylogeny for the tribe Euglossini (Ramírez *et al*., 2010) which included 80 species of *Euglossa s*. *lat*., uncertainty had always existed about the monophyly of the subgeneric groups in use. The only prior phylogenetic study in *Euglossa s*. *lat*. based on morphology, corresponded to a species level study of the *cordata* species group [group XII of Dressler (1978b)] in *Euglossa s*. *str*. (Bembé, 2007). In the mentioned study, the inclusion of male genitalic characters showed to be of particular help.

The previously cited relocation of species involving *Glossura*, *Glossurella*, and *Glossuropoda* (Roubik, 2004; Faria and Melo, 2007) is an example of taxonomic problems after the evaluation of the groups based on the external characters as originally defined, raising concerns about monophyly of the subgenera. The absence of monophyletic confidence for the subgenera or the species groups made it hard to evaluate the possible phylogenetic relationships among the same, however Dressler (1978b) with cautious diffidence made some assertion on this respect. Among other things he mentioned the possibility of *E*. *intersecta* being a link between *Euglossa s. lat*. and *Eufriesea*, making *Glossura* (including *Glossurella* and *Glossuropoda* as he originally envisioned it) “primitive” (basal) in the genus; he erected *Dasystilbe* to maintain the stability of both *Glossura* and

Euglossella as he saw it as intermediate between them, and finally he raised some concern on the naturalness (monophyly) of *Euglossa s. str*.

Despite the seeming problematic position of some species (particularly *E. nigrosignata*) and the perceived intergradation of *Glossura* and *Glossurella*, as well as the already cited dubious monophyly of *Euglossa s. str.*, all the recently described species of the genus have been assigned to the existing subgenera and, in most cases, to one of the described species groups, nonetheless Michener (2007) prefered to avoid the use of any subgeneric taxa, regarding all the subgenera as synonyms.

Here a systematic approach is taken to provide a phylogenetic framework to analyze and re-evaluate the existing subgeneric classification for *Euglossa*, along with the proposed species groups, using external morphology of the males, plus features from the male genital structures. A comparison is provided with the molecular phylogenetic hypothesis of Ramírez *et al*. (2010), which was published while the present study was in its final stages.

METHODS

In order to assess the naturalness of the currently in use subgeneric division of *Euglossa*, a phylogenetic analysis was here undertaken. A selection of at least two species belonging to each species group as presented in table 3.1 was made. The type species for the six subgenera were selected to be part of the analysis. Other criteria taken into account to select the species to be included in the analysis, were: 1) availability of specimens in the collection of the Division of

Entomology, Natural History Museum and Biodiversity Research Center, University of Kansas, 2) when possible, non overlapping geographical distribution for the different representative species per species group, and 3) species with a controversial position in the current classification. Species of the other three euglossine genera to which *Euglossa* has appeared as sister in those phylogenetic analyses available as of 2009 (Kimsey, 1982, 1987; Michener, 1990; Engel, 1999; Michel-Salzat *et al.*, 2004; Oliveira, 2006) were selected as outgroups. Two species of *Eufriesea* and *Eulaema* each were selected to represent basal and derived groups within these genera (see Kimsey, 1982; Oliveira, 2006).

A list of the species included in the analysis is presented in Table 3.2

Table 3.2. Species selected for the phylogenetic analysis. Five species are included as outgroups and 41 species of *Euglossa* representing the subgenera and species groups.

Outgroups		
	Eufriesea auripes	
	Eufriesea caerulescens	
	Eulaema polychroma	
	Eulaema speciosa	
	Exaerete smaragdina	
Subgenus *Dasystilbe*		
	species group VI	*E. villosa*
Subgenus *Euglossa*		
	species group VIII	*E. cognata* *E. iopyrrha* *E. mixta*
	species group IX	*E. viridissima*
	species group X	*E. cybelia* *E. nigropilosa*
	species group XI	*E. hansoni* *E. townsendi*
	species group XII	*E. cordata*

	E. deceptrix *E. tridentata*
Subgenus *Euglossella*	
species group VII	*E. bigibba* *E. cyanura* *E. decorata* *E. jacquelinae* *E. viridis*
Subgenus *Glossura*	
species group III	*E. imperialis* *E. piliventris*
species group IV	*E. annectans* *E. stellfeldi*
Subgenus *Glossurella*	
species group Va	*E. parvula* *E. sapphirina*
species group Vb	*E. gorgonensis* *E. hyacinthina* *E. oleolucens* *E. stilbonota* *E. trinotata*
species group Vc	*E. dodsoni* *E. obtusa*
species group Vd	*E. augaspis* *E. bursigera*
species group Ve	*E. laevicincta* *E. macrorhyncha* *E. turbinifex*
species group Vf	*E. allosticta* *E. asarophora* *E. viridifrons*
Subgenus *Glossuropoda*	
species group I	*E. intersecta* *E. nigrosignata* *E. rugilabris*

The phylogenetic analysis was based exclusively on external morphology of the male bees, since the vast majority of the taxonomic distinctions among the species groups and subgenera are based in those characters. Although for a good number of species both sexes are known, there is regularly uncertainty on the pairing of both sexes as members of the same species, since the external morphology of the females tends to be conservative. There must certainly be external morphological characters of the females that can provide phylogenetic information. However due to the uncertainty on the association of sexes and the almost universal use of male characters as diagnostics of the different species, the analysis was based on these last. Most of the characters discussed by Dressler (1978b) as of particular taxonomic value (i.e. secondary sexual features) were taken into account to build the matrix. An additional and important source of characters came from the analysis of the male genitalic structures (hidden sterna and genital capsule), which were obtained from observations of dissected specimens of all the species (except *E*. *stellfeldi*). Nomenclature for the external characters was mainly based on Michener (2007) and Dressler (1978b), while for the genitalic structures the nomenclature provided in the previous chapter (Hinojosa-Díaz, 2008) was applied. The taxonomic identity of every specimen was verified previous to its use as a source of characters. When gathering character information for the external features (other than genitalic structures), several specimens of every species were observed under a dissecting microscope, to record possible polymorphisms, except for species for which only one specimen was available (i.e. *E*. *stellfeldi*). The genitalic characters were based for the most part in one dissected specimen, although revision of exposed genital capsules in mounted specimens was used to look for possible variation or polymorphism. The dissecting technique applied corresponds to the one described in the previous chapter (Hinojosa-Díaz, 2008). Some of the characters recorded corresponded to synapomorphies for the ingroup (*Euglossa s. lat.*), but the majority of them represented the variation among the species groups and subgenera as stated above. The different character states per character were assigned codes starting with "0" (zero), and following an incremental sequence with no particular order (except for number of mandibular teeth which was coded directly as the respective number of teeth per species). Characters and taxa for which no information could be gathered (i.e. genitalic characters for *E. stellfeldi*) were coded as question mark while for non applicable characters a dash sign was used. A total of 79 characters were selected for the analysis. A list of the characters and character states is provided in Appendix 2. Illustrations of some of the characters are shown in Appendix 3.

A matrix was created using the program WinClada (Nixon, 1999) (see Appendix 4). The phylogenetic analysis was performed with the program Nona (Goloboff, 1999), through its interface with WinClada (Nixon, 1999). The taxa in the matrix were arranged with the outgroups at the beginning, as required by the program. The final version of the matrix had *Eulaema speciosa* as the first outgroup; varying the position of the species functioning as outgroup had no impact on the results of the analysis. All the characters were set as non-additive and all equally weighted. The analysis was run multiple times using the Ratchet (Island Hopper) module in the Analyze menu of the WinClada/Nona interface with the parameters as shown in Fig. 3.29. The analysis was run at least five times under 10,000 iteration/rep, and a few other times under 20,000 and 30,000 iteration/rep to ensure that results were stable. All the equally optimal trees were combined in a strict consensus tree.

Two measures of tree support values for the strict consensus tree were calculated, Jackknife through WinClada/Nona, and Bremer support through TNT (Goloboff *et al*., 2000). The Jackknife values were obtained by running the Jackknife module of the Analyze menu in WinClada/Nona with 1000 replications (Fig. 3.30). Bremer support values were produced by running the Traditional search module of the Analyze menu in TNT (1000 replications) retaining suboptimal trees up to ten steps longer, and mapping the Bremer support values on the strict consensus tree previously produced in the phylogenetic analysis (Fig. 3.31).

To test the phylogenetic significance of the characters that have been heavily used in the taxonomic arrangement of the genus (i.e. secondary sexual characters), special attention was devoted to their changes of character states when mapped on the strict consensus tree. The same procedure was applied to those characters that showed particular phylogenetic significance as a result of the analysis proper.

The existing classification of the genus (as described above) was evaluated in terms of the phylogenetic hypothesis here produced and possible changes to the infrageneric arrangement were proposed.

Based on information from the known distribution of the species used in the analysis, either from available literature or observed specimens, the consensus tree was used to make some inferences on the historical biogeography of the genus. For this the species were assigned to four regions based on the main biogeographic components previously identified for the Neotropical region (Amorim and Pires, 1996; Camargo, 1996; Camargo and Pedro, 2003), plus the northern Andes as a different region. A similar assessment of the orchid relationship of the different species and the phylogenetic result was carried out by tracing the known orchid visiting records by orchid subtribe (according to Cameron *et al*., 1999) per bee species.

RESULTS

A total of 64 equally optimal trees were produced every time ratchet analysis was performed, one of these trees is shown in Fig. 3.32. The statistics for these trees were:

Length = 335

Ci = 39

Ri = 72

The strict consensus tree was produced by collapsing seven nodes of the combined 64 optimal trees (Figs. 3.33, 3.34).

The tree support values (Jackknife and Bremer values) for the strict consensus tree are shown in Fig. 3.35.

Of the five non-monotypic subgeneric groups, *Euglossa s*. *str*. and *Euglossella* appear separately as monophyletic species assemblages in the consensus tree (Fig. 3.36). *Glossura* is recovered as a paraphyletic group with some *Glossurella* species, and *Glossuropoda* (except *E. nigrosignata*) nested within. The monotypic *Dasystilbe* shows up as sister to this "*Glossura*-some *Glossurella*-most *Glossuropoda*" group, while most of the *Glossurella* species [with *E*. (*Glossuropoda*) *nigrosignata* inserted there] appear as a grade (Fig. 3.36).

The known distribution for each species in the analysis, as mapped in the consensus tree, is shown in Fig. 3.37. The available information for male bee visitation of orchid plants and pollination records of the same is shown in the phylogenetic hypothesis in Fig. 3.38.

DISCUSSION

When describing the genus *Euglossa* (and all euglossines as then understood), Latreille (1802) emphasized the morphology of the mouthparts, noticeably the length of the labiomaxillary complex (as long or longer than the body), not surprisingly the generic name chosen reflects that feature. The first infrageneric splitting of *Euglossa s*. *lat*. came from the appreciation of the very same feature, as Cockerell (1917), continuing on the mouthpart-naming fashion, proposed *Glossura* to group those species with mouth-parts extending far beyond the apex of the metasoma. After *Euglossella* was initially defined by Moure (1967) in terms of tridentate male mandibles (leaving *Euglossa s*. *str*. as the remaining bidentate male group), the attention for the creation of subgeneric divisions went to the secondary sexual features of the males, heavily expressed in the structure of the meso and meta legs, and the second metasomal sternum. With this approach, Dressler (1978b, 1982c) rearranged *Euglossella* and *Euglossa s*. *str*., and erected *Glossurella* and *Dasystilbe,* this last based on its “distinctiveness”. Moure (1989) followed, using features from the legs to erect *Glossuropoda*. All of these taxonomic works based on very distinctive features rendered a concise arrangement that is seemingly coherent in terms of species assignation. Nonetheless problems have arisen in terms of the interpretation of some of the characters or combinations of them when considering some species, as is the case of *E*. *nigrosignata* and *E*. *stellfeldi*, which have recently been subgenerically relocated (Roubik, 2004, Faria and Melo, 2007) based on the observation of the character combinations. Accordingly, just by the consideration of these two cases, concern should arise in the delimitation of the three subgenera involved in these two cases (*Glossurella*, *Glossura* and *Glossuropoda*). It seems of course necessary to interpret this under a phylogenetic approach which could possibly give a natural arrangement of the infrageneric groups and a set of features to identify them. Since the reinterpretation of some of the secondary sexual features of the males have driven to uncertainty the subgeneric affiliation of some species, the addition of the genital characters to the analysis gives extra information to elucidate the nature of the subgeneric groups and their respective supportive characters.

General phylogenetic results .— The results produced by the phylogenetic analysis as presented in Figs. 3.33-3.36, show that besides the monotypic *Dasystilbe*, only *Euglossella* and *Euglossa s*. *str*. are supported as natural (monophyletic) groups. The other three subgenera (*Glossura*, *Glossurella*, and *Glossuropoda* – although look below for *Glossuropoda*), as in use to date, are not supported as natural assemblages. There is enough confidence that the taxon sampling in this study is a robust representation of the diversity within the whole genus, and that likewise, the set of external morphological characters conveys a good picture of the variation across the species in the genus. This is not to say that addition of species or additional sets of characters (or even a reinterpretation of the set of characters here used) would not produce different hypotheses, but as it is presented here, the resulting phylogenetic hypothesis provides a good picture of the evolutionary history of the group.

The status of *Glossura*, *Glossurella*, and *Glossuropoda* in the resulting philogenetic hypothesis, is non-coincidentally consistent with the unstable classification of *E*. *nigrosignata* and *E*. *stellfeldi* (as described above). These species are a good example of the problems faced by different authors when interpreting the sets of characters that delimit each of those subgenera. Faria and Melo (2007) when dealing with *E*. *stellfeldi*, realized the similarities of this species with what had been characterized by Dressler (1982c) as the subgenus *Glossurella*, and specifically with *E*. *laevicincta* [species group Ve of Dressler (1982c) according to the terminology used here], and the noteworthy absence of some of the features more commonly found in *Glossura* (labiomaxillary complex longer extending well beyond apex of metasoma, and second metasomal sternum bearing cowled slits: *sensu* Roubik, 2004) . The classification of the species in question in either one of the involved subgenera was a matter of judgement based on the sets of characters used before the present work. Similar observations regarding intergradation of characters between *Glossura* and *Glossurella* were noticed by Parra-H *et al*. (2006) when describing *E*. *natesi*, a species closely associated with the Vf species group of *Glossurella*, assemblage composed of species with an external morphology that combines characters from both *Glossura* and *Glossurella*, and genitalic features definitely closer to *Glossura,* as was addressed in the previous chapter (Hinojosa-Díaz, 2008). All the authors of the referred works stated that the subgeneric assignation of the respective species and the validity of either *Glossura* or *Glossurella* would only become clear under a phylogenetic perspective.

Glossuropoda as treated here, following Roubik (2004), includes *E*. *nigrosignata*, this sole species addition to the *Glossuropoda* assemblage makes a huge impact on the naturalness of it. Dressler (1982c) originally assigned *E*. *nigrosignata* to the Vb group of *Glossurella* prior to the erection of *Glossuropoda* by Moure (1989) this last defined by a set of characters, for the most part absent in *E*. *nigrosignata*. The presence of characters other than those used by Moure (1989), in all species of group I (as here understoodd) and in *E*. *nigrosignata* persuaded Roubik (2004) to expand the definition of *Glossuropoda* to accommodate this last species, these characters will be discussed later. The inclusion or exclusion of *E*. *nigrosignata* in *Glossuropoda*, although important to determine the monophyletic nature of the assemblage either as proposed by Moure (1989) or as redefined by Roubik (2004), is ultimately moot given their position in the phylogenetic hypothesis here presented. As taken from the consensus tree, besides *Dasystilbe* (natural on its own) and *Euglossella* plus *Euglossa s*. *str*. recovered as monophyletic, the "*Glossura*-some *Glossurella*-most *Glossuropoda* [*Glossuropoda* as viewed by Moure (1989)]" derived clade is here referred as a natural group that should be regarded as *Glossura* with a new definition, as is discussed later.

Previous phylogenetic statements.—The original approach that Dressler (1978b) took to subdivide *Euglossa s. lat*. was in his own view intended to serve as a starting point to develop a classification system that would be re-evaluated by adding characters not in use or available at the time of his proposal. The monophyly of the groups as he proposed them and re-modeled them in his subsequent work necessarily implied his understanding of them as natural (monophyletic) groups, even with his cautious assertions on future re-evaluations. The nature of these groups in respect to the phylogenetic hypothesis in the present study has already been discussed in the previous paragraphs. The only phylogenetic assertions of Dressler involving relationships between the subgenera as he originally proposed them, involved the positions of *Glossura* (at that moment including *Glossurella* and *Glossuropoda*) and *Dasystilbe* (Dressler, 1978b). Later he also made reference to a link between *Euglossa s. str*. and *Glossura* (Dressler, 1982b). It was of his opinion (Dressler, 1978b) that *Glossura* might be relatively “primitive” in relation to all other *Euglossa s. lat*., this based on the seeming similarity of *E. intersecta* to some species of *Eufriesea*, especially in coloration as well as in some meso and meta-tibial features. He advanced the possibility of *E. intersecta* as the representative of a distinct subgenus [as indeed was later brought up by Moure (1989)], but hesitated to split it as *E. rugilabris*, sharing a number of features with *E.*

intersecta, had also close resemblance to species of his species group III (*Glossura* in its strictest sense, i.e. minus *Glossurella* and *Glossuropoda*). *E*. *intersecta* does bear some coloration resemblance to some *Eufriesea*, particularly to *Eufriesea pulchra* with which it appears to form a mimetic complex that includes two other *Euglossa s*. *lat*. species, *E*. *cyanochlora* and *E*. *tiputini* (Roubik, 2004). The leg features cited by Dressler (1978b) as links between *E*. *intersecta* and *Eufriesea* are difficult to appreciate; the "eyelash-like" fringe of mesotibial michrotrichia is indeed present in some species of *Eufriesea*, but not in all, and the shape of the metatibia is not necessarily comparable. *Eufriesea* species in general show a metatibia with ovoid or prolate spheroid shape, while *E*. *intersecta* (and all other *Glossuropoda*) have a triangular metatibia (Figs. 3.22, 3.23). The characterization of *Glossuropoda* by Moure (1989) includes very particular characters in the mesotarsal segments, which were not noted by Dressler (1978b) as features of the species group I to which he assigned *E*. *intersecta* (type species of *Glossuropoda*). Instead, he noted features that are found only in *E*. *intersecta* and *E*. *cyanochlora*, which besides the coloration and the "eyelash" setae of the mesotibia included the noticeably lamellar projection of the malar area. There are some other unusual morphological features of *E*. *intersecta* and *E*. *cyanochlora* that indeed make them look somewhat "aberrant" [using words of Dressler (1978b)], all of which added to the numerous distinctive characters of *Glossuropoda* (sensu Moure) make them rather distinctive. However, contrary to what Dressler (1978b) suggested in terms of *Glossura*, and particularly of *Glossuropoda* as a subgroup of his original view of the former, they appear as a derived clade as can be seen in the consensus tree of the phylogenetic hypothesis here presented (Figs. 3.33, 3.4, 3.36). *Dasystilbe* was formulated by Dressler (1978b) as a very distinctive monotypic subgenus, with *E*. *villosa* bearing a mixture of *Glossura* and *Euglossella* characters, so in order to preserve the integrity of these two groupings, the only solution was to erect a new subgenus. Indeed the external morphology of *E*. *villosa* is a mosaic of *Glossura* and *Euglossella*, having as stated by Dressler (1978b) the facial and prothoracic distinctive features of the latter, while at the same time having the distinctive sternal cowled slits of the species of Dressler's group III (*Glossura* in its strict sense, i.e. minus *Glossurella* and *Glossuropoda*). The morphology of the male genitalia of *E*. *villosa* reveals also a mosaic of characters. While features of the gonostylus link it clearly to *Glossura*, most features of the hidden sterna are not *Glossura* like. In the phylogenetic hypothesis here presented *Dasystilbe* appears as sister to the "*Glossura*-some *Glossurella*-most *Glossuropoda*" derived clade, although in earlier versions of the analysis, with different combinations of characters, it appeared as basal to all *Euglossa s. lat*., and in the phylogenetic hypothesis based on molecular data of Ramírez *et*. *al* (2010), it appears as sister to all *Euglossella* (see further comments on this later). As it appears, Dressler had a good appreciation of the intermediacy of *E*. *villosa*, as is corroborated by its phylogenetic position in the present study and in the hypothesis of Ramírez *et al*. (2010). When describing *E*. *annectans*, Dressler (1982b) associated it with *E*. *stellfeldi*, and commented on the combinations of features of these two species in such a way that they must represent a link between *Euglossa s. str*. and *Glossura*. Morphologically both species certainly have some resemblance to some species in *Euglossa s. lat*., particularly in characters of the metafemur; however, those characters rather support the major clade in the consensus tree that contains *Euglossa s. str*., some *Glossurella*, *Glossura*, *Dasystilbe* and *Glossuropoda*.

"Old" subgenera in the phylogeny.—The classificatory subdivision of *Euglossa s. lat*. should reflect the natural arrangement of the infrageneric assemblages, as such, following the results here produced, of the six subgenera in use to date, only three of them as defined by their authors comply with the phylogeny as natural groups, *Dasystilbe*, *Euglossa s. str*., and *Euglossella* and they should be kept as valid subgeneric names. Regardless of its phylogenetic position *Dasystilbe*, as a monotypic entity, will always come out as a natural taxon. If it appears as nested into a seemingly natural broader subgenus, its validity as a subgeneric category would be challenged. However that is not the case in terms of the phyoglenetic hypothesis here presented.

Dasystilbe appears as sister to the "*Glossura*-some *Glossurella*-most *Glossuropoda*" clade (Fig. 3.36). Besides the characters cited by Dressler (1978b), of which most of them are rather ambiguous or non exclusive, characters used in this study that are unique for *Dasystilbe* include the emargination of the posterior margin of the second mesotarsomere (Fig. 3.16), the strongly concave ventral margin of the inner surface of the metafemur (Fig. 3.19), and the rounded shape of the lateral area of the gonostylar process of the gonocoxite (Fig. 3.27). As mentioned before, the gonostylus of *Dasystilbe* strongly resembles that of species of *Glossura*, while some of the facial and prothoracic features are more like those of *Euglossella* species. The presence of the notorious cowled slits on the second metasomal sternum is a convergent character as is discussed later.

Euglossa s. str. should be kept as a valid subgeneric category since it appears as a natural group in the phylogeny (Fig. 3.36). Of the various features cited by Dressler (1978b), the most relevant are the shape of the anterior mesotibial tuft and the shape of the metatibia. He referred the first as notched, comma-like or bilobed, and the second as subtriangular and obtuse (in reference to the posterior ventral apex), or usually rhomboid. Here only the second character is recovered as a unique feature of this subgenus, although instead of using the overall shape of the metatibia as a character, this was subdivided into different characters according to the variation in shape and/or length of the different margins that make up the metatibial shape (Characters # 50, 51). This avoids the ambiguous characterization of the shapes described by Dressler. The character as used here defines species of *Euglossa s. str*. as having a noticeable furrow on the posterodorsal margin of the metatibia. This is better appreciated if the metatibia is viewed from its inner surface (Figs. 3.3, 3.20. 3.22). This "furrow", in the same position as the basal section of the metatibial organ slit, marks a division of the posterodorsal margin, producing the shape referred to as rhomboid by Dressler. This is the only exclusive character of *Euglossa s. str*., although its combination with a few others gives support to the monophyly of the subgenus. Two of the other characters that appear in the consensus tree as supporting *Euglossa s. str*. were also mentioned by Dressler (1978b) in his outline of the subgenera. These are, short labiomaxillary complex, never surpassing the fourth metasomal sternum when in repose, and absence of integumental modifications on the second metasomal sternum (only setal patches present). Additionally, the presence of an additional fold on the dorsal sector of the lateral section of the gonostylus appears at the base of the *Euglossa s. str*. clade; this is shared with the species of *Glossura* as in use previous to this study. The shape of the anterior mesotibial tuft (notched, comma-like, or bilobed in words of Dressler, with a cleft in the anterior margin as defined here) according to this analysis (Fig. 3.14) is a feature of *Euglossa s. str*. minus *E. viridissima*, which in the consensus tree appears as sister to all other *Euglossa s. str*.

Euglossella as defined by Dressler (1978b), is perhaps the most stable and distinctive subgenus of *Euglossa s. lat*., and it stays as a natural assemblage, highly supported by a good number of characters, in the consensus tree (Fig. 3.33). Besides the rather ambiguous particularities of coloration, habitus, and punctuation cited by Dressler as features of *Euglossella*, and the references to the metatibial shape and the lack of features present in other groups (most of these plesiomorphic features), he emphasized the tridentate mandibles, the acute pronotal dorsolateral angles and the long and narrow midbasitarsus, for the males. While all of these characters cited by Dressler are certainly as a group features that can be used to recognize *Euglossella* as a distinctive group, none of them is exclusive (synapomorphic). Certainly the habitus and metatibial shape can be appreciated, but in terms of assignation of characters it is not very straightforward to assign these to particular character states. The seemingly distinctive long and narrow mesobasitarsus also falls in a rather ambiguous character group. In the present study, the characters that appear as exclusive to *Euglossella* are, presence of a blunt bumpy projection on the ventral margin of the inner metatibial surface adjacent to the spur (Fig. 3.21); the remaining characters are present in the genital structures, being, truncate lateral area of gonostylar process of conocoxite (Fig. 3.27), presence of long and complete striae on surface of the spatha, convex shape of the setose dorsal sector of the latreral section of the gonostylus, distinctive plumose setae on this area, and thumb-like membranous ventral lobe of the gonostylus (Fig. 3.28). It can be seen that although distinctive, the external (non-genital) morphology of *Euglossella* species is in general a set of plesiomorphic features for *Euglossa s.lat*., while the genital structures bear more distinctive features that define the subgenus. *Euglossella* appears, according to this study, as sister to all other *Euglossa s. lat*., a position that (as discussed above) Dressler (1978b) thought would correspond to *Glossura*. However the unique genital features of *Euglossella* together with the absence of modifications in the mesotarsi (which is a feature of all other *Euglossa s. lat*.) (Fig. 3.16), contribute to the phylogenetic position of *Euglossella* as separate from all other species in the genus.

A variety of conditions, as evidenced in the phylogenetic analysis, affect the validity of the remaining three subgenera (*Glossura*, *Glossurella*, and

Glossuropoda) as natural assemblages. As mentioned before, *Glossuropoda* corresponds to the original species groups I and II (here fused in group I) as outlined by Dressler (1978b), then given subgeneric status by Moure (1989), and broadened by Roubik (2004) with the addition of *E*. *nigrosignata*. This last definition of *Glossuropoda* [i.e. *sensu* Roubik (2004)] is the one used here for the subgeneric assignation of *E*. *nigrosignata*. Moure's (1989) conception of *Glossuropoda* was based on the very distinctive morphology of the mesotarsi, especially the basitarsus, and the absence of a mesotibial spur. Of these characters, *E*. *nigrosignata* only shares the absence of mesotibial spur. Roubik (2004) brought up a different set of characters for his deffinition of the subgenus (i.e. to include *E*. *nigrosignata* in it), besides the lack of the mesotibial spur these included long probasitarsal setae, triangular and acute metatibia with a deep short furrow, wide paraocular lines, inflated mesotibia, and only 11 jugal comb blades. He also mentioned the slender hidden seventh metasomal sternum as a possible feature of all *Glossuropoda*, although he also referred to the absence of information on genital structures for most *Euglossa s*. *lat*. *Glossuropoda* in terms of Moure is monophyletic, strongly supported by 24 characters, five of these synapomorphic (Fig. 3.33), while *Glossuropoda* as redefined by Roubik (2004) is a polyphyletic group since *E*. *nigrosignata* appears associated with *E*. *gorgonensis*, *E*. *oleolucens*, and *E*. *trinotata* in a clade sister to all other *Euglossa s*. *lat*. beyond the exclusion of *Euglossella*. Of all the characters cited by Roubik as "synapomorphies" for *Glossuropoda*, only the length of the probasitarsal setae, and the inflated mesotibia are unique to *Glossuropoda* if *E*. *nigrosignata* is included. *Glossuropoda* as conceived by Moure is indeed a natural group, and would be a perfectly valid subgenus if not for its position in the consensus tree. The status of *Glossura* has to be discussed in order to clarify the situation of *Glossuropoda*.

Glossura was the very first subgenus erected within *Euglossa s. lat*. (Cockerell, 1917), but as used until the present study, it was what remained after *Glossurella* and *Glossuropoda* were subtracted from it (Dressler, 1982c, Moure 1989). *Glossura* remained composed by Dressler's species groups III and IV, and instead of being defined by the exceedingly long labiomaxillary complex (beyond the metasomal apex) and the biconvex scutellum (Cockerell 1917), it would be defined by the common features of the two remaining groups, which in terms of Dressler are united by the presence of a keel on the inner surface of the mesobasitarsus. The position of all species of *Glossura* in the consensus tree make the subgenus a paraphyletic group, rendered as such by the position of *Glossuropoda* (*sensu* Moure) plus some species of *Glossurella*, all of them belonging to the species group Vf (Fig. 3.36). The inclusion of *Glossuropoda* (*sensu* Moure) as part of the *Glossura* clade is completely congruent with the original subgeneric proposal of Dressler (1978b). When creating *Glossurella* by taking species group V away from *Glossura*, Dressler realized the affinities between *E. asarophora*, *E. allosticta*, and *E. viridifrons* (species group Vf). In the description of *E. natesi*, Parra-H *et al*. (2006) alluded to the morphological similarities of this species group to *Glossura*, and the genitalic characters of were already mentioned in the previous chapter of this work (Hinojosa-Diaz, 2008).

The monophyletic nature of this clade including *Glossura*, *Glossurella* species group Vf and *Glossuropoda* sensu Moure, is supported only by the characteristic convexity of the ventral margin of the inner metafemoral surface (only synapomorphic character unique to this clade), the presence of a biconvex mesoscutum produced by a well developed furrow short of the scutellar margin), a characteristic triangular metatibia about 1.5 times as long as its maximum width, the absence of setae on the eighth metasomal sternum, and the presence of an additional fold on the dorsal sector of the lateral section of the gonostylus. It is here proposed that this clade should be regarded as the subgenus *Glossura*, having nomenclatural priority over *Glossuropoda* (with *E*. *intersecta* as type species), and the type species of *Glossurella* (*E*. *bursigera*) not belonging to the species group Vf.

Glossurella as it appears in the consensus tree is mainly a paraphyletic assemblage if the species group Vf is included in *Glossura*. The heterogeinity of this non natural group is reflected by the original mention of the six informal species assemblages by Dressler (1982c). As can be appreciated in the consensus tree, only two of these species groups are recovered as monophyletic (Vc and Vd). The main character that Dressler referred to when creating *Glossurella* was the presence of semicircular depressions on the second metasomal sternum. However this feature is absent in several of the species in the *Glossurella* grade. The genitalic structures of all of these species reveal a variety of morphological conditions, notably *E*. *laevicincta* shares gonostylar features with the new *Glossura* clade. *E*. *bursigera* (type species of *Glossurella)* along with *E*. *augaspis* seem to have intermediate morphological (genitalic and non-genitalic) features between all other *Glossurella* species and the more derived *Euglossa s*. *str.* and *Glossura*. If *Glossurella* as a subgeneric name is to remain in use, it should only be applied to those species closely allied to *E*. *bursigera*, such as *E*. *augaspis* and *E*. *prasina* (the latter not included in this analysis), or otherwise considered a synonym of *Euglossa s.lat*. Alternatively additional subgeneric names can be created for every monophyletic group present in the *Glossurella* grade. The first approach is favored here, *i*.*e*. preserve *Glossurela* as exclusive for *E*. *bursigera* and allies. Only one of the monophyletic assemblages within the *Glossurella* grade, species group Vb plus *E*. *nigrosignata*, is here proposed to be considered as a new subgenus described below. The remaining species in the grade, should remain as *incertae sedis* in terms of subgeneric assignation.

New subgenus.— Among the six informal species groups into which Dressler (1982c) subdivided *Glossurella*, the species group Vb originally included *E*. *fuscifrons*, *E*. *gorgonensis*, *E*. *hyacinthina*, *E*. *nigrosignata*, *E*. *stilbonota*, and *E*. *trinotata*. Additionally, *E*. oleolucens although omitted in the species group assignation, was described originally (Dressler, 1978a) as closely allied to *E*. *gorgonensis*. Finally, *E*. *paisa* (Ramírez, 2005) and *E*. *samperi* (Ramírez, 2006) were added to this group by morphological association. Besides the coloration and punctuation features, Dressler mentioned the narrow metatibia as a feature of this group. The problematic taxonomic position of *E*. *nigrosignata* without a phylogenetic hypothesis, has already been discussed above, but despite its relocation to *Glossuropoda* by Roubik (2004), the number of external morphological features that it shares with the species group Vb make for a better case of taxonomic association even without a phylogenetic framework. The addition of genitalic characters greatly reinforces this, and finally the results of the present study give phylogenetic validation to this. This group (with the inclusion of *E*. *nigrosignata*) is recovered as a monophyletic clade except for the position of *E*. *stilbonota* (Fig. 3.36). The clade is sister to all other *Euglossa s*. *lat*., except *Euglossella* which is the basalmost clade. The structure of the lateral section of the gonostylus constitutes a charactersistic feature of this clade, it is projected in a compressed blade-like shape (Fig. 3.28), much as it appears in *Eufriesea* and *Eulaema* (outgroups) standing on a more or less sagittal orientation in respect to the body plane. This orientation of the lateral section of the gonostylus is shared by the outgroups and *Euglossella*, and it seems to have a degree of transitional variation in most other species of the *Glossurella* grade, towards the diagonal orientation of this structure in *Euglossa s*. *str*., *Glossura* (as the new definition here proposed) and *Dasystilbe*. There are some other external characters that, although not restricted to this clade, in combination support its monophyletic nature. Some species have a small third mesotibial patch contiguous with the posterior patch (Fig. 3.15) (e.g. *E. trinotata*, *E*. *oleolucens*). *E*. *nigrosignata* on the other hand has no patches at all. The dorsolateral angle of the pronotum in most species of *Euglossa s*. *lat*. has an obliquely truncate shape, while in this clade (and a few other species) it is rather obtuse and with no projections. All species in the clade have a very distinctive knob on the inner surface of the metafemur near the trochanter joint (Fig. 3.19). This last character is perhaps the easiest way to recognize the species of this clade, although it is also shared by *E*. *stilbonota*. This situation brings back to the original inclusion by Dressler of *E*. *stilbonota* in the species group Vb. Indeed *E*. *stilbonota*, could well be part of this clade, since besides the peculiar metafemoral morphology it has three mesotibial patches, as several species in the clade including most of the species of the Vb species group. The general habitus of *E*. *stilbonota* also bears resemblance to the species of this clade, however the gonostylar morphology seems closer to that of *E*. *bursigera*, as well as the metatibial shape. Dressler alluded to the "narrowness" of the metatibia as a feature of the Vb species group, and although is not clear what body plane he referred to in defining the narrowness, the metatibial morphology is indeed characteristic, as it is triangular and with an anterior margin about 1.5 times the length of the ventral margin. This is very similar to some *Glossura* species, and especially to those in *Glossuropoda*. *E*. *stilbonota* has a metatibia more like that of *E*. *bursigera*, in which the anterior margin is shorter than 1.5 times the length of the ventral one. It must be stated that the position of *E. stilbonota* is in no way conclusive, and it might certainly be part of this clade as has been supported by the recent molecular phylogenetic study of Ramírez *et al*. (2010). Another species originally designated by Dressler as part of the Vb species group, *E*. *hyacinthina*, has definitely features that separate it from that clade. Regradless of the inclusion of this species, the monophyletic clade including most of the species of the Vb species group is here regarded as a new subgenus within *Euglossa s. lat*., based on the distinctive gonostylar, prothoracic and metafemoral morphology of the males. As proposed here the new subgenus includes the following species: *E. fuscifrons*, *E. gorgonensis*, *E. oleolucens*, *E. paisa*, *E. samperi*, *E. stilbonota*, *E. trinotata*, with *E. oleolucens* as type species (Fig. 3.46). Additionally there are two undescribed species that belong to this proposed new subgenus (author's personal observation). Most species in this clade have distributions restricted to southern Central America, northwestern South America (Choco and northern Andes), and the western Amazon basin near the Andean eastern slope.

Character evolution.— The characters used to segregate and distinguish the subgenera as known before this study, are mainly those already mentioned as the secondary sexual characters of the males, expressed for the most part in the morphology of the legs. Most species descriptions have also used combinations of those characters. Dressler (1978b) outlined his subgeneric classification mainly based on the presence/absence and variation of shape and size of the mesotibial setose tufts, presence/absence and structure of the integumental modifications on the second metasomal sternum, shape of the metatiba, length of the labiomaxillary complex in respect to the body, structure of the dorsolateral angle of the prothorax, number of mandibular teeth, and punctuation. He even addressed the possibility of a functional correlation between the absence of modifications in the second metasomal sternum and the absence of posterior tuft on the metatibia of several species of his original species group V (later elevated to subgeneric status as *Glossurella*). *Glossuropoda* was segregated by Moure

(1989) by emphasizing the mesotarsal particularities of *E*. *intersecta* and allies, although again Roubik brought up a different combination of characters in order to make place for *E nigrosignata* in it. These included the lack of the mesotibial spur, long probasitarsal setae, shape of the metatibia, presence of wide paraocular lines, inflated mesotibia, and hind wing with only 11 jugal comb blades. Faria and Melo (2007) observed the presence of a circular depression on the inner surface of the metatibia of both *E*. *stellfeldi* (*Glossura*) and *E*. *laevicincta* (*Glossurella*), which added to the overall morphological resemblance between these two species. This led them to propose that *Glossura* would be more homogeneous if *E*. *stellfeldi* was removed. Most of these characters used by different authors to infer either the subgenera or species-group membership, were included in this study; the only feature not examined in terms of a phylogenetic character was punctation. The philogenetic relevance of the characters necessarily varies, and although some of them indeed appear as synapomorphic for clades coincident with the subgenera as used before this study, they are more relevant in the general configuration of the phylogenetic pattern.

The elongated labiomaxillary complex has left its mark in the naming of the genus and the subgenera. It was the very first feature used to segregate a subgenus (Cockerell, 1917). Other euglossines have a labiomaxillary complex that only reaches midway in the metasoma. In *Euglossa s*. *lat*., the length seem to have initially evolved to be as long as the whole body when folded in repose, then followed two pathways, reversion to be as short as in the outgroups (notably in *Euglossa s. lat.*), or becoming even more elongated as in most *Glossura*, specially long in *Glossuropoda* and some species of the new subgenus here proposed (Chararcter # 12, Fig. 3.39).

The shape of the metatibia, much used by Dressler (1978b, 1982c) in his subgeneric delineation, is relevant in terms of the phylogeny. However, it was found difficult to use a straightforward character, since there are intergradations between the triangular and rhomboidal shapes (Fig. 3.22, 3.23) cited by Dressler. The posterior angle of the metatibia, where the ventral and posterodorsal margins meet, was used also by Dressler. However this is a hard feature to categorize, since in several species, especially in *Euglossa s. str.* although not restricted to them, the posterodorsal margin is curved on its posterior half, such that it meets the ventral margin on a different angle than the one it would have if the posterodorsal margin kept its original orientation. A way to account for the metatibial shape as referred to by Dressler was to express it as a messure of the the ratio “metatibial anterior margin length/metatibial ventral margin length” (Char. # 51), and also as the presence or absence of a deep furrow in the posterodorsal margin (Char. # 37). The metatibia of all other euglossine genera, although expanded, does not have a ventral margin as elongated (posteriorly) in respect to the anterior margin as in *Euglossa s. lat.*, thus causing the rather triangular shape of the metatibia. The basal clades of *Euglossa s. lat.*, including *Euglossella*, the new subgenus here proposed and the clade including *E. dodsoni* and *E. obtusa*, all have a metatibia with an anterior margin at least 1.4 times the length of the ventral margin. In the remaining species of the

Glossurella grade, and most of the species of *Euglossa s*. *lat*., as well as in *Dasystilbe*, the metatibial ventral margin expands, so the ratio anterio margin/ventral margin is less than 1.4. This characters reverts to a ratio of 1.4 or more in *Glossura* (as of the new definition here proposed), with *E*. *imperialis* as an exception. The deep furrow in the posterodorsal margin is a synapomorphy of *Euglossa s*. *lat*.

The structure of the mesotibial tufts is of great relevance for the taxonomy of *Euglossa s*. *lat*. It is useful in species identification, and especially to segregate species assemblages. The velvety surface of the mesotibia of the other euglossine genera has a single tuft. The presence of a second tuft (the posterior one) is a synapomorphy of *Euglossa s*. *lat*. (Char. # 22, Fig. 3.40). The posterior tuft sits on a concavity at the posterobasal extreme of the velvety area. In few species within the genus the posterior tuft is secondarily lost, either completely (e.g. *E*. *asarophora*), or as a non-setose cavity (i.e. *E*. *nigrosignata*). The posterior tuft is smaller than the anterior one in most major clades, while it becomes relatively larger (although never larger than the anterior one) in most *Glossura* species, and definitely larger than the anterior tuft in *E*. *viridissima* (Char. # 24, Fig. 3.41). The shape of the anterior tuft (Char. # 27) is quite variable (Fig. 3.14). However the most notable feature is the presence of a cleft on its anterior margin, causing it to look either rheniform, bilobed or even divided. These feature appears only once in *Euglossa s*. *lat*., as a synapomorphy for *Euglossa s*. *str*. (Fig. 3.42).

The integumental modifications on the second metasomal sternum (Char. # 54), along with the setose patches on the sternal margin (Char. # 55), are not present in other euglossines. Those species that have no integumental modifications at all, have no setose patches either (e.g. *E*. *gorgonensis*, *E*. *nigrosignata*, *E*. *dodsoni*, *E*. *obtusa*, *E. parvula*, *E*. *allosticta*, *E*. *viridifrons*, *E*. *asarophora*). A good number of the species included in the analysis have setose patches with no identifiable integumental modifications [all *Euglossella* (except *E*. *bigibba*), all *Euglossa s*. *str*. (except *E*. *viridissima*)] , but all species with modifications, also have setae. It is possible that the setae appeared first on the sternal margin, and the integument followed to be modified as shallow “omega-like” depressions, as bumpy areas where the patches grew (false cowled slits), or the tipical cowled slits present in *E*. *piliventris* and allies (Fig. 3.43). The cowled slits are present also in one species of *Euglossella*, *E*. *polita* (not included in the analysis) and in *E*. *villosa* (Dasystilbe), giving evidence of a tendency to develop this structure even in groups phylogenetically distant.

The shape of the dorsolateral angle of the prothorax (Char. # 13), and the presence/absence of projections on it (Char. # 14) (Fig. 3.8), were already mentioned when discussing the characters of the new subgenus. In the three subgenera used as outgroups, the dorsolateral angle is cut in an oblique truncate fashion, as in the majority of *Euglossa s*. *str*. (except species group VIII), *Glossura*, and most of the *Glossurella* grade except the new subgenus here proposed. In this last group, in *Dasystilbe*, the species group VIII within *Euglossa s*. *str*., *E*. *bigibba* (*Euglossella*), and *E*. *hyacinthina* (*Glossurella* grade), the contour of the prothoracic margin is a rather obtuse rounded angle toward the lateral area. The projections of this angle appeared at least thrice in the evolution of the genus, but is a notorious feature of *Euglossella*, in which most of the species have a lamellar projection coming off the dorsolateral angle. This feature seems to have appeared independently in *E*. *cognata* (*Euglossa s*. *str*.) and *Dasystilbe*.

The mandible of male euglossines (Char. # 1, Fig. 3.4) seems to be plesiomorphycally two toothed. Three toothed mandibles have evolved several times, and are characteristic of *Euglossella*, while also present in *E*. *bursigera* and allies, and in some *Euglossa s*. *str*., notably species belonging to group VIII (*E*. *analis* and allies) and *E*. *tridentata*.

The set of morphological particularities of the mesotarsi used by Moure (1989) to erect *Glossuropoda* are characters that appeared only in this highly derived group nested in *Glossura*. The most notable of these is, the notch or dramatic emargination of the posterior margin of the mesobasitarsus (Char. # 30, Fig. 3.16). The second, third and forth mesotarsal segments also have a particular morphology in these group of bees (Chars. # 32, 35). This peculiar tarsal morphology is part of the expression of an evolutionary trend involving morphological changes in the male euglossine legs (especially meso and meta), all of them related to the recollection, handling, storage and spraying of aromatic compounds (Kimsey, 1984; Eltz *et al*., 2005). The least modified mesotarsi are found in the outgroups, in which all segments beyond the basitarsus are similarly shaped as in the other legs. In *Euglossa s*. *lat*. a deep invagination on the distal inner margin of the mesobasitarsus appears (Char. # 31), making the mesobasitarsus-second mesotarsomere joint much more movable inwards. A good number of *Euglossa s. lat*. male museum specimens have the mesotarsi coiled inwards in both legs (author's personal observation), perhaps adopting the position in which these are held while transferring the collected chemicals (Kimsey, 1984). While all species in *Euglossella* have unmodified second mesotarsomeres (as in the outgroups), a further modification appears in all other *Euglossa s. lat*. in which the anterior margin is emarginate proximally (Char. # 33). This modification is obviously lost or obscured in *Glossuropoda* due to the compression of the second mesotarsomere. In *Dasystilbe* the posterior margin of the second tarsomere is also emarginate proximally (Char. # 34). It is possible that the observed evolutionary tendency to acquire extra mobility in the mesotarsal segments, as well as adopting a morphology possibly more suitable for the successful handling of the collected chemicals, have contributed to make *Euglossa s. lat*. the most diverse genus in the tribe.

Back to the characters that support *Glossuropoda*, the suite of characters cited by Roubik (2004) in his redefinition of the group, appear for the most part several times in different groups in the genus. A notable example is the width of the paraocular lines (Char. # 2, Fig. 3.5). Only the length of the probasitarsal setae, and the inflated mesotibia appear both in the original *Glossuropoda* of Moure and in *E. nigrosignata*, but yet again, the genitalia and the mesotarsi of the latter have no resemblance to *Glossuropoda*.

The character mentioned by Faria and Melo (2007) to challenge the subgeneric position of *E*. *stellfeldi*, *i.e*. presence of a circular depression in the inner surface of the metatibia (Char. # 44), is only present in a few species in the genus, and besides its support of the species group of *E*. *bursigera* and allies, it is mot useful for the subgeneric assignation of *E*. *stellfeldi* (Fig. 3.44).

Some other characters used in this study provide valuable phylogenetic information. Most species in the genus have almost the same upper and lower interocular separation (Char. # 9, Fig. 3.6). The most notable change in this character occurs in the derived *Glossura* clade (beyond *E*. *annectans*), in which the lower paraocular distance is slightly, but noticeably longer than the upper one, just as is observed in the outgroups. This reversal towards a widening of the lower facial area occurred perhaps to accommodate the mouth parts associated with a particularly long labiomaxillary complex. Although the characters here analyzed pertain exclusively to males, measurements of female specimens confidently identified as belonging to *Glossura* and other *Euglossa s*. *lat*. groups, show the same upper and lower interorbital distances as the males. This is relevant in terms of determining the possible taxonomic affiliation of the oldest fossil *Euglossa*, *E. moronei* (Engel, 1999), which is a female specimen. Measurements of the interocular distances of pictures of the specimen show the same character state as species of *Glossura*. Although more evidence would be necessary to accurately assign this fossil specimen to any subgenus, it is quite possible that it belongs to *Glossura*. It must be mentioned that the only extant orchid bee in the West Indies, *E*. *jamaicensis*, is a species of *Glossura*.

Among the genitalic characters, the seventh metasomal sternum, in most species in the genus, as well as in the outgroups, bears setae on the discal edge (Char. # 57), but this condition changes to a totally bare sternum four times in the tree, most notably in the *Glossura* clade.

The morphology of the eighth metasomal sternum (Fig. 3.28), is very informative. The lateral edges of the posterior section of this sternum are strongly projected both in the outhgroups, *Euglossella* and in the new subgenus here proposed. The shape of this changes in all the remaining groups of the *Glossurella* grade (except *E*. *stilbonota*) as well as in all *Euglossa s*. *str*., in these the posterior section has a rather triangular shape with lobes not so projected. A revertion to the deeply invaginated lobes occurs in the *Glossura* clade (Fig. 3.45).

The gonostylar morphology seems to have evolved from a lateral section on the gonocoxite oriented sagittaly as it is in *Eufriesea* and *Eulaema*. This is the way it is in *Euglossella*, and in the new subgenus, even when in *Euglossella* it is broadly membranous and in the new subgenus it is quite compressed and laminar (Char. # 70, Fig. 3.28). As mentioned above in the section discussing the new subgenus, most other species of the *Glossurella* grade, *Euglossa s*. *str*., *Glossura* (as the new definition here proposed) and *Dasystilbe* have a lateral gonostylar section oriented diagonally on the gonocoxite. The main evolutionary pathway of the gonostylus, as seen in this study, would then be from a condition in which the orientation of the lateral section is initially sagittal, membranous but not thickened. Then it took two evolutionary pathways, one in *Euglossella* in which it the lateral section conserved the sagittal orientation, but became broadly membranous with various degrees of dorsal convexity densely covered with plumose setae. a second pathway showing a gradual change towards a broadly membranous lateral section that creates an inwards oriented and concave surface bearing the seta. In species of *Euglossa s. str*., *Dasystilbe* the *Glossura* clade, and notably in *E. laevicincta* (*Glossurella* grade), the second pathway produces an extended, diagonal, membranous lateral gonostylar section that in combination with the ventral lobe produces the "bilobed" appearance referred to by Ospina-Torres *et al*. (2006). The vast majority of the species in the genus could be described as having a bilobed gonostilar lateral section, because one only has to vary the visual plane to appreciate this structure.

Recent molecular phylogeny of Euglossini.— Ramírez *et al*. (2010) recently published a comprehensive molecular phylogeny of the tribe Euglossini, based on four loci (cytochrome oxidase, elongation factor 1-α, arginine kinase and RNA polymerase II). They included 80 species of *Euglossa s. lat*. (including 33 of the ones used in the present study), with all the subgenera and species groups represented. Their analysis produced a highly resolved phylogenetic tree based on parsimony and Bayesian (single and multiple models of sequence evolution for each loci) implementations, both yielding congruent phylogenetic hypotheses. *Euglossa* like all the other euglossine genera, was highly supported as monophyletic, with *Euglossa* appearing in a terminal node as sister to *Eulaema*. The internal structure of their consensus tree for *Euglossa* is highly resolved, and in many instances congruent with the phylogenetic hypothesis here presented as can be seen by comparison of the trees in Fig. 3.46. Both studies recover

Euglossella and *Euglossa s*. *str*. as monophyletic, besides the monotypic *Dasystilbe*. The molecular analysis also shows *Glossura* and *Glossurela* as paraphyletic, and in the case of *Glossura* even the same clades (i.e. *Glossuropoda* and *Glossura* Vf species group) are nested within it, rendering it paraphyletic. Their tree, however, shows *Dasystilbe* as sister to *Euglossella* in a clade sister to the rest of the genus, although they state that this position comes when using a locus-specific model of evolution, while when a single model of evolution is used for all loci, *Dasystilbe* is recovered as sister to all other *Euglossa s*. *lat*. Either position in the basal section of the tree is at odds with the position of *Dasystilbe* in the present morphology-based analysis. Interestingly this conflict supports the original opinion of Dressler (1978b) who referred to *E*. *villosa* as intermediate between *Euglossella* and *Glossura*, a finding that made him erect a different subgenus for this species. Pure morphological characters show also a mosaic of features in *Dasystilbe*, although the genital structures, especially the gonostylus, are much more like the derived situation found in *Euglossa s*. *str*. and the *Glossura* clade. Only more information or combined analysis of both morphological and molecular data could possibly give a more conclusive solution to the phylogenetic position of *Dasystilbe*. *Glossuropoda* is stated to be monophyletic by Ramírez *et al*. (2010), but missing *E*. *nigrosignata* as part of the analysis, leaves *Glossuropoda* in the sense of Roubik (2004) non comparable with the results of the present study where that definition of *Glossuropoda* is not recovered as monophyletic. So the monophyletic *Glossuropoda* recovered in the molecular study corresponds only to the definition of Moure (1989). The heterogeinity of *Glossurella* is evident also in the molecular tree. In their analysis, no distinction is made for the different informal species groups mentioned by Dressler (1982c) when erecting *Glossurella*.

The new subgenus here proposed is not completely congruent with the molecular phylogenetic hypothesis of Ramírez *et al*. (2010). From the Vb species group of Dressler (1982c), they included *E*. *fuscifrons*, *E*. *gorgonensis*, *E*. *oleolucens*, *E*. *paisa*, *E*. *samperi* and *E*. *stilbonota*, only three of them coincident with the sampling in the present study. As can be seen in Fig. 3.46, the clade that would be equivalent to the new subgenus in the molecular tree includes *E*. *stilbonota*, and excludes *E*. *gorgonensis*. The position of *E*. *stilbonota* as sister to all other species of this clade in the molecular tree can be defended from a morphological perspective as features of the mesotibial patches, and the morphology of the metafemur are shared by all of these species. The exclusion of *E*. *gorgonensis* from this clade seen from a morphological point of view is more controversial, since this species bears not only external morphological features shared by all species in the clade as recovered in the morphology tree, but also genitalic features not found elsewhere in the tree. The new subgenus as proposed above is obviously based on morphological features, and although susceptible to change by subsequent analyses, it is here argued that it must include *E*. *gorgonensis*, for the reasons just mentioned.

The molecular phylogenetic hypothesis of Ramírez *et al*. (2010), was also used by the authors to infer lineage diversification using fossil-calibrated molecular clocks, for which they used *Euglossa moronei*, a fossil from Dominican amber (Engel, 1999) to determine the minimum age constraint for *Euglossa s*. *lat*. Although there is no absolute certainty of the subgeneric association for this fossil, as was discussed above, it is quite possible that it belongs to *Glossura*, making it part of a rather derived group within the genus, thus clearly having an impact on the consideration of minimum age.

It should not be overlooked that besides the mentioned conflicts in phylogenetic positions of some groups, notably *Dasystilbe*, the molecular phylogenetic hypothesis of Ramírez *et al*. (2010) is remarkably coincident with the one presented in this morphological study. A combination of both sources of information would likely reinforce the phylogenetic hypothesis that both separately support, and would give a more conclusive outcome.

Phylogeny and distribution.— The distribution of every species used in the analysis was associated with their positions in the consensus tree in order to determine possible associations of particular clades with any of the previously detected biogeographical components of the Neotropical region. Species not included do not contradict the distributional information as shown. As can be seen in Fig. 3.37, the two non monotypic subgenera previously established, and supported in this study as monophyletic, *Euglossella* and *Euglossa s*. *str*., plus *Glossura* as proposed in this study, include species found in all the areas, either as exclusive or widespread. On the other hand, the new subgenus here proposed consists of species mainly distributed in Central America and the Choco (northwesten southmerica on the Pacific shore). Additionally, *E*. *paisa* is found only in the Colombian Andes, while *E*. *fuscifrons* is distributed in the western Amazonian region. The Andean species could perhaps be considered as part of the Central America-Choco region, but *E*. *fuscifrons* would constitute the only Amazonian member of this clade. If *E*. *stilbonota* is included in this new subgenus, as suggested by some morphological features and by the molecular tree of Ramírez *et al*. (2010), a northern Amazonian element would also be added to this group. Interestingly the remaining species of the *Glossurella* grade are also distributed only in the Central America-Choco area and the Northern Amazon. Ramírez *et al*. (2010) by using model-based likelihood inferences over their molecular phylogeny, somehow suggest an Amazonian origin for most groups with repeated dispersion towards the other regions. Phylogenetic comprehensive analysis of each monophyletic assemblage would be necessary in order to propose more accurately the area or areas where each clade originated.

Phylogeny and orchids.— Perhaps the most remarkable biological feature of euglossine bees is the collecting behavior of the males, in which they visit floral and non-floral sources gathering perfumes that are later exposed during mating behavior (Dressler, 1982a; Eltz *et al*., 2005). Orchid flowers seem to be the main source of aromatic compounds for the male bees, hence the common name "orchid bees". The pollinator services of the bees are necessary for the reproduction of the orchids, but the bees are not limited to these plants as sources of the chemical compounds (Ackerman, 1983; Whitten *et al*., 1993;

Pemberton and Wheeler, 2006; Cappellari *et al*., 2009). The perfumes are also known to be gathered from non-floral sources such as rotting plant tissue, fruits, seeds, leaf litter, bark wounds, insecticides, fragrant leaves and terrestrial mushrooms (Roberts *et al*., 1982; Ackerman, 1983; Whitten *et al*., 1989, 1993; Pemberton and Wheeler, 2006; Capellari and Harter-Marques, 2010). The floral records for orchids visited by the species included in the phylogenetic study show that most species for which records exist (Fig. 3.38), visit a varied taxonomic array of orchid plants, which only strengthens the idea of non-dependence on a single source by the bees.

Conclusion.— The external morphological set of characters used in this study, a combination of the broadly used, noteworthy secondary sexual features, and the, for the first time assessed, internal genitalic characters of the males of *Euglossa s*. *lat*., provide a significant source of information that produced a comprehensive, highly resolved phylogenetic hypothesis for the genus. The major impact of this study is in respect to the naturalness of the six subgeneric taxa previously created. Of these, besides the monotypic *Dasystilbe*, only two (*Euglossella* and *Euglossa s*. *str*.) are supported as natural groups, keeping their definitions as before this study. *Glossura*, which is recovered as paraphyletic, is here redefined to include *Glossuropoda* (*sensu* Moure) and a species group of *Glossurella*, which together compose a monophyletic assemblage. *Glossurella* is recovereded as a heterogeneus paraphyletic group, and this subgeneric name should only be applied to the species group encompassing *E*. *bursigera* and allies, which form a monophyletic assemblage. A new subgenus is here proposed for the basal monophyletic clade of the *Glossurella* grade, based on the distinctive morphology of the gonostylus and the metafemur. The remainder of the species of the paraphyletic array, previously regarded as part of *Glossurella*, remain as *Euglossa incertae sedis*, until a more conprehensive phylogenetic study, including all the relevant known species, is undertaken. The phylogenetic hypothesis here presented also allows elucidation of the evolution of characters previously outweghted in the creation of subgeneric groups. A series of changes in the morphology of the legs, notoriously including shape of the felty patches, shape of the mesotarsal segments, and shape of the metatibia are reveal by the phylogenetic hypothesys here produced. Also noteworthy is the sequence of evolutionary changes in the morphology of the eighth metasomal sternum and the lateral section of the gonostylus. Most of the phylogenetic information provided by this morphological analysis is corroborated by the recent molecular phylogenetic study by Ramírez *et al*. (2010).

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CHAPTER 4

Potential distribution of orchid bees outside their native range: The cases of *Eulaema polychroma* (Mocsáry) and *Euglossa viridissima* Friese in the United States (Hymenoptera: Apidae)

INTRODUCTION

An appealing bright metallic coloration is a trademark of the orchid bees (Euglossini), notwithstanding the fact that they owe their name to the remarkable behaviour of males, which visit flowers of Orchidaceae to gather fragrances offered by the plants in exchange for pollination services. Aside from their aesthetic and catchy floral preferences, the phylogenetic position and concomitant biogeographical implications of the group are deserving of attention. According to morphological, behavioural, and palaeontological evidence, as well as combined analyses with DNA sequences, euglossines are the modern (crown group) representatives of the basal lineage of the corbiculate clade (e.g., Schultz *et al*., 1999, 2001; Engel, 2001a, 2001b; Noll, 2002; Cardinal and Packer, 2007). They are also the only strictly neotropical corbiculate tribe (Michener, 1979, 2007). The just over 200 currently recognized species (Ramírez *et al*., 2002; Roubik and Hanson, 2004; Nemésio and Silveira, 2007) are encompassed in five genera, of which the monotypic and cleptoparasitic *Aglae* is restricted to South America (with an uncertain report from eastern Panama), while the remainder (*Eufriesea*, *Euglossa*, *Eulaema*, and the cleptoparasitic *Exaerete*) range from the tropical areas of Mexico to northern Argentina and subtropical southeastern Brazil (Michener, 2007; Rebêlo, 2001; Roubik and Hanson, 2004). The modern bee fauna of the Caribbean Islands is practically devoid of orchid bees except for the presence in Jamaica of the endemic *Euglossa jamaicensis* Moure (Engel, 1999; Rebêlo 2001).

As in any diverse group of organisms, some species exhibit widespread distributional ranges, while others are restricted to varying degrees to certain areas. A good example of a euglossine with a widespread distribution is *Eulaema polychroma* (Mocsáry), which can be found from northern Mexico to the Amazon Basin (e.g. Roubik and Hanson, 2004), on the other hand *Euglossa viridissima* Friese, distributed from northern Mexico to Costa Rica, while not dramatically restricted is nonetheless notably absent from South America. These two species of orchid bees have in recent years attracted attention in regard to their distribution since specimens of both have been collected outside of their natural areas of occurrence although each species under different circumstances. The northern limit for viable populations of both species has been reported at around 27° N in the Mexican states of Sonora and Chihuahua at the base of the Sierra Madre Occidental where tropical deciduous forest, among other types of vegetation, can be found in the lowlands (Búrquez, 1996). In April of 1994 a sole male of *E. polychroma* was captured in Silverbell, Arizona at 32° N, far into the Sonoran Desert, over 550 km North of the supposed natural limit of the species (Minckley and Reyes, 1996). *Euglossa viridissima* appears to have been accidentally introduced to southeastern Florida where some males were first collected in Fort Lauderdale during the summer of 2003 and seemingly have become established (Skov and Wiley, 2005; Pemberton and Wheeler, 2006). Aside from an old record of *E. polychroma* in southernmost Texas (Minckley and Reyes, 1996; Michener, 2007), both cases represent the only records of euglossines in the United States. While there have not been other collections of

E. *polychroma* in Arizona besides the male mentioned above, and the presence of *E*. *viridissima* in Florida clearly corresponds most likely to an accidental introduction, both represent the occurrence of the respective species outside of their natural ranges. The reported tight association between euglossine males and specific orchid species in their native range has been addressed as a constraint for the successful colonization of new areas by orchid bees (or their orchid counterpart), and as such the nearly absolute absence of euglossines in the Caribbean Islands has been explained in this context (e.g. Janzen, 1975; Rebêlo, 2001). However, the established population of *E*. *viridissima* in Florida, where the orchids to which this species is associated in its native range do not occur, challenges this point of view (Pemberton and Wheeler, 2006). There are a large variety of ecological and historical factors that affect the distribution of an organism, and as such hypotheses on the impact of any given observed factor can be ventured (in this case the supposed euglossine-orchid interdependence) at risk of disregarding other variables or the interaction of all of them (Soberón and Peterson, 2005). However, comprehensive approaches that unite the diversity of biotic, abiotic, as well as historical factors shaping the distribution of a species, such as species distribution modelling (SDM), assuredly give a more accurate account of those parameters determining the presence/absence of the habitat of an organism in different geographic regions. SDM has already been applied to predict the potential distribution of *Megachile sculpturalis* Smith, another adventive bee species in the United States (Hinojosa-Díaz *et al*., 2005). Here SDM is applied to predict the potential distribution of *E*. *polychroma* and *E*.

viridissima in the United States, the first as a possible extension of the northernmost natural range of the species, and the second as an already established adventive species.

METHODS

Distributional data.— A database of 88 locality records for *E. viridissima* and 104 for *E. polychroma* was compiled based on historical specimens from different collections and existing literature. Locality data of the natural range for both species were taken from specimens deposited in the following collections: Snow Entomological Collection, Division of Entomology, University of Kansas Natural History Museum, Lawrence, KS, USA; Colección Himenopterológica, Museo de Zoología “Alfonso L. Herrera”, Departamento de Biología Evolutiva, Facultad de Ciencias, UNAM, México D.F., México. Published records were taken from Búrquez (1996), and Oliveira (2000). Prior to modelling, in order to avoid the use of imprecise taxonomic or distributional information, all specimens were taxonomically reviewed and all historical records without geographical coordinates were georeferenced via consultation of online resources (http://www.fallingrain.com/world/, and http://www.tageo.com/index.htm) and Google Earth (http://earth.google.com/).

The data occurrence for *E. viridissima* in Florida were taken from Skov and Wiley (2005) while the locality information for the only record of *E. polychroma* in Arizona was taken from the specimen deposited at the Snow Entomological Collection, Division of Entomology, University of Kansas Natural History

Museum. The locality data of the old record from Brownsville, Texas for *E. polychroma* was taken from Minckley and Reyes (1996). None of these records were included in the set of data used to produce the model.

Potential distribution.— Using occurrence data and environmental variables, Species Distribution Models (SDM) can be used to model the potential distribution of species by characterizing the environment where the species can potentially occur in geographic space (Araújo and Guisan, 2006; Kearney, 2006). This is based on the assumption that the known distribution of a species (i.e., recorded presence and/or absence) provides sufficient information to characterize its environmental requirements. A great variety of methods for modelling species' potential distributions exist (Guisan and Zimmerman, 2000; Elith *et al*., 2006). Here Maxent is used (Phillips *et al*., 2004; 2006) to estimate the potential geographic distributions for our target species, since recent studies have shown that Maxent performs better at discriminating between suitable and unsuitable areas than do other SDM methods (Elith *et al*., 2006; Phillips *et al*., 2006; Hernandez *et al*., 2006). While Peterson *et al*. (2007) suggest that Maxent may overfit the data, Phillips (2008) noted that while they show overfitting in one circumstance this does not mean that the same will be true for different sets of data. Presently, there are at least 16 different methods for predicting species distributions (SDM). The data were also run using GARP and showed no significant difference from the results produced with Maxent, so the general results are discussed based on this.

Maxent makes predictions using only species presences (Phillips *et al*., 2004; 2006). Maxent models a probability distribution (potential distribution) of habitat suitability over the study area. The modelled potential distribution must agree with everything that is known about the environment and the known distributional data of the species, thereby avoiding placement of any unfound constraints. The best potential distribution of the species is the one that is closest to uniform (i.e., nearest to having equal probabilities of occurrence across the entire study area) subject to the constraint that the expectation for each environmental variable included in the modelled distribution must match its empirical average over the

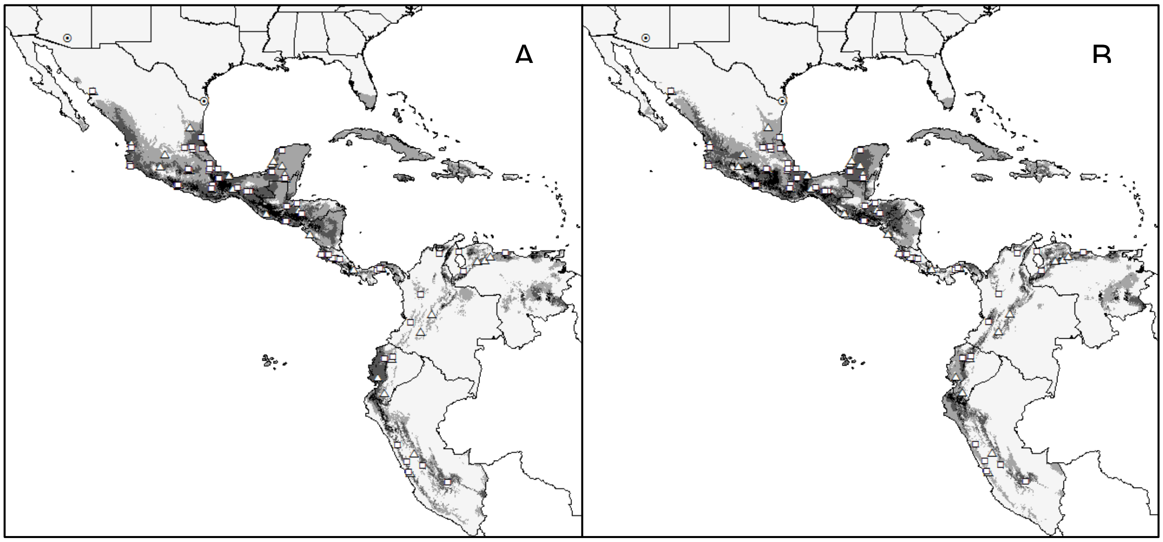

Fig. 4.1. Training data to obtain the potential distribution of *Eulaema polychroma* (Mocsáry). **A**. Prediction based on subset 1 (squares), tested with subset 2 (triangles). **B**. Prediction based on subset 2 (triangles), tested with subset 1 (squares). The dotted circles represent the two localities in the United States, presumably out of the natural range for the species (similarity scale as in Fig. 4.2).

known distributional data. Thus, the probability calculated in Maxent is not one of occurrence, but rather a value representing the relative suitability of the environmental conditions for the target species in each pixel in the study area as a function of the environment in all of the known distributional units. Maxent assigns a probability of habitat suitability per each grid cell in the study area, ranging from 0 to 1. The cell with a value of 1 is the most suitable, while cells close to 0 are the least suitable within the study area. (See Phillips *et al*. (2006) for a detailed mathematical formulation of Maxent).

The estimation of the potential distribution of *E. viridissima* and *E. polychroma* was produced using Maxent version 3.0 -- (http://www.cs.princeton.edu/~schapire/maxent/), with the default modelling parameters (convergence threshold = 10^5, maximum iterations = 500, regularization value β = auto) following Phillips *et al*. (2006). 19 Bioclimatic variables were used as predictors. The bioclimatic variables result from global land area interpolation of climate point data (years 1950–2000) at a spatial resolution of 2.5 arc-min (Hijmans *et al*., 2005; available at http://www.worldclim.org). All variables were clipped to include North, Central, and South America, along with the Antilles at a resolution of approximately 1x1 km^2 grid cells. All the probability thresholds of the potential distributions were considered in order to analyze the habitat suitability of the areas in the USA where the species have been recorded. Model results were processed and visualized using the GIS software ArcView 3.2 (ESRI, 1999) and ArcGIS 9.1 (ESRI, 2005).

Model predictivity was evaluated by splitting available occurrence data for the species in their native ranges. Following Peterson *et al*. (2008) a random sample of 50% of the known occurrences was set aside in order to evaluate (testing data) the first predictive map that was modelled with the remaining 50% of known data. This analysis was run twice for each species, the first with subset 1 predicting subset 2 and vice versa (Fig. 4.1). Model predictions were evaluated by overlaying the random 50% of known occurrence points that had been set aside (testing data) prior to using Maxent. We calculated the Area Under the Curve (AUC) in Receiver Operating Characteristics plots (ROC) (Fielding and Bell, 1997) to evaluate the models. ROC is a threshold–independent measure that evaluates the sensitivity (probability that the model produces a positive result in a positive locality) versus the specificity (probability that the model produces a negative result in a negative locality) of a model when presented with new data. A ROC plot is obtained by plotting all sensitivity values on the *y*–axis against their equivalent (1–specificity) values for all available decision thresholds on the *x*–axis. The theoretically perfect result is AUC = 1, whereas a test performing no better than random yields AUC = 0.5. AUC and ROC plots were calculated in SPSS 12.0. Since AUC calculations require absences, we created "pseudo-absences" using the random point generator extension in ArcView 3.2 (ESRI, 1999). Pseudo-absences (used in model evaluation only) were defined in areas where the species has not been recorded. The number of pseudo-absences was equal to the number of known occurrence data that was used to run the models.

Furthermore, AUC scores obtained by splitting the data to run the models is another way to demonstrate that a powerful SDM is being used.

GARP is a machine–learning procedure based on a genetic algorithm (Genetic Algorithm for Rule set Production; Stockwell and Noble, 1992), which divides distributional data into training and test data sets and works in an iterative process to develop a set of rules through evaluation, testing, and incorporation or rejection. Rules that come from one or more types (atomic rules, logistic regression, and BIOCLIM rules) are chosen, applied to the training data; rules develop or evolve through random processes until the best possible model is found or a set of iterations are performed. For each of these runs, GARP randomly selected 70% of the training dataset presences to generate the models that were validated with the remaining 30%. Because each run results in a different prediction due to the genetic–based algorithm, a final map product was based on the best results from 100 runs for each species. Following Anderson *et al*. (2002) the 20 best models were selected from the 100 runs. The best–subset models included runs which had no more than 5% omission error (false negative) using validation data held back by GARP. These runs were sorted with less than 5% omission error by their commission index (false positives) and selected 10 models on each side of the median commission index. These 20 best–subset models were exported as ArcGIS grids, where they were summed to obtain a unique map per species with cell values from 0 to 20 (20 indicates that all 20 models scored the species as present in that cell). The GARP predictions were produced with DesktopGARP (http://www.lifemapper.org/desktopgarp/).

RESULTS

Maxent.—The potential distribution maps obtained based on the two random subsets of available occurrence data for both *E. polychroma* and *E. viridissima* were similar in their general predictions (e.g., Fig. 4.1). Model evaluation showed high scores of performance (AUCs > 0.9). Thus, we considered the global geographic implications, that is, the species probability of habitat suitability, and therefore potential distribution in non-native ranges in the United States.

The probability distributions for both species after running Maxent are shown in figures 2–4. For *E*. *polychroma*, of the two localities in the United States, Silverbell, Arizona shows zero probability of habitat suitability between known and potential habitats (a complete absence of suitable environmental conditions for the species – note that in figure 3 the lightest shading covers the range from 0–0.09 but for the Arizona locality the actual value is zero), while Brownsville in the southernmost tip of Texas presents a probability of habitat suitability in terms of known and potential habitats of 0.1 (probability of habitat suitability slightly higher west towards the coast), a value outside the range of probabilities of other known occurrences. Besides the two tested localities, the analysis shows suitable conditions for the species in southern Florida (Fig. 4.2). According to our model, there are areas potentially suitable for *E*. *polychroma* to inhabit as far north as around 29° N following the pacific slope of the Sierra Madre Occidental in Mexico (Fig. 4.3). The descriptive statistics based on Maxent probability of habitat suitability for *E. polychroma* were: mean = 0.6531; standard deviation =

0.176; minimum value = 1.3 (Costa Rica, Torrialba Olivera), maximum value = 0.93 (Mexico, Hidalgo, 1 km Tlanchinol-Apantlazol).

The potentiality of *E*. *viridissima* to be distributed in southern Florida, according to probability of habitat suitability as predicted by Maxent, corresponds to a maximum of around 0.3 in the southwestern section of the peninsula with lower similarity both eastward and northward. Nowhere else in the continental United States does the model predict suitable habitat for this species (Fig. 4.4). The descriptive statistics based on Maxent probability of habitat suitability for *E. viridissima* were: mean = 0.6679; standard deviation = 0.2084; minimum value = 1.9 (Mexico, Hidalgo, Actopan), maximum value = 0.93 (Mexico, Guerrero, Chilpancingo de los Bravo Omiltemi).

Garp.—The potential distributions for both species as predicted by Garp are mainly coincident with the results produced with Maxent. For *Eulaema polychroma* eight of the 20 best models predicted similarity between the known and predicted habitats for Brownsville. No similarity was found for Silverbell, Arizona (Fig. 4.5).

For *Euglossa viridissima* 16 of the 20 best models predicted the presence of this species in Florida (Fig. 4.6).

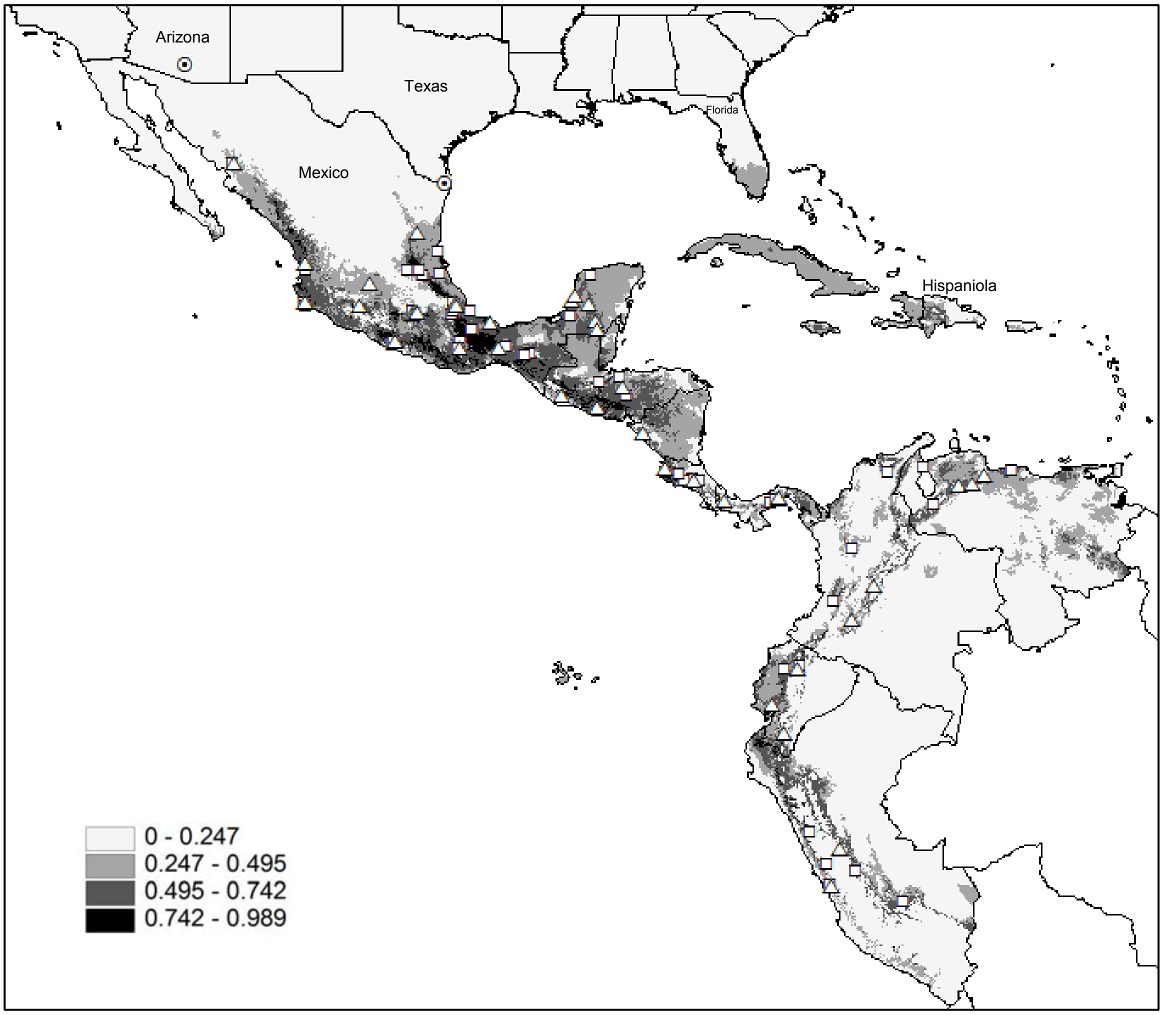

Fig. 4.2. Final potential distribution of *Eulaema polychroma* (Mocsáry) according to Maxent based on all of the data and accordingly used for interpretative purposes (a test of this model is depicted in Fig. 4.1). The squares and triangles correspond to the totality of the locality data entry used to generate the model. The dotted circles represent the two localities in the United States, presumably out of the natural range for the species. The gray scale indicates the similarity between known and predicted habitats, darkest grey represents areas with the highest probability of habitat suitability and lighter grey shows lower probabilities of habitat suitability.

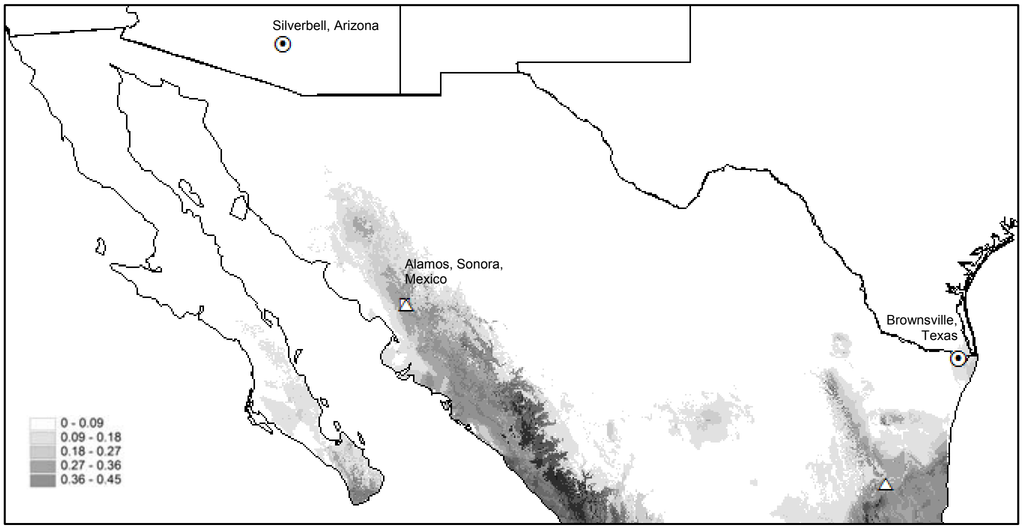

Fig. 4.3. Northernmost section of the potential distribution of *Eulaema polychroma* (Mocsáry) according to Maxent (a magnification of Fig. 4.2 focusing on the specific target area using a different gradient scale to emphasize the exceedingly low probability of habitat suitability within the United States, the actual values for Arizona are zero). The squares and triangles correspond to a subset of the locality data entry used to generate the model across all the natural range of the species. The dotted circles represent the two localities in the United States, presumably out of the natural range for the species.

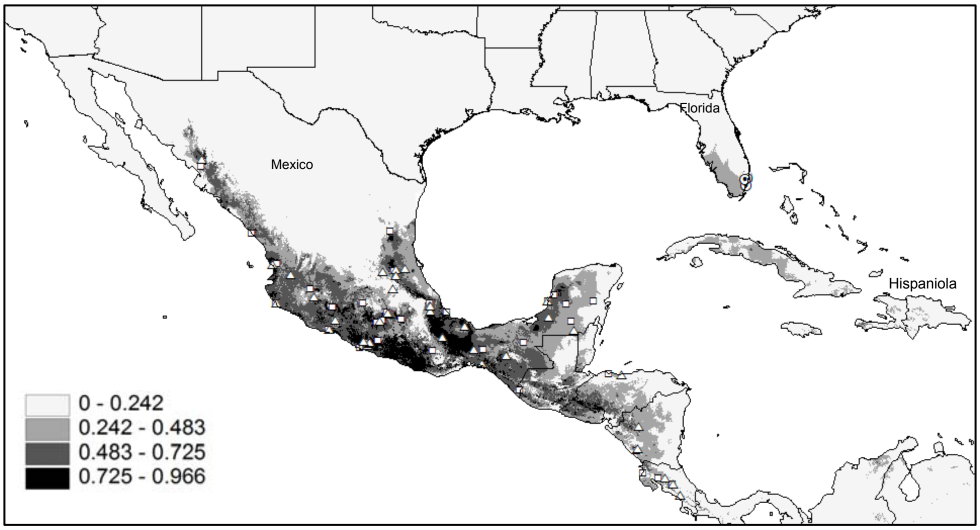

Fig. 4.4. Final potential distribution of *Euglossa viridissima* Friese according to Maxent based on all the data (an identical test as described for *E*. *polychroma*, Fig. 4.1, was undertaken for *E*. *viridissima* but not illustrated). The squares and triangles correspond to the totality of the locality data entry used to generate the model. The dotted circles represent the records for the adventive population of the species in Florida.

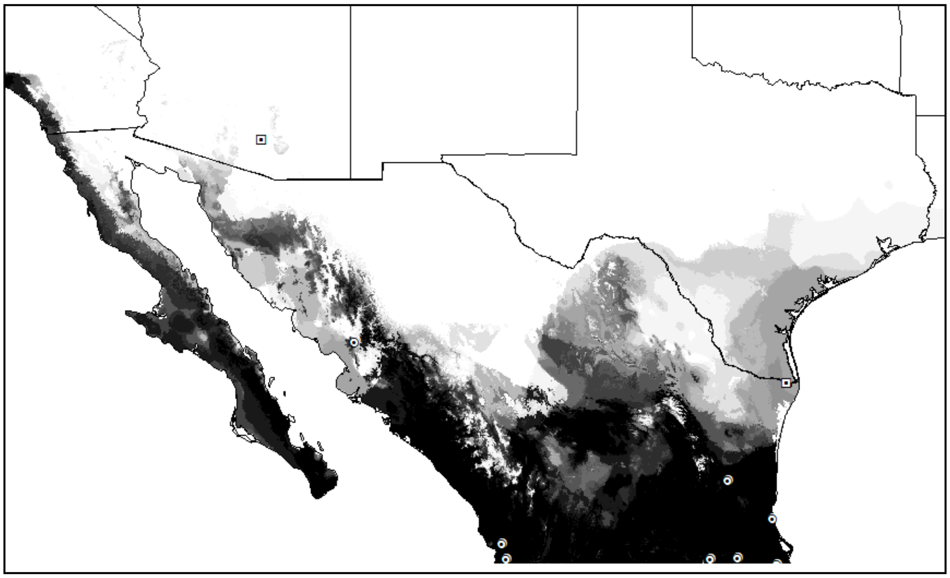

Fig. 4.5. Final potential distribution of *Eulaema polychroma* (Mocsáry) according to Garp based on all of the data. The dotted circles correspond to the totality of the locality data entry used to generate the model. The dotted squares represent the two localities in the United States, presumably out of the natural range for the species.

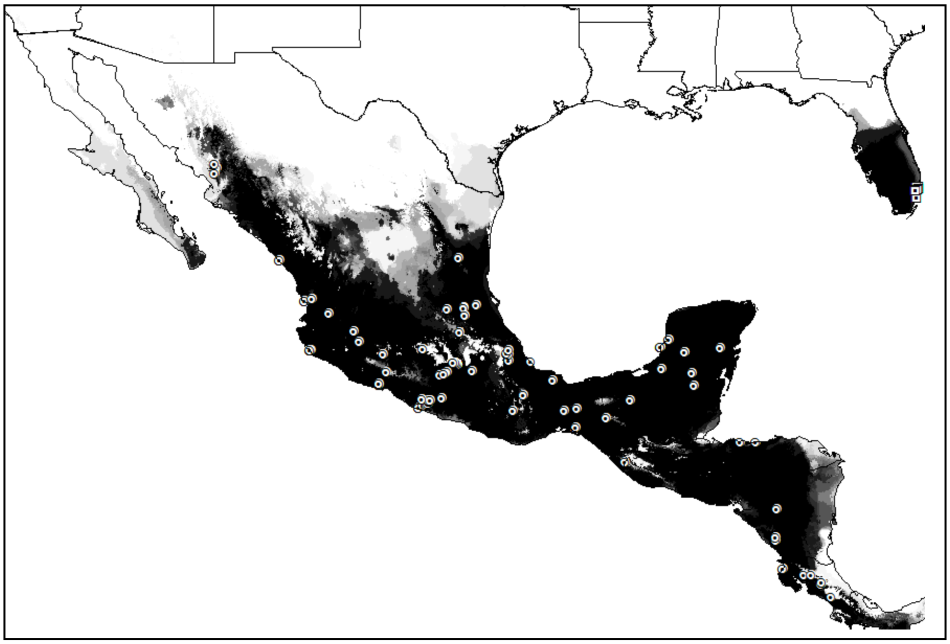

Fig.4. 6. Final potential distribution of *Euglossa viridissima* Friese according to Garp based on all of the data. The dotted circles correspond to the totality of the locality data entry used to generate the model. The dotted squares represent the adventive population of the species in Florida..

DISCUSSION

Euglossine bees stand out visually as a component of local bee faunas because of the metallic bright coloration of most of the species and the robust bodies of several of them; as such they would not be overlooked if they appear in areas where they are not normally found. The cases of *E*. *polychroma* and *E*. *viridissima* are relevant in these terms as both exemplify cases of records well beyond their known natural boundaries. The presence of both species in the continental United States (with peculiarities corresponding to each species) has interesting implications not only in terms of the current distribution of the organisms, but also in respect to their biogeographical history and biology.

In regard to *E*. *polychroma*, the male collected at Silverbell, Arizona corresponds, according to our analysis, to an anomalous isolated case since there is null similarity between the known and the predicted habitat, meaning there is a complete absence of suitable environment for stable populations of the species to persist in the zone. This is in agreement with the earlier interpretation of Minckley and Reyes (1996). This anomalous record has to be attributed to the highly vagile nature of euglossine bees (Janzen, 1971; Dressler, 1982), as the specimen must have undergone a long distance solitary dispersal from some of the northern areas of subtropical vegetation of the Sierra Madre Occidental in Mexico, a situation supported by the heavily worn wings of this particular bee specimen (Minckley and Reyes, 1996). It is also noticeable that no other records of *E*. *polychroma* have been reported from southern Arizona despite the annual bee surveys as part of the “Bee Course” offered at the Southwestern Research

Station, near Portal, Arizona, or the continuous bee (and other pollinator) monitoring activities at the University of Arizona, Tucson. All of this corroborates the ideas that persistent populations of this bee species in southern Arizona are entirely absent. It seems plausible that the specimen captured in Silverbell, Arizona came from the northernmost sections of the natural range of the species or was accidentally introduced from elsewhere. The old record from Brownsville, Texas, on the other hand, could well belong to a persistent population since, according to our model, there are environmental conditions for the species to subsist there. However, the known versus predicted probability of habitat suitability is rather low in that region and is shown as an isolated spot separated from the remainder of the potential distribution area; interestingly the male of *E*. *polychroma* from Brownsville was collected 100 years ago (Minckley and Reyes, 1996), and no other record from the area (either in the United States or in Mexico) is known. As such it would not be surprising that this male could have also just flown away from some of the areas south of the Brownsville region along the Mexican coast or on the eastern face of the Sierra Madre Oriental as our model predicts higher probability of habitat suitability in those regions (Fig. 4.3). It will be interesting to sample the Brownsville area using baits known to attract males of *E*. *polychroma* (e.g., Ramírez *et al*., 2002), as well as sample flower patches to look for the presence of females to corroborate if there are populations of the species there. The same should be done in the areas of the Pacific slope of the Sierra Madre Occidental at around 29° N to assert if that region would be the absolute northernmost range of *E*. *polychroma* and concomitantly of any euglossine bee. Interestingly enough the southern part of the peninsula of Baja California has, according to our model, suitable conditions for this species, especially the southernmost tip (Fig. 4.3). However, this species has never been reported from there and, although in terms of linear distance, this area is somewhat similarly distant from known natural areas of occurrence of the species as is Silverbell, Arizona, the Gulf of California certainly represents a fundamentally different form of barrier. Nonetheless, it would be revealing to sample the southern tip of the peninsula to clarify the absence or presence of *E. polychroma*.

Euglossa viridissima was introduced to southeastern Florida most likely accidentally (Skov and Wiley, 2005) and it has become naturalized there (Pemberton and Wheeler, 2006). Skov and Wiley (2005), predicted a spread of the species from this localized area, stating that there seem to be all the required environmental conditions for the species to subsist, although they did not venture to give more details on their prediction or the area encompassed. Our model of potential distribution of the species projected over Florida and nearby areas in the southeastern United States shows suitable conditions for *E. viridissima* in roughly half of the Florida peninsula (Fig. 4.4). Interestingly, the area where the species was initially established with success is not the one with the highest probability of habitat suitability (the southwest of the peninsula shows the highest, as mentioned above). According to this, *E. viridissima* will potentially spread to occupy the whole tip of the Florida Peninsula, south of a roughly diagonal boundary line between nearby 27° N on the eastern side and around

28°45’ N on the western side. These predictions are initially a base from which the actual future distribution of the species could vary. Our results do not show any possibility for the species to spread out of Florida. Naturally, it will be interesting to test this prediction with careful monitoring of the species as it continues to reach new areas within Florida.

The distributional patterns of orchid bees in general are deserving of attention. Regarding their phylogenetic position, as sister to all other corbiculate apines [according to several analyses (e.g., Schultz *et al*., 1999, 2001; Engel, 2001a, 2001b; Noll, 2002; Cardinal and Packer, 2007)], their restriction to the neotropics is interesting as the other tribes are either Pantropical, Palaearctic, Ethiopian, and/or Oriental. No biogeographical studies of euglossines under a phylogenetic umbrella exist; however, there are some noticeable differences in faunistic composition of different neotropical areas. At the generic level the most obvious is the restriction of *Aglae* to the Amazon basin, while based on a quantitative approach Nemésio and Silveira (2007) have shown the differential composition of the euglossine fauna in different areas of the neotropics. One interesting point is the virtual absence of orchid bees in the Caribbean Islands, as Jamaica harbours the only extant euglossine among those islands. Some dubious records of two other species have been reported also from Jamaica, but they most likely correspond to misidentifications or mislabelling of specimens. Beyond the dispersal capacities of euglossines, which obviously play a central role in their ability to colonize new areas, some authors have invoked the seemingly tight euglossine-orchid association as a handicap for the bees to establish in the

Antilles, as they would not succeed unless their orchid counterparts existed on the target island or colonize it nearly simultaneously (e.g. Janzen, 1975; Rebêlo, 2001). The presence and naturalization of *E. viridissima* in Florida attests to the non-binding dependence on orchids as perfume sources for the bees, since perfume orchids do not occur naturally in that region and it has been shown that the male bees gather chemicals from other plant sources, not necessarily flowers, to acquire an assemblage of chemicals similar to what they amass in their natural range (Pemberton and Wheeler, 2006). Other evidence exists of euglossines gathering chemicals from sources other than orchid flowers (Dressler, 1979; Whitten *et al*., 1993; Roubik, 1998), but as Pemberton and Wheeler (2006) assert, the persistence of *E. viridissima* in Florida shows for the first time the ability of the bees to live in an environment without perfume orchids. In terms of the Caribbean Islands, this demonstrates that euglossines could have persisted there beyond the presence or absence of perfume orchids and the current low euglossine diversity should be explained more accurately by historical events. The potential distribution models produced here for our two orchid bee species subjects show indeed the presence of suitable habitat for both in most of the Caribbean Islands (Figs. 2, 4). Despite the current absence of orchid bees on the island of Hispaniola, the fossil record reveals the presence of two species (one *Euglossa*, one *Eufriesea*) as late as the Early Miocene (Engel, 1999), but the different geological components of the modern island were subject to major tectonic events that might have resulted or played a significant component in the extinction of these species from the region.

The cases of the adventive presence of both *E*. *polychroma* and *E*. *viridissima* show how historical and ecological variables act to influence the distribution of this lineage of bees. The presence of suitable environment for *E*. *viridissima* in Florida as predicted by our model coincides entirely with the “naturalization” of the species there and aids our understanding of the ecological requirements of the species (no strict dependence on perfume orchids). In turn, the absence of suitable habitat for *E*. *polychroma* in Arizona as our model predicts, matches the lack of further records for the species beyond the isolated case here described. In any case, the persistence and future spread of *E*. *viridissima* in Florida, as well as the continuous monitoring of the bee fauna in southern Arizona and southernmost Texas will be the ultimate arbiter of our models. It is also hoped that these methodological tools may be employed to analyze other recent records of euglossines collected outside of their previously understood distributions (e.g., Nemésio and Silveira, 2004; Anjos-Silva, 2008).

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Appendix 1. List of species studied in the comparative genital morphology section (Chapter 2).

Species studied by direct examination of dissected specimens.

E. (*Dasystilbe*) *villosa* Moure

E. (*Euglossella*) *bigibba* Dressler
E. (*Euglossella*) *cosmodora* Hinojosa-Díaz & Engel
E. (*Euglossella*) *cyanea* Friese
E. (*Euglossella*) *cyanura* Cockerell
E. (*Euglossella*) *decorata* F. Smith
E. (*Euglossella*) *granti* Cheesman
E. (*Euglossella*) *mandibularis* Friese
E. (*Euglossella*) *perpulchra* Moure & Schlindwein
E. (*Euglossella*) *perviridis* Dressler
E. (*Euglossella*) *polita* Ducke
E. (*Euglossella*) *viridis* (Perty)

E. (*Glossurella*) *allosticta* Moure
E. (*Glossurella*) *asarophora* Moure
E. (*Glossurella*) *augaspis* Dressler
E. (*Glossurella*) *bursigera* Moure
E. (*Glossurella*) *dodsoni* Moure
E. (*Glossurella*) *gorgonensis* Cheesman
E. (*Glossurella*) *hyacinthina* Dressler
E. (*Glossurella*) *laevicincta* Dressler
E. (*Glossurella*) *macrorhyncha* Dressler
E. (*Glossurella*) *nigrosignata* Moure
E. (*Glossurella*) *obtusa* Dressler
E. (*Glossurella*) *oleolucens* Dressler
E. (*Glossurella*) *parvula* Dressler
E. (*Glossurella*) *sapphirina* Moure
E. (*Glossurella*) *stilbonota* Dressler
E. (*Glossurella*) *turbinifex* Dressler
E. (*Glossurella*) *viridifrons* Dressler

E. (*Glossura*) *annectans* Dressler
E. (*Glossura*) *imperialis* Cockerell
E. (*Glossura*) *piliventris* Guérin
E. (*Glossura*) *stellfeldi* Moure

E. (*Glossuropoda*) *intersecta* Latreille
E. (*Glossuropoda*) *rugilabris* Moure

E. (*Euglossa*) *cognata* Moure
E. (*Euglossa*) *cordata* (Linnaeus)
E. (*Euglossa*) *cybelia* Moure

E. (*Euglossa*) *deceptrix* Moure
E. (*Euglossa*) *hansoni* Moure
E. (*Euglossa*) *iopyrrha* Dressler
E. (*Euglossa*) *mixta* Friese
E. (*Euglossa*) *nigropilosa* Moure
E. (*Euglossa*) *townsendi* Cockerell
E. (*Euglossa*) *tridentata* Moure
E. (*Euglossa*) *viridissima* Friese

Aglae caerulea Lepeletier de Saint Fargeau & Audinet-Serville
Eufriesea caerulescens (Lepeletier de Saint Fargeau)
Eufriesea auripes Gribodo
Eulaema polychroma (Mocsáry)
Eulaema speciosa (Mocsáry)
Exaerete smaragdina (Guérin-Méneville)
Exaerete frontalis (Guérin-Méneville)

Bombus sp.
Anthophora dufourii Lepeletier de Saint Fargeau
Centris birkmanii Friese
Centris poecila Lepeletier de Saint Fargeau
Centris labrosa Friese
Epicharis metatarsalis Friese

Groups consulted in the Literature (for each work cited, all species depicted were used for comparative statements in this study)

Eufriesea spp. (Kimsey, 1982)
Eulaema spp. (Oliveira, 2006)
Exaerete spp. (Kimsey, 1979)
Bombus spp. (Ito, 1985; Michener, 1990, 2007)
Anthophora spp. (Brooks, 1988)
Centridini spp. (Ayala, 1998)

Appendix 2. List of male external morphological characters included in the phylogenetic analysis of the genus *Euglossa*. Characters are arranged following their location on the body from head to genitalic structures and numbered as they appear in the matrix created for the analysis (see Appendix 4). Character numbers appear (in bold face) and character state codes appear before description of either.

Head

1.- Number of mandibular teeth

2	Two teeth
3	Three teeth

2.- Presence/absence and structure of paraocular lines

0	Absent or barely seen as thin marks
1	Present, incomplete (not reaching horizontal section of epistomal sulcus)
2	Present, complete (reaching horizontal section of epistomal sulcus), if wider apically (proximal to horizontal section of epistomal sulcus) no more than twice basal width (near antennal socket)
3	Present, wider apically, more than twice as base, but not reaching vertical section of epistomal sulcus
4	Present, covering all the area between compound eye and horizontal and vertical section of epistomal sulcus

3.- Presence/absence of translucent spots on labrum (labral windows)

0	Absent
1	Present

4.- Ratio labrum width/labrum length

0	0.9<Ratio<1.1 (labrum almost square)
1	Ratio>=1.1 (labrum wider than long)
2	Ratio<=0.9 (labrum longer than wide)

5.- Presence/absence and structure of medial ridge of labrum

0	Present, complete
1	Present, incomplete (terminating at midpoint)
2	Absent

6.- Presence/absence of dark integumental spot on labrum (labral macula)

0	Absent
1	Present

7.- Ratio width of malar area/diameter of third flagellomere

0	Ratio<=0.9 (malar area narrower than third flagellomere)
1	0.9<Ratio<1.1(malar area around as wide as third flagellomere)
2	Ratio>=1.1 (malar area wider than third flagellomere)

8.- Color of malar area

0	White (with some dark-brown areas on acetabulum and condyle)
1	Dark-brown

9.- Ratio upper interocular distance/lower interocular distance

0	0.9<Ratio<1.1 (upper and lower interocular distances equal)
1	Ratio>=1.1 (upper interocular distance wider)
2	Ratio<=0.9 (lower interocular distance wider)

10.- Ratio length of first flagellomere/combined length of second and third flagellomeres

0 Ratio<1.4 (first flagellomere about as long as second and third flagellomeres combined)
1 Ratio>=1.4 (first flagellomere considerably longer than second and third flagellomeres combined)

11.- Color of lateral parts of clypeus

0 White
1 Same as facial integument color (blue, green, red, etc.)

12.- Length of labiomaxillary complex relative to body

0 Noticeably shorter than body, at most reaching fourth sternum
1 About as long as body, sometimes slightly shorter (tip reaching at least posterior margin of fifth sternum)
2 Noticeably longer than body (exceeding apex of metasoma)

Mesoma

13.- Shape of dorsolateral angle of pronotum

0 Obliquely truncate
1 Not obliquely truncate

14.- Presence/absence and structure of projections on dorsolateral angle of pronotum

0 Not projected, usually orthogonal, or obtuse (when obliquely truncate)
1 Projected as an acute prong
2 Projected as a lamella

15.- Mesoscutellar shape

0 Lateral margins relatively parallel, arching distally to posterior mesoscutellar margin
1 Lateral margins tapering, arching basally converging to shortened posterior scutellar margin

16.- Presence/absence and structure of mesoscutellar furrow

0 Absent or poorly developed (at most seen as a very weak channel)
1 Well developed, shallow
2 Well developed as a deep median channel, not reaching anterior scutellar margin
3 Well developed as a deep median channel, reaching anterior scutellar margin

Legs

17.- Length of setae on posterior surface of probasitarsus

0 No longer than combined length of second to fifth probasitarsomer
1 Several setae longer than combined length of second to fifth probasitarsomer

18.- Presence/absence of mesotibial spur

0 Present
1 Vestigial, only seen with microscope
2 Absent, only socket present

19.- Shape of mesotibial border on area where spur is generally found

0 Unmodified, no projections present
1 Projected in a spine or lamella-like structure noticeably emarginated

20.- Relative width of depressed-tuberculate surface (posterior) of mesotibia respect to velvety surface (outer-lateral surface covered with microtrichia) of mesotibia

0	Depressed-tuberculate surface noticeably narrower than velvety surface
1	Both surfaces about the same width
2	Depressed-tuberculate surface noticeably wider than velvety face

21.- Mesotibial build

0	Not conspicuously inflated, with a noticeable deep depressed-tuberculate area adjacent to posterior carina of velvety area
1	Inflated, tuberculate area adjacent to posterior carina of velvety area not deepened but leveled with adjacent integumental surfaces

22.- Presence/absence of posterior tuft of mesotibia

0	Absent, velvety area (dense microtrichia) occupying the surface where the posterior tuft would appear in those species that have it
1	Absent, anterior and unique tuft extended over the surface where the posterior tuft would appear in those species that have it
2	Present as a structure composed of dense setae sitting in a cavity
3	Present as a structure composed solely of a cavity
4	Absent, integument unmodified and bare over the surface where the posterior tuft would appear in those species that have it

23.- Depth of cavity of posterior tuft of mesotibia

0	Tuft cavity shallow, its integument is just slightly lower than the surrounding integument
1	Tuft cavity deep, excavated

24.- Relative size of posterior tuft (or cavity) respect to anterior tuft in mesotibia

0	Posterior tuft at most half the length (measured as following longitudinal axis of mesotibia) of anterior tuft
1	Posterior tuft more than half as long or as long (but no longer) than anterior tuft
2	Posterior tuft longer than anterior tuft

25.- Shape of posterior tuft of mesotibia

0	Roughly circular
1	Roughly oval, longer axis following longitudinal axis of mesotibia
2	Polygonal, longer axis following longitudinal axis of mesotibia
3	Roughly oval, longer axis perpendicular to longitudinal axis of mesotibia

26.- Presence/absence of knob on anterior margin of posterior tuft of mesotibia

0	Absent
1	Present

27.- Shape of anterior tuft of mesotibia

0	Variable shape but never presenting a cleft in anterior margin
1	Variable shape but always with a cleft in anterior margin, sometimes producing two lobes

28.- Third mesotibial tuft between anterior and posterior tufts of mesotibia

0	Absent
1	Present

29.- Presence/absence of keel, ridge, carina or elevation on inner surface of mesobasitarsus

0	Absent
1	Present

30.- Shape of posterior margin of mesobasitarsus
0 Slightly convex, gradually curving distad (looking straight in some species)
1 Notched, forming a conspicuous emargination in distal half

31.- Shape of distal inner margin of mesobasitarsus at joint with second mesotarsomere
0 Shallow or no invagination on distal inner margin of basitarsus
1 Very deep invagination on distal inner margin of basitarsus

32.- Structure of second mesotarsomere
0 Not compressed
1 Compressed (widened in such a way that looks somewhat laminar)

33.- Shape of anterior margin of second mesotarsomere
0 Unmodified, straight or slightly curved
1 Emarginate proximally

34.- Shape of posterior margin of second mesotarsomere
0 Unmodified, straight or slightly curved
1 Emarginate proximally

35.- Presence/absence of lamellae on margins of third and fourth mesotarsomers
0 Absent
1 Present

36.- Structure of metatibial organ slit (differentiation or not into proximal and distal sections)
0 Longitudinally uniform, not differentiated proximally
1 Proximal oval section differentiated by deviating (towards the posterodorsal margin of the tibia) from the remaining section

37.- Presence/absence of furrow (noticeable deepening) on posterodorsal margin of metatibia
0 Absent
1 Present

38.- Width of connection of basal and distal sections of metatibial organ slit
0 As wide as contiguous basal section
1 Narrower than contiguous basal section

39.- Shape of metabasitarsus
0 Roughly triangular, anterior and posterior margins not parallel, or if parallel only on distal third of metabasitarsus
1 Roughly rectangular, anterior and posterior margins parallel, at least on most of its length
2 Trapezoidal, posterior margin concave

40.- Shape and alignment of ventral margin of metabasitarsus
0 Oblique, posteriorly projected in a sharp acute angle
1 Oblique, posteriorly projected as an even convexity
2 Roughly straight respect sagittal body plane, appearing truncate and without noticeable projections of posterior margin

41.- Shape of ventral margin of inner surface of metafemur
0 Straight
1 Slightly concave

2 Strongly concave
3 Slightly evenly convex
4 Convex, specially mid-proximally (bulging)
5 Evenly convex

42.- Presence/absence of knob on inner surface of metafemur proximal to trochanter joint
0 Absent
1 Present

43.- Shape of dorsal margin of inner surface of metafemur
0 Rather straight
1 Evenly convex (convexity slightly pronounced proximally)
2 Convex, specially mid-proximally, (bulging)

44.- Structure of inner surface of metatibia (presence/absence of depressed areas)
0 All surface even, no noticeable depressions present
1 Presence of circular depression near basitarsal joint
2 Presence of triangular depression near basitarsal joint

45.- Structure of pilosity on inner face of metatibia
0 All surface evenly setose
1 Setae absent (or less dense) only along mesal area of anterior margin
2 Setae absent (or less dense) only along anterior margin and ventro-mesally on a semicircle (of varying size)
3 As previous state plus completely bare at circular area near basitarsal joint

46.- Presence/absence of projection on ventral margin of inner surface of metatibia, immediately posterior to spur
0 Absent or inconspicuous
1 Present, strongly projected as a compressed plate (sometimes slightly obtuse)
2 Present, strongly projected as a plate but also bulging as a bump
3 Present as a blunt bump (either rounded or obtuse)

47.- Shape of anterior margin of distal section of metatibial organ slit
0 Evenly convex
1 Spur shaped
2 Straight, following posterodorsal margin of metatibia

48.- Shape of basal section of metatibial organ slit
0 Oval shaped
1 Basally oval but with an acute anterior projection

49.- Extension of metatibial organ slit (distal section) respect ventral margin of metatibia
0 Reaching ventral margin
1 Separated from ventral margin by less than length of distal section of organ slit
2 Separated from ventral margin by at least the length of distal section of organ slit

50.- Shape-alignment of metatibial ventral margin respect body axis
0 Rather straight
1 Convex
2 Concave

51.- Ratio metatibial anterior margin length/metatibial ventral margin length
0 >=1.4 (equal or more than 1.4)
1 < 1.4 (less than 1.4)

Wings

52.- Average number of jugal comb blades on forewing

0	More than ten
1	Ten or less

Hidden Sterna

53.- Shape of posterior margin of second metasomal sternum

0	Straight
1	Sinuate, forming two mid-lateral emarginations

54.- Presence/absence and structure of Integumental modifications of second metasomal sternum

0	Absent
1	Present as two contiguous cowled slits with openings towards posterior margin of sternum
2	Present as two contiguous elevations with no openings (false cowled slits)
3	Present as two integumental depressions with a variation of an omega-like shape

55.- Presence/absence of setal patches on second metasomal sternum

0	Absent
1	Present

56.- Presence/absence of incision (noticeable emargination) on posterior margin of disc of seventh metasomal sternum

0	Absent
1	Present

57.- Presence/absence of setae on posterior margin of disc of seventh metasomal sternum

0	Present
1	Absent

58.- Shape of lateral edges of posterior section of eighth metasomal sternum

0	Not or shallowly invaginated, lobes not strongly projected
1	Deeply invaginated, lobes strongly projected

59.- Lateral width of posterior section (midway between lobes and apex) of eighth metasomal sternum

0	Almost as wide (often as wide or wider) as lateral width of anterior section (mid-anterior)
1	Noticeably narrower than lateral width of anterior section (mid-anterior)

60.- Distribution of setae on posterior section of eighth metasomal sternum

0	Not covering basal lobes
1	Covering basal lobes

61.- Length of setae on posterior section of eighth metasomal sternum

0	None of them as long or longer than apex of the posterior section
1	At least some as long or longer than the apex of the posterior section

62.- Structure of setae on posterior section of eighth metasomal sternum

0	All simple
1	At least some of them plumose

Genital Capsule

63.- Alignment of posterior margin of apical process of gonocoxite

0	Perpendicular to sagittal plane
1	Oblique (inner-posterior corner posterior to outer-posterior corner)
2	Oblique (outer-posterior corner posterior to inner-posterior corner)

64.- Shape of Lateral area of gonostylar process of gonocoxite

0	Prongued
1	Acute
2	Fused to gonostylus (indistinguishable)
3	Truncate
4	Rounded

65.- Presence/absence of elevation (bump) on inner area of gonostylar process

0	Absent
1	Present, in frontal view well separated from posteromesal prongs
2	Present, in frontal view contiguous (or almost) to posteromesal prongs

66.- Presence/absence of proximal notch (emargination) on posterior margin of outer blade of penis valve

0	Absent
1	Present, shallow
2	Present, strong

67.- Structure of spatha

0	Present as just a brief narrow band on border of bridge of the PV's
1	Present, wider than short (width measured on bridge of the PV's
2	Present, roughly as long as wide
3	Present, evidently longer than wide

68.- Presence/absence and length of central axis of the spatha

0	Absent
1	Present, running only mid-distance or less on spatha
2	Present, running complete
3	Present, complete and wide invagination near the apex

69.- Ornamentation of the spatha

0	Mainly smooth, no evident pattern of wrinkles
1	Smooth along mid section, some transversal or diagonal wrinkles
2	Strong longitudinal wrinkles running along most of the surface

70.- Structure of lateral section of gonostylus

0	Compressed, appearing flattened on ventral or dorsal views (blade-like)
1	Thickened, or not so compressed, such that there is a broadened area usually where setae develop

71.- Structure of cuticular lamina connecting ventral and lateral sections of gonostylus

0	Present only in a narrow dorsal area
1	Present on most of the posterior face of ventral section

72.- Arrangement of setose area on dorsal sector of lateral section of gonostylus

0	Absent
1	Present covering flat inner face
2	Projected as a convexity on a dome-like shape
3	Present as a concave area on inner face

73.- Differentiation between ventral lobe and dorsal sector of lateral section of gonostylus

0	Both well differentiated
1	Fused for the most part, so only the tip of the ventral lobe is free

74.- Shape/orientation of ventral lobe of gonostylus

0	Conical
1	Compressed (along with dorsal sector)
2	Thick membranous, thumb-like
3	Thick membranous, tube-like, semiflattened ventro-laterally
4	Thick membranous proximally but compressed on most of its length, flattened area facing ventrally

75.- Presence/absence of additional dorsal-most fold on dorsal sector of lateral section of gonostylus

0	Absent
1	Present

76.- Structure of main setae of lateral section of gonostylus

0	Simple (not branched)
1	Plumose

77.- Length of main setae of lateral section of gonostylus

0	Short, not passing the distal end of the apical process of gonocoxite
1	Long, passing apical process of gonocoxite but barely or not reaching ventral blades of penis valves
2	Longer, reaching ventral blades of penis valve, sometimes passing them

78.- Presence/absence of setae on inner face of ventral lobe

0	Absent
1	Present

79.- Ratio width/length of ventral surface of ventral sector of gonostylus

0	Less than half as wide as long
1	Width more than half of length

Appendix 3. Figures associated with the phylogenetic analysis of the genus *Euglossa*. Figures appear grouped in three sections as follows:

> Figs. 3.1–3.28. Illustrations of the general morphology of males of *Euglossa s*. *lat*., plus illustrations of some characters used in the phylogenetic analysis. Generic names other than *Euglossa* are never abbreviated, all generic abbreviations refer to *Euglossa s*. *lat*.
>
> Figs. 3.29–3.31. Screenshots of the programs and settings used to run the phylogenetic analysis.
>
> Figs. 3.32–3.47. Illustrations of the results of the phylogenetic analysis, and information derived from it.

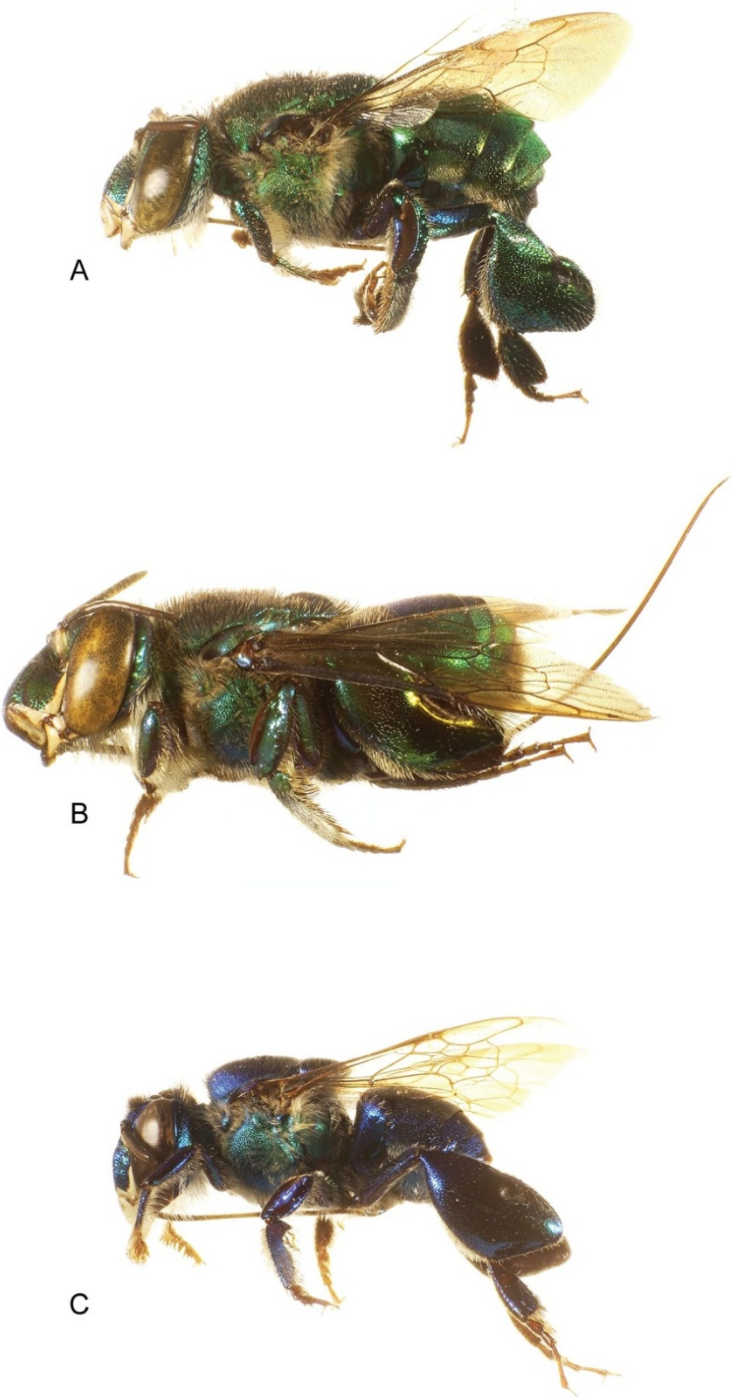


Fig. 3.1. Lateral habitus of males of the type species of the six subgenera of *Euglossa* as used before this work. **A**. *Euglossa* (*Euglossa*) *cordata*. **B**. *E*. (*Glossura*) *piliventris*. **C**. *E*. (*Euglossella*) *viridis* (continues in next page).

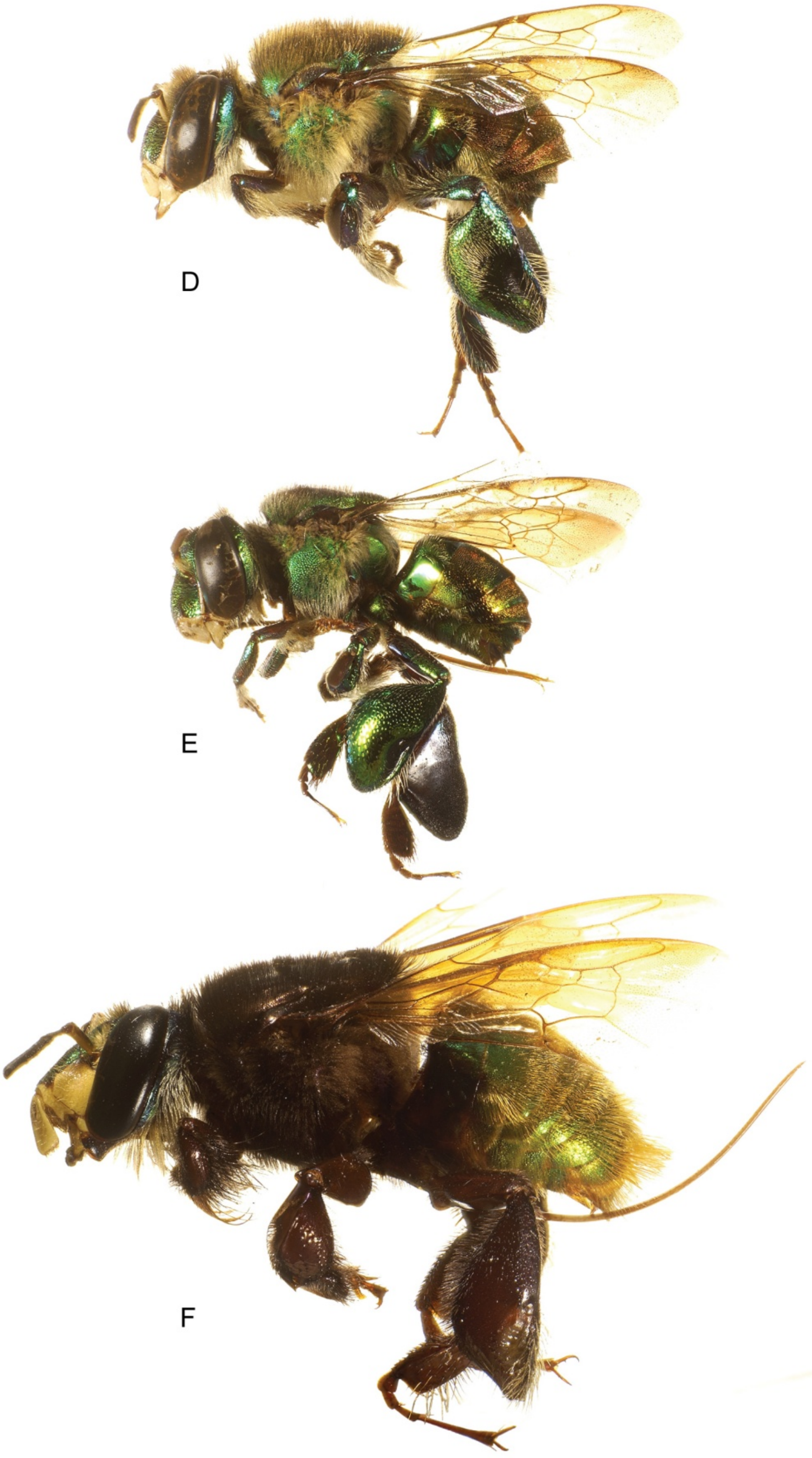


Fig. 3.1. (continues from previous page) **D**. *Euglossa* (*Dasystilbe*) *villosa*. **E**. *E*. (*Glossurella*) *bursigera*. **F**. *E*. (*Glossuropoda*) *intersecta*.

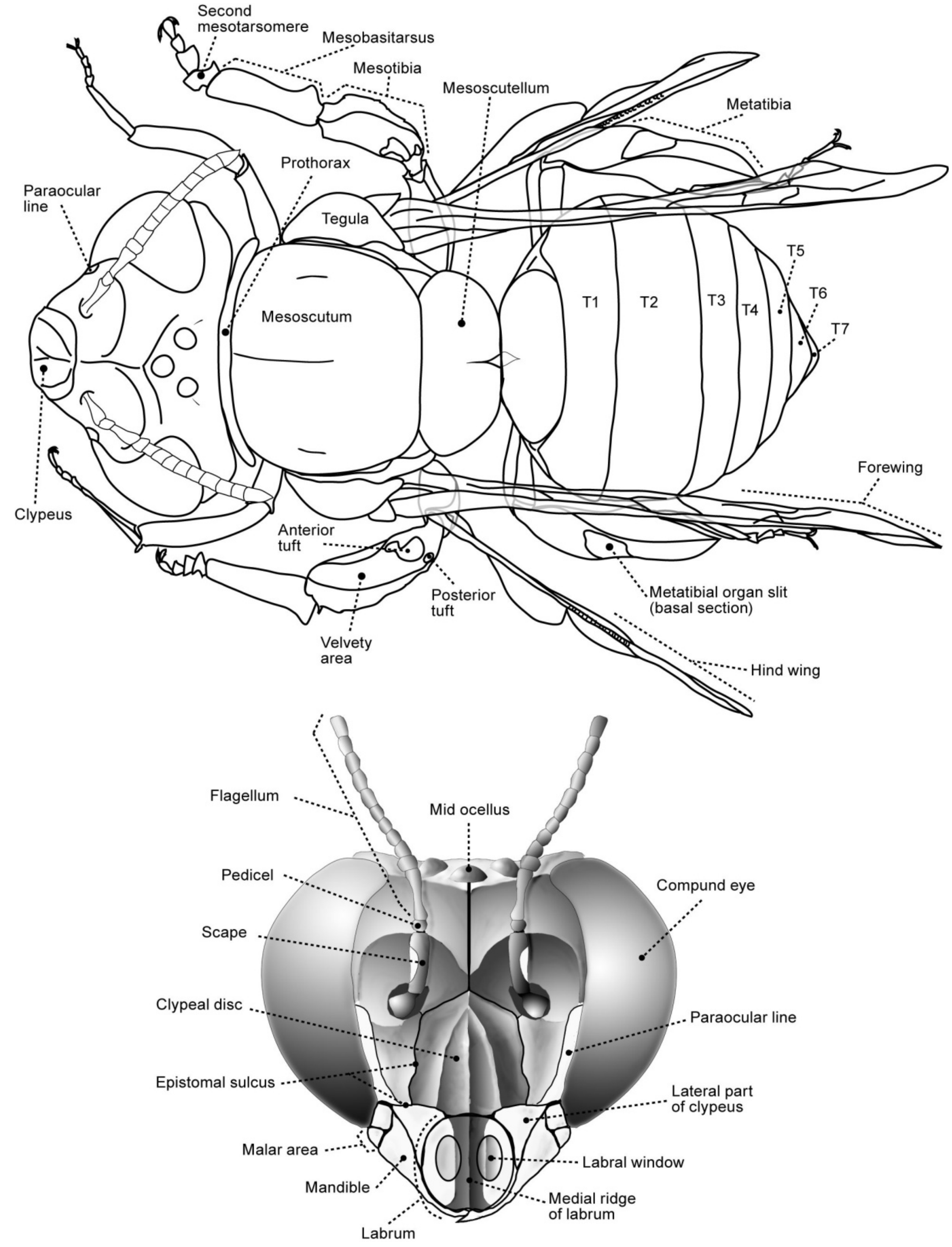
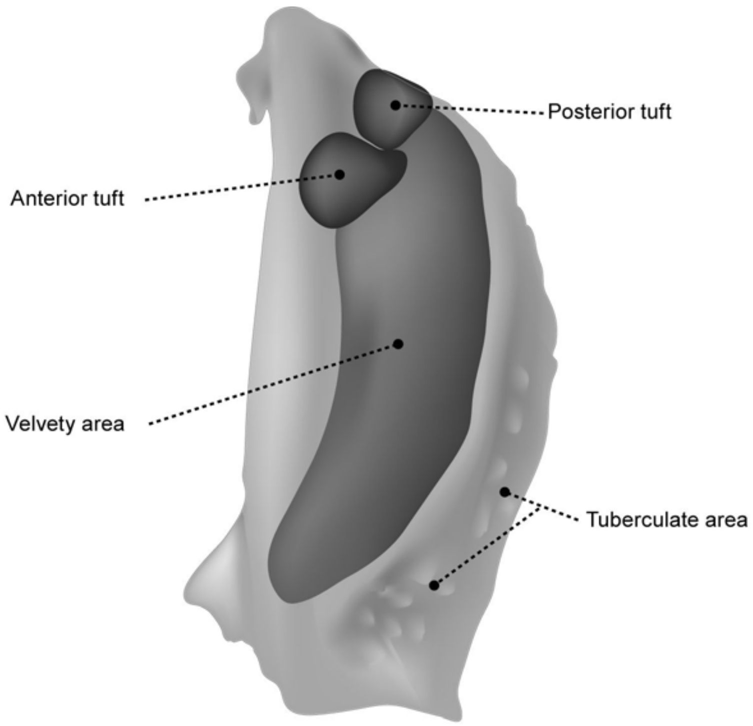


Fig. 3.2. Nomenclature of the principal external morphological features of *Euglossa s. lat*. Above, schematic dorsal habitus based on views of *E*. *townsendi* and *E*. *hansoni*. Below, facial features of *E*. *piliventris*. Vestiture and sculpturing omitted in both views.



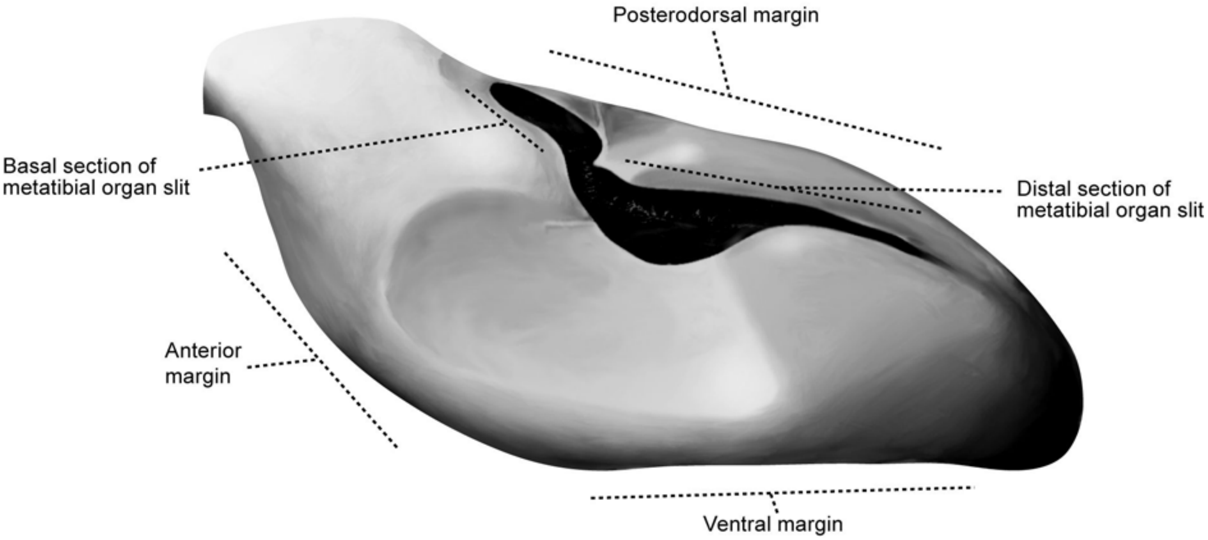


Fig. 3.3. Nomenclature of some of the principal features of the mesotibia and metatibia of *Euglossa s. lat*. Above, mesotibia of *E. rugilabris*. Below, metatibia of *E. cotylisca* [modified from Hinojosa-Díaz and Engel (2007)]. Vestiture and sculpturing omitted.

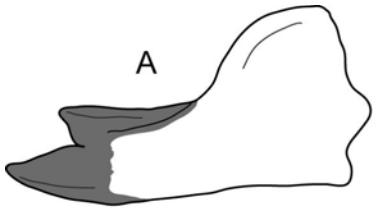


Fig. 3.4. Mandibles of *Euglossa imperialis* (**A**), and *E*. *cyanura* (**B**), showing bidentate and tridentate conditions.

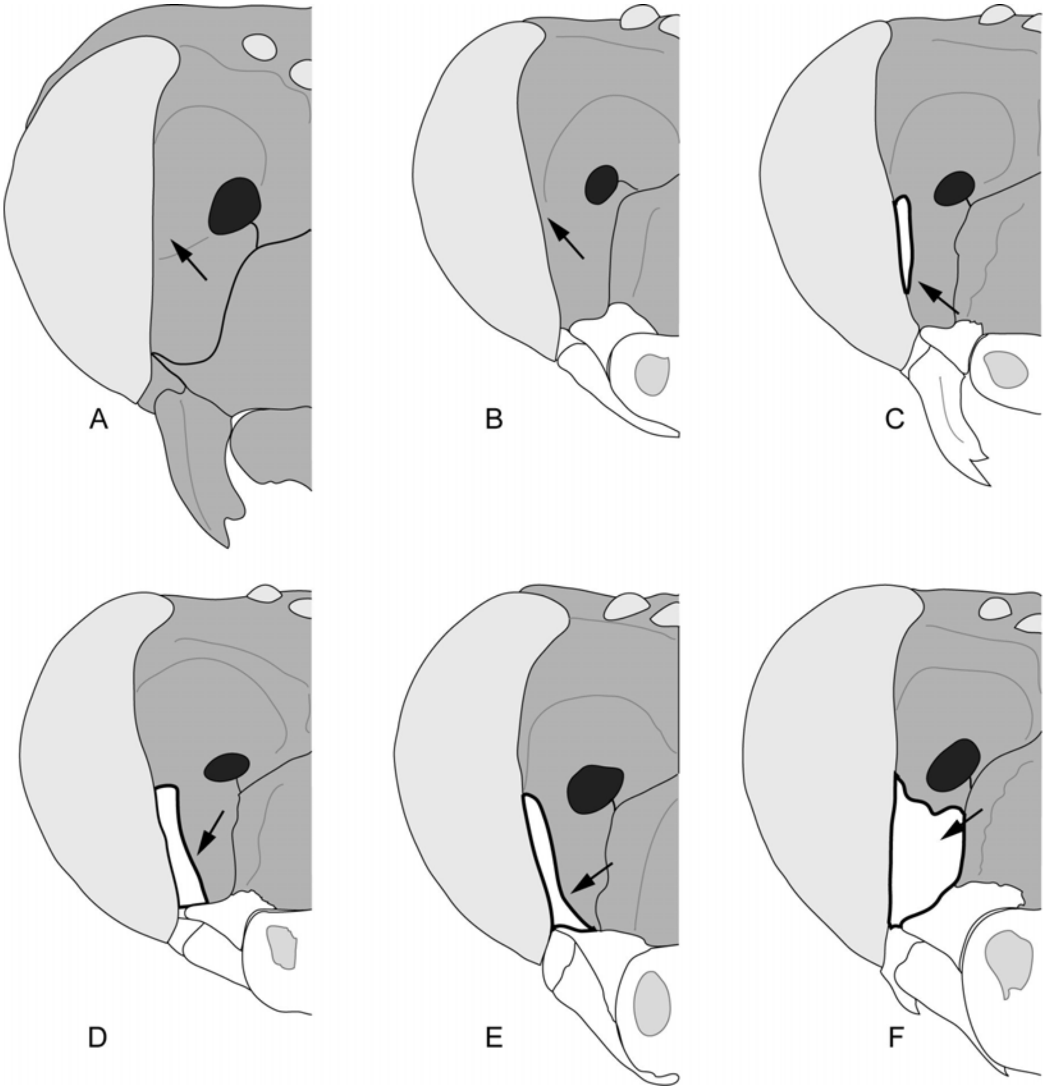


Fig. 3.5. Faces of *Eufriesea auripes* (**A**), *Euglossa oleolucens* (**B**), *E*. *gorgonensis* (**C**), *E*. *viridissima* (**D**), *E*. *piliventris* (**E**), and *E*. *intersecta* (**F**), showing the structure of the paraocular lines (or their absence) as indicated by the arrows.

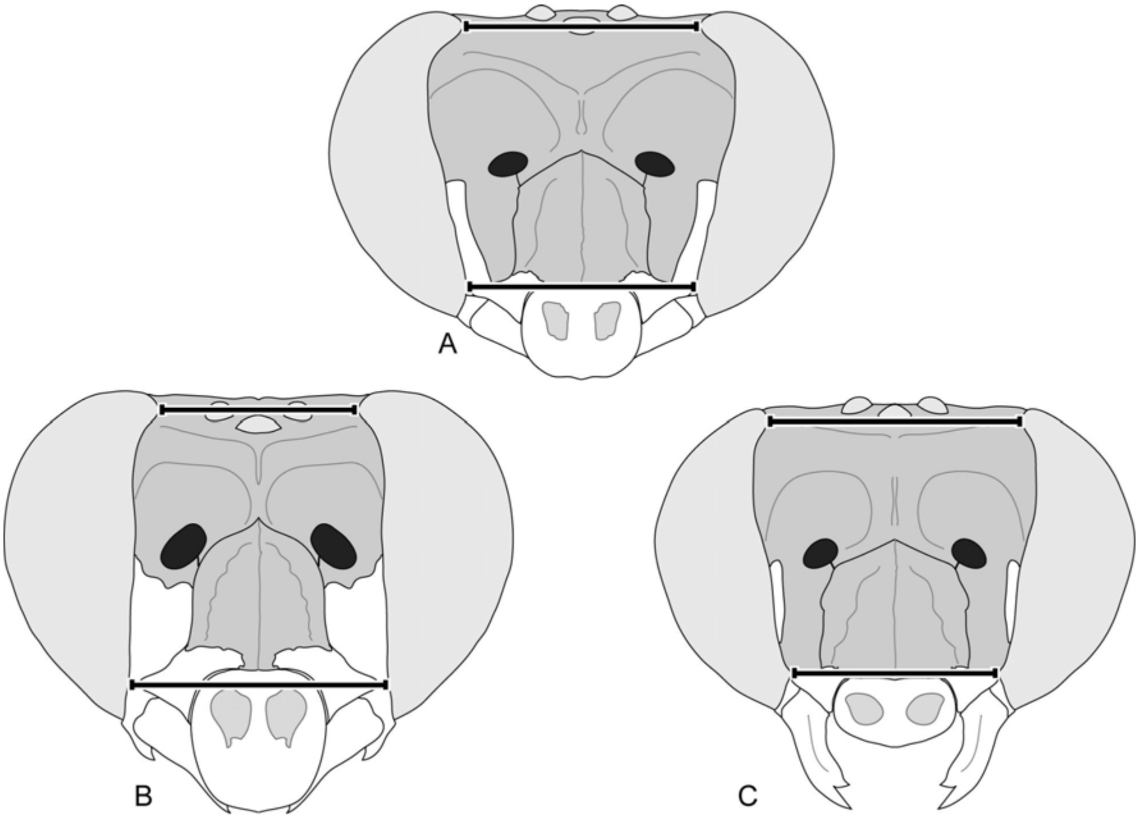


Fig. 3.6. Faces of *Euglossa viridissima* (**A**), *E*. *intersecta* (**B**), and *E*. *gorgonensis* (**C**), showing upper and lower interocular distances. In *E*. *viridissima* both are rather the same, in *E*. *intersecta* the lower is slightly wider, in *E*. *gorgonensis* the upper is slightly wider.

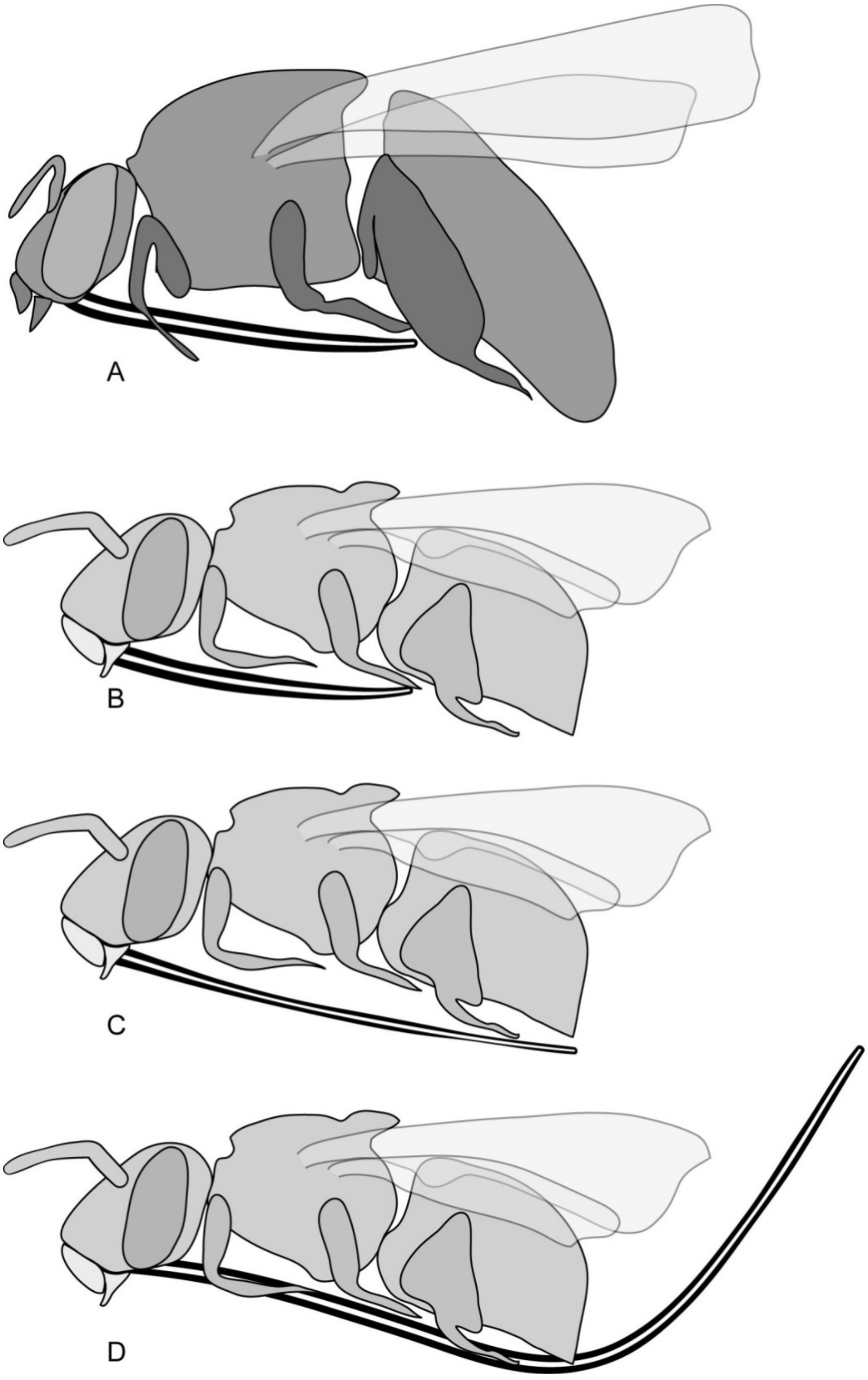


Fig. 3.7. Schematic representation of the length of the labiomaxillary complex relative to the body in *Eulaema* (**A**), and *Euglossa s. lat.* (**B, C**, **D**). A and B represent a labiomaxillary complex noticeably shorter than the body, C a labiomaxillary complex as long as the body, and D a labiomaxillary complex noticeably longer than the body.

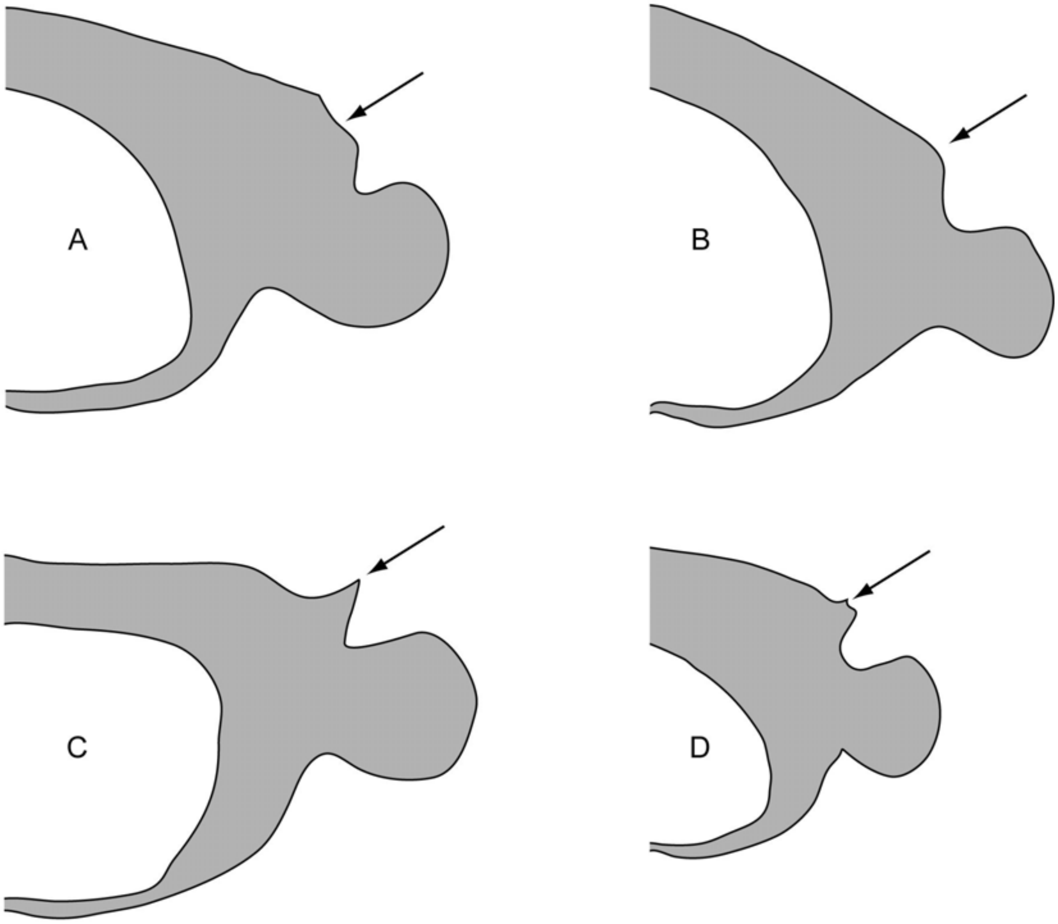


Fig. 3.8. Schematic representation of the dorsolateral angle of the pronotum, as indicated by the arrows. **A**. *Euglossa maculilabris* with an obliquely truncate angle. **B**. *E*. *gorgonensis* with an obtuse angle. **C**. *E*. *villosa* with an acute prong. **D**. *E*. *decorata* with a lamellar projection.

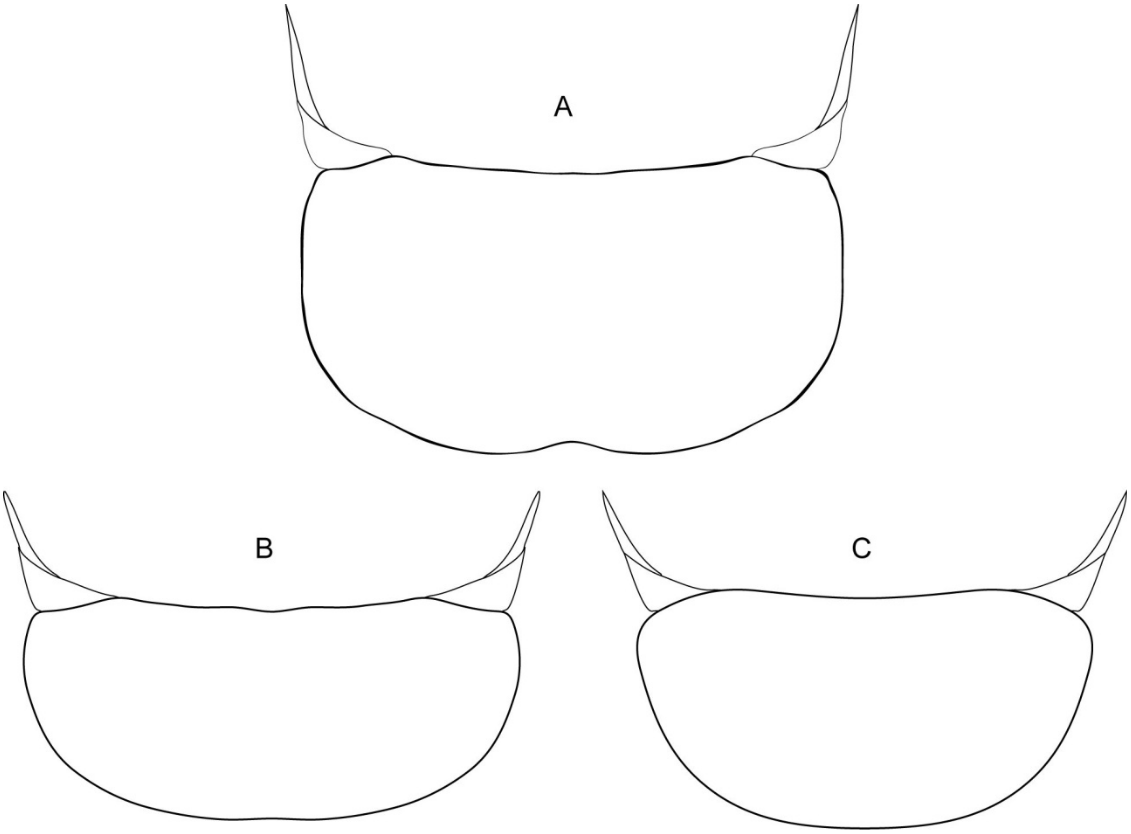


Fig. 3.9. Dorsal view of mesoscutellum. **A**. *Euglossa cognata*. **B**. *E*. *townsendi*. **C**. *E*. *gorgonensis*.

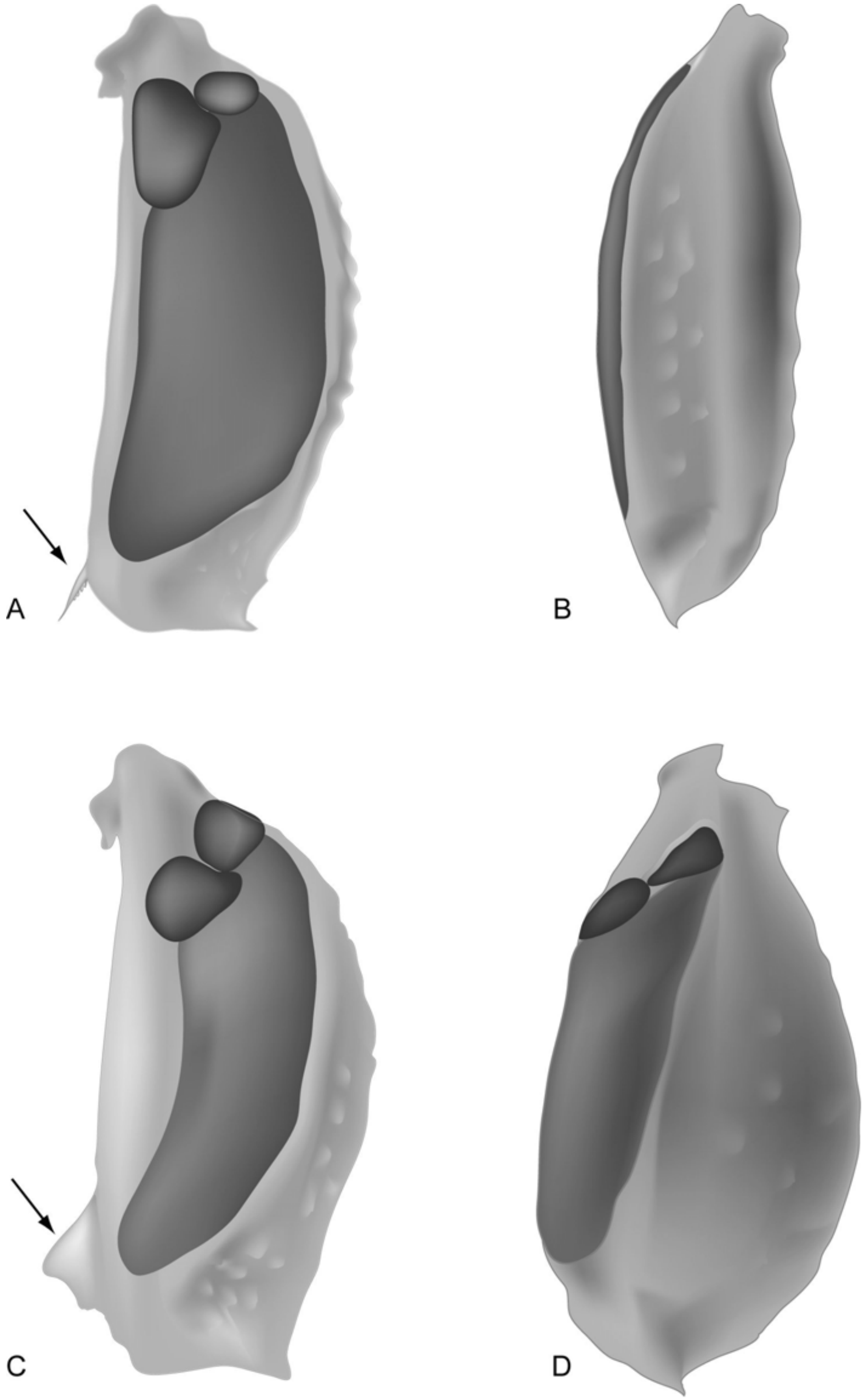


Fig. 3.10. Outer and posterior surfaces of metatibia. **A**-**B**, *Euglossa villosa*. **C**-**D**, *E. rugilabris*. The arrows show the presence of mesotibial spur (A), or integumental projection and absence of spur (C).

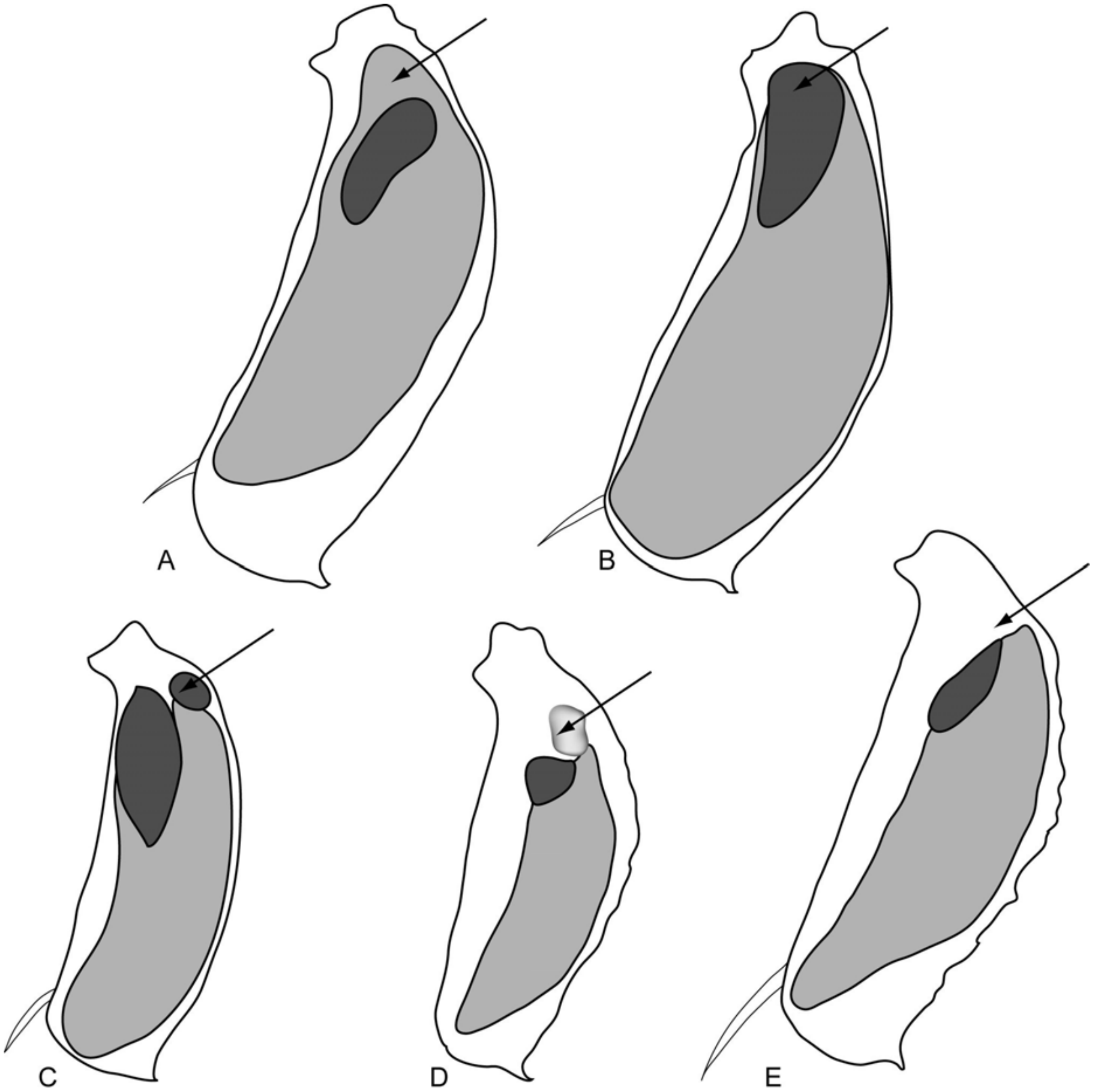


Fig. 3.11. Schematic representation of the structure of the posterior tuft of the mesotibia (or the absence of it), as shown by the arrows. **A**. *Eulaema polychroma* with no posterior tuft, velvety area extended basally. **B**. *Exaerete smaragdina* with no posterior tuft, unique tuft (homologous to the anterior tuft) extended basally. **C**. *Euglossa cyanura*, posterior tuft present. **D**. *E*. *allosticta*, cavity representative of posterior tuft present. **E**. *E*. *asarophora*, posterior tuft absent, setae absent from basal area.

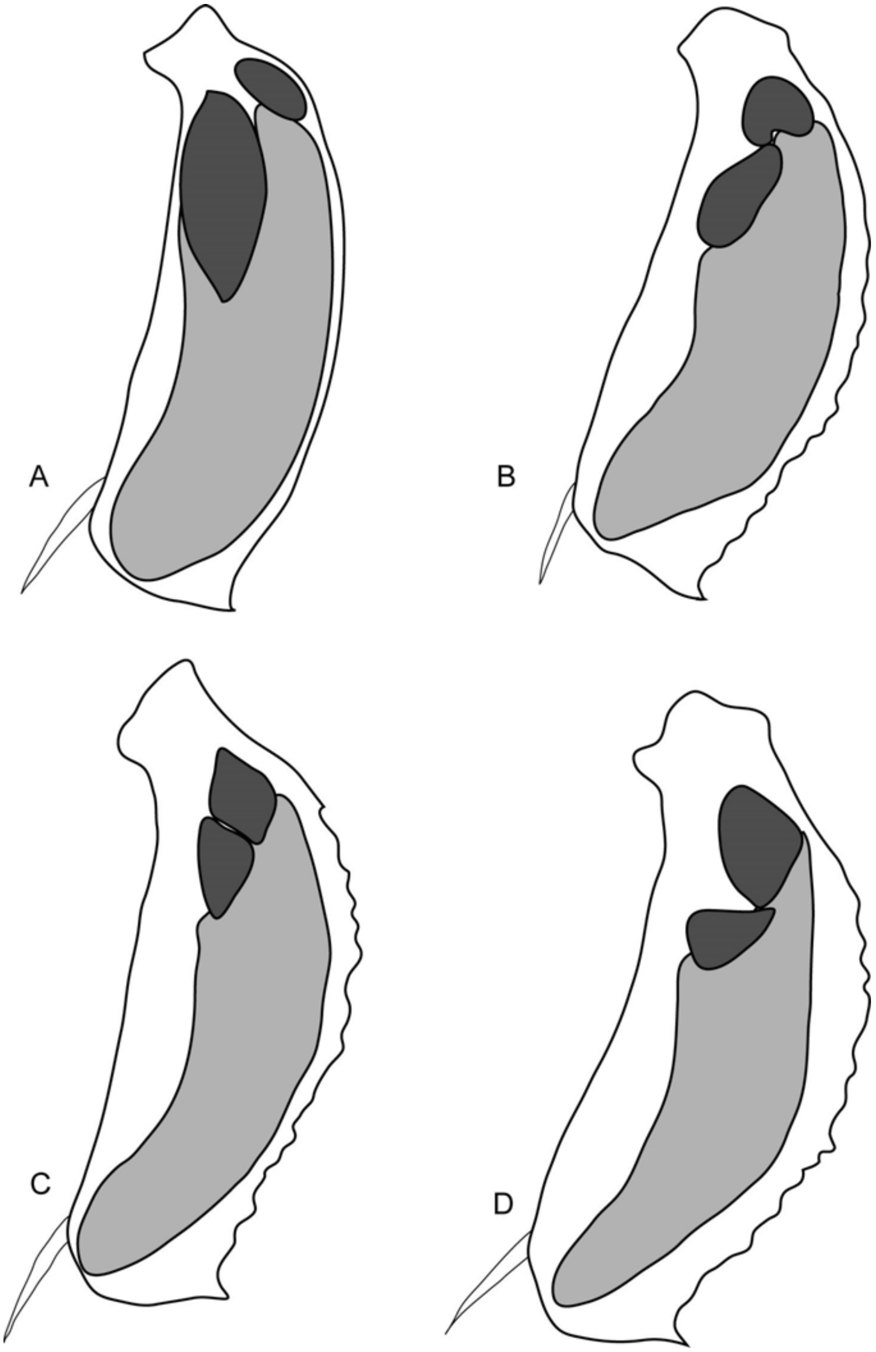


Fig. 3.12. Schematic representation of the relative size of the posterior tuft respect the anterior tuft of the mesotibia. **A**. *Euglossa cyanura*. **B**. *E*. *macrorhyncha*. **C**. *E*. *imperialis*. **D**. *E*. *viridissima*. A with posterior tuft at most ½ the length of anterior tuft, B and C with posterior tuft more than ½ the length of the anterior, but never longer. D with posterior tuft longer than anterior.

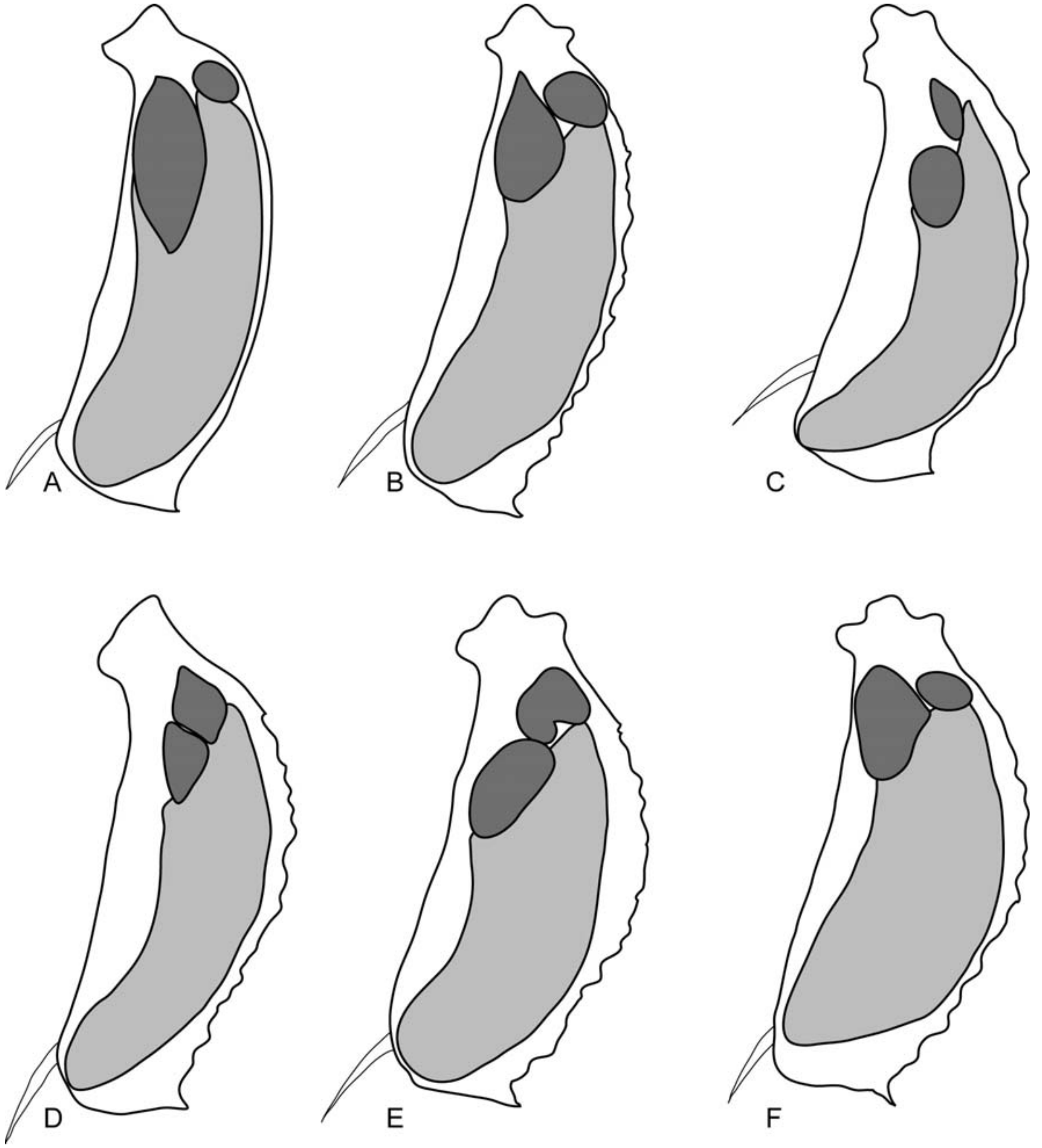


Fig. 3.13. Schematic representation of the variation of shape in the posterior tuft of the mesotibia. **A**. *Euglossa cyanura*. **B**. *E*. *decorata*. **C**. *E*. *gorgonensis*. **D**. *E*. *imperialis*. **E**. *E*. *hyacinthina*. **F**. *E*. *villosa*. A and B roughly circular, C longitudinally oval longitudinal, D and E polygonal, F perpendicularly oval.

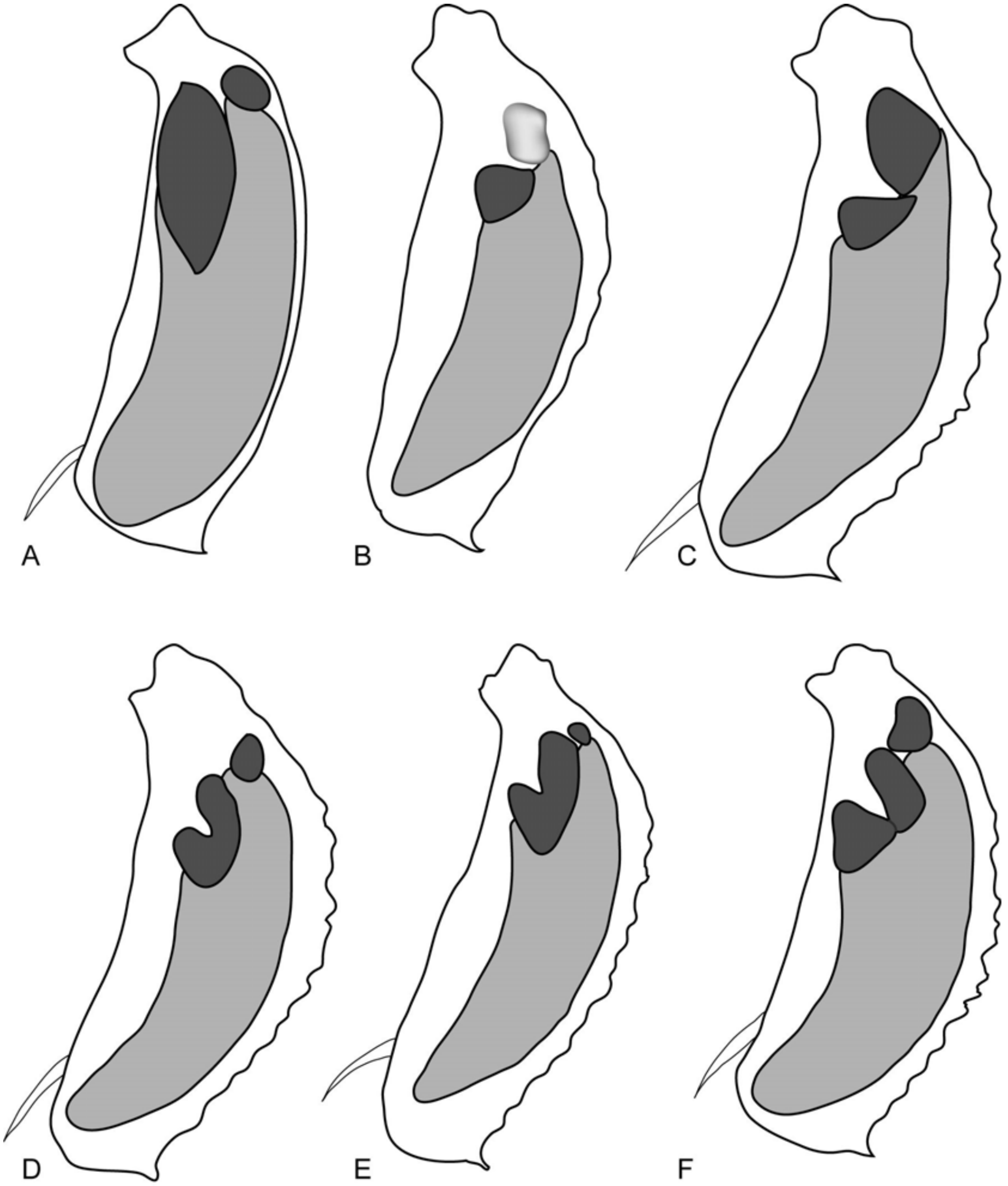


Fig. 3.14. Schematic representation of the variation of shape in the anterior tuft of the mesotibia. **A**. *Euglossa cyanura*. **B**. *E*. *allosticta*. **C**. *E*. *viridissima*. **D**. *E*. *cordata*. **E**. *E*. *iopyrrha*. **F**. *E*. *hansoni*. D, E and F with anterior cleft.

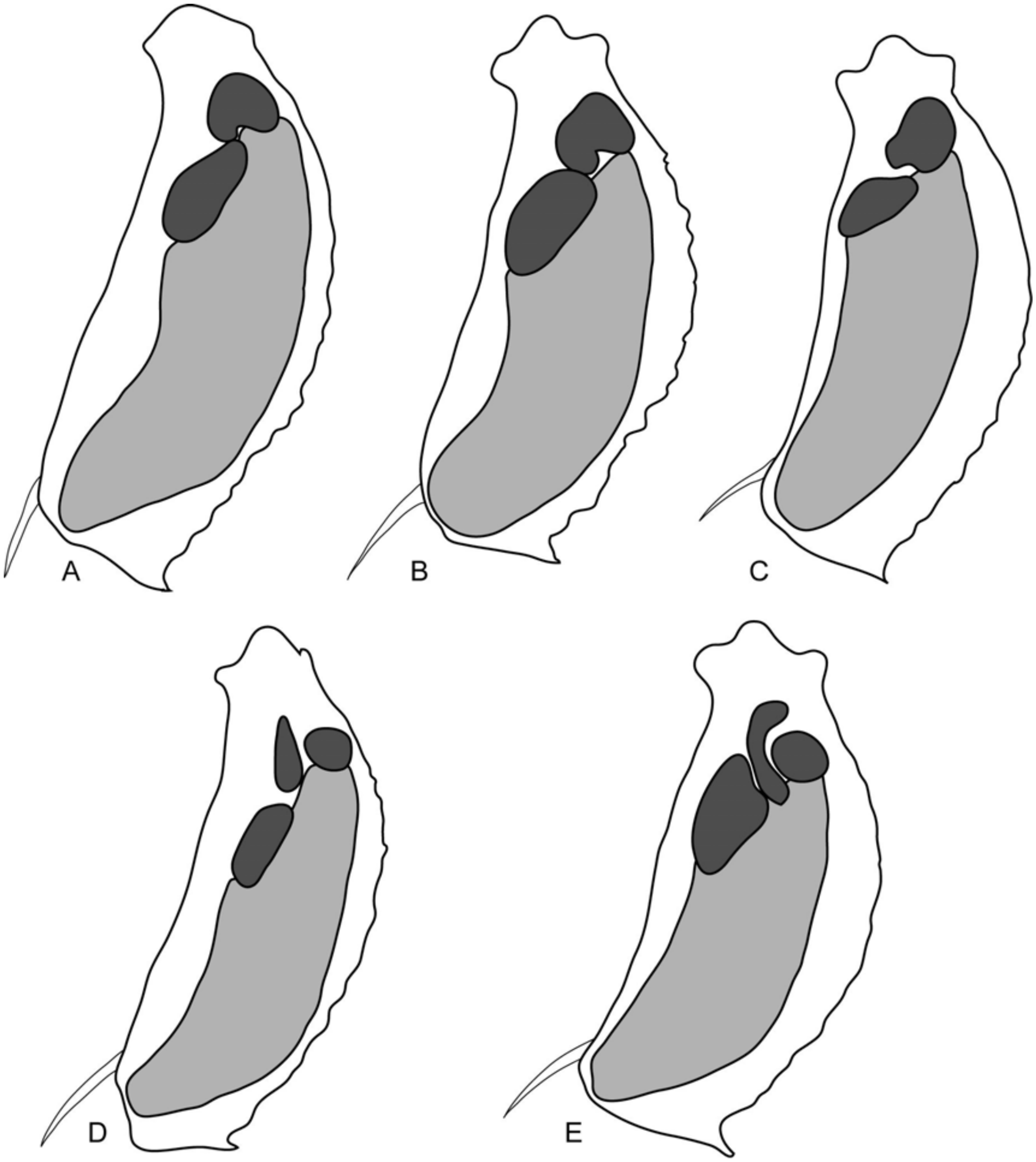


Fig. 3.15. Schematic representation of the variation of shape in the anterior tuft of the mesotibia, and third tuft . **A**. *Euglossa macrorhyncha*. **B**. *E*. *hyacinthina*. **C**. *E*. *turbinifex*. **D**. *E*. *trinotata*. **E**. *E*. *stilbonota*. A, B and C with a knob on posterior tuft, D and E with a third tuft.

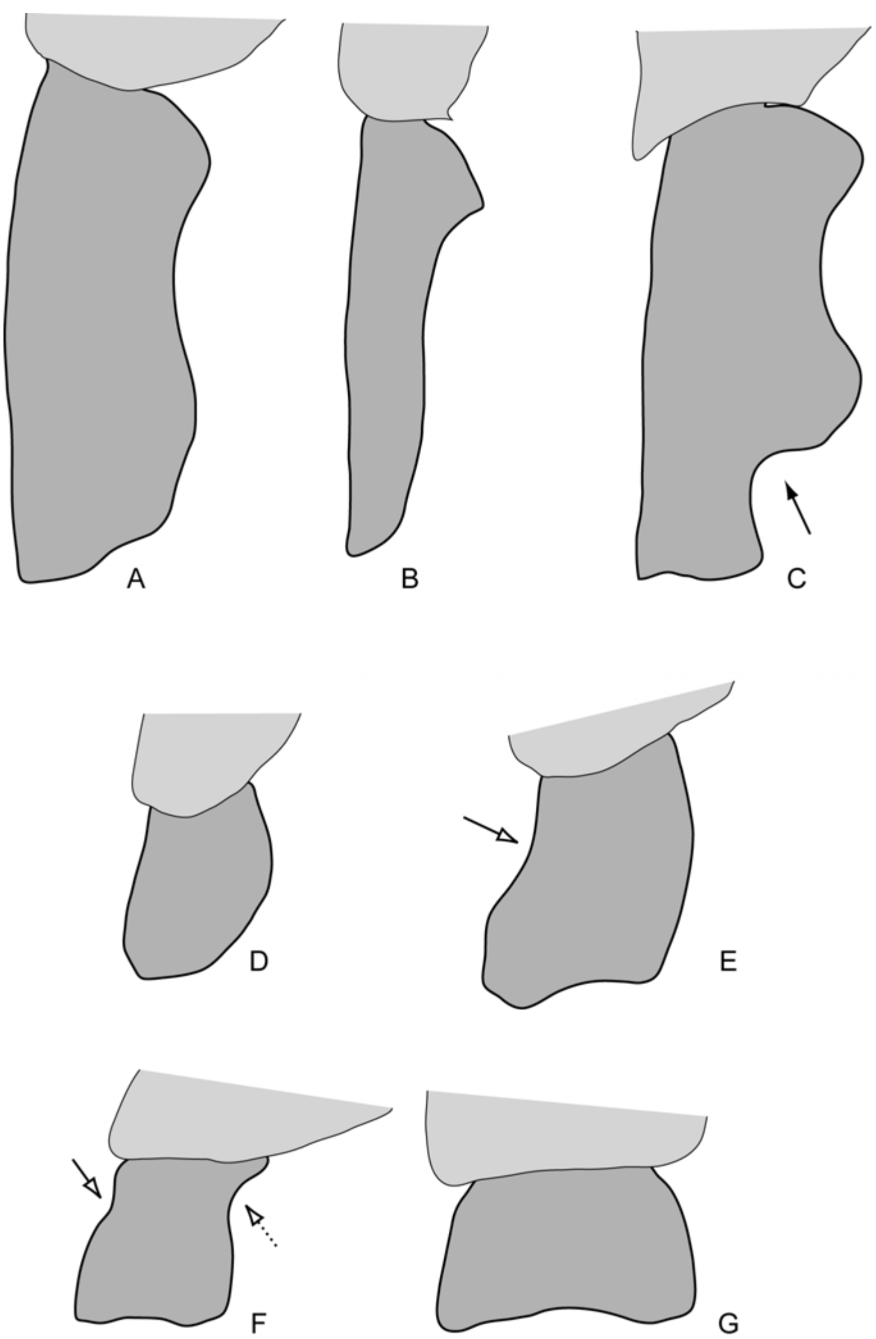


Fig. 3.16. Schematic representation of the mesobasitarsus and second mesotarsomere. **A**. Mesobasitarsus of *Euglossa stilbonota*. **B**. Mesobasitarsus of *E*. *viridis*. **C**. Mesobasitarsus of *E*. *intersecta*. **D**. Second mesotarsomere of *E*. *decorata*. **E**. Second mesotarsomere of *E*. *tridentata*. **F**. Second mesotarsomere of *E*. *villosa*. **G**. Second mesotarsomere of *E*. *rugilabris*. Arrow in C showing conspicuous mesobasitarsal emargination. Solid arrows in E and F showing anterior emargination of second mesotarsomere. Dashed arrow in F showing posterior emargination of second mesotarsomere.

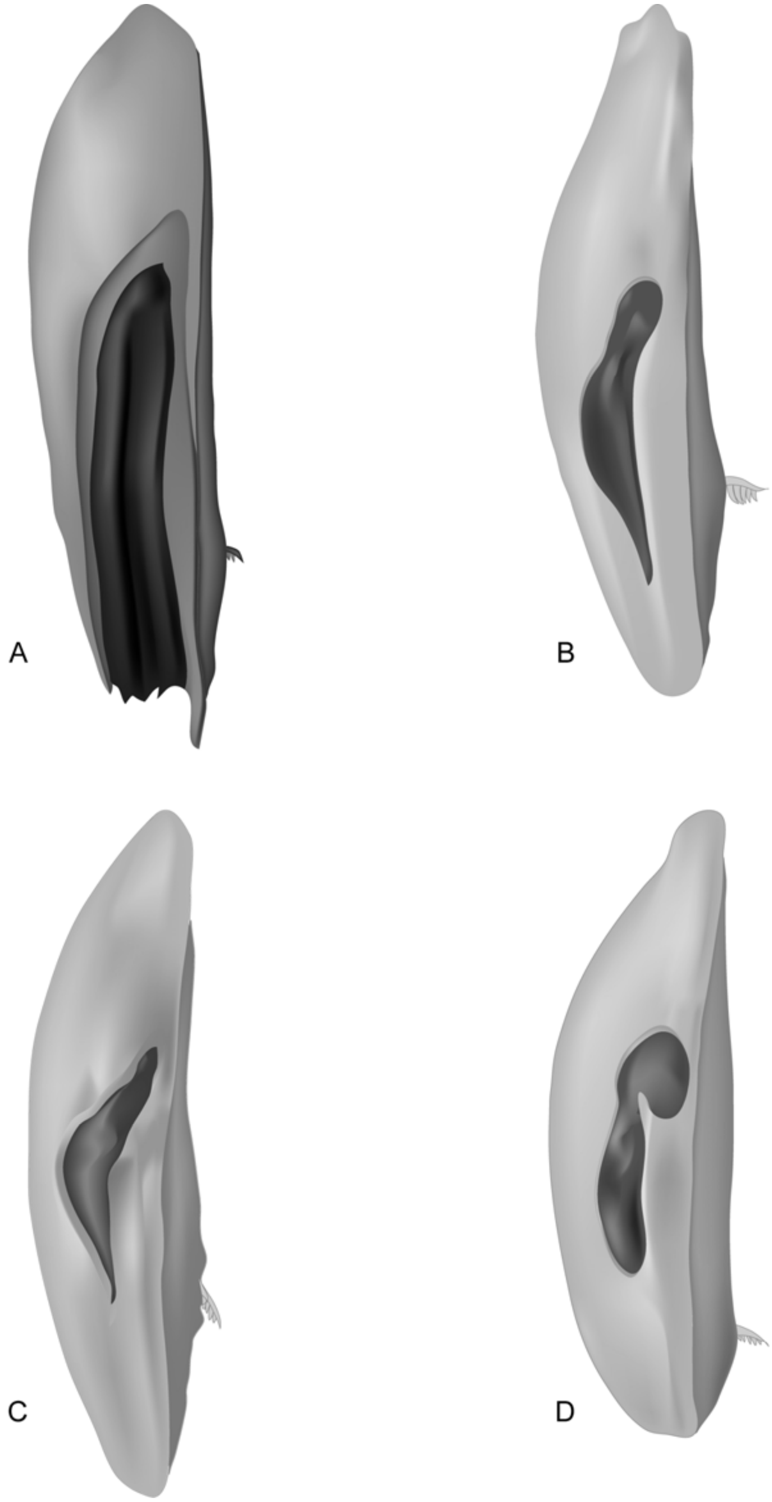


Fig. 3.17. Dorsal view of metatibial organ slit. **A**. *Eulaema polychroma*. **B**. *Euglossa asarophora*. **C**. *E*. *tridentata*. **D**. *E*. *decorata*.

Fig. 3.18. Schematic representation of the metabasitarsus. **A**. *Euglossa oleolucens*. **B**. *E*. *intersecta*. **C**. *E*. *bigibba*. **D**. *E*. *piliventris*. Arrows showing projected posterior angle, A evenly convex, D acute.

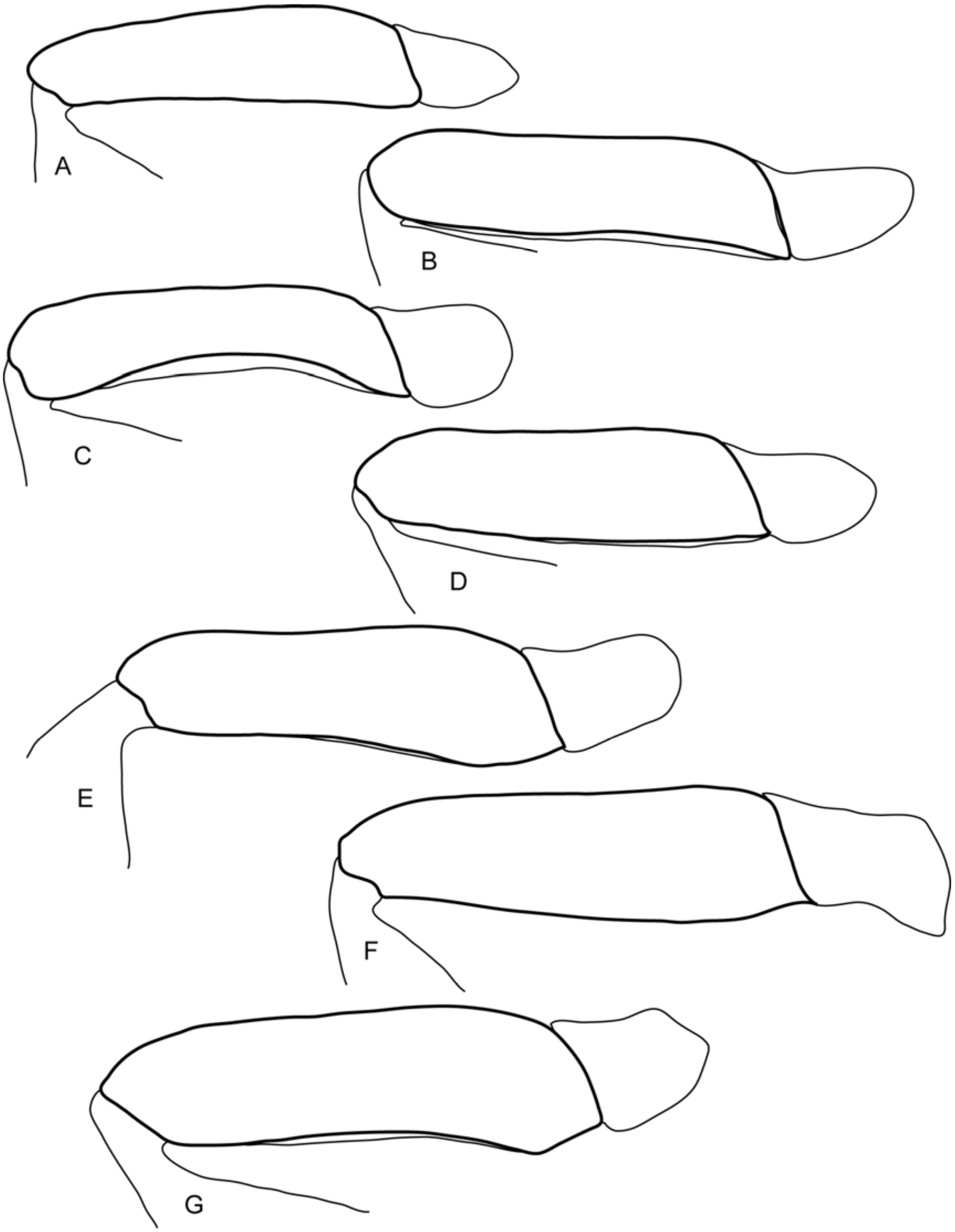


Fig. 3.19 Schematic representation of the variation of shape of the metafemur. **A**. *Euglossa cyanura*. **B**. *E*. *hansoni*. **C**. *E*. *villosa*. **D**. *E*. *imperialis*. **E**. *E*. *piliventris*. **F**. *E*. *intersecta*. **G**. *E*. *trinotata*.

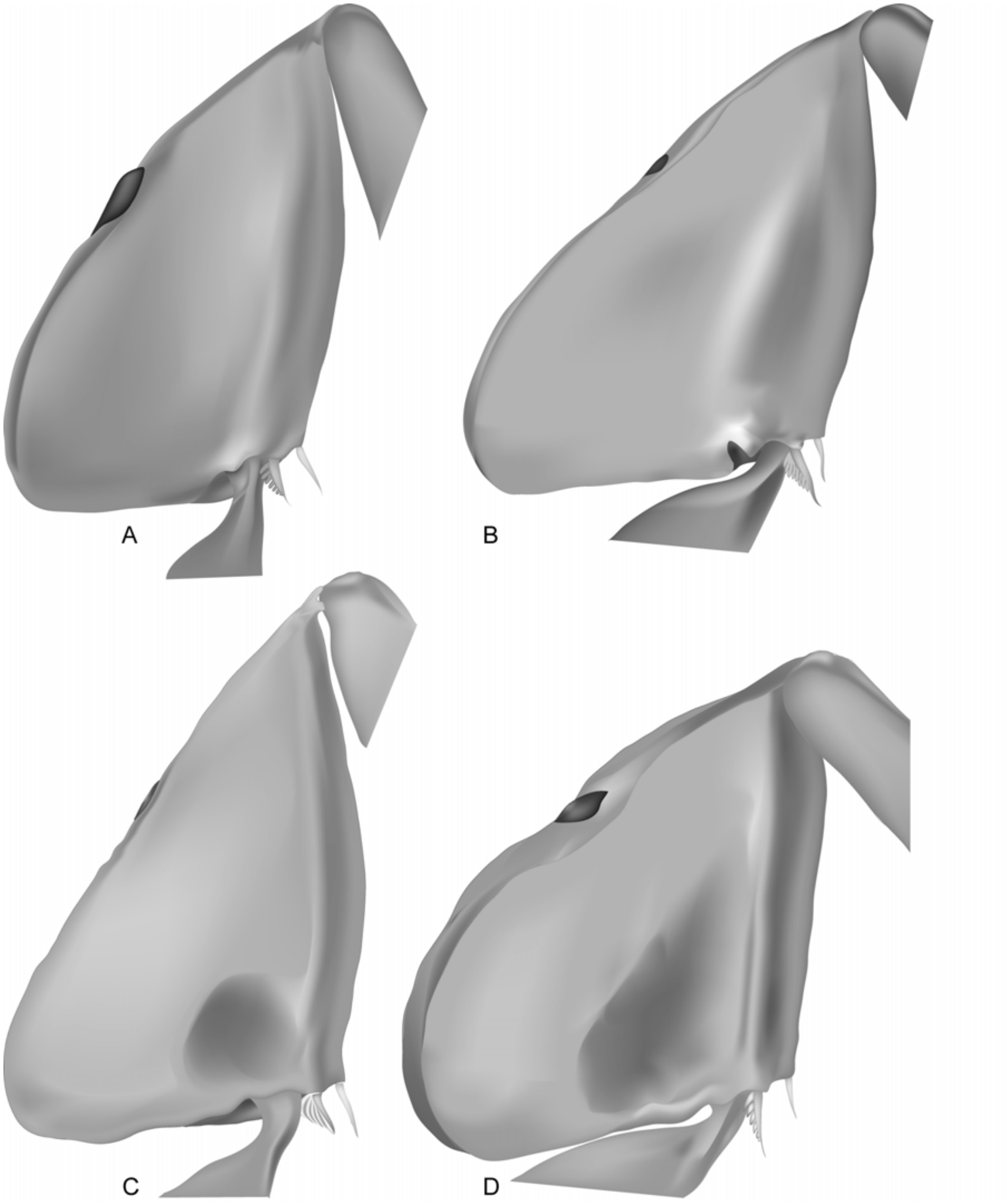


Fig. 3.20. Metatibial inner surface. **A**. *Euglossa decorata*. **B**. *E*. *imperialis*. **C**. *E*. *trinotata*. **D**. *E*. *mixta*. C and D with conspicuous depressions. Vestiture and sculpturing omitted.

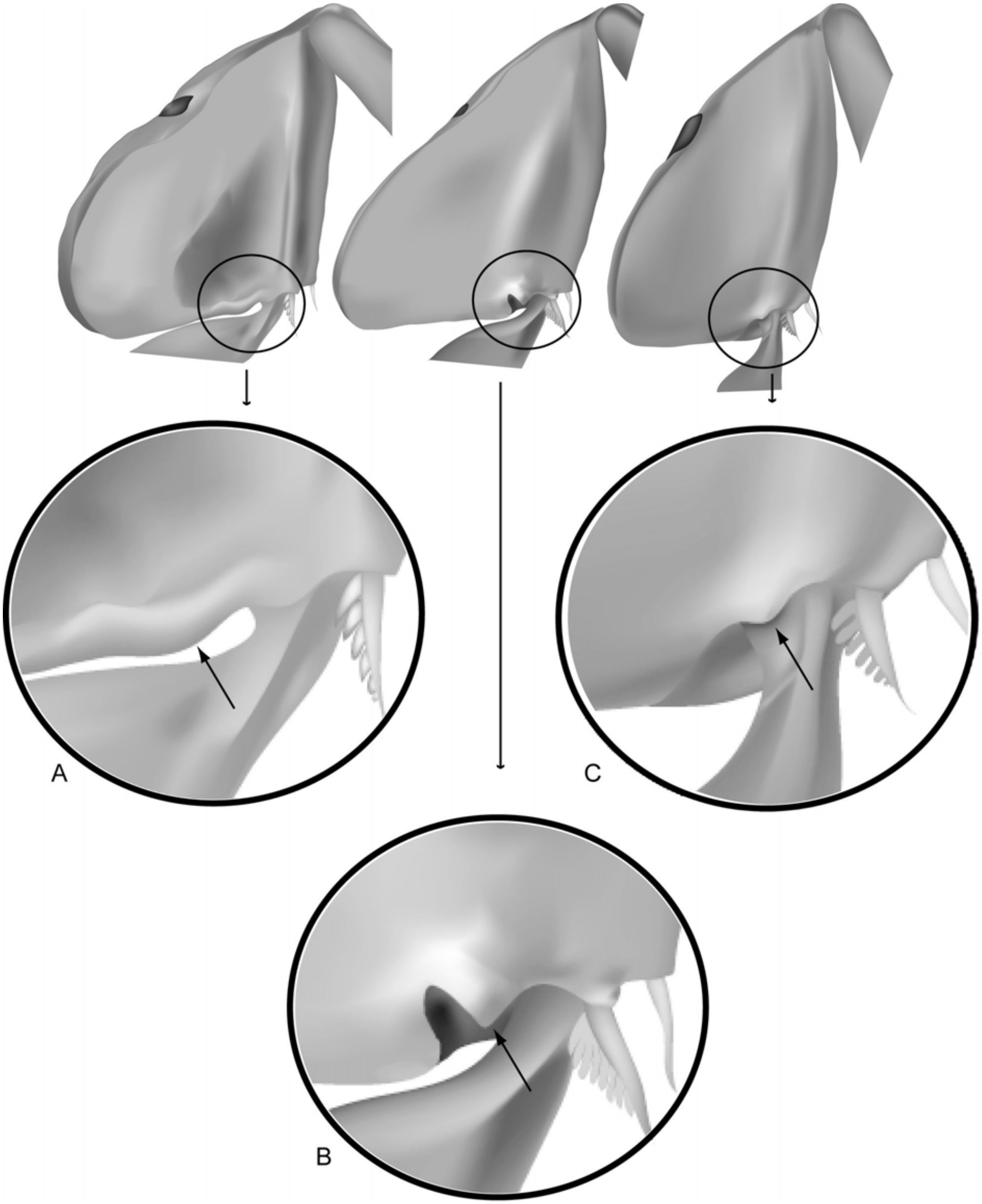


Fig. 3.21. Projections on ventral margin of inner surface of metatibia. **A**. *Euglossa mixta*. **B**. *E*. *imperialis*. **C**. *E*. *decorata*. Arrows pointing to the projections posterior to the inner spur.

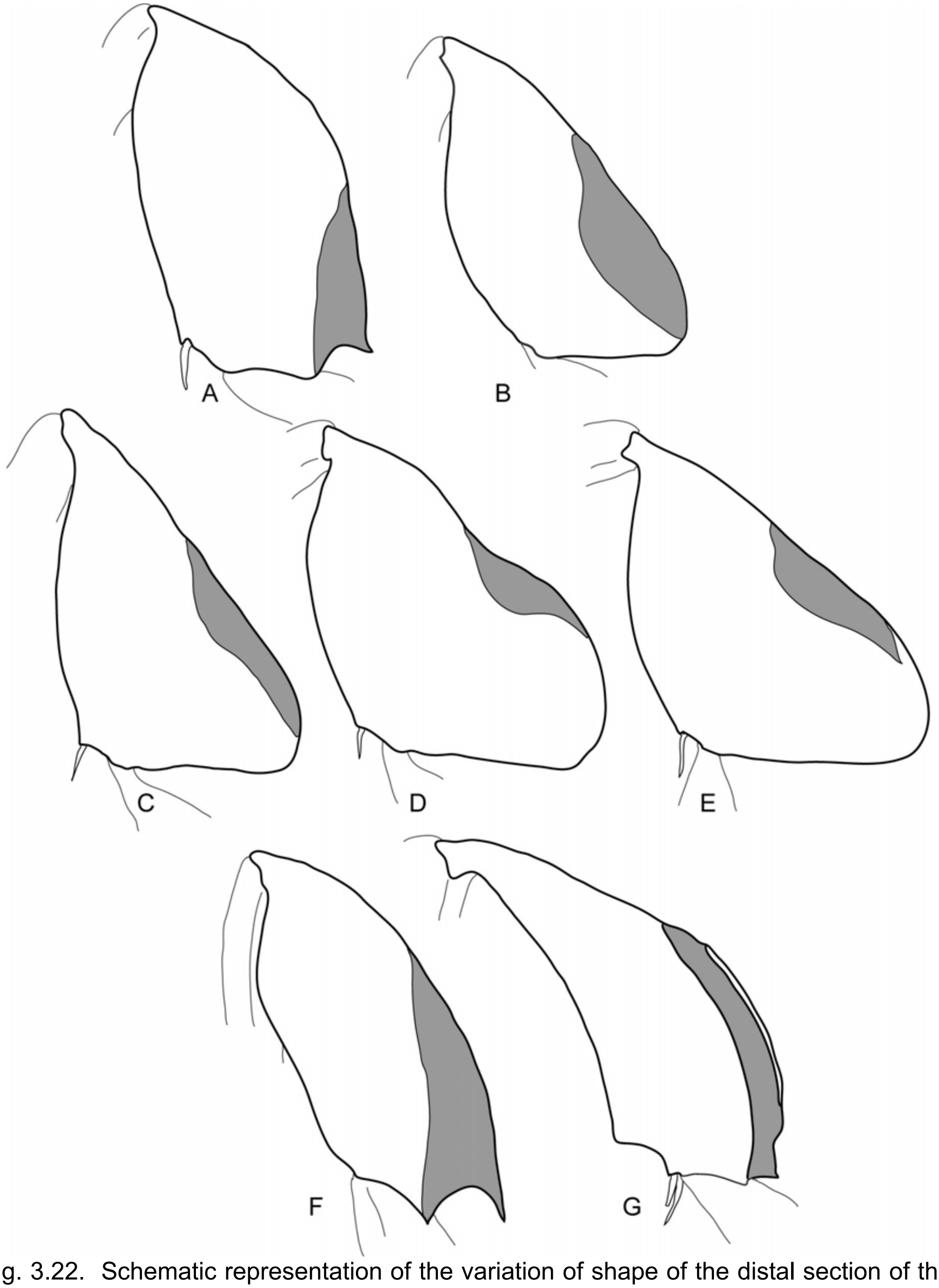


Fig. 3.22. Schematic representation of the variation of shape of the distal section of the metatibial organ slit. **A**. *Eufriesea caerulescens*. **B**. *Euglossa decorata*. **C**. *E. trinotata*. **D**. *E. townsendi*. **E**. *E. macrorhyncha*. **F**. *Eulaema polychroma*. **G**. *Exaerete smaragdina*.

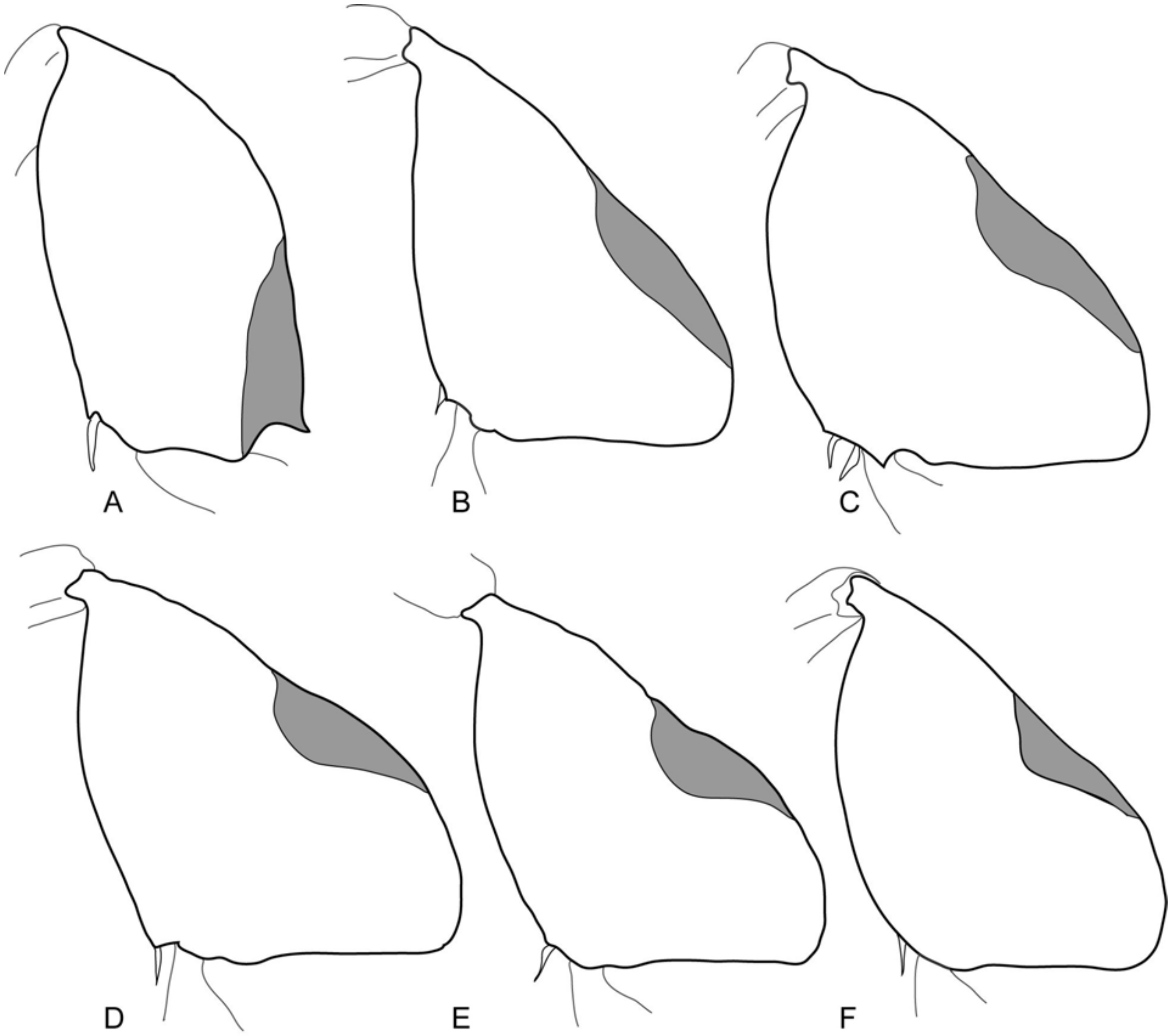


Fig. 3.23. Schematic representation of the variation in extension of the distal section of the metatibial organ slit. **A**. *Eufriesea caerulescens*. **B**. *Euglossa imperialis*. **C**. *E*. *villosa*. **D**. *E*. *cordata*. **E**. *E*. *tridentata*. **F**. *E*. *dodsoni*.

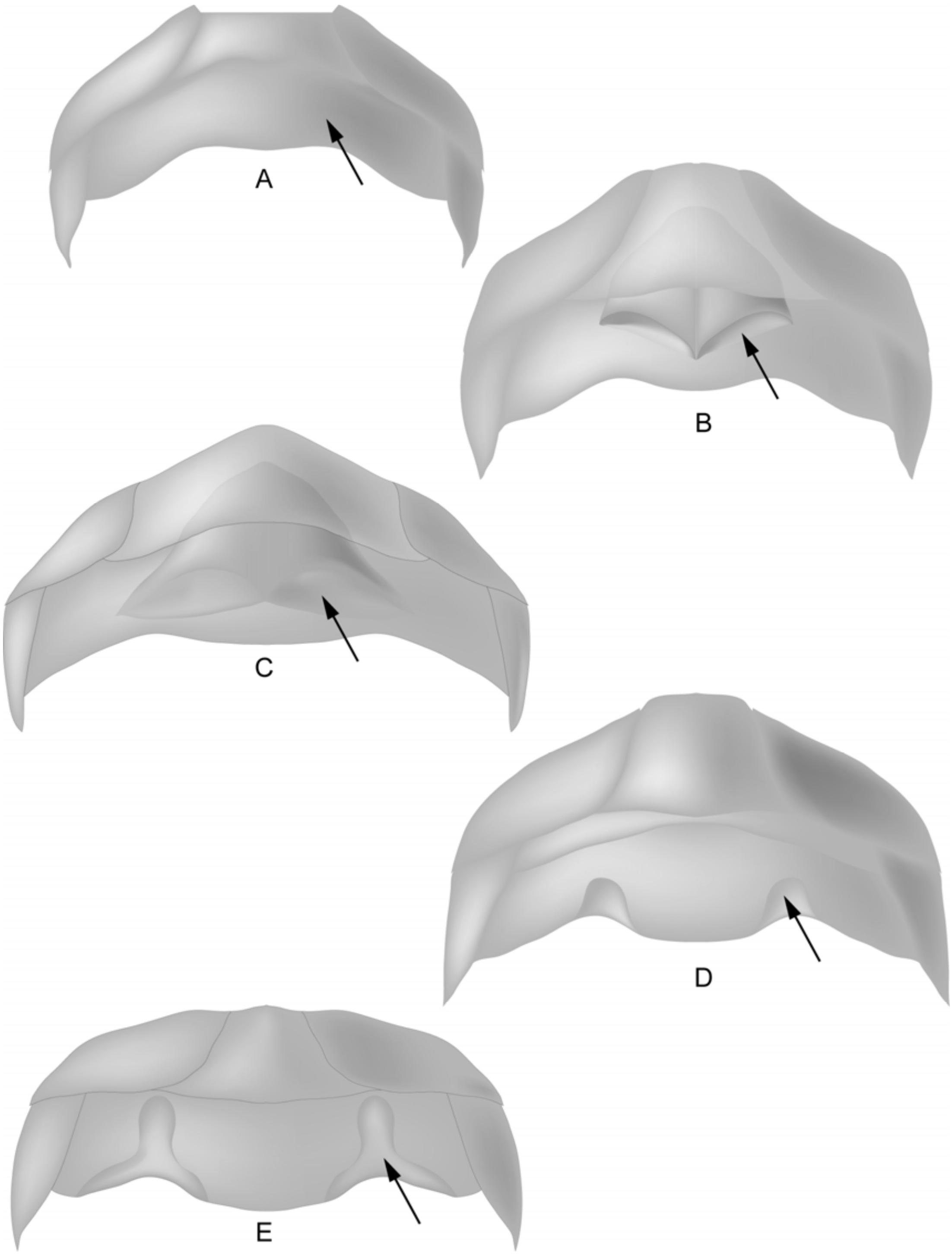


Fig. 3.24. Structure of Integumental modifications of second metasomal sternum. **A**. *Euglossa gorgonensis*. **B**. *E*. *imperialis*. **C**. *E*. *intersecta*. **D**. *E*. *oleolucens*. **E**. *E*. *bursigera*. Arrows showing the absence of integumental modifications (A), presence of cowled slits (B), false cowled slits (C), or omega-like depressions (D and E). Vestiture and ornamentation omitted.

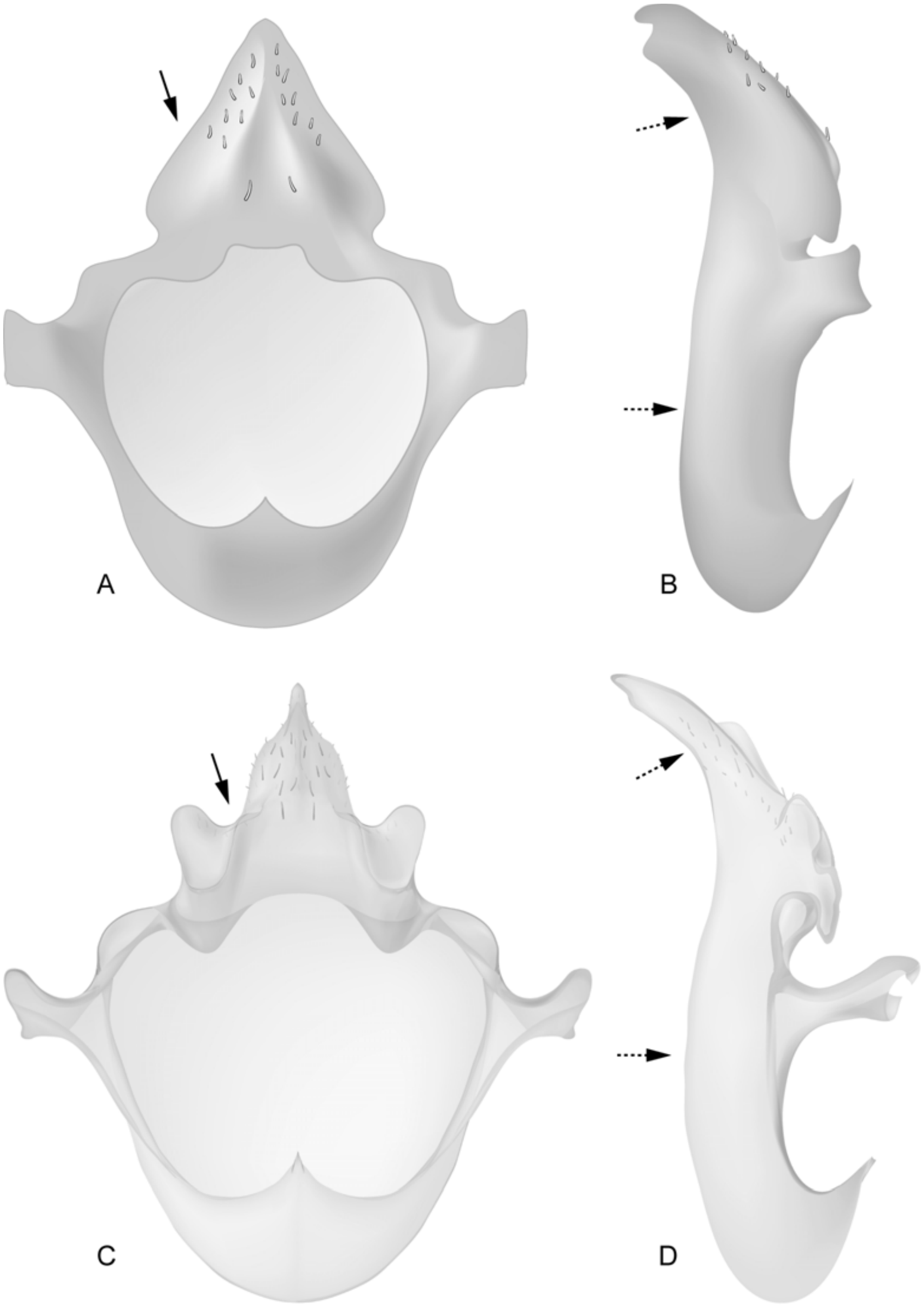


Fig. 3.25. Structure of eighth metasomal sternum. **A**-**B**. *Euglossa obtusa*. **C**-**D**. *E. imperialis* [modified from Hinojosa-Díaz (2008)]. Solid arrows showing the presence (A) or absence (C) of invaginations and lobes. Dotted arrows showing the relative width of posterior and anterior sections (posterior located above).

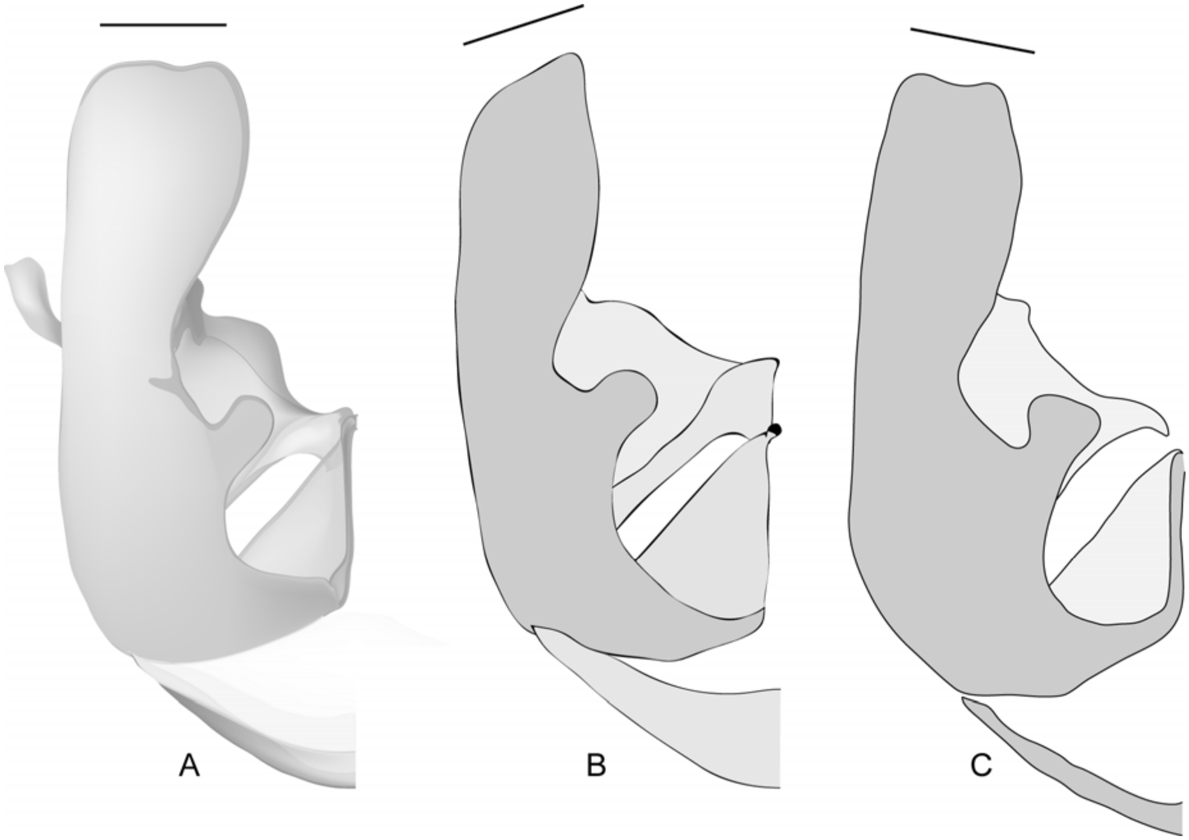


Fig. 3.26. Schematic representation of the alignment of the posterior margin of apical process of gonocoxite. **A**. *Euglossa imperialis* [modified from Hinojosa-Díaz (2008)]. **B**. *E*. *urarina* [modified from Hinojosa-Díaz and Engel (2007)] (species not included in the analysis). **C**. *E*. *rugilabris*.

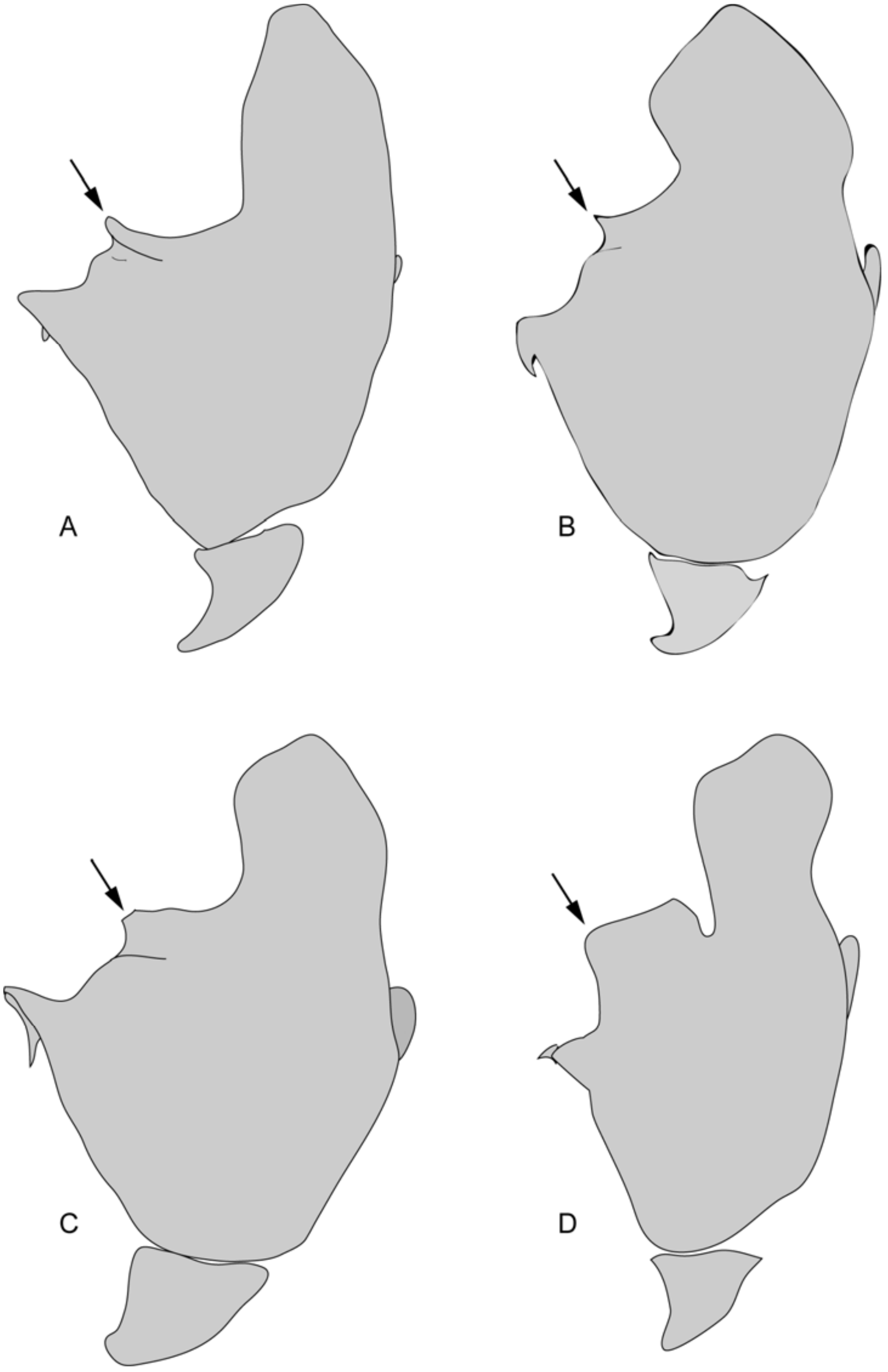


Fig. 3.27. Schematic representation of lateral view of the gonocoxite. **A**. *Euglossa bursigera*. **B**. *E*. *cosmodora* [modified from Hinojosa-Díaz and Engel (2007)]. **C**. *E*. *azurea*. **D**. *E*. *villosa*. Arrows pointing to the different modifications of the lateral area of the gonostylar process.

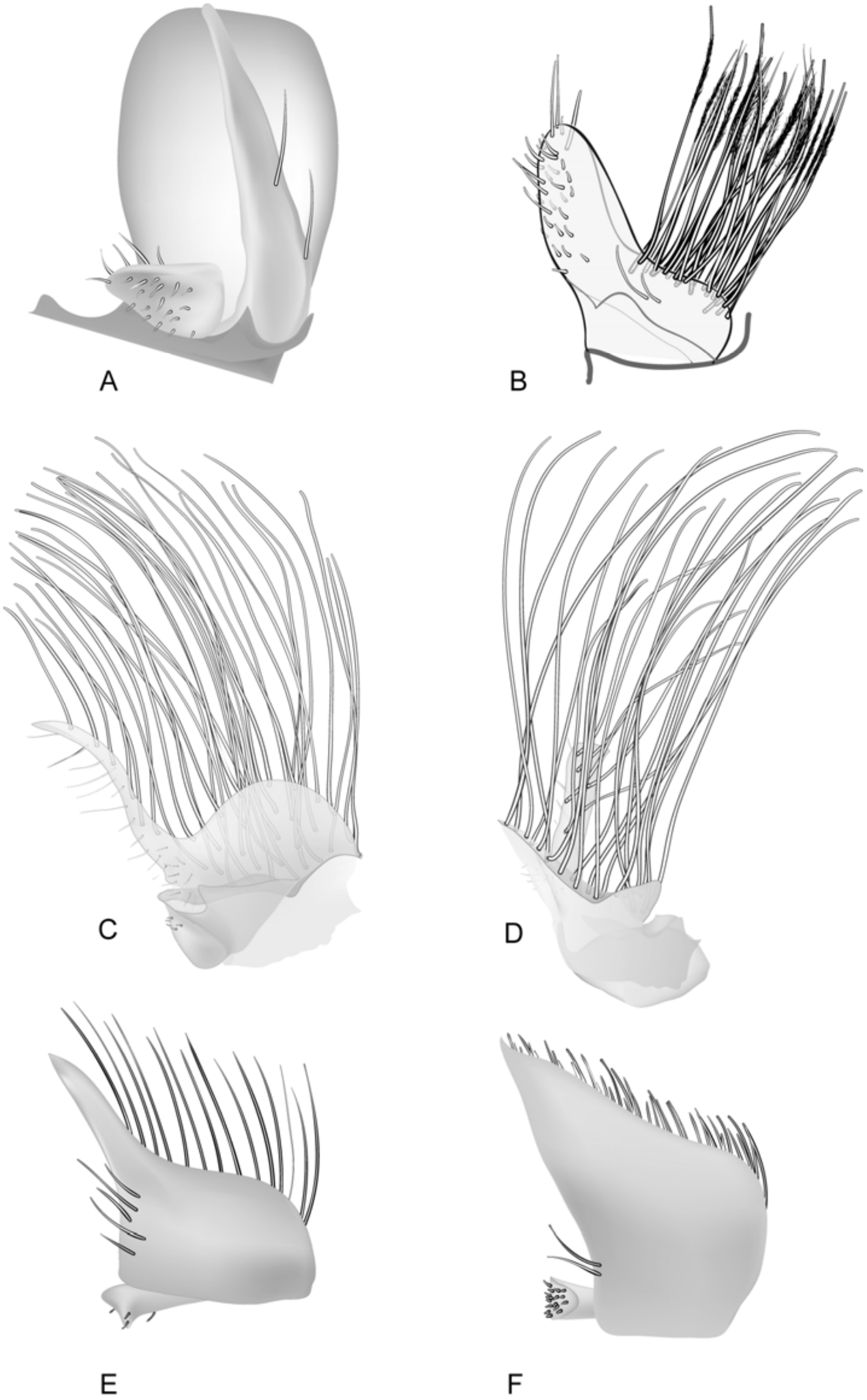
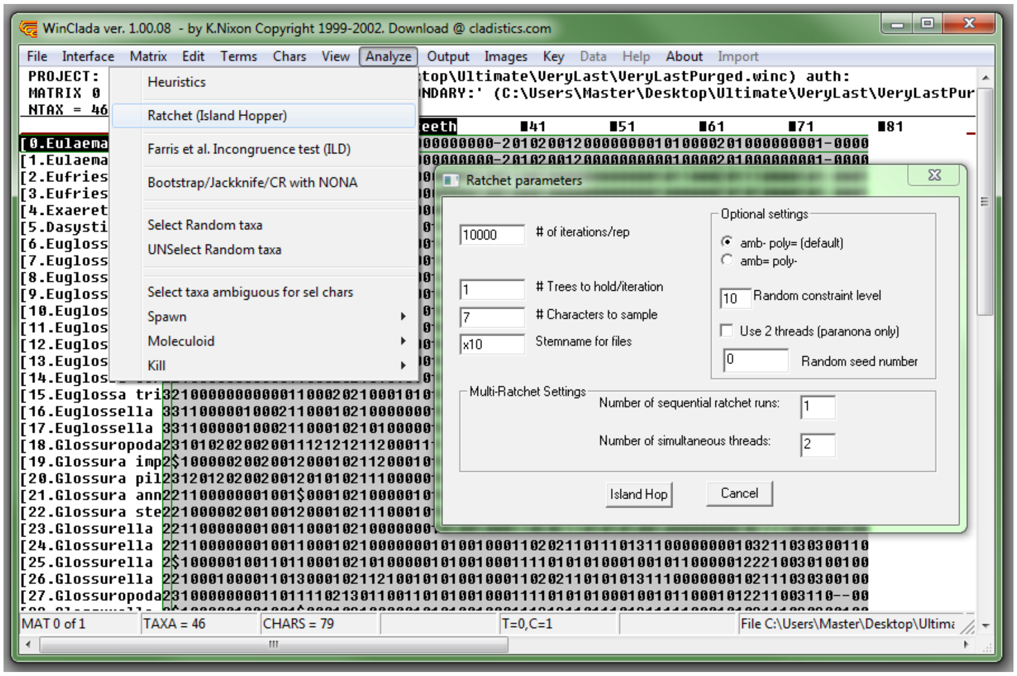


Fig. 3.28. Schematic representation of lateral section of gonostylus. **A**. *Euglossa oleolucens*, ventral view. **B**. *E*. *cosmodora*, lateral view. **C**-**D**. *E*. *imperialis*, lateral and dorsal views [modified from Hinojosa-Díaz (2008)]. **E**. *E*. *stilbonota*, lateral view. **F**. *E*. *dodsoni*, lateral view.



```
[24.Glossurella 22110000000100110001021000000010100100011020211011101311000000001032110303000110
[25.Glossurella 2$1000001001101100010210100000101001000111101010100010010110000012221003010010 0
[26.Glossurella 22100010000110130001021121001010100100011020211010101311100000001021110303000100
[27.Glossuropoda23100000000110111102130110011010100100011110101010001001011000101221100311 0--00
```

Ratchet parameters

10000 # of iterations/rep

1 # Trees to hold/iteration

7 # Characters to sample

x10 Stemname for files

Optional settings

- ● amb- poly= (default)
- ○ amb= poly-

10 Random constraint level

☐ Use 2 threads (paranona only)

0 Random seed number

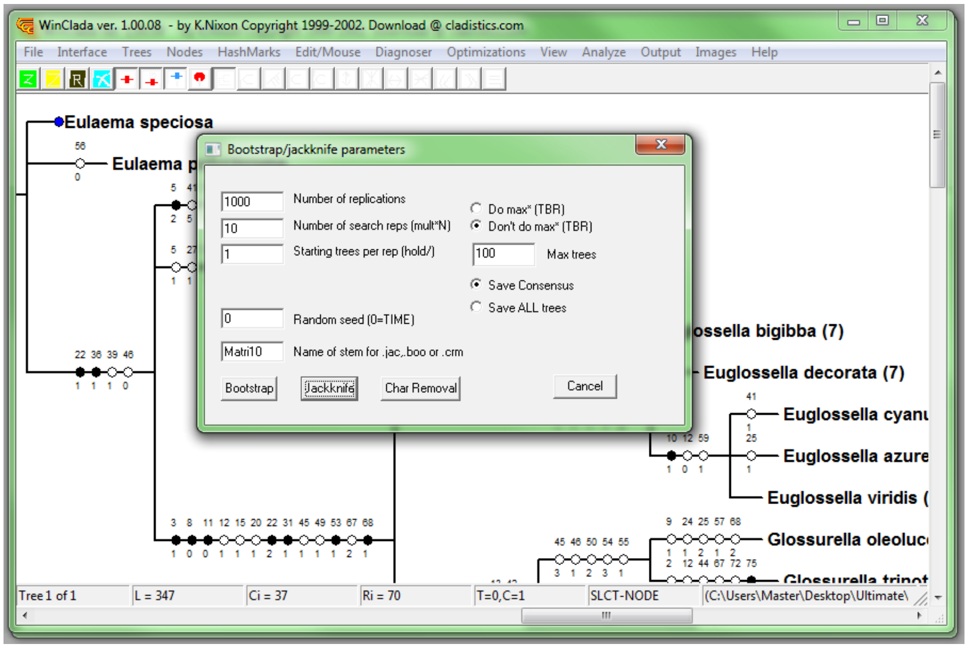
Multi-Ratchet Settings

Number of sequential ratchet runs: 1

Number of simultaneous threads: 2

MAT 0 of 1 | TAXA = 46 | CHARS = 79 | T=0,C=1 | File C:\Users\Master\Desktop\Ultima

Fig. 3.29. Screen view of WinClada (Nixon 1999), showing the parameters in the Ratchet module as used in the phylogenetic analysis.



Bootstrap/jackknife parameters

1000 Number of replications

10 Number of search reps (mult*N)

1 Starting trees per rep (hold/)

- ○ Do max* (TBR)
- ● Don't do max* (TBR)

100 Max trees

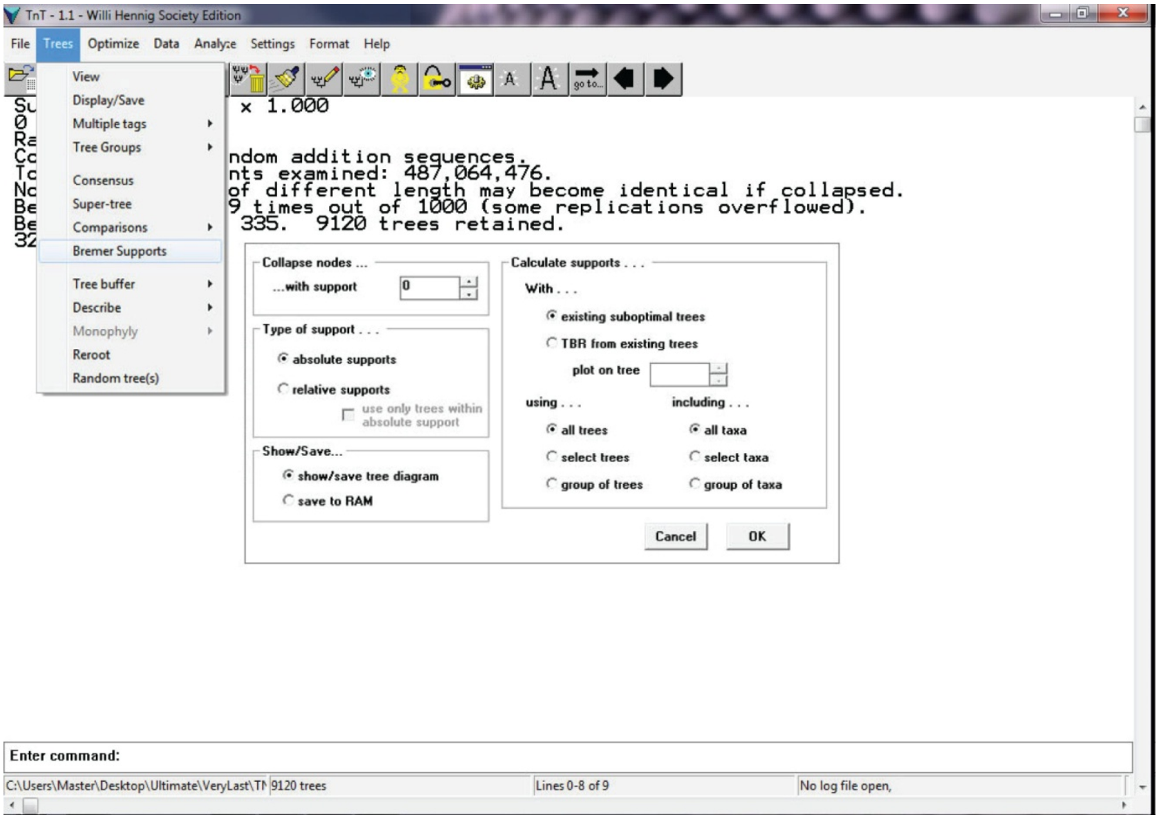
- ● Save Consensus
- ○ Save ALL trees

0 Random seed (0=TIME)

Matri10 Name of stem for .jac,.boo or .crm

Tree 1 of 1 | L = 347 | Ci = 37 | Ri = 70 | T=0,C=1 | SLCT-NODE | (C:\Users\Master\Desktop\Ultimate\

Fig. 3.30. Screen view of WinClada (Nixon 1999), showing the parameters in the Bootstrap/Jackknife module as used to calculate Jacknife support values for the strict consensus tree.



```
Su                  x 1.000
0
Ra
Co                ndom addition sequences.
To                nts examined: 487,064,476.
No                of different length may become identical if collapsed.
Be                9 times out of 1000 (some replications overflowed).
Be                 335.  9120 trees retained.
32
```

Collapse nodes ...
...with support 0

Type of support . . .
● absolute supports
○ relative supports
☐ use only trees within absolute support

Show/Save...
● show/save tree diagram
○ save to RAM

Calculate supports . . .
With . . .
● existing suboptimal trees
○ TBR from existing trees
plot on tree

using . . .
● all trees
○ select trees
○ group of trees

including . . .
● all taxa
○ select taxa
○ group of taxa

C:\Users\Master\Desktop\Ultimate\VeryLast\TI | 9120 trees | Lines 0-8 of 9 | No log file open,

Fig. 3.31. Screen view of TNT (Goloboff *et al*. 2000), showing the options used to calculate Bremer support values.

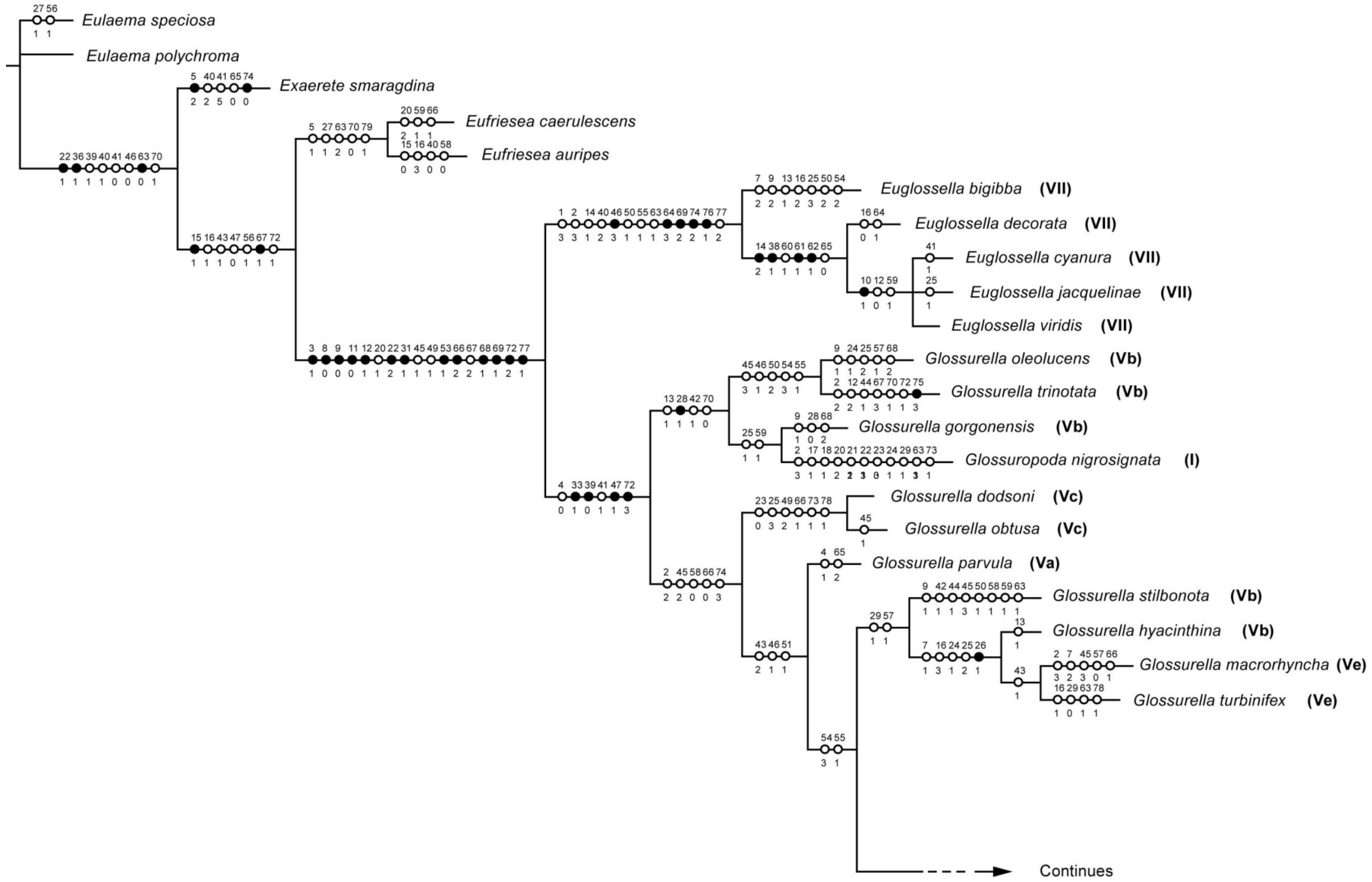


Fig. 3.32. One of the 64 most parsimonious trees produced by the phylogenetic analysis. Length = 335, Ci = 39, Ri = 72. Black circles represent unreversed changes; white circles represent homoplastic character transitions. Character numbers indicated above the branch, character states below. Character changes presented in fast optimization. *Eulaema speciosa*, *Eulaema polychroma*, *Exaerete smaragdina*, *Eufriesea caerulescens* and *Eufriesea auripes* are the outgroups. Names of the species of the ingroup (*Euglossa s. lat*) bear the subgeneric name along with the specific epithet. Parentheses after the ingroup taxa names refer to the species groups as listed in Table 3.2 (continues in next page).

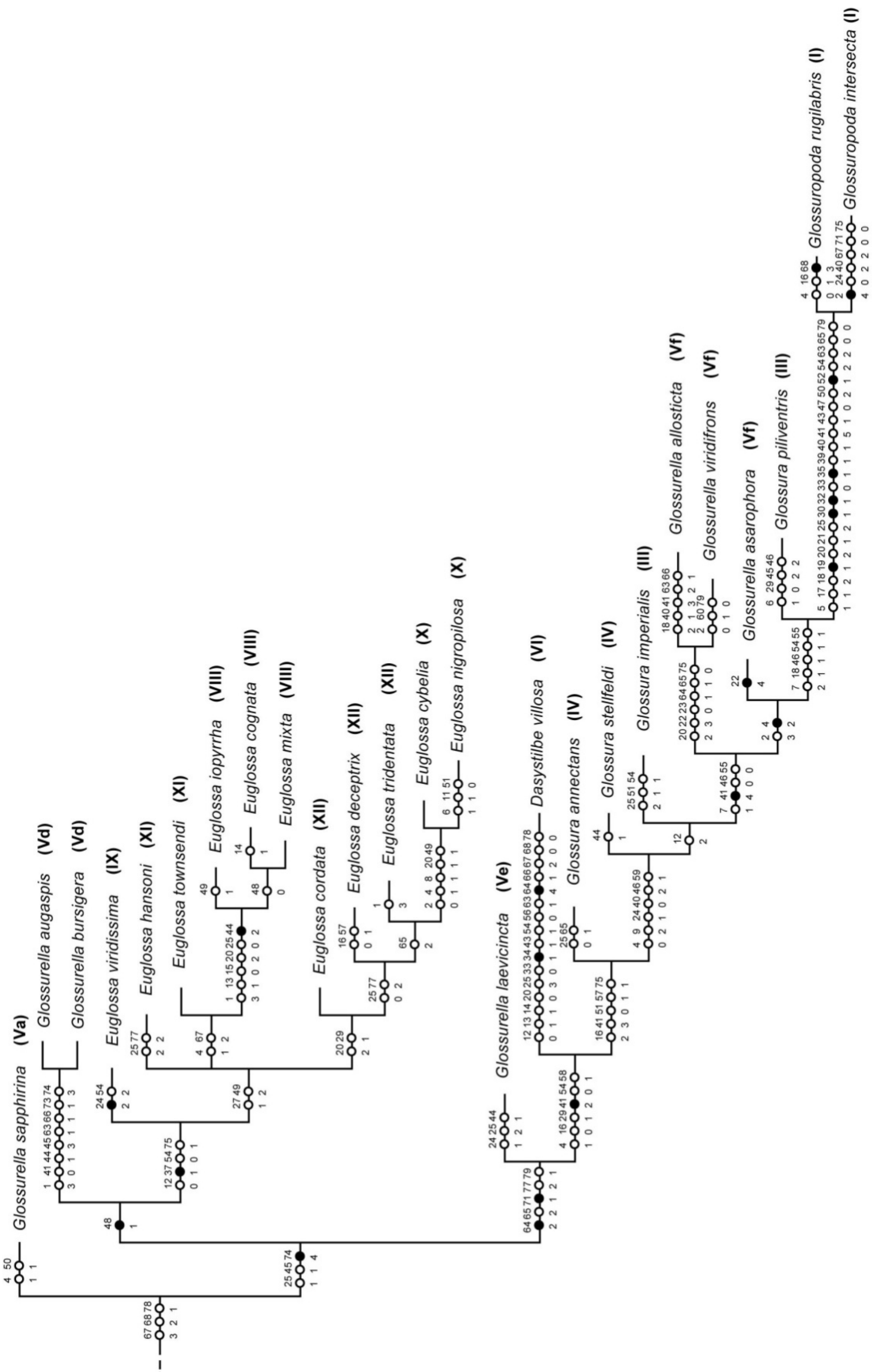


Fig. 3.32. (continues from previous page)

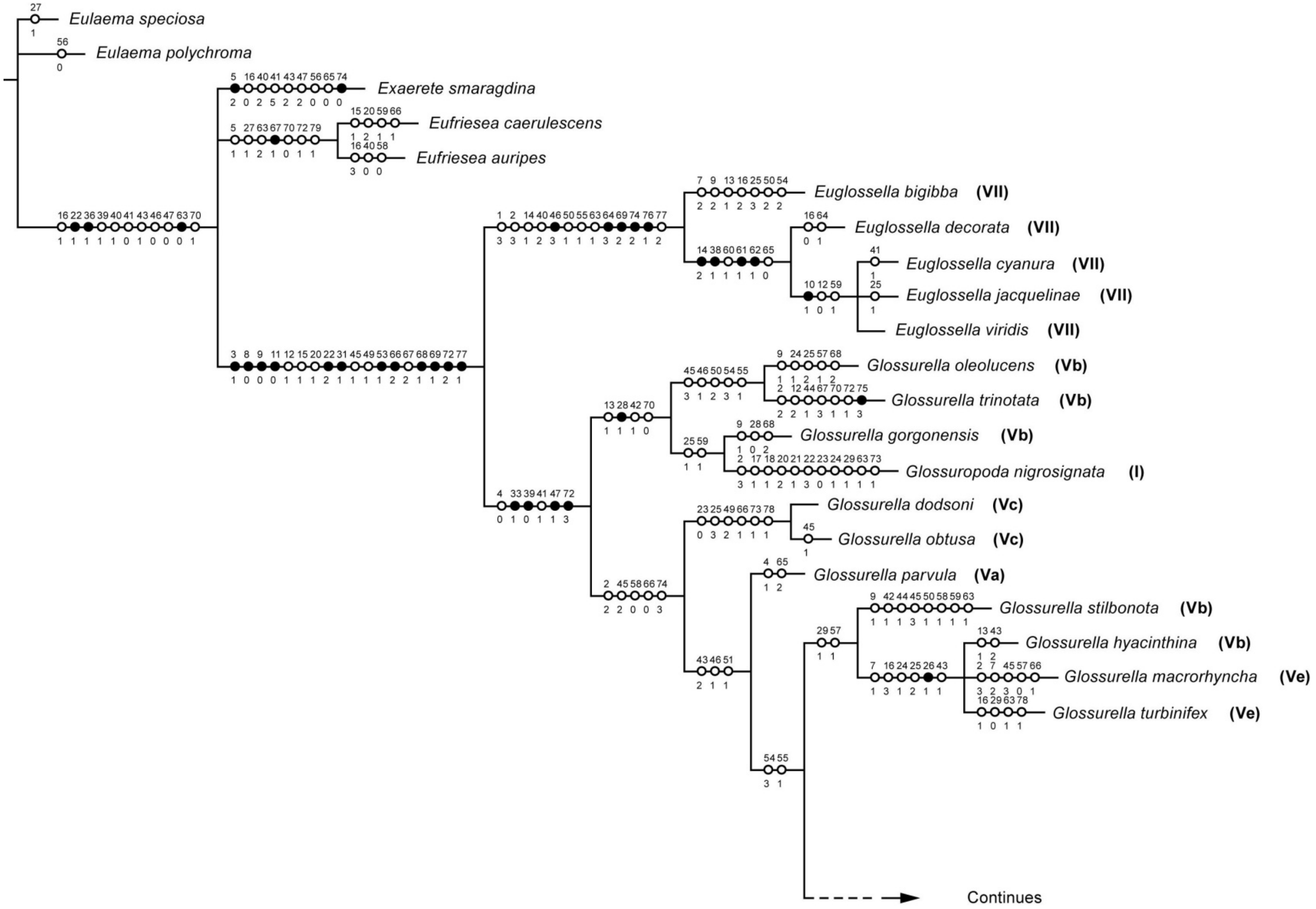


Fig. 3.33. Strict consensus of the 64 most parsimonious trees produced by the phylogenetic analysis. Length = 347, Ci = 37, Ri = 70. Black circles represent unreversed changes; white circles represent homoplastic character transitions. Character numbers indicated above the branch, character states below. Character changes presented in fast optimization. *Eulaema speciosa*, *Eulaema polychroma*, *Exaerete smaragdina*, *Eufriesea caerulescens* and *Eufriesea auripes* are the outgroups. Names of the species of the ingroup (*Euglossa s. lat*) bear the subgeneric name along with the specific epithet. Parentheses after the ingroup taxa names refer to the species groups as listed in Table 3.2 (continues in next page).

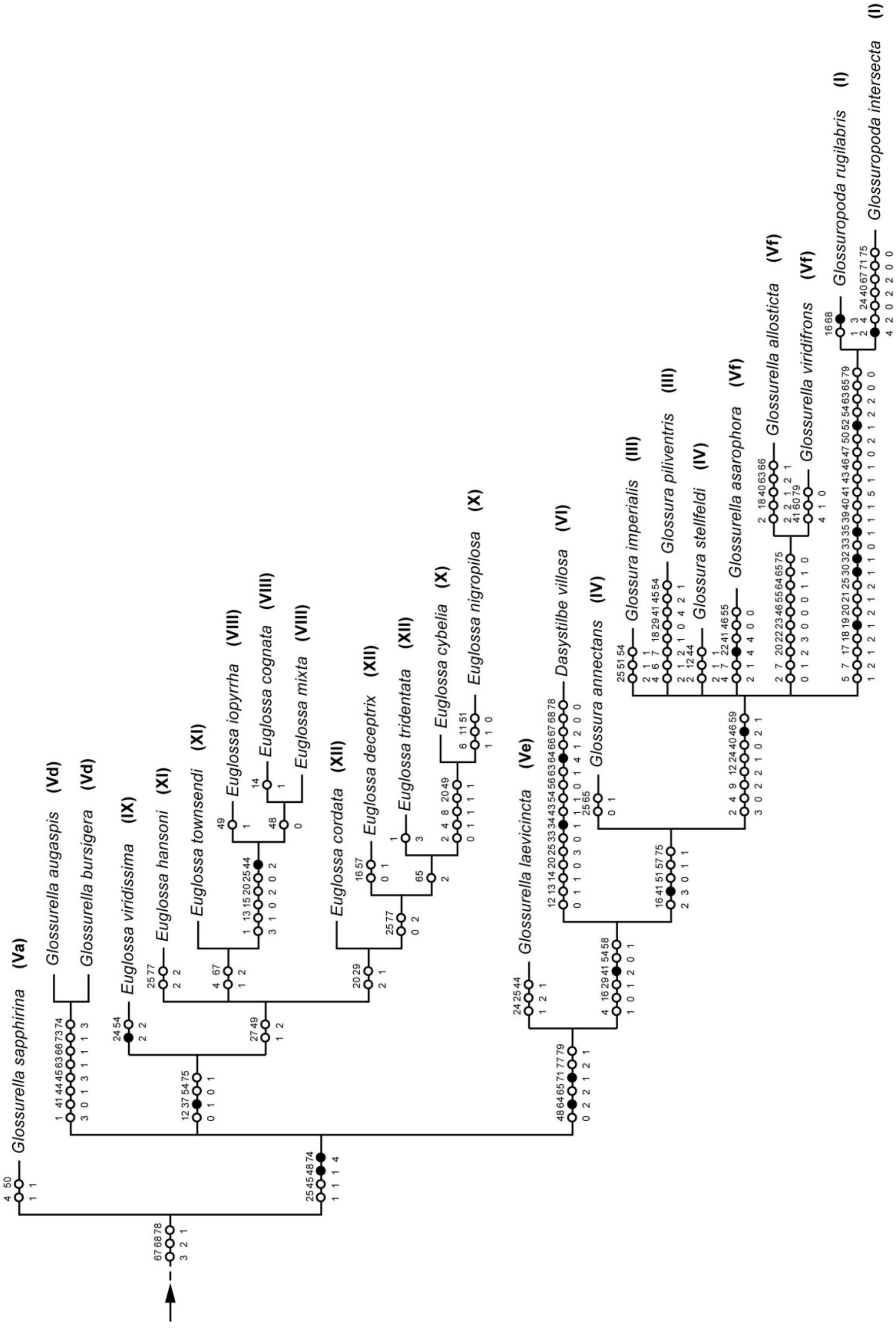


Fig. 3.33. (continues from previous page)

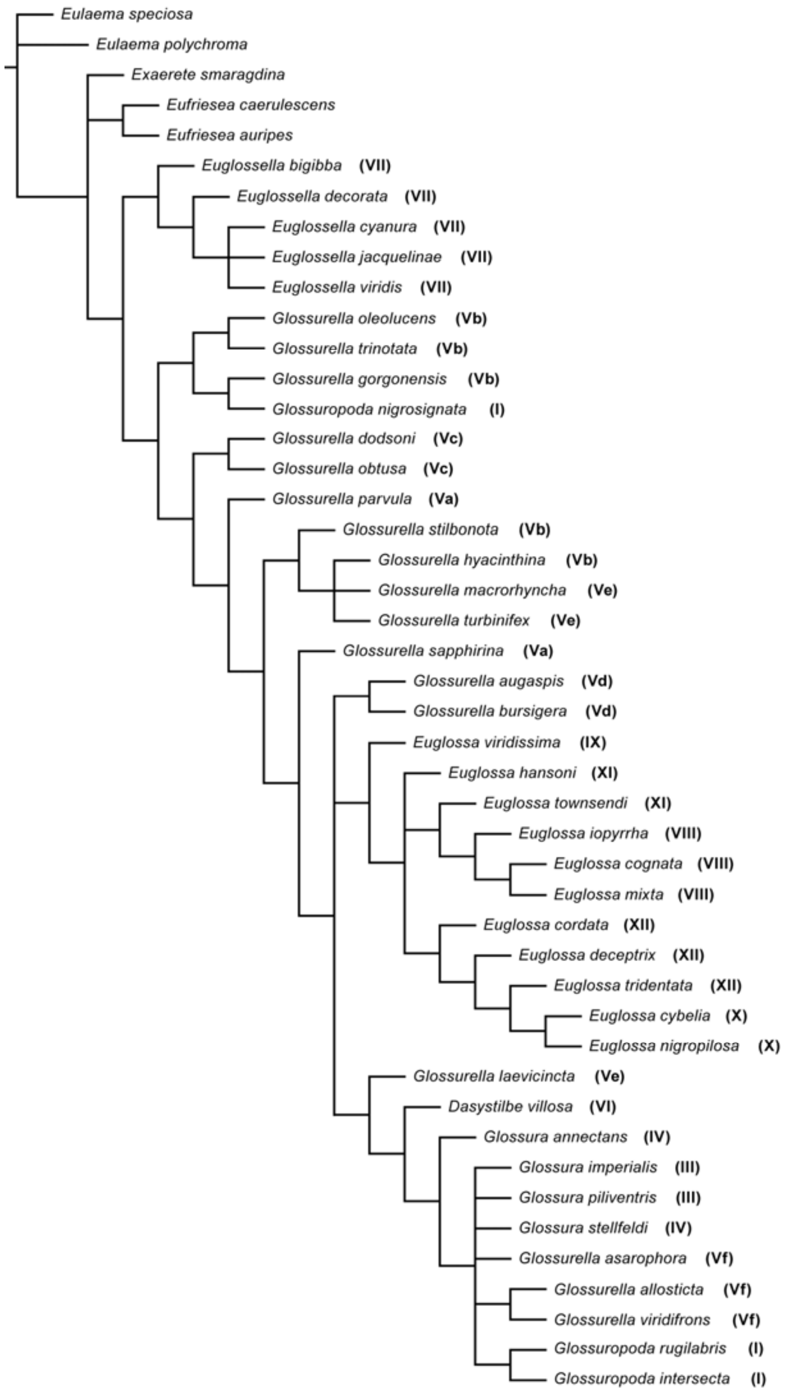


Fig. 3.34. Strict consensus of the 64 most parsimonious trees produced by the phylogenetic analysis. *Eulaema speciosa*, *Eulaema polychroma*, *Exaerete smaragdina*, *Eufriesea caerulescens* and *Eufriesea auripes* are the outgroups. Names of the species of the ingroup (*Euglossa s. lat*) bear the subgeneric name along with the specific epithet. Parentheses after the ingroup taxa names refer to the species groups as listed in Table 3.2.

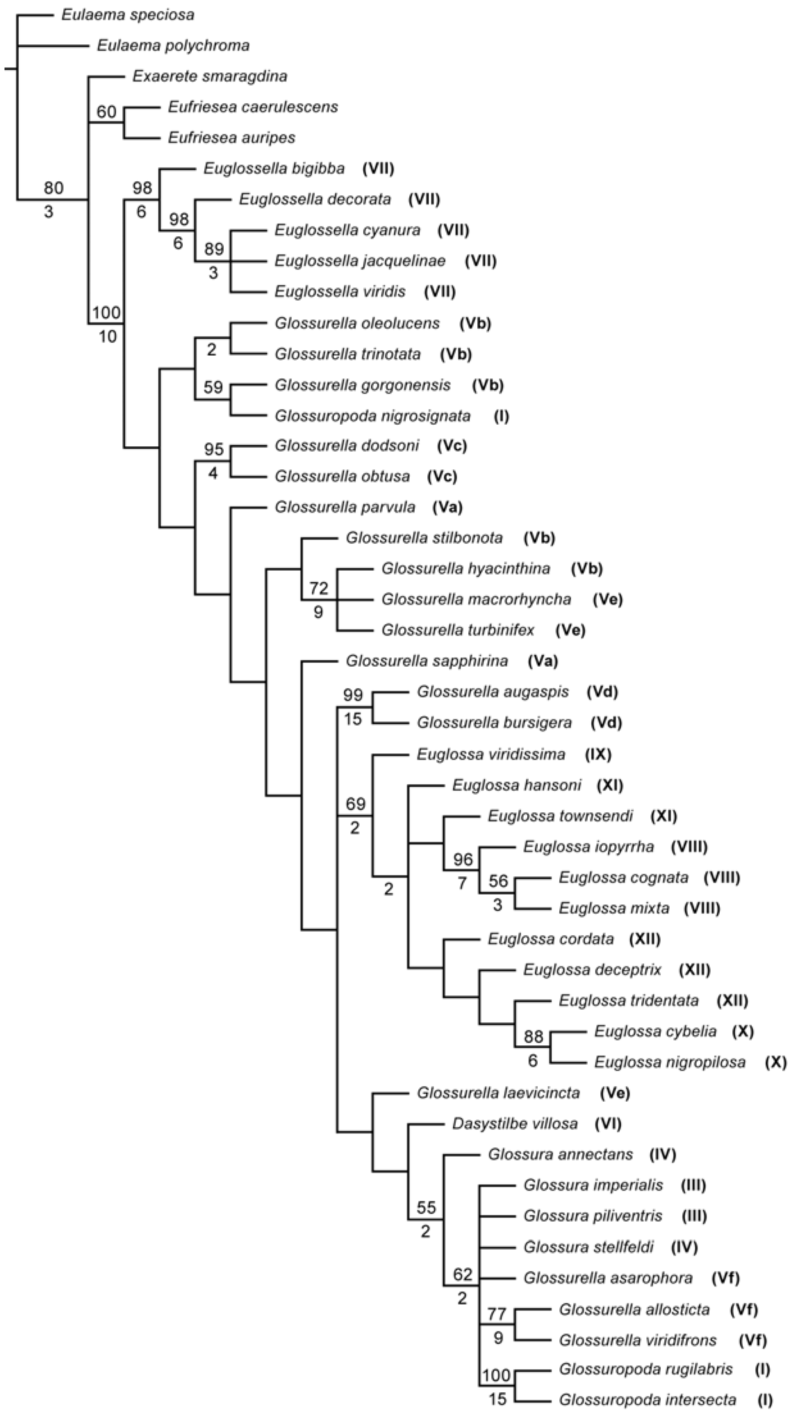


Fig. 3.35. Support values for the strict consensus tree produced by the phylogenetic analysis. Jacknife values shown above the internodes (values below 50 ommited), Bremer support values below (only values higher than one shown). *Eulaema speciosa*, *Eulaema polychroma*, *Exaerete smaragdina*, *Eufriesea caerulescens* and *Eufriesea auripes* are the outgroups. Names of the species of the ingroup (*Euglossa s. lat*) bear the subgeneric name along with the specific epithet. Parentheses after the ingroup taxa names refer to the species groups as listed in Table 3.2.

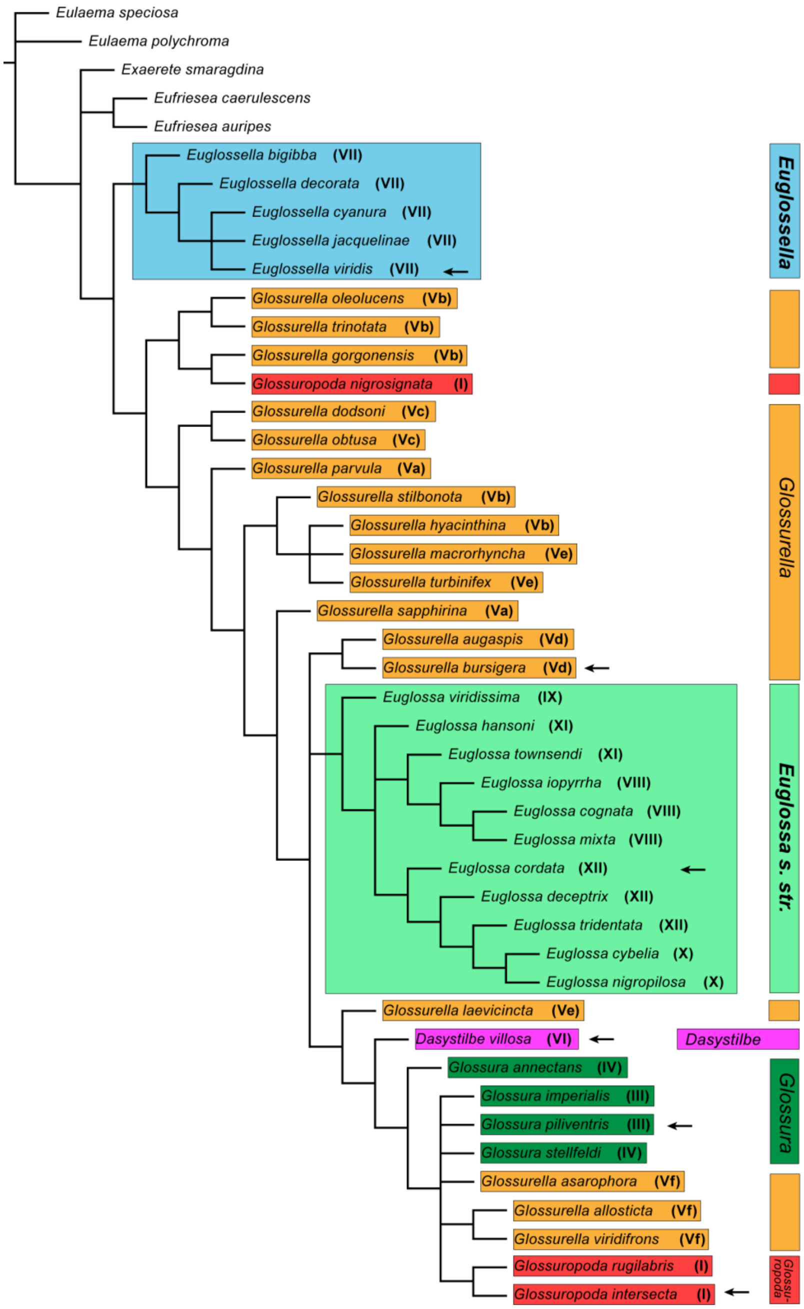


Fig. 3.36. Subgeneric association of the species of *Euglossa s. lat*. in the strict consensus tree, showing the nature of the different subgenera as in use before the present study. Only the groups recovered as monophyletic (*Euglossella* and *Euglossa s. str*.) are enclosed in a single rectangle, others are either monotypic (*Dasystilbe*) or not recovered as monophyletic (*Glossurella*, *Glossura* and *Glossuropoda*). Names of the species of the ingroup (*Euglossa s. lat*) bear the subgeneric name along with the specific epithet. Parentheses after the ingroup taxa names refer to the species groups as listed in Table 3.2. The arrows indicate type species status.

Fig. 3.37. Distributional association of the species of *Euglossa s. lat.* in the strict consensus tree. Color boxes after the species names indicate their association with the four major areas (plus the Andes) as illustrated in the map.

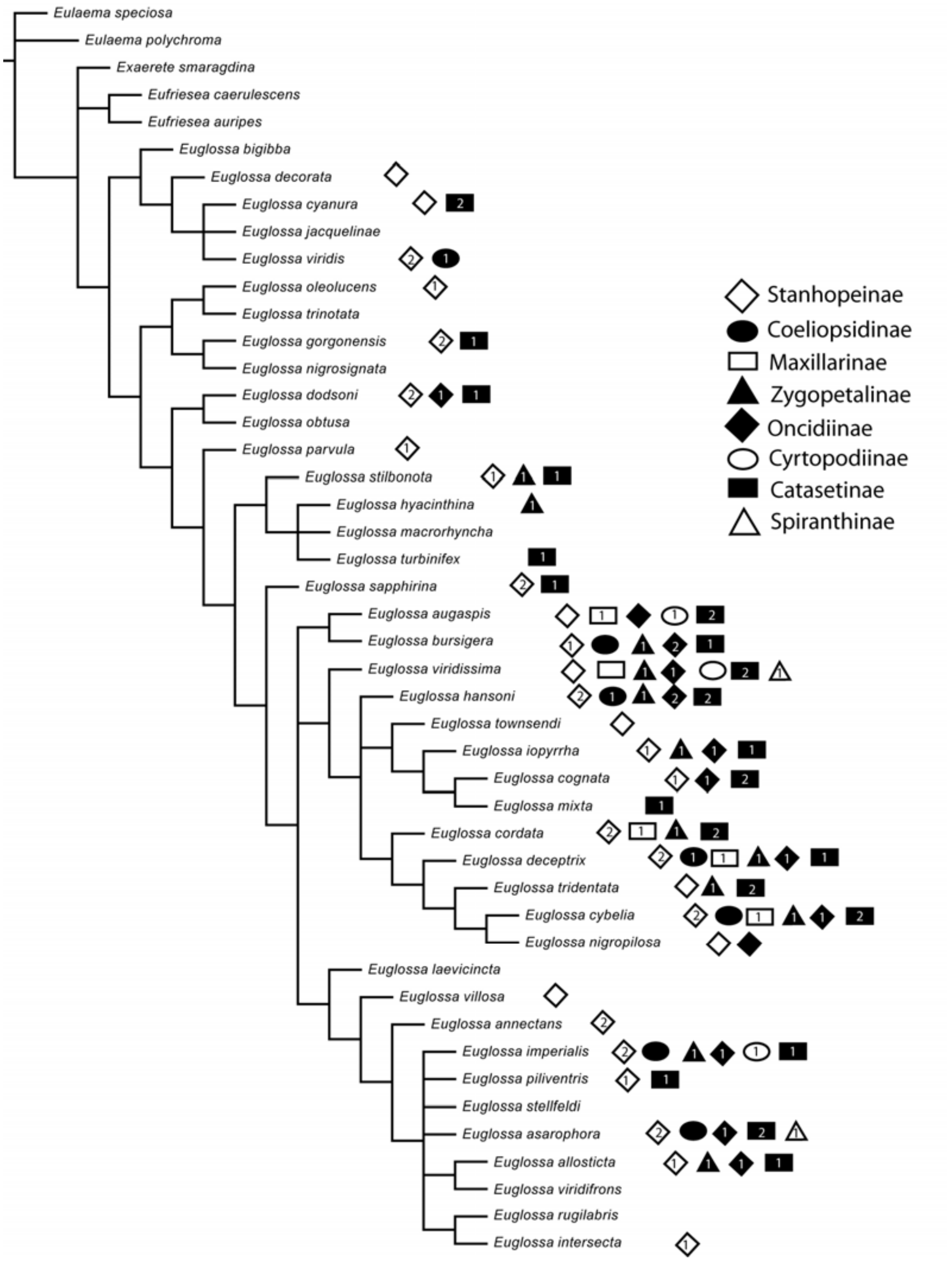


Fig. 3.38. Association with orchids pollinated and species of *Euglossa s. lat*. included in the phylogenetic analysis, as they appear in the strict consensus tree. Symbols after the species names indicate pollination records for the corresponding orchid subfamily as they appear in the legend. Numbers inside the symbol represent records of bees carrying orchid pollinium of the orchid subfamily (1), or both, records of direct observation of pollination event plus records of bees carrying orchid pollinium of the orchid subfamily (2).

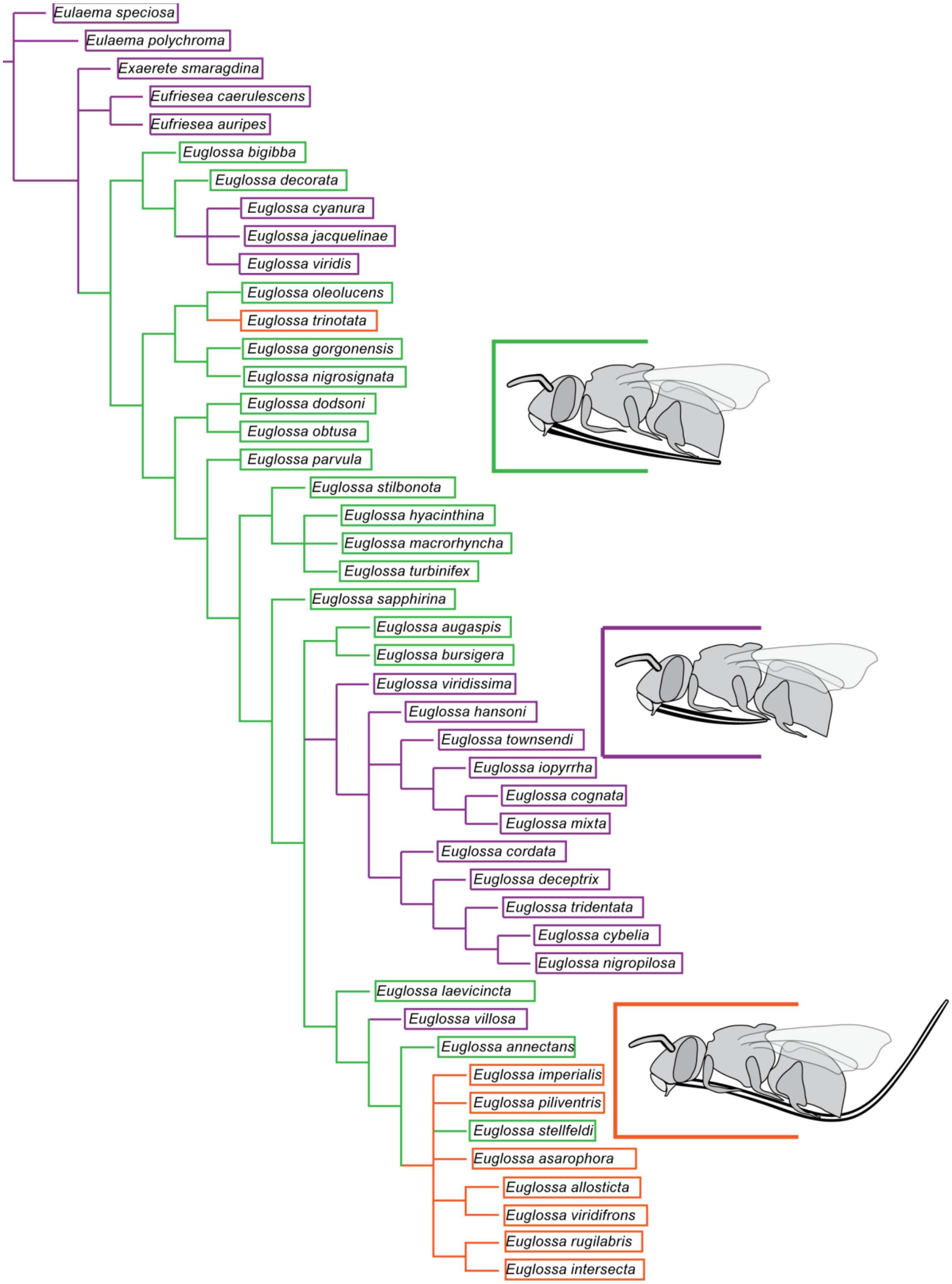


Fig. 3.39. Evolution of the Length of labiomaxillary complex relative to body (Char. # 12), as traced on the strict consensus tree under fast optimization.

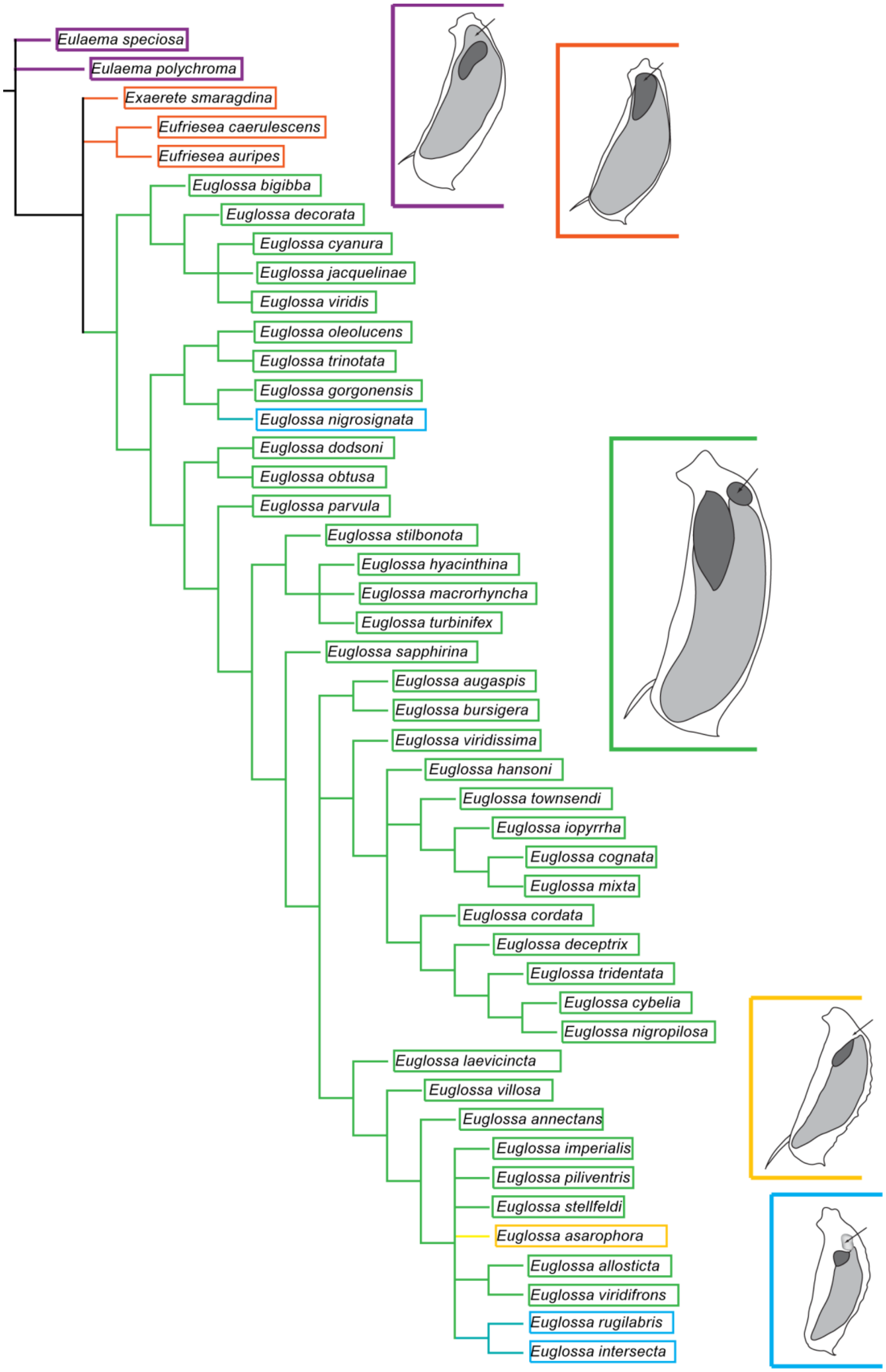


Fig. 3.40. Evolution of the basal area of the outer face of the mesotibia, emphasizing the presence/absence of the posterior tuft (Char. # 22), as traced on the strict consensus tree under fast optimization.

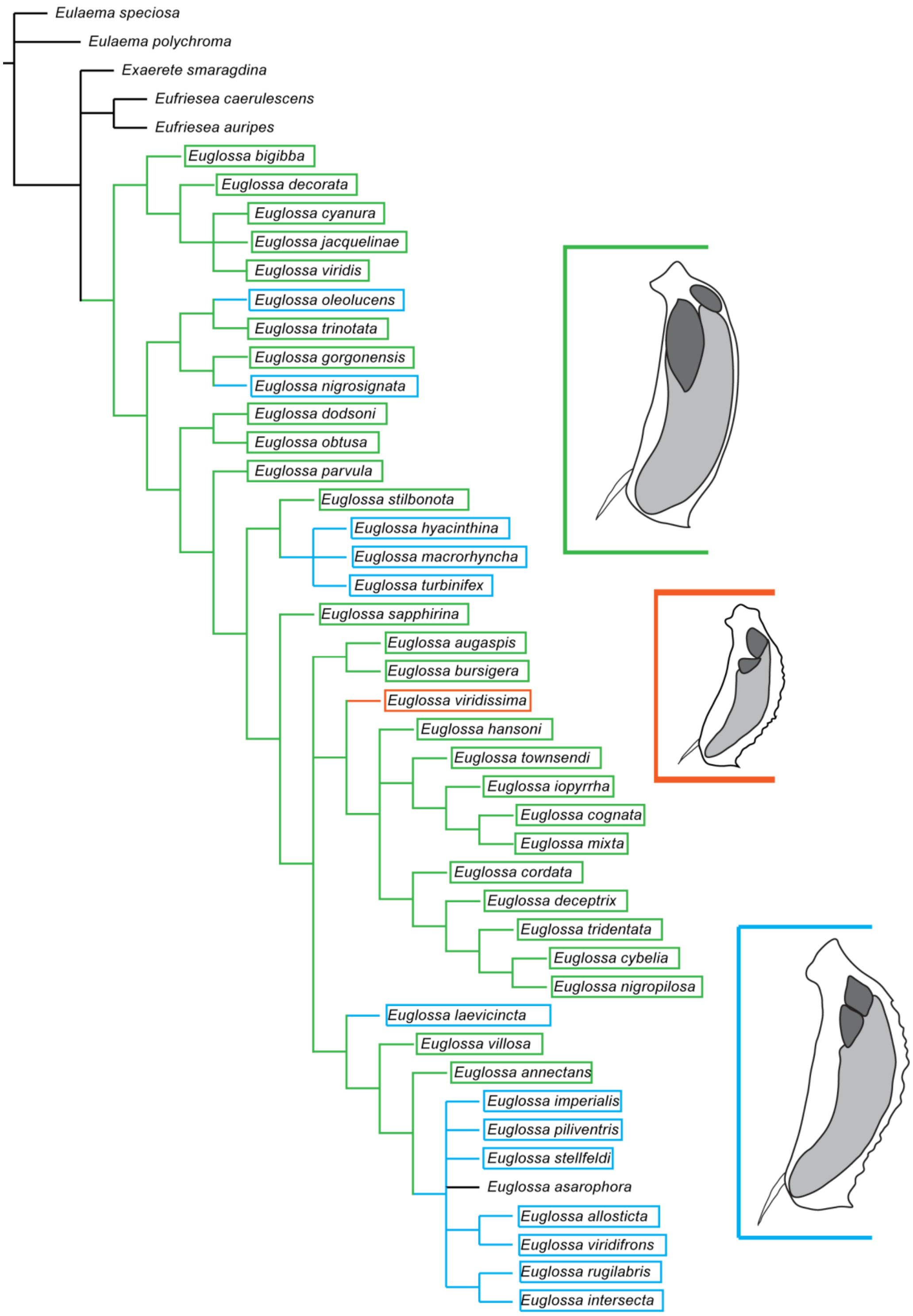


Fig. 3.41. Evolution of the relative size of the posterior and anterior tufts of the mesotibia (Char. # 24), as traced on the strict consensus tree under fast optimization.

Fig. 3.42. Evolution of the shape of the anterior tuft of the mesotibia (Char. # 27), as traced on the strict consensus tree under fast optimization.

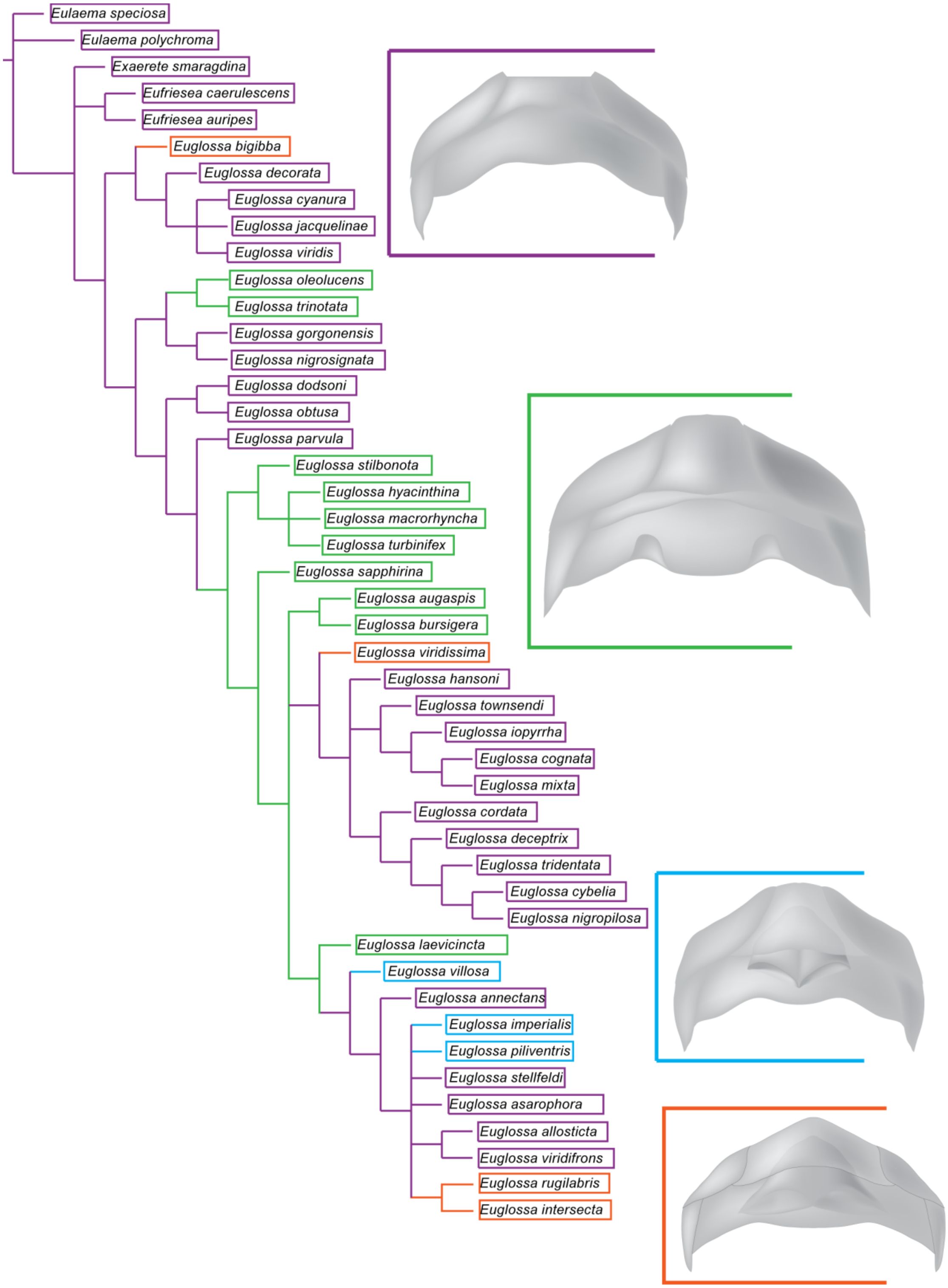


Fig. 3.43. Evolution of the integumental modifications of the second metasomal sternum (Chra. # 54), as traced on the strict consensus tree under fast optimization.

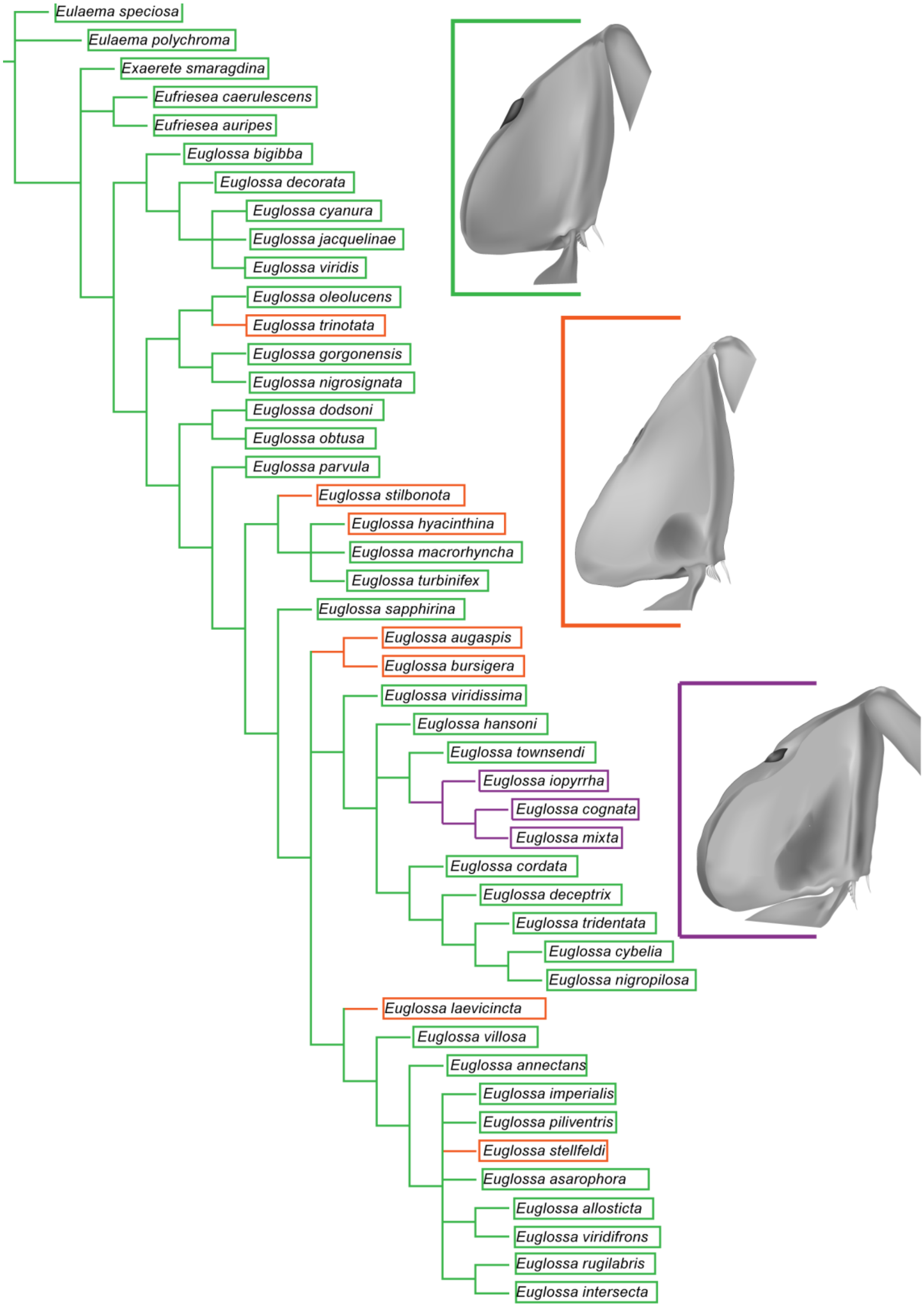


Fig. 3.44. Evolution of the inner surface of the metastibia, emphasizing the presence/absence of depressed areas (Char. # 54), as traced on the strict consensus tree under fast optimization.

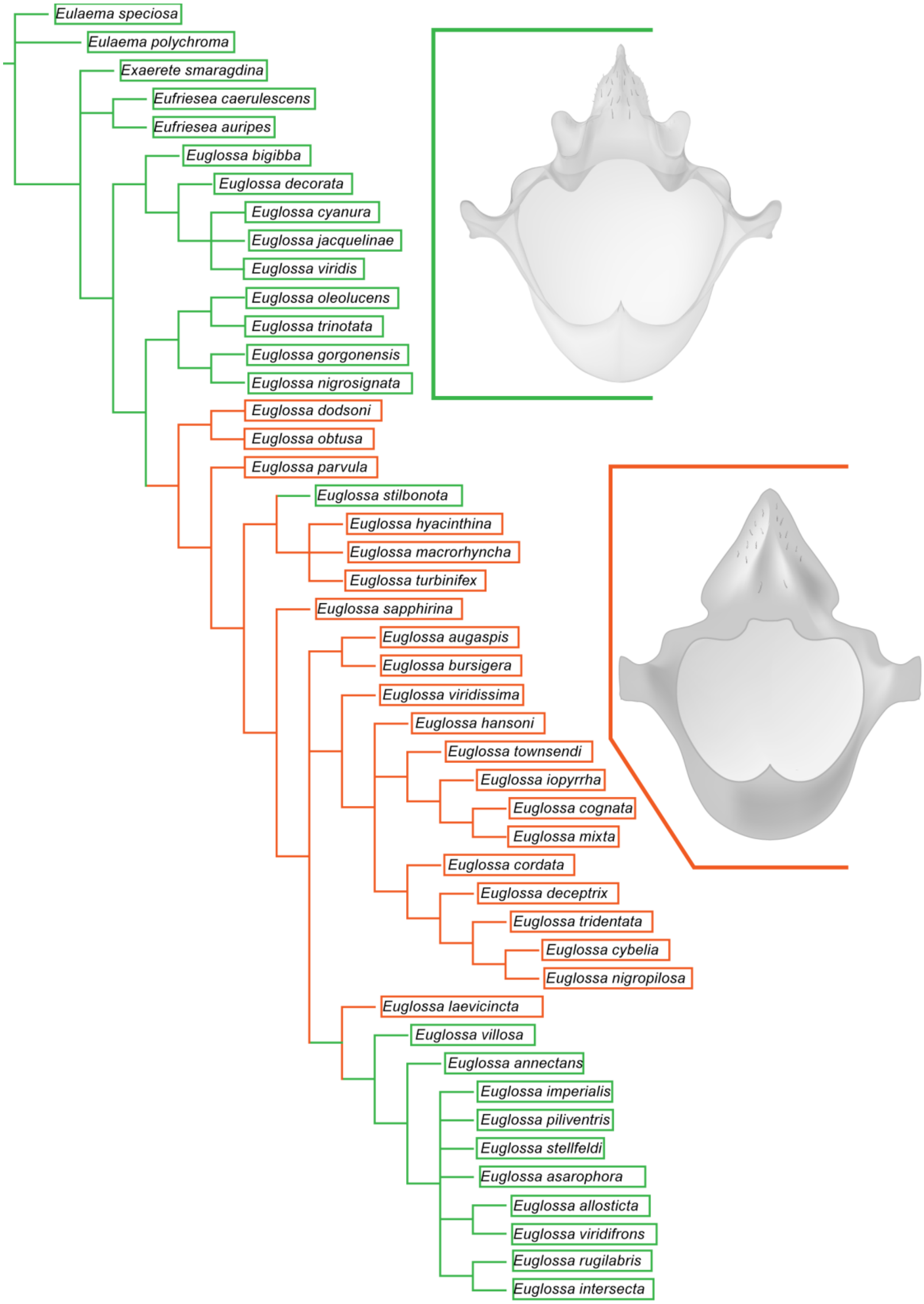


Fig. 3.45. Evolution of the morphology of the eighth metasomal sternum, emphasizing the shape of the posterior section (Char. # 58), as traced on the strict consensus tree under fast optimization.

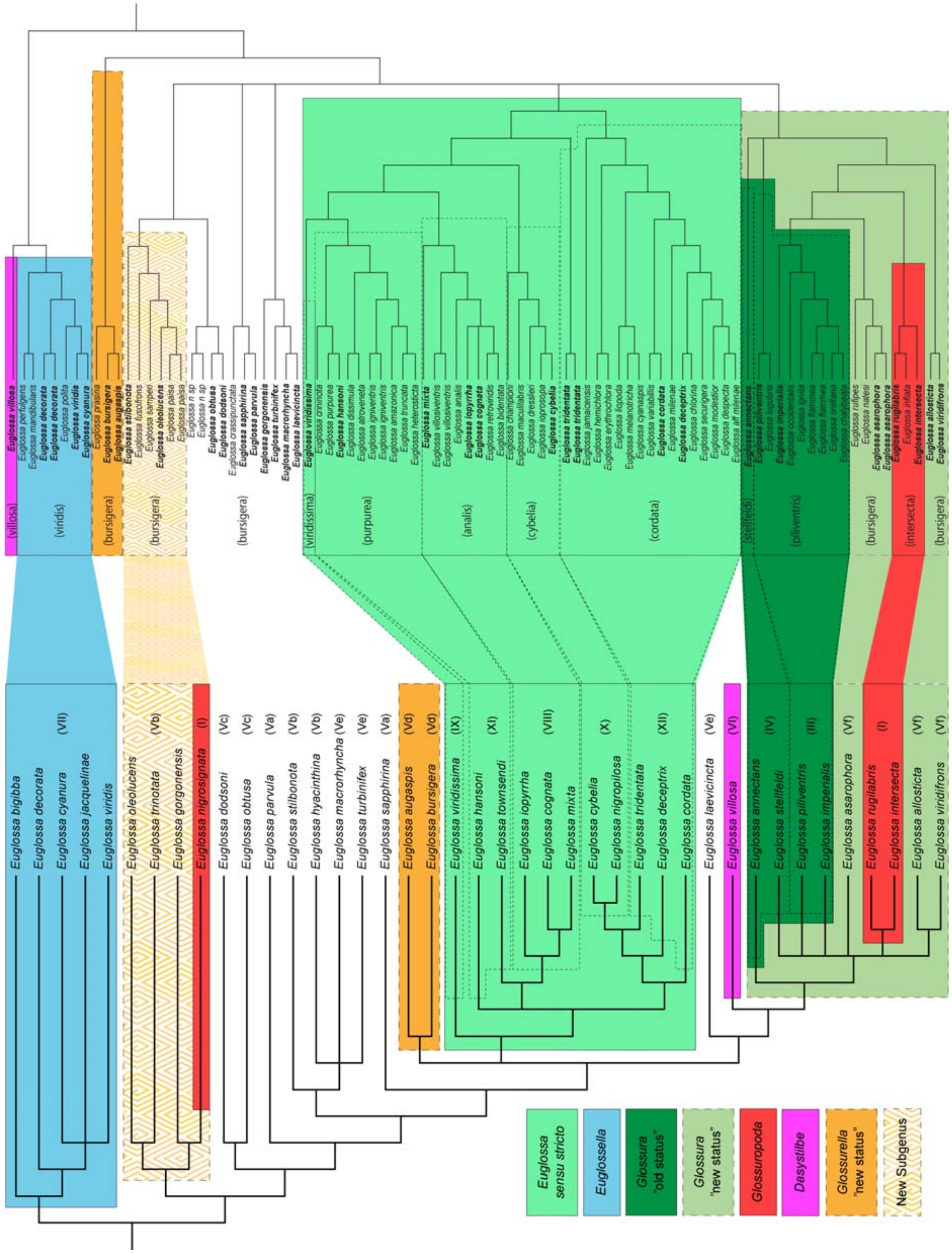

Euglossa villosa
Euglossa perfulgens
Euglossa mandibularis
Euglossa decorata
Euglossa decorata
Euglossa polita
Euglossa viridis
Euglossa cyanura
Euglossa prasina
Euglossa bursigera
Euglossa augaspis
Euglossa stilbonota
Euglossa fuscifrons
Euglossa samperi
Euglossa oleolucens
Euglossa paisa
Euglossa paisa
Euglossa n sp
Euglossa n sp
Euglossa obtusa
Euglossa dodsoni
Euglossa crassipunctata
Euglossa sapphirina
Euglossa parvula
Euglossa gorgonensis
Euglossa turbinifex
Euglossa macrorhyncha
Euglossa laevicincta
Euglossa viridissima
Euglossa crininota
Euglossa purpurea
Euglossa hansoni
Euglossa dissimula
Euglossa atroveneta
Euglossa igniventris
Euglossa igniventris
Euglossa amazonica
Euglossa truncata
Euglossa heterosticta
Euglossa mixta
Euglossa villosiventris
Euglossa villosiventris
Euglossa analis
Euglossa iopyrrha
Euglossa cognata
Euglossa retroviridis
Euglossa bidentata
Euglossa championi
Euglossa maculilabris
Euglossa dressleri
Euglossa ioprosopa
Euglossa cybelia
Euglossa tridentata
Euglossa tridentata
Euglossa jamaicensis
Euglossa hemichlora
Euglossa erythrochlora
Euglossa liopoda
Euglossa melanotricha
Euglossa cyanaspis
Euglossa variabilis
Euglossa cordata
Euglossa milenae
Euglossa deceptrix
Euglossa chlorina
Euglossa securigera
Euglossa modestior
Euglossa despecta
Euglossa aff milenae
Euglossa annectans
Euglossa piliventris
Euglossa Iugubris
Euglossa imperialis
Euglossa occidentalis
Euglossa ignita
Euglossa flammea
Euglossa flammea
Euglossa orellana
Euglossa chalybeata
Euglossa rufipes
Euglossa natesi
Euglossa asarophora
Euglossa asarophora
Euglossa rugilabris
Euglossa inflata
Euglossa intersecta
Euglossa allosticta
Euglossa viridifrons
(villosa)
(viridis)
(bursigera)
(bursigera)
(bursigera)
(viridissima)
(purpurea)
(analis)
(cybelia)
(cordata)
(stellfeldi)
(piliventris)
(bursigera)
(intersecta)
(bursigera)
Euglossa bigibba
Euglossa decorata
Euglossa cyanura
Euglossa jacquelinae
Euglossa viridis
(VII)
Euglossa oleolucens
Euglossa trinotata
Euglossa gorgonensis
Euglossa nigrosignata
(Vb)
(I)
Euglossa dodsoni
Euglossa obtusa
Euglossa parvula
Euglossa stilbonota
Euglossa hyacinthina
Euglossa macrorhyncha
Euglossa turbinifex
Euglossa sapphirina
(Vc)
(Vc)
(Va)
(Vb)
(Vb)
(Ve)
(Ve)
(Va)
Euglossa augaspis
Euglossa bursigera
(Vd)
(Vd)
Euglossa viridissima
Euglossa hansoni
Euglossa townsendi
Euglossa iopyrrha
Euglossa cognata
Euglossa mixta
Euglossa cybelia
Euglossa nigropilosa
Euglossa tridentata
Euglossa deceptrix
Euglossa cordata
(IX)
(XI)
(VIII)
(X)
(XII)
Euglossa laevicincta
(Ve)
Euglossa villosa
(VI)
Euglossa annectans
Euglossa stellfeldi
Euglossa piliventris
Euglossa imperialis
(IV)
(III)
Euglossa asarophora
(Vf)
Euglossa rugilabris
Euglossa intersecta
(I)
Euglossa allosticta
Euglossa viridifrons
(Vf)
(Vf)
Euglossa sensu stricto
Euglossella
Glossura "old status"
Glossura "new status"
Glossuropoda
Dasystilbe
Glossurella "new status"
New Subgenus

Fig. 3.46. (See previous page) Comparison of the strict consensus trees produced by phylognetic analyses based on external morphology (this study; left tree), and DNA (Ramírez *et al*., 2010; right tree) for the genus *Euglossa*. The color boxes represent the subgeneric groups as recovered by both analyses. With the exception of *Glossurella*, the subgenera according to their definitions before the present study are enclosed by solid line boxes. New status for already existing subgeneric names, and new subgeneric proposals are enclosed by dotted line boxes. The species in bold face in the molecular tree correspond to species also included in the present study. The molecular tree as presented here is modified from Ramírez *et al*. (2010) by rotating some nodes to facilitate comparison.

Fig. 3.47. Lateral habitus of male of *E*. *oleolucens*, type species of the new subgenus here proposed.

Appendix 4. Matrix of 79 external morphological characters for 41 species of *Euglossa* (abbreviated as *E*. in most species names) and five species as outgroups. The matrix is presented in three sections for ease of reading, species appear in rows in no particular order (outgroups first) and characters in colums (two numbers in the cell = polymorphism, “?” = missing value, “-“ = character non-applicable).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Eulaema speciosa	2	0	0	1	0	0	0	1	2	0	1	0	0	0	0	0	0	0	0	0	0	0	-	-	-	0	1
Eulaema polychroma	2	0	0	1	0	0	0	1	2	0	1	0	0	0	0	0	0	0	0	0	0	0	-	-	-	0	0
Eufriesea caerulescens	2	0	0	1	1	0	0	1	2	0	1	0	0	0	1	1	0	0	0	2	0	1	-	-	-	0	1
Eufriesea auripes	2	0	0	1	1	0	0	1	2	0	1	0	0	0	0	3	0	0	0	0	0	1	-	-	-	0	1
Exaerete smaragdina	2	0	0	1	2	0	0	1	2	0	1	0	0	0	0	0	0	0	0	0	0	1	-	-	-	0	0
Euglossa villosa	2	2	1	1	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	2	1	0	3	0	0
E. cognate	3	2	1	1	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	2	0	2	1	0	0	0	1
E. iopyrrha	3	2	1	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	2	0	2 3	1	0	0	0	1
E. mixta	3	2	1	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	2	0	2	1	0	0	0	1
E. viridissima	2 3	2	1	0 1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	2	1	2	1	0	0
E. cybelia	2	0	1	1	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	1	0	2	1	0	0	0	1
E. nigropilosa	2	0	1	1	0	1	0	1	0	0	1	0	0	0	1	1	0	0	0	1	0	2	1	0	0	0	1
E. hansoni	2	2	1	0	0	0	0	0	0	0	0	0	0	0	1	0 1	0	0	0	1	0	2	1	0	2	0	1
E. townsendi	2	2	1	1	0	0	0	0	0	0	0	0	0	0	1	0 1	0	0	0	1	0	2	1	0	1	0	1
E. cordata	2	2	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	2	0	2	1	0	1	0	1
E. tridentata	3	2	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	2	0	2	1	0	0	0	1
E. cyanura	3	3	1	1	0	0	0	0	0	1	0	0	0	2	1	1	0	0	0	1	0	2	1	0	0	0	0
E. jacquelinae	3	3	1	1	0	0	0	0	0	1	0	0	0	2	1	1	0	0	0	1	0	2	1	0	1	0	0
E. rugilabris	2	3	1	0	1	0	2	0	2	0	0	2	0	0	1	1	1	2	1	2	1	2	1	1	2	0	0
E. imperialis	2	2 3	1	0	0	0	0	0	2	0	0	2	0	0	1	2	0	0	0	1	0	2	1	1	2	0	0
E. piliventris	2	3	1	2	0	1	2	0	2	0	0	2	0	0	1	2	0	1	0	1	0	2	1	1	1	0	0
E. annectans	2	2	1	1	0	0	0	0	0	0	0	1	0	0	1	1 2	0	0	0	1	0	2	1	0	0	0	0
E. stellfeldi	2	2	1	0	0	0	0	0	2	0	0	1	0	0	1	2	0	0	0	1	0	2	1	1	1	0	0
E. parvula	2	2	1	1	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	1	0	2	1	0	0	0	0
E. sapphirina	2	2	1	1	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	1	0	2	1	0	0	0	0
E. gorgonensis	2	0 1	1	0	0	0	0	0	1	0	0	1	1	0	1	1	0	0	0	1	0	2	1	0	1	0	0
E. hyacinthina	2	2	1	0	0	0	1	0	0	0	0	1	1	0	1	3	0	0	0	1	0	2	1	1	2	1	0
E. nigrosignata	2	3	1	0	0	0	0	0	0	0	0	1	1	0	1	1	1	1	0	2	1	3	0	1	1	0	0
E. stilbonota	2	2 3	1	0	0	0	0	0	1	0	0	1	0	0	1	1 2	0	0	0	1	0	2	1	0	0	0	0
E. dodsoni	2	2	1	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	1	0	2	0	0	3	0	0
E. obtusa	2	2	1	0	0	0	0	0	0	0	0	?	0	0	1	1	0	0	0	1	0	2	0	0	3	0	0
E. augaspis	3	2	1	0	0	0	0	0	0	0	0	1	0	0	1	?	0	0	0	1	0	2	1	0	1	0	0
E. bursigera	3	2	1	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	1	0	2	1	0	1	0	0
E. laevicincta	2	2	1	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	1	0	2	1	1	2	0	0
E. macrorhyncha	2	3	1	0	0	0	2	0	0	0	0	1	0	0	1	3	0	0	0	1	0	2	1	1	2	1	0
E. turbinifex	2	2	1	0	0	0	1	0	0	0	0	1	0	0	1	1	0	0	0	1	0	2	1	1	2	1	0
E. allosticta	2	2	1	0	0	0	1	0	2	0	0	2	0	0	1	2	0	2	0	2	0	3	0	1	1	0	0
E. asarophora	2	3	1	2	0	0	1	0	2	0	0	2	0	0	1	2	0	0	0	1	0	4	-	-	-	0	0
E. viridifrons	2	0	1	0	0	0	1	0	2	0	0	2	0	0	1	2	0	0	0	2	0	3	0	1	1	0	0
E. oleolucens	2	0	1	0	0	0	0	0	1	0	0	1	1	0	1	1	0	0	0	1	0	2	1	1	2	0	0
E. intersecta	2	4	1	2	1	0	2	0	2	0	0	2	0	0	1	2	1	2	1	2	1	2	1	0	2	0	0
E. bigibba	3	3	1	1	0	0	2	0	2	0	0	1	1	1	1	2	0	0	0	1	0	2	1	0	3	0	0
E. decorata	3	3	1	1	0	0	0	0	0 1	0	0	1	0	2	1	0	0	0	0	1	0	2	1	0	0	0	0
E. deceptrix	2	2	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	0	2	1	0	0	0	1
E. viridis	3	3	1	1	0	0	0	0	0	1	0	0	0	2	1	1	0	0	0	1	0	2	1	0	0	0	0
E. trinotata	2	2	1	0	0	0	0	0	0	0	0	2	1	0	1	1	0	0	0	1	0	2	1	0	0	0	0

	2	2	3	3	3	3	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	4	5	5	5	5
	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3
Eulaema speciosa	-	0	0	0	0	0	0	0	0	0	-	2	0	1	0	2	0	0	1	2	0	0	0	0	0	0
Eulaema polychroma	-	0	0	0	0	0	0	0	0	0	-	2	0	1	0	2	0	0	1	2	0	0	0	0	0	0
Eufriesea caerulescens	-	0	0	0	0	0	0	0	1	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0
Eufriesea auripes	-	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Exaerete smaragdina	-	0	0	0	0	0	0	0	1	0	0	1	2	5	0	2	0	0	0	2	0	0	0	0	0	0
Euglossa villosa	0	1	0	1	0	0	1	0	1	0	0	0	1	2	0	1	0	1	1	1	0	1	0	1	0	1
E. cognata	0	0	0	1	0	1	0	0	1	1	0	0	1	1	0	2	2	1	1	1	0	2	0	1	0	1
E. iopyrrha	0	0	0	1	0	1	0	0	1	1	0	0	1	1	0	2	2	1	1	1	1	1	0	1	0	1
E. mixta	0	0	0	1	0	1	0	0	1	1	0	0	1	1	0	2	2	1	1	1	0	2	0	1	0	1
E. viridissima	0	0	0	1	0	1	0	0	1	1	0	0	1	1	0	2	0	1	1	1	1	1	0	1	0	1
E. cybelia	0	1	0	1	0	1	0	0	1	1	0	0	1	1	0	2	0	1	1	1	1	1	0	1	0	1
E. nigropilosa	0	1	0	1	0	1	0	0	1	1	0	0	1	1	0	2	0	1	1	1	1	1	0	0	0	1
E. hansoni	0	0	0	1	0	1	0	0	1	1	0	0	1	1	0	2	0	1	1	1	1	2	0	1	0	1
E. townsendi	0	0	0	1	0	1	0	0	1	1	0	0	1	1	0	2	0	1	1	1	1	2	0	1	0	1
E. cordata	0	1	0	1	0	1	0	0	1	1	0	0	1	1	0	2	0	1	1	1	1	2	0	1	0	1
E. tridentata	0	1	0	1	0	1	0	0	1	1	0	0	1	1	0	2	0	1	1	1	1	2	0	1	0	1
E. cyanura	0	0	0	1	0	0	0	0	1	0	1	1	2	1	0	1	0	1	3	0	0	1	1	0	0	1
E. jacquelinae	0	0	0	1	0	0	0	0	1	0	1	1	2	0	0	1	0	1	3	0	0	1	1	0	0	1
E. rugilabris	0	1	1	1	1	0	0	1	1	0	0	1	1	5	0	1	0	1	1	0	0	1	2	0	1	1
E. imperialis	0	1	0	1	0	1	0	0	1	0	0	0	0	3	0	2	0	1	2	1	0	1	0	1	0	1
E. piliventris	0	0	0	1	0	1	0	0	1	0	0	0	0	4	0	2	0	2	2	1	0	1	0	0	0	1
E. annectans	0	1	0	1	0	1	0	0	1	0	0	0	1	3	0	2	0	1	1	1	0	1	0	0	0	1
E. stellfeldi	0	1	0	1	0	1	0	0	1	0	0	0	0	3	0	2	1	1	2	1	0	1	0	0	0	1
E. parvula	0	0	0	1	0	1	0	0	1	0	0	0	1	1	0	2	0	2	1	1	0	1	0	1	0	1
E. sapphirina	0	0	0	1	0	1	0	0	1	0	0	0	1	1	0	2	0	2	1	1	0	1	1	1	0	1
E. gorgonensis	0	0	0	1	0	1	0	0	1	0	0	0	1	1	1	1	0	1	0	1	0	1	0	0	0	1
E. hyacinthina	0	1	0	1	0	1	0	0	1	0	0	0	1	1	0	2	0	2	1	1	0	1	0	1	0	1
E. nigrosignata	1	1	0	1	0	1	0	0	1	0	0	0	1	1	1	1	0	1	0	1	0	1	0	0	0	1
E. stilbonota	0	1	0	1	0	1	0	0	1	0	0	0	1	1	1	2	1	3	1	1	0	1	1	1	0	1
E. dodsoni	0	0	0	1	0	1	0	0	1	0	0	0	1	1	0	1	0	2	0	1	0	2	0	0	0	1
E. obtusa	0	0	0	1	0	1	0	0	1	0	0	0	1	1	0	1	0	1	0	1	0	2	0	0	0	1
E. augaspis	0	0	0	1	0	1	0	0	1	0	0	0	1	0	0	2	1	3	1	1	1	1	0	1	0	1
E. bursigera	0	0	0	1	0	1	0	0	1	0	0	0	01	0	0	2	1	3	1	1	1	1	0	1	0	1
E. laevicincta	0	0	0	1	0	1	0	0	1	0	0	0	1	1	0	2	1	1	1	1	0	1	0	1	0	1
E. macrorhyncha	0	1	0	1	0	1	0	0	1	0	0	0	1	1	0	1	0	3	1	1	0	1	0	1	0	1
E. turbinifex	0	0	0	1	0	1	0	0	1	0	0	0	1	1	0	1	0	2	1	1	0	1	0	1	0	1
E. allosticta	0	1	0	1	0	1	0	0	1	0	0	0	1	3	0	2	0	1	0	1	0	1	0	0	0	1
E. asarophora	0	1	0	1	0	1	0	0	1	0	0	0	0	4	0	2	0	1	0	1	0	1	0	0	0	1
E. viridifrons	0	1	0	1	0	1	0	0	1	0	0	0	0	4	0	2	0	1	0	1	0	1	0	0	0	1
E. oleolucens	1	0	0	1	0	1	0	0	1	0	0	0	1	1	1	1	0	3	1	1	0	1	2	0	0	1
E. intersecta	0	1	1	1	1	0	0	1	1	0	0	1	2	5	0	1	0	1	1	0	0	1	2	0	1	1
E. bigibba	0	0	0	1	0	0	0	0	1	0	0	1	2	0	0	1	0	1	3	0	0	1	2	0	0	1
E. decorata	0	0	0	1	0	0	0	0	1	0	1	1	2	0	0	1	0	1	3	0	0	1	1	0	0	1
E. deceptrix	0	1	0	1	0	1	0	0	1	1	0	0	1	1	0	2	0	1	1	1	1	2	0	1	0	1
E. viridis	0	0	0	1	0	0	0	0	1	0	1	1	2	0	0	1	0	1	3	0	0	1	1	0	0	1
E. trinotata	1	0	0	1	0	1	0	0	1	0	0	0	1	1	1	1	1	3	1	1	0	1	2	0	0	1

	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79
Eulaema speciosa	0	0	1	0	1	0	0	0	0	2	0	1	0	0	0	0	0	0	0	0	1	-	0	0	0	0
Eulaema polychroma	0	0	0	0	1	0	0	0	0	2	0	1	0	0	0	0	0	0	0	0	1	-	0	0	0	0
Eufriesea caerulescens	0	0	1	0	1	1	0	0	0	2	0	1	1	1	0	0	0	0	1	0	1	-	0	0	0	1
Eufriesea auripes	0	0	1	0	0	0	0	0	0	2	0	1	0	1	0	0	0	0	1	0	1	-	0	0	0	1
Exaerete smaragdina	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	1	0	0	-	0	-	0	0	0	0
Euglossa villosa	1	1	0	0	1	0	0	0	0	1	4	2	1	2	0	1	1	1	3	0	4	0	0	2	0	1
E. cognata	0	1	1	0	0	0	0	0	0	0	0	1	0	2	2	1	1	0	3	0	4	1	0	1	1	0
E. iopyrrha	0	1	1	0	0	0	0	0	0	0	0	1	0	2	2	1	1	0	3	0	4	1	0	1	1	0
E. mixta	0	1	1	0	0	0	0	0	0	0	0	1	0	2	2	1	1	0	3	0	4	1	0	1	1	0
E. viridissima	2	1	1	0	0	0	0	0	0	0	0	1	0	3	2	1	1	0	3	0	4	1	0	1	1	0
E. cybelia	0	1	1	0	0	0	0	0	0	0	0	2	0	3	2	1	1	0	3	0	4	1	0	2	1	0
E. nigropilosa	0	1	1	0	0	0	0	0	0	0	0	2	0	3	2	1	1	0	3	0	4	1	0	2	1	0
E. hansoni	0	1	1	0	0	0	0	0	0	0	0	1	0	3	2	1	1	0	3	0	4	1	0	2	1	0
E. townsendi	0	1	1	0	0	0	0	0	0	0	0	1	0	2	2	1	1	0	3	0	4	1	0	1	1	0
E. cordata	0	1	1	0	0	0	0	0	0	0	0	1	0	3	2	1	1	0	3	0	4	1	0	1	1	0
E. tridentata	0	1	1	0	0	0	0	0	0	0	0	2	0	3	2	1	1	0	3	0	4	1	0	2	1	0
E. cyanura	0	1	1	0	1	1	1	1	1	1	3	0	2	2	1	2	1	0	2	0	2	-	1	2	0	0
E. jacquelinae	0	1	1	0	1	1	1	1	1	1	3	0	2	2	1	2	1	0	2	0	2	-	1	2	0	0
E. rugilabris	2	1	1	1	1	1	0	0	0	2	2	0	0	3	3	1	1	1	3	0	4	1	0	2	1	0
E. imperialis	1	1	1	1	1	1	0	0	0	0	2	2	0	3	2	1	1	1	3	0	4	1	0	2	1	1
E. piliventris	1	1	1	1	1	1	0	0	0	0	2	2	0	3	2	1	1	1	3	0	4	1	0	2	1	1
E. annectans	0	1	1	1	1	0	0	0	0	0	2	1	0	3	2	1	1	1	3	0	4	1	0	2	1	1
E. stellfeldi	0	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
E. parvula	0	0	1	0	0	0	0	0	0	0	0	2	0	2	1	1	1	0	3	0	3	0	0	1	0	0
E. sapphirina	3	1	1	0	0	0	0	0	0	0	0	1	0	3	2	1	1	0	3	0	3	0	0	1	1	0
E. gorgonensis	0	0	1	0	1	1	0	0	0	0	0	1	2	2	2	1	0	0	3	0	1	0	0	1	0	0
E. hyacinthina	3	1	1	1	0	0	0	0	0	0	0	1	0	2	1	1	1	0	3	0	3	0	0	1	0	0
E. nigrosignata	0	0	1	0	1	1	0	0	0	1	0	1	2	2	1	1	0	0	3	1	1	0	-	-	0	0
E. stilbonota	3	1	1	1	1	1	0	0	0	1	0	1	0	2	1	1	1	0	3	0	3	0	0	1	0	0
E. dodsoni	0	0	1	0	0	0	0	0	0	0	0	1	1	2	1	1	1	0	3	1	3	0	0	1	1	0
E. obtusa	0	0	1	0	0	0	0	0	0	0	0	1	1	2	1	1	1	0	3	1	3	0	0	1	1	0
E. augaspis	3	1	1	0	0	0	0	0	0	1	0	1	1	3	2	1	1	0	3	1	3	0	0	1	1	0
E. bursigera	3	1	1	0	0	0	0	0	0	1	0	1	1	3	2	1	1	0	3	1	3	0	0	1	1	0
E. laevicincta	3	1	1	0	0	0	0	0	0	0	2	2	0	3	2	1	1	1	3	0	4	0	0	2	1	1
E. macrorhyncha	3	1	1	0	0	0	0	0	0	0	0	1	1	2	1	1	1	0	3	0	3	0	0	1	0	0
E. turbinifex	3	1	1	1	0	0	0	0	0	1	0	1	0	2	1	1	1	0	3	0	3	0	0	1	1	0
E. allosticta	0	0	1	1	1	1	0	0	0	2	1	1	1	3	2	1	1	1	3	0	4	0	0	2	1	1
E. asarophora	0	0	1	1	1	1	0	0	0	0	2	2	0	3	2	1	1	1	3	0	4	1	0	2	1	1
E. viridifrons	0	0	1	1	1	1	1	0	0	0	1	1	0	3	2	1	1	1	3	0	4	0	0	2	1	0
E. oleolucens	3	1	1	1	1	0	0	0	0	0	0	1	2	2	2	1	0	0	3	0	1	0	-	-	0	0
E. intersecta	2	1	1	1	1	1	0	0	0	2	2	0	0	2	2	1	1	0	3	0	4	0	0	2	1	0
E. bigibba	2	1	1	0	1	0	0	0	0	1	3	1	2	2	1	2	1	0	2	0	2	-	1	2	0	0
E. decorata	0	1	0 1	0	1	0	1	1	1	1	1	0	2	2	1	2	1	0	2	0	2	-	1	2	0	0
E. deceptrix	0	1	1	1	0	0	0	0	0	0	0	1	0	3	2	1	1	0	3	0	4	1	0	2	1	0
E. viridis	0	1	1	0	1	1	1	1	1	1	3	0	2	2	1	2	1	0	2	0	2	-	1	2	0	0
E. trinotata	3	1	1	0	1	0	0	0	0	0	0	1	2	3	1	1	1	0	1	0	1	3	0	1	0	0