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Phylogenetic analysis of the tribe Dufouriini (Diptera: Tachinidae) using a total evidence approach based on adult and immature stages

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Abstract

Dufouriini are a worldwide distributed tachinid tribe comprised of 51 species in 13 genera, made up of parasitoids of adult Coleoptera. The systematic positioning of Dufouriini has been controversial. Currently, it is placed within Dexiinae, but was previously placed in Phasiinae and Voriinae, and has even had the status of subfamily. Delimitation and composition of Dufouriini has also been debated, whether it is a single tribe or divided into two (Dufouriini and Freraeini) or three (Dufouriini, Oestrophasiini and Freraeini) tribes. Herein, we present the first phylogenetic analysis of Dufouriini based on total evidence using morphological data from adult and immature stages. The taxonomic sampling included all genera in Dufouriini (including Oestrophasiini) and also the genus belonging to Freraeini, a historically related tribe. Data matrix comprised 35 species and 22 genera in the ingroup, and 185 characters constructed from eggs, first instar larvae, puparia and adults, including female and male terminalia and spermathecae. The phylogenetic analysis recovered Dexiinae as paraphyletic in relation to Phasiinae, since the clade (Freraeini (Dufouriini + Oestrophasiini)) is more closely related to Phasiinae than Dexiinae. Dufouriini, Oestrophasiini and Freraeini are recovered as separate monophyletic tribes, strongly supported by a number of synapomorphies. Oestrophasiini is revalidated. A new synonymy is proposed: *Comyopsis* Townsend **syn. nov.** of *Ebenia* Macquart. Accordingly, *Ebenia fumata* (Townsend, 1919) is *nomen preoccupatum* by *Ebenia fumata* (Wulp, 1891), thus we change its specific epithet by designation of the new replacement name *Ebenia neofumata* Santis and Nihei [*nomen novum*]. The genera *Mesnilana* and *Rhinophoroides* are removed from Dufouriini and tentatively placed into Palpostomatini. Finally, *Cenosoma* **stat. rev**., previously a subgenus of *Oestrophasia*, is revalidated as genus.

Keywords

cladistics, Dexiinae, larvae, morphology, puparium

1. Introduction

Tachinidae is one of the largest Diptera families, with 8547 described species worldwide (O'Hara et al. 2020). Four subfamilies have traditionally been recognized in Tachinidae: Exoristinae, Phasiinae, Tachininae and Dexiinae (Herting and Dely-Draskovits 1993; Tschorsnig and Richter 1998; O'Hara and Wood 2004; Cerretti et

Table 1. Genera belonging to Dufouriini sensu lato (Dufouriini + Oestrophasiini) prior to the present study.

Genus	Geographic distribution
Chetoptilia Rondani, 1862	Afrotropical, Australasian, Palaearctic, Oriental
Comyops Wulp, 1891	Neotropical
Comyopsis Townsend, 1919	Neotropical
Dufouria Robineau-Desvoidy, 1830	Nearctic, Palaearctic
Ebenia Macquart, 1846	Neotropical
Eugymnopeza Townsend, 1933	Palaearctic
Euoestrophasia Townsend, 1892	Neotropical
Jamacaria Curran, 1928	Neotropical
Mesnilana Emden, 1945	Afrotropical
Microsoma Macquart 1855	Palaearctic
Oestrophasia Brauer and Bergenstamm, 1889	Nearctic, Neotropical
Pandelleia Villeneuve, 1907	Afrotropical, Palaearctic
Rhinophoroides Barraclough, 2005	Afrotropical
Rondania Robineau-Desvoidy, 1850	Australasian, Palaearctic, Nearctic

al. 2014; Stireman et al. 2019), although two other subfamilies have been proposed: Voriinae (Mesnil 1966; Richter 1987) and Dufouriinae (Verbeke 1962; Crosskey 1976, 1980). Of these, only Dexiinae were supported by a traditionally putative synapomorphy found in the male genitalia – an aedeagus with basiphallus and distiphallus articulated to each other (Tschorsnig 1985; Verbeke 1962; Wood 1987). However, in the first and only morphological phylogeny of Tachinidae by Cerretti et al. (2014), this feature did not prove to be a synapomorphy. Additionally, while the subfamily Phasiinae was recovered as monophyletic, Dexiinae, Exoristinae and Tachininae were recovered as paraphyletic. In the comprehensive molecular phylogeny of Tachinidae by Stireman et al. (2019), Phasiinae, Exoristinae and Dexiinae were recovered as monophyletic and Tachininae as paraphyletic.

The subfamily Dexiinae are a large and morphologically diverse group that is distributed worldwide, whose larvae predominantly parasitizes Coleoptera or Lepidoptera immatures. It contains 1323 species in 259 genera and approximately 12 tribes (Cantrell and Crosskey 1989; Crosskey 1976; Guimarães 1971; Herting and Dely-Daskovits 1994; O'Hara and Wood 2004; O'Hara and Cerretti 2016). The paraphyly of Dexiinae in Cerretti et al. (2014) occurred because Dufouriini was more closely related to Phasiinae than to the rest of Dexiinae and Eutherini were placed within Exoristinae. Sampled with the following genera: *Chetoptilia*, *Dufouria*, *Rondania*, *Pandelleia*, *Freraea* and *Eugymnopeza*, Dufouriini was recovered as paraphyletic. Five (all Palaearctic) out of 12 tribes of Dexiinae were sampled and only Dexiini and Eutherini (within Exoristinae) were recovered as monophyletic. In the molecular phylogeny of Phasiinae by Blaschke et al. (2018), Dexiinae was recovered as monophyletic and sister group to Phasiinae. However, no taxa from Dufouriini and Freraeini (and the formerly recognized tribe Oestrophasiini) were sampled in that analysis. Stireman et al. (2019) also supported Dexiinae as monophyletic. Dufouriini was sampled with one species each of *Oestrophasia*, *Rondania*, *Microsoma*, *Dufouria* and *Ebenia*. Freraeini was considered a distinct monotypic tribe, separated from Dufouriini by its type genus *Freraea*. The resulting tree showed a polyphyletic Dufouriini split into two groups: 1) with *Microsoma* forming a clade with Freraeini, sister group of some Palpostomatini genera; and 2) the other genera of Dufouriini (*Oestrophasia*, *Rondania*, *Dufouria* and *Ebenia*) forming a clade nested with some clades of Voriini and Telothyriini. The authors stressed that the tribal classification of Dexiinae requires major revision and that the phylogenetic resolution was unsatisfying in several parts of the tree.

Dufouriini is one of the 12 tribes of Dexiinae, distributed worldwide, and is composed of 52 species in 13 genera (Table 1), including its last described species: *Pandelleia crosskeyi* Santis and Nihei, 2021. This large and heterogenous (Figs 1, 2) tribe was expanded by Tschorsnig (1985), who incorporated taxa from the formerly valid tribe Oestrophasiini (Figs 1A, 2A). Additionally, the tribe Freraeini (Figs 1C, 2C), initially composed with *Freraea* and *Eugymnopeza* (Mesnil, 1975), had its members included in Dufouriini by Herting (1984), because he considered a broad definition of this tribe that put Freraeini in synonymy with Dufouriini. Yet, O'Hara and Wood (2004) preferred to consider Freraeini as valid, but only with *Freraea*, by arguing over the differences in the male and female genitalia with Dufouriini, a position maintained by O'Hara et al. (2020). Dufouriini (Figs 1B, 2B) have experienced several changes in its systematic position and have been included in four different subfamilies by different authors: **(1)** In Phasiinae: Mesnil (1939) revived Robineau-Desvoidy's Dufouriidae group (1830), with subtribe Dufouriina of Phasiini, while Emden (1945) raised this subtribe to tribe (Dufouriini). **(2)** In Dexiinae: Herting (1957) constructed a new concept of Dexiinae with Dufouriini as tribe because of the absence of syntergite 9 + 10 and later Herting (1984) disagreed with Mesnil (1975) and considered his subtribes Dufouriina and Freraeina in a single group Dufouriini (agreeing somehow with Verbeke). Tschorsnig (1985) defined Dexiinae with putative synapomorphies of the basiphallus and distiphallus and included Oestrophasii-

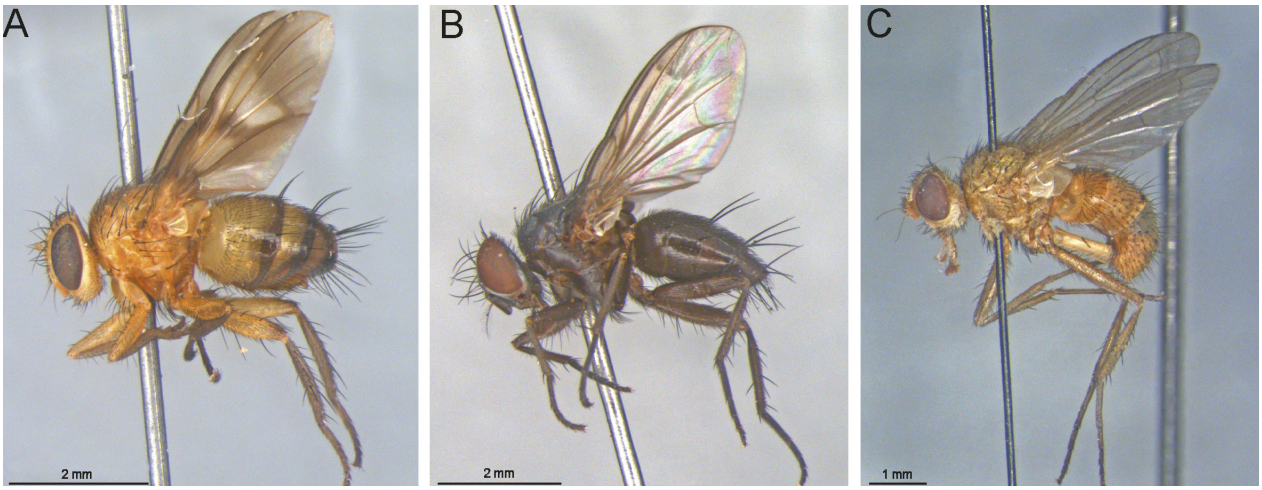

Figure 1. Habitus images of representative taxa used in the phylogenetic analysis. **A**: Oestrophasiini, *Euoestrophasia* sp. ♀; **B**: Dufouriini, *Ebenia* sp. ♀; **C**: Freraeini, *Pandelleia otiorrhynchi* Villeneuve, 1922 ♀.

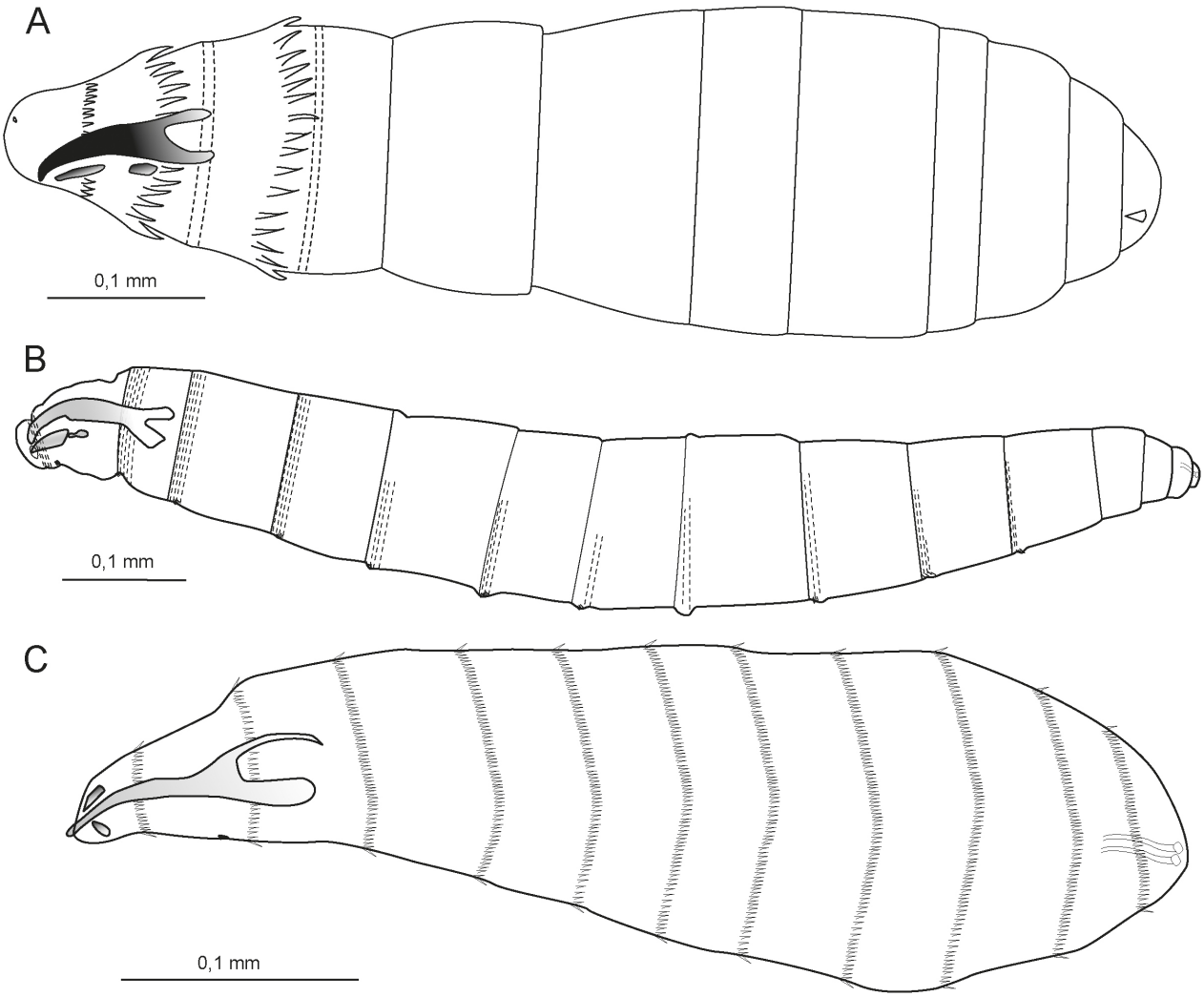

Figure 2. First instar larvar of representative taxa used in the phylogenetic analysis. **A**: Oestrophasiini, *Oestrophasia calva* Coquillett, 1902; **B**: Dufouriini, *Chetoptilia puella* (Rondani, 1962); **C**: Freraeini, *Microsoma exiguum* (Meigen, 1824).

ni within Dufouriini. Since then, this concept has been maintained by subsequent authors and is followed herein (O'Hara and Wood 2004; Cantrell and Burwell 2010; Cerretti et al. 2014; O'Hara and Cerretti 2016; Stireman et al. 2019; O'Hara et al. 2020; Table 1). **(3)** In Dufouriinae: Verbeke (1962, 1963) raised Dufouriini to subfamily rank and considered it phylogenetically close to Phasiinae; Crosskey (1976, 1980) considered Dufouriinae

with only Imitomyiini and Dufouriini and argued that they do not belong to either Dexiinae or Phasiinae. **(4)** In Voriinae: Mesnil (1966) divided the group into three subtribes of Voriini: Dufouriina (with only *Dufouria*), Campogastrina (with five genera: *Chetoptilia*, *Afrophasia* (= *Pandelleia*), *Pandelleia*, *Rondania* and *Microsoma*) and Freraeina (using the same concept of Freraeini sensu Townsend (1936), with *Eugymnopeza* Townsend and *Freraea*). Richter (1987) proposed a classification that was very similar to that of Mesnil (1966), with Dufouriini as part of Voriinae.

The current concept of Dufouriini (Table 1) (= after Herting 1984; Tschorsnig 1985; Cantrell and Burwell 2010; O'Hara and Wood 2004; Cerretti et. al. 2014; Stireman et al. 2019; O'Hara et al. 2020), called "sensu lato" herein, includes the genera that were historically recognized in the tribe, as well as the genera from Oestrophasiini, and *Eugymnopeza*, *Microsoma* and *Pandelleia*. Although most of the Palaearctic Dufouriini and Neotropical genera belonging to former Oestrophasiini are well delimited and revised, their phylogenetic relationships are poorly resolved and suprageneric delimitations are unclear. For all recorded species, members of Dufouriini are characterized as parasitoids of adult beetles. Most genera present modified ovipositors with diverse forms (Herting 1957) to parasitize their hosts through different strategies, e.g., perforating the epithelium to introduce larvae in natural openings as in *Microsoma*; using its ovipositor to inject first instar larvae directly into the mouth of its host (Fluiter and Blijdorp 1935) as in *Rondania*; and depositing microtype eggs into leaves that are swallowed by the host as in *Oestrophasia* (*Cenosoma*) sp. (Grillo and Alvarez 1984).

In Tachinidae systematics, adult morphology (excluding male or female terminalia) had initially been used as the primary, and in most cases, unique criterium for their classification (e.g., Villeneuve 1924; Mesnil 1939). Later, male terminalia had its taxonomic value accepted and progressively added, becoming ever since one of the most important character sources in Tachinidae (e.g., Verbeke 1962, 1963; Tschorsnig 1985), as has occurred for many other insect groups (Song and Bucheli 2010). The use of different character sources other than adult morphology and male terminalia has been revealed and encouraged by a number of authors over time for Tachinidae systematics. The relevance of larval morphology as a valuable source of data for the classification of tachinids was discussed in several articles by Thompson (1914–1967). While Herting (1957) was the first to emphasize the importance of female terminalia and eggs. Considering the importance of Thompson and Herting's discoveries, Mesnil (1966) recognized that an appropriate classification of Tachinidae would only be possible using other data sources, and then revised his early classification using characters from larvae, and male and female terminalia. Later, Herting (1983) discussed the main groups of Tachinidae and concluded that (p. 2 therein): "The most reliable indicators of phylogenetic relationships appear to be the biologically-adaptive characteristics that are pronounced in the female ovipositor, in the structure of the egg membrane and the morphology of the first ínstar larva". In a comprehensive study, Ziegler (1998) described and discussed the phylogenetic significance of characters from puparia and larval cephaloskeleton for 261 tachinid species, defining putative synapomorphies for the family and, whenever possible, for tribes. Barraclough (1992: p.1149), reinforced these viewpoints by stating: "This broad-based approach is preferable, since it is particularly unwise in the Tachinidae to give undue weighting to particular characters or suites of characters.".

In the present study, we carried out a phylogenetic analysis including a complete sampling of all genera belonging to Dufouriini and all genera belonging to Freraeini, a tribe that has historically been related to and controversial for Dufouriini, in order to clarify the internal relationships and monophyly of the tribe Dufouriini and its supra-tribal relationships. Our phylogenetic analysis was based on Hennig's concept of holomorphology (Hennig 1966), i.e., the integration of data from different life cycle stages (semaphoronts), embodied in the light of the 'requirement of total evidence'. This requires that all relevant evidence be used for an appropriate inductive or abductive inference (Fitzhugh 2006). Therefore, the higher the number and more sources of characters, the greater the degree of being a natural group, i.e., ontologically realistic taxa (Rieppel 2005). We examined a large number of morphological characters from adult (external morphology, male and female terminalia, spermathecae) and immature stages (e.g., eggs, larvae, puparia). Herein, morphology from the puparia is included in a phylogenetic analysis of Tachinidae for the first time.

2. Material and methods

2.1. Studied material

A total of 223 specimens were examined belonging to the following institutions: **ARC** – Arthropod Research Collection, Michigan State University, Michigan, USA; **DZUP** – Coleção de Entomologia Pe. Jesus Santiago Moure, Curitiba, Brazil; **MNCR** – Museo Nacional de Costa Rica [formerly Instituto Nacional de Biodiversidad – INBio], Santo Domingo de Heredia, Costa Rica; **MZSP** – Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil; **NHMUK** – Natural History Museum, London, England; **ZMHB** – Berlin Museum für Naturkunde der Humboldt-Universität, Berlin, Germany. Other repositories cited in the text are: **NMSA** – Natal Museum, Department of Arthropoda, Pietermaritzburg, South Africa; and **USNM** – National Museum of Natural History, Washington DC, USA. Specimens collected in the Brazilian states of Mato Grosso, Mato Grosso do Sul and Rondônia from the SISBIOTA-Diptera Project (CNPq-FAPESP), coordinator Carlos Lamas, vice-coordinator Silvio Nihei, were also examined.

2.2. Morphological study and terminology

To study adult morphology, dried and pinned specimens were examined under a Leica EZ4 stereomicroscope. A Leica DM2500 optical microscope was used to analyze the postabdomen, first instar larvae and spermathecae.

To study the male postabdomen, the specimens were carefully dissected from the fifth segment to avoid damaging the sixth tergite and to maintain the integrity of the abdomen as much as possible. To study the female abdomen and obtain the spermathecae, first instar larvae and/or eggs, the abdomen was dissected from the fourth segment and rarely in the third. The male terminalia were bleached in 10% potassium hydroxide solution (KOH) for four minutes in boiling water, neutralized with 5% acetic acid solution and washed with water. The female terminalia, larvae, spermathecae and eggs were subjected to a similar procedure, except they were heated for 10 minutes in 10% KOH solution. At the end of the procedure, the material was preserved in glycerin, packed in microplastic vials and pinned with the respective specimen.

The terminology of adult and spermathecae morphology follows Cumming and Wood (2017). The terminology used for the wing structure and trace of M_2 vein is taken from Crosskey (1976). For male terminalia, we follow Tschorsnig (1985). The terminology of the first instar larva follows Thompson (1963), with some modifications discussed by Cantrell (1988). The term "cephaloskeleton" from Courtney et al. (2000) was used. The terminology for the puparium follows Ziegler (1998) and that for the eggs follows Gaponov (2003).

2.3. Selection of taxa

To select the terminals of the ingroup, three premises were considered: (1) the availability of adult specimens for morphological study; (2) the availability of immature stage material (e.g., first instar larvae); and (3) differences in geographic distribution and morphology. All 13 genera included in Dufouriini (sensu Herting 1984) (Table 1) were studied, and 11 were sampled, including those genera from the formerly valid Oestrophasiini. Additionally, the sole genus currently assigned to Freraeini (*Freraea*) (O'Hara et al. 2020) was included, as it has historically been related to Dufouriini. The ingroup included 26 species from 13 genera. In light of the results of Cerretti et al. (2014) (where Dexiinae is paraphyletic in relation to Phasiinae) and Stireman et al. (2019) (Dufouriini polyphyletic, split into two lineages), some additional representative tribes were chosen as outgroup taxa. The basis of paraphyly of Dexiinae derived from historically problematic taxa: Strongygastrini, Imitomyiini and Catharosiini, besides other Phasiinae (*Cylindromyia*) were included. We also included species of Dexiini and Voriini concerning Dufouriini monophyly and relationships. *Xanthozona* (Tachinini) was selected as the root for the analyses. The outgroup included a total of nine species from Phasiinae, Dexiinae and Tachininae. Supplementary file 1 shows the terminals included in the cladistic analysis with geographical distribution, data source and observed structures (whether personal observation or literature data).

2.4. Phylogenetic analysis and character coding

The study of phylogenetic relationships was based on morphological characters of adults (including female and male genitalia and spermathecae), first instar larva, egg and puparium, which was based on parsimony as the optimality criterion. Whenever possible, the characters were constructed according to the proposal of Sereno (2007), with preference for the contingent coding (Forey and Kitching 2000). Characters are scored with "–" in case of inapplicability (usually taxon lacking the character-bearing structure), and with "?" in case of lacking observation. The data and putative synapomorphies of the male terminalia presented by Tschorsnig (1985) were reanalyzed and included within a cladistic framework. Characters from the literature, e.g., Cerretti et al. (2014), have been properly indicated in the character list.

The polarization was conducted using the method of outgroup comparison (Nixon and Carpenter 1993). The matrix of characters was built with Mesquite 3.04 software (Madison and Madison 2015). For the parsimony analysis using equal and implied weighing, the TNT 1.1 software (Goloboff et al. 2008) and the strategies of the New Search Technology (Ratchet, Drift, Tree Fusion and Sectorial Searches) were used. The analysis was performed according to the following parameters: random seed = 1; number of replicates = 10,000; number of trees saved per replication = 10. The software Winclada 1.00.08 (Nixon 2002) was used to display the trees with the transformation series of each character, in addition to its optimization. For the MP tree under equal weights, we provide the total length (L), the consistency index (CI) (Kluge and Farris 1969) and the retention index (RI) (Farris 1989), calculated from all characters.

The parsimony criterion of Fitch (1971), which treats the characters as unordered (or non-additive), was used in this study. Autapomorphic characters of single terminals were maintained in the analysis because they are part of cladistic results (Yeates 1992). Implied weighting (Goloboff 1993) was used to observe how the characters behave in different weighing schemes, based on the fit measure of each character and its overall fit of the topology. The k-values of 1, 2, 3, 5 and 10 were tested. Branch support was checked using Bremer support (1994), with the "Bremer.run" script provided in the TNT Software Wiki (http://phylo.wdfiles.com).

Character optimization is often performed following the proposal of De Pinna (1991), which argues that ACCTRAN is preferable to DELTRAN because it preserves more primary homology hypotheses of. However, Agnarsson and Miller (2008) argue that they do not see theoretical components that make ACCTRAN more preferable than DELTRAN. Amorim (2002) argues that it is

more reasonable to analyze the evolution of the characters case by case and to explicitly explain the reason for using ACCTRAN or DELTRAN rather than using only one optimization for all characters. Thus, in some cases (e.g., when there are terminals with non-observable or inapplicable state) ACCTRAN would consider it a spurious synapomorphy, whereas DELTRAN does not perform this transformation, considering an apomorphy for the taxa that have the given state only. Thus, it is safer to adopt the latter. The preference of each optimization was explicitly indicated in the character list.

2.5. Illustration

Most characters were illustrated using photographs and line drawings to facilitate identification of different character states. The photographs were taken with a Leica DFC420 digital camera coupled to a Leica MZ16 stereomicroscope. The images were obtained through the software LAS V4.1, then stacked in the software Helicon Focus 5.3.14 and edited in the software Adobe Photoshop CS6 and Adobe Illustrator CS6. The microphotographs of eggs and puparia were processed in Balzers CPD 030, and later were metallized in the Balzers SCD 050 for analysis using the scanning electron microscope, Zeiss DSM 940. In addition, drawings were made using the Leica DM2500 optical microscope with its coupled camera. Subsequently, these drawings were vectored and edited in Adobe Illustrator CS6 software.

3. Results

3.1. List of characters used in the cladistic analysis

A total of 185 characters were constructed, 5 of the egg, 22 of the first instar larva, 7 of the puparium (posterior spiracle), 67 of the external morphology (except terminalia) 53 of the male terminalia, 23 of the female terminalia, and 8 of the spermatheca. The optimizations of the ambiguous characters will be discussed. When relevant, comments will be made for some characters. The characters from literature will be properly referenced with the statement of the author and/or first observer.

EGG

1. **Eggs**: membranous (0); macrotype (Fig. 3A) (1); microtype (Fig. 3B) (2). — Egg types are morphologically and functionally defined (Gaponov 2003) and traditionally used for delineation of some groups, e.g., macrotype in Phasiinae and Exoristini and microtype in Goniini (Herting 1960; Gaponov 2003). Following the ideas of Townsend (1934) and Mesnil (1966), for the first time, microtype eggs was shown to be found outside the Exoristinae (Goniini and some Blondeliini), in the tribe Oestrophasiini, and this character is resolved as a synapomorphy for this tribe, confirming the importance of eggs for the classification of Tachinidae. — L = 2; CI = 100; RI = 100.
2. **Microtype egg, stalk with hooks**: absent (0); present (Fig. 3C) (1). — The presence of this stalks with hooks of unknown function, is only found in the genus *Euoestrophasia*. — L = 1; CI = 100; RI = 100.
3. **Microtype egg, chorion surface**: smooth (Fig. 3B) (0); prominent, i.e., with reticulate surface (1); polygonal network (Fig. 4A) (2). — Ambiguous character, however ACCTRAN or DELTRAN optimization are shown to be equal in this clade, i.e., state 1 is synapomorphic for *Euoestrophasia* and 2 is synapomorphic for *Oestrophasia*. We chose DELTRAN in this case. — L = 2; CI = 100; RI = 100.
4. **Microtype egg, exochorion, pigmentation**: without pigmentation (Fig. 4C) (0); with pigmentation (Fig. 4B) (1). — Ambiguous character, however ACCTRAN or DELTRAN optimization are shown to be equal in this clade, i.e., state 1 is synapomorphic for *Oestrophasia*. We chose DELTRAN in this case. — L = 1; CI = 100; RI = 100.
5. **Microtype egg, pores**: present (Fig. 4A) (0); absent (1). — Ambiguous character, however ACCTRAN or DELTRAN optimization are shown to be equal in this clade, i.e., state 1 is synapomorphic for clade 11. We chose DELTRAN in this case. — L = 1; CI = 100; RI = 100.

LARVA (1st instar)

6. **Short rod-shaped sensorium, dorsally**: absent (0); present (1) (Fig. 5C). — Character after Thompson (1954); this structure is found only in *Strongygaster*. — L = 1; non-informative.
7. **Dermal cuticle, type**: dark-colored plates and scales (Fig. 5A) (0); colorless with a weak granular-scale structure (Fig. 5B) (1); spiniform (Fig. 5C) (2). — The type of dermal cuticle comprises important biological characteristics in relation to form of host infection. State 0 is found in *Xanthozona* and in species that perform the sit-and-wait strategy to find the host, which is often a Lepidopteran larva, and as soon as this larva moves, the Lepidopteran larva is infected; in addition, during this time the first instar larvae does not suffer desiccation while waiting for its host because the larva has these dermal plates. State 1 is found in *Billaea*, *Dexia* and *Prophorostoma*, and is characteristic of Dexiini (clade 2), which actively seek their host, mostly larvae of beetles and such granular scales help in this search, providing friction against the substrate, which may be the ground or within trunks of plants. State 2 is found in many other tachinids, where larvae do not undergo major morphological modifications, possessing several other forms of host infection. Ambiguous character, however ACCTRAN or DELTRAN optimization are shown to be equal in this case, i.e., state

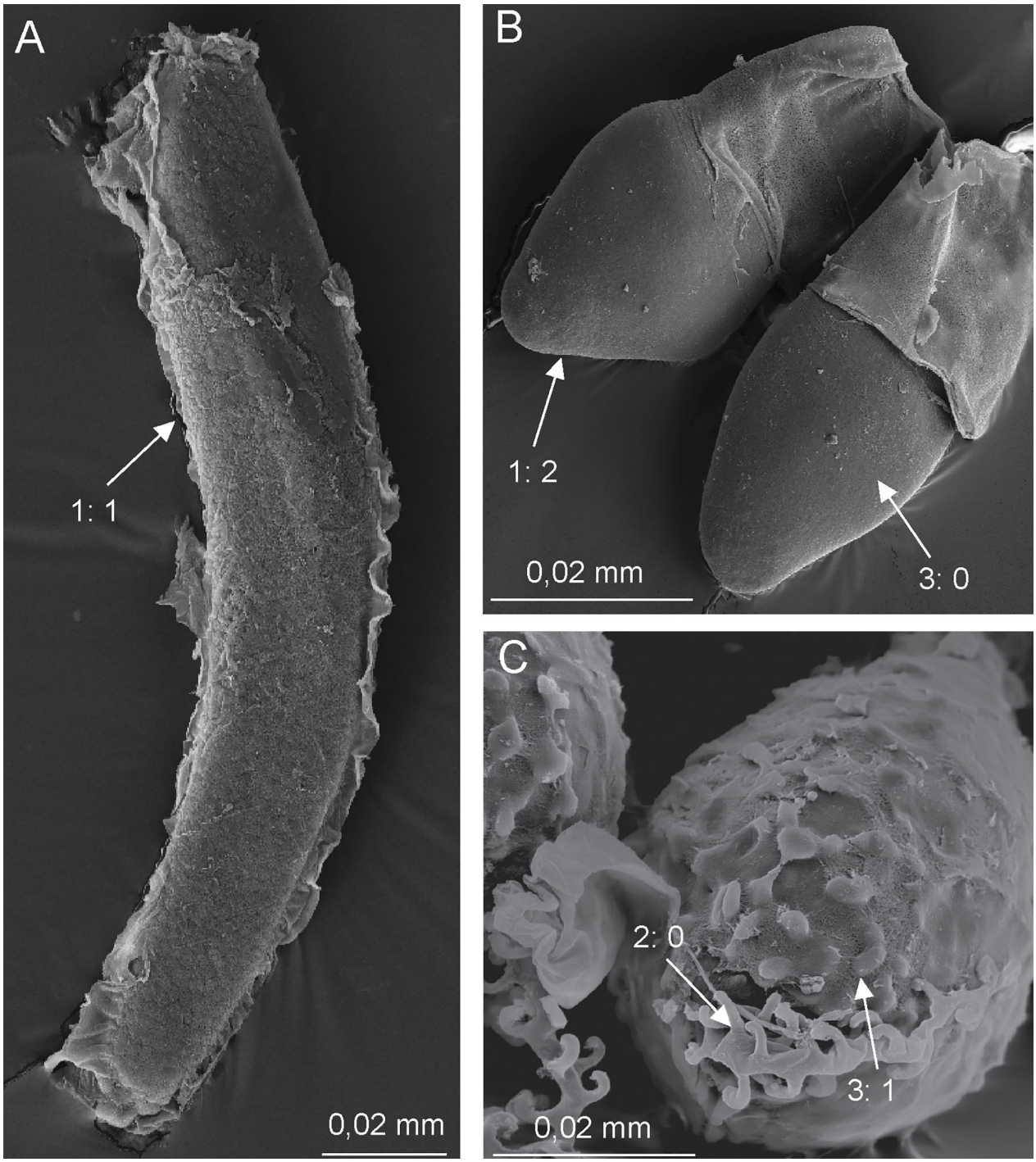

Figure 3. Transmission electron microscopy of eggs. **A**: *Strongygaster triangulifera* (Loew, 1863); **B**: *Cenosoma thompsoni* Guimarães, 1977; **C**: *Euoestrophasia plaumanni* Guimarães, 1977. Arrows identify characters and states (enclosed in parentheses) discussed in text.

2 is synapomorphic for clade 4 and (*Voria* (*Dexia* (*Billaea* + *Prophorostoma*))). We chose DELTRAN in this case. — L = 2; CI = 100; RI = 100.

8. **Segment I, antenna**: absent (0); present and developed (1); present, but reduced (Fig. 6B) (2). — Ambiguous character, however ACCTRAN or DELTRAN optimization are shown to be equal in this case, i.e., state 1 is synapomorphic of clade 4 + (*Voria* (*Dexia* (*Billaea* + *Prophorostoma*))). We chose DELTRAN in this case. — L = 4; CI = 50; RI = 80.

9. **Segment I, antenna shape**: flattened (Fig. 6C) (0); convex, i.e., small bump (1); conical (Fig. 5B) (2). — Ambiguous character. When ACCTRAN optimization is performed, state 2 becomes a synapomorphy of clade 5 which contains Freraeni, Oestrophasiini and Dufouriini s.s., but in Oestrophasiini and Freraeini it is coded as not applicable "-", and in this optimization this synapomorphy is spurious. When the DELTRAN optimization is performed, this state appears as a synapomorphy of *Ebenia*, *Dufouria* and *Chetoptilia* (clade 15), reflecting the

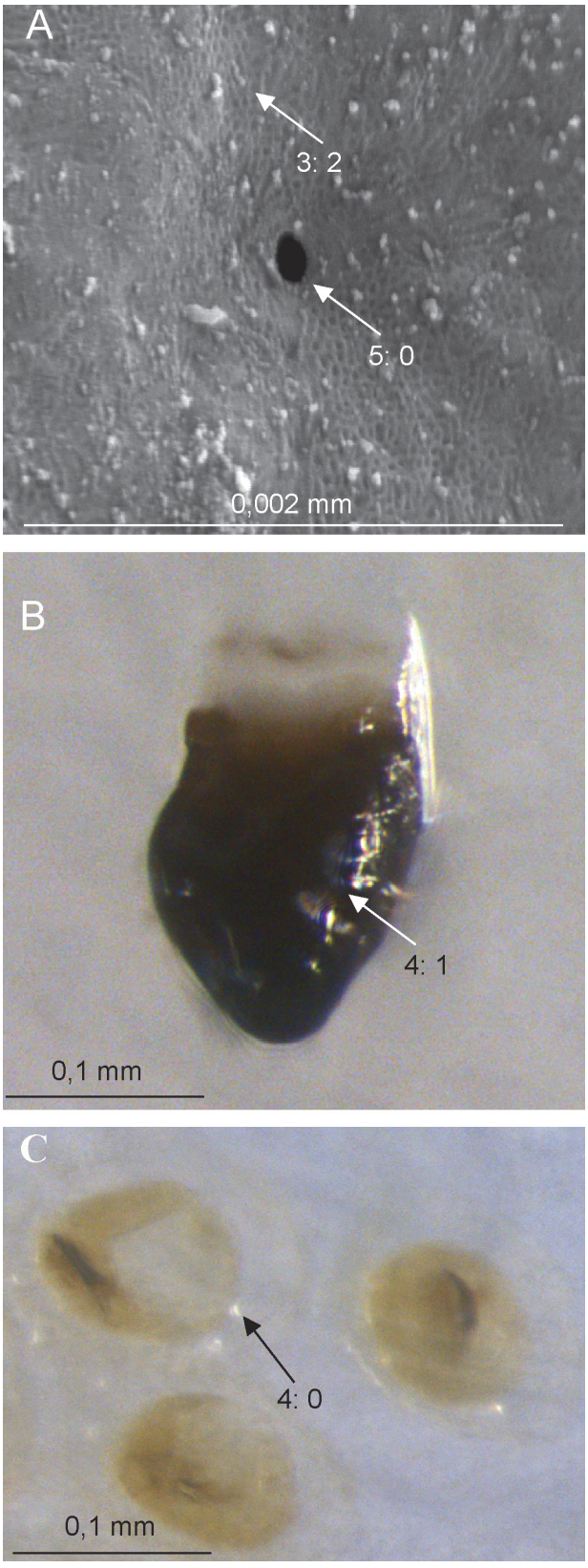

Figure 4. Egg characters. **A** (transmission electron microscopy), **B**: *Oestrophasia calva* Coquillett, 1902; **C**: *Euoestrophasia plaumanni* Guimarães, 1977. Arrows identify characters and states (enclosed in parentheses) discussed in text.

correct coding, with no spurious results, so DELTRAN was used in this case. — L = 2; CI = 100; RI = 100.

10. **Segment I, dorsal sclerotized structure**: absent (0); present (Fig. 6A) (1). — State 1 is autapomorphic *Freraea*. — L = 1; non-informative.
11. **Segment I, spines**: present (Fig. 6B) (0); absent (1). — L = 2; CI = 50; RI = 75.
12. **Segment I-XII, creeping welts or spines**: absent (Fig. 6C) (0); present (Fig. 6A) (1). — L = 1; CI = 100; RI = 100.
13. **Segment II, spines, development in relation to length of adjacent microtrichia**: twice length (Fig. 6B) (0); thrice length (Fig. 6A) (1). — L = 1; CI = 100; RI = 100.
14. **Segment IV, microtrichia**: present (0); absent (1). — L = 2; CI = 50; RI = 50.
15. **Segment V, spines, localization**: dorsal and ventral (0); ventral (1). — L = 2; CI = 50; RI = 0.
16. **Segment XII, shape**: rounded (Fig. 6B) (0); conical (Fig. 6C) (1). — Ambiguous character. In ACCTRAN optimization state 1 is a synapomorphy for Dufouriini s.s., but since there is no known larval data for *Rondania*, this synapomorphy becomes spurious. In DELTRAN this state becomes a synapomorphy of clade 14 formed by *Ebenia*, *Dufouria*, *Chetoptilia* and *Comyops*, representing the codification for this character, therefore being preferred. — L = 1; CI = 100; RI = 100.
17. **Segment XII, pseudopods**: absent, (0); present (1) (Fig. 5C). — Character after Thompson (1954). State 1 autapomorphic for *Strongygaster*. — L = 1; non-informative.
18. **Segment XII, sensorial stylus**: absent (0); present (1) (Thompson 1960: fig. 2). — L = 2; CI = 50; RI = 0.
19. **Posterior spiracle, felt chambers, shape**: tubular (Fig. 6A) (0); conical (Fig. 6B) (1); vestigial (reduced) (2). — L = 2; CI = 100; RI = 100.
20. **Cephaloskeleton, sclerite of salivary gland, shape**: reduced to narrow strip (Fig. 5A) (0); narrow anteriorly, wide posteriorly (Fig. 7C) (1); oval (Fig. 7B) (2); rectangular (Fig. 7A) (3). — Ambiguous character. In ACCTRAN optimization, state 2 is a synapomorphy for Dufouriini s.s. In DELTRAN, this state is a synapomorphy of clade 14, formed by *Ebenia*, *Dufouria*, *Chetoptilia* and *Comyops*, representing the codification for this character, therefore being preferred. — L = 3; CI = 100; RI = 100.
21. **Cephaloskeleton, accessory sclerite, shape**: narrow anteriorly, wide posteriorly (Fig. 5A) (0); reduced to narrow strip (Fig. 7B) (1); triangular (Fig. 7A) (2); unciform (Fig. 5C) (3); falciform (Fig. 7C) (4). — Ambiguous character. In ACCTRAN optimization, state 4 is a synapomorphy for Dufouriini s.s. In DELTRAN, this state becomes a synapomorphy of clade 14, formed by *Ebenia*, *Dufouria*, *Chetoptilia* and *Comyops*, representing the codification for this character, therefore being preferred. — L = 4; CI = 100; RI = 100.
22. **Cephaloskeleton, mouthhook, shape**: truncate apically (0); unciform (Fig. 5B) (1). — L = 1; CI = 100; RI = 100.

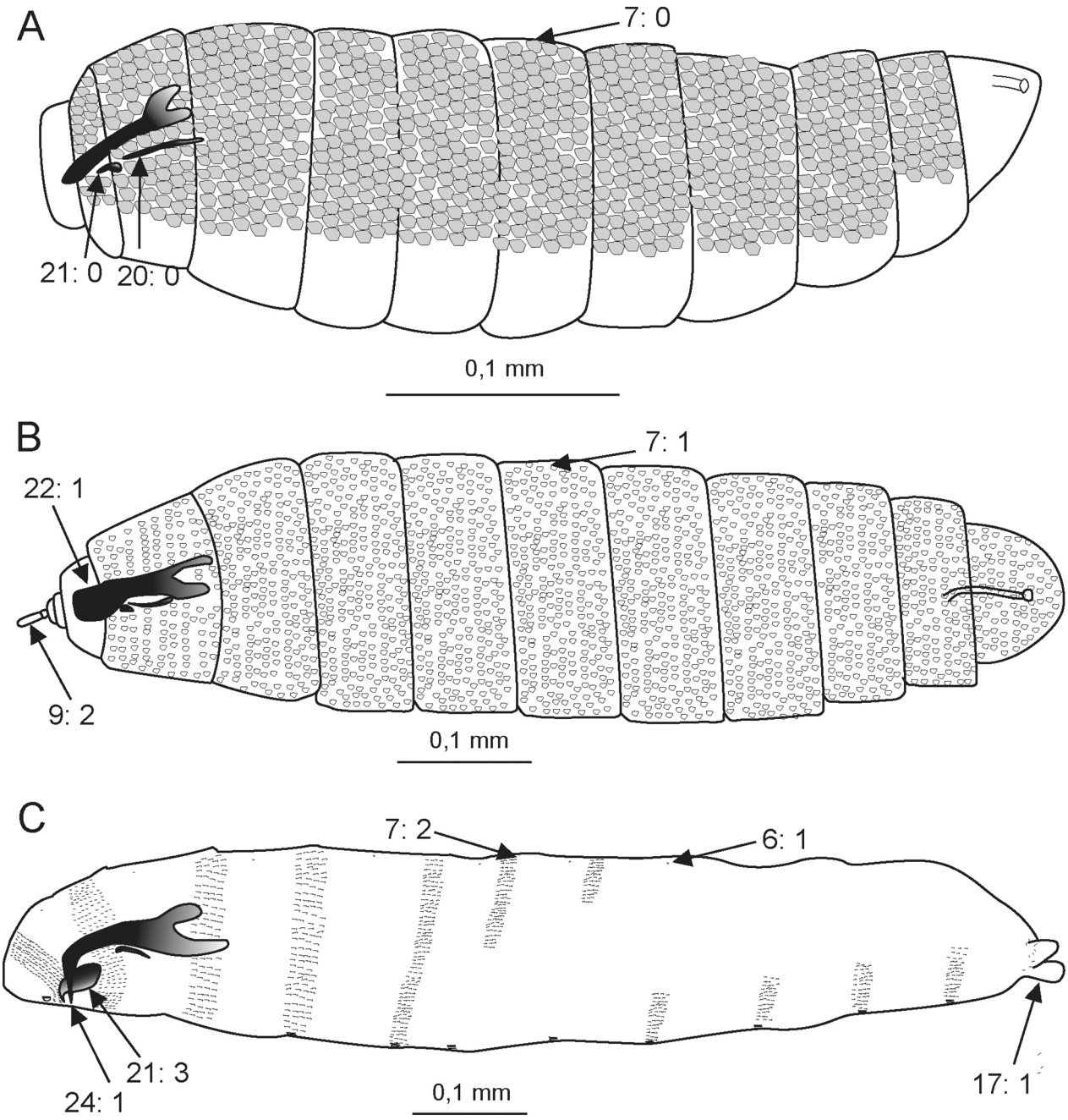

Figure 5. First instar larval characters. **A**: *Xanthozona melanopyga* (Wiedmann, 1830); **B**: *Prophorostoma pulchra* Townsend, 1927; **C**: *Strongygaster triangulifera* (Loew, 1863). Arrows identify characters and states (enclosed in parentheses) discussed in text.

23. **Cephaloskeleton, mouthhook, width of base in relation to dorsal cornu**: broader (Fig. 7B) (0); same width (Fig. 7A) (1). — L = 1; CI = 100; RI = 100.
24. **Cephaloskeleton, mouthhook, direction**: antero-ventral (0); ventral (Fig. 5C) (1). — State 1 is autapomorphic for *Strongygaster*. — L = 2; CI = 50; RI = 0.
25. **Cephaloskeleton, accessory sclerite, position with regard to sclerite of salivary gland**: apical (Fig. 7C) (0); ventral (Fig. 7A) (1). — L = 1; CI = 100; RI = 100.
26. **Cephaloskeleton, intermediate region, median enlargement (as a slope)**: absent (Fig. 7B) (0); present (Fig. 7C) (1). — Ambiguous character. In ACCTRAN optimization, state 1 is a synapomorphy for Dufouriini s.s., but *Rondania* does not have known larval data. In DELTRAN, this state is a synapomorphy of clade 14, formed by *Ebenia*, *Dufouria*, *Chetoptilia* and *Comyops*, representing the codification for this character, therefore being preferred. — L = 1; CI = 100; RI = 100.
27. **Cephaloskeleton, dorsal cornu, length compared to mouthhook**: longer (0); shorter (1). — L = 1; CI = 100; RI = 100.

PUPARIUM (posterior spiracle)

28. **Peritreme, paired structure divided into two parts, i.e., two ventrally and two dorsally**: absent

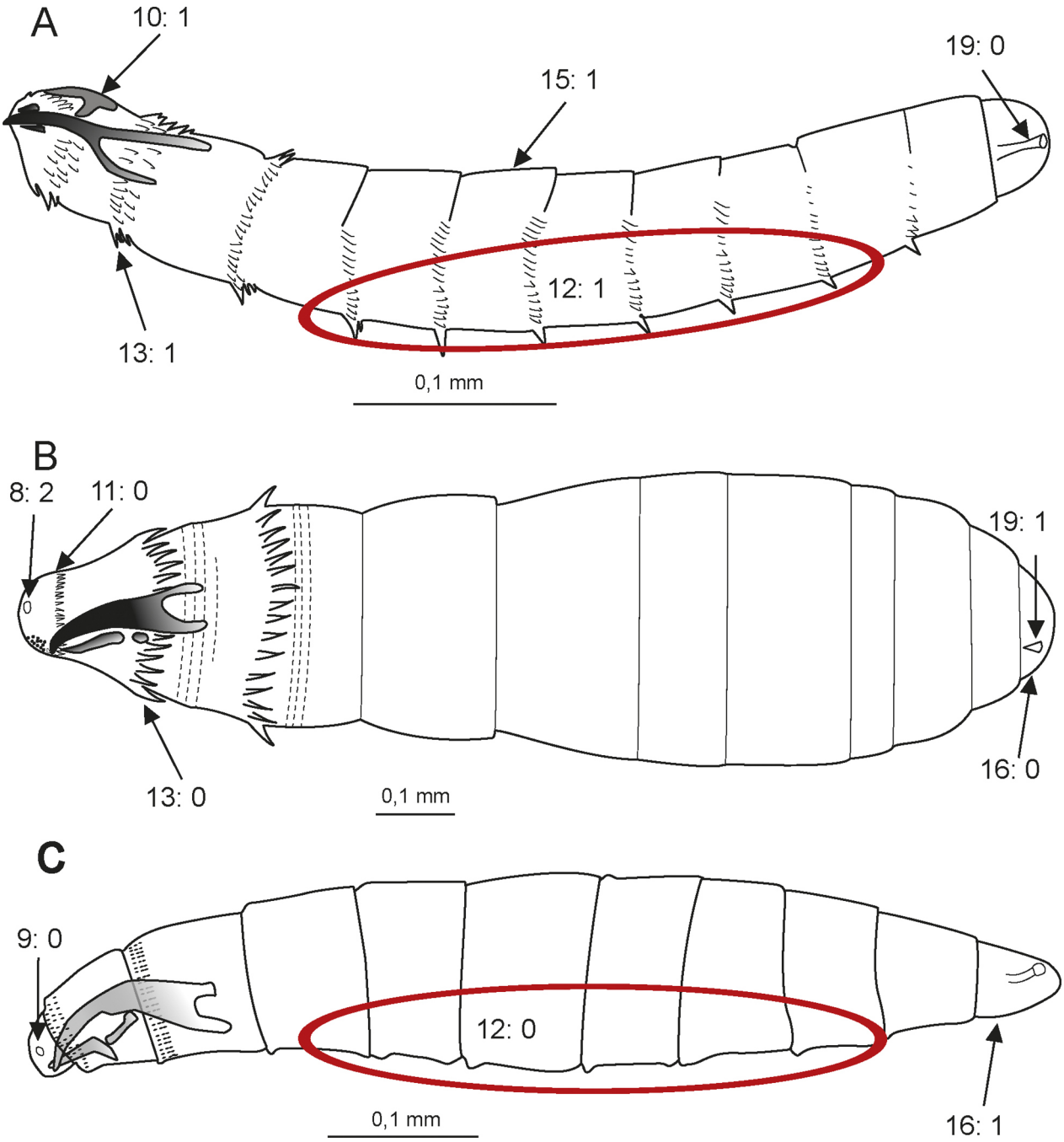

Figure 6. First instar larval characters. **A**: *Freraea gagatea* Robineau-Desvoidy, 1830; **B**: *Cenosoma thompsoni* Guimarães, 1977; **C**: *Dufouria chalybeata* (Meigen, 1824). Arrows and red circle identify characters and states (enclosed in parentheses) discussed in text.

(Thompson 1961: fig. 8) (0); present (Thompson 1961: fig. 11) (1). — State 1 is autapomorphic for *Voria*. — L = 1; non-informative.

29. **Spiracular plate, number of fusions**: 1 region (Draber-Monko 1994: figs 3, 7) (0); 2 regions (Rabaud and Thompson 1914: fig. 2) (1); 3 regions (Cerretti and Mei 2001: fig. 17) (2). — L = 5; CI = 40; RI = 40.
30. **Peritreme, completely fused (forming single structure, unpaired)**: absent (Draber-Monko 1994: fig. 4) (0); present (Fig. 8A) (1). — State 1 is synapomorphic for the tribe Oestrophasiini. The shape of the posterior spiracle (with fully fused peritreme) is unique among known tachinids. — L = 1; CI = 100; RI = 100.
31. **Spiracular opening ["Stigmenwulst" of Ziegler (1998)], hook shape structure**: absent (0); present (Thompson 1954: fig. 7) (1). — State 1 is autapomorphic for *Strongygaster*. — L = 2; non-informative.
32. **Spiracular opening ["Stigmenwulst" of Ziegler (1998)], shape**: undifferentiated (Rabaud and Thompson 1914: fig. 2) (0); rounded (Cerretti and Mei 2001: fig. 17) (1); elliptical (Draber-Monko 1994: fig. 6) (2); irregular (Fig. 8B), (3). — Ambiguous character. In ACCTRAN optimization, state 1 is a synapomorphy for Freaeini, but as the puparium

of *Pandelleia* is unknown. In DELTRAN, this state is a synapomorphy for the clade of *Freraea*, *Microsoma* and *Eugymnopeza* representing the codification for this character, therefore being preferred. — L = 5; CI = 60; RI = 80.

33. Spiracular opening, shape: sinuous (Rabaud and Thompson 1914: fig. 2) (0); arborescent (Fig. 8B) (1); round (Cerretti and Mei 2001: fig. 17) (2); rectilinear (Draber-Monko 1994: fig. 12) (3); small and irregular (Draber-Monko 1994: fig. 11) (4). — Ambiguous character. In ACCTRAN optimization, state 2 is a synapomorphy for Freaeini, but as the puparium of *Pandelleia* is unknown. In DELTRAN, this state becomes a synapomorphy for the clade of *Freraea*, *Microsoma* and *Eugymnopeza* representing the codification for this character, therefore being preferred. — L = 6; CI = 66; RI = 81.

34. Cicatrix, insertion position: peripheral (Fig. 8A) (0); central (1). — State 1 is autapomorphic for *Xanthozona*. — L = 1; non-informative.

ADULT: Head

35. Eyes, sexual dimorphism, holoptic males with dichoptic females: absent (Fig. 9C) (0); present (Fig. 9B) (1). — In state 0, both male and female are either holoptic or dichoptic. — L = 2; CI = 50; RI = 80.

36. Flattening, i.e., in form of "discal head", in profile: absent (0); present (1). — L = 1; CI = 100; RI = 100.

37. Eye, ommatrichia: absent (0); present (Fig. 9A) (1). — L = 2 CI = 50; RI = 80.

38. Vertex, ocellar triangle: protuberant (Fig. 9C) (0); not protuberant (Fig. 9F), (1). — L = 1; CI = 100; RI = 100.

39. Postocellar seta: present (0); absent (Fig. 10C), (1). — L = 1; CI = 100; RI = 100.

40. Fronto-orbital plate, elevated in profile at antennal axis: absent (Fig. 10D) (0); present (Fig. 10C) (1). — L = 4; CI = 25; RI = 62.

41. Fronto-orbital plate, ground color, in males: silver (Fig. 9C) (0); black (Fig. 9E) (1); yellow (Fig. 9B) (2); golden (3). — L = 8; CI = 37; RI = 61.

42. Fronto-orbital plate, setulae along orbital setae: absent (0); present (1). — L = 1; CI = 100; RI = 100.

43. Fronto-orbital plate, setae on the ptilinal fissure region: absent (0); present (Fig. 9A) (1). — L = 1; CI = 100; RI = 100.

44. Fronto-orbital plate, orbital setae, females: present (0); absent (1). — L = 3; CI = 33; RI = 50.

45. Fronto-orbital plate, proclinate orbital setae, females: two (0); forming row of several setae (Fig. 10A) (1); three (2). — L = 2; CI = 100; RI = 100.

46. Frontal vitta, width in relation to ocellar triangle, females: broader (0); narrower (1). — L = 2; CI = 50; RI = 66.

47. Frontal vitta, width at upper third, males: broad (frontal vitta visible) (Fig. 9D) (0); narrow (vitta invisible) (Fig. 9E) (1). — L = 6; CI = 16; RI = 44.

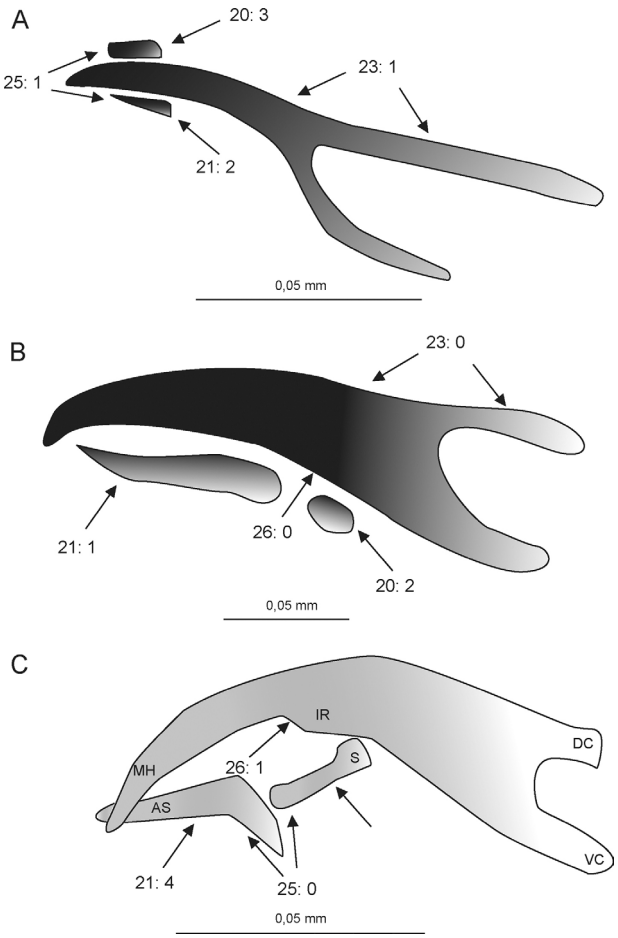

Figure 7. First instar larval characters. **A**: *Freraea gagatea* Robineau-Desvoidy, 1830; **B**: *Cenosoma thompsoni* Guimarães, 1977; **C**: *Dufouria chalybeata* (Meigen, 1824). Arrows identify characters and states (enclosed in parentheses) discussed in text. (Abbreviations: AS, accessory sclerite; DC, dorsal cornu; IR, intermediate region; MH, mouth hook; S, sclerite of salivary gland; VC, ventral cornu).

48. Frontal vitta, interfrontal setae: absent (0); present (Fig. 9D) (1). — L = 2; CI = 50; RI = 88.

49. Parafacial, setulae: absent (0); present (1). — L = 1; CI = 100; RI = 100.

50. Parafacial, swollen: absent (Fig. 10B) (0); present (Fig. 10A) (1). — L = 1; CI = 100; RI = 100.

51. Face, lunule, setulae: absent (0); present (1). — L = 2; CI = 50; RI = 83.

52. Face, facial carina: absent (Fig. 10A) (0); present (Fig. 10B) (1). — State 1 is traditionally recognized as common to some members of Dexiini. *Imitomyia* presents this character, hence it had its systematic placement controversial, e.g., the proposition that it would be a highly modified Dexiinae (Crosskey 1976). — L = 2; CI = 50; RI = 66.

53. Antennae, degree of approximation: separated (Fig. 9E) (0); close to each other (Fig. 9C) (1). — L = 2; CI = 50; RI = 66.

54. Antenna, postpedicel, shape: subcylindrical (5X the ratio of length to width) (Fig. 10D) (0); rounded (2X the ratio of length to width) (Fig. 9D) (1). — L = 4; CI = 25; RI = 62.

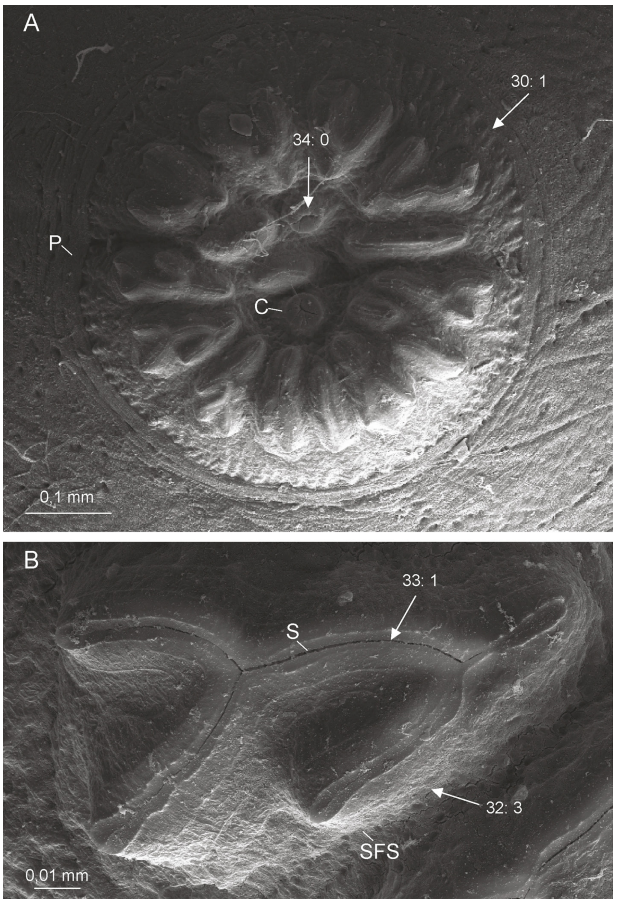

Figure 8. Transmission electron microscopy of puparial characters. **A**, **B**: *Euoestrophasia panamensis* Guimarães, 1977. Arrows identify characters and states (enclosed in parentheses) discussed in text. (Abbreviations: C, cicatrix; S, spiracular slits; P, peritreme; SFS, small fragment of spiracle).

55. **Antenna, arista, setulosity**: pubescent (Fig. 10C) (0); micropubescent (Fig. 10E) (1); plumose (Fig. 10D) (2). — L = 3; CI = 66; RI = 92.
56. **Vibrissa, degree of differentiation from supravibrissals**: differentiated (Fig. 9F) (0); undifferentiated (Fig. 10A) (1). — L = 2; CI = 50; RI = 66.
57. **Vibrissa, length**: long (longer than antenna) (Fig. 10D) (0); short (shorter than antenna) (Fig. 9D) (1). — L = 3; CI = 33; RI = 85.
58. **Facial ridge, region of insertion of vibrissae, setulae**: only at base (Fig. 10B) (0); along facial ridge (Fig. 9A) (1). — L = 1; non-informative.
59. **Palpus, color with sexual dimorphism, females**: same colour as male (0); different from male (1). — L = 3; CI = 33; RI = 60.
60. **Proboscis, prementum, length relative to head height**: subequal (0); twice (1). — L = 2; non-informative.
61. **Occiput, setula, coloration**: black (0); silver (Fig. 10F) (1). — L = 4; CI = 25; RI = 40.

ADULT: Thorax

62. **Seta, i.e., major setae on thorax, shape**: thin (0); robust (Fig. 11B) (1). — L = 1; CI = 100; RI = 100.
63. **Postpronotal lobe, number of setae**: 6 (0); 2 (1); 3 (Fig. 11C), (2); 4 (3); 5 (4); 1 (5) (Fig. 11B). — L = 7; CI = 71; RI = 71.
64. **Postpronotal lobe, pruinosity**: present (Fig. 11C) (0); absent (Fig. 11A) (1). — L = 4; CI = 25; RI = 76.
65. **Notopleuron, number of setae**: 2 (0); 3 (1). — State 1 is autapomorphic for *Xanthozona*. — L = 1; non-informative.
66. **Scutum, color in males**: dark brown (Fig. 11A) (0); yellow with black spots (1); brown with strips of silver pruinosity (2); entirely yellow (Fig. 11D) (3). — L = 4; CI = 75; RI = 88.
67. **Scutum, presutural region, supra-alar setae**: 1 (0); 2 (1). — L = 1; CI = 100; RI = 100.
68. **Scutum, postsutural region, dorsocentral setae**: 4 (0); 3 (1); 2 (Fig. 11D) (2); 1 (Fig. 11A) (3). — L = 10; CI = 30; RI = 58.
69. **Scutum, postalar callus, number of setae**: 3 (0); 2 (1). — L = 2; CI = 50; RI = 0.
70. **Scutellum, shape**: rounded (Fig. 11D) (0); triangular (Fig. 11E) (1). — Ambiguous character. Since there are no missing or inapplicable data, the two optimizations do not provide spurious results. Both forms being considered, thus, in ACCTRAN state 1 is a homoplasy for *Chetoptilia*, *Comyops* and *Ebenia*, with a revertion to state 0 and another in *Dufouria*. In DELTRAN, state 1 is a homoplasy for *Chetoptilia* and clade 16 (*Comyops* + *Ebenia*). — L = 2; CI = 50; RI = 80.
71. **Scutellum, subapical seta**: present (Fig. 11F) (0); absent (1). — L = 3; CI = 33; RI = 33.
72. **Scutellum, discal seta**: present (Fig. 11F) (0); absent (1). — L = 3; CI = 33; RI = 33.
73. **Postnotum, color**: black (Fig. 11F) (0); yellow (1). — L = 3; CI = 33; RI = 0.
74. **Prosternum, setulae**: absent (0); present (Fig. 12A) (1). — Ambiguous character. Since there are no missing or inapplicable data, the two optimizations do not provide spurious results. Both forms being considered, thus, in ACCTRAN state 1 is a homoplasy to *Comyops* + *Ebenia*, with a reversion to state 0 in *Ebenia fumata*. Now in DELTRAN is a homoplasy for *Comyops* and *E. claripennis* and *E.* sp1. — L = 2; CI = 50; RI = 50.
75. **Anterior spiracle**: slit closed by fringes of hairs (Fig. 12B) (0); slit not closed by fringes of hairs (Fig. 12C) (1). — Ambiguous character. Since there are no missing or inapplicable data, the two optimizations do not provide spurious results. In ACCTRAN, state 1 is a synapormophy for (Freraeini ((Oestrophasiini + Dufouriini)) with clade 3 (Phasiinae) except *Imitomyia*, with a reversion to state 0. In DELTRAN, state 1 is a homoplasy for clade *Strongygaster*, *Catharosia* and *Cylindromyia* and clade 5 (Freraeini (Oestrophasiini + Dufouriini)). — L = 2; CI = 50; RI = 80.
76. **Katepisternum, number of setae**: 3 (in position 1 + 1 + 1) (0); 2 (in position 1 + 1, Fig. 12D) (1); 1 (posterior seta) (2). — L = 2; CI = 100; RI = 100.

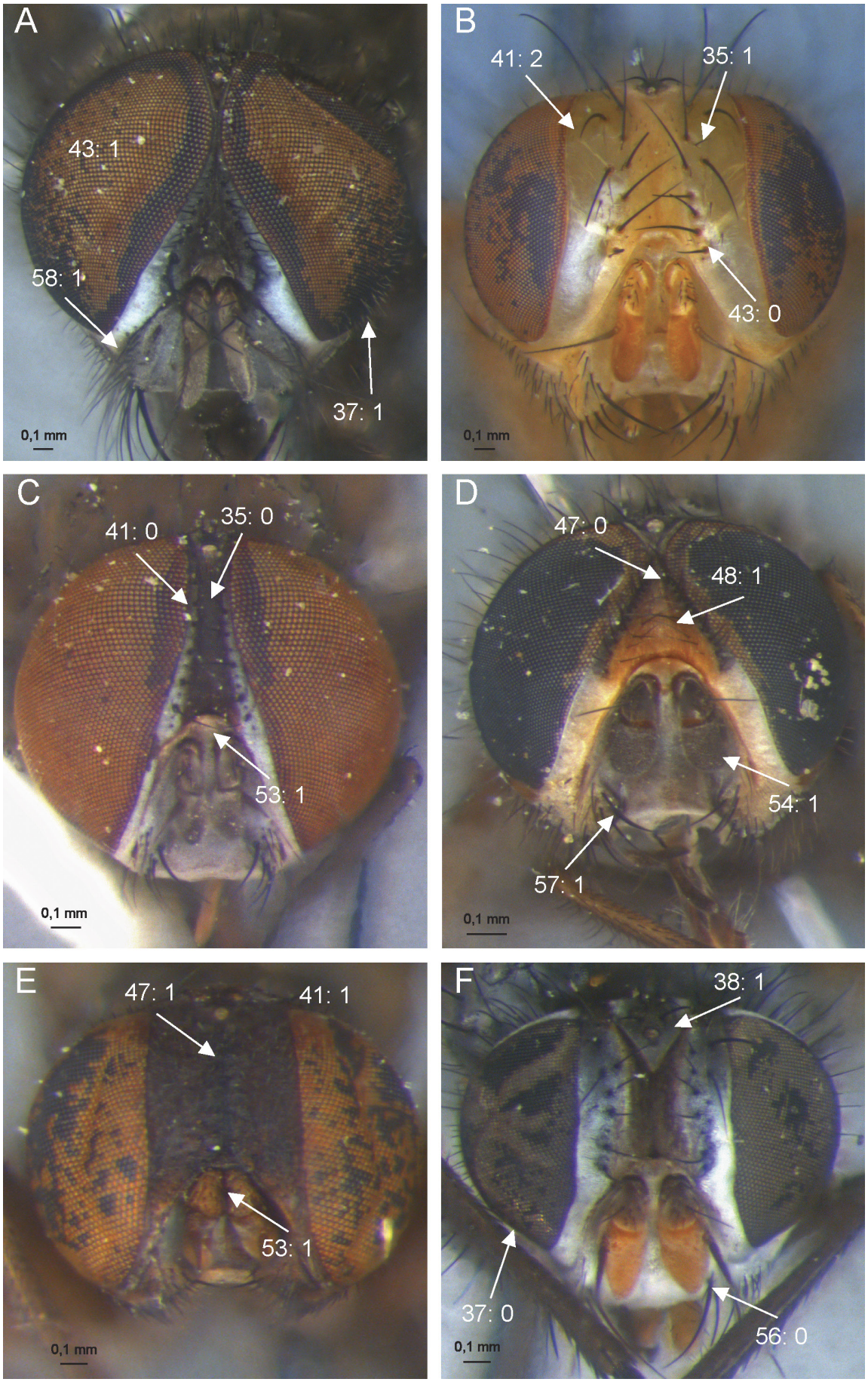

Figure 9. Head characters. **A**: *Comyops nigripennis* Wulp, 1891 ♂; **B**: *Euoestrophasia plaumanni* Guimarães, 1977 ♀; **C**: *Strongygaster triangulifera* (Loew, 1863) ♂; **D**: *Rondania fasciata* (Macquart, 1834) ♀; **E**: *Freraea gagatea* Robineau-Desvoidy, 1830 ♀; **F**: *Microsoma exiguum* (Meigen, 1824) ♀. Arrows identify characters and states (enclosed in parentheses) discussed in text.

77. **Anepimeron, setae, degree of development**: strong (broad diameter) (0); slim (narrow diamenter) (Fig.12D) (1); fine hair (2). — Ambiguous character, however ACCTRAN or DELTRAN optimization are shown to be equal in this clade, i.e., state 1 is a synapomorphy of clade 5. We chose DELTRAN in this case. —L = 5; CI = 40; RI = 81.
78. **Posterior spiracle, arrangement of fringes**: Mainly on the posterior region (Fig. 12E) (0); equally distributed on both sides (Fig. 12F) (1). — O'Hara (2002) observed that state 1 is often associated with small-sized tachinids, but in the present study both small (*Freraea*) and large-sized (*Dufouria*) taxa possess this character state. — L = 2; CI = 50; RI = 91.

ADULT: Leg

79. **Femur II, submedian anterodorsal setae, females**: 4 (0); 2 (1); 3 (Fig. 12D) (2); 1 (3); absent (4). — L = 8 CI = 50; RI = 73.

ADULT: Wing

80. **Membrane, macules**: absent (0); present (Fig. 13B) (1). — L = 1; CI = 100; RI = 100.
81. **Membrane, color, smoky**: present (0); absent (1). — L = 2; CI = 50; RI = 50.
82. **Tegula, color**: dark brown (Fig. 14A) (0); yellow (1). — L = 3; CI = 33; RI = 33.
83. **Costal vein, setulae, degree of development**: developed (0); poorly developed (Fig. 13A) (1). — L = 1; CI = 100; RI = 100.
84. **Costal spine**: absent (0); present (Fig. 13C) (1). — L = 4; CI = 25; RI = 81.
85. **Rs node, dorsal setulosity**: present (Fig. 13C) (0); absent, (1). — L = 2; CI = 50; RI = 75.
86. **Rs node, ventral setulosity**: absent (0); present (1). — L = 4; CI = 25; RI = 25.
87. **R_{4+5} vein, dorsal setulosity**: only on Rs node (0); beyond Rs node (Fig. 13C) (1). — L = 4; CI = 25; RI = 62.
88. **Bend of M, strongly angled**: present (0); absent, i.e., almost straight (Fig. 13A) (1). — L = 1; CI = 100; RI = 100.
89. **M_2**: absent (0); present (1). — State 1 is autapomorphic for *Imitomyia*. — L = 1; non-informative.
90. **Crossvein dm-cu, form**: straight (Fig. 13B) (0); sinuose (1). — L = 2; CI = 50; RI = 87.

ADULT: Abdomen

91. **Tergites, 1 to 5, length**: at least one different in size (0); all equal in size (1). Character after Mesnil (1975). — L = 1; CI = 100; RI = 100.
92. **Syntergite 1 + 2, median excavation length**: until the posterior margin (0); until 7/8 of the posterior margin (Fig. 14B) (1); until half way to the posterior margin (Fig. 14D) (2). — Adapted from Cerretti et. al. (2014). — L = 3; CI = 66; RI = 75.
93. **Tergites, pruinosity**: absent (Fig. 14C), (0); all tergite (1); only on anterior margin (2); on anterior margin, but only laterally (3). — L = 9; CI = 33; RI = 50.
94. **Setae, whole abdomen**: present (0); absent, i.e., just setulae (Fig. 14C) (1). — L = 1; CI = 100; RI = 100.
95. **Setae. whole abdomen, organization**: marginal lateral, marginal median (0); entire tergite (Fig. 14D) (1). — L = 3; CI = 33; RI = 60.
96. **Tergites 1 to 5, small brownish black round spots, dorsally**: absent (0); present (1). — L = 1; CI = 100; RI = 100.
97. **Syntergite 1 + 2, marginal lateral seta**: present (Fig. 14F) (0); absent (1). — L = 4; CI = 25; RI = 72.
98. **Tergite 3, setae**: one pair of lateral marginal and median marginal (Fig. 14F) (0); row of marginals (1); median discals (Fig. 14E) (2). — L = 2; CI = 100; RI = 100.
99. **Tergite 4, setae**: row of marginals (0); row of median discals (Fig. 14E) (1); one pair of median discals (2); widely distributed (3). — L = 7; CI = 42; RI = 66.
100. **Tergite 5, pair of dark brown rounded spots on ventral posterolateral region**: absent (0); present (Fig. 14F) (1). — L = 1; CI = 100; RI = 100.
101. **Tegument, ground color, yellow**: absent (0); present (1). — L = 1; CI = 100; RI = 100.

ADULT: Male terminalia

102. **Tergite 5, fusion with tergite 6**: not fused (0); medially fused (1). — State 1 is autapomorphic for *Catharosia*. — L = 1; non-informative.
103. **Tergite 5, connection with segment 6 + 7**: separate (Fig. 15A) (0); fused (1); fused, but with visible suture (median dividing line present) (2); fused, but with distinguishable limits (from lateral prominences) (Fig. 15B) (3). — L = 6; CI = 50; RI = 82.
104. **Tergite 6, in form of two degenerate hemitergites**: absent (0); present (1). Character after Tschorsnig (1985). — State 1 is autapomorphic for *Voria*. — L = 1; non-informative.
105. **Sternite 5, membranous lateral line**: present (Fig. 15C) (0); absent (Fig. 15D) (1). — In the dichotomous key of male terminalia, Tschorsnig (1985) reported that in almost all Dufouriini s.l. and Phasiinae, the membranous lateral line is absent. In the present analysis, this absence is a synapomorphy for the clade 4 (Phasiinae (Dufouriini + Oestrophasiini)), undergoing reversals in clade 7 (*Microsoma* (*Freraea* + *Eugymnopeza*)) and in the clade 14 (*Chetoptilia* (*Dufouria* (*Comyops* + *Ebenia*))). In the cladistic analysis of Cerretti et al. (2014), this character resulted as one of the two homoplasies that joined Dufouriini s.l. with Phasiinae, however, when the species of *Chetoptilia* and *Dufouria* were observed, we found a coding error. This basal membranous "window" in sternite 5 (character 90:1 of

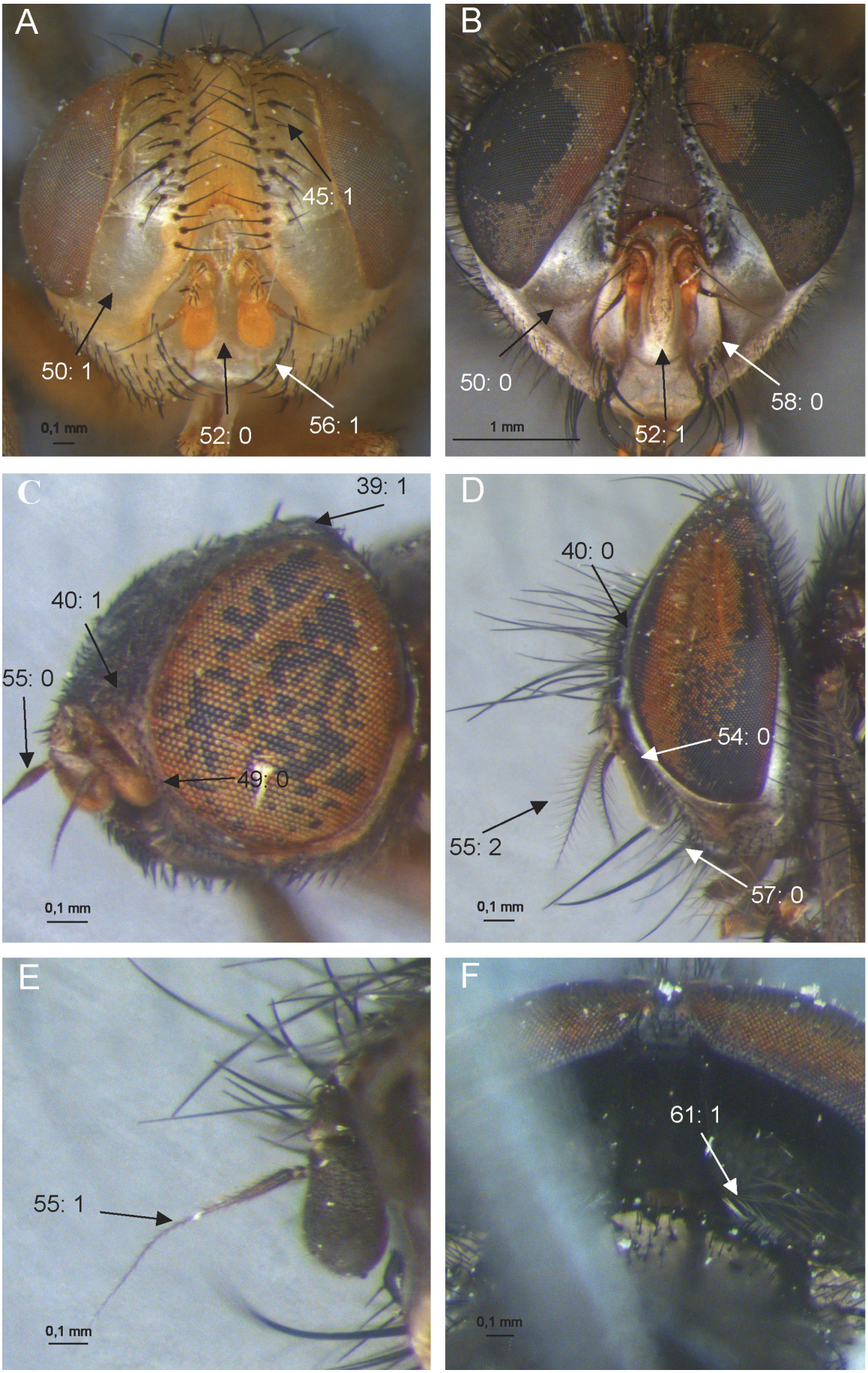

Figure 10. Head characters. **A**: *Oestrophasia calva* Coquillett, 1902 ♀; **B**: *Prophorostoma pulchra* Townsend, 1927 ♂; **C**: *Freraea gagatea* Robineau-Desvoidy, 1830 ♀; **D**: *Comyops nigripennis* Wulp, 1891 ♂; **E**: *Dufouria chalybeata* (Meigen, 1824) ♂; **F**: *Ebenia claripennis* Macquart, 1846 ♂. Arrows identify characters and states (enclosed in parentheses) discussed in text.

Cerretti et al. 2014) is present in both genera; however, in the character matrix was coded as absent. — L = 3; CI = 33; RI = 87.

106. **Sternite 5, lobules**: present (Fig. 16C) (0); absent (Fig. 16D) (1). — L = 2; CI = 50; RI = 87.
107. **Sternite 5, lobules, development**: well-developed (Fig. 16C) (0); poorly-developed (Fig. 15E) (1). — L = 2; CI = 50; RI = 75.
108. **Sternite 5, sensilla "*trichodea*"**: absent (0); present (Fig. 16C) (1). — L = 2; CI = 50; RI = 85.
109. **Sternite 6, superimposed with segment 7 at right side**: absent (0); present (1). — Character after Tschorsnig (1985). State 1 is autapomorphic for *Voria*. — L = 1; non-informative.
110. **Epandrium, fusion with segment 7 + 8**: absent (0); present (Fig. 15F) (1). — L = 1; CI = 100; RI = 100.
111. **Epandrium, lateral lobes**: absent (0); present (1). — In his dichotomous key of the male terminalia, Tschorsnig (1985) reported that almost no member of Dufouriini s.l. possess these lateral lobes. Here, this characteristic was recovered as a synapomorphy of clade 9 (Dufouriini + Oestrophasiini). — L = 1; CI = 100; RI = 100.
112. **Epandrium, posterior projection zone**: absent (Fig. 15F) (0); present (Fig. 16B) (1). — L = 1; CI = 100; RI = 100.
113. **Cerci, fusion**: partial (0); absent (Fig. 16B) (1); complete (2). — L = 3; CI = 66; RI = 0.
114. **Cerci, dorsally, globose expansion**: absent (0); present (Fig. 16A) (1). — L = 1; CI = 100; RI = 100.
115. **Cerci, curvature of the distal region, profile view**: anterior (0); posterior (1). — L = 1; CI = 100; RI = 100.
116. **Surstylus**: present (0); absent (Rubtzov 1951: fig. 87) (1). — State 1 is autapomorphic for *Catharosia*. — L = 1; non-informative.
117. **Surstylus, shape**: broad, massive (0); narrow, thin (Fig. 15A) (1). — Character after Tschorsnig (1985). — L = 3; CI = 33; RI = 50.
118. **Surstylus, fusion to epandrium**: absent (0); present (1). — State 1 is autapomorphic for *Strongygaster*. — L = 1; non-informative.
119. **Surstylus, lateral setae length**: short (0); long (Fig. 16A) (1). — L = 1; CI = 100; RI = 100.
120. **Surstylus, apical spines**: absent (0); present (Fig. 16B) (1). — L = 2; CI = 50; RI = 75.
121. **Hypandrial arms**: present (Fig. 16F) (0); absent (1). — Character after Tschorsnig (1985). — L = 2; CI = 50; RI = 75.
122. **Hypandrial arms, opening**: absent (closed) (Fig. 16E) (0); present (1). — State 0 is autapomorphic for *Xanthozona*. — L = 1; non-informative.
123. **Hypandrial apodeme, boundary with the central plate**: poorly developed (incomplete boundary) (Fig. 15E) (0); developed (1); indistinct (Fig. 15F) (2). — L = 3; CI = 66; RI = 85.
124. **Hypandrium, central plate, length**: short (Fig. 16C) (0); elongated (Fig. 16D) (1). — Character after Tschorsnig (1985). The elongated central plate of the hypandrium was the only putative synapomorphy for Phasiinae found by Tschorsnig (1985). Here, this character state was confirmed as a synapomorphy for clade 3, with the Phasiinae included (*Imitomyia*, *Strongygaster*, *Catharosia* and *Cylindromyia*). — L = 1; CI = 100; RI = 100.
125. **Processus longus, shape**: rod-shaped (Fig. 16D) (0); plate-shaped (1); sinuose (2). — L = 2; CI = 50; RI = 50.
126. **Phallapodeme, fan-shaped apex**: absent (0); present (Fig. 17B) (1). — L = 1; CI = 100; RI = 100.
127. **Phallapodeme, length, relative to hypandrium**: equal length (0); larger than hypandrium (Fig. 17A) (1). — L = 1; CI = 100; RI = 100.
128. **Phallapodeme, dorsal central depression, along extention**: present (0); absent (1). — L = 2; CI = 50; RI = 66.
129. **Aedeagus, sclerotization, shape**: well differentiated in distiphallus and basiphallus (0); reduced in basal and dorsal rings (Fig. 17C) (1). — Character after Tschorsnig (1985). State 1 is autapomorphic for *Strongygaster*. — L = 1; non-informative.
130. **Membranous connection between basiphallus (dorsal sclerite) and distiphallus**: absent (Fig. 17D) (0); present (Fig. 17E) (1). — Verbeke (1962, 1963) was the first to recognize the systematic value of this character, which separated his subfamilies Dexiinae, Voriinae and Dufouriinae from the other tachinids by the presence of a membranous connection between basiphallus and distiphallus. Described as "indirect and mobile" (Type II). Tschorsnig (1985) recognized this character as a putative synapomorphy of Dexiinae, which contained the tribes Dexiini, Voriini, and Dufouriini sensu lato. Based on this character, Wood (1987) and subsequent authors, considered Dexiinae as a possible monophyletic group within Tachinidae. However, in the first cladistic analysis of the family (Cerretti et al. 2014), it was recovered as a reversal in Phasiinae, not confirming the monophyly of Dexiinae. This putative synapomorphy of Dexiinae was also not found herein, appearing in Dexiinae and in Dufouriini s.l., with a reversion in Phasinae. Thus, confirming that it is a homoplastic character. — L = 2; CI = 50; RI = 66.
131. **Membranous connection between basiphallus (dorsal sclerite) and distiphallus, 180º movement capacity**: immovable (Fig. 17D), (0); movable (Fig. 17E), (1). — One of putative synapomorphies of Dexiinae, Voriinae and Dufouriinae (Dexiinae sensu Herting [1984]) suggested by Verbeke (1962; 1963) would be that the membranous connection of the basiphallus (dorsal sclerite) with the distiphallus would be associated with the movement capacity of the distiphallus. However, some taxa with uncertain systematic position, such as *Imitomyia*, have this membranous connection, but without movement (in 180°). This is an ambiguous character, since there are no missing or inapplicable data, the two optimizations do not provide spurious results. Both forms being considered, thus, in ACCTRAN, state

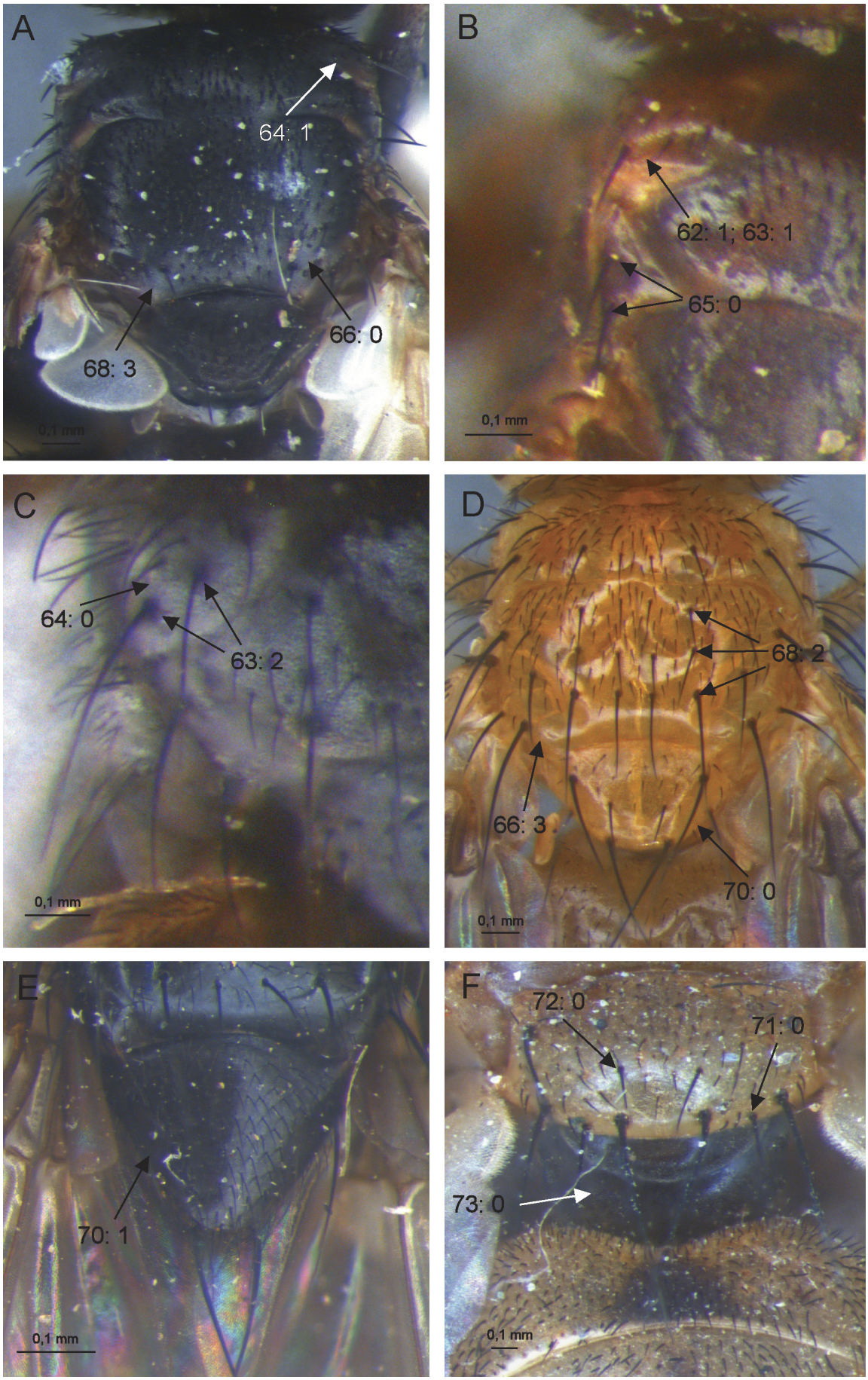

Figure 11. Thorax characters. **A**, **B**: *Freraea gagatea* Robineau-Desvoidy, 1830 ♀; **C**: *Microsoma exiguum* (Meigen, 1824) ♀; **D**: *Euoestrophasia panamensis* Guimarães, 1977 ♂; **E**: *Ebenia claripennis* Macquart, 1846 ♂; **F**: *Euoestrophasia aperta* Brauer and Bergenstamm, 1889 ♂. Arrows identify characters and states (enclosed in parentheses) discussed in text.

1 is a synapomorphy for Dexiinae (clade 1) and for (Freraeini (Oestrophasiini + Dufouriini)) (clade 5), with a reversion to state 0 in Phasiinae (clado 3). In DELTRAN, state 1 is a homoplasy for Phasiinae (clade 3) and for Freraeini, Oestrophasiini and Dufouriini s.l. (clade 5). — L = 2; CI = 50; RI = 75.

132. **Basiphallus, dorsally segmented, i.e., fragmented**: absent (0); present (Fig. 17F) (1). — L = 1; CI = 100; RI = 100.
133. **Basiphallus, length, in relation to distipallus**: long, 4x times longer (Fig. 17D) (0); short, 2x times longer (1). — L = 2; CI = 50; RI = 83.
134. **Epiphallus**: present (Fig. 17F) (0); absent (Fig. 17A), (1). — L = 2; CI = 50; RI = 50.
135. **Epiphallus, length, in relation to basiphallus**: short, at most 1/8 the length (0); long, about half the length (Fig. 17F) (1). — L = 2; CI = 50; RI = 0.
136. **Distiphallus, segmentation**: trisegmented (0); unisegmented (1). — State 0 is autapomorphic for *Xanthozona*. — L = 1; non-informative.
137. **Distiphallus, extension of dorsal sclerite, length relative to median bar**: less than half (0); more than half (Fig. 17E) (1); same length (2). — L = 2; CI = 100; RI = 100.
138. **Distiphallus, extension of dorsal sclerite, fusion with median bar**: absent (0); present (1). — L = 1; CI = 100; RI = 100.
139. **Distiphallus, ventral sclerite dorsal projection**: absent (0); present (Fig. 17F) (1). — L = 1; CI = 100; RI = 100.
140. **Distiphallus, granular structure**: absent (0); present (1). — Character after Tschorsnig (1985). In the dichotomous key of male terminalia, Tschorsnig (1985) reported that in almost all Dexiini this granular structure is present. This putative synapomorphy was confirmed here too, as a synapomorphy for the Dexiini (*Billaea*, *Prophorostoma*, *Dexia*). In Cerretti et al. (2014), this character state was also confirmed as a synapormorphy for the Palaearctic Dexiini. — L = 1; CI = 100; RI = 100.
141. **Distiphallus, asymmetry**: absent (0); present (1). — State 1 is autapomorphic for *Catharosia*. — L = 1; non-informative
142. **Distiphallus, anterior margin, sclerotization**: strong (0); weak, with anterior margin completely sclerotized (1); weak, with anterior margin partially sclerotized (2). — L = 2; CI = 100; RI = 100.
143. **Distiphallus, microtrichia**: present (0); absent (1). — Verbeke (1962, 1963) defined the POS type [= *Phasia*, *Ocyptera*, *Strongygaster*] as having no microtrichia in the distiphallus (143:1) and this absence would be observed only in Phasiinae. However, as Tschorsnig (1985) observed, this is not a good character for the subfamily, being confirmed in this study, since it is absent in *Rondania* (which does not belong to Phasiinae). — L = 2; CI = 50; RI = 80.
144. **Distiphallus, distal portion**: absent (0); present (1). — L = 2; CI = 100; RI = 100.
145. **Ejaculatory apodeme**: present (0); absent (cf. Tschorsnig 1985: fig. 160) (1). — Character after Tschorsnig (1985). State 1 is autapomorphic for *Strongygaster*. — L = 1; non-informative.
146. **Ejaculatory apodeme, shape**: narrow (0); fan-shaped (Fig. 18A) (1). — L = 1; CI = 100; RI = 100.
147. **Pregonite, fusion with postgonite**: absent (Fig. 18D) (0); present (Fig. 18C) (1). — Rubtzov (1951) was first to remark that the fusion of the gonites would be characteristic of Phasiinae. All the Phasiinae included here possess this character state, except *Imitomyia*, although the gonites are very close and articulated to each other. — L = 2; CI = 50; RI = 66.
148. **Pregonite, insertion in hypandrial arms**: anterior (0); posterior (1). — Character after Tschorsnig (1985). — L = 1; CI = 100; RI = 100.
149. **Pregonite, strong curvature**: present (0); absent (1). — State 0 is autapomorphic for *Xanthozona*. — L = 2; CI = 50; RI = 0.
150. **Pregonites, fusion**: separated from each other (Fig. 18C) (0); partially fused (Fig. 18E) (1); fully fused (Fig. 18F) (2). — Following the viewpoint of Tschorsnig (1985), O'Hara and Wood (2004) restricted the definition of the Nearctic Dufouriini s.s. (including Oestrophasiini, see Table 1) only for taxa that possess the fused pregonites, thus excluding genera traditionally considered in the tribe, such as *Freraea* and *Microsoma*. This character was analyzed and redefined to include one more state: whether the fusion is complete (Oestrophasiini synapomorphy) or incomplete (Dufouriini s.s. synapomorphy). This is an ambiguous character, since there are no missing or inapplicable data, and the two optimizations do not provide spurious results. Both forms being considered, thus, in ACCTRAN, state 2 is a synapomorphy for Oestrophasiini and Dufouriini s.s., with a reversion to state 1 in Dufouriini s.s. In DELTRAN, state 1 is a synapomorphy for Dufouriini s.s. and state 2 is a synapomorphy for Oestrophasiini. — L = 2; CI = 100; RI = 100.
151. **Pregonite, when fused together, downwards directed apex**: present (Fig. 18E) (0); absent (Fig. 18F) (1). — L = 1; CI = 100; RI = 100.
152. **Pregonite, posterior margin fused to hypandrium**: absent (0); present (Fig. 16C) (1). — L = 2; CI = 50; RI = 50.
153. **Postgonite, anterior margin, sclerotization**: weak (Fig. 18B) (0); strong (1). — L = 1; CI = 100; RI = 100.
154. **Postgonite, articulation with pregonite**: not articulated (0); articulated (Fig. 18D) (1). — State 1 is autapomorphic for *Imitomyia*. — L = 1; non-informative.

ADULT: Female terminalia

155. **Tergite 5, short spines**: absent (0); present (Rubtzov 1951: fig. 92). — State 1 is autapomorphic for *Catharosia*. — L = 1; non-informative.
156. **Tergite 6**: present (0); absent (1). — L = 1; CI = 100; RI = 100.

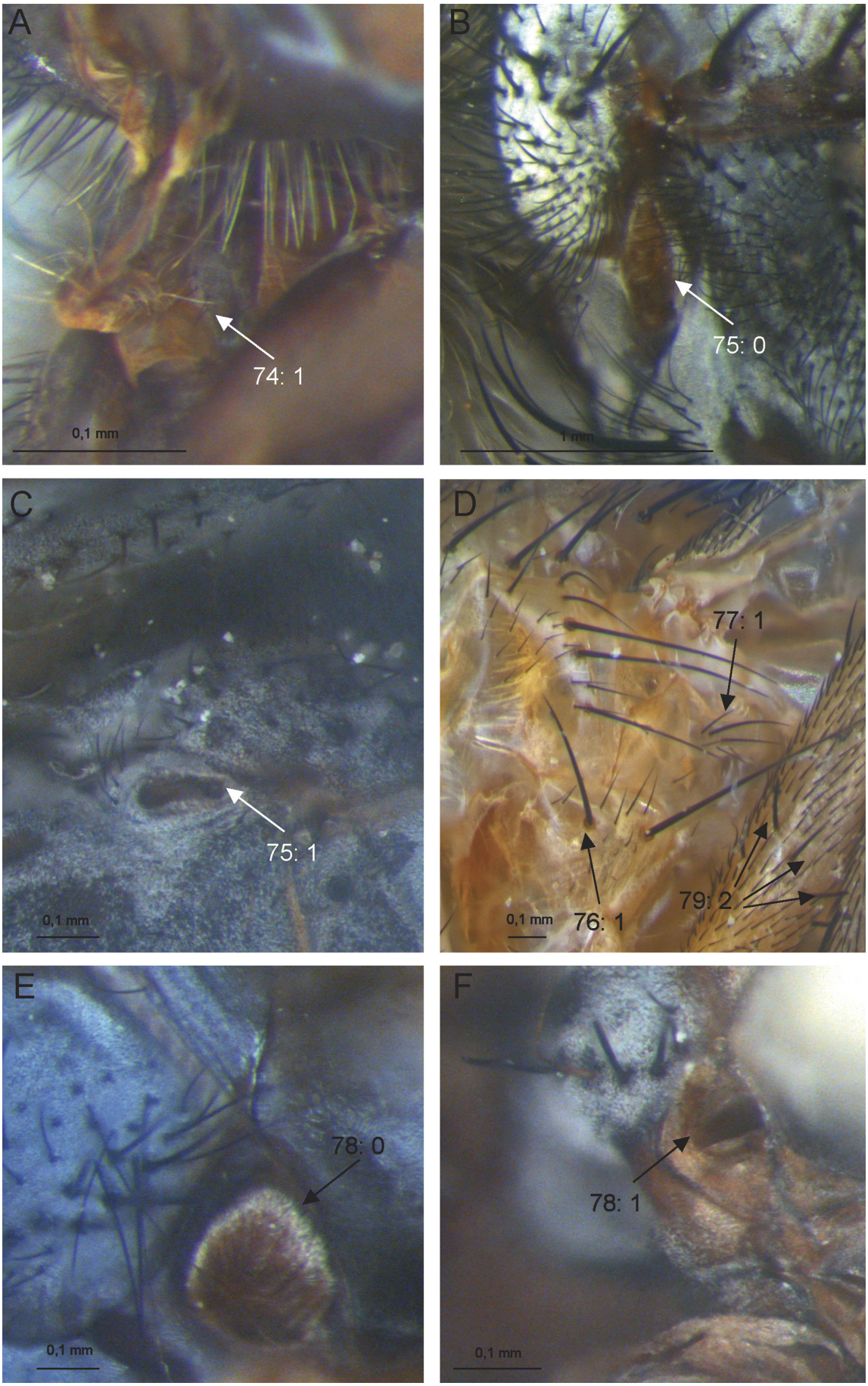

Figure 12. Thoracic characters. **A**: *Ebenia claripennis* Macquart, 1846 ♂; **B**, **E**: *Prophorostoma pulchra* Townsend, 1927 ♂; **C**, **F**: *Rondania dimidiata* (Meigen, 1824) ♀; **D**: *Euoestrophasia plaumanni* Guimarães, 1977 ♀. Arrows identify characters and states (enclosed in parentheses) discussed in text.

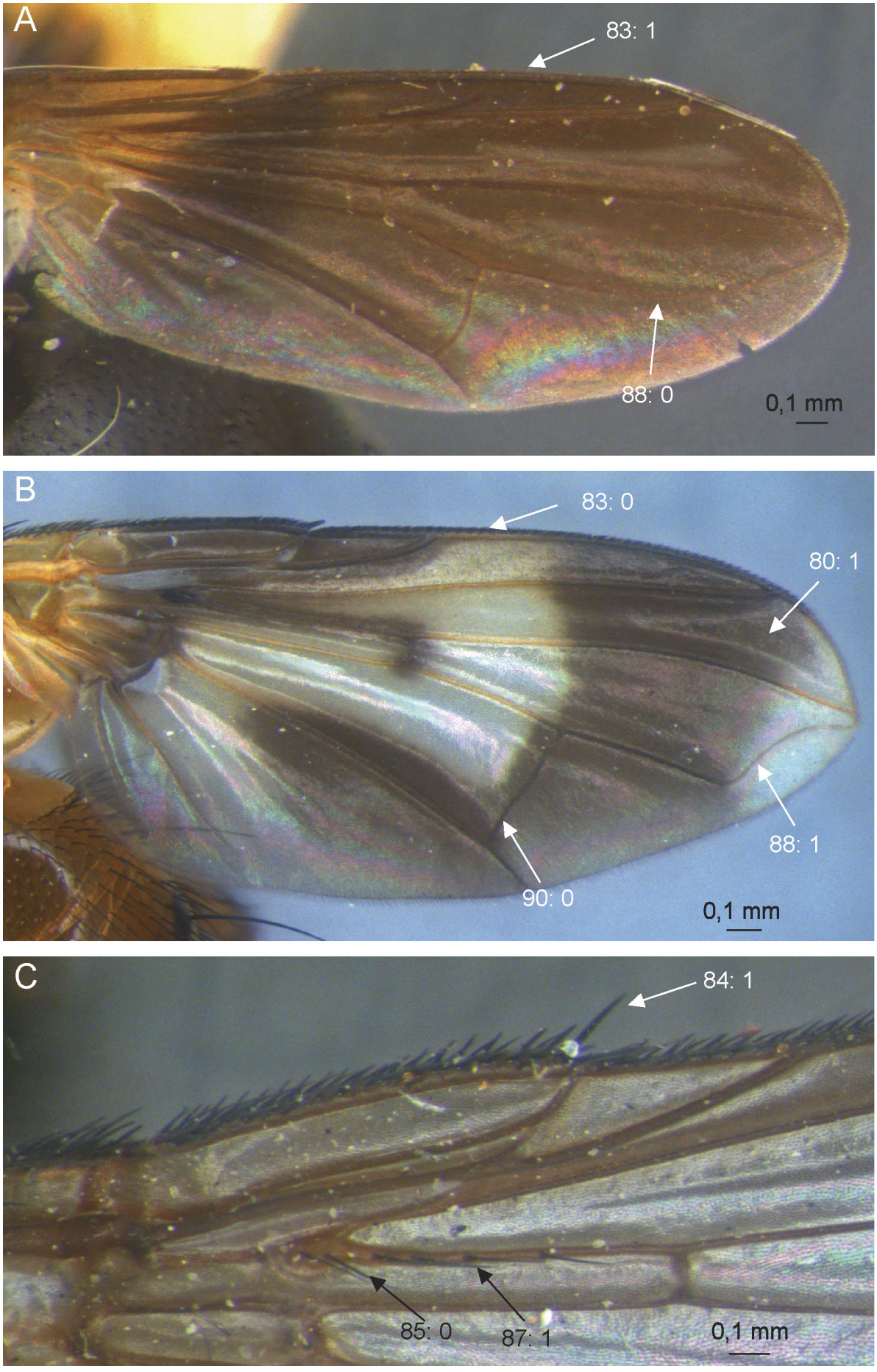

Figure 13. Wing characters. **A**: *Freraea gagatea* Robineau-Desvoidy, 1830 ♀; **B**: *Euoestrophasia plaumanni* Guimarães, 1977 ♀; **C**: *Dufouria chalybeata* (Meigen, 1824) ♂. Arrows identify characters and states (enclosed in parentheses) discussed in text.

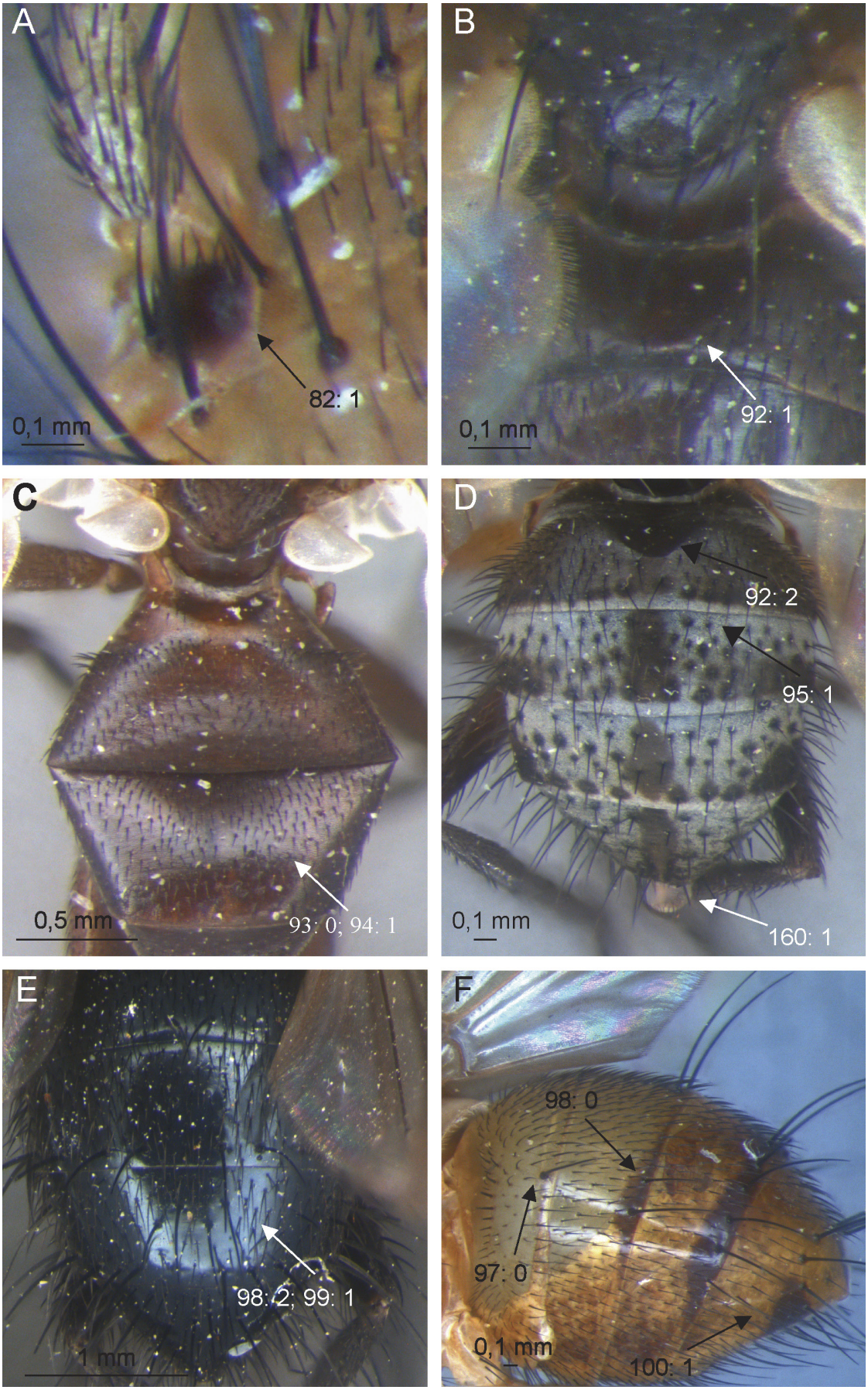

Figure 14. Abdominal characters. **A**: *Euoestrophasia panamensis* Guimarães, 1977 ♂; **B**: *Chetoptilia puella* (Rondani, 1962) ♀; **C**: *Freraea gagatea* Robineau-Desvoidy, 1830 ♀; **D**: *Rondania dimidiata* (Meigen, 1824) ♀; **E**: *Dufouria chalybeata* (Meigen, 1824) ♂; **F**: *Euoestrophasia plaumanni* Guimarães, 1977 ♀. Arrows identify characters and states (enclosed in parentheses) discussed in text.

157. Tergite 6, elongated dorsally: absent (0); present (Fig. 19A) (1). — L = 1; non-informative.

158. Tergite 6, setae: present (0); absent (1). — L = 1; CI = 100; RI = 100.

159. Syntergosternite 6: separated (0); partially fused (1); completely fused (Fig. 19B) (2). — L = 3; CI = 66; RI = 85.

160. Tergite 6, direction: anterior (bent forward) (0); posterior (1). — Ambiguous character. In ACCTRAN optimization, state 1 is a synapomorphy for Oestrophasiini and Dufouriini s.l., but they are inapplicable for this character; thus that synapomorphy is spurious. In DELTRAN, this state becomes a synapomorphy for *Rondania*, representing the codification for that character, so it was used. — L = 1; CI = 100; RI = 100.

161. Tergite 7, well-developed plate (covering other posterior segments): absent (0); present (Fig. 20C) (1). — State 1 is autapomorphic for *Imitomyia*. — L = 1; non-informative.

162. Tergite 7, spines: absent (0); present (Fig. 20C) (1). — State 1 is autapomorphic for *Imitomyia*. — L = 1; non-informative.

163. Sternite 7, bipartite: absent (0); present (Herting 1957: fig. 16D) (1). — Character after Herting (1957). State 1 is autapomorphic for *Catharosia*. — L = 1; non-informative.

164. Syntergosternite 7: absent (0); present (Fig. 19B) (1). — L = 5; CI = 20; RI = 66.

165. Syntergosternite 7: tube (Fig. 19B) (0); ring (1). — L = 1; CI = 100; RI = 100.

166. Tergite 7: present (Fig. 20C) (0); absent (1). — L = 2; CI = 50; RI = 0.

167. Tergite 7, shape, when free: wide plate (0); narrow plate (1); curved tube (Fig. 20C) (2); elongated (Fig. 19B) (3); filiform (Herting 1957: fig. 16D) (4). — Ambiguous character, but in ACCTRAN or DELTRAN optimizations are shown to be equal in this clade, that is, state 3 is synapomorphic of clade 5 (Oestrophasiini, Freraeini and Dufouriini s.s.). We chose DELTRAN in this case. — L = 4; CI = 100; RI = 100.

168. Tergite 8, fusion with sternite 8: absent (0); present (Fig. 20A), (1). — Character after Herting (1957). — L = 1; CI = 100; RI = 100.

169. Tergite 8, form of fusion with sternite 8: cone shape (posteriorly facing) (Fig. 20A) (0); peak shape (ventrally facing) (Fig. 20B) (1). — Character after Herting (1957). Ambiguous character, however the ACCTRAN or DELTRAN optimizations are shown to be equal in this clade, that is, state 1 is a synapomorphy of *Chetoptilia*. We chose DELTRAN in this case. — L = 1; CI = 100; RI = 100.

170. Tergite 8, fusion of sternite 8 with sternite 9: absent (0); present (Fig. 19A) (1). — Herting (1957) considered sternite 9 as reminiscent, and called this structure lingulae. — L = 1; CI = 100; RI = 100.

171. Sternite 8: single piece (0); paired piece (1). — State 1 is autapomorphic for *Strongygaster*. — L = 1; non-informative.

172. Sternite 8, shape: subsquared (0); sharp (Fig. 19A) (1); elongated (Fig. 19B) (2); bulbous (Fig. 19C) (3). — Ambiguous character. In ACCTRAN optimization, state 1 is the synapomorphy for *Jamacaria* and *Cenosoma*. In DELTRAN, this state becomes a synapomorphy for *Cenosoma*, representing the coding for that character, so it was used. — L = 4; CI = 75; RI = 90.

173. Syntergite 9 + 10: present (0); absent (1). — Dexiinae was defined by Herting (1957) with absence of syntergite 9 + 10 (end tergite, *sec* Herting 1957). This inference was confirmed herein in part, since a member of Phasiinae (*Imitomyia*) also does not have this structure. Although all members of Dexiinae (clade 1) and Freraini, Oestrophasiini and Dufouriini s.s. (clade 5) also do not present this structure and ratify the author's proposal. — L = 2; CI = 50; RI = 66.

174. Sternite 10, shape: square (0); narrow and long (Fig. 20B) (1); narrow and short (Fig. 19B) (2); reduced (3); sharp and curved (Fig. 19C) (4); sharp and rectilinear (5). — Ambiguous character, however the ACCTRAN or DELTRAN optimizations are shown to be equal in this clade, that is, state 3 is a homoplasy for clade 5. We chose DELTRAN in this case. — L = 8; CI = 62; RI = 82.

175. Sternite 9: present (0); absent (1). — State 1 was elaborated from the observation of sternite 8, which is longitudinally elongated and has no visible sternite 9. The unobservable sternite 9 is considered to have occurred due to a complete fusion with sternite 8. Then, in state 0, sternite 9 is always easily differentiated from other structures (usually very close to sternite 8). — L = 2; CI = 50; RI = 80.

176. Cercus, length: elongated (longer than sternite 8) (0); short (in relation to sternite 8). — (L = 2; CI = 50; RI = 83).

177. Spiracle, number: 2 (Fig. 20A) (0); 1 (Fig. 19C) (1). — Ambiguous character. In ACCTRAN optimization, state 1 is a synapomorphy for *Jamacaria* and *Cenosoma*, but as this character is inapplicable in *Jamacaria*, the indicated synapomorphy becomes spurious. In DELTRAN, this state becomes a synapomorphy for *Cenosoma*, representing the correct transformation for that character, so it was used. — L = 1; CI = 100; RI = 100.

ADULT: Spermathecae

178. Number of spermathecae: 3 (0); 2 (1). — *Microsoma exiguum* and *Freraea gagatea* have only two spermathecae, however, in Cerretti et al. (2014) both species were coded as having three (Cerretti et al. 2014, character 135: 0). — L = 1; CI = 100; RI = 100.

179. Pores on spermathecae: absent (0); present (Fig. 21C) (1). — L = 1; CI = 100; RI = 100.

180. Surface of spermathecae: striated (Fig. 22B) (0); low roughness (Fig. 21C) (1); high roughness (Fig. 21A) (2); smooth (3). — L = 4; CI = 75; RI = 75.

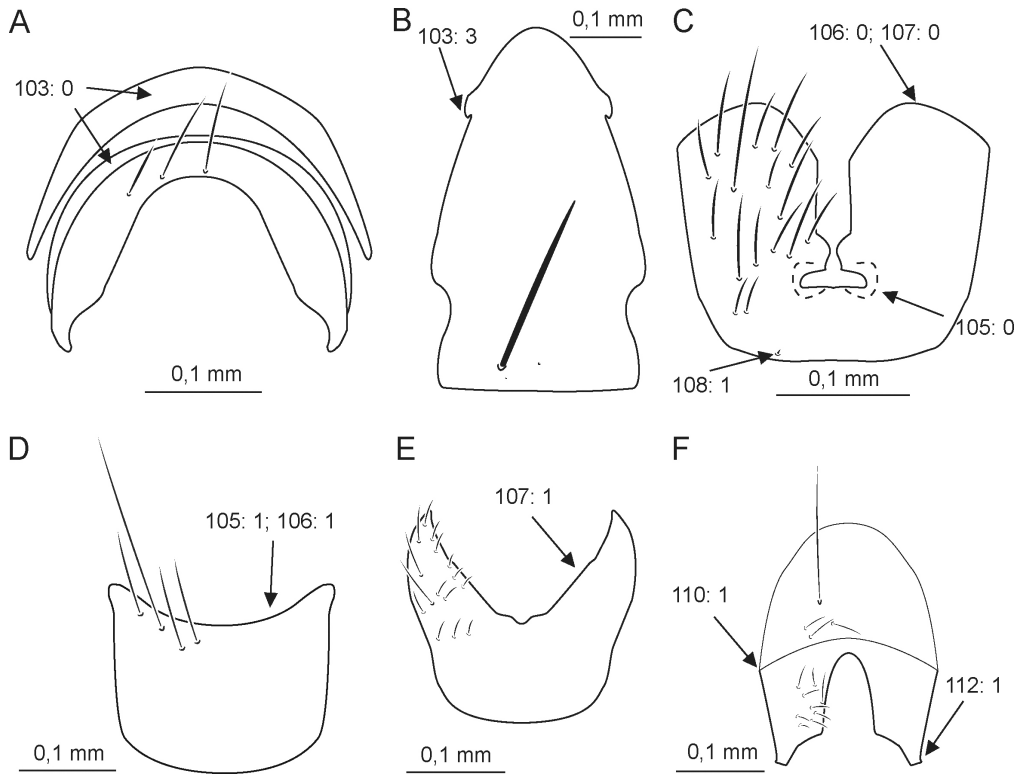

Figure 15. Male terminalia characters. **A**: *Ebenia neofumata* Santis and Nihei, **nom. nov.**; **B**, **D**: *Oestrophasia calva* Coquillett, 1902; **C**: *Microsoma exiguum* (Meigen, 1824); **E**, **F**: *Pandelleia crosskeyi* Sanits and Nihei, 2021. Arrows identify characters and states (enclosed in parentheses) discussed in text.

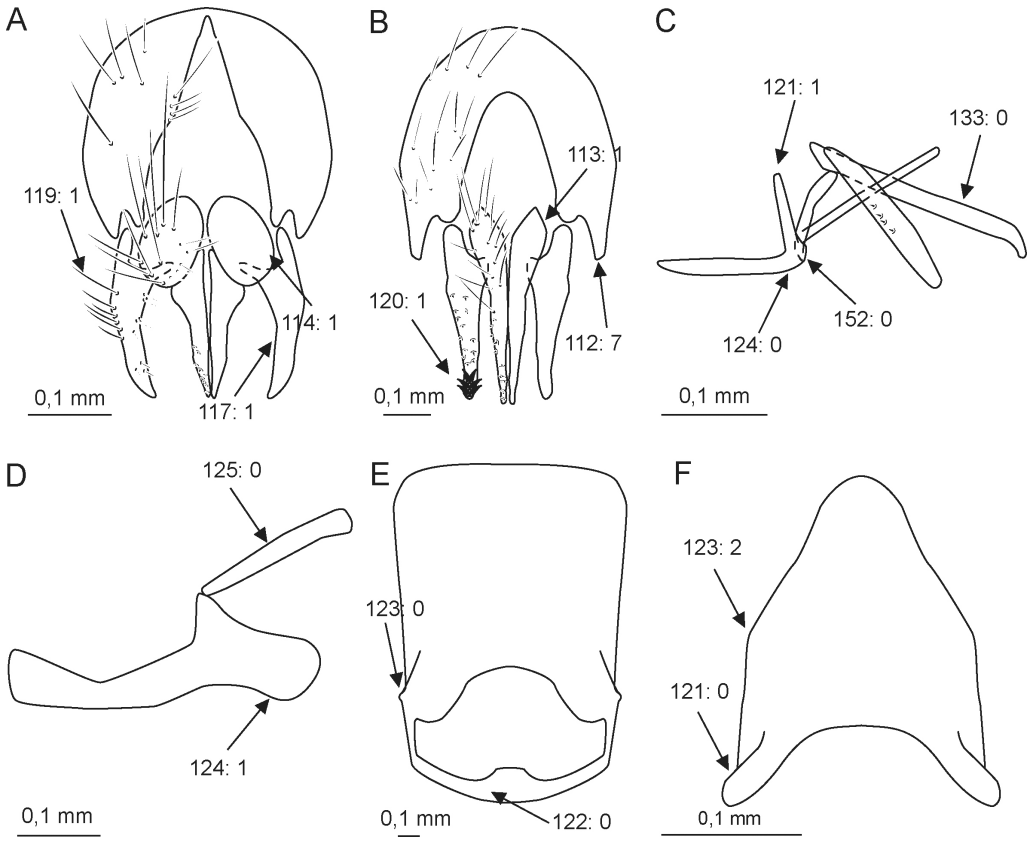

Figure 16. Male terminalia characters. **A**: *Ebenia neofumata* Santis and Nihei, **nom. nov.**; **B**: *Dufouria chalybeata* (Meigen, 1824); **C**: *Freraea gagatea* Robineau-Desvoidy, 1830; **D**: *Imitomyia sugens* (Loew, 1863); **E**: *Xanthozona melanopyga* (Wiedmann, 1830); **F**: *Euoestrophasia panamensis* Guimarães, 1977. Arrows identify characters and states (enclosed in parentheses) discussed in text.

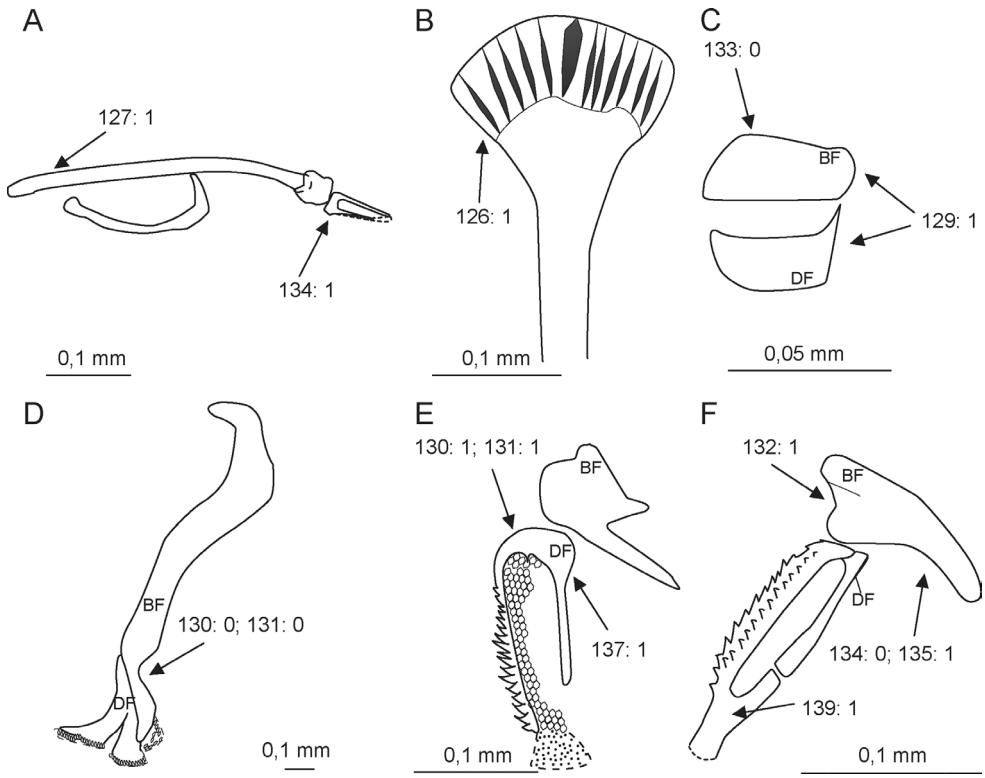

Figure 17. Male terminalia characters. **A**: *Rondania fasciata* (Macquart, 1834); **B**: *Dufouria chalybeata* (Meigen, 1824); **C**: *Strongygaster triangulifera* (Loew, 1863); **D**: *Xanthozona melanopyga* (Wiedmann, 1830); **E**: *Oestrophasia uncana* (Fabricius, 1805); **F**: *Comyops nigripennis* Wulp, 1891. Arrows identify characters and states (enclosed in parentheses) discussed in text. (Abbreviations: BF, basiphallus; DF, distiphallus).

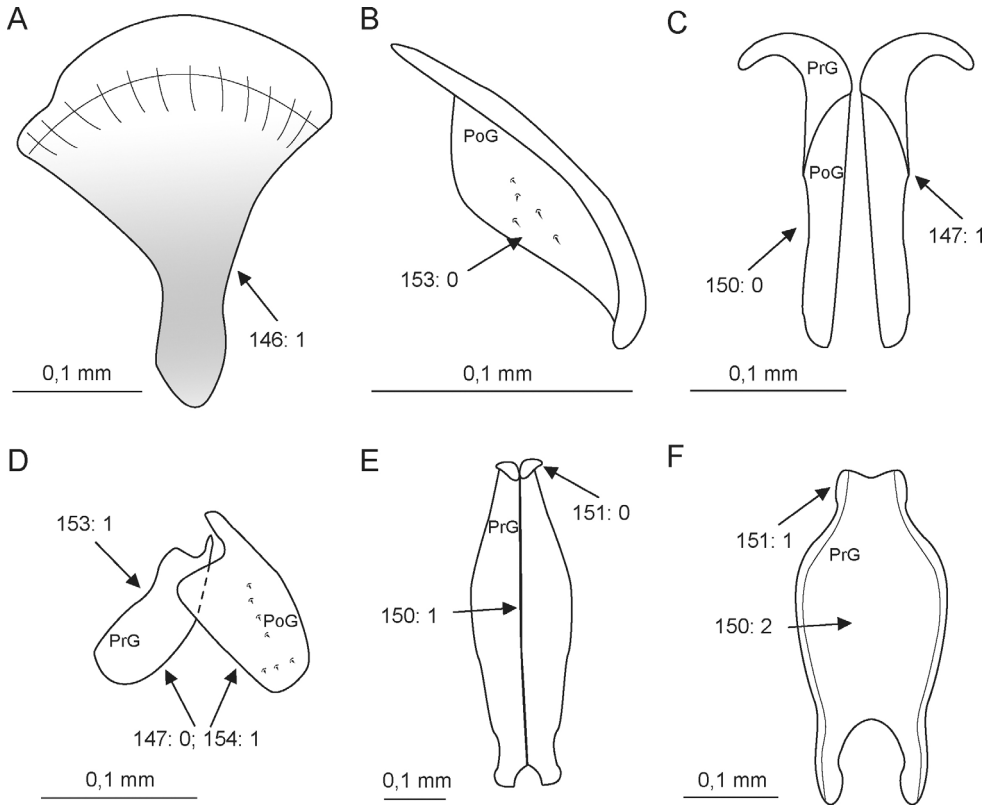

Figure 18. Male terminalia characters. **A**: *Comyops nigripennis* Wulp, 1891; **B**: *Oestrophasia uncana* (Fabricius, 1805); **C**: *Microsoma exiguum* (Meigen, 1824); **D**: *Imitomyia sugens* (Loew, 1863); **E**: *Chetoptilia puella* (Rondani, 1962); **F**: *Euoestrophasia aperta* Brauer and Bergenstamm, 1889. Arrows identify characters and states (enclosed in parentheses) discussed in text. (Abbreviations: PoG: postgonite; PrG, pregonite).

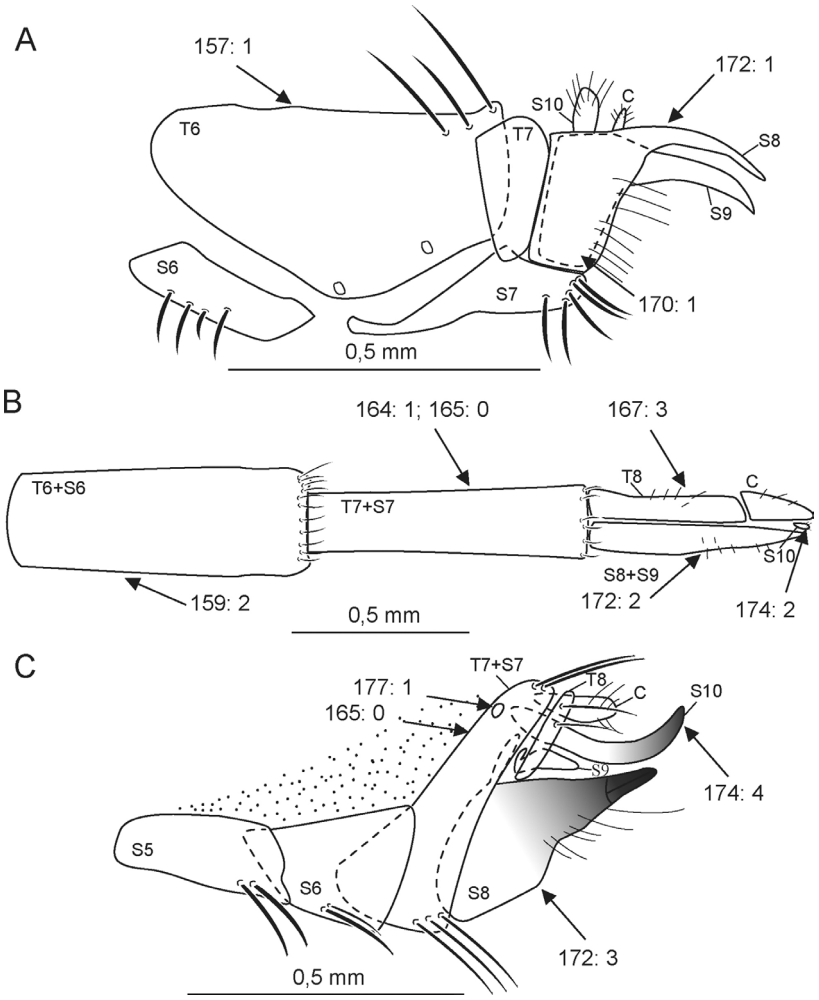

Figure 19. Female terminalia characters. **A**: *Microsoma exiguum* (Meigen, 1824); **B**: *Rondania fasciata* (Macquart, 1834); **C**: *Cenosoma thompsoni* Guimarães, 1977. Arrows identify characters and states (enclosed in parentheses) discussed in text. (Abbreviations: C, cercus; S, sternite; T, tergite).

181. Fringes on spermathecae: absent (0); present (Fig. 21B) (1). — L = 2; CI = 50; RI = 83.

182. Concavity, i.e., in at least one spermatheca: absent (0); present (Fig. 21B) (1). — L = 2; CI = 50; RI = 83.

183. Asymmetry between spermathecae: absent (0); present (Fig. 21B) (1). — Ambiguous character. In ACCTRAN optimization, state 0 is a synapomorphy for *Jamacaria* and *Cenosoma*. In DELTRAN, this state is a synapomorphy for *Cenosoma*, representing the coding for that character, so it was used. — L = 3; CI = 33; RI = 77.

184. Shape (when there is no asymmetry): round (0); pear-shaped (1); reniform (Fig. 22A) (2). — L = 3; CI = 66; RI = 0.

185. Setulae: absent (0); present (Fig. 22C) (1). — State 1 is autapomorphic for *Imitomyia*. — L = 1; non-informative.

3.2. Phylogenetic analysis

Our study included 35 species and 22 genera, with 26 species and 13 genera in the ingroup. All genera of Dufouriini (including Oestrophasiini and excluding *Mesnilana* and *Rinophoroides*, see more in Discussion) and Freraeini were sampled. Our holomorphological analysis included a total of 185 characters from the egg (5 characters), first instar larva (22), puparium (7), adult external morphology (67, excl. terminalia), female terminalia (23), male terminalia (53) and spermatheca (8). The data matrix is provided in Supplementary file 2.

Cladistic analysis with equal weights resulted in a single, most parsimonious tree (L = 400; CI = 61; RI = 83) (Fig. 23). The implied weighting analysis resulted in a single tree with the same length and topology as the equal weighted analysis, but with differences in the optimization of some characters. The single most parsimonious tree with equal weighting will be used in the discussion with unambiguous characters optimized and clades numbered (Fig. 24). Cladograms with ACCTRAN and DELTRAN character optimization, in addition to the Bremer support of each clade, are provided in Supplementary file 3.

Dufouriini, as defined prior to this study (Table 1), was considered paraphyletic. In the present analysis, the genera *Microsoma* and *Pandelleia* form a clade with (*Freraea* + *Eugymnopeza*), supported by eight synapo-

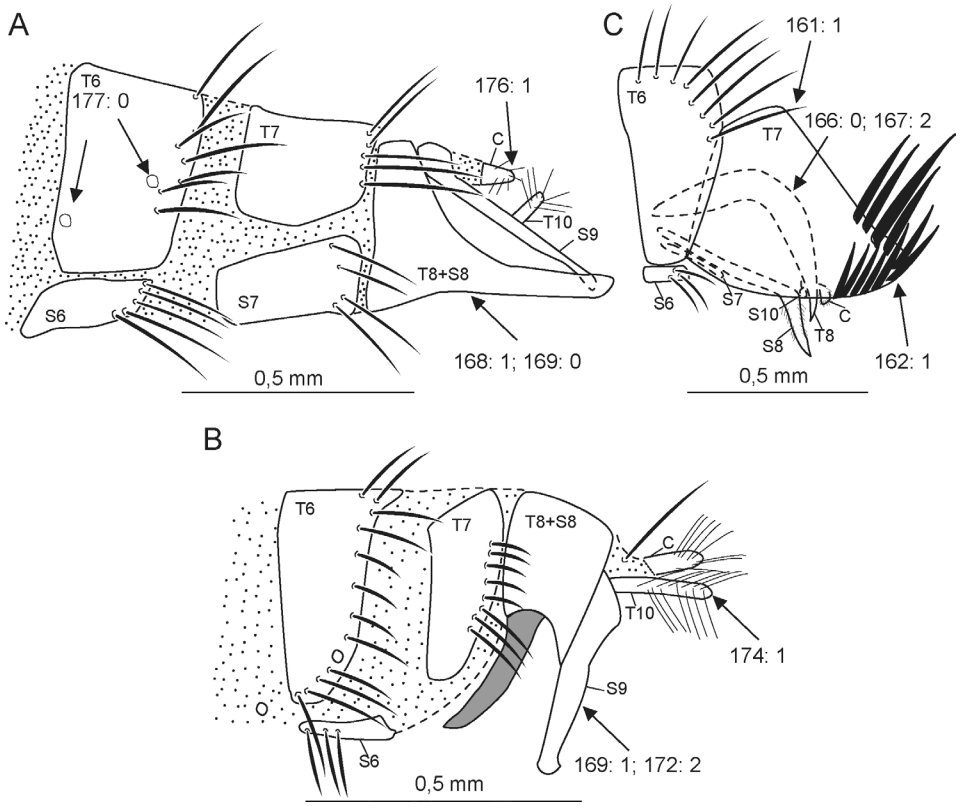

Figure 20. Female terminalia characters. **A**: *Dufouria chalybeata* (Meigen, 1824); **B**: *Chetoptilia puella* (Rondani, 1962); **C**: *Imitomyia sugens* (Loew, 1863). Arrows identify characters and states (enclosed in parentheses) discussed in text. (Abbreviations: C, cercus; S, sternite; T, tergite).

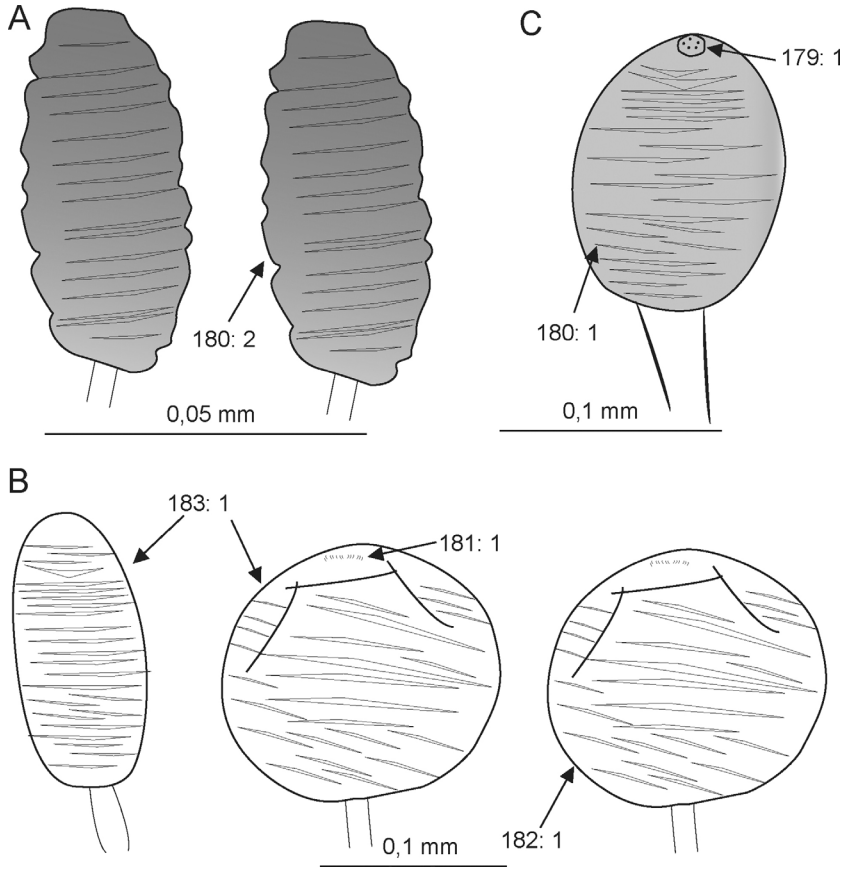

Figure 21. Spermathecal characters. **A**: *Microsoma exiguum* (Meigen, 1824); **B**: *Euoestrophasia plaumanni* Guimarães, 1977; **C**: *Dufouria chalybeata* (Meigen, 1824). Arrows identify characters and states (enclosed in parentheses) discussed in text.

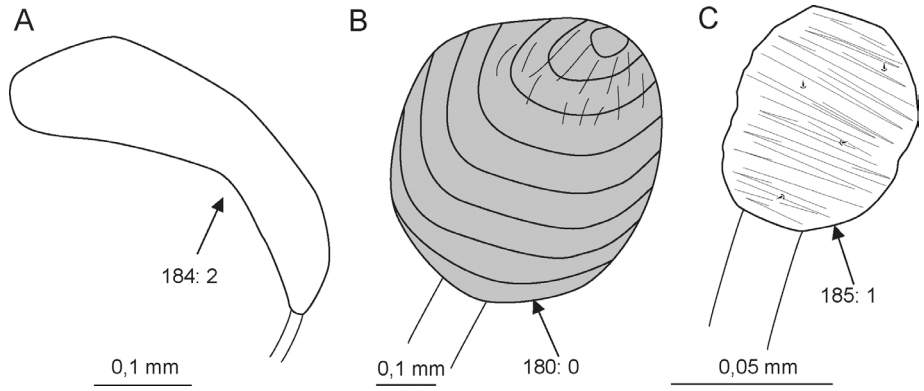

Figure 22. Spermathecal characters. **A**: *Billaea claripalpis* (Wulp, 1895); **B**: *Xanthozona melanopyga* (Wiedmann, 1830); **C**: *Imitomyia sugens* (Loew, 1863). Arrows identify characters and states (enclosed in parentheses) discussed in text.

morphies (clade 6), constituting the new definition of the tribe Freraeini. Thus, *Eugymnopeza*, *Microsoma* and *Pandelleia*, prior to this analysis as Dufouriini, were recovered in a clade within Freraeini; while the remaining genera of Dufouriini, i.e., composed only of *Rondania*, *Chetoptilia*, *Dufouria*, *Comyops* and *Ebenia* clustered in their own clade (clade 13), i.e., Dufouriini. The former Oestrophasiini genera *Cenosoma*, *Euoestrophasia*, *Jamacaria* and *Oestrophasia* are grouped in a strongly supported clade defined by 19 unambiguous synapomorphies (clade 10). This clade is sister group to Dufouriini s.s. (clade 13), and defined by three synapomorphies. Based on these results, we are here considering Oestrophasiini as a valid tribe separate from Dufouriini.

Dufouriini s.s. (clade 13) as here defined is composed by five genera: *Rondania*, *Chetoptilia*, *Dufouria*, *Comyops* and *Ebenia*. It is supported by three synapomorphies: antennae with micropubescent arista (55:1); spermathecae with pores (179:1); and male terminalia with distiphallus with anterior margin partially sclerotized (142:2 under DELTRAN); and one homoplasy: female terminalia with elongate sternite 8 (172:2).

In the internal resolution of Dufouriini s.s., *Rondania* is sister group to the clade grouping all other genera. This clade (14) is supported by seven synapomorphies: first instar larva with segment I with flattened antenna (9:0 in DELTRAN); conical segment XII (16:1 in DELTRAN); sclerite of the salivary gland narrow anteriorly and wide posteriorly (20:1 in DELTRAN); accessory sclerite falciform (21:4); intermediate region with median enlargement (26:1 in DELTRAN); female terminalia with tergite 8 fused with sternite 8 (168:1); sternite 10 sharp and curved (174:1). *Chetoptilia* is sister group to (*Dufouria* (*Comyops* + *Ebenia*) (clade 15), supported by four unambiguous synapomorphies: fronto-orbital plate with several setae on the antennal socket (43:1); male terminalia with phallapodeme with fan-shaped apex (126:1); distiphallus with ventral sclerite dorsal projection (139:1) and distiphallus with distal portion (144:1) and two unambiguous homoplasies. *Dufouria* is sister group to clade 16 (*Comyops* + *Ebenia)* supported by one unambiguous synapomorphy: male terminalia with surstylus with lateral setae (119:1), and four unambiguous homoplasies. Along with *Comyops*, we also sampled *Comyopsis*, represented by its type species, *C. fumata*, which is sister group to *Ebenia* species supported by one unambiguous synapomorphy, and therefore *Comyopsis* is here synonymized with *Ebenia* (see discussion below).

Dufouriini s.s. is sister group to Oestrophasiini (clade 9) based on seven unambiguous synapomorphies: epandrium with lateral lobes (111:1); epandrium with posterior projection zone (112:1); phallapodeme larger than hypandrium (127:1); basiphallus dorsally segmented (132:1); ejaculatory apodeme fan-shaped (146:1); anterior margin of postgonite with weak sclerotization (153:0); asymmetric spermathecae (183:1). Oestrophasiini (clade 10) as here defined and revalidated is formed by *Cenosoma*, *Euoestrophasia*, *Jamacaria* and *Oestrophasia*, as recognized by Guimarães (1977), and supported by 19 unambiguous synapomorphies: microtype egg (1:2); first instar larva with antena present, but reduced (8:2); segment II with spines with triple development in relation to length of adjacent microtrichia (13:1); posterior spiracle with conical felt chambers (19:1); sclerite of salivary gland rounded (20:2); puparia with completely fused peritreme (30:1); spiracular openings arborescent (33:1); fronto-orbital plate yellow in males (41:2); face with setulae on lunule (51:1); scutum yellow with black spots (66:1); femur II with 3 submedian anterodorsal setae in females (79:2); wing membrane with macules (80:1); tergite 5 with pair of dark brown rounded spots on ventral posterolateral region (100:1); yellow tegument (101:1); male terminalia with tergite 6 fused, but with distinguishable limits (from lateral prominences) segment 7 + 8 (103:3); pregonite fused together with downwards directed apex (151:1); female terminalia with bare tergite 6 (158:0); syntergosternite 7 ring-shaped (165:1); tergite 8 with narrow plate shape (167:1); besides seven unambiguous homoplasies.

Freraeini, by including *Pandelleia*, *Eugymnopeza* and *Microsoma*, formely in Dufouriini, provide evidence of this newly delimited clade as monophyletic (clade 6). This redesigned tribe is composed and related as follows: (*Pandelleia* (*Microsoma* (*Freraea* + *Eugymnopeza*). Here, this newly delimitation of Freraeini is supported by eight unambiguous synapomorphies: first instar larva with spines on segments I–XII (12:1); rectangular sclerite of salivary gland (20:3); triangular accessory sclerite (21:2); mouth-

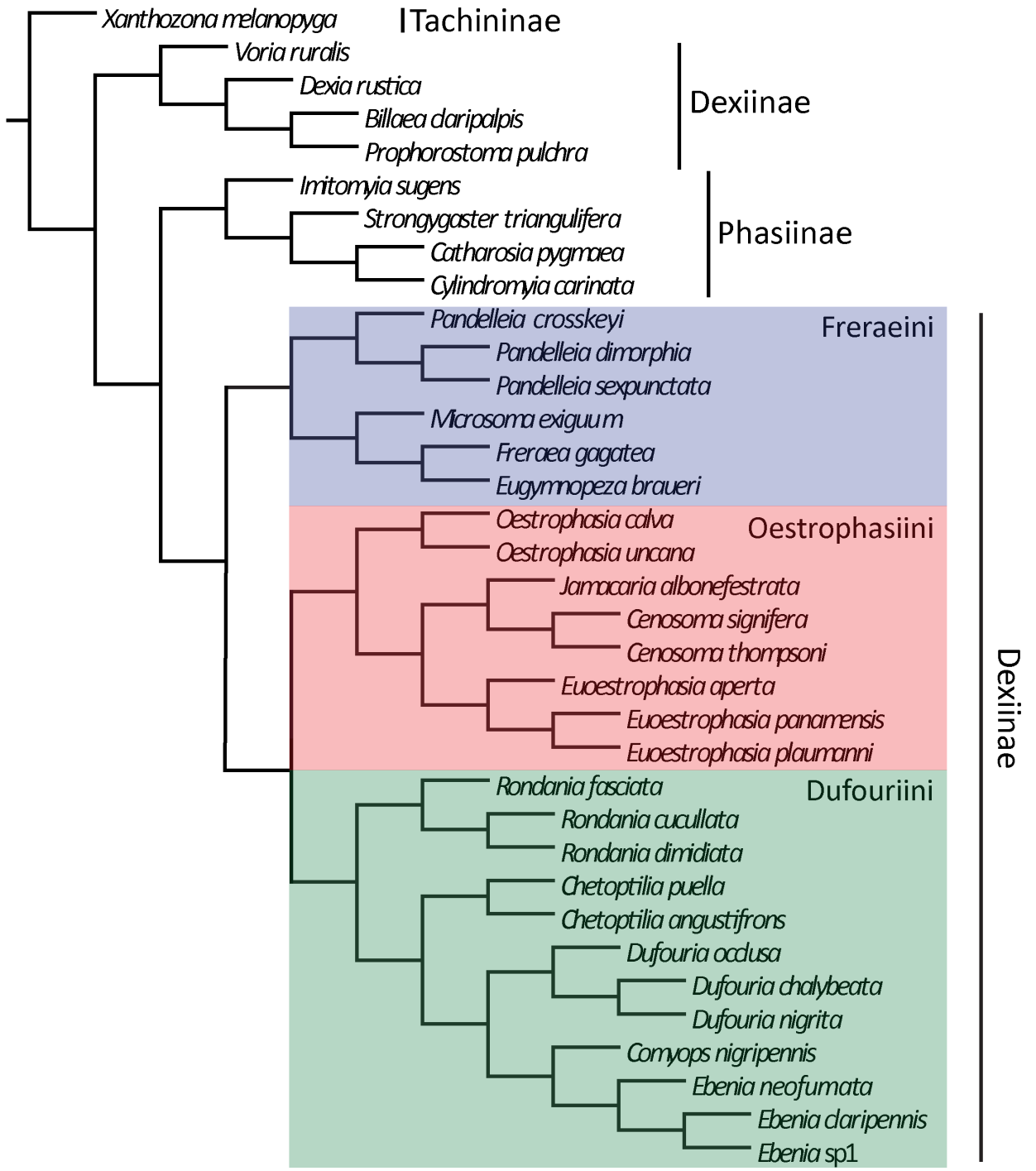

Figure 23. Most parsimonious cladogram resulting from the cladistic analysis with equal weighing analysis. See text for discussion of new nomenclatural acts summarized on the tree.

hook with same basal thickness as dorsal cornu (23:1); accessory sclerite with ventral position with regard to sclerite of salivary gland (25:1); dorsal cornu shorter in length compared to mouthhook (27:1); vertex with protuberant ocellar triangle not protuberant (38:1); and female terminalia with tergite 8 fused with sternite 8 and 9 (170:1); in addition to five unambiguous homoplasies.

All members of Dufouriini s.s., Oestrophasiini and Freraeini form a monophyletic clade (clade 5) outside the Dexiinae and sister group to the Phasiinae exemplars included herein (clade 3). Clade 5 is supported by four unambiguous synapomorphies: anepimeron with fine setae (77:2); male terminalia with hypandrial apodeme with boundary with central plate indistinct (123:2); distiphallus with extension of dorsal sclerite more than half length of median bar (137:1); and female tergite 8 elongated, when free (167:3), in addition to three homoplasies: holoptic male with dichoptic female (35:1); female terminalia with syntergosternite 7 (tergite 7 fused with sternite 7) present (164:1) and sternite 10 reduced (174:3).

The Phasiinae was recovered as sister group (clade 4) of the tribes Dufouriini s.s., Freraeini and Oestrophasiini (clade 5), being supported by six synapomorphies and two homoplasies.

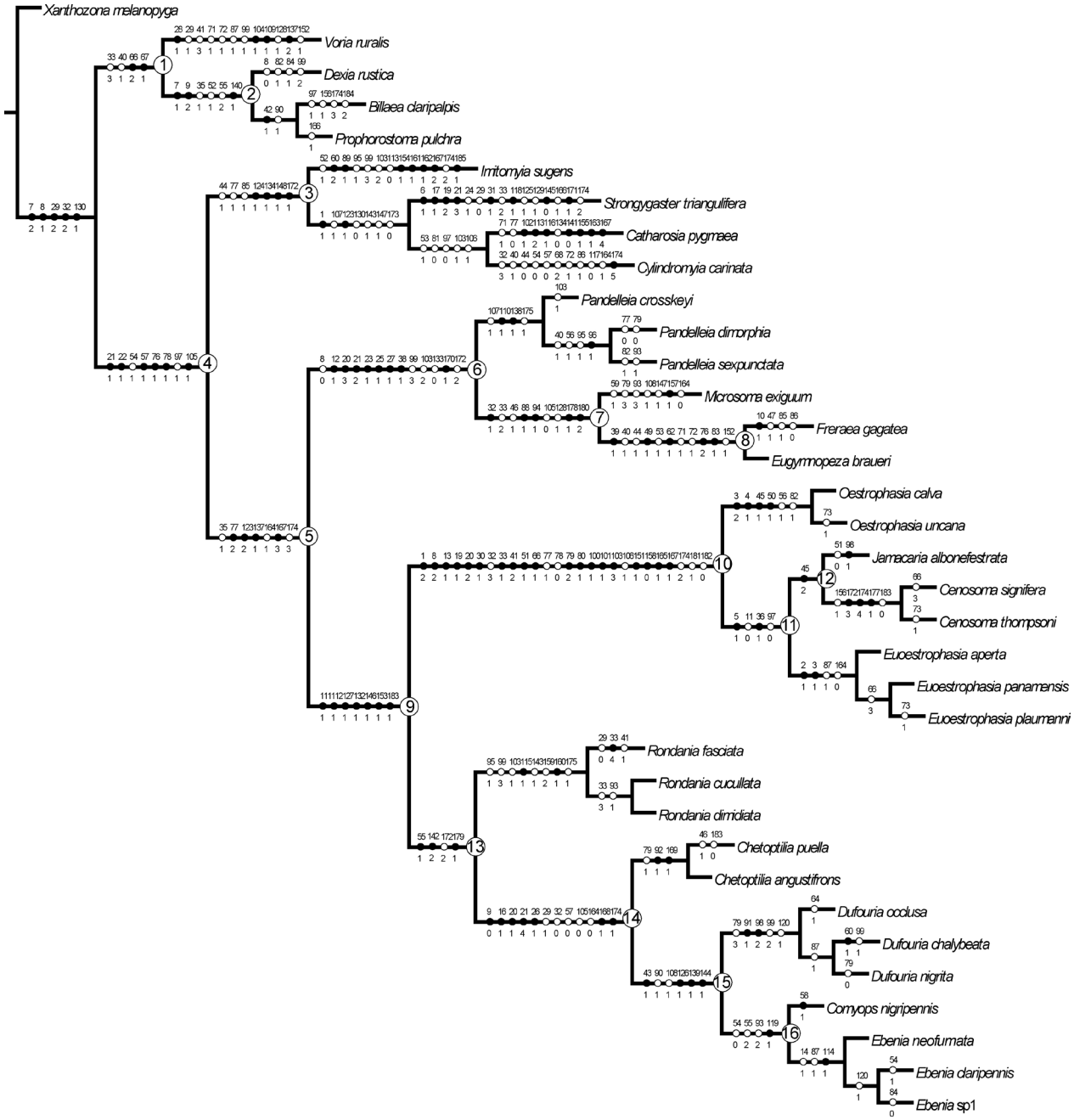

Figure 24. Most parsimonious cladogram resulting from the cladistic analysis with equal weighting analysis under unambiguous optimization. Numbers on nodes of each clade associate discussions in the text. See text for discussion of new nomenclatural acts summarized on the tree.

4. Discussion

4.1. Dufouriini, Oestrophasiini and Freraeini as separate tribes

Dufouriini was recovered as paraphyletic, confirming earlier results by Ziegler (1998), Barraclough (2005), Cantrell and Burwell (2010) and Cerretti et al. (2014); except by Stireman et al. (2019) that recovered Dufouriini as polyphyletic. A broad definition of Dufouriini including *Microsoma* and *Pandelleia*, as well as the four genera of Oestrophasiini, was not supported here. Our analysis supports splitting the previous delimitation of Dufouriini, i.e. sensu lato (Table 1), into three strongly supported and closely related tribes (clade 5): Dufouriini s.s. (hereafter, just Dufouriini), Oestrophasiini and Freraeini. Dufouriini is composed of five genera and defined by the synapomorphies mentioned above in the Results section.

Cerretti et al. (2014) considered Dufouriini in the broadest sense, comprising all the genera from Dufouriini, Oestrophasiini and Freraeini. Their six Palaearctic genera sampled (*Chetoptilia*, *Dufouria*, *Rondania*, *Pandelleia*, *Freraea* and *Eugymnopeza*) were paraphyletic and graded with a monophyletic Phasiinae. Stireman et al. (2019), on the other hand, considered Dufouriini (incl.

Oestrophasiini) and Freraeini separately. The former was sampled with five genera (*Oestrophasia*, *Rondania*, *Microsoma*, *Dufouria* and *Ebenia*), while the latter with one (*Freraea*). Their recovered Dufouriini (with four genera of Dufouriini and Oestrophasiini) and Freraeini (with two genera) as not closely related, but instead intergraded by Telothyriini and by small clades of Voriini and Palpostomatini.

Although our analysis was based on a complete generic sampling of Dufouriini, Oestrophasiini and Freraeini and considered a comprehensive and detailed morphological study of adult and immatures stages (totaling 185 characters), our results might be limited, especially concerning supratribal relationships. On one hand, those three tribes were strongly supported by comprehensive morphological evidence and based on thorough sampling of each tribe. On the other hand, to obtain a reliable intertribal relationship, a more comprehensive sampling of other tribes of Dexiinae (and perhaps Phasiinae) is recommended and desired. Our outgroup sampling was composed of taxa of Phasiinae, that were found to be closely related to Dufouriini (Cerretti et al. 2014), and Dexiinae, wherein Dufouriini are considered to belong, so it is expected that these closely related taxa to Dufouriini present the greatest potential to access the robustness of its monophyly (Grant 2019). Thus, we believe this sampling was sufficient for establishing the monophyly of Dufouriini, Oestrophasiini and Freraeini, as we are not inferring its placement within Tachinidae. Finally, we are confident that our choice of outgroups provides a crucial test of the ingroup topology – by evaluating the ingroup character-state transformations (Grant 2019) – as it can reliably answer our question within this paper, i.e., what are the relationships among the genera and the tribes Dufouriini, Oestrophasiini and Freraeini.

Given the size, diversity and distribution of Tachinidae, taxonomic sampling in Stireman et al. (2019) was far from complete, but was enough to shed light on several questions. In this sense, their findings were a step forward since they indicated that the delimitation and relationships of Dexiinae groupings remains unclear and puzzling. Now, as it will be discussed, our study adds some more evidence to the classification of Tachinidae by supporting that Dufouriini is not a single tribe, but, in fact, three separate tribes.

4.2. Redefining the tribe Dufouriini

Herting (1957, 1960) grouped the taxa with modified ovipositor (syntergite 9 + 10) in Dufouriini s.l., composed of the following Palaearctic genera: *Chetoptilia*, *Dufouria*, *Eugymnopeza*, *Freraea*, *Microsoma*, *Pandelleia* and *Rondania*. However, the three synapomorphies and one homoplasy for the tribe found herein (clade 13) were not from the female terminalia. Besides, the homology among their ovipositors was not conclusively demonstrated (O'Hara and Wood 2004), and in the present analysis some structures were considered non-homologous. For example, *Rondania* has a posteriorly directed tube-shaped ovipositor, completely fused syntergosternite 6 + 7 and lacks sternite 9, while in *Freraea* and *Eugymnopeza* the tube-shaped ovipositor is directed anteriorly, has a partially fused syntergosternite 6 + 7 and well-developed sternite 9.

The configuration of genera recovered here highly agrees with Verbeke (1962), with the *Dufouria* group within his Dufouriines, containing the Palaearctic genera *Chetoptilia*, *Dufouria* and *Rondania*. Accordingly, herein, *Rondania* is sister group to the genera *Chetoptilia*, *Dufouria*, *Comyops* and *Ebenia*. The last two genera mentioned, *Comyops* (including *Comyopsis*) and *Ebenia*, pertained to the Neotropical tribe "Ebeniini". This tribe, currently invalid and formerly composed of 11 genera, was an assemblage of many unrelated taxa that was put together by Townsend (1936). The remaining genera of the former tribe Ebeniini of Townsend (1936) are currently placed in different tribes, like Voriini, and even subfamilies, like Palpostomatini in Tachininae (O'Hara et al. 2020). However, when better studied, some of the "Ebeniini", i.e., *Ebenia*, *Comyops* and *Comyopsis*, showed affinities with the Dufouriini as discussed by Thompson (1963) and placed formally in Dufouriini by O'Hara et al. (2020). Thompson (1963) argued for a probable relationship between *Comyops*, *Comyopsis* and *Ebenia* with *Dufouria* based on larval anatomy and cephaloskeleton (similar to *Dufouria chalybeata* Meigen), as well as male terminalia (*Comyopsis* resembling *Dufouria occlusa* (Robineau-Desvoidy, 1863)). This relationship was confirmed here with *Comyops* and *Ebenia* as sister group to *Dufouria* (clade 15). Additionally, *Comyopsis* is considered a junior synonym of *Ebenia* herein (see below).

Mesnil (1975) delimited Dufouriini into three subtribes: Dufouriina with *Dufouria*; Campogastrina with *Chetoptilia*, *Pandelleia*, *Rondania* and *Microsoma*; and Freraeina with *Eugymnopeza* and *Freraea*. His classification was not recovered herein, with some genera of Campogastrina placed in Dufouriini (*Chetoptilia*, *Rondania*) and others in Freraeini (*Pandelleia* and *Microsoma*). Ziegler (1998) assigned *Rondania* and *Dufouria* to Voriini s.l. based on the 3rd instar larva cephaloskeleton, along with Stireman et al. (2019), where Dufouriini taxa were graded within Voriini (in addition to Palpostomatini and Telothyriini). O'Hara and Wood (2004) characterized members of Dufouriini (*Dufouria*, *Rondania* and *Oestrophasia*) as having a fused pregonite, thus setting apart *Microsoma* and *Freraea*. This character (150:1) was confirmed as an ambiguous homoplasy grouping Dufouriini and Oestrophasiini (clade 9). Before comparing our results with the phylogenetic hyphotheses of Cerretti et al. (2014) and Stireman et al. (2019), it is worth noting that recently O'Hara et al. (2020) placed *Kambaitimyia* Mesnil, 1953 in Dufouriini. This genus, known from two species from Myanmar, was originally assigned to Dufouriinae (Dufouriini, in part) by Mesnil (1953). However, later he changed his mind (Mesnil 1966) and placed this genus within his subtribe Ptilopsinina near Macquartini and Leskiini in Tachininae, only to be placed again in Dufouriini by Crosskey (1976) - by relying only on

the similar external adult facies with other taxa placed in this tribe by him, including the Macquartini (Tachininae) genus, *Anthomyiopsis* Townsend, 1916. Verbeke (1962) was the first author who examined the male terminalia of *Kambaitimyia* (*K. carbonata* Mesnil, 1953), and concluded that the presence of a reduced distiphallus inserted on an U-shaped basiphallus is very close to the *Strongygaster* group and it would be best placed in a group including genera like *Imitomyia*, *Strongygaster* and *Rondaniooestrus* (all currently placed in Phasiinae). Later, Tschorsnig (1985) confirmed Verbeke's (1962) conclusion and placed *Kambaitimyia* in Strongygastrini (Phasiinae). Herein, by examining and dissecting a male of *K. carbonata* from NHMUK, we further confirm the peculiar and strong resemblance of the male terminalia of members of the genus *Strongygaster* and confirm the conclusion of Verbeke (1962) and Tschorsnig (1985) that *Kambaitimyia* is conclusively not a Dufouriini and is probably best placed in Strongygastrini.

The results of Cerretti et al. (2014) with Dufouriini s.l. as paraphyletic and closely related to Phasiinae was partially confirmed herein. Our analysis confirms the close relationship between Dufouriini s.l. and Phasiinae, but both were monophyletic and sister groups. The clade with Dufouriini s.l. + Phasiinae was supported by a single homoplasy (character 45:0 of Cerretti et al. 2014): presutural acrostichal seta absent; which is not a reliable character, since it appears independently in several other taxa within Tachinidae and other muscoestroid families. Besides, the genera that were restricted to Dufouriini did not group together. Stireman et al. (2019) recovered part of Dufouriini s.l. forming a clade with (*Ebenia* (*Dufouria* (*Rondania* + *Oestrophasia*), which was confirmed here, but included a less comprehensive sampling. We support both Dufouriini and Oestrophasiini as monophyletic and sister group to each other (clade 9).

4.3. Tribe Freraeini

Our results diverge from Herting (1957, 1960, 1984), who brought together *Eugymnopeza* and *Freraea* in Dufouriini based on the structure of the ovipositor, thereby invalidating Freraeini. Herein, Freraeini (clade 6) was supported by two ovipositor characters: one synapomorphy (tergite 8 fused with sternites 8 and 9 (170:1)) and one homoplasy (sternite 8 elongated (172:2)). On the other hand, Freraeini defined herein agrees partially with Verbeke (1962) and his *Freraea*-group (containing *Freraea*, *Litophasia* and *Microsoma*) and *Pandelleia*-group (with *Pandelleia*). Verbeke based these groups on male terminalia: the former group has thin and elongated distiphallus and the latter a reduced and subrectangular basiphallus. We obtained two homoplasies from the male terminalia supporting Freraeini: tergite 6 fused but with visible suture (median dividing line present) in segment 7 + 8 (103:2) and long basiphallus (133:0). Excluding *Litophasia* (see below), Verbeke's (1962) proposal to leave these genera outside the *Dufouria*-group was accurate according to the present study, because Dufouriini (clade 13) does not include *Freraea*, *Eugymnopeza*, *Pandelleia* and *Microsoma*. These three genera in turn belong to Freraeini (clade 6), similar to his *Pandelleia*-group plus *Freraea*-group (except for *Eugymnopeza*, which Verbeke did not study).

Mesnil (1975) considered his subtribe Freraeina with the same genera as Townsend (1936), with *Freraea* and *Eugymnopeza* only. Herein, this subtribe was monophyletic (clade 8). He commented that *Microsoma* is very closely related to Freraeina, and we confirm here *Microsoma* as sister group of *Freraea* + *Eugymnopeza* (clade 7). O'Hara and Wood (2004) transferred *Freraea* from Dufouriini to Freraeini. Later *Eugymnopeza* was too placed in Freraeini by O'Hara et al. (2009), agreeing with Townsend (1936) and Mesnil (1975), a relationship that was confirmed in the present study; however, in clear contrast, O'Hara et al. (2020) changed the placement of this genus one more time, and returned it to Dufouriini. Furthermore, the character used for this transfer, presence of fused pregonite (our character 150:1), was confirmed as a synapomorphy for clade 9 (Oestrophasiini + Dufouriini). Cerretti et al. (2014) recovered a clade with most Freraeini genera ((*Pandelleia* + *Rondania*) (*Microsoma* (*Eugymnopeza* + *Freraea*))) supported by one character from the female terminalia, tergite 6 long and tubular (Cerretti et al. 2014, character 128:1). However, when scrutinized, this character shows differences among these genera. Although both *Pandelleia* and *Rondania* have a long and tubular tergite 6, it is anteriorly directed (160:0) in *Pandelleia*, while it is posteriorly directed (160:1) in *Rondania*. Additionally, only *Rondania* possesses fully telescoped terminalia. The relationships found by these authors is nearly identical to those found herein, differing only by the presence of *Rondania*, which was placed in Dufouriini herein (clade 13). Finally, the presence of the six unique synapomorphies of the first instar larva (as listed in Results), in addition to the unique synapomorphy found on the female terminalia – tergite 8 fused with sternites 8 and 9 (170:1) – are compeling evidence for the unique habit of host infection that evolved in Freraeini. As this tribe, in the same way of Oestrophasiini and Dufouriini, attacks adult Coleoptera, the functional solution to overcome this challenge was developed by some of its members (*Pandelleia*, *Eugymnopeza* and *Freraea*). Thus, they place the eggs inside the beetles with their terminalia in order to infect them; *Microsoma*, distinctively, avoided this problem by piercing the sclerite of the beetle with its sharp terminalia. This strategy, even if functionally equivalent to some Dufouriini (*Chaetoptilia*, *Dufouria*, *Ebenia* and *Comyops*), is morphological different in *Microsoma*, particularly the larva and the female terminalia, as it happens to the other members of Freraeini. It differs considerably from those genera of Dufouriini as pointed by the synapomorphies above, and clearly indicate a unique solution to infect their hosts. Thus, our preference to maintain this tribe as unique and separate from Dufouriini (clade 13).

The clade (*Microsoma* (*Eugymnopeza* + *Freraea*) of Cerretti et al. (2014) supported by one synapomorphy (anteriorly curved tergite 5, character 126:1) was also recovered here (clade 7) and supported by five synapo-

morphies. *Eugymnopeza* + *Freraea* was supported by two homoplasies in Cerretti et al. (2014): ocellar seta present or absent (polymorphic) (character 17:1/2) and short chaetotaxy, thin and reclined bristles that covers most of its surface or reaching at least the lower half (character 24:4). It was recovered here (clade 8) with one unambiguous autapomorphy and three homoplasies. Accordingly, Stireman et al. (2019) recovered *Freraea* as sister group of *Microsoma*, but *Eugymnopeza* was not sampled.

Litophasia is a very special case, as it has been considered in Catharosiini (Phasiinae) (Cerretti et al. 2014) and recently as an unplaced Dexiinae (Blaschke et al. 2018; Stireman et al. 2019). While a definitive placement for *Litophasia* is unknown, some clues might signal a possible relationship with Freraeini. Moreover Stireman et al. (2019) have indicated *Litophasia* as close to *Freraea*.

4.4. Tribe Oestrophasiini revalidated

Guimarães (1971) considered the Neotropical genera of Glaurocarini sensu Townsend (1936) as the new tribe Oestrophasiini. Moreover, Mesnil (1973) mentioned that Townsend (1936) erroneously classified *Oestrophasia* and *Cenosoma* in Glaurocarini and these genera are related to *Dufouria*, with a connection with his subtribe Campogastrina near *Chetoptilia*. Our results partially agree with Mesnil's (1973) since members of his Campogastrina, namely, *Chetoptilia*, *Pandelleia* and *Rondania*, but not *Microsoma*, are placed in Dufouriini and sister group to Oestrophasiini (clade 9). Guimarães (1977), in his revision of Oestrophasiini, discussed a likely relationship of this tribe with the Old World Dufouriini based on Verbeke's (1962: pl. X) genitalia drawings of *Chetoptilia*, *Dufouria* and *Rondania*.

Tschorsnig (1985) formally considered Oestrophasiini as belonging to Dufouriini. O'Hara and Wood (2004) agreed with Tschorsnig (1985) based on the presence of a fused pregonite. This character was used here (character 150:1) and appeared to be an ambiguous homoplasy grouping Oestrophasiini and Dufouriini. Despite the importance of the pregonite, a relevant synapomorphy for Oestrophasiini is the presence of microtype eggs (character 1:2). Thus far, this feature had only been considered to be present in Goniini and some Blondeliini (Gaponov 2003), however we found and characterized it as present in Oestrophasiini based on the evidence provided by Gaponov (2003) and Salked (1980) for the eggs, the internal morphology of the female and the larva by Thompson (1924, 1963). This is so because these eggs are very small in size (less than 0.4 mm in length); are placed on leaves and are accidentally ingested by the host, which are thus infected (Grillo and Alvarez 1984); are present in high quantity (between 2,000 and 3,000); the female ovary have more than 100 ovarioles (Grillo and Alvarez 1984); while the larvae have extremely reduced antennae and posterior spiracles; transparent and colourless cuticle, with rows of spines at the posterior end of the first two thoracic segments; segment I extremely well-developed and pigmented, with the rest of the body without spines. Accordingly, the important biological significance of the presence of microtype eggs in Oestrophasiini, which indicates a very specific and complex adaptation to host infection (Gaponov 2003; Thompson 1963), in addition to the posterior spiracles of the puparia, with the peritreme completely fused (character 30:1) – constituting a unique characteristic within Tachinidae, unknown elsewhere in the family (Ferrar 1987; Greene 1921; Ziegler 1998) – confirm that this tribe is best ranked as a separate tribe from Dufouriini. Moreover, an additional 17 unambiguous synapomorphies are shared by Oestrophasiini and separate them from Dufouriini.

Still within Oestrophasiini, Wood (1987) synonymized *Cenosoma* with *Oestrophasia*, an act that was maintained by O'Hara and Wood (1998, 2004). Here the synonymy was not supported, with *Oestrophasia* monophyletic and supported by four autapomorphies and two homoplasies, and sister group of *Cenosoma*, *Euoestrophasia* and *Jamacaria* (clade 11). Based on this evidence, *Cenosoma* and *Oestrophasia* are considered as distinct genera herein. *Jamacaria* is a monotypic genus that is sister group to *Cenosoma*. Finally, our analysis did not support the placement of *Cenosoma thompsoni* as unplaced species of *Oestrophasia* (sensu O'Hara et al. 2020) as done by O'Hara et al. (2020). Contrarily, our phylogenetic analysis places *Cenosoma thompsoni* conclusively within *Cenosoma* as proposed by Guimarães (1977).

4.5. *Comyopsis* as synonym of *Ebenia*

Herein, *Comyopsis* Townsend, 1919 is conclusively transferred from the former tribe Ebeniini to Dufouriini, confirming the proposal of Thompson (1963), and most recently by Stireman et al. (2019) and O'Hara et al. (2020). Additionally, following our phylogeny, we also propose *Comyopsis* as a junior synonym of *Ebenia* Macquart, 1846. Furthermore, our work does not confirm the proposition of O'Hara et al. (2020) that, oddly, placed *Comyopsis* in Voriini. In our analysis however, *Comyopsis fumata* is sister group of *E. claripennis* + *Ebenia* sp. 1 (within clade 16). Unlike Townsend's (1927: 234) key, *C. fumata* does have a costal spine and vein R_{4+5} with setulae reaching crossvein r-m, as well as *Ebenia* species. In Thompson's key (1963: 342), the couplet separating *Ebenia* and *Comyopsis* uses the length of the costal spine (long in *Comyopsis*, short in *Ebenia*) and wing membrane pigmentation (smoky in *Comyopsis*, and totally hyaline in *Ebenia*). After examining some species of *Ebenia*, we found that the only characteristic distinguishing these genera is the setulose prosternum in *Ebenia*. We considered this character as very unsubstantial to justify generic separation. Besides, there is no significant difference between their male terminalia, therefore, we propose a synonymy between *Comyopsis* and *Ebenia*. The only species of *Comyopsis*, *C. fumata* Townsend, 1919 (type-locality: Nicaragua, Chinandega) is consequently transferred to *Ebenia*. However, when O'Hara et al. (2020) placed the previously unplaced species of "Ebeniini" (Guimarães 1971), *Ebenia fumata* (van der Wulp, 1891) in *Ebenia*,

our new synonymy, *E. fumata* (Townsend, 1919), constitutes a junior secondary homonomy. In order to resolve this issue, we herein propose a new name for this new combination: *Ebenia neofumata* Santis and Nihei ***nomen novum*** for *Ebenia fumata* (Townsend, 1919) [*nomen preoccupatum*].

4.6. Systematic placement of *Mesnilana* and *Rhinophoroides*

The Afrotropical genera *Mesnilana*, with one single species *M. bevisi* Emden, 1945, and *Rhinophoroides*, also with one single species *R. minutus* Barraclough, 2005, were originally included in Dufouriini. Emden (1945) erected *Mesnilana* for a female from South Africa and included it by considering the classification of Mesnil (1939), which was then in Phasiinae. In the generic description, he commented (1945: 414): "*The longer antenna would seem to approach this genus to the Ocypterini* [Cylindromyiini, in part]*, but the general appearance, genitalia, dark occipital hairs, etc. make it more closely related to* Diplopota [= *Imitomyia* Townsend]". Thus, *Imitomyia*, currently placed in its own tribe (Imitomyiini) in Phasiinae (Tschorsnig 1985) or uncertain position (Stireman et al. 2019), would be the closest genus of *Mesnilana*. Later, Crosskey (1980, 1984) maintained *Mesnilana* in Dufouriini, but in the subfamily Dufouriinae. Barraclough (2005) described the new genus *Rhinophoroides* and placed it in Dufouriini and subfamily Dexiinae, because of its great resemblance with *Mesnilana*. Actually, Barraclough reported that he did not observe any close relationships between any other Afrotropical genera of Dufouriini and included *Rhinophoroides* in this tribe by relying only on the general similarity with *Mesnilana*. In the Afrotropical Catalogue (O'Hara and Cerretti 2016), *Mesnilana* was tentatively placed in Dufouriini and it was pointed that *Rhinophoroides* could be a junior synonym of *Mesnilana*.

The female holotype of *Mesnilana bevisi* deposited at NHMUK was recently examined by MDS, and by carefully observing the descriptions provided by Barraclough (2005), we found that the observed features do not correspond to the Dufouriini as redefined herein, nor with any of the related tribes, Freraeini and Oestrophasiini. Some of these characters include the bare facial ridge, three katepisternal setae and anepimeron with a well-developed seta. In addition to external morphology, more evidence seems to provide an important biological insight: both genera were collected in light traps, suggesting nocturnal hosts (Barraclough 2005). This is not known from other members of Dufouriini and is uncommon in Tachinidae (occurring, for instance, in the cricket parasitoid tribe Ormiini, Tachininae). Some of the characters found in both *Mesnilana* and *Rhinophoroides* are the small and tongue-shaped lower calyptra that diverges from the scutellum and the parafacial with several setulae; these traits are also found in the coleopteran parasitoid tribe Palpostomatini. Besides these traits, the general appearance (abdominal chaetotaxy and head proportions) is very similar to some Palpostomatini, mainly the genus *Palpostoma* (e.g., *Palpostoma subsessile* Malloch, 1931). Based on these observations, *Mesnilana* and *Rhinophoroides* are removed from Dufouriini and tentatively considered as Palpostomatini, until additional evidence becomes available. In Stireman et al. (2019), Palpostomatini was a polyphyletic group, with one part forming a clade with Imitomyiini and sister to all other Dexiinae + Phasiinae, and another part as sister to Freraeini.

4.7. Dufouriini or Dufouriinae?

For a long time, Dufouriini was considered a tribe or subtribe of Phasiinae. It was initially allocated as a subtribe of Phasiini by Mesnil (1939), and then as tribe of Phasiinae by Emden (1945, 1950) based mainly on chaetotaxy. Verbeke (1962, 1963) considered it as a new subfamily: Dufouriinae, including two tribes, Dufouriini and Macquartiini (the latter currently in Tachininae), based mainly on postgonites of the intermediate type (in relation to the sensory and the connective Type II) and distiphallus DEG subtype. Verbeke also noted similarities in the male postabdomen shared by Dufouriinae and Phasiinae and was the first to suggest a close relationship between Dufouriinae and Phasiinae. Finally, the specializations of the female terminalia which allow Dufouriini to parasitize adult Coleoptera, as well as Phasiinae to parasitize adult Heteroptera, support the proximity between the two groups (Verbeke 1962). In contrast, in Dexiini hosts are actively sought out by first instar larvae deposited by females near the host and females possess a simple and short terminalia, with larvae completing their development in the host (Barraclough 1992). Later, Crosskey (1976, 1980) also recognized the subfamily Dufouriinae with the tribes Imitomyiini and Dufouriini, as these two would be excluded from Phasiinae and Dexiinae, respectively.

Following Herting (1984), Tschorsnig (1985) considered Dufouriini as a tribe of Dexiinae, with this subfamily as probably monophyletic, being supported by characters of the male terminalia; however, he recognized it as very inconsistent considering its biology and adult external characters. As discussed previously, the main putative synapomorphy discussed by Tschorsnig (1985) – aedeagus with basiphallus and distiphallus articulated to each other – was not recovered as a synapomorphic character herein, agreeing with Cerretti et al. (2014). The state 1 of character 130 is a synapomorphy shared by clade 1 (Dexiini + Voriini) and clade 4 ((Freraeini (Oestrophasiini + Dufouriini)) + Phasiinae) but undergoes a reversal in Phasiinae. Cerretti et al. (2014) proposed the paraphyly of Dufouriini s.l. in relation to Phasiinae, providing more evidence for a close phylogenetic relationship between these groups. Furthermore, Tschorsnig (1985) also recognized a number of similarities between the male terminalia of Dufouriini and Phasiinae, reporting that only the pregonite and phallus would place Dufouriini near Dexiinae. Considering his dichotomous key of the male terminalia of Tachinidae (Tschorsnig 1985), several shared characteristics can be found in the couplet of Dufouriini

and Phasiinae: sternite 5 without lobes and without lateral membranous line; membranous connection between sternites 5 and 6; tergite 6 fused to segment 7 + 8. In the same line, Cantrell (1988: 147) stated: "The affinities of the Dufouriinae appear to be intermediate between those of the Phasiinae and Dexiinae and deserve further study." Barraclough (1992) reported that the Palaearctic Dufouriini would not belong to Dexiinae, considering modifications in the female terminalia (elongated tergite 8 forming dorsal lamellae). He then affirmed: "[T]he Dufouriini belong in neither the Phasiinae nor Dexiinae." (1992: 1152).

Our phylogenetic results support the proximity between the clade (Freraeini (Oestrophasiini + Dufouriini) and Phasiinae, as previously suggested by Verbeke (1962, 1963), Crosskey (1976, 1980) and Cerretti et al. (2014). Furthermore, Verbeke (1962, 1963), Crosskey (1976, 1980), Cantrell (1988) and Barraclough (1992) indeed argued for Dufouriini as a separate subfamily, i.e., Dufouriinae. Despite our results, other relevant phylogenetic results (Cerretti et al. 2014; Stireman et al. 2019) were not conclusive in supporting (or rejecting) the ideas of a clade formed by Dufouriini, Oestrophasiini and Freraeini or a close relationship between this clade and Phasiinae. Our taxonomic sampling, with five out of 12 Dexiine tribes (Dexiini, Voriini, Dufouriini, Freraeini and Oestrophasiini), does not allow any conclusions at the subfamily level. Hence, we included a comprehensive sampling for Dufouriini, Oestrophasiini and Freraeini, but a reduced and critical sampling of other Dexiine tribes. Cerretti et al. (2014) sampled five tribes (Dexiini, Dufouriini, Eutherini, Freraeini, Voriini) and Stireman et al. (2019) included representatives from all Dexiine tribes, and Dufouriini + Oestrophasiini was not closely related to Freraeini, nor was it close to Phasiinae. However, many tribes were not monophyletic (namely Dexiini, Voriini, Palpostomatini, and Dufouriini), perhaps indicating the need for more information (e.g., phylogenomic approaches and a detailed morphological analysis; and/or the need for better sampling of each tribe). This matter is completely open to debate with Dexiinae deserving further studies to reach a better conclusion about the systematic ranking and placement of Dufouriini, Oestrophasiini and Freraeini. Only time and more empirical data will tell whether these three tribes should be better elevated to subfamily level (the Dufouriinae of Verbeke 1962, 1963).

4.8. New classification proposal

We propose a new classification for Dufouriini based on our phylogenetic results (see Supplementary file 4). The tribe Dufouriini is redefined and restricted now to five genera only: *Chetoptilia*, *Comyops*, *Dufouria*, *Ebenia* and *Rondania*. *Comyopsis* is proposed as a **junior synonym** of *Ebenia*, and *Ebenia neofumata* Santis and Nihei **nom. nov.** is transferred from *Comyopsis* to *Ebenia*. The other genera formerly recognized in Dufouriini are allocated to Freraeini and Oestrophasiini. The tribe Freraeini is redefined and broadened to include *Microsoma*, *Eugymnopeza* and *Pandelleia*, along with the type genus, *Freraea*. The tribe Oestrophasiini sensu Guimarães (1977) is **revalidated**, including four genera: *Cenosoma*, *Jamacaria*, *Oestrophasia* and *Euoestrophasia*, all removed from Dufouriini. *Cenosoma* **stat. rev.**, previously a subgenus of *Oestrophasia* is **revalidated** as genus. Finally, although not included in the phylogenetic analysis, *Mesnilana* and *Rhinophoroides* are removed from Dufouriini and are tentatively transferred to Palpostomatini.

5. Conclusions

This is the first phylogenetic study to include all genera of Dufouriini s.l. (Dufouriini, Oestrophasiini) and Freraeini. Our study supported the monophyly and taxonomic validity of Dufouriini, Oestrophasiini and Freraeini, each defined by several synapomorphies. Furthermore, the three tribes formed a sister group clade to Phasiinae sharing six synapomorphies. Despite the most recent efforts, phylogenetically supported definitions of tachinid groupings remain uncertain at all levels. At the subfamily level, morphological data only recovered Phasiinae as monophyletic (Cerretti et al. 2014), whereas molecular data recovered Phasiinae and Exoristinae (Stireman 2002; Tachi and Shima 2010; Blaschke et al. 2018; Stireman et al. 2019), in addition to Dexiinae more recently (Stireman et al. 2019).

The present study carried out a holomorphological phylogenetic analysis based on total evidence of morphological characters from eggs, puparium, larvae and adults (including male and female terminalia, and spermathecae). Morphological characters of adults along with male terminalia are traditionally used as main character sources in Tachinidae systematics and this study demonstrated that characters from eggs, larvae, puparia, female terminalia and spermathecae have great systematic importance, as they mutually supported clades and resulted in important synapomorphies for several taxonomic levels. The clade grouping Dufouriini, Oestrophasiini and Freraeini was supported by three unambiguous synapomorphies from adult external morphology, male terminalia and spermathecae, and one homoplasy from female terminalia. The eight unambiguous synapomorphies supporting Freraeini were from first instar larvae (six synapomorphies), adult external morphology (1) and female terminalia (1). Oestrophasiini is a separate case, being supported by characters from all sources of evidence, with synapomorphies from the egg (1), first instar larva (4), puparium (3), adult external morphology (10), male terminalia (5), female terminalia (5) and spermatheca (2). The use of other character sources to infer phylogenetic relationships besides the traditional adult external morphology and male terminalia has been discussed and emphasized by a number of authors that dealt withTachinidae classification (e.g., Thompson 1954, 1960, 1961, 1963; Herting 1957, 1983; Mesnil 1966; Richter 1987; Ferrar 1987; Barraclough 1992; Ziegler 1998; Cerretti et al. 2014), and our study is a confirmation of their views.

We hope that, besides contributing to the phylogeny and classification of Dufouriini, Oestrophasiini and Freraeini, our study also highlights the need for more detailed morphological studies of Tachinidae taxa. Our study demonstrates that little is known about the basic morphology and biology of this group. For example, microtype eggs were previously described and recognized only in Goniini and some Blondeliini (Gaponov 2003), being a synapomorphy for Goniini (Cerretti et al. 2014), but herein were also recognized in Oestrophasiini. Therefore, we wonder how many trivial discoveries are still hidden inside the drawers just waiting for our curiosity.

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Supplementary mateiral 1

Terminals used in cladistic analysis

Authors: Santis and Nihei (2021)
Data type: .docx
Explanation note: Terminals used in cladistic analysis with their respective distribution, data source and discrimination regarding if the structure was observed directly (• = examined; - = not examined) or from literature data (reference given).

Link: https://doi.org/10.3897/asp.80.e69618.suppl1

Supplementary mateiral 2

Morphological character matrix

Authors: Santis and Nihei (2021)
Data type: .docx
Explanation note: Morphological character matrix showing 185 characters for 35 terminals (including nine outgroup taxa).

Link: https://doi.org/10.3897/asp.80.e69618.suppl2

Supplementary mateiral 3

Cladograms

Authors: Santis and Nihei (2021)
Data type: .docx
Explanation note: Cladograms showing ACCTRAN and DELTRAN optimizations and bremer support.

Link: https://doi.org/10.3897/asp.80.e69618.suppl3

Supplementary mateiral 4

New classification proposal

Authors: Santis and Nihei (2021)
Data type: .docx
Explanation note: New classification proposal for Dufouriini, Oestrophasiini and Freraeini.

Link: https://doi.org/10.3897/asp.80.e69618.suppl4