

Research article

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Simulium (*Trichodagmia*) (Diptera, Simuliidae) phylogeny revisited: the Neotropical and Afrotropical connection

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Abstract. *Simulium* (*Trichodagmia*) Enderlein, 1934 has an unstable classification system. The broader concept of the subgenus includes five species-groups resulting from an extensive history of synonymisations, often outside of a phylogenetic framework. This concept also ignores relationship hypotheses with the Afrotropical subgenera *S. (Anasolen)* Enderlein, 1930, *S. (Freemanellum)* Crosskey, 1969, *S. (Xenosimulium)* Crosskey, 1969, and the Oviedo species-group, with several Neotropical species of *S. (Trichodagmia)*. We performed a morphological phylogenetic analysis to test the monophyly of *S. (Trichodagmia)*, its species-groups, and their relationship with the above-mentioned subgenera and Oviedo species-group. We analysed a data matrix with 69 terminal taxa and 62 characters under parsimony implied weights, with a range of concavities (k1–100), finding three categories of k. Our analysis concludes that *S. (Trichodagmia)* is not monophyletic, since Oviedo and the Afrotropical subgenera group with its species-groups Tarsatum and Orbitale. Therefore, we propose a new classification for *S. (Trichodagmia)* by restricting it to the Orbitale species-group, revalidating *S. (Hearlea)* Vargas *et al.*, 1946, *S. (Hemicnetha)* Enderlein, 1934, *S. (Obuchovia)* Rubtsov, 1947 and *S. (Shewellomyia)* Peterson, 1975, synonymising *S. (Xenosimulium)* with *S. (Anasolen)*, and erecting a new subgenus, *S. (Disculter)* subgen. nov. for Oviedo. The geographical distribution of the groups involved is discussed.

Keywords. Systematics, morphology, black flies, Neotropical Region, Afrotropical Region.

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Introduction

Simuliidae Newman, 1834 (Diptera), commonly known as black flies, has 2331 recognized species distributed in 31 genera, *Simulium* Latreille, 1802 being the most diverse with 1905 species, 37 subgenera,

and 110 species-groups (Adler 2020). However, the relationships among *Simulium* supra-specific groups are scarcely studied and the diagnoses of several of its subgenera are unsatisfactory (Gil-Azevedo *et al.* 2012). This is especially noticeable in the Neotropical *Simulium*, for which several authors have proposed different systematic arrangements in the last decades (e.g., Py-Daniel & Moreira-Sampaio 1994; Coscarón & Coscarón-Arias 2007; Shelley *et al.* 2010).

The subgenus *Simulium* (*Trichodagmia*) Enderlein, 1934 is a good example of this problem. It's so-far unstable classification is a result of an extensive taxonomic history, as various authors have attempted to define it (e.g., Coscarón 1987; Coscarón & Coscarón-Arias 2007; Shelley *et al.* 2010; Hernández 2011). The splitter's concept of *S. (Trichodagmia)* [= *S. (Trichodagmia)* s. str.] (Coscarón & Coscarón-Arias 2007; Coscarón *et al.* 2008) comprises only seven species from the Neotropical Region, and accepts *S. (Hearlea)* Vargas, Martínez Palacios & Díaz Nájera, 1946, *S. (Hemicnetha)* Enderlein, 1934, and *S. (Thrysopelma)* Enderlein, 1934 as valid subgenera.

The lumpers' concept of *S. (Trichodagmia)* (Shelley *et al.* 2010; Hernández 2011) consists of synonyms of preexisting *Simulium* subgenera with *S. (Trichodagmia)*, treated as species-groups: *Simulium (Hearlea)* [= Canadense species-group]; *S. (Shewellomyia)* Peterson, 1975 [= Pictipes species-group]; *S. (Hemicnetha)* [Tarsatum species-group]; *S. (Thrysopelma)* together with *S. (Trichodagmia)* s. str. [= Orbitale species-group]; *S. (Obuchovia)* Rubtsov, 1947 [= Albellum species-group]. Altogether, it includes 78 valid species distributed in diverse biogeographical regions in six species-groups: the Albellum species-group (Palearctic, 15 species), the Canadense species-group (Nearctic and Neotropical, 20 species), the Orbitale species-group (Neotropical, 21 species), the Pictipes species-group (Nearctic, three species), and the Tarsatum species-group (Nearctic and Neotropical, 19 species) (Adler 2020; Nascimento *et al.* 2020). According to this classification, the subgenus is the fifth largest among *Simulium* subgenera in terms of number of species and one of the most widespread in the world.

Crosskey (1969) raised an important consideration concerning the taxonomic limits of *S. (Trichodagmia)*. He suggested that the Afrotropical subgenera *S. (Anasolen)* Enderlein, 1930, *S. (Freemanellum)* Crosskey, 1969, and *S. (Xenosimulium)* Crosskey, 1969 each share several morphological and ecological similarities with species of *S. (Trichodagmia)* s. str. (e.g.: pupal cocoon shape, pupal gills form (in *S. (Anasolen)* and *S. (Xenosimulium)*), similar larval body shape, larval posterior proleg with numerous hooked rows and absence of ventral papillae). Miranda-Esquivel & Coscarón (2001) corroborated this relationship through a phylogenetic analysis including representatives of *S. (Anasolen)* (three species), *S. (Freemanellum)* (one species), *S. (Xenosimulium)* (one species), *S. (Trichodagmia)* s. str. (seven species), *S. (Thrysopelma)* (six species), *S. (Hearlea)* (three species), and *S. (Hemicnetha)* (two species). All these groups, together with the Neotropical Oviedo species-group (form by *S. oviedo* Ramírez-Pérez 1971 and *S. rivasi* Ramírez-Pérez 1971), formed a clade based on two synapomorphies: larval anal sclerite with more than 150 hooks; pupal tergites VI to IX without a basal spine comb. Additionally, *S. (Freemanellum)* groups with [*S. (Anasolen)* + *S. (Xenosimulium)*] and [*S. (Trichodagmia)* s. str. + *S. (Thrysopelma)*], as proposed by Crosskey (1969), sharing three synapomorphies: pupal gills filaments with sharp tips; pupal gills with strong sclerotization (dark tips); female with the basal sector of radial vein hairy. However, none of these relationships were considered by Shelley *et al.* (2010) and Hernández (2011) in their lumpers' concept of *S. (Trichodagmia)*.

In the present paper, we performed a cladistic analysis to test the monophyly of *S. (Trichodagmia)* and to investigate its phylogenetic position, considering the possible relationship with the Afrotropical subgenera *S. (Anasolen)*, *S. (Freemanellum)*, *S. (Xenosimulium)*, and the Neotropical Oviedo species-group.

Material and methods

Terminal taxa and specimens

The present study included 69 terminal taxa. The ingroup was composed of representatives of *S. (Trichodagmia)* (32 species, 41% of the subgenus), *S. (Anasolen)* (five species, 45%), *S. (Freemanellum)* (four species, 57%), *S. (Xenosimulium)* (five species, 100%), as well as *S. oviedoii*, and *S. rivasi*, including the type species of each of the mentioned subgenera (Table 1). The outgroup was composed of 21 representatives of other Afrotropical, Nearctic, Neotropical, and Palearctic *Simulium* subgenera. The analysis was rooted with *Metacnephia bilineata* (Rubtsov, 1940), a species from a genus considered to be the sister-group of *Simulium* (Adler *et al.* 2004; Gil-Azevedo 2010).

We chose the terminal taxa according to the following criteria: **A.** Inclusion of endemic subgenera from the Afrotropical, Nearctic, Neotropical, and Palearctic regions to investigate possible phylogenetic relationships; **B.** Inclusion of subgenera and representatives of species-groups synonymized under *S. (Trichodagmia)* but considered as valid by other authors, such as *S. (Hemicnetha)* (species-groups Brachycladum (four species), Mexicanum (two species), and Paynei (four species)), *S. (Hearlea)* (species-groups Carolinae (two species) and Juarezi (three species)), *S. (Thrysopelma)* (five species); **C.** Inclusion of subgenera shown as related to *S. (Trichodagmia)* in previous phylogenies (Miranda-Esquivel & Coscarón 2001; Adler *et al.* 2004; Hernández 2011; Gil-Azevedo *et al.* 2012).

The specimens examined are detailed in the Supp. file 3 and are deposited in the following entomological collections:

CSIOC = Coleção de Simulídeos, Instituto Oswaldo Cruz (Fiocruz), Rio de Janeiro, Brazil
MLP = Museo de La Plata, La Plata, Argentina
MNRJ = Museu Nacional, Universidade Federal do Rio de Janeiro, Brazil
NHMUK = Natural History Museum, London, England

Terminology and characters

The terminology used follows that of Adler *et al.* (2004). To support the morphological study, we used the original descriptions of the species used as terminal taxa, all works cited in the introduction, and the following works: De-Meillon (1930, 1953); Gibbins (1935, 1937, 1938, 1939); Fain (1949, 1950, 1951); Grenier & Douget (1949); Freeman & De-Meillon (1953); Dalmat (1955); Grenier & Grjébine (1958); Shewell (1959); Crosskey (1960, 1967); Lewis (1961); Díaz-Najera & Vulcano (1962); Grenier & Le Roy-Moret (1970); Ovazza & Ovazza (1970); Ramírez-Pérez (1971, 1983); Lewis & Disney (1972); Fain & Elsen (1973, 1980); Rubtsov (1956, 1974); Fain *et al.* (1982); Car (1983); Currie & Adler (1985); Maia-Herzog *et al.* (1985); Crosskey & Santos-Grácio (1985); Bouzidi & Giudicelli (1987); Peterson *et al.* (1988); Coscarón & Py-Daniel (1989); Shelley *et al.* (1989); Adler (1990); Luna-Dias *et al.* (2004); Strieder (2004); Hernández & Shelley (2005); Py-Daniel *et al.* (2005); Hernández *et al.* (2007); Díaz *et al.* (2015); De Moor (2016); Nascimento *et al.* (2017a, 2017b).

Based on an extensive morphological study of adults, pupae, and last instar larvae, we described the characters and proposed hypotheses of primary homology (Pinna 1991). To code the characters used (Supp. file 1), we adopted the logic of Sereno (2007) and summarized these in a data matrix (Supp. file 2) using the program Mesquite (Maddison & Maddison 2017).

Phylogenetic analyses

We submitted the data set to a parsimony analysis under equal weights (EW) and implied weights (IW) in TNT (Goloboff *et al.* 2008a). The IW analysis carried the searches out under RAS+TBR, with a wide range of concavities (k3 to k100). The approach used here derives from Goloboff *et al.* (2008b), being

Table 1 (continued on next page). In-group of the cladistics analyses. Abbreviations: AF = Afrotropical; NA = Nearctic; NT = Neotropical; PA = Palearctic.

Subgenera (lumper concept)	Species groups	Subgenera (splitter concept)	Species in analysis (with status)	Distribution
<i>S. (Anasolen)</i> Enderlein, 1930			<i>dentulosum</i> Roubaud, 1915 (Type of <i>Anasolen</i>)	AF
			<i>kauntzeum</i> Gibbins, 1938	AF
			<i>masabae</i> Gibbins, 1934	AF
			<i>nili</i> Gibbins, 1934	AF
			<i>ngabogei</i> Fain, 1950	AF
<i>S. (Freemanellum)</i> Crosskey, 1969			<i>berghei</i> Fain, 1949 (Type of <i>Freemanellum</i>)	AF
			<i>hirsutilateris</i> De Meillon, 1937	AF
			<i>debegene</i> De Meillon, 1934	AF
			<i>manense</i> Elsen & Escaffre, 1976	AF
<i>S. (Trichodagmia)</i> Enderlein, 1934	Albellum	<i>S. (Obuchovia)</i> Rubtsov, 1947	<i>margaritae</i> Rubtsov, 1956	PA
			<i>auricoma</i> Meigen, 1818	PA
			<i>galloprovinciale</i> Giudicelli, 1963	PA
			<i>ibericum</i> Crosskey & Santos Grácio, 1985	PA
	Canadense	<i>S. (Hearlea)</i> Vargas, Martínez Palacios & Díaz Nájera, 1946	<i>ayrozai</i> Vargas, 1945	NA
			<i>canadense</i> Hearle, 1932 (Type of <i>Hearlea</i>)	NA
			<i>capricorne</i> De León, 1945	NA
			<i>larvispinosum</i> De León, 1948	NA
			<i>carolinae</i> De León, 1945	NA
	Pictipes	<i>S. (Shellewomyia)</i> Peterson, 1975	<i>claricentrum</i> Adler, 1990	NA
			<i>innoxium</i> Comstock & Comstock, 1895	NA
			<i>pictipes</i> Hagen, 1880 (Type of <i>Shewellomyia</i>)	NA
	Orbitale	<i>S. (Trichodagmia)</i> s. str.	<i>huairayacu</i> Wygodzinsky, 1953	NT
			<i>lahillei</i> (Paterson & Shannon, 1927)	NT
			<i>nigrimanum</i> Macquart, 1838	NT
			<i>townsendi</i> Malloch, 1912 (Type of <i>Trichodagmia</i>)	NT
			<i>guianense</i> Wise, 1911	NT
		<i>S. (Thyrsopelma)</i> Enderlein, 1934	<i>hirtipupa</i> Lutz, 1910	NT
			<i>itaunense</i> D'Andretta & Dolores González, 1964	NT
			<i>orbitale</i> Lutz, 1910 (Type of <i>Thyrsopelma</i>)	NT
<i>scutistriatum</i> Lutz, 1909			NT	
<i>brachycladum</i> Lutz & Pinto, 1932			NT	
Tarsatum	<i>S. (Hemicnetha)</i> Enderlein, 1934	<i>briceno</i> i Vargas, Martínez Palacios & Díaz Nájera, 1946	NT	

Table 1 (continued).

Subgenera (lumper concept)	Species groups	Subgenera (splitter concept)	Species in analysis (with status)	Distribution
			<i>cristalinum</i> Coscarón & Py-Daniel, 1989	NT
			<i>earlei</i> Vargas, Martínez Palacios & Díaz Nájera, 1946	NA + NT
			<i>hieroglyphicum</i> Peterson, Vargas & Ramírez-Pérez, 1988	NT
			<i>lobatoi</i> Luna Dias, Hernández, Maia-Herzog & Shelley, 2004	NT
			<i>paynei</i> Vargas, 1942 (Type of <i>Hemicnetha</i>)	NA + NT
			<i>pulverulentum</i> Knab, 1915	NA + NT
			<i>rubrithorax</i> Lutz, 1909	NT
			<i>tarsatum</i> Macquart, 1846	NT
			<i>virgatum</i> Coquillett, 1902	NA + NT
<i>S. (Xenosimulium)</i> Crosskey, 1969			<i>ambositrae</i> Grenier & Grjebine, 1959	AF
			<i>imerinae</i> Roubaud, 1905 (Type of <i>Xenosimulium</i>)	AF
			<i>griveaudi</i> Ovazza & Ovazza, 1970	AF
			<i>iphias</i> De Meillon, 1951	AF
			<i>neireti</i> Roubaud, 1905	AF
Oviedo species-group			<i>oviedo</i> Ramírez-Pérez, 1971	NT
			<i>rivasi</i> Ramírez-Pérez, 1971	NT

the basic strategy to explore different parameters of the concavity constant (k) and group the searches when the Most-Parsimonious Trees (MPTs) are found, to investigate the effect of character weighting on hypotheses of relationship (Gil-Azevedo & Coscarón 2020). The parameters used in all searches were 5000 TBR replications with 1000 trees saved per replication. To calculate the nodal support, we used TNT through bootstrap Poisson independent re-weighting and relative Bremer support (Goloboff & Farris 2001). We used Winclada to map and optimize the characters (Nixon 2002).

Results

Phylogenetic analyses

The data matrix used comprised 69 terminal taxa and 62 characters from adults (31), pupae (11), and last instar larvae (20). The searches by IW phylogenetic analyses using k3 to k100 found the same three MPTs. Disagreement among trees was limited to the placement of some species in the *Albellum* species-group. We chose the strict consensus tree under k3 (Fig. 1, Fit = 26.33977, CI = 0.24, RI = 0.75) for graphical representation, to map the support values (bootstrap and Bremer), get character scores, and as a basis for the discussion that follows. Table 2 shows the in-group synapomorphies common to all trees. The EW analysis resulted in 16 MPTs (each with CI = 0.24, RI = 0.76), including the three MPTs found with the IW analysis. A strict consensus cladogram of these 16 trees is shown in Figure 2. Disagreement among EW and IW analyses was limited to the placement of the species-groups *Albellum*, *Canadense*, *Pictipes*, and *Oviedo* (Figs 1–2).

Our analysis shows that *S. (Trichodagmia)*, *S. (Anasolen)*, *S. (Freemanellum)*, *S. (Xenosimulium)*, and *Oviedo* form a monophyletic group based on three synapomorphies (Fig. 1, Table 2, node 1). Therefore,

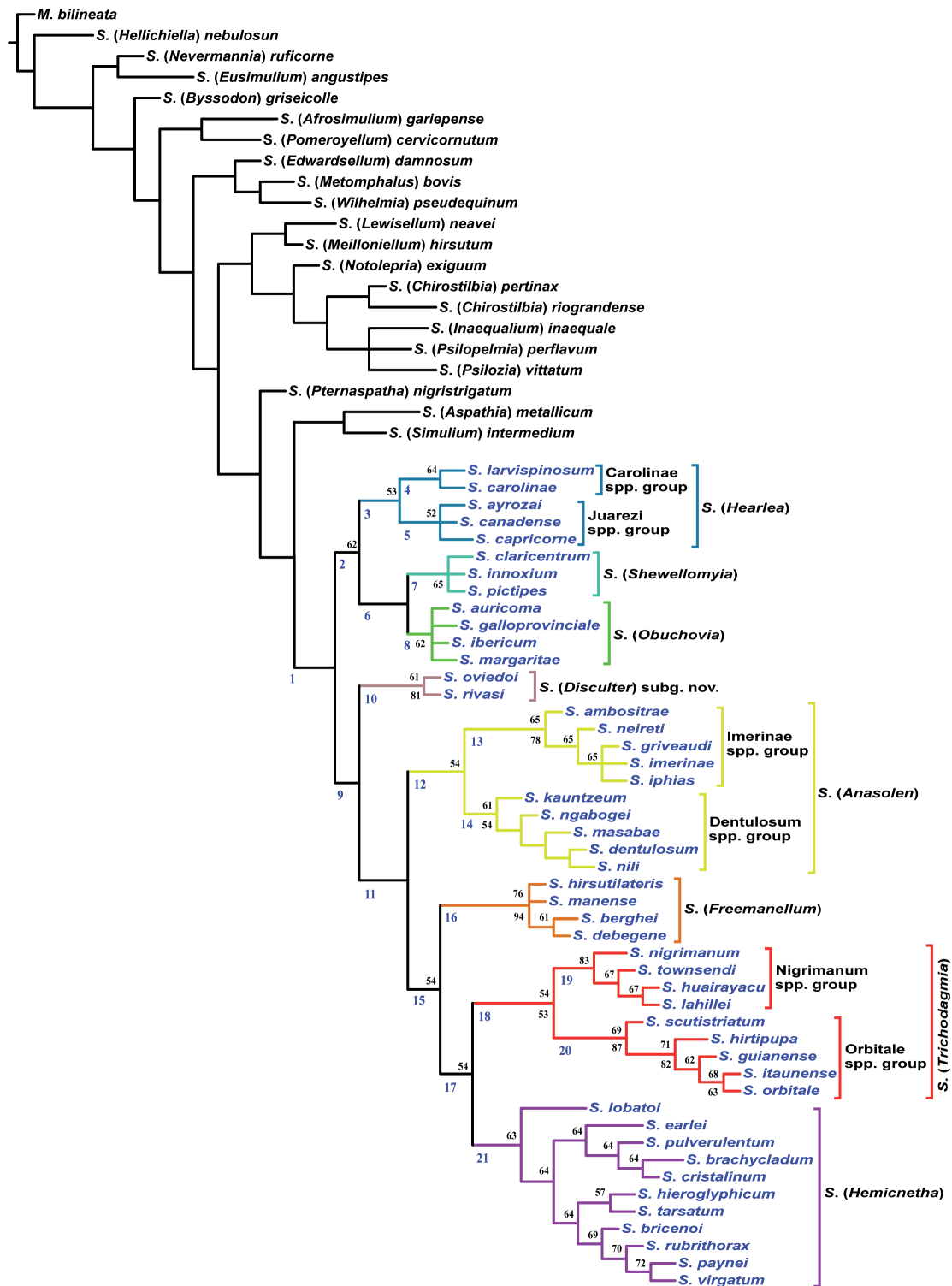


Fig. 1. Strict consensus of the three most parsimonious trees that resulted from the analysis under Implied Weights with $K = 3$ (Fit = 26.33977; Ci = 0.24; Ri = 0.75). Bremer support values (> 50) mapped above branches and bootstrap values over 50% are given below the nodes. Blue numbers on nodes (1–21) represent clades. Blue terminal taxa are the original ingroup included in the analysis. Colored branches show the proposed classification. *Simulium* (*Disculter*) subgen. nov. correspond to old Oviedoii species-group.

Table 2. Synapomorphies of the three trees obtained with implied weights from k3 to k100 (Fig. 1) used to construct the final hypothesis. Bold are non-homoplastic apomorphies.

Node	Node name	Synapomorphies	Bremer	Bootstrap
1	<i>S. (Trichodagmia)</i> + Oviedoï + <i>S. (Xenosimulium)</i> + <i>S. (Anasolen)</i> + <i>S. (Freemanellum)</i>	46 (2); 59 (1); 60 (0)	< 50	< 30
2	Canadense + Pictipes + Albellum	14 (1)	62	< 30
3	Canadense [= <i>S. (Hearlea)</i>]	22 (2); 46 (1); 55 (1)	53	< 30
4	Carolinae species-group of <i>S. (Hearlea)</i>	40 (0); 42 (0); 51 (0)	64	45
5	Juarezi species-group of <i>S. (Hearlea)</i>	24 (0); 28 (1); 34 (1)	52	30
6	Pictipes + Albellum	7 (0); 17 (0); 42 (2); 43 (1)	< 50	42
7	Pictipes [= <i>S. (Shewellomyia)</i>]	24 (0); 28 (1); 54 (0)	< 50	65
8	Albellum [= <i>S. (Obuchovia)</i>]	53 (2)	< 50	62
9	Oviedoï + <i>S. (Xenosimulium)</i> + <i>S. (Anasolen)</i> + <i>S. (Freemanellum)</i> + Orbitale + Tarsatum	16 (1)	< 50	< 30
10	Oviedoï	8 (0); 21 (0); 22(2); 23(0); 32(1); 57 (0)	61	81
11	<i>S. (Xenosimulium)</i> + <i>S. (Anasolen)</i> + <i>S. (Freemanellum)</i> + Orbitale + Tarsatum	43 (1); 47 (1); 53 (0); 55 (1)	< 50	< 30
12	<i>S. (Xenosimulium)</i> + <i>S. (Anasolen)</i>	10 (1); 11 (0); 15(1); 48 (0); 58 (1)	54	< 30
13	<i>S. (Xenosimulium)</i>	9 (1); 31 (0); 44 (0); 45 (1) ; 46 (0); 52 (0); 54 (0); 62 (0)	65	78
14	<i>S. (Anasolen)</i>	38 (1); 53 (2)	61	54
15	<i>S. (Freemanellum)</i> + Orbitale + Tarsatum	13 (1) ; 50 (0)	54	< 30
16	<i>S. (Freemanellum)</i>	4 (2); 8 (0); 22 (1); 23 (0); 35 (0) ; 36 (0); 44 (0); 52 (0); 56 (1)	76	94
17	Orbitale + Tarsatum	16 (0); 25 (1)	54	< 30
18	Orbitale [= <i>S. (Trichodagmia)</i> s.str. + <i>S. (Thyrsopelma)</i>]	1 (1); 2 (0); 10 (2) ; 19 (0) ; 21 (0); 30 (1) ; 49 (0); 54 (0)	54	53
19	<i>S. (Trichodagmia)</i> s. str. [= part of Orbitale]	32 (1); 42 (0); 53 (1)	83	< 30
20	<i>S. (Thyrsopelma)</i> [= part of Orbitale]	3 (1) ; 7 (0); 11 (0); 38 (1); 44 (0); 52 (0); 58 (1)	69	87
21	Tarsatum [= <i>S. (Hemicnetha)</i>]	20 (0) ; 47 (0); 51 (0); 53 (2)	63	< 30

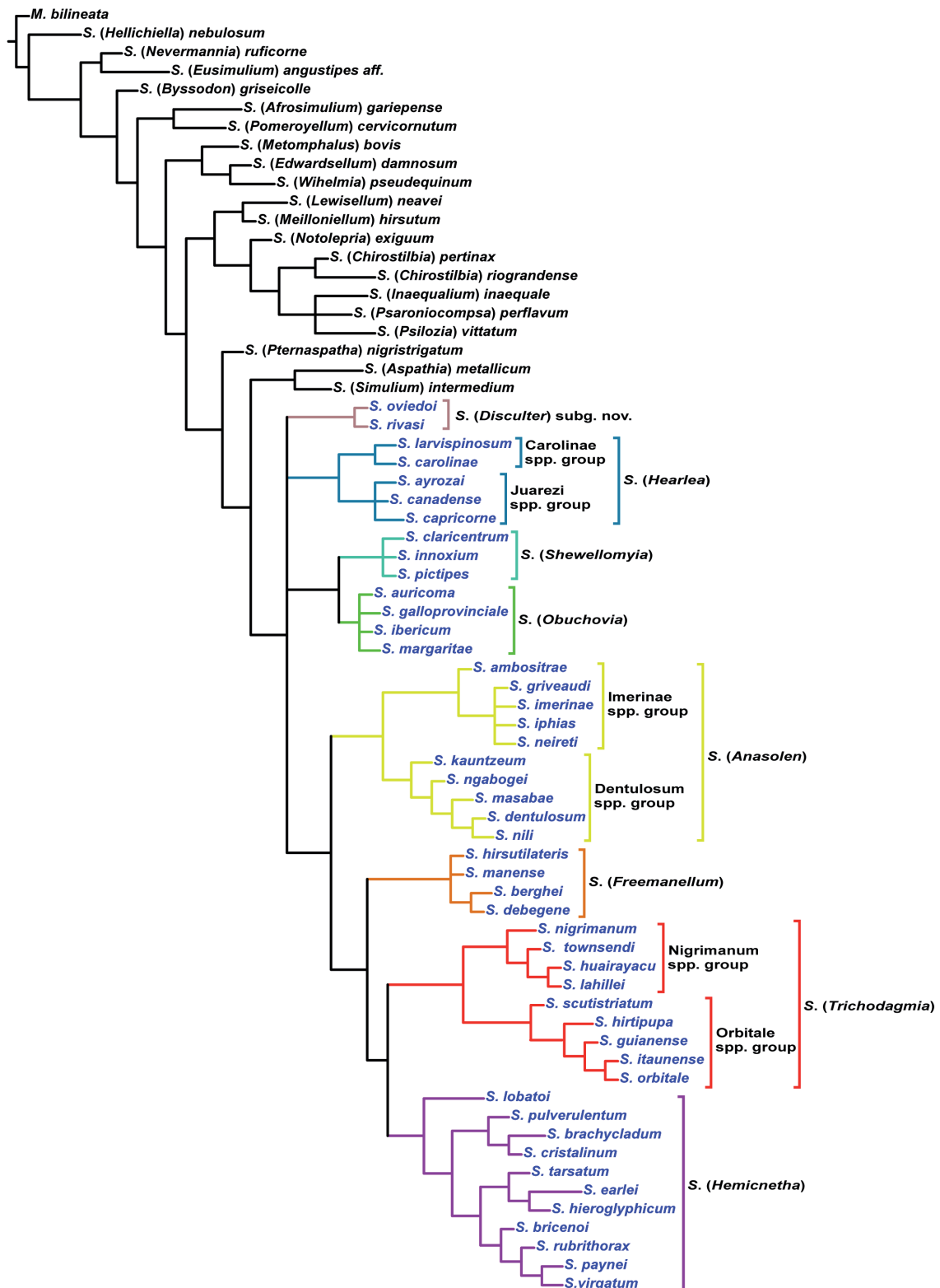


Fig. 2. Strict consensus of the 16 most parsimonious trees that resulted from the analysis under Equal Weights (Fit = 16.87, Ci = 0.22, Ri = 0.74). Blue terminal taxa are the ingroup species included in the analysis. Colored branches show the proposed classification. *Simulium* (*Disculter*) subgen. nov. correspond to old Oviedo species-group.

the lumpers' concept of *S. (Trichodagmia)* is not a monophyletic group because his species-groups Tarsatum and Orbitale are closer to *S. (Anasolen)*, *S. (Freemanellum)*, *S. (Xenosimulium)*, and Oviedo than to the other species-groups, based on one synapomorphy (Fig. 1, Table 2, node 9).

Simulium (Anasolen), *S. (Freemanellum)*, *S. (Xenosimulium)*, and Oviedo were each recovered as monophyletic, and defined by two, nine, eight and six synapomorphies, respectively (Fig. 1, Table 2, nodes 14, 16, 13, 10). *Simulium (Anasolen)* grouped with *S. (Xenosimulium)* based on five synapomorphies (Fig. 1, Table 2, node 12), whereas *S. (Freemanellum)* grouped with [Orbitale+Tarsatum] based on two synapomorphies (Fig. 1, Table 2, node 15).

All species-groups of *S. (Trichodagmia)* were recovered as monophyletic (Fig. 1): Albellum, supported by one synapomorphy (Table 2, node 8); Pictipes by three (Table 2, node 7); Canadense by three (Table 2, node 3); Orbitale by eight (Table 2, node 18); and Tarsatum by four (Table 2, node 21). In addition, they formed two separated clades: [Canadense+[Albellum+Pictipes]] (Fig. 1, node 2) and [Orbitale+Tarsatum] (Fig. 1, node 17). The first one based on one synapomorphy (Table 2, node 2) and the second one based on two (Table 2, node 17). Within the first clade, [Albellum+Pictipes] was supported by four synapomorphies (Table 2, node 6).

Taxonomy

Based on our results, there are two possible classifications for *S. (Trichodagmia)*: (1) to synonymize *S. (Anasolen)*, *S. (Freemanellum)*, *S. (Xenosimulium)*, and Oviedo with *S. (Trichodagmia)*; (2) to redefine the taxonomic limits of *S. (Trichodagmia)*. We believe the second option is preferable because of the low phylogenetic support for the clade [*S. (Trichodagmia)* s. lat. + *S. (Xenosimulium)* + *S. (Anasolen)* + *S. (Freemanellum)* + Oviedo] (Fig. 1, Table 2, node 1), and because all the subgenera subsumed as species-groups in *S. (Trichodagmia)* were recovered as monophyletic and supported with robust diagnostic characters. Therefore, we restrict *S. (Trichodagmia)* to the Orbitale species-group (Fig. 1, node 18) and revalidate *S. (Hearlea)* (Fig. 1, node 3), *S. (Hemicnetha)* (Fig. 1, node 21), *S. (Shewellomyia)* (Fig. 1, node 7), and *S. (Obuchovia)* (Fig. 1, node 8).

We also propose the synonymy of *S. (Xenosimulium)* with *S. (Anasolen)* (Fig. 1, node 12), and create a new subgenus to accommodate the species-group Oviedo (Fig. 1, node 10).

Class Insecta Linnaeus, 1758
Order Diptera Linnaeus, 1758
Family Simuliidae Newman, 1834
Genus *Simulium* Latreille, 1802

Simulium (Trichodagmia) Enderlein, 1934

Figs 13–15, 21, 28–30, 45–47, 64–68, 86–88, 104–106, 145–147, 156–157

Trichodagmia Enderlein, 1934a: 288 (as genus). Type species: *Trichodagmia latitarse* Enderlein, 1934 [= *Simulium townsendi* Malloch, 1912].

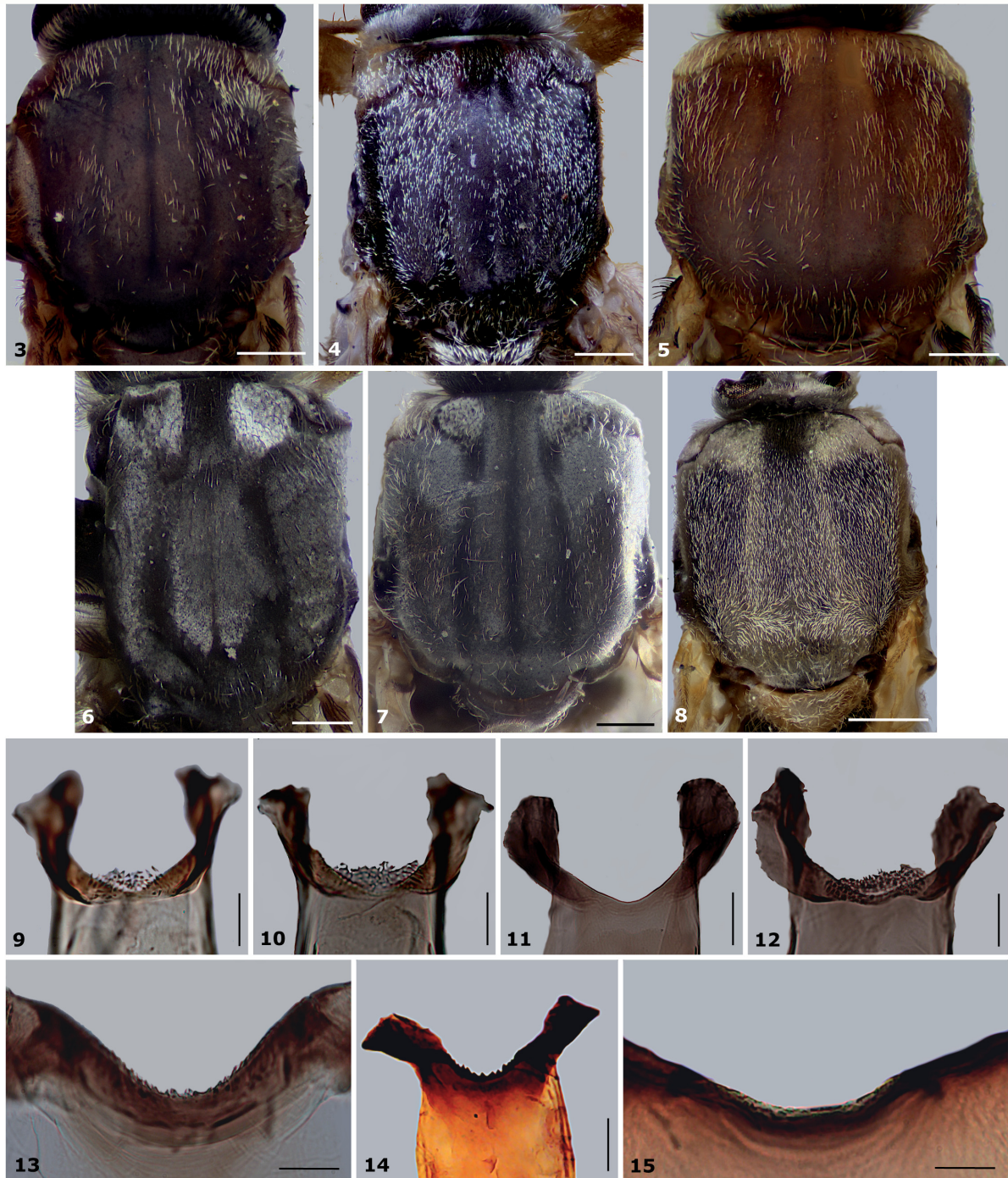
Thyrsopelma Enderlein, 1934a: 284 (as genus). Type species: *Thyrsopelma brasiliense* Enderlein, 1934 [= *Simulium orbitale* Lutz, 1910].

Grenieriella Vargas & Díaz Nájera, 1951: 141, figs 14–22 (as subgenus of *Simulium*). Type species: *Grenieriella lahillei* Vargas & Díaz Nájera, 1951 [= *Simulium lahillei* (Paterson & Shannon, 1927)].

Diagnosis

Female

Scutum blackish; scutal pattern present (except in *S. orbitale*). Cibarium pigmented anteromedially (Figs 13–15), medial area armed with sharp teeth or with tubercle-like prominences. Tarsal claw with or



Figs 3–15. Females. **3–8.** Thorax, dorsal view. **3–4.** *Simulium* (*Anasolen*) Enderlein, 1930. **3.** *neireti* Roubaud, 1905. **4.** *nili* Gibbins, 1934. — **5.** *S. (Freemanellum) berghei* Fain, 1949. — **6.** *S. (Hearlea) canadense* Hearle, 1932. — **7.** *S. (Shewellomyia) pictipes* Hagen, 1880. — **8.** *S. (Obuchovia) margaritae* Rubtsov, 1956. — **9–15.** Cibarium of females. **9–12.** *S. (Hemicnetha)* Enderlein, 1934. **9.** *brachycladum* Lutz & Pinto, 1932. **10.** *crystalinum* Coscarón & Py-Daniel, 1989. **11.** *tarsatum* Macquart, 1846. **12.** *pulverulentum* Knab, 1915. — **13–15.** *S. (Trichodagmia)* Enderlein, 1934. **13.** *lahillei* (Paterson & Shannon, 1927). **14.** *nigrimanum* Macquart, 1838. **15.** *scutistriatum* Lutz, 1909. Scale bars: 3–8 = 0.25 mm; 9–12, 14 = 0.05 mm; 13, 15 = 0.02 mm.

without subbasal tooth. Hypoginial valves subovoid, shorter than sternite VIII width, directed forwardly or inwardly (Fig. 21). Anal lobe subrectangular, being two times longer or shorter than cercus base width.

Male

Ventral plate two times longer than wide, with or without anterior notch; lateral shoulders projected or not; posteromedial process present (except in *S. nigrimanum*), being cylindrical or globular. Gonocoxite wider than long, with base almost equal or less in width than gonostylus width at their point of intersection (Figs 64–68). Gonostylus hooked-shaped, distinctly longer than gonocoxite, with acute apex, and apical spinule (Figs 64–68). Parameres with very small to absent spines.

Pupa

Cocoon aperture upward directed, separated from substratum, not corbicular (Figs 86–88). Gill shorter than pupa body, with trunk development, free or with the base enclosed by the cocoon anterior margin; with 12–50 filamentous arranged in different planes (tridimensional); filaments stiff or flexible, cylindrical, and with acute apices darkened or not; inferior branch multibranching. Thoracic trichomes simple or multibranching.

Larva (last instar)

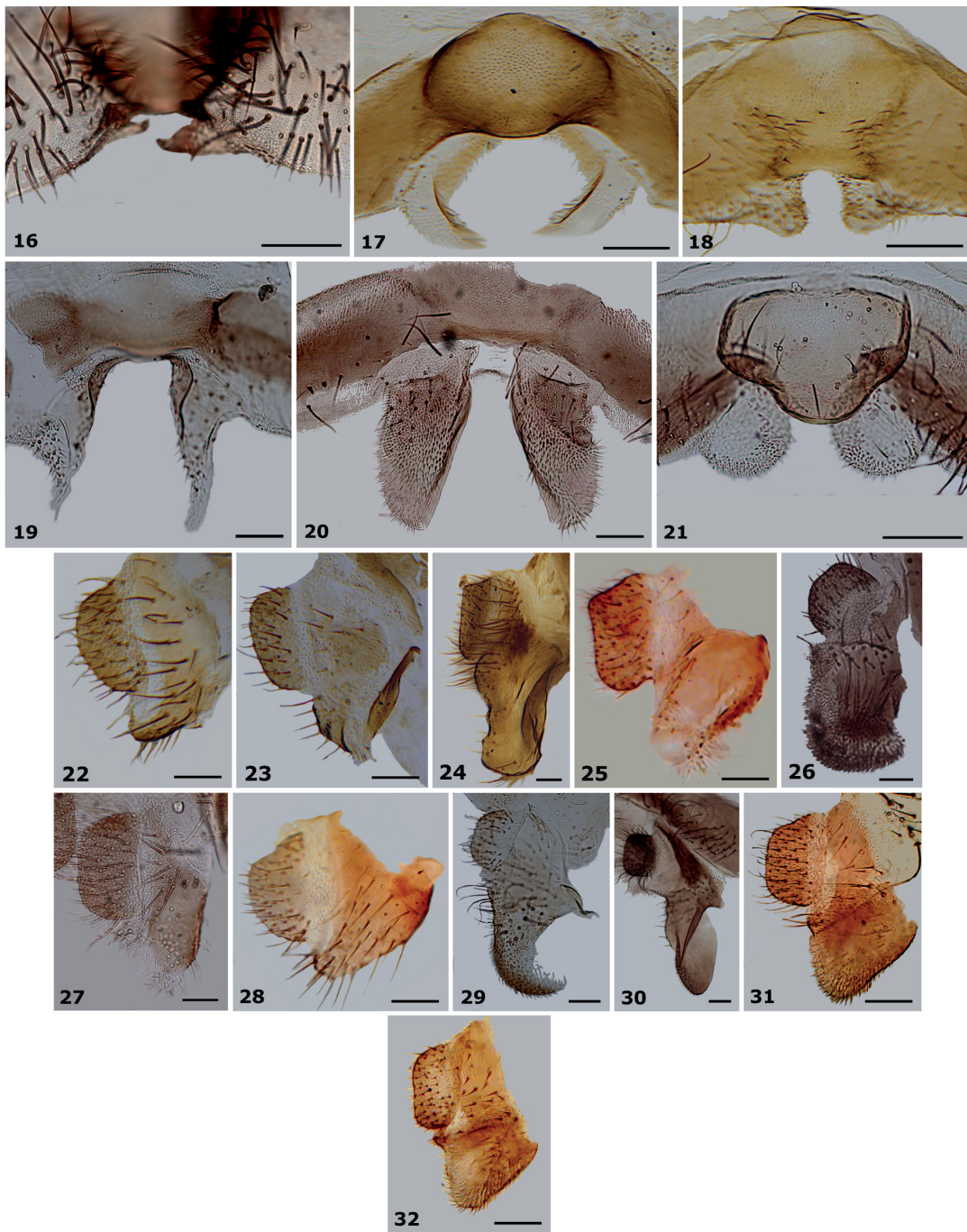
Cephalic apotome spots insertion area pigmented. Postocciput extended over the cervical sclerites or not; Antenna ranging from equal or shorter, to longer than labral fan stalk, without secondary annulations. Mandibles with preapical teeth from shorter or as long as the apical tooth; two mandibular serrations of different sizes. Postgenal cleft triangular or rounded; postgenal bridge length less than hypostomal length (Figs 145–147). Hypostoma anterior margin straight; hypostomal teeth extended below or beyond anterior margin of ventral wall of hypostoma; medial tooth as prominent as lateral teeth or reduced; lateral serrations absent or much reduced; paralateral teeth absent. Body surface glabrous or hairy. Abdomen gradually expanding posteroventrally to last segments, then abruptly contracting to posterior proleg. Anal sclerite not encircling base of posterior proleg. Posterior portion of abdomen without sclerotized accessory plates. Without 1+1 ventral tubercles. Rectal papillae with three multi-branched lobes.

Remarks

We recognize *S. (Trichodagmia)* (Fig. 1, node 18) just as the Orbitale species-group of *S. (Trichodagmia)* sensu Adler (2020), which includes 21 species. So defined, this subgenus was recovered as monophyletic and well supported based on eight synapomorphies (Table 2, node 18). According to the clades found, we divided *S. (Trichodagmia)* into two species-groups: Nigrimanum [= *S. (Trichodagmia)* s. str. (Fig. 1, node 19)], and Orbitale [= *S. (Thyrsopelma)* (Fig. 1, node 20)]. Nigrimanum is comprised of nine species and supported by three synapomorphies (Table 2, node 19). Orbitale is comprised of the remaining seven species and is supported by seven synapomorphies (Table 2, node 20), one of them unique: female cibarium armed with tubercle-like prominences (3 (1)) (Fig. 15).

These two groups are considered as different subgenera by other authors, but previous studies showed its close relationship as recovered here (Coscarón 1987; Miranda-Esquivel & Coscarón 2001). Both species-groups can be differentiated by: female tarsal claws, cibarium, hypoginial valves orientation, and anal lobe length; male ventral plate morphology; pupal gills; larval body tegument, antenna, postocciput, and hypostomal teeth orientation.

Simulium (Trichodagmia) includes anthropophilic species with medical-economic importance: *Simulium guianense* Wise, 1911 is the primary vector of onchocerciasis in the Brazilian Amazonia focus of the disease (Shelley *et al.* 2010). *Simulium nigrimanum* Macquart, 1838, is a potential vector of onchocerciasis in the Minaçu focus disease of Brazil (Shelley *et al.* 2000) and is also suspected to be the responsible for causing endemic pemphigus foliaceus in Brazil, an autoimmune skin reaction in response



Figs 16–32. Females. **16–21.** Hypoginial valves. **16–17.** *Simulium* (*Anasolen*) Enderlein, 1930. **16.** *dentulosum* Roubaud, 1915. **17.** *neireti* Roubaud, 1905. — **18.** *S.* (*Freemanellum*) *berghei* Fain, 1949. — **19–20.** *S.* (*Hemicnetha*) Enderlein, 1934. **19.** *crystalinum* Coscarón & Py-Daniel, 1989. **20.** *virgatum* Coquillett, 1902. — **21.** *S.* (*Trichodagmia*) *scutistriatum* Lutz, 1909. — **22–32.** Anal lobe and cercus. **22–23.** *S.* (*Anasolen*). **22.** *masabae* Gibbins, 1934. **23.** *neireti*. — **24.** *S.* (*Freemanellum*) *hirsutilateris* De-Meillon, 1937. — **25.** *S.* (*Hearlea*) *ayrozai* Vargas, 1945. — **26.** *S.* (*Hemicnetha*) *tarsatum* Macquart, 1846. — **27.** *S.* (*Shewellomyia*) *claricentrum* Adler, 1990. — **28–30.** *S.* (*Trichodagmia*). **28.** *guianense* Wise, 1911. **29.** *lahillei* (Paterson & Shannon, 1927). **30.** *scutistriatum*. — **31–32.** *S.* (*Disculter*) subgen. nov. **31.** *rivasi* Ramírez-Pérez, 1971. **32.** *oviedo* Ramírez-Pérez, 1971. Scale bars: 16–21 = 0.1 mm; 22–32 = 0.05 mm.

to the female's bites (Eaton *et al.* 1998). *Simulium lahillei* (Paterson & Shannon, 1927), *S. orbitale* Lutz, 1910 and *S. scutistriatum* Lutz, 1909 are nuisance species to humans and cattle (Miranda-Esquivel & Coscarón 2001).

Nigrimanum species-group

Species

Simulium chinguazaense Díaz, Moncada, Lotta, Matta & Adler, 2015
S. huairayacu Wygodzinsky, 1953
S. lahillei (Paterson & Shannon, 1927)
S. muiscorum Bueno, Moncada & Muñoz de Hoyos, 1979
S. nigrimanum Macquart, 1838
S. nunesdemelloi Hamada, Pepinelli & Hernández, 2006
S. sumapazense Coscarón & Py-Daniel, 1989
S. townsendi Malloch, 1912
S. wygodzinskyorum Coscarón & Py-Daniel, 1989

Diagnosis

Female

Tarsal claw with a subbasal tooth shorter than a third of the claw length. Cibarium medial area armed with sharp teeth (Figs 13–14). Hypoginial valves forwardly directed. Anal lobe twice as long as cercus width at base (Fig. 29).

Male

Ventral plate not notched; lateral shoulders not projected; posteromedial process cylindrical with length lower or equal than ventral plate length (Fig. 46).

Pupa

Gills free from cocoon (Fig. 86); filaments with apices concolorous with rest of gill (Fig. 104) (except *S. nunesdemelloi*). Thoracic trichomes simple.

Larva (last instar)

Antenna equal or shorter than labral fan stalk. Postocciput not extended over the cervical sclerites. Postgenal cleft triangular (e.g., *S. huairayacu*, *S. lahillei* (Fig. 146)) or rounded (e.g., *S. townsendi*, *S. nigrimanum* (Fig. 147)). Hypostomal teeth arranged beyond anterior margin of ventral wall of hypostoma; medial tooth as prominent as lateral teeth (Fig. 157). Body glabrous.

Distribution

The group is distributed in Argentina, Bolivia, Brazil (southern and central regions), Colombia, Ecuador, Paraguay, Peru, and Venezuela (Adler 2020).

Orbitale species-group

Species

Simulium coscaroni Nascimento, Hamada & Adler, 2017
S. criniferum Nascimento, Hamada, Andrade-Souza & Adler, 2017
S. duodenicornium Pepinelli, Hamada & Trivinho-Strixino, 2005
S. guianense Wise, 1911
S. hirtipupa Lutz, 1910

S. itajara Nascimento, Hamada & Pepinelli, 2020
S. itaunense d'Andretta & Dolores González, 1964
S. jeteri (Py-Daniel, Darwich, Mardini, Strieder & Coscarón, 2005)
S. litobranchium Hamada, Pepinelli, Mattos-Glória & Luz, 2010
S. orbitale Lutz, 1910
S. perplexum Shelley, Maia-Herzog, Luna Dias & Couch, 1989
S. scutistriatum Lutz, 1909

Diagnosis

Female

Tarsal claw without subbasal tooth. Cibarium armed (except in *S. hirtipupa*) with tubercle-like prominences (Fig. 15). Hypoginial valves inwardly directed (Fig. 21). Anal lobe shorter than the cercus base width (Fig. 28) (exceptions: *S. scutistriatum* (Fig. 30), *S. criniferum*, *S. itajara*).

Male

Ventral plate with a shallow notch (except on *S. scutistriatum* and *S. itajara*, which do not have notch, and *S. criniferum* and *S. hirtipupa*, in which the notch is deep as half the length of ventral plate body); lateral shoulders projected (Fig. 45) (except in *S. scutistriatum* (Fig. 47) and *S. itajara*); posteromedial process cylindrical (e.g., *S. hirtipupa*, *S. scutistriatum* (Fig. 47), *S. itajara*) or globular (e.g., *S. criniferum*, *S. itaunense* (Fig. 45), *S. orbitale*), longer than ventral plate length (except *S. scutistriatum* (Fig. 47) and *S. itajara*).

Pupa

Gill base enclosed by cocoon anterior margin (Figs 87–88) with filament apices darkened (Figs 105–106) (except in *S. coscaroni*). Thoracic trichomes simples.

Larva (last instar)

Antenna with ½ of distal antennomere surpassing the labral fan stalk. Postocciput extended over the cervical sclerites. Postgenal cleft rounded (Fig. 145). Hypostomal teeth arranged below anterior margin of ventral wall of hypostoma; medial tooth shorter than lateral teeth (Fig. 156). Body covered with multiple setae (e.g., *S. scutistriatum* and *S. itajara*).

Distribution

The group is mostly found in Brazil, but with some species also recorded from Colombia, French Guiana, Guyana, Paraguay, Suriname, and Venezuela (Adler 2020).

Simulium (*Hearlea*) Vargas, Martínez Palacios & Díaz Nájera, 1946
Figs 6, 25, 39–40, 57–59, 78–79, 98, 110, 121, 136–139

Hearlea Rubtsov, 1940: 154 (unav., nom. nud.).

Hearlea Vargas, Martínez Palacios & Díaz Nájera, 1946: 106, figs 19–34 (as subgenus of *Simulium*).
Type species: *Simulium canadense* Hearle, 1932.

Diagnosis

Female

Scutum blackish to dark brown; scutal pattern present (Fig. 6). Cibarium unpigmented anteromedially and unarmed. Tarsal claws with subbasal tooth. Hypoginial valves subtriangular, shorter than sternite VIII width and forwardly directed. Anal lobe subrectangular with ventral extension shorter than cercus base width (Fig. 25).



Figs 33–49. Males. Ventral plate. **33–37.** *Simulium* (*Anasolen*) Enderlein, 1930. **33.** *dentulosum* Roubaud, 1915. **34.** *imerinae* Roubaud, 1905. **35.** *kauntzeum* Gibbins, 1938. **36.** *masabae* Gibbins, 1934. **37.** *nili* Gibbins, 1934. — **38.** *S.* (*Freemanellum*) *debegene* De-Meillon, 1934. — **39–40.** *S.* (*Hearlea*) Vargas *et al.* 1946. **39.** *ayrozai* Vargas, 1945. **40.** *larvispinosum* De León, 1948. — **41–42.** *S.* (*Hemicnetha*) Enderlein, 1934. **41.** *bricenoi* Vargas *et al.*, 1946. **42.** *tarsatum* Macquart, 1846. — **43.** *S.* (*Shewellomyia*) *claricentrum* Adler, 1990. — **44.** *S.* (*Obuchovia*) *galloprovinciale* Giudicelli, 1963. — **45–47.** *S.* (*Trichodagmia*) Enderlein, 1934. **45.** *itaunense* D’Andretta & Dolores González, 1964. **46.** *lahillei* (Paterson & Shannon, 1927). **47.** *scutistriatum* Lutz, 1909. — **48–49.** *S.* (*Disculter*) subgen. nov. **48.** *rivasi* Ramírez-Pérez, 1971. **49.** *oviedo* Ramírez-Pérez, 1971. Scale bars = 0.1 mm.

Male

Ventral plate with sub-equal width and length, with or without anterior notch; lateral shoulders projected or not; posteromedial process present or absent. Gonocoxite wider than long, with base double in width than gonostylus width at their point of intersection (Figs 57–59). Gonostylus cylindrical, distinctly longer than gonocoxite, with blunt apex and apical spinule (Figs 57–59). Parameres with both distinctly large and small accessory spines.

Pupa

Cocoon aperture upward directed or not, not corbicular. Gills shorter than pupa body, with trunk developed, and free or with the base enclosed by the cocoon anterior margin; with 2–12 filaments arranged in the same or in different planes (tridimensional); filaments stiff, swollen, with blunt or acute apices concolorous with rest of gill; inferior branch not multibranching. Thoracic trichomes simples.

Larva (last instar)

Cephalic apotome spots insertion area not pigmented (Fig. 121). Postocciput not extended over the cervical sclerites (Fig. 121). Antenna with $\frac{1}{2}$ of distal antennomere surpassing the labral fan stalk, without secondary annulations. Mandibles with preapical teeth as long as or longer than apical tooth; two mandibular serrations of same sizes. Postgenal cleft triangular; postgenal bridge length equal or less than hypostomal length. Hypostomal anterior margin convex or straight; hypostomal teeth extended beyond anterior margin of ventral wall of hypostoma; medial tooth as prominent as lateral ones or longer; lateral serrations well-developed, sharp; hypostomal paralaral teeth present or absent. Body surface glabrous. Abdomen gradually expanding posteroventrally to last segments, then abruptly contracting to posterior proleg (Fig. 110). Anal sclerite encircling posterior posterior proleg or not. Posterior portion of abdomen with or without sclerotized accessory plates. Without 1 + 1 ventral tubercles. Rectal papillae with three multi-branched lobes.

Remarks

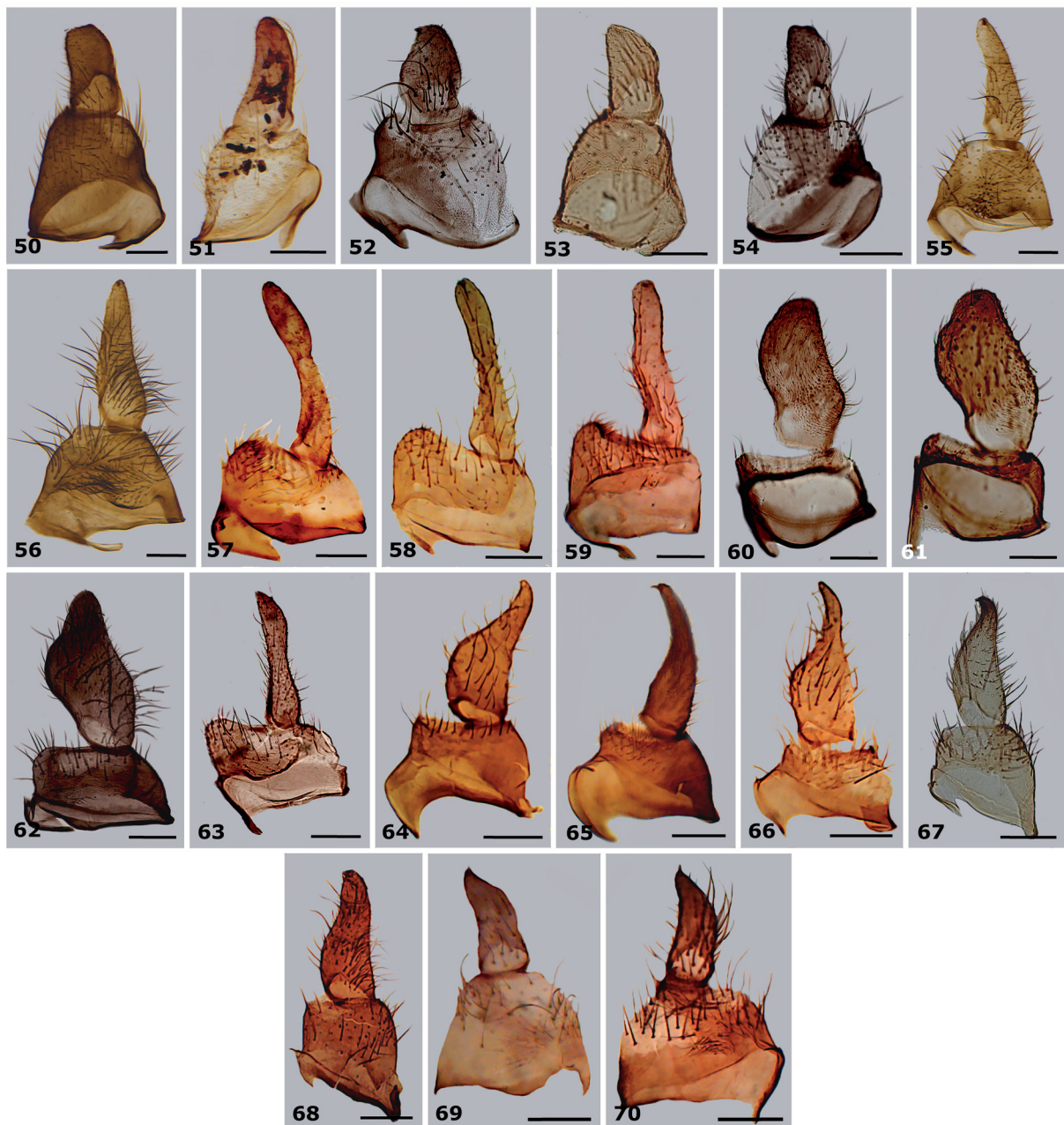
Simulium (*Hearlea*) was recovered based on three synapomorphies (Fig. 1, Table 2, node 3), so we propose its revalidation. Coscarón *et al.* (2004) divided *S.* (*Hearlea*) into the species-groups Carolinae and Juarezi. We included two species of Carolinae and three of Juarezi in our analysis and, despite the low number of representatives, both groups were recovered, each defined by three synapomorphies and highly supported (Fig. 1, Table 2, nodes 4, 5).

Based to our results, we maintain *S.* (*Hearlea*), which comprises 19 species and is divided into the species-groups Carolinae (Fig. 1, node 4) (seven species) and Juarezi (Fig. 1, node 5) (12 species), as defined by Coscarón *et al.* (2004). Both species-groups can be differentiated by: male ventral plate; pupal gills and cocoon morphology; larval hypostoma, abdominal posterior sclerotized accessory plates, and anal sclerite.

Carolinae species-group

Species

- Simulium carolinae* De León, 1945
- S. gorirossiae* Vargas & Díaz Nájera, 1957
- S. johnsoni* Vargas & Díaz Nájera, 1957
- S. larvispinosum* De León, 1948
- S. menchacai* Vargas & Díaz Nájera, 1957
- S. paracarolinae* Coscarón, 2004
- S. temascalense* Díaz Nájera & Vulcano, 1962



Figs 50–70. Males. Gonocoxite and gonostyle. **50–54.** *Simulium* (*Anasolen*) Enderlein, 1930. **50.** *dentulosum* Roubaud, 1915. **51.** *imerinae* Roubaud, 1905. **52.** *kauntzeum* Gibbins, 1938. **53.** *masabae* Gibbins, 1934. **54.** *nili* Gibbins, 1934. — **55–56.** *S.* (*Freemanellum*) Crosskey, 1969. **55.** *hirsutilateris* De-Meillon, 1937. **56.** *manense* Elsen & Escaffre, 1976. — **57–59.** *S.* (*Hearlea*) Vargas *et al.* 1946. **57.** *ayrozai* Vargas, 1945. **58.** *canadense* Hearle, 1932. **59.** *capricorne* De León, 1945. — **60–62.** *S.* (*Hemicnetha*) Enderlein, 1934. **60.** *brachycladum* Lutz & Pinto, 1932. **61.** *crystalinum* Coscarón & Py-Daniel, 1989. **62.** *tarsatum* Macquart, 1846. — **63.** *S.* (*Shewellomyia*) *claricentrum* Adler, 1990. — **64–68.** *S.* (*Trichodagmia*) Enderlein, 1934. **64.** *guianense* Wise, 1911. **65.** *hirtipupa* Lutz, 1910. **66.** *itaunense* D’Andretta & Dolores González, 1964. **67.** *lahillei* (Paterson & Shannon, 1927). **68.** *nigrimanum* Macquart, 1838. — **69–70.** *S.* (*Disculter*) subgen. nov. **69.** *rivasi* Ramírez-Pérez, 1971. **70.** *oviedo* Ramírez-Pérez, 1971. Scale bars = 0.1 mm.

Diagnosis

Male

Ventral plate not notched; lateral shoulders not projected; posteromedial process absent (Fig. 40).

Pupa

Cocoon aperture next to the substratum; with 2–12 gill filaments, stiff, swollen, of same thickness, with accessory small branches, and oriented in different planes (tridimensional).

Larva (last instar)

Hypostomal anterior margin convex; hypostomal medial tooth distinctly longer than lateral ones. Abdomen with posterior sclerotized accessory plates. Anal sclerite totally encircling base of posterior proleg (e.g., *S. carolinae*, *S. gorirossiae*, *S. johnsoni*, *S. larvispinosum*, *S. menchacai*, *S. temascalense*) or almost (e.g., *S. paracarolinae*).

Distribution

The group is found exclusively in central and southern Mexico and Guatemala (Adler 2020).

Juarezi species-group

Species

- Simulium ayrozai* Vargas, 1945
- S. burchi* Dalmat, 1951
- S. canadense* Hearle, 1932
- S. capricorne* De León, 1945
- S. contrerense* Díaz Nájera & Vulcano, 1962
- S. dalmati* Vargas & Díaz Nájera, 1948
- S. delatorrei* Dalmat, 1950
- S. estevezi* Vargas, 1945
- S. ethelae* Dalmat, 1950
- S. juarezi* Vargas & Díaz Nájera, 1957
- S. microbranchium* Dalmat, 1949
- S. nigricorne* Dalmat, 1950

Diagnosis

Male

Ventral plate shallowly notched; lateral shoulders projected; posteromedial process present (Fig. 39).

Pupa

Cocoon aperture upward-directed (Figs 78–79); with 2–3 gill filaments, stiff, swollen, of different thickness and length, without accessory small branches, and oriented in the same plane (Fig. 98).

Larva (last instar)

Hypostomal anterior margin straight; hypostomal medial tooth at same level as lateral ones. Abdomen without posterior sclerotized accessory plates. Anal sclerite not encircling base of posterior proleg.

Distribution

Most members of this group occur in central and southern Mexico and Guatemala; *S. canadense* occurs in Canada and the USA and *S. ethelae* occurs in Costa Rica and Panama (Adler 2020).

***Simulium (Hemicnetha)* Enderlein, 1934**

Figs 9–12, 19–20, 26, 41–42, 60–62, 80–81, 99–102, 111, 122–123, 140–143, 153–154

Hemicnetha Enderlein, 1934b: 190 (as genus). Type species: *Hemicnetha mexicana* Enderlein, 1934 [= *Simulium paynei* (Vargas, 1942) (subst. name) nom. nov.]

Dyarella Vargas, Martínez Palacios & Díaz Nájera, 1946: 105, figs 8–28 (as subgenus of *Simulium*). Type species: *Simulium mexicanum* Bellardi 1982 [= *Simulium tarsatum* Macquart, 1846 (1844)].

Species

Simulium brachycladum Lutz & Pinto, 1932

S. bricenoi Vargas, Martínez Palacios & Díaz Nájera, 1946

S. cristalinum Coscarón & Py-Daniel, 1989

S. earlei Vargas, Martínez Palacios & Díaz Nájera, 1946

S. freemani Vargas & Díaz Nájera, 1949

S. guerrerense Vargas & Díaz Nájera, 1956

S. hieroglyphicum Peterson, Vargas & Ramírez-Pérez, 1988

S. hinmani Vargas, Martínez Palacios & Díaz Nájera, 1946

S. hippovororum Malloch, 1914

S. lobatoi Luna Dias, Hernández, Maia-Herzog & Shelley, 2004

S. paynei Vargas, 1942

S. pulverulentum Knab, 1915

S. rubrithorax Lutz, 1909

S. smarti Vargas, 1946

S. solarii Stone, 1948

S. tarsale Williston, 1896

S. tarsatum Macquart, 1846

S. virgatum Coquillett, 1902

S. yepocapense Dalmat, 1949.

Diagnosis

Female

Scutum black to dark brown (e.g., *S. pulverulentum*, *S. earlei*, *S. hieroglyphicum*, *S. bricenoi*, *S. tarsatum*) or red to light brown (e.g., *S. brachycladum*, *S. cristalinum*, *S. lobatoi*, *S. paynei*, *S. rubrithorax*, *S. virgatum*); scutal pattern present. Cibarium unpigmented anteromedially, armed with sharp teeth (Figs 9–10, 12) or unarmed (Fig. 11). Tarsal claw with subbasal tooth shorter than a third of claw length (except in *S. lobatoi*, in which the tooth has about half of claw length). Hypoginial valves fine and pointed (Fig. 19) or subtriangular (Fig. 20), longer than sternite VIII width (except in *S. lobatoi*, in which are shorter), forwardly directed. Anal lobe subrectangular (except in *S. lobatoi*, in which is hemispheric with curved distal border), twice as long as cercus width at base. (Fig. 26).

Male

Ventral plate two times longer than wide or with sub-equal width and length, not notched (except in *S. arlei*); lateral shoulders absent (Fig. 42) or present (Fig. 41), in this case, not projected (except in *S. lobatoi*); posteromedian process absent (Fig. 42) or present (Fig. 41), in this case, is cylindrical and longer than ventral plate width (except in *S. lobatoi*, in which is smaller). Gonocoxite longer than wide, with base almost equal in width to gonostylus width at their point of intersection (Figs 60–62). Gonostylus cylindrical, with medial region wider than basal region; distinctly longer than gonocoxite, with blunt apex and apical spicule (Figs 60–62). Parameres with both distinctly large and small accessory spines.



Figs 71–90. Pupae. 71–75. *Simulium* (*Anasolen*) Enderlein, 1930. 71. *ambositrae* Grenier & Grjebine, 1959. 72. *dentulosum* Roubaud, 1915. 73. *imerinae* Roubaud, 1905. 74. *iphias* De-Meillon, 1951. 75. *nili* Gibbins, 1934. — 76–77. *S.* (*Freemanellum*) Crosskey, 1969. 76. *debegene* De-Meillon, 1934. 77. *manense* Elsen & Escaffre, 1976. — 78–79. *S.* (*Hearlea*) Vargas *et al.* 1946. 78. *canadense* Hearle, 1932. 79. *capricorne* De León, 1945. — 80–81. *S.* (*Hemicnetha*) Enderlein, 1934. 80. *paynei* Vargas, 1942. 81. *rubrithorax* Lutz, 1909. — 82–83. *S.* (*Shewellomyia*) Peterson, 1975. 82. *claricentrum* Adler, 1990. 83. *pictipes* Hagen, 1880. — 84–85. *S.* (*Obuchovia*) Rubtsov, 1947. 84. *albellum* Rubtsov, 1947. 85. *galloprovinciale* Giudicelli, 1963. — 86–88. *S.* (*Trichodagmia*) Enderlein, 1934. 86. *nigrimanum* Macquart, 1838. 87. *orbitale* Lutz, 1910. 88. *scutistriatum* Lutz, 1909. — 89–90. *S.* (*Disculter*) subgen. nov. 89. *rivasi* Ramírez-Pérez, 1971. 90. *oviedo* Ramírez-Pérez, 1971. Scale bars = 1 mm.

Pupa

Cocoon aperture upward directed (Figs 80–81), separated from substratum, corbicular (Fig. 81) or not. Gill shorter than pupa body, with trunk developed and base enclosed by cocoon anterior margin (Figs 80–81) (except in *S. lobatoi*, in which the gills are totally free); with 6–90 filaments arranged in different planes (tridimensional); filaments flexible, cylindrical, with apices blunt (e.g., *S. hieroglyphicum*, *S. bricenoi*, *S. paynei*, *S. rubrithorax*, *S. virgatum*, *S. tarsatum*) or acute (e.g., *S. brachycladum*, *S. cristalinum*, *S. lobatoi*, *S. pulverulentum*, *S. earlei*), concolorous with rest of gill; inferior branch multi-branched (Figs 99–100) or single-branched (Figs 100–101). Thoracic trichomes generally simple.

Larva (last instar)

Cephalic apotome spots insertion area pigmented (Figs 122–123) (except in *S. lobatoi*). Postocciput extended (Fig. 122) over the cervical sclerites, or not (Fig. 123). Antenna ranging from equal in length to or shorter than labral fan stalk (e.g., *S. earlei*, *S. bricenoi*, *S. paynei*, *S. rubrithorax*, *S. tarsatum*), to longer (e.g., *S. brachycladum*, *S. cristalinum*, *S. lobatoi*, *S. pulverulentum*, *S. hieroglyphicum*), without secondary annulations. Mandibles with preapical teeth as long as apical tooth; two mandibular serrations of different sizes. Postgenal cleft triangular; postgenal bridge length less than hypostomal length (Figs 140–143). Hypostoma anterior margin convex (Fig. 154) or straight (Fig. 153); hypostomal teeth extended beyond anterior margin of ventral wall of hypostoma; median tooth distinctly longer than lateral ones (Fig. 154) or at same level (Fig. 153); lateral serrations well-developed, blunt (except in *S. pulverulentum*, in which are sharp); hypostomal paralateral teeth present (except in *S. brachycladum*). Body surface glabrous. Abdomen gradually expanding posteroventrally to last segments, then abruptly contracting to posterior proleg (Fig. 111). Anal sclerite no encircling base of posterior proleg. Posterior portion of abdomen without sclerotized accessory plates. Without 1+1 ventral papillae. Rectal papillae with three multi-branched lobes.

Remarks

Simulium (*Hemicnetha*) was recovered as monophyletic based on four synapomorphies (Fig. 1, Table 2, node 21), one of them unique: male gonostylus with medial region larger than basal region (20 (0), Figs 60–62). We therefore propose the revalidation of the subgenus, which comprises 19 species.

Coscarón & Coscarón-Árias (2007) divided *S.* (*Hemicnetha*) into four species-groups: Paynei, Brachycladum, Mexicanum, and Oviedo. We included the following representatives in our analysis: Paynei (five species), Brachycladum (four species), Mexicanum (two species), and Oviedo (two species). *Simulium* (*Hemicnetha*) sensu Coscarón & Coscarón-Árias (2007) was not recovered, because Oviedo does not group with the remaining species-groups. Moreover, except for Oviedo, none of the previously defined species-groups in this subgenus were recovered. We therefore revalidate *S.* (*Hemicnetha*), but without Oviedo and without a subdivision into species-groups pending a more comprehensive investigation on the subgenus.

Distribution

Simulium (*Hemicnetha*) is mostly distributed in Mesoamerica and South America, being recorded from the following countries: Argentina, Belize, Bolivia, Brazil, Canada, Colombia, Costa Rica, Dominica, Ecuador, El Salvador, French Guiana, Guadeloupe, Guatemala, Guyana, Honduras, Mexico, Nicaragua, Panama, Paraguay, Peru, Saint Vincent, Suriname, Trinidad and Tobago and Venezuela. However, *S. bricenoi*, *S. freemani*, *S. paynei*, *S. solarii*, and *S. virgatum* are present in the USA, and *S. hippovororum* occurs in Canada and the USA (Adler 2020).

***Simulium* (*Shewellomyia*) Peterson, 1975**

Figs 7, 27, 43, 63, 82–83, 112, 124–125

Hagenomyia Shewell, 1959: 83, figs 1–9 (as subgenus of *Simulium*) (preoc.). Type species: *Simulium pictipes* Hagen, 1880. nom. praeoc.

Shewellomyia Peterson, 1975: 111 (as subgenus of *Simulium*). Type species: *Simulium pictipes* Hagen, 1880. nom. nov.

Species

Simulium claricentrum Adler, 1990

S. innoxium Comstock & Comstock, 1895

S. pictipes Hagen, 1880

Diagnosis

Female

Scutum black to dark brown; scutal pattern present (Fig. 7). Cibarium unpigmented anteromedially and unarmed. Tarsal claw without subbasal tooth. Hypoginial valves subtriangular, shorter than sternite VIII width, forwardly directed. Anal lobe subrectangular with ventral extension shorter than the cercus base width (Fig. 27).

Male

Ventral plate (Fig. 43) two times longer than wide, deeply notched (reaching about half of the ventral plate body width); lateral shoulders projected; posteromedial process absent. Gonocoxite wider than long, with base double in width than gonostylus width at their point of intersection (Fig. 63). Gonostylus cylindrical, distinctly longer than gonocoxite, with blunt apex and without apical spinule (Fig. 63). Parameres with both distinctly large and small accessory spines.

Pupa

Cocoon aperture upward directed, separated from substratum, not corbicular (Figs 82–83). Gills shorter than pupa body, with trunk development, and totally enclosed by cocoon anterior margin; with nine filaments arranged in different planes (tridimensional); filaments flexible, cylindrical, with blunt apices concolorous with rest of gill; inferior branch multibranched. Thoracic trichomes simples.

Larva (last instar)

Cephalic apotome spots insertion area pigmented (Figs 124–125). Postociput extended over the cervical sclerites. Antenna equal or shorter than labral fan stalk, without secondary annulations. Mandibles with pre-apical teeth shorter than apical tooth; two mandibular serrations of different sizes. Postgenal cleft triangular; postgenal bridge length equal or less than hypostomal length. Hypostomal anterior margin straight; hypostomal teeth extended beyond anterior margin of ventral wall of hypostoma; medial tooth at same level as lateral ones; lateral serrations well-developed, sharp; paralateral teeth absent. Body surface glabrous. Abdomen gradually expanding posteroventrally to last segments, then abruptly contracting to posterior proleg (Fig. 112). Anal sclerite no encircling base of posterior proleg. Posterior portion of abdomen without sclerotized accessory plates. Without 1+1 ventral tubercles. Rectal papillae with three multi-branched lobes.

Remarks

Simulium (*Shewellomyia*) was recovered as monophyletic and stable, based on three synapomorphies (Fig. 1, Table 2, node 7), therefore we propose its revalidation.



Figs 91–106. Gills of pupae. **91–94.** *Simulium* (*Anasolen*) Enderlein, 1930. **91.** *bisnovem* Gibbins, 1938. **92.** *dentulosum* Roubaud, 1915. **93.** *imerinae* Roubaud, 1905. **94.** *masabae* Gibbins, 1934. — **95–97.** *S.* (*Fremanellum*) Crosskey, 1969. **95.** *debegene* De-Meillon, 1934. **96.** *manense* Elsen & Escaffre, 1976. **97.** *hessei* Gibbins, 1941. — **98.** *S.* (*Hearlea*) *canadense* Hearle, 1932. — **99–102.** *S.* (*Hemicnetha*) Enderlein, 1934. **99.** *crystalinum* Coscarón & Py-Daniel, 1989. **100.** *earlei* Vargas *et al.*, 1946. **101.** *paynei* Vargas, 1942. **102.** *virgatum* Coquillett, 1902. — **103.** *S.* (*Obuchovia*) *galloprovinciale* Giudicelli, 1963. — **104–106.** *S.* (*Trichodagmia*) Enderlein, 1934. **104.** *nigrimanum* Macquart, 1838. **105.** *hirtipupa* Lutz, 1910. **106.** *scutistriatum* Lutz, 1909. Scale bars: 91–104, 106 = 0.2 mm; 105 = 0.05 mm.

Distribution

Simulium (*Shewellomyia*) is distributed in the Nearctic Region, recorded from the central-eastern regions of Canada and the USA (Adler 2020).

Simulium (*Obuchovia*) Rubtsov, 1947
Figs 8, 44, 84–85, 103, 113, 126, 144, 155

Obuchovia Rubtsov, 1947: 90 (as subgenus of *Simulium*). Type species: *Simulium albellum* Rubtsov, 1947.

Species

Simulium adornatum (Rubtsov, 1956)
S. albellum Rubtsov, 1947
S. auricoma Meigen, 1818
S. biseriatum Rubtsov, 1940
S. brevifile (Rubtsov, 1956)
S. continii (Rivosecchi & Cardinali, 1975)
S. emiliae (Rubtsov, 1976)
S. galloprovinciale Giudicelli, 1963
S. ibericum Crosskey & Santos Grácio, 1985
S. karasuae (Panchenko, 1998)
S. margaritae (Rubtsov, 1956)
S. marocanum Bouzidi & Giudicelli, 1987
S. popowae Rubtsov, 1940
S. segusinum (Couvert, 1968)
S. transcaspicum Enderlein, 1921

Diagnosis

Female

Scutum black to dark brown; scutal pattern present (Fig. 8) or absent (e.g., *S. auricoma*, *S. galloprovinciale*). Cibarium unpigmented anteromedially and unarmed. Tarsal claw without subbasal tooth. Hypoginial valves subtriangular, shorter than sternite VIII width, forwardly directed. Anal lobe subrectangular with ventral extension shorter than cercus base width.

Male

Ventral plate two times longer than wide, rectangular, not notched; lateral shoulders not projected (Fig. 44); posteromedial process absent. Gonocoxite wider than long, with base double in width than gonostylus width at their point of intersection. Gonostylus cylindrical, distinctly longer than gonocoxite, with blunt apex and without apical spurt. Parameres with both distinctly large and small accessory spines.

Pupa

Cocoon aperture upward directed, separated from substratum, not corbicular (Figs 84–85). Gills shorter than pupal body with main trunk development and totally enclosed by cocoon anterior margin; with six filaments arranged in different planes (tridimensional); filaments flexible, cylindrical, with blunt apices concolorous with rest of gill; inferior branch multibranched (Fig. 103). Thoracic trichomes simples.

Larva (last instar)

Cephalic apotome spots insertion area pigmented (Fig. 126). Postocciput not extended over the cervical sclerites. Antenna equal or shorter than labral fan stalk, without secondary annulations. Mandibles with pre-



Figs 107–127. 107–113. Larvas (last instar). 107–108. *Simulium* (*Anasolen*) Enderlein, 1930. 107. *dentulosum* Roubaud, 1915. 108. *imerinae* Roubaud, 1905. — 109. *S.* (*Freemanellum*) *manense* Elsen & Escaffre, 1976. — 110. *S.* (*Hearlea*) *canadense* Hearle, 1932. — 111. *S.* (*Hemicnetha*) *paynei* Vargas, 1942. — 112. *S.* (*Shewellomyia*) *pictipes* Hagen, 1880. — 113. *S.* (*Obuchovia*) *auricoma* Meigen, 1818. — 114–126. Last instar larvae. Head, dorsal view. 114–118. *S.* (*Anasolen*). 114. *ambositrae* Grenier & Grjebine, 1959. 115. *dentulosum*. 116. *imerinae*. 117. *masabae* Gibbins, 1934. 118. *nili* Gibbins, 1934. — 119–120. *S.* (*Freemanellum*). 119. *manense*. 120. *hirsutilateris* De-Meillon, 1937. — 121. *S.* (*Hearlea*) *canadense*. — 122–123. *S.* (*Hemicnetha*). 122. *brachycladum* Lutz & Pinto, 1932. 123. *paynei*. — 124–125. *S.* (*Shewellomyia*). 124. *claricentrum* Adler, 1990. 125. *pictipes*. — 126. *S.* (*Obuchovia*) *ibericum* Crosskey & Santos Grácio, 1985. — 127. Antenna of *S.* (*Anasolen*) *ambositrae*. Scale bars: 107–113 = 1 mm; 114–126 = 0.5 mm; 127 = 0.2 mm.

apical teeth shorter than apical tooth; two mandibular serrations of same (e.g., *S. obuchovia*, *S. margaritae*) or different sizes (e.g., *S. auricoma*, *S. ibericum*). Postgenal cleft triangular; postgenal bridge length equal or less than hypostomal length (Fig. 144). Hypostomal anterior margin straight; hypostomal teeth extended beyond anterior margin of ventral wall of hypostoma (Fig. 155); medial tooth longer than lateral teeth; lateral serrations well-developed, sharp; paralateral teeth present. Body surface glabrous. Abdomen gradually expanding posteroventrally to last segments, then abruptly contracting to circlet (Fig. 113). Anal sclerite no encircling base of posterior proleg. Posterior portion of abdomen without sclerotized accessory plates. Without 1+1 ventral tubercles. Rectal papillae with three multi-branched lobes.

Remarks

Simulium (*Obuchovia*) was recovered as monophyletic and stable, based on one synapomorphy (Fig. 1, Table 2, node 8). We therefore propose its revalidation.

Distribution

Simulium (*Obuchovia*) is distributed in the Palearctic Region, ranging from North Africa through south-central and southern Europe to the eastern Mediterranean area, the Caucasus and Transcaucasus, to south-west Asia, being recorded from the following countries: Afghanistan, Algeria, Andorra, Armenia, Austria, Azerbaijan, Bulgaria, Croatia, Cyprus, Czech Republic, France, Georgia, Germany, Greece, Iran, Italy, Kazakhstan, Lebanon, Morocco, Pakistan, Poland, Portugal, Romania, Russia, Serbia, Slovakia, Spain, Tajikistan, Turkey, Ukraine, and Uzbekistan (Adler 2020).

Simulium (*Anasolen*) Enderlein, 1930

Figs 3–4, 16–17, 22–23, 33–37, 50–54, 71–75, 91–94, 107–108, 114–118, 127–131, 150–151

Anasolen Enderlein, 1930: 94 (as genus). Type species: *Anasolen adolffriedericianus* Enderlein, 1930 [= *Simulium dentulosum* Roubaud, 1915].

Xenosimulium Crosskey, 1969: 86, figs 129, 161, 200–201, 277 (as subgenus of *Simulium*). Type species: *Simulium imeriniae* Roubaud, 1905.

Diagnosis

Female

Scutum black to dark brown; scutal pattern present (Figs 3–4). Cibarium unpigmented anteromedially and unarmed. Tarsal claw with subbasal tooth shorter than a third of claw length. Hypoginial valves fine and pointed, shorter or longer than sternite VIII width, inwardly directed. Anal lobe subrectangular, shorter than the cercus base width (Figs 22–23).

Male

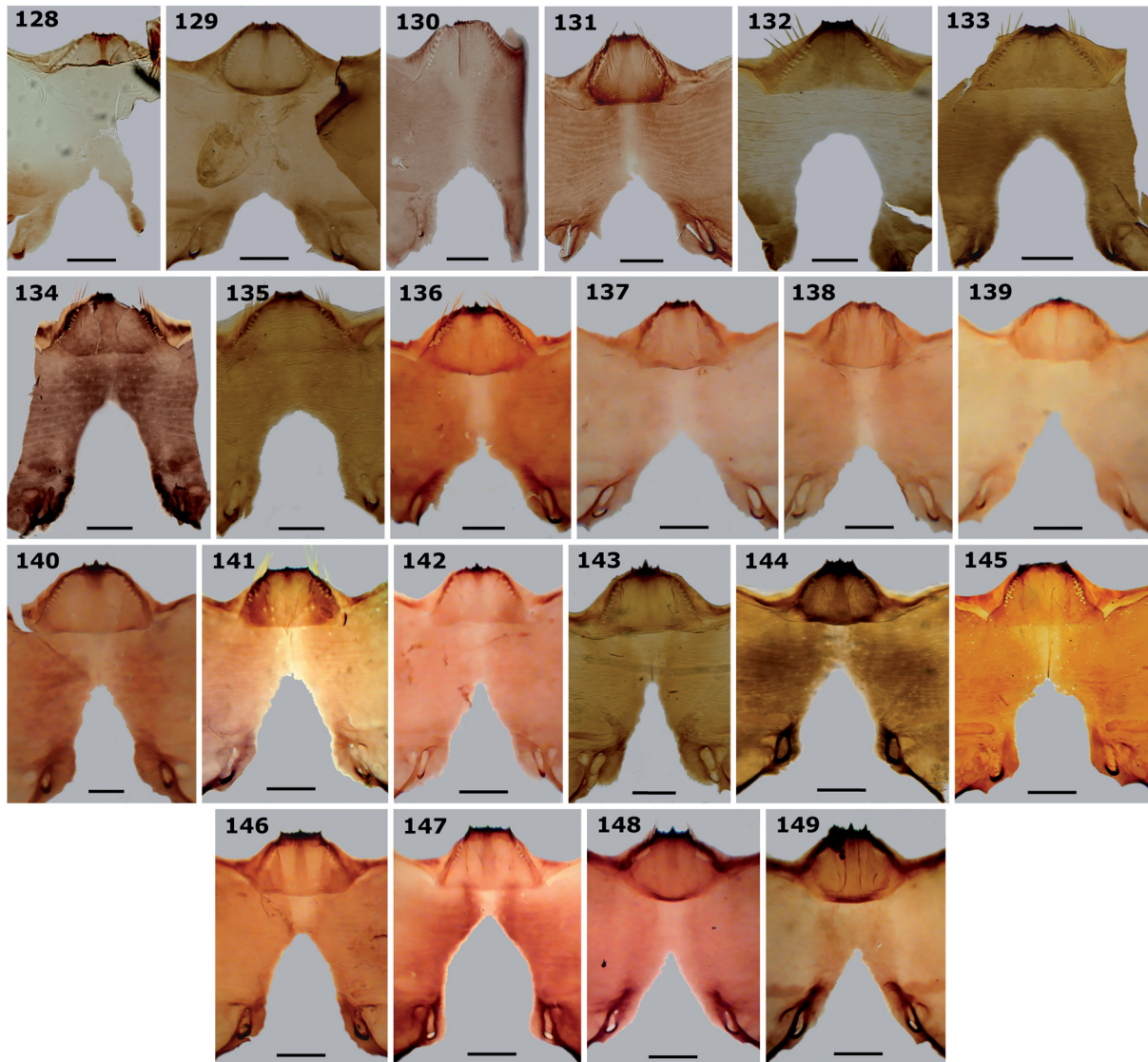
Ventral plate two times longer than wide or with sub-equal width and length, not notched; lateral shoulders projected or not; posteromedial process absent or present. Gonocoxite longer than wide, with base almost equal in width than gonostylus width at their point of intersection (Figs 50–54). Gonostylus cylindrical, with medial region equal in width than basal region; sub-equal or shorter than gonocoxite, with blunt apex and apical spinule (Figs 50–54). Parameres with spines of equal size, or with both distinctly large and small accessory spines.

Pupa

Cocoon aperture upward directed, separated from substratum, not corbicular (Figs 71–75). Gill much shorter than pupa body, with trunk developed, and totally free or with base enclosed by cocoon anterior margin; with 8–19 filaments arranged in different planes (tridimensional); filaments stiff, cylindrical, with acute apices of different or same color than rest of the gill; inferior branch multibranched. Thoracic trichomes simples.

Larva (last instar)

Cephalic apotome spots insertion area pigmented (Figs 114–118). Postocciput extended over the cervical sclerites or not. Antenna ranging from equal or shorter, to longer than labral fan stalk, with medial antennomere annulated or not. Mandibles with preapical teeth shorter, equal, or longer than apical tooth; with two to up to five mandibular serrations of different sizes. Postgenal cleft rounded; postgenal bridge



Figs 128–149. Larva (last instar). Postgenal cleft. **128–131.** *Simulium* (*Anasolen*) Enderlein, 1930. **128.** *ambositrae* Grenier & Grjebine, 1959. **129.** *dentulosum* Roubaud, 1915. **130.** *imerinae* Roubaud, 1905. **131.** *nili* Gibbins, 1934. — **132–135.** *S.* (*Freemanellum*) Crosskey, 1969. **132.** *berghei* Fain, 1949. **133.** *hirsutilateris* De-Meillon, 1937. **134.** *debegene* De-Meillon, 1934. **135.** *manense* Elsen & Escaffre, 1976. — **136–139.** *S.* (*Hearlea*) Vargas *et al.*, 1946. **136.** *ayrozai* Vargas, 1945. **137.** *canadense* Hearle, 1932. **138.** *capricorne* De León, 1945. **139.** *larvispinosum* De León, 1948. — **140–143.** *S.* (*Hemicnetha*) Enderlein, 1934. **140.** *bricenoi* Vargas *et al.*, 1946. **141.** *crystalinum* Coscarón & Py-Daniel, 1989. **142.** *paynei* Vargas, 1942. **143.** *rubrithorax* Lutz, 1909. — **144.** *S.* (*Obuchovia*) galloprovinciale Giudicelli, 1963. — **145–147.** *S.* (*Trichodagmia*) Enderlein, 1934. **145.** *hirtipupa* Lutz, 1910. **146.** *lahillei* (Paterson & Shannon, 1927). **147.** *nigrimanum* Macquart, 1838. — **148–149.** *S.* (*Disculter*) subgen. nov. **148.** *oviedo* Ramírez-Pérez, 1971. **149.** *rivasi* Ramírez-Pérez, 1971. Scale bars = 0.2 mm.

longer than hypostomal length (Figs 128–131). Hypostoma anterior margin straight; hypostomal teeth extended beyond or below hypostomal anterior margin; medial tooth longer than lateral ones or at same level; lateral serrations absent or present; paralateral teeth present or absent. Body surface mostly glabrous. Abdomen gradually expanding posteroventrally to last segments, then abruptly contracting to posterior proleg (Figs 107–108). Anal sclerite no encircling base of posterior proleg. Posterior portion of abdomen without sclerotized accessory plates. Without 1+1 ventral tubercles. Rectal papillae with three multi-branched or simple lobes.

Remarks

Simulium (*Anasolen*) and *S.* (*Xenosimulium*) were both recovered as monophyletic and well supported based on two and eight synapomorphies, respectively (Fig. 1, Table 2, nodes 14, 13). In *S.* (*Xenosimulium*), two of them are unique: larval antenna annulated (45 (1), Fig. 127) and larval rectal papillae with undivided lobes (62 (0)).

Both groups form a clade based on five synapomorphies (Fig. 1, Table 2, node 12), some of them unique among the Afrotropical black flies: hypoginial valves of similar shape and orientation (Figs 16–17), similar anal lobe shape (Figs 22–23), and shape of pupal gills (Figs 91–94). Therefore, we propose to subsume *S.* (*Xenosimulium*) as the Imerinae species-group (Fig. 1, node 13) (five species) in *S.* (*Anasolen*), and treat the species already included in *S.* (*Anasolen*) as the Dentulosum species-group (Fig. 1, node 14) (11 species). Both species-groups can be differentiated by: female hypoginial valves; male ventral plate and parameres; pupal gill morphology; larval antenna, hypostoma and rectal papillae.

Dentulosum species-group

Species

Simulium bisnovem Gibbins, 1938
S. dentulosum Roubaud, 1915
S. heptaspicae Gouteux, 1977
S. kauntzeum Gibbins, 1938
S. masabae Gibbins, 1934
S. ngabogei Fain, 1950
S. nili Gibbins, 1934
S. octospicae Gibbins, 1937
S. rhodesiense De-Meillon, 1942
S. shoae Grenier & Ovazza, 1956
S. voltae Grenier, Ovazza & Valade, 1960

Diagnosis

Female

Hypoginial valves shorter than sternite VIII width (Fig. 16).

Male

Ventral plate with sub-equal width and length; lateral shoulders present (Fig. 35) or absent (Figs 33, 36–37); posteromedial process absent. Parameres with both distinctly large and small accessory spines.

Pupa

Gill filaments with apices darkly pigmented, contrasting in color with the rest of the gill (Figs 91–92, 94).

Larva (last instar)

Postocciput extended over the cervical sclerites (Figs 115, 117–118). Antenna equal or shorter than labral fan stalk; medial antennomere not annulated. Mandibles with preapical teeth shorter (e.g., *S. dentulosum*, *S. nili*) or as long as apical tooth (e.g., *S. kauntzeum*, *S. ngabogei*); two mandibular serrations of equal or different sizes. Hypostomal (Fig. 151) teeth extended beyond hypostomal anterior margin; medial tooth distinctly longer than lateral ones; lateral serrations well-developed, sharp; paralateral teeth present. Rectal papillae with three multi-branched lobes.

Distribution

Dentulosum is distributed in the following countries of the Afrotropical Region: Angola, Burkina Faso, Cameroon, Democratic Republic of Congo, Equatorial Guinea, Eritrea, Ethiopia, Ghana, Guinea, Ivory Coast, Kenya, Lesotho, Malawi, Nigeria, Rwanda, São Tomé and Príncipe, Saudi Arabia, Sierra Leone, South Africa, Sudan, Swaziland, Tanzania, Togo, Uganda, Yemen and Zimbabwe (Adler 2020).

Imerinae species-group

Species

Simulium ambositrae Grenier & Grjebine, 1959

S. griveaudi Ovazza & Ovazza, 1970

S. imerinae Roubaud, 1905

S. iphias De-Meillon, 1951

S. neireti Roubaud, 1905

Diagnosis

Female

Hypoginial valves longer than sternite VIII width (Fig. 17).

Male

Ventral plate two times longer than wide; lateral shoulders projected (e.g., Fig. 34); posteromedial process present (e.g., *S. griveaudi*, *S. iphias*, *S. neireti*), cylindrical and lower or equal than ventral plate length, or absent (Fig. 34). Parameres with all spines equal in size.

Pupa

Gill filaments with apices concolorous with rest of gill (Fig. 93).

Larva (last instar)

Postocciput extended over the cervical sclerites (Fig. 116). Antenna with distal antennomere and ½ of medial antennomere surpassing labral fan stalk; medial antennomere secondarily annulated (Fig. 127). Mandibles with preapical teeth equal or longer than apical tooth; supernumerary mandibular serrations (except in *S. ambositrae*) of different sizes. Hypostomal (Fig. 150) teeth very reduced and extended below hypostomal anterior margin; lateral serrations blunt (e.g., *S. ambositrae*, *S. neireti*) or absent (e.g., *S. griveaudi*, *S. imerinae*, *S. iphias*); paralateral teeth absent. Rectal papillae without secondary lobules.

Distribution

The Imerinae group is endemic to Madagascar but *S. imerinae* is also recorded from the Comoro Islands (Adler 2020).

***Simulium* (*Freemanellum*)** Crosskey, 1969

Figs 5, 18, 24, 38, 55–56, 76–77, 95–97, 109, 119–120, 132–135, 152

Freemanellum Crosskey, 1969: 92, figs 91, 110–112, 131, 147, 163, 205–206, 254 (as subgenus). Type species: *Simulium berghei* Fain, 1949.

Species

- Simulium berghei* Fain, 1949
- S. debegene* De-Meillon, 1934
- S. empopomae* De-Meillon, 1937
- S. hessei* Gibbins, 1941
- S. hirsutilateris* De-Meillon, 1937
- S. manense* Elsen & Escaffre, 1976
- S. spinulicorne* Fain & Elsen, 1980

Diagnosis

Female

Scutum red to light brown; scutal pattern present (Fig. 5). Cibarium unpigmented anteromedially and unarmed. Tarsal claw with subbasal tooth of about half of claw length. Hypoginial valves subtriangular, shorter than sternite VIII width, forwardly directed (Fig. 18). Anal lobe rectangular, longer than cercus width (Fig. 24).

Male

Ventral plate two times wider than longer, not notched, without lateral shoulders or posteromedial process (Fig. 38). Gonocoxite of equal length and width, with base almost equal in width than gonostylus width at their point of intersection (Figs 55–56). Gonostylus cylindrical with medial region equal in width than basal region, distinctly longer than gonocoxite, with blunt apex and apical spinule (Figs 55–56). Parameres with both, large and small accessory spines.

Pupa

Cocoon aperture upward directed, separated from substratum and not corbicular (Figs 76–77). Gill shorter than pupal body with trunk very reduced and with base enclosed by cocoon anterior margin (Figs 95–97); with four filaments arranged in different planes (tridimensional); filaments flexible, cylindrical, and with blunt apices concolorous with rest of gill; inferior branch single (Figs 95–97). Thoracic trichomes simples.

Larva (last instar)

Cephalic apotome spots insertion area pigmented (Figs 119–120). Postocciput extended over the cervical sclerites. Labral fan stalk shorter than antenna, but longer than antennomeres 1+2. Antenna without secondary annulations. Mandibles with preapical teeth as long as apical tooth; supernumerary mandibular serrations of different sizes. Postgenal cleft rounded; postgenal bridge length nearly equal than hypostomal length (Figs 132–135). Hypostomal anterior margin straight; hypostomal teeth very reduced and extended below anterior margin of ventral wall of hypostoma (Fig. 152); lateral serrations well-developed, blunt; paralateral teeth present. Body surface glabrous. Abdomen gradually expanding posteroventrally to last segments, then abruptly contracting to posterior proleg (Fig. 152). Anal sclerite no encircling base of posterior proleg. Posterior portion of abdomen without sclerotized accessory plates. Without 1+1 ventral tubercles. Rectal papillae with three multi-branched lobes.

Remarks

Simulium (*Freemanellum*) was recovered as monophyletic and stable based on nine synapomorphies (Fig. 1, Table 2, node 16), one of them unique: pupal gill trunk reduced (35 (0), Figs 95–97). *Simulium* (*Freemanellum*) groups with *S.* (*Trichodagma*) and *S.* (*Hemicnetha*) based on two synapomorphies (Fig. 1, Table 2, node 15), one of them unique: female anal lobe at least twice as long as cercus width at base twice as long as cercus width at base (13 (1), Figs 24, 26, 29–30), but with reversals in most species of the Orbitale species-group of *S.* (*Trichodagma*) (e.g., Fig. 28).

Distribution

Simulium (*Freemanellum*) is distributed in the following countries of the Afrotropical Region: Cameroon, Democratic Republic of Congo, Ghana, Guinea, Ivory Coast, Liberia, Malawi, Nigeria, Sierra Leone, South Africa, Tanzania, Togo, Uganda, and Zimbabwe.

Simulium (*Disculter*) subgen. nov.

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Figs 31–32, 48–49, 69–70, 89–90, 148–149

Type species

Simulium oviedo Ramírez-Pérez, 1971.

Diagnosis

Female

Scutum black; scutal pattern present. Cibarium unpigmented anteromedially and unarmed. Tarsal claw with subbasal tooth of about half of claw length. Hypoginial valves subtriangular, shorter than sternite VIII width, forwardly directed. Anal lobe subrectangular and shorter than the cercus base width (Figs 31–32).

Male

Ventral plate (Figs 48–49) with sub-equal width and length, not notched; lateral shoulders absent; posteromedian process absent. Gonocoxite length equal to width, with base almost equal in width than gonostylus width at their point of intersection (Figs 69–70). Gonostylus cylindrical, with medial region equal in width than basal region; longer than gonocoxite, with acute apex and apical spinule (Figs 69–70). Parameres with both distinctly large and small accessory spines.

Pupa

Cocoon aperture upward directed, separated from substratum, not corbicular (Figs 89–90). Gills shorter than pupa body, with trunk development, and base enclosed by cocoon anterior margin; with six filaments arranged in different planes (tridimensional); filaments cylindrical, with blunt apices concolorous with rest of gill; inferior branch multibranching. Thoracic trichomes present or absent.

Larva (last instar)

Cephalic apotome spots insertion area not pigmented. Postocciput not extended over the cervical sclerites. Antenna shorter than labral fan stalk, without secondary annulations. Mandibles with preapical teeth shorter than apical tooth; two mandibular serrations of same sizes. Postgenal cleft triangular; postgenal bridge length nearly equal than hypostomal length (Figs 148–149). Hypostomal anterior margin straight; hypostomal teeth extended beyond anterior margin of ventral wall of hypostoma; medial tooth as prominent as lateral ones; lateral serrations well-developed, sharp; paralateral teeth present. Body glabrous. Abdomen gradually expanding posteroventrally to last segments, then abruptly contracting to posterior proleg. Anal sclerite not encircling base of posterior proleg. Posterior portion of

abdomen without sclerotized accessory plates. Without 1+1 ventral tubercles. Rectal papillae with three multi-branched lobes.

Etymology

The name *Disculter* derives from Latin prefix ‘dis’, meaning ‘apart’, plus the Latin ‘culter’, meaning ‘knife or razor’, in reference to the gonostylus shape, which differs from those of other related subgenera, such as *S.* (*Trichodagmia*) and *S.* (*Hemicnetha*). The gender is masculine.

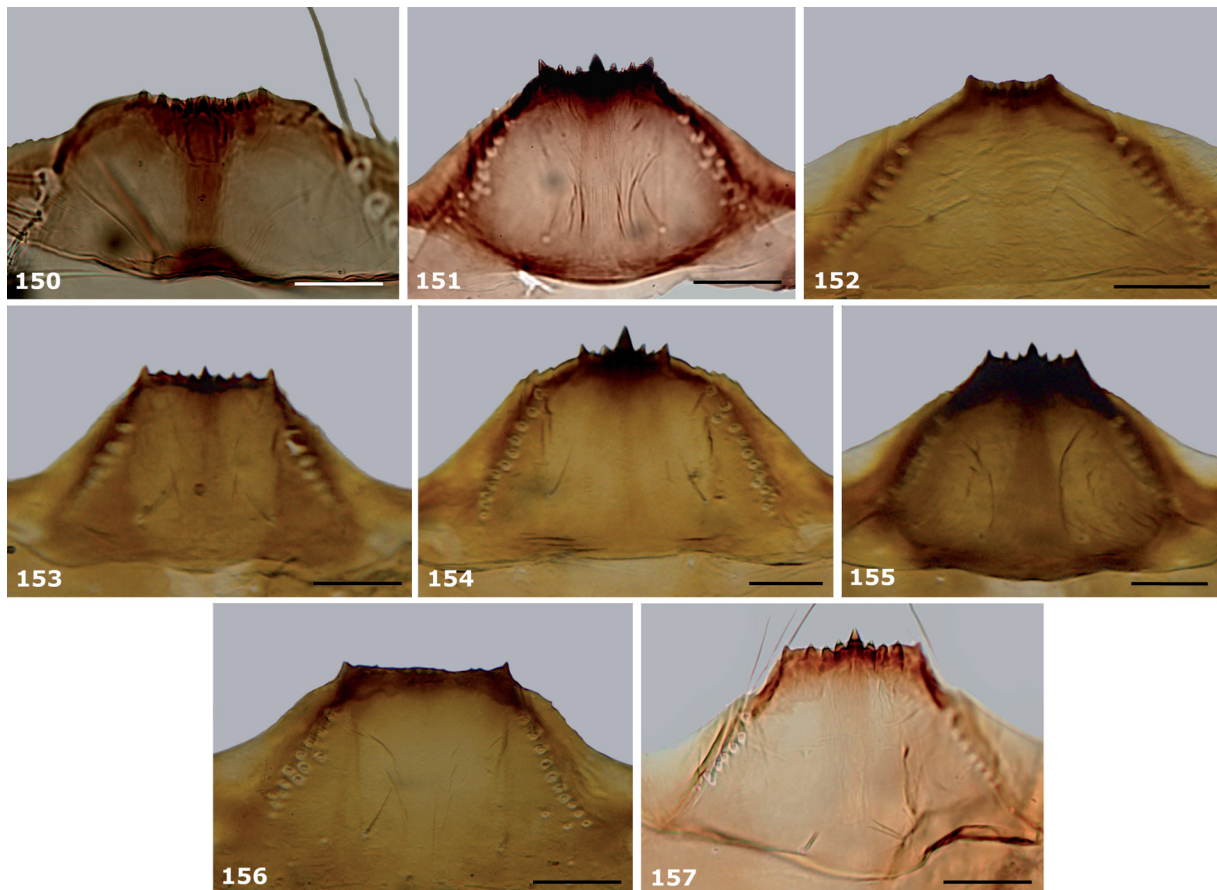
Species

Simulium oviedo Ramírez-Pérez, 1971

S. rivasi Ramírez-Pérez, 1971

Remarks

Oviedo species-group in Coscarón (1987) (not included in any *Simulium* subgenera). Oviedo species-group of *Simulium* (*Hemicnetha*) in Coscarón & Coscarón-Arias (2007). As part of the Orbitale



Figs 150–157. Larva (last instar). Hypostoma. **150–151.** *Simulium* (*Anasolen*) Enderlein, 1930. **150.** *ambositrae* Grenier & Grjebine, 1959. **151.** *nili* Gibbins, 1934. — **152.** *S.* (*Freemanellum*) *manense* Elsen & Escaffre, 1976. — **153–154.** *S.* (*Hemicnetha*) Enderlein, 1934. **153.** *brachycladum* Lutz & Pinto, 1932. **154.** *rubrithorax* Lutz, 1909. — **155.** *S.* (*Obuchovia*) *galloprovinciale* Giudicelli, 1963. — **156–157.** *S.* (*Trichodagmia*) Enderlein, 1934. **156.** *hirtipupa* Lutz, 1910. **157.** *lahillei* (Paterson & Shannon, 1927). Scale bars = 0.1 mm.

species-group of *S. (Hemicnetha)* in Shelley *et al.* (2010). As part of the Bicoloratum species-group of *S. (Psilopelmia)* in Hernández (2011).

Simulium oviedo and *S. rivasi* were first described and included in *S. (Hemicnetha)* by Ramírez-Pérez (1971). Coscarón (1987) created the group Oviedo, not including it in any *Simulium* subgenus, but regarding it as close to *S. (Hemicnetha)* based on the general aspect of the adult and last instar larva. Coscarón & Coscarón-Arias (2007) placed Oviedo as a species-group within *S. (Hemicnetha)* based on the female cibarium, nudiocular triangle, frontoclypeal base, basal section of the Radius, pupal cocoon, and shape of pupal gill. Even these authors considered that *S. (Hemicnetha)* offered the closest fit for *S. oviedo* and *S. rivasi*, and pointed out that the female and male genitalia of the Oviedo species-group differ from those of most other species of *S. (Hemicnetha)*. Shelley *et al.* (2010) transferred both species from *S. (Hemicnetha)* to the Orbitale species-group of *S. (Trichodagmia)* (sensu Shelley *et al.* 2010), but did not explain the reason for this taxonomic change. Hernández (2011) transferred them to the Bicoloratum species-group of *S. (Psilopelmia)* based on the female cibarium, male ventral plate shape, gonocoxite/gonostyle length ratio, and larval hypostomal teeth. Gil-Azevedo & Coscarón (2020) confirmed that Oviedo failed to group with *S. (Psilopelmia)* and recovered it as closer to *S. (Hemicnetha)*.

According to our analysis, Oviedo is close to the clade [*S. (Anasolen)* + *S. (Freemanellum)* + *S. (Hemicnetha)* + *S. (Trichodagmia)*] (Fig. 1, node 9), but did not fit in any of these groups or in any other Neotropical *Simulium* subgenus. Therefore, we here propose a new subgenus, *S. (Disculter)* subgen. nov. (Fig. 1, node 10) to accommodate the species in this group.

Both species are almost identical and can be differentiated only by the trichomes and tubercles of the pupal frontoclypeus and thorax. *Simulium oviedo* has trichomes with 3–5 ramifications and spinous tubercles, whereas *S. rivasi* does not have trichomes and has rounded tubercles.

Distribution

The subgenus is endemic to the state of Mérida, Venezuela (Adler 2020).

Discussion

Phylogenetic analysis

According to the topology found, *S. (Hearlea)*, *S. (Shewellomyia)*, and *S. (Obuchovia)* (Fig. 1, Table 2, node 2) form a stable clade supported by a single, unique synapomorphy: gonocoxite base twice as wide as gonostylus at their point of intersection (14 (1). Figs 57–59, 63). However, we prefer to consider each group as an independent subgenus due to their stability and morphological differences in the pupal gills and male ventral plate.

Crosskey (1969) pointed out that *S. (Anasolen)*, *S. (Freemanellum)*, and *S. (Xenosimulium)* should form a monophyletic group. He considered these groups as an isolated segregate without affinity with any other Afrotropical, Holarctic, Oriental or Australasian *Simulium* group, but instead related to the Neotropical *Simulium lahillei*, type species of *S. (Grenierella)* [= Nigrimanum species-group of *S. (Trichodagmia)*]. In our analysis, we included representatives of all Neotropical and Afrotropical *Simulium* subgenera except *S. (Phoretomyia)* Crosskey, 1969, and found that *S. (Anasolen)*, *S. (Freemanellum)*, and *S. (Xenosimulium)* are indeed more related to Neotropical subgenera than to Afrotropical ones, as they grouped with *S. (Trichodagmia)* and *S. (Hemicnetha)* based on four synapomorphies (Fig. 1, Table 2, node 11).

According to the consensus cladogram of the EW analysis (Fig. 2), *Simulium (Disculter)* subgen. nov. is in an undetermined position. It could group with (1) *S. (Hearlea)*; (2) [*S. (Shewellomyia)* + [*S. (Obuchovia)*];

or with (3) [*S. (Anasolen)* + *S. (Freemanellum)* + *S. (Trichodagmia)* + *S. (Hemicnetha)*]. The possibility (3), the same found in the consensus cladogram of the IW analysis (Fig. 1, node 9), would be the most likely. The basal position of *S. (Disculter)* subgen. nov. in this clade, and the fact that it has unique features and do not fit the diagnosis of any other group, justifies it as a separate subgenus.

The clade [*S. (Disculter)* subgen. nov. + *S. (Anasolen)* + *S. (Freemanellum)* + *S. (Hemicnetha)* + *S. (Trichodagmia)*] (Fig. 1, Table 2, node 9) is supported by a single synapomorphy: gonocoxite longer than or as long as wide (16 (1). Figs 50–60, 69–70), albeit with a reversal in [*S. (Hemicnetha)* + *S. (Trichodagmia)*] (Fig. 1, Node 17. Figs 60–62, 64–68). These relationships must be further investigated.

Biogeographical and ecological considerations

The present analysis reveals two well-defined groups, one comprising Nearctic and Palearctic species: [*S. (Hearlea)* + *S. (Shewellomyia)* + *S. (Obuchovia)*] (northern clade) (Fig. 1, node 2), another comprising Neotropical and Afrotropical species: [*S. (Disculter)* subgen. nov. + *S. (Anasolen)* + *S. (Trichodagmia)* + *S. (Freemanellum)* + *S. (Hemicnetha)*] (southern clade) (Fig. 1, node 9).

In the northern clade, *S. (Obuchovia)* is restricted to the Palearctic Region with a distribution ranging from North Africa and the Iberian Peninsula, through central-southern and southern Europe to the eastern Mediterranean area, the Caucasus and Transcaucasus, and Western Asia (Crosskey & Santos-Gracio 1985; Bouzidi & Giudicelli 1987). *Simulium (Shewellomyia)* is distributed in the Nearctic Region, being found in Canada and the USA (Adler *et al.* 2004; Adler 2020). *Simulium (Hearlea)* is distributed at the intersection between the Nearctic and Neotropical regions, being mostly confined to northern and central Mexico (also reaching southern Guatemala), albeit with a few species in the USA and (northern) Canada (Coscarón *et al.* 2004; Coscarón & Coscarón-Arias 2007).

The relationship of *S. (Hearlea)* as the sister group of [*S. (Shewellomyia)* + *S. (Obuchovia)*] disagrees with Coscarón & Coscarón-Arias (1995), who argued that the Mesoamerican black fly fauna has a stronger affinity with the Neotropical fauna than with the Nearctic fauna, citing the specific case of *S. (Hearlea)* and *S. (Hemicnetha)*. The distributions of these two subgenera overlap in Mesoamerica, where both may occur in sympatry. According to our analysis, *S. (Hemicnetha)* is closer to the southern clade, whereas *S. (Hearlea)* is closer to the northern clade.

In the southern clade, *S. (Trichodagmia)* is Neotropical, being recorded from south-eastern Brazil to Amazonia, Guyana, and Suriname, and from Venezuela to central Argentina (Miranda-Esquivel & Coscarón 2001; Coscarón & Coscarón-Arias 2007). *Simulium (Hemicnetha)* is the subgenus with the widest distribution, occurring from Canada to Argentina (Coscarón & Coscarón-Arias 1995, 2007). Both subgenera occur in sympatry in South America. *Simulium (Disculter)* subgen. nov. is endemic to Venezuela (Ramírez-Pérez 1971; Coscarón & Coscarón-Arias 2007).

Simulium (Freemanellum) is distributed in East, West, and southern Africa, reaching from north-eastern Congo and Uganda to the Cape of Good Hope (Crosskey 1969). Within *S. (Anasolen)*, the *Dentulosum* species-group is widespread in the Afrotropical Region (Crosskey 1969), whereas the *Imerinae* species-group is endemic to Madagascar and the Comoro Islands (Crosskey 1969; Ovazza & Ovazza 1970; De Moor 2016).

The clade [*S. (Disculter)* subgen. nov. + *S. (Anasolen)* + *S. (Trichodagmia)* + *S. (Freemanellum)* + *S. (Hemicnetha)*] (Fig. 1, node 9) is supported by Miranda-Esquivel & Coscarón (2001, 2003) and Coscarón *et al.* (2004), who suggested that it might constitute a Gondwanan lineage together with *S. (Hearlea)*. We cannot estimate when the groups that form this clade separated, because of the current lack of a fossil record; however, despite that, Miranda-Esquivel & Coscarón (2003), suggested that

the subgenera that forms this clade separated due to a vicariance event in the early Cretaceous, with Amazonia s. lat. (defined as Guyana + Amazonia + Cerrado + SE Brazil (sensu Coscarón & Coscarón-Arias 1995) + Africa as its ancestral area. However, the clade [*S. (Disculter)* subgen. nov. + *S. (Anasolen)* + *S. (Trichodagmia)* + *S. (Freemanellum)* + *S. (Hemicnetha)*] could be considered as a model group to understand the Gondwanian black fly distribution.

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Supplementary material

Supp. file 1. List of characters. <https://doi.org/10.5852/ejt.2021.773.1517.5131>

Supp. file 2. Data matrix for the cladistic analyses. Symbols: “-” = codes for inapplicable data; “?” = for unavailable data. <https://doi.org/10.5852/ejt.2021.773.1517.5135>

Supp. file 3. Material examined. <https://doi.org/10.5852/ejt.2021.773.1517.5137>