

# The phylogeny of *Simulium* (*Chirostilbia*) (Diptera: Simuliidae) and perspectives on the systematics of the genus in the Neotropical Region

Leonardo Henrique Gil-Azevedo<sup>1,2/+</sup>, Sixto Coscarón<sup>3</sup>, Marilza Maia-Herzog<sup>2</sup>

<sup>1</sup>Departamento de Zoologia, Instituto de Biologia, Universidade do Estado do Rio de Janeiro, Rua São Francisco Xavier 524, 20550-013 Rio de Janeiro, RJ, Brasil <sup>2</sup>Laboratório de Simulídeos e Oncocercose, Instituto Oswaldo Cruz-Fiocruz, Rio de Janeiro, RJ, Brasil <sup>3</sup>Facultad de Ciencias Naturales y Museo, La Plata, Buenos Aires, Argentina

Recently, knowledge of Neotropical Simuliidae has been accumulating quickly. However, information about supra-specific relationships is scarce and diagnoses of *Simulium* subgenera are unsatisfactory. To investigate the relationships among *Simulium* (*Chirostilbia*) species and test the subgenus monophyly, we performed a cladistic analysis. The ingroup included all species of this subgenus and the outgroup included representatives of the 17 species groups of Neotropical *Simulium* and three Holarctic species. The study was based on a data matrix with 31 terminal taxa and 45 morphological characteristics of adult, pupa and larva. The phylogenetic analysis under equal weights resulted in eight most-parsimonious trees (length = 178, consistency index = 34, retention index = 67). The monophyly of the *S.* (*Chirostilbia*) was not supported in our analysis. The *Simulium* subpallidum species group was closer to *Simulium* (*Psilopelmia*) and *Simulium* (*Ectemnaspis*) than to the *Simulium* *pertinax* species group. Additionally, we describe the three-dimensional shape of the terminalia of male and female of *Simulium* (*Chirostilbia*) for the first time and provide comments about the taxonomic problems involving some species of the subgenus: *Simulium* *acarayense*, *Simulium* *papaveri*, *S.* *pertinax*, *Simulium* *serranum*, *Simulium* *striginotum* and *S.* *subpallidum*.

Key words: black fly - Culicomorpha - cladistics - morphology - taxonomy

Systematic knowledge of the Neotropical Simuliidae has been accumulating quickly and there are 351 valid Neotropical species (Coscarón et al. 2008, Adler & Crosskey 2010, Hamada et al. 2010). Over the past 10 years, 67 taxonomic papers involving 20 researchers, as well as two extensive manuals, have been published (Coscarón & Coscarón-Arias 2007, Shelley et al. 2010), resulting in the description of 18 new species (Adler & Crosskey 2010, Hamada et al. 2010). Consequently, the Neotropical black fly became relatively well known and, unlike most of the other Neotropical animal taxa, a basic lack of data no longer is an issue (Rocha & Boerger 2009).

Eleven subgenera of *Simulium* Latreille, 1802 have been found in the Neotropical Region (Adler & Crosskey 2010). Most of the species in these subgenera are well described and well represented in institutional collections. However, knowledge of supraspecific group relationships is very scarce and subgenera diagnoses are unsatisfactory. In addition, there are problems in the delimitation of the identity of some species. As a result of these problems, divergent systematic arrangements exist in the literature (Py-Daniel & Sampaio 1995, Coscarón et al. 2008, Adler & Crosskey 2010, Shelley et al. 2010) and loose synonymies of species are often proposed, which tend to appear and disappear in the literature.

In the literature, all systematic decisions were made exclusively based on personal opinions, without any clear methodology or hypothesis; as a result, one cannot rationally reject or accept the changes proposed. There have been a few morphological phylogeny studies, but they have not targeted major subjects such as the monophyly of subgenera, the relationship amongst the subgenera or the species identity (e.g., Coscarón & Coscarón-Arias 1997, Strieder & Py-Daniel 2002). In addition, there are limited molecular, cytogenetic and morphometric works dealing with Neotropical taxa (e.g., Coscarón-Arias 1998, Scarpassa & Hamada 2003, Hamada et al. 2008, 2010). Therefore, the real problem underlying current Neotropical Simuliidae systematics is the organisation of the massive amount of data produced over the years.

*Simulium* (*Chirostilbia*) Enderlein, 1921 is a good case study because it is a group endemic to the Neotropics and has remained taxonomically stable. For example, no researchers have ever questioned the monophyly of the group; however, as with the other subgenera, the position of the group within *Simulium* is unknown and the diagnoses made for this group are too broad and are inadequate to clearly identify the subgenus (Coscarón 1981, Coscarón & Coscarón-Arias 2007, Hernandez et al. 2008).

In the present study, a cladistic analysis was performed to test the monophyly of *Simulium* (*Chirostilbia*) and to evaluate the phylogenetic structure within the subgenus. We also provide comments about the taxonomic problems of some species of *Simulium* (*Chirostilbia*).

*Historical background* - *Chirostilbia* was created by Enderlein (1921a: 199) as a genus, with *Chirostilbia flavifemur* Enderlein, 1921, as the type species and was fully described by Enderlein in a different publication (1921b: 79). Subsequently, *Chirostilbia* was considered a subge-

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+ Corresponding author: lhgazevedo@yahoo.com.br

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nus of *Simulium* (Pinto 1932) and Lane and Porto (1939) considered *Simulium flavifemur* as a synonym for *Simulium pertinax* Kollar, 1832. Coscarón (1981) established the subgenus, which contained five species. Coscarón (1982) proposed the *Simulium subpallidum* species group with three species later included in *Simulium* (*Chirostilbia*) (Coscarón 1987). He considered *Simulium* (*Chirostilbia*) to have two species groups: *S. pertinax* and *S. subpallidum*. Coscarón and Coscarón-Arias (1997) performed the only phylogenetic analysis of the subgenus, with 18 characters, 12 taxa in the ingroup and *Simulium* (*Inaequalium*) as the outgroup. Coscarón et al. (2008) considered 15 species in *Simulium* (*Chirostilbia*), 12 in *S. pertinax* and three in the *S. subpallidum* species group. The division into these species groups was contested by Hernández et al. (2008), who do not consider Coscarón's characters valid.

#### MATERIALS AND METHODS

*Terminal taxa and specimens* - The ingroup taxa consisted of all the species of *Simulium* (*Chirostilbia*) except for *Simulium striginotum* (Enderlein, 1933), which is known only by a female, for a total of 14 terminal taxa. The chosen outgroups were as follows: *Metacnephia saileri* (Stone, 1952), representatives of two *Simulium* subgenera from the Holarctic Region, *Simulium* (*Hellichella*) *nebulosum* Currie & Adler, 1986 and *Simulium* (*Eusimulium*) *aureum* Fries, 1824 and the 17 species groups of Neotropical *Simulium* recognised by Adler and Crosskey (2010).

The specimens studied herein are deposited in the following institutions: the Museo de La Plata (MLP) (Argentina), the Museu de Zoologia de São Paulo (Brazil), the Faculdade de Saúde Pública da Universidade de São Paulo (Brazil), the Coleção Histórica de Adolpho Lutz, Instituto Oswaldo Cruz (Brazil), the Coleção do Laboratório de Simulídeos e Oncocercose, Instituto Oswaldo Cruz (Brazil), the Instituto Nacional de Pesquisa da Amazônia (Brazil) and the Royal Ontario Museum (Canada). Whenever possible, we examined specimens of three developmental stages (larva, pupa and adult). The techniques used to prepare and observe the specimens and specimen-related material were previously described in Gil-Azevedo et al. (2005). Additionally, the preparations involved and the techniques used to observe the three-dimensional shape of the terminalia in a drop of glycerin on a depression slide were described in Adler et al. (2004).

*Terminology and characters* - We followed the morphological terminology of Adler et al. (2004), with the addition of terms from Gil-Azevedo and Maia-Herzog (2007). Based on an extensive morphological study, we identified the characters and proposed hypotheses of primary homology (Pinna 1991) (Supplementary data). The logic employed in the preparation of characters followed Sereno (2007). We codified these hypotheses in a data matrix using the program Mesquite (Maddison et al. 1997, Maddison & Maddison 2007) (Supplementary data). Characters were preferentially coded reductively (Wilkinson 1995) and were treated as unordered (Fitch 1971). When two or more states of a character were found in one terminal taxon, the character was treated as polymorphic and both states were coded in the matrix.

*Phylogenetic analyses* - The dataset was analysed with maximum parsimony equal weights. The searches were carried out through heuristic algorithms. The parameters of the search were as follows: 3,000 replications and Tree bisection and reconnection with 1,000 trees saved per replication. The branch support was evaluated by bootstrap (Felsenstein 1985) with 5,000 replications by decay index (Bremer 1994), through the search of suboptimal trees and by a sensitivity analysis using analyses under implied weights (Goloboff et al. 2008b) ( $k = 3, 6, 10, 15, 20, 25$ ). We used the TNT program for all searches (Goloboff et al. 2008a). Character optimisation, mapping and comparisons among the obtained trees were carried out with WinClada (Nixon 2002).

#### RESULTS

The data matrix was composed of 34 terminal taxa and 45 characters obtained from adult (25), pupa (12) and larva specimens of the last instar (8) (Supplementary data). The list of characters used in this analysis is given in Supplementary data.

The phylogenetic analysis under equal weights resulted in eight most-parsimonious trees with 178 steps, consistency index = 0.34 and retention index = 0.67 (Fig. 1, Table I). The obtained hypothesis indicates that *Simulium* (*Chirostilbia*) is not a monophyletic group because the *S. subpallidum* species group is closer to *Simulium* (*Psilopelmia*) Enderlein, 1934 + *Simulium* (*Ectemnaspis*) Enderlein, 1934, than to the *S. pertinax* species-group (Fig. 1). In spite of the low support from the bootstrap analysis and the decay index, the strict consensus tree has a good resolution and the clades are also found in the implied weights analyses (Fig. 1, Table II). We chose one of the shortest trees for character distribution and optimisation (Fig. 2).

*Description of the terminalia of Simulium* (*Chirostilbia*) based on a three-dimensional view - Female terminalia (Fig. 3): the hypogynial valves are small and subtriangular with slightly divergent external margins. The anal lobe is long and subtriangular, approximately 2.5-fold longer than wide in the lateral view. The internal sides of the anal lobes are concave and facing each other, resembling a pair of long spoons. Therefore, when the anal lobe is fixated in a slide, it may either be wider and less curved or narrower and more curved, depending on whether the dorsal axis of the anal lobe is central or lateral, respectively (Hernandez et al. 2007, Figs 118, 121, respectively). The anal lobe is approximately three-fold longer than the cerci. The cerci are hemispheric and distinctly longer than wide in the lateral view. The internal sides of the cerci are concave and face each other like brackets. The genital fork stem is as long as the lateral arms and the spermatheca are oval; male terminalia (Fig. 4): the gonocoxites are subtrapezoidal to subquadrangular. The gonostyles are subtriangular and curved towards the aedeagus. In the *S. subpallidum* species group and *Simulium jefersoni* Hamada et al., 2006 the height of the gonostyle is distinctly longer than the base, whereas in the others the height and the base are similar sizes. The apical spur of the gonostyle is absent, but there is a sclerotized longitudinal ridge along the external border. The longitudinal ridge likely has the

same function as the apical spur in other groups. The ventral plate is wider than it is long, with little curvature seen in profile, and is not very narrow apically. The median sclerite is widened with a strong median concavity. A dorsal sclerite is present as a sclerotized thin line. The paramere is elongated with developed teeth and connected to the apex of the ventral plate arms.

Below we give some remarks on some taxonomic species of *Simulium* (*Chirostilbia*).

The *S. pertinax* Kollar, 1832 has a complex taxonomic history, as explained in Shelley et al. (1984), but is not particularly complicated taxonomically, as suggested by Hernandez et al. (2008). There are six junior synonyms of *S. pertinax*, but these are easily explained. This species has a continental distribution and is a vicious human biter; as a result, it is easy to find and has been collected by different and isolated authors, who went on to describe it as a new species. *S. pertinax* is homogeneous based on morphological (Gil-Azevedo et al. 2004) and cytological (Campos et al. 1996, 2001) data and there is no reference in the literature to delimitation problems for this species. Such problems are common for other *Simulium* species (e.g., *S. subpallidum* Lutz, 1910 and *Simulium incrustatum* Lutz, 1910). Hernandez et al. (2008) recorded this species in Bo-

livia (north of Santa Cruz; 16.V.1989, JJ McGrande coll.) based only on two biting females. We did not consider this a valid occurrence because those females could have been from any *Simulium* (*Chirostilbia*) species and we prefer to wait until someone collects additional exemplars associated with pupae to confirm this occurrence.

*Simulium serranum* Coscarón, 1981 - Originally, Coscarón (1981) named this species *S. serranus*, but *Simulium* is neutral in gender and *serranus* is an adjective in the masculine form. According to the International Code of Zoological Nomenclature, the species-group name should agree in gender with the genus (ICZN 1999: 31.2); thus, the corrected name is now *S. serranum*.

Hernandez et al. (2007) observed the holotype of *S. striginotum* (Enderlein, 1933) [1 female, Bolivia, Department of La Paz, Mapiro, Sarampione (14.II.1903)] and synonymised this species with *Simulium spinibranchium* based on the scutum colouration, morphology of the cibarium, nudiocular area, wing setation, leg colouration and the structure of the genitalia, especially the cercus and anal lobe. We do not accept this synonymy because the colouration of exemplars collected over a century ago is not reliable. In addition, wing setation and the structures of genitalia are very similar in all *Simulium* (*Chirostilbia*) species. Moreover, the Department of La Paz is located in the Bolivian Altiplano, at a high altitude and very far from locations associated with any records of *Simulium* (*Chirostilbia*) species; therefore, this is likely a valid separate species. Although we agree with Hernandez et al. (2007) that this species is most likely within the *S. pertinax* species group of *S. (Chirostilbia)*, it is necessary to collect fresh material of all stages at the type-locality to define the identity of the species properly.

Hernandez et al. (2007) synonymised *Simulium acarayense* Coscarón & Wygodzinsky, 1972 with *S. subpallidum* and we agree that there is only one distinctive character between both species, the scutum colouration, but the species also differ in pupal and larval continuous characters that are related to environmental variables: the length of the pupal gill, the number of rays in the

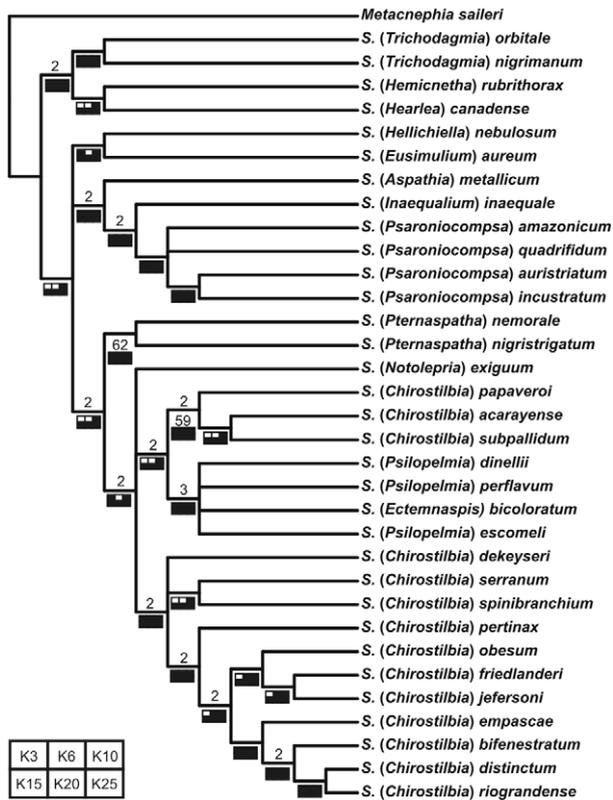


Fig. 1: reconstructed phylogeny of *Simulium* (*Chirostilbia*). Strict consensus of the eight most parsimonious trees resulted from the analysis under equal weights, with 178 steeps (consistency index = 0.34, retention index = 0.67). Branch support: decay index (up) and bootstrap (down). Decay index below two and bootstrap below 50% are not shown. The boxes represent the permanence of the clades in the analyses under implied weights with different concavities (k = 3, 6, 10, 15, 20, 25).

TABLE I

Number of most parsimonious trees (MPTs), length, consistency index (CI), retention index (RI) and fit from trees found in the analyses, with equal weights (EW) and implied weights (IW)

Analysis	MPTs	Length	CI/RI	Fit
EW	8	178	0.34/0.67	-
IW k3	2	183	0.33/0.66	17.94
IW k6	6	180	0.33/0.67	12.16
IW k10	1	178	0.34/0.67	8.56
IW k15	2	178	0.34/0.67	6.25
IW k20	2	178	0.34/0.67	4.92
IW k25	2	178	0.34/0.67	4.07

TABLE II

Differences between *Simulium pertinax* and *Simulium subpallidum* species-groups based on characters of the cladistic analyses

Characters	<i>S. pertinax</i> species group	<i>S. subpallidum</i> species group
Claw sub-basal tooth	Present	Absent
Abdomen, general colouration	Dark colour	Light colour
Male terminalia, length ratio gonostyle/gonocoxite	Gonocoxite distinctly greater <sup>a</sup>	Equal or gonostyle greater
Larval antenna, segment 3	Equal to or shorter than 2 <sup>b</sup>	Longer than 2
Larval hypostoma, anterior margin	Straight <sup>c</sup>	Concave
Larval post genal cleft	Subtriangular	Oval
Cocoon, shape	Boot-shaped or intermediate	Clearly shoe-shaped

a: except *Simulium jefersoni*; b: except *Simulium serranum*, *Simulium spinibranchium* and *S. jefersoni*; c: except *S. serranum* and *Simulium bifenestratum*.

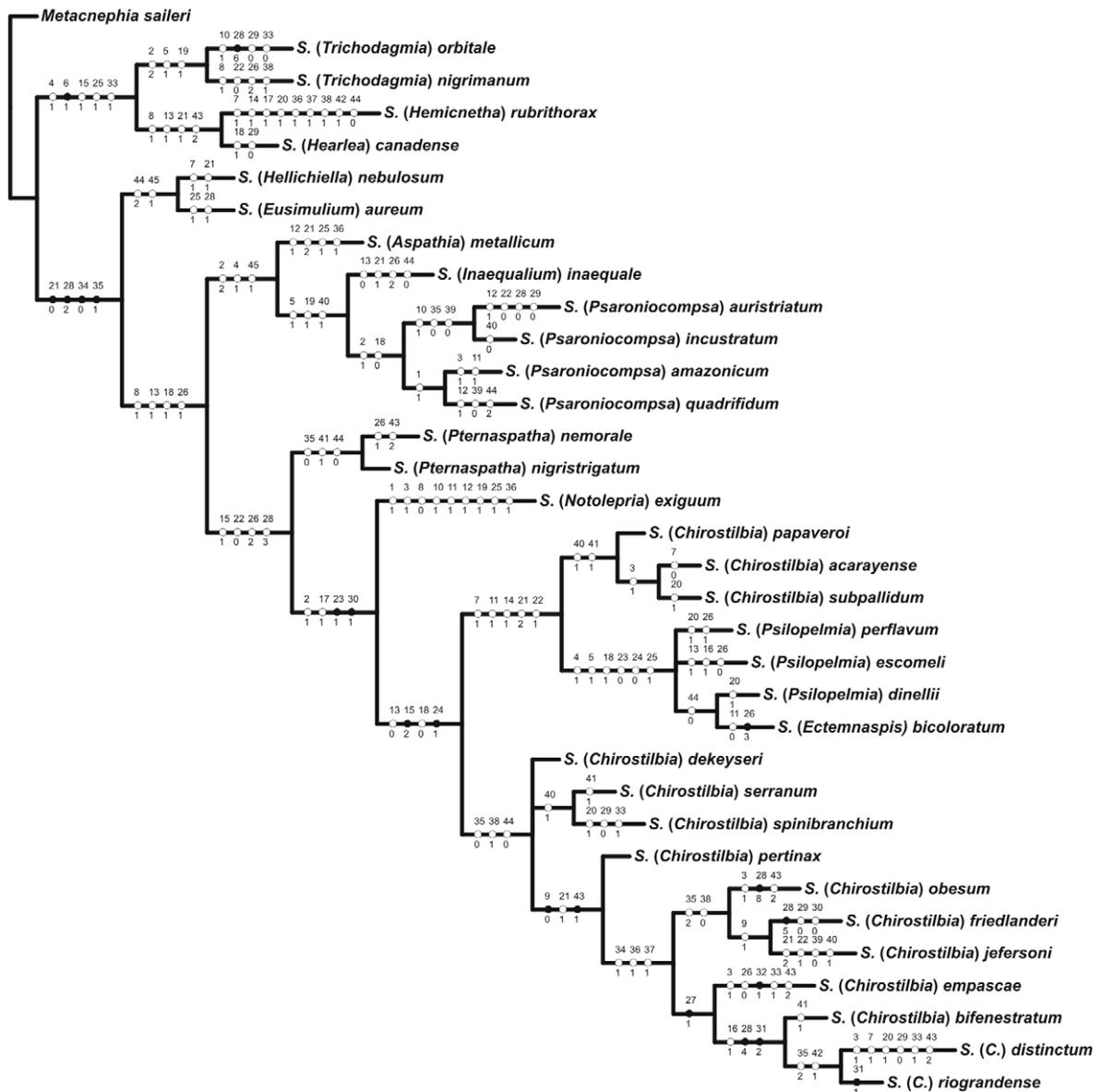


Fig. 2: one of eight trees obtained with maximum parsimony chosen to represent the character distribution. Circles represents apomorphies (white: homoplasy; black: no homoplasy). The numbers above the circles represent characters and the numbers below refer to character states. Character with ambiguous optimization: 18, 24, 26 and 33: ACCTRAN; 8, 11, 38, 39, 45: DELTRAN.

larval cephalic fan, the number of hook rows of the larval anal ring and the number of lobes in the larval rectal papillae. We accept this synonymy for now, but future works based on morphometry, molecular or cytological data are necessary to properly define the identity of the species.

Hernandez et al. (2008) indicate that *Simulium papaveroi* Coscarón, 1982 is morphologically similar to *Simulium dekeyseri* Shelley & Py-Daniel, 1981 and *S. seranum* based only on a few female and pupal characters, mainly scutum colouration, female terminalia and pupal gills. These characters are very similar in all species of *Simulium* (*Chirostilbia*) *sensu lato* and likely for this reason, these authors did not recognise the *S. subpallidum* species group. However, a careful observation of the specimen characters, including male terminalia, pupal cocoons and larval heads, indicates that this species is closer to *S. subpallidum*.

### DISCUSSION

The monophyly of the *Simulium* (*Chirostilbia*) was not supported in our analysis because the *S. subpallidum* species group was closer to *Simulium* (*Psilopelmia*) Enderlein, 1934 + *Simulium* (*Ectemnaspis*) Enderlein, 1934 than to the *S. pertinax* species group (Figs 1, 2). These analytical results may explain the weak diagnoses of the subgenus throughout the literature (e.g., Coscarón 1981, 1991, Hernandez et al. 2008). These results also indicate the importance of performing cladistic analyses in taxa reviews, especially when proposing systematic changes. Based on our data, we see two possibilities: restricting *Simulium* (*Chirostilbia*) to the *S. pertinax* species group and creating a new subgenus for the *S. subpallidum* species group, or including and synonymising *Simulium* (*Psilopelmia*) and *Simulium* (*Ectemnaspis*) with *Simulium* (*Chirostilbia*). We believe the second option is preferable, but our analyses do not have enough bootstrap or Bremer support to make us comfortable proposing these changes at this time. A more complete analysis of *Simulium* is necessary before implementing a deeper rearrangement of *Simulium* subgenera.

The division of *S. (Chirostilbia)* into species groups, as proposed by Coscarón (1991), was contested by Hernandez et al. (2008). The latter authors argue that “the species do not clearly fit into groups because of the variation and overlap of morphological characters, especially the colouration of the thorax and the form of the male gonostyle” (Hernandez et al. 2008). We agree that these two characters do not justify the species groups; however, Hernandez et al. (2008) ignore other characters, such as sub-basal claw teeth, cocoon shape and larval head characters that differentiate the species groups (Table II). In fact, our analyses indicate that the two groups are monophyletic and may not be sister groups (Figs 1, 2).

The *S. pertinax* species group was recovered in all of our analyses. In our basal branches, the resolution is poor and the relationship amongst *S. dekeyseri*, *S. seranum*, *S. spinibranchium* and the major branch requires further investigation. However, the resolution within the major branch is clear. The major branch presents, as synapomorphies, female scutum patterns with blurred stripes, a male gonostyle more than two-thirds the length of the gonocoxite and larvae with 8 + 8 hyposomal setae or more. *S. pertinax* is in the base of the major branch opposed to the group formed by boot-shaped cocoon species (the only exception is *Simulium bifenestratum* Hamada & Pepinelli, 2004 that has a highly derivative cocoon and was treated as inapplicable for this character). Inside the boot-shaped cocoon clade, there are two branches: (*Simulium obesum* Vulcano, 1959 (*Simulium friedlanderi* Py-Daniel, 1987 + *S. jefersoni*)) and (*Simulium empascae* Py-Daniel, Souza & Caldas, 1988 (*S. bifenestratum* (*Simulium distinctum* Lutz, 1910 + *Simulium riograndense* Py-Daniel, Souza & Caldas, 1988))). The synapomorphies of these branches are as follows: the larvae with positive apoteme and cocoon fabric with visible thick threads are synapomorphic with the first branch and the pupa with cephalic trichomes that are small and thin and are synapomorphic with the second. In addition, the clade (*S. bifenestratum* (*S. dis-*

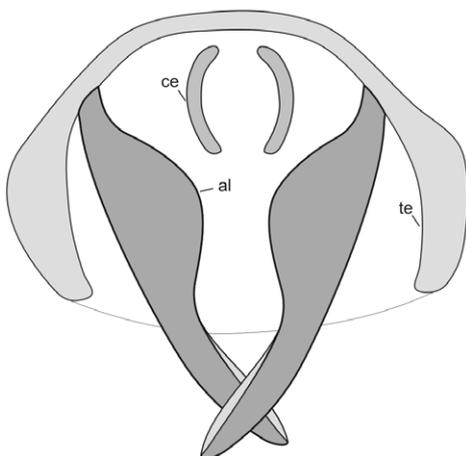


Fig. 3: scheme of female terminalia of *Simulium* (*Chirostilbia*) in ventrodistal view. al: anal lobe; ce: cerci; te: tergite.

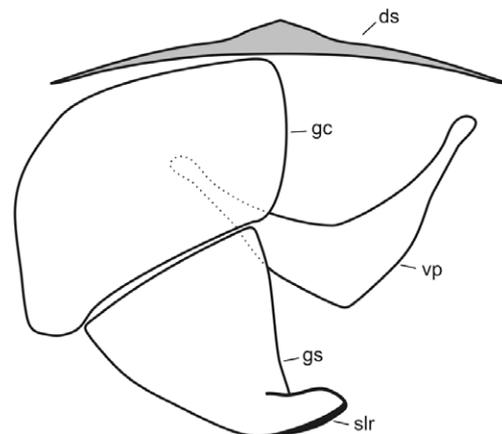


Fig. 4: scheme of male terminalia of *Simulium* (*Chirostilbia*) in ventrodistal view. ds: dorsal sclerite; gc: gonocoxite; gs: gonostyle; slr: sclerotized longitudinal ridge; vp: ventral plate.

*tinctum* + *S. riograndense*) is well defined and presents three synapomorphies: female with the distal apex of the anal lobe pointed, pupal gill with 10 filaments and gill filaments arrangement two-dimensionally and forming an almost complete circle.

Our tree is similar to the tree found by Coscarón and Coscarón-Arias (1997). Both trees recovered the *S. pertinax* and *S. subpallidum* species-groups, but the 1997 paper did not test the *Simulium* (*Chirostilbia*) monophyly because only one outgroup, *Simulium* (*Inaequalium*) Coscarón & Wygodzinsky, 1984, was used. In addition, the 1997 tree indicates two distinct clades in the *S. pertinax* species group: *S. dekeyseri* (as *Simulium laneportoi* Vargas, 1941) + *S. pertinax* + *S. serranum* + *S. spinibranchium*, and *S. distinctum* + *S. empascae* + *S. friedlanderi* + *S. obesum* + *S. riograndense*. However, our tree presents the first group as paraphyletic and the second with more resolution. These differences may result from the inclusion of more characters (45 against 18 for the 1997 tree) and the inclusion of the more recently described taxa, *S. bifenestratum* and *S. jefersoni*.

The representatives of the Neotropical *Simulium* subgenera were used here to test the monophyly of *Simulium* (*Chirostilbia*). However, the trees obtained provide insights about the relationships among these subgenera. Some groups were formed with large numbers of non-ambiguous synapomorphies and were recovered in implied weights analyses. We comment on these clades following.

*Simulium* (*Hearlea*) + *Simulium* (*Hemicnetha*) + *Simulium* (*Trichodagmia*) - *Simulium* (*Hemicnetha*) Enderlein, 1934 and *Simulium* (*Hearlea*) Vargas, Martínez Palacios & Díaz Nájera, 1946, were synonymised by Adler et al. (2004) based on a phylogeny of Nearctic simuliids. Shelley et al. (2010) proposed the synonymy of both with *Simulium* (*Trichodagmia*) Enderlein, 1934, based on Enderlein's papers (1934a, b, 1936) but did not discuss more modern definitions of the subgenera (e.g., Coscarón 1991, Miranda-Esquível 2001). The group was recovered in our phylogeny with five synapomorphies (Fig. 2) and it is likely that these subgenera are closely related. Moreover, some Afrotropical *Simulium* subgenera, such as *Simulium* (*Anasolen*) Enderlein, 1930, *Simulium* (*Freemanellum*) Crosskey, 1969, and *Simulium* (*Xenosimulium*) Crosskey, 1969 are likely also related to this clade (Miranda-Esquível & Coscarón 2001) and an analysis with all *Simulium* subgenera is necessary to test these hypotheses.

*Simulium* (*Inaequalium*) + *Simulium* (*Psaroniocompsa*) - Shelley et al. (2010) proposed the synonymy of *Simulium* (*Psaroniocompsa*) Enderlein, 1934 and *Simulium* (*Inaequalium*) based on their personal opinion concerning the characters of the original diagnosis of *Simulium* (*Inaequalium*) (“... none is considered by us to be of subgeneric value.”). *Simulium* (*Inaequalium*) and *Simulium* (*Psaroniocompsa*) are sister groups, as proposed by Coscarón and Wygodzinsky (1984), and were recovered here with three synapomorphies (Fig. 2). Both subgenera are very distinct and have an easily recognisable morphology. We also disagree that there are “good

or bad” characters for specific hierarchical categories. Therefore, we think that this synonymy is unnecessary.

*Simulium* (*Notolepria*) + [*Simulium* (*Psilopelmia*) + *Simulium* (*Ectemnaspis*) + *Simulium* (*Chirostilbia*)] - The relationship amongst these subgenera has not been proposed before and the group has four synapomorphies (Fig. 2). The clade *Simulium* (*Chirostilbia*) + *Simulium* (*Psilopelmia*) + *Simulium* (*Ectemnaspis*) presents four synapomorphies (Fig. 2) and brings back a forgotten issue in the Neotropical black fly history. Stone (1963) proposed the synonymy of *Chirostilbia* and *Ectemnaspis* based on similarities of female terminalia of the type species; Wygodzinsky (1971) argued that the species were quite different when comparing male and pupa but stressed that the “*Ectemnaspis-Chirostilbia* complex”, as he denominated it, needed further investigation. We agree with that suggestion.

In the literature, there is no consensus regarding the limits of *Simulium* (*Psilopelmia*) and *Simulium* (*Ectemnaspis*) (Coscarón et al. 2008, Adler & Crosskey 2010) and Shelley et al. (2010) proposed the synonymy of both based on that. In our phylogeny, these species groups were recovered as a monophyletic group with four synapomorphies and *Simulium* (*Psilopelmia*) was paraphyletic without *Simulium* (*Ectemnaspis*), corroborating their synonymy. However, it is still necessary to examine the relationship between the group and *Simulium* (*Psilozia*) Enderlein, 1936 (Adler et al. 2004).

Some characters that have been traditionally used in the systematic classification of Neotropical simuliids were tested in the present analysis, including the scutum colouration of adults, the number of branches of pupal trichomes and the number of hypostomal setae of larvae. The phylogenetic signal of these characters was weak, with low consistency and retention indexes. Thus, these characters are not reliable for defining groups and require further investigation.

Many systematic problems in the Neotropical *Simulium* species are still unsolved and the traditional morphology seems to be inconclusive in some respects. A broad approach is important, using chromosomal, molecular biology and morphology data, as has been shown in the studies of Nearctic *Simulium* (Adler et al. 2004). Studies using these techniques are incipient in Neotropical Region and this needs to change. At the same time, the morphological studies in the region have not been fully explored. For example, in the present work, a three-dimensional view of a Neotropical Simuliidae terminalia was described for the first time, a character that has been used for Nearctic fauna since the 1970s. In addition, studies using scanning electron microscopy and/or morphometry in the region are rare.

Whatever the tool used, systematic changes must be grounded in a methodology, whether cladistic or probabilistic. Systematics without a basic methodology and based on key features or personal opinion only lead us into a vicious circle. Such systematics proposals are not based on a clear method and lack hypotheses. Therefore, they cannot be rejected or corroborated by other researchers and need to be avoided.

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Characters and states	Character list			Summary
	L	CI	RI	
	Adult - female			
1 Nudiocular area: (0) present; (1) absent.	2	0.50	0.50	Cited by Coscarón (1987).
2 Mandible teeth: (0) well developed in both sides; (1) developed in the internal side and scarce developed on external side; (2) developed just on the internal side.	4	0.50	0.77	Modified from character 7 of Gil-Azevedo and Maia-Herzog (2007: 43).
3 Palp, ration between the length of the segments IV and V: (0) V about twice or more than the IV; (1) V distinctly shorter than the double of the IV.	7	0.14	0.14	Character 8 of Gil-Azevedo and Maia-Herzog (2007: 43).
4 Cibarium, anteromedial border, colouration: (0) unpigmented or slightly pigmented; (1) distinctly pigmented.	3	0.33	0.84	Cited by Coscarón (1987).
5 Cibarium, anteromedial border, surface: (0) unarmed; (1) distinctly armed <sup>a</sup> .	3	0.33	0.80	Character 203 of Adler et al. (2004: 157).
6 Cibarium, arms, shape: (0) large, about two times longer than wide; (1) short, as long as wide.	1	1.00	1.00	-
7 Thorax, scutum, colouration: (0) dark colour - black to dark brown; (1) light colour - light brown to yellow.	5	0.20	0.50	Modified from character 1 and 3 of Coscarón and Coscarón-Arias (1997: 114) and character 12 of Gil-Azevedo and Maia-Herzog (2007: 43).
8 Thorax, scutum, pattern: (0) absent; (1) adorned with silvery spots and/or stripes.	4	0.25	0.25	Cited by Coscarón (1987).
9 Thorax, scutum, pattern, stripes definition: (0) blurred; (1) well-marked.	2	0.50	0.80	-
10 Thorax, scutum, hairs, distribution: (0) homogeneous; (1) in groups (tuft).	3	0.33	0.33	Character 2 of Coscarón and Coscarón-Arias (1997: 113).
11 Hind leg, basal or sub-basal tooth: (0) present; (1) absent.	4	0.25	0.57	Character 5 of Coscarón and Coscarón-Arias (1997: 114) and 142 of Adler et al. (2004: 152) and 17 of Gil-Azevedo and Maia-Herzog (2007: 44).
12 Wing, vein Sc <sub>2</sub> setae: (0) present; (1) absent.	4	0.25	0.00	Character 15 of Miranda-Esquivel and Coscarón (2001: 430).
13 Wing, basal sector of the vein R, setae: (0) present; (1) absent.	5	0.20	0.60	Character 4 of Coscarón and Coscarón-Arias (1997: 114) and 24 of Gil-Azevedo and Maia-Herzog (2007: 45).
14 Abdomen, general colouration: (0) dark colour - black to dark brown; (1) light colour - light brown to yellow.	2	0.50	0.85	Cited by Coscarón (1987).
15 Anal lobe, shape: (0) hemispheric with curved distal border; (1) sub-square; (2) triangular and base smaller than high.	3	0.66	0.92	Modified from character 6 of Coscarón and Coscarón-Arias (1997: 114) and 18 of Miranda-Esquivel and Coscarón (2001: 430).
16 Triangular anal lobe, distal apex, shape: (0) blunt; (1) pointed.	2	0.50	0.66	Cited by Coscarón (1991).
17 Anal lobe, internal border, shape: (0) straight or convex; (1) concave.	2	0.50	0.92	Cited by Coscarón (1991).
18 Genital fork, shape: (0) stem as long as lateral arms; (1) stem about twice longer or more than lateral arms.	5	0.20	0.55	Character 36 of Gil-Azevedo and Maia-Herzog (2007: 48).
19 Spermatheca, shape: (0) oval, (1) spherical.	3	0.33	0.71	Character 37 of Gil-Azevedo and Maia-Herzog (2007: 48).

Characters and states	L	CI	RI	Summary
Adult - male				
20 Scutum, colouration: (0) dark colour - black to dark brown; (1) light colour - light brown to yellow.	6	0.16	0.00	Modified from character 12 of Gil-Azevedo and Maia-Herzog (2007: 43).
21 Male terminalia, length ratio between gonostyle and gonocoxite: (0) gonostyle less than 2/3 of gonocoxite; (1) gonostyle more than 2/3 of gonocoxite but distinctly smaller than gonocoxite; (2) gonostyle about as long as the gonocoxite to distinctly greater.	8	0.25	0.70	Character 43 of Gil-Azevedo and Maia-Herzog (2007: 49).
22 Gonostylus, length ratio between height and base: (0) similar sizes; (1) the height distinctly longer than base.	5	0.20	0.71	Modified from character 9 of Coscarón and Coscarón-Arias (1997: 114) and character 45 of Gil-Azevedo and Maia-Herzog (2007: 49).
23 Gonostylus, apex, spurs: (0) present; (1) absent or very reduced.	3	0.50	0.92	Character 8 of Coscarón and Coscarón-Arias (1997: 114) and 46 of Gil-Azevedo and Maia-Herzog (2007: 49).
24 Gonostylus, longitudinal ridge: (0) absent; (1) present.	2	0.50	0.92	Cited by Coscarón (1991) and Hernandez et al. (2008).
25 Ventral plate, median keel projected apically: (0) absent; (1) present.	5	0.20	0.60	Cited by Coscarón (1991).
Pupa				
26 Pupal trichomes, branches [number (maximum)]: (0) 1; (1) 2; (2) 3-6; (3) 25-40.	9	0.33	0.53	Cited by Coscarón (1987).
27 Cephalic trichomes, shape: (0) large and thick; (1) small and thin.	1	1.00	1.00	Cited by Coscarón (1991).
28 Gill filaments (number): (0) 2-3; (1) 4; (2) 6; (3) 8; (4) 10; (5) 11; (6) 12; (7) about 20; (8) about 120.	12	0.66	0.50	Modified from character 12 of Coscarón and Coscarón-Arias (1997: 114).
29 Gill filaments, shape: (0) thick; (1) thin.	6	0.16	0.00	Modified from character 12 of Coscarón and Coscarón-Arias (1997: 114).
30 Gill filaments, arrangement, spatial distribution: (0) three-dimensional; (1) two-dimensional.	2	0.50	0.92	Cited by Gil-Azevedo et al. (2005).
31 Gill filaments, two-dimensional branch, arrangement: (0) about 1/4 of a circle; (1) about a semi-circle; (2) almost a whole circle.	2	1.00	1.00	Cited by Gil-Azevedo et al. (2005).
32 Gill, main-branch, base, spines: (0) absent; (1) present.	uninformative			Cited by Gil-Azevedo et al. (2005).
33 Abdominal tergites, rows of short spines: (0) present; (1) absent.	5	0.20	0.20	Character 44 of Adler et al. (2004: 135) and 75 of Gil-Azevedo & Maia-Herzog (2007: 53).
34 Cocoon, shape in lateral view: (0) slipper-shaped (anterior opening next to the substratum); (1) boot-shaped (anterior opening separated from substratum, upward directed) <sup>b</sup> .	2	0.50	0.90	Character 89 of Gil-Azevedo and Maia-Herzog (2007: 54).
35 Cocoon, fabric, structure: (0) homogeneous without distinct threads; (1) with visible fine threads; (2) with visible thick threads.	6	0.33	0.76	Cited by Gil-Azevedo et al. (2005).
36 Cocoon, anterior rim, shape: (0) thickened; (1) not thickened.	5	0.20	0.60	Character 178 of Adler et al. (2004: 154) and 91 of Gil-Azevedo and Maia-Herzog (2007: 55).
37 Cocoon, extension: (0) covering partially the thorax and gill; (1) covering the entire pupa.	2	0.50	0.85	Character 10 of Coscarón and Coscarón-Arias (1997: 114).



Characters and states	L	CI	RI	Summary
				Larva
38 Cephalic apoteme, colouration, pattern: (0) positive, light background; (1) negative, dark background.	4	0.25	0.66	Cited by Crosskey (1990).
39 Antenna, segment 1, length ratio: (0) longer than 2; (1) equal to or smaller than 2.	3	0.33	0.33	Character 98 of Gil-Azevedo and Maia-Herzog (2007: 56).
40 Antenna, segment 3, length ratio: (0) equal to or shorter than 2; (1) longer than 2.	5	0.20	0.50	Modified of character 99 of Gil-Azevedo and Maia-Herzog (2007: 56).
41 Hypostoma, anterior margin shape: (0) straight; (1) concave.	4	0.25	0.50	Character 162 of Adler et al. (2004: 153).
42 Hypostoma, para-lateral teeth: (0) present; (1) absent.	2	0.50	0.50	Character 50 of Adler et al. (2004: 163) and 104 of Gil-Azevedo and Maia-Herzog (2007: 58).
43 Hypostoma, hypostomal setae, number: (0) 7 + 7 or less; (1) 8 + 8; (2) 10 + 10 or more.	6	0.33	0.55	Cited by Coscarón (1991).
44 Postgenal cleft, shape: (0) subtriangular; (1) oval; (2) subsquare.	7	0.28	0.64	Character 109 of Gil-Azevedo and Maia-Herzog (2007: 58).
45 Abdomen, 1 + 1 ventral tubercles: (0) absent; (1) present.	2	0.50	0.85	Character 113 of Gil-Azevedo and Maia-Herzog (2007: 58).

*a*: here we considered all the armed cibarium homologous, but there are some structural and teeth distribution differences that should be better observed in the future. In our analysis, the cibarium distinctly armed appears three times independently: *Simulium (Inaequalium)* + *Simulium (Psaroniocompsa)*, *Simulium (Ectemnaspis)* + *Simulium (Psilopelmia)* and *Simulium (Trichodagmia)*; *b*: *Simulium dekeyseri*, *Simulium pertinax*, *Simulium serranum* and *Simulium spinibranchium* have an intermediate cocoon that sometimes appears as slipper-shaped and other as boot-shaped. In this case we coded those species with both states. *Simulium bifenestratum* that has a bizarre cocoon and was treated as inapplicable for this character; CI: consistency index; L: length; RI: retention index.

Data matrix for the cladistic analyses

	1										2										3										4																	
	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5			
<i>Metacnephia saileri</i>	0	0	1	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	7	1	0	-	0	0	1	0	1	0	0	1	0	0	0	0	-	0				
<i>Hellichella nebulosum</i>	0	0	0	0	0	0	1	0	-	0	0	0	0	0	0	-	0	0	0	0	1	1	0	0	0	0	2	1	0	-	0	0	0	1	0	0	0	1	0	0	0	0	2	1				
<i>Eusimulium aureum</i>	0	0	0	0	0	0	0	0	-	0	0	0	0	0	0	-	0	0	0	0	0	1	0	0	1	0	0	1	1	0	-	0	0	0	1	0	0	0	1	0	0	0	0	2	1			
<i>Aspathia metallicum</i>	0	2	0	1	0	0	0	1	1	0	0	1	1	0	0	-	0	1	0	0	2	1	0	0	1	1	0	2	1	0	-	0	0	0	1	1	0	0	1	0	0	0	0	1	1			
<i>Hemicnetha rubrithorax</i>	0	0	0	1	0	1	1	1	1	0	0	0	1	1	1	-	1	0	0	1	1	1	0	0	1	0	0	3	1	0	-	0	1	1	0	1	1	1	1	0	0	1	2	0	0			
<i>Notolepria exiguum</i>	1	1	1	0	0	0	0	0	-	1	1	1	1	0	1	-	1	1	1	0	0	0	1	0	1	2	0	3	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	1	0			
<i>Inaequalium inaequale</i>	0	2	0	1	1	0	0	1	-	0	0	0	0	0	0	-	0	1	1	0	1	1	0	0	0	2	0	2	1	0	-	0	0	0	1	0	0	0	1	1	0	0	0	0	1			
<i>Psaroniocompsa amazonicum</i>	1	1	1	1	1	0	0	1	1	0	1	0	1	0	0	-	0	0	1	0	0	1	0	0	0	1	0	3	1	0	-	0	0	0	1	0	0	0	1	?	0	0	0	1	1			
<i>Psaroniocompsa auristriatum</i>	0	1	0	1	1	0	0	1	-	1	0	1	1	0	0	-	0	0	1	0	0	0	0	0	0	1	0	0	0	-	-	0	0	0	0	0	0	0	0	1	0	0	0	1	1			
<i>Psaroniocompsa incustratum</i>	0	1	0	1	1	0	0	1	-	1	0	0	1	0	0	-	0	0	1	0	0	1	0	0	0	1	0	2	1	0	-	0	0	0	0	0	0	0	0	0	0	0	0	1	1			
<i>Psaroniocompsa quadrifidum</i>	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	-	0	0	1	0	0	1	0	0	0	1	0	1	1	0	-	0	0	0	1	0	0	0	0	1	0	0	0	2	1			
<i>Psilopelmia dinellii</i>	0	1	0	1	1	0	1	1	-	0	1	0	0	1	2	0	1	1	0	1	2	1	0	0	1	2	0	3	1	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0		
<i>Psilopelmia perflavum</i>	0	1	0	1	1	0	1	1	-	0	1	0	0	1	2	0	1	1	0	1	2	1	0	0	1	1	0	3	1	1	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0
<i>Ectemnaspis bicolouratum</i>	0	1	0	1	1	0	1	1	-	0	0	0	0	1	2	0	1	1	0	0	2	1	0	0	1	3	0	3	1	1	0	0	0	0	1	0	0	0	1	?	0	0	0	0	0	0		
<i>Psilopelmia escomeli</i>	0	1	0	1	1	0	1	1	1	0	1	0	1	1	2	1	1	1	0	0	2	1	0	0	1	0	0	3	1	1	0	0	0	0	1	0	0	0	1	?	0	0	0	1	0			
<i>Pternaspatha nemorale</i>	0	0	0	0	0	0	0	1	1	0	0	0	1	0	1	-	0	1	0	0	0	0	0	0	0	1	0	3	1	0	-	0	0	0	0	0	0	-	1	0	1	0	2	0	0			
<i>Pternaspatha nigristrigatum</i>	0	0	0	0	0	0	0	1	1	0	0	0	1	0	1	-	0	1	0	0	0	0	0	0	0	2	0	3	1	0	-	0	0	0	0	0	0	-	1	0	1	0	0	0	0			
<i>Trichodagmia orbitale</i>	0	2	0	1	1	1	0	0	-	1	0	0	0	0	1	-	0	0	1	0	2	1	0	0	0	1	0	6	0	0	-	0	0	1	0	0	0	-	1	0	0	0	0	1	0			
<i>Trichodagmia nigrimanum</i>	0	2	0	1	1	1	0	1	-	0	0	0	0	0	1	-	0	0	1	0	2	0	0	0	1	2	0	7	1	0	-	0	1	1	0	0	0	1	1	0	0	0	0	1	0			
<i>Hearlea canadense</i>	0	0	0	1	0	1	0	1	1	0	0	0	1	0	1	-	0	1	0	0	1	1	0	0	1	0	0	0	0	-	-	0	1	1	0	0	0	0	1	?	0	0	2	1	0			
<i>Chirostilbia acarayense</i>	0	1	1	0	0	0	0	1	1	0	1	0	0	1	2	0	1	0	0	0	2	1	1	1	0	2	0	3	1	1	0	0	0	0	1	0	0	0	1	1	1	0	0	1	0			
<i>Chirostilbia bifenestratum</i>	0	1	0	0	0	0	0	1	0	0	0	0	0	0	2	1	1	0	0	0	1	0	1	1	0	2	1	4	1	1	2	0	0	-	0	1	1	1	1	0	1	0	1	0	0			
<i>Chirostilbia distinctum</i>	0	1	1	0	0	0	1	1	0	0	0	0	0	0	2	1	1	0	0	1	1	0	1	1	0	2	1	4	0	1	2	0	1	1	2	1	1	1	1	0	0	1	2	0	0			
<i>Chirostilbia empascae</i>	0	1	1	0	0	0	0	1	0	0	0	0	0	0	2	0	1	0	0	0	1	0	1	1	0	0	1	3	1	1	0	1	1	1	0	1	1	1	1	0	0	0	2	0	0			
<i>Chirostilbia friedlanderi</i>	0	1	0	0	0	0	0	1	1	0	0	0	0	0	2	0	1	0	0	0	1	0	1	1	0	2	0	5	0	0	-	0	0	1	2	1	1	0	1	0	0	0	1	0	0			
<i>Chirostilbia jefersoni</i>	0	1	0	0	0	0	0	1	1	0	0	0	0	0	2	0	1	0	0	0	2	1	1	1	0	2	0	3	1	1	0	0	0	1	2	1	1	0	0	1	0	0	1	0	0			
<i>Chirostilbia dekeyseri</i>	0	1	0	0	0	0	0	1	1	0	0	0	0	0	2	0	1	0	0	0	0	0	0	1	1	0	2	0	3	1	1	0	0	0	<b>a</b>	0	0	0	1	1	0	0	0	0	0	0		
<i>Chirostilbia obesum</i>	0	1	1	0	0	0	0	1	0	0	0	0	0	0	2	0	1	0	0	0	1	0	1	1	0	2	0	8	1	1	0	0	0	1	2	1	1	0	1	0	0	0	2	0	0			
<i>Chirostilbia papaveroi</i>	0	1	0	0	0	0	1	1	1	0	1	0	0	1	2	0	1	0	0	0	2	1	1	1	0	2	0	3	1	1	0	0	0	0	1	0	0	0	1	1	1	0	0	1	0			
<i>Chirostilbia pertinax</i>	0	1	0	0	0	0	0	1	0	0	0	0	0	0	2	0	1	0	0	0	1	0	1	1	0	2	0	3	1	1	0	0	0	<b>a</b>	0	0	0	1	1	0	0	0	1	0	0			
<i>Chirostilbia riograndense</i>	0	1	0	0	0	0	0	1	0	0	0	0	0	0	2	1	1	0	0	0	1	0	1	1	0	2	1	4	1	1	1	0	0	1	2	1	1	1	1	0	0	1	1	0	0			
<i>Chirostilbia serranum</i>	0	1	0	0	0	0	0	1	1	0	0	0	0	0	2	0	1	0	0	0	0	0	0	1	1	0	2	0	3	1	1	0	0	0	<b>a</b>	0	0	0	1	1	1	1	0	0	0	0		
<i>Chirostilbia spinibranchium</i>	0	1	0	0	0	0	0	1	1	0	0	0	0	0	2	0	1	0	0	1	0	0	1	0	0	1	1	0	2	0	3	0	1	0	0	1	<b>a</b>	0	0	0	1	1	1	0	0	0	0	
<i>Chirostilbia subpallidum</i>	0	1	1	0	0	0	1	1	1	0	1	0	0	1	2	0	1	0	0	1	2	1	1	1	0	2	0	3	1	1	0	0	0	0	1	0	0	0	1	1	1	0	0	1	0			

A: 0&amp;1; -: inapplicable data; ?: unavailable.