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New synonymy to *Simulium (Inaequalium) diversibranchium* Lutz, with comments on the Inaequale species-group (Diptera, Simuliidae)



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ABSTRACT

Simulium (Inaequalium) Coscarón and Wygodzinsky, 1984 is a homogeneous subgenus widely spread on the Neotropical Region, which the adults can only be reliably identified from linked-reared specimens. Despite many revisionary works only a single character of the pupal gill separates *Simulium diversibranchium* Lutz, 1910 from *S. mariavulcanoae* Coscarón and Wygodzinsky, 1984. Specimens of both species show that *S. mariavulcanoae* is a junior synonym of *S. diversibranchium*. The relationship between *S. diversibranchium* and *S. subnigrum* Lutz, 1910 needs further investigation. Some features of the pupal gill often used as diagnosis in *Simulium (Inaequalium)* species, such as the direction of gill filaments or the height of the bifurcation of the secondary or tertiary branches of the pupal gill are highly polymorphic, making its use as diagnostic character not reliable.

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Introduction

Simulium (Inaequalium) Coscarón and Wygodzinsky, 1984 is a subgenus widely distributed across the Neotropical Region. Shelley et al. (2010) proposed the synonymy of *Simulium (Psaroniocompsa)* Enderlein, 1934 and *S. (Inaequalium)* based on a subjective criterion, which was the lack of “subgeneric value” in the characters used in the diagnosis of *S. (Inaequalium)*. Indeed *S. (Inaequalium)* and *S. (Psaroniocompsa)* are sister groups, they share similarities, as the adult female cibarium shape; larval antenna shape; and presence of 1 + 1 ventral tubercles on the larval abdomen (Gil-Azevedo et al., 2012). But both subgenera are probably monophyletic and have easily recognizable diagnostic characters, as adult wing with setae on basal sector of radial vein; pupal trichomes with 3–6 branches; and larval postgenal cleft subtriangular in *S. (Inaequalium)*. On the other hand, *S. (Psaroniocompsa)* has wing without setae on basal sector of radial vein; pupal trichomes only with two branches; and larval postgenal cleft oval (Gil-Azevedo et al., 2012). We do not accept this synonymy of the subgenera based on currently available data.

The *S. (Inaequalium)* species are morphologically homogeneous and are divided in two species-groups, Botulibranchium and Inaequale (Coscarón and Wygodzinsky, 1984), which are broadly accepted (Coscarón et al., 2008; Shelley et al., 2010; and Adler and

Crosskey, 2016). However, this classic division was challenged in the 2000s with the description of four species which do not fit easily into any of both species-groups: *S. maranguapense* (Pessoa et al., 2005); *S. margaritatum* Pepinelli et al., 2006; *S. lundi* (Py-Daniel and Barbosa, 2007); and *S. marins* Pepinelli et al., 2009 (see the original descriptions for more details). Py-Daniel and Barbosa (2007) proposed another species-group, Maranguapense, to accommodate the first three species (from now on referred to as the ‘2000s species’), which was criticized by Adler and Crosskey (2011). Anyway, we believe that this issue should be better and deeper investigated in the future.

Adults of the Inaequale species-group (sensu Adler and Crosskey, 2016) are almost identical. However, the pupal gill is a quite variable structure, and all the species of the group have diagnoses based primarily on it. The adults can only be reliably identified from linked-reared specimens (Coscarón and Coscarón-Arias, 2007; Shelley et al., 2010), with prominent exceptions such as the ‘2000s species’ (Pessoa et al., 2005; Pepinelli et al., 2006; Py-Daniel and Barbosa, 2007). This problematic combination makes most species of the Inaequale species-group identified from only a few characters of gill filaments. This has resulted in 21 named species, of which only ten are currently valid (Adler and Crosskey, 2016; Coscarón et al., 2008).

Of those ten valid species of the Inaequalium species-group, the ‘2000s species’ are the exception, as they are easily distinguishable, and have restricted distribution, to very isolated high grassland areas of Northeastern and Northern Regions of Brazil. The other seven species have a wider geographical distribution and

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are frequently found in Atlantic Forest streams, and form a much more morphologically homogenous group (Adler and Crosskey, 2016). From those species, only *Simulium travassosi* Vulcano and d'Andretta, 1947 lacks six gill filaments in their pupa, instead they bear three thick filaments. In this group, the immatures of most species prefer forested low order streams, whereas *S. inaequale* (Paterson and Shannon, 1927) prefer streams in open and deforested areas, and *S. rappae* Py-Daniel & Coscarón, 1982 seems to be generalist. These last two species also have a more distinguishable pupa: *S. inaequale* – all gill branches bifurcate near the base and cocoon bear small anterodorsal projection; *S. rappae* – despite its known gill variation, they always have thick gill filaments (Hernández-Triana et al., 2007b).

Therefore, four species of Inaequale species-group with indistinct adults and similar pupae (with six paired gill filaments) occur in streams running inside forested areas: *S. clavibranchium* Lutz, 1910, *S. subnigrum* Lutz, 1910, *S. diversibranchium* Lutz, 1910, and *S. mariavulcanoae* Coscarón and Wygodzinsky, 1984. The immatures of the two first species are common and abundant, and can be found on most of the creeks of the Atlantic Forest, whereas the other two species are quite rare.

Coscarón and Wygodzinsky (1984) considered *S. diversibranchium* and *S. mariavulcanoae* close species that could be differentiated from each other, and from the remaining species of the subgenus by the configuration of the gill branches. Those authors also pointed out mild differences between the male and female terminalia of both species, and also among the remaining Inaequalium species-group species. But, most of the works posterior Coscarón and Wygodzinsky (1984) considered the adults of Inaequalium species-group morphologically indistinguishable (e.g. Coscarón and Coscarón-Arias, 2007; Hernández-Triana et al., 2007b).

In this work we propose that *S. mariavulcanoae* is a junior synonym of *S. diversibranchium*. We also highlight that the relationship between other species of Inaequalium species-group needs further investigation because some features of the pupal gill, often used as diagnosis of its species, are not reliable.

Material and methods

In order to obtain images, we used focus stacking, using CombineZP (Hadley, 2013), with photographs taken using a microscope. We followed the morphological nomenclature of Gil-Azevedo et al. (2005). The collections mentioned in this paper and their respective acronyms are: Museo de La Plata, La Plata, Argentina (MLP); Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil (MZSP); Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro (CSIOC); Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro (MNRJ); Instituto Nacional de Pesquisas da Amazônia, Manaus (INPA). Geographical distribution information was based on the labels of the analyzed material and records obtained from the literature (Coscarón, 1991; Coscarón et al., 2008; Gil-Azevedo and Maia-Herzog, 2010; Strieder, 2004).

Examined material

Simulium diversibranchium Lutz, 1910: LECTOTYPE (designated by Hernández-Triana et al., 2007b): BRAZIL, SP, São José do Barreiro, Serra da Bocaina, Fazenda do Bonito: 1 pupa (slide) (CSIOC #12,097 Lutz collection). PARALECTOTYPES: same data of Lectotype: 4 pupae (CSIOC # 12,095 and 12,098 Lutz collection).

ARGENTINA, Misiones, Campiña: 3 pupae (slide) (MLP 21/VIII/1972, S. Coscarón col.). BRAZIL, SP, Boracéia: 1 pupa (spirit) (MLP 31/X/1979, S. Coscarón col.). Campos do Jordão: 4 pupae (spirit) (CSIOC #3,774; and 3,780). Casagrande: 1 pupa

(spirit) (MLP 25/VII/1979, S. Coscarón col.). SP?: 2 pupae (spirit) (CSIOC #3,737).

Simulium mariavulcanoae Coscarón and Wygodzinsky, 1984: HOLOTYPE: BRAZIL, SP, Salesópolis, Boracéia: 1 female, with pupal exuvia (slide) (MZSP #115-212). PARATYPES: BRAZIL, SP, Salesópolis, Boracéia: 1 male, with pupal exuvia (slide) (MZSP #115-47, “allotype”); 3 pupae (slides) (MLP #115-263 and #115-232).

BRAZIL, MG, Bocaina de Minas: 1 pupa (spirit) (MNRJ #60,020). SC, São Bento: 1 male, with pupal exuvia (pinned) (CSIOC #4,581). SP, Campos do Jordão: 2 females, with pupal exuviae (pinned) (CSIOC #4,446; and 4,448). Serra da Bocaina: 3 adults, with pupal exuviae (pinned) (CSIOC #3,986; 4,404; and 4,469). SP?: 1 female, with pupal exuvia (pinned) (CSIOC #4,132).

Simulium subnigrum Lutz, 1910: ARGENTINA, Misiones, Pto. Leoni: 1 pupa (slide) (MLP 07/VII/2002, S. Coscarón col.). BRAZIL, MG, Bocaina de Minas: 3 females, with pupal exuvia+1 male, with pupal exuvia+3 pupae (spirit) (MNRJ #60,002; #60,006; #60,007; #60,010; #60,012; and #60,024). Pedralva: 5 pupae (CSIOC #438; 440; and 442). RJ, Teresópolis: 1 female, with pupal exuvia+8 pupae (spirit) (MNRJ #60,027; 60,029; and 60,032). RS, Vale dos Sinos, Arroio Cascatinha: 1 pupa (spirit) (MLP 22/X/2000, S. Coscarón col.). Brazil?: 1 male with pupal exuvia (pinned) (CSIOC #5,702); 1 female with pupal exuvia (pinned) (CSIOC #5,727).

Taxonomy

Simulium (Inaequalium) diversibranchium Lutz, 1910 (Figs. 1–5; 10–12)

Simulium missionum Coscarón, 1976

Simulium mariavulcanoae Coscarón & Wygodzinsky, 1984 **syn. n.**

Taxonomic remarks. Despite many revisionary works about *Simulium (Inaequalium)* (Coscarón, 1991; Coscarón and Coscarón-Arias, 2007; Coscarón and Wygodzinsky, 1984; Hernández-Triana et al., 2007b; Shelley et al., 2010; Strieder and Py-Daniel, 2000, 2002), still only one character of pupal gill shape separates *S. diversibranchium* from *S. mariavulcanoae*: “secondary branches of ventral primary branch downwards and posteriorly directed”; in contrast with the other species in which those branches are anteriorly directed. But those authors agree that both species are close, and could be grouped by sharing a single character of the pupal gill shape: “dorsal primary branch thicker than other primary branches”. On the other hand, *S. mariavulcanoae* lacks unique exclusive characters, making the species meaningless.

This single character currently separating *S. diversibranchium* and *S. mariavulcanoae* is not consistent and could be caused by environmental influences in the pupal development. There is, however, not many specimens for examination, the species being rare. But we found an interesting pupa from Pedralva (MG), which could be identified either as *S. diversibranchium*, or as *S. mariavulcanoae* depending if the left (Fig. 3), or the right (Fig. 4) side is examined. This is evidence that the direction of gill filaments is not a reliable character.

Lutz (1910) described *S. diversibranchium* based on pupae syntypes. In a review of *Simulium (Inaequalium)*, Hernández-Triana et al. (2007b) chose one of the pupae syntypes and raised it to lectotype status of the species. We examined this, and the gill does not have the secondary branches of ventral primary branch downward and posteriorly directed, but all branches are anteriorly directed (Fig. 1). The lectotype of *S. diversibranchium* does not match the description of this species in the literature, but with the description and the types of *S. mariavulcanoae* (Coscarón and Wygodzinsky, 1984) (Fig. 2). So, both names refer to the same



Fig. 1. Pupal gill branches of *Simulium (Inaequalium) diversibranchium* (= *S. mariavulcanoae*), in slides (1–5; 10–12): (1) CSIOC #12,097 Lutz collection (Lectotype); (2) MLP #115,232 (paratype of *S. mariavulcanoae*); (3) CSIOC #3,109 left gill; (4) CSIOC #3,109 right gill; (5) MNRJ #60,020; (10) CSIOC #3,780; (11) CSIOC #3,986; (12) CSIOC #4,448. Pupal gill branches of *Simulium (Inaequalium) subnigrum*, in slides (6–9): (6) MNRJ #60,002; (7) MNRJ #60,007; (8) MNRJ #60,010; (9) MNRJ #60,012.

entity, and we propose that *S. mariavulcanoae* is a junior synonym of *S. diversibranchium*.

Diagnosis (based only on pupa) (modified from Coscarón and Coscarón-Arias, 2007: 286). Frontoclypeus and thorax with abundant tubercles; trichomes of frontoclypeus with 2–6 branches, thoracic trichomes with 5–8 branches. Gill with 6 filaments in three primary branches: dorsal primary branch distinctly thicker than medial and ventral primary branch; the most ventral secondary branch (from ventral primary branch) could be downward and posteriorly directed, or directed as the other secondary branches; gill length 2.8–3.5 mm. Cocoon with thin weave, with few evident threads, reinforced edge and slowly projected ventrally; cocoon length on base 3.2–4.0 mm, on dorsum 2.6–3.5 mm.

Distribution. Argentina, *Misiones*: Campiña. Brazil, *RS*: Caxias do Sul; Bom Jesus. *SC*: São Bento do Sul; Joinville. *SP*: Selesópolis, Estação Biológica de Boraceia; Itu; Capivari; Bragança Paulista; Campos do Jordão; São José do Barreiro, Serra da Bocaina, Fazenda do Bonito; Ocaçu (as Casagrande). *RJ*: Guapimirim; Petrópolis; Itatiaia. *MG*: Bocaina de Minas.

Discussion

Some authors suggested that the level of the bifurcation of the secondary or tertiary branches of the pupae could be a good diagnostic character for neotropical *Simulium* species (e.g. Coscarón and Coscarón-Arias, 2007; Hernández-Triana et al., 2007a). However, this character is questionable, because it always shows many intermediary states. Especially in *S. (Inaequalium)* species, in which that problem is well documented, e.g.: *S. clavibranchium* “Variation in the branching of the dorsal primary branch and the enlargement of the pupal gill filaments (...) have been seen” (Hernández-Triana

et al., 2007a: p.18); *S. rappaee*: “Gill configuration very variable with filaments branching at different heights even within same specimen.” (Hernández-Triana et al., 2007b: p.43; see also Pepinelli et al., 2009: p.735); *S. subnigrum*: “gill branching at different distances from base (...) with relative variability of distances in different specimens (Coscarón and Coscarón-Arias, 2007: p. 278; see also Hernández-Triana et al., 2007b: p. 47). And many species have been synonymized based on the weakness of that feature, e.g.: *S. subclavibranchium* Lutz, 1910; *S. nogueirai* d’Andretta and Dolores González, 1964; *S. beaupertuyi* Ramírez-Pérez, Rassi, Ramírez, 1977; and *S. leopoldense* (Strieder and Py-Daniel, 2000), all considered junior synonym of *S. subnigrum* (Hernández-Triana et al., 2007a: p. 18–20; see also Shelley et al., 2010). Observation of the available material shows a great variation in the height of the bifurcation of the secondary or tertiary branches of the pupal gill (Figs. 1–12), bringing new evidence that gill filaments in *S. (Inaequalium)* species is a polymorphic character and its use as a diagnostic character for this subgenus is debatable.

Only a single character separates *S. diversibranchium* sensu this paper and *S. subnigrum*. The former species has a “pupal gill with dorsal primary branch thicker than other primary branches”; in contrast with *S. subnigrum* in which all primary branches are similar (Coscarón, 1991; Coscarón and Coscarón-Arias, 2007; Coscarón and Wygodzinsky, 1984; Strieder and Py-Daniel, 2000, 2002). We examined material of both species, and found that some specimens are difficult to determine if the dorsal primary branch diameter is thicker or similar to the others. Once again, there are intermediary states, as we can see in the examined material, especially from Bocaina de Minas (Figs. 5–9).

Whereas *S. subnigrum* has a wide distribution from northeastern Argentina to Trinidad (Adler and Crosskey, 2016), and is abundant

in forested areas in all its geographical range, *S. diversibranchium* has punctual distribution and is quite rare. In fact, it draws our attention that there are very few specimens of *S. diversibranchium* in collections, even in the three most representative Neotropical simuliid collections (MLP, CSIOC, and INPA).

The very low frequency of *S. diversibranchium*, always found in areas where *S. subnigrum* is abundant, allied that the two species can only be differentiated by one pupal gill character, suggests that *S. diversibranchium* is a variation of *S. subnigrum*. Maybe those are aberrant variations on the gill that appears in some populations of *S. subnigrum*, caused by environmental variables and/or mutations. Or *S. diversibranchium* is just a very rare species. Morphometric and molecular studies, based on large number of specimens should be necessary to answer it. Additionally, larvae could be reared under different velocity conditions to see what affect that had on the gill.

Conflicts of interest

The authors declare no conflicts of interest.

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