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Taxonomy and polytene chromosomes of the Neotropical black fly *Simulium perplexum* (Diptera: Simuliidae)

Nayra Gomes da Silva[a,1], Neusa Hamada[a,*], Peter H. Adler[b]

[a] Instituto Nacional de Pesquisas da Amazônia (INPA), Coordenação de Biodiversidade (CoBio), Divisão de Curso de Entomologia (DiEnt), Avenida André Araújo 2936, CEP 69067-375 Manaus, AM, Brazil

[b] Department of Plant and Environmental Sciences, Clemson University, Clemson, SC, 29634, USA

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ABSTRACT

Simulium perplexum Shelley, Maia-Herzog, Luna Dias & Couch is structurally similar in the pupal stage to *Simulium guianense* Wise, the main vector in the onchocerciasis foci of Amazonian Brazil and Venezuela. We report *S. perplexum* for the first time beyond its type locality (Guyana, Potaro River), describe its larva, redescribe its pupa, and provide a chromosomal comparison with *S. guianense* and other morphologically similar species. We collected it in two rivers in Rurópolis municipality, Pará state, Brazil. The larvae can be distinguished from those of related species by having body cuticle with microscopic, translucent, and lanceolate setae. Chromosomal comparisons of *S. perplexum* and similar Brazilian species with available chromosome information (*S. guianense*, *S. hirtipupa* Lutz, and *S. litobranchium* Hamada, Pepinelli, Mattos-Glória & Luz), using *S. guianense* Cytoform A as the standard, show that *S. perplexum* has the nucleolar organizer uniquely in the middle of the short arm of chromosome I, whereas the other three species have this marker at the base of the long arm of chromosome I. All chromosome arms, except IIS and IIIS, of *S. perplexum* are rearranged, compared with *S. guianense* Cytoform A, suggesting that it is not closely related to this species or to *S. litobranchium*, as suggested by some authors, based on morphological features.

1. Introduction

Classification of the Simuliidae at the subgeneric level remains controversial for the Neotropical Region (*e.g.* Coscarón and Coscarón-Arias, 2007; Shelley et al., 2010; Adler and Crosskey, 2016), in part, owing to a high degree of morphological uniformity and lack of comprehensive phylogenetic analyses. Nontraditional taxonomic tools, such as chromosomal and molecular analyses, integrated with morpho-taxonomy, can be useful in resolving some of these issues (Rivera and Currie, 2009; Hamada et al., 2010). Polytene chromosomes, for example, have provided a means for discovering cryptic species (*e.g.*, Hamada and Adler, 1999) and improving the identification and classification of black flies (*e.g.*, Ríos-Velásquez et al., 2002; Adler et al., 2004; Alvan-Aguilar et al., 2005; Tangkawanit et al., 2009).

Simulium perplexum Shelley et al. (1989) was described from a series of specimens, originally identified as *Simulium guianense* Wise, 1911, which had been collected in Guyana and deposited in the Natural History Museum in London, England (Shelley et al., 1989). Smart (1940) collected these specimens in the Potaro River, Guyana, but identified them as *S. guianense* (Shelley et al., 1989), probably due to the similarity in the pupal stage. *Simulium guianense* is the main vector of the causal agent of onchocerciasis in the Amazonian focus of the disease in Brazil and Venezuela (Charalambous et al., 1996; Shelley et al., 1997; Grillet et al., 2005). The morphological characterization of all life stages of *S. perplexum* is, therefore, essential for correct identification of the vector of the disease agent and to provide information about the relationships with the closest species. The pupae of both species are similar, having 12 pointed gill filaments, but the adults are distinguishable by their scutal patterns and genitalia (Shelley et al., 1989).

We provide the first description of the larva of *S. perplexum*, redescribe the pupa, compare the polytene chromosomes with those of structurally similar species, and record the species for the first time beyond its type locality in Guyana, including its first occurrence in Brazil.

2. Materials and methods

Simulium perplexum was collected in two rivers in the Amazon forest region of Brazil: the Tambor River, Cachoeira do Grim (04°07′20.7″S;

* Corresponding author.
E-mail addresses: nhamada@inpa.gov.br (N. Hamada), padler@clemson.edu (P.H. Adler).
[1] The first author passed away in August 2014.

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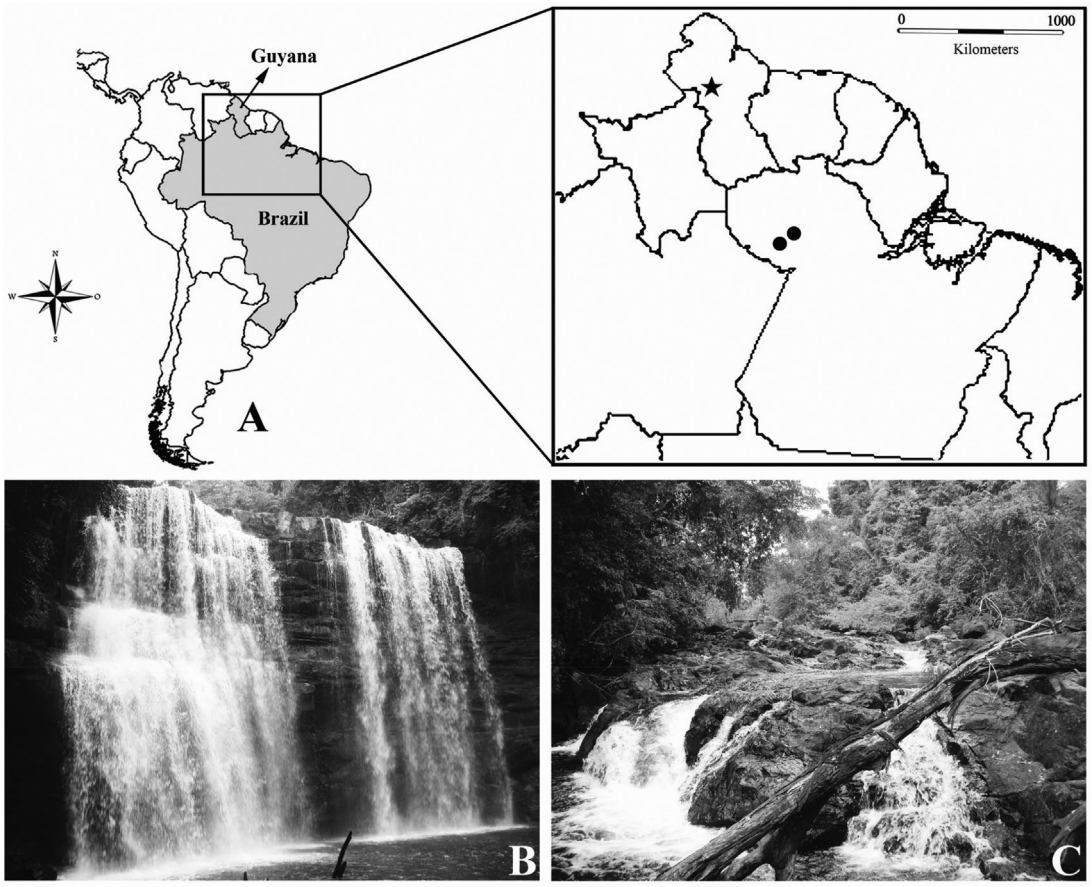

Fig. 1. A: Map of South America with detailed area indicating the Kayeteur falls, Potaro River (star), type locality of *Simulium perplexum* (Diptera: Simuliidae), in Guyana and location of two rivers (black circles) where this species is first recorded in Brazil. B: Tambor River, Cachoeira do Grim. C: Cupari River tributary, Cachoeira do 100 (*S. perplexum* habitat in Rurópolis municipality, Pará state, Brazil).

54°57′27.2″W) and a tributary of Cupari River, Cachoeira do 100 (04°03′40.7″S; 54°56′38.4″W) in Rurópolis municipality, Pará state (Fig. 1). Voucher specimens are deposited in the Invertebrate Collection of the Instituto Nacional de Pesquisas da Amazônia (INPA).

Pupae and some last-instar larvae were fixed in absolute ethanol; remaining larvae were fixed in Carnoy's solution (3 parts absolute ethanol: 1 part glacial acetic acid) for chromosomal analysis. Specimens were maintained at −20 °C in the laboratory until analysis. Pupae were reared individually to adults in 2-mL plastic vials with damp filter paper.

Adults were fixed in 80% ethanol 24 h after emergence (Hamada et al., 2010); in the laboratory, they were dehydrated using the Sabrosky (1966) technique and glued at the tip of a pinned triangle.

Polytene chromosomes of last-instar larvae were prepared and analyzed following the technique of Charalambous et al. (1996). Chromosomal nomenclature follows that of Rothfels (1988). *Simulium guianense* is the only structurally similar species (in the pupal stage) with published chromosome maps (Charalambous et al., 1996). Consequently, we chose *S. guianense* cytoform A of Charalambous et al. (1996) as the sequence to compare the polytene chromosomes of *S. perplexum*. Unpublished chromosome maps of *S. hirtipupa* Lutz, 1910 and *S. litobranchium* Hamada et al. (2010) in the Laboratório de Citotaxonomia e Insetos Aquáticos at INPA were also compared with the banding sequence of *S. perplexum*.

Larval carcasses from chromosomal analysis, and pupae fixed in ethanol, were clarified with hot 85% lactic acid, dissected, and slide mounted in Euparal® for morphological study (Hamada et al., 2010). The larval and pupal descriptions follow the terminology of Adler et al. (2004). Measurements are given as minimum-maximum or mean ± standard deviation, unless otherwise indicated. Adult females and males were identified by examining their genitalia and scutal patterns and by comparing them with the original description (Shelley et al., 1989).

Morphological features were photographed using a Leica M165C stereomicroscope with an attached Leica DFC 72 camera – the Leica Automontage program was used to combine the photographs – and an Olympus BX51 optical microscope with the Cell-D (Olympus) program. Polytene chromosomes were observed and photographed, using immersion oil, with an Olympus digital camera and the Cell-D computer program.

2.1. Material examined

BRAZIL, Pará state, Rurópolis municipality, Tambor River, Cachoeira do Grim (04°05′35.6″S 55°00′27.2″W), 23.X.2007, N. Hamada & L.M. Fusari, 30 last-instar larvae dissected on slides (3 larvae on each slide), 6 pupae dissected on slides, 10 larvae and 10 pupae in absolute ethanol, 2 males and 2 females (pinned); tributary of Cupari