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A systematic study on *Endotribelos* Grodhaus (Diptera: Chironomidae) from Brazil including DNA barcoding to link males and females

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Abstract

Six new species of *Endotribelos* from Brazil are described and illustrated as male, female, pupa and larva: *E. bicolor* **sp. n.**, *E. fulvidus* **sp. n.**, *E. jaragua* **sp. n.**, *E. jiboia* **sp. n.**, *E. semibruneus* **sp. n.** and *E. sublettei* **sp. n.** The female of *E. calophylli* Roque & Trivinho-Strixino and the larvae of four unknown morphotypes are also described. Keys including males and larvae of all known species of *Endotribelos* are provided. Adults' males and females from five species were linked using DNA Barcoding mtCOI sequences.

Key words: *Endotribelos*, chironomid, key, DNA barcoding, Neotropical

Introduction

Until 2013, the genus *Endotribelos* Grodhaus was composed by seven species (Grodhaus 1987; Sublette & Sasa 1994; Spies & Reiss 1996; Roque *et al*. 2005; Roque & Trivinho-Strixino 2008) occurring from the southernmost part of the Nearctic Region (south USA) to southeastern of Brazil, mostly in the Neotropical region. Surprisingly, in 2013 it was recorded in China, [see the description of *Endotribelos redimiculum* Qi, Shi, Lin and Wang (Qi *et al*. 2013)], which brought new perspectives on the geographical distribution of the genus, now increased far to the oriental region. All, except the Chinese species, have its immature stages known.

Several captures made in the last years in different localities from Brazil, supported by rearing specimens, provided evidence of six more new species and other four larval morphotypes. The DNA barcode region of the cytochrome c oxidase I (COI) mitochondrial gene has been extensively used in Chironomidae studies (e.g. Brodin *et al*. 2013; Ekrem *et al*. 2007; Ekrem *et al*. 2010; Trivinho-Strixino *et al*. 2012; Laurindo *et al*. 2012) and here it was used to associate males and females.

In the present study, six new species are described as male, female and immature stages. The larvae of four unknown morphotypes are also described. Keys including males and larvae of all known species of *Endotribelos* are provided.

Material and methods

The larvae were collected in streams using a hand net, transported and reared in the laboratory to obtain the associated pupal exuviae and adults. Larvae associated to aquatic macrophyte were also isolated in vials for the same purpose. Moreover, litter bags containing leaf and fruit detritus were installed on the bed of streams for colonization by larvae. These bags were transferred to lab and the mining larvae reared individually for emergence of adults. The emerged adults were fixed with ethanol 96% together with their larval and pupal exuviae.

Larvae, pupae and adult specimens were mounted in Euparal medium after being cleared in 10% potassium hydroxide solution, for morphological analyses. Adults male and female slides were mounted with associated larval and pupal exuviae if available. Some specimens, mainly the ones whose immature were not associated with

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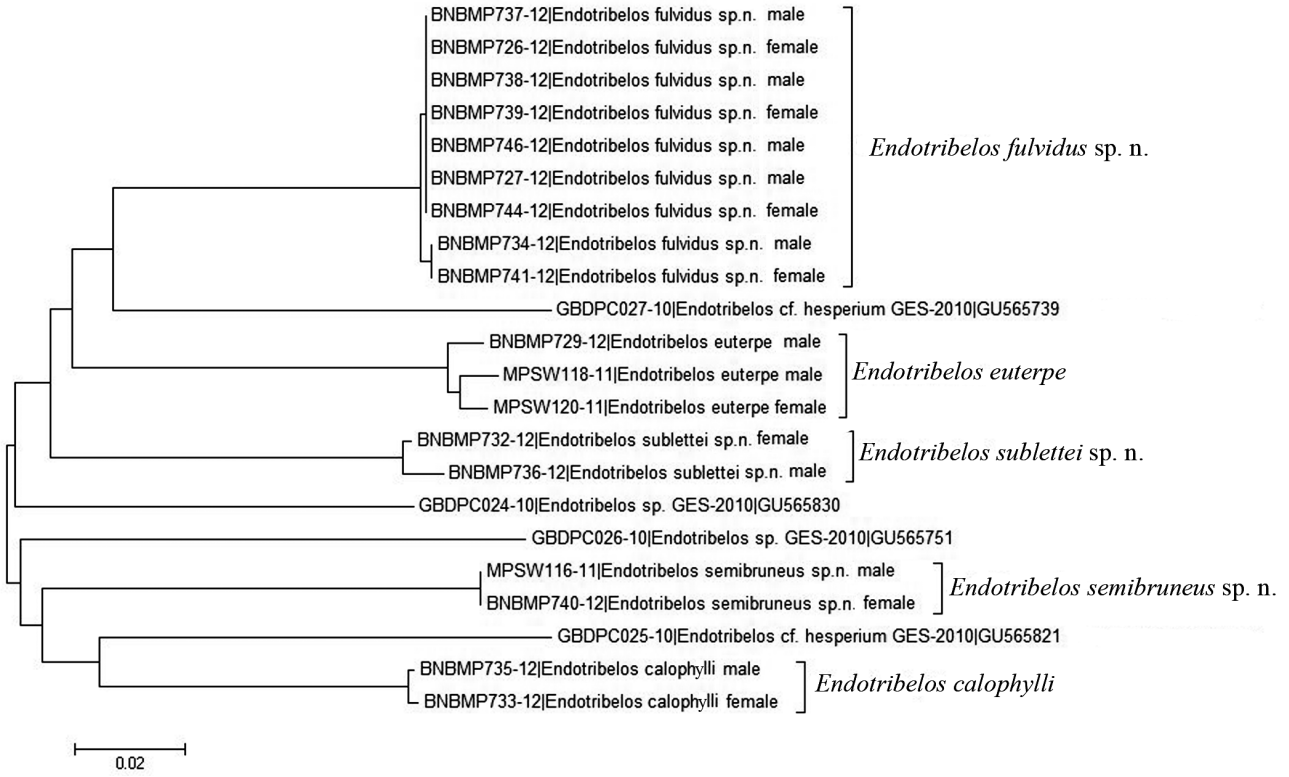

FIGURE 22. A Kimura two-parameter neighbor-joining tree of *Endotribelos* species.

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