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Taxonomy and Systematics

New species of *Psychoda* Latreille (Diptera: Psychodidae) from the Brazilian Amazon with sexual association using molecular data

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Abstract. *Psychoda* Latreille is one of the largest genera of Psychodinae, with around 450 species, from which more than 130 are known from only one of the sexes. This results from the high diversity, sympatry of closely related species and scarcity of diagnostic characters to accurately associate males and females in this genus. Here we describe a new species, *Psychoda dactyla* **sp. n.**, from Brazil, Amazonas, São Gabriel da Cachoeira with morphological description of male and female specimens associated by DNA-barcoding.

Keywords: Amazonia; COI; drainfly; integrative taxonomy; mothflies.

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Psychoda Latreille, the type genus of family Psychodidae, has gone through several changes concerning its delimitation. The broad delimitation of Quate (1959; 1996) encompasses around 450 species around the globe (Salmela *et al*. 2012), while a more restrictive delimitation (Ježek 1984; 2007; Ježek & van Harten 1996; 2005) splits the genus into 13 genera. Bravo *et al*. (2006) and Cordeiro *et al*. (2011) discussed the complex variation of characters in the Neotropical species, which conflicts with the diagnosis of the more restrictive proposal for the delimitation of genera in this group. They suggested that the broad *Psychoda sensu lato* is still useful to allow the description of species in this megadiverse and neglected genus, especially when dealing with tropical fauna.

Beyond the diversity and morphological complexity of these insects, the issue of associating males and females worsens the taxonomic problems of this genus. This difficulty is long known (Quate 1962), and the main causes are the sympatry of several related species in a single locality and the scarcity of characters to accurately associate males and females and separate them from closely related species. As a result, more than 130 species are known from only one of the sexes, also contributing to the inertia of *Psychoda* taxonomy.

A recent review of studies on the molecular taxonomy of phlebotomine sand flies demonstrates how molecular data can be a powerful tool for distinguishing nominal species and male-female association in psychodids (Rodrigues & Galati 2023). In Psychodinae, Kvifte & Andersen (2012) were the first to use the DNA-barcoding approach (Hebert *et al*. 2003) for species identification and sexual association. In the Neotropics, the first DNA-barcodes of Psychodinae are from Jaume-Schinkel & Kvifte (2022) and Jaume-Schinkel (2023), but there is still no published molecular data from Neotropical *Psychoda*. Here we describe a new species from the Brazilian Amazon based on male and female specimens associated with molecular data.

MATERIAL AND METHODS

The specimens were stored at 99% ethanol. For the molecular analysis, the thorax and legs of the specimens were used for DNA extraction. Head, wings and abdomen were slide mounted in Canadian Balsam, after clarification with 10% KOH. For description, the morphological terminology follows Cumming & Wood (2017). Palpal formula was calculated with the length of first palpal segment as the reference (equivalent to 1.0) and the lengths of the following segments calculated in relation to the first segment. The specimens are deposited at the invertebrate collection of the Instituto Nacional de Pesquisas da Amazônia (INPA).

DNA extraction was performed using AutoGenprep 965 (Autogen, Holliston, MA, USA) with the standard protocol. The amplification reactions (PCR) were performed with the primers LCO1490 and HCO2198 (Folmer *et al*. 1994), with the following parameters: one cycle at 94 °C for 3 minutes, 35 cycles at 94 °C for 30 seconds, 48 °C for 30 seconds and 72 °C for 40 seconds, and finally one cycle at 72 °C for 5 minutes. The PCR products were purified

with ExoSAP-IT® (USB Corporation, Cleveland, Ohio, USA), sequence reactions were performed with Big Dye terminator Kit® (PE Applied BioSystems, Warrington, England) and read at sequencer ABI 3730 (PE Applied BioSystems). The sequences were checked and aligned at Software Sequencher® (version 5.1, Gene Codes Corporation, Ann Arbor, MI USA, https://www.genecodes.com), using Clustal (Chenna *et al.* 2003), with default parameters. At Software MacClade 4.08 the sequences were checked for codon position and stop codons. Sequences were compared with the database of NCBI (National Center of Biotechnology Information) using BLASTn (Standard Nucleotide Basic Local Alignment Search Tool). The genetic divergence was calculated using the K2P model (Kimura 1980) at Software MEGA4 (Tamura *et al.* 2007). The sequences are publicly available in GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

RESULTS AND DISCUSSION

Taxonomy

Psychoda dactyla **sp. n.**

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(Figs. 1A-H)

Diagnosis: antenna with 14 flagellomeres, 12-14th reduced, 14th smaller than the others; palpus short, 1º segment of palpus slightly longer than the others; male hypandrium with an inverted V shape; male gonostylus with median finger-like projection; female hypogynium with pilosity not restricted to the lobes, with a wide bridge connecting the apical lobes to the base of the hypogynium.

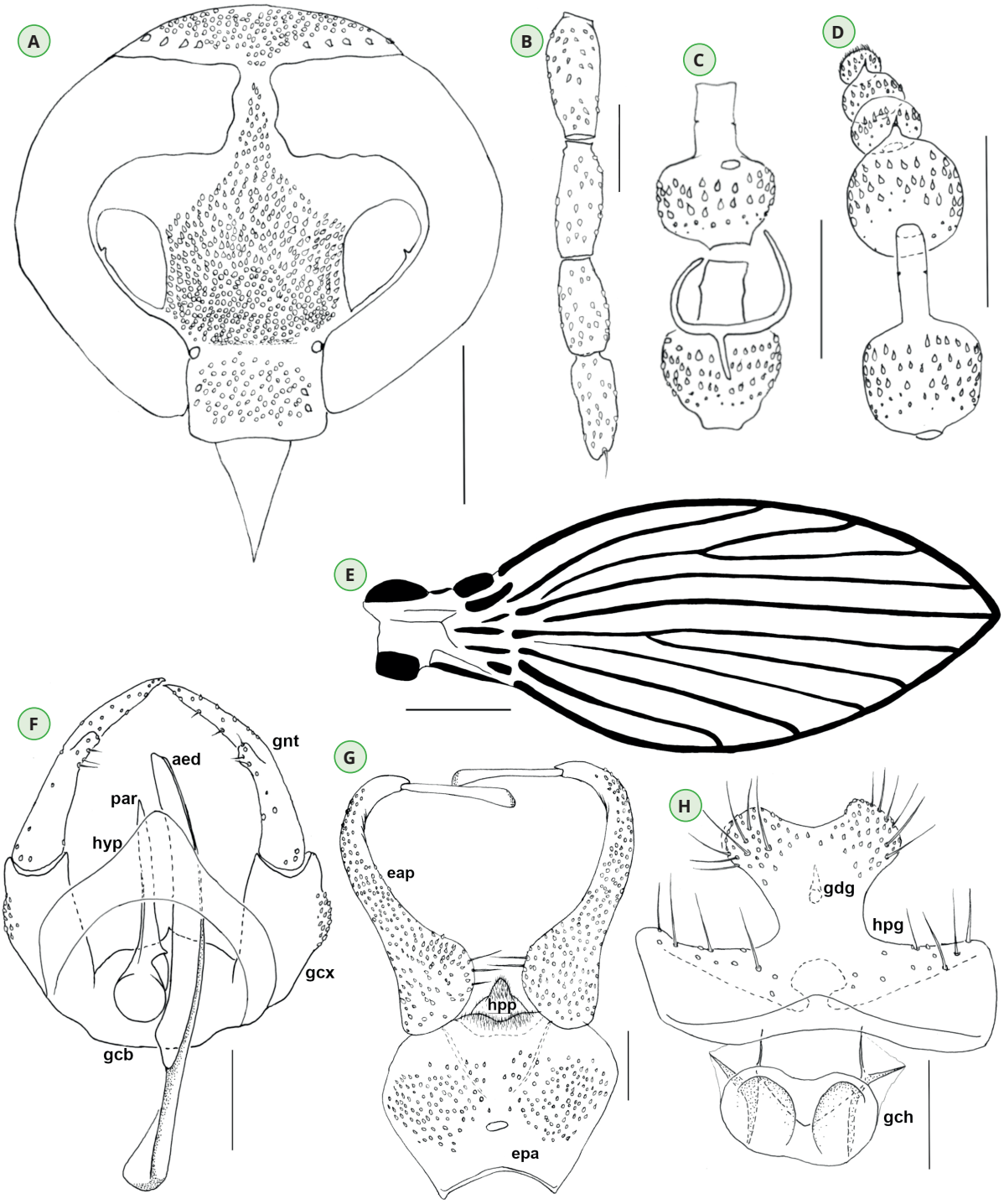

Figure 1A-H. *Psychoda dactyla* **sp. n.** A. head, frontal view. B. Palpus. C. Basal flagellomeres showing one ascoid. D. Flagellomeres, 10-14th. E. Wing. F. Male Terminalia: hypandrium, gonopods aedeagus and paramere, dorsal view. G. Male Terminalia: epadrium, epandrial appendage and hypoproct, ventral view. H. Female hypogynium and genital chamber. Abbreviations: aed-aedeagus; eap-epandrial appendage; epa-epadrium; gcb-gonocoxal bridge; gch-genital chamber; gcx-gonocoxite; gdg-genital digit; gnt-gonostyle; hpg-hypogynium; hpp-hypoproct; hyp-hypandrium; par-paramere. Scale bars: 0.2 mm (E), 0.1 mm (A), 0.05 mm (B-D, F-H).

Description: Head (Figure 1A). Vertex, frons and clypeus pilose; frons hair patch extending posteriorly between eyebridge, reaching first row of facets, eyebridge with four facets; eyes separated by 1.3 (male) and 1.7 (female) times the diameter of a facet; vertex hair patch separated from occipital hair patch; 5-6 supraocular setae, 3 occipital strong alveoli on the posterior margin of eye; interocular suture absent; clypeus with two conspicuous lateral alveoli; frontoclypeal suture absent; antenna with scape cylindrical, 1.4 times the length of the subspherical pedicel, and with 14 flagellomeres, 12-14th reduced, subspherical and fused, 14th smaller (Figure 1D), apico-lateral spine present on 11th and 13th flagellomeres; ascoids Y shaped (Figure 1C); palpal formula 1.0:0.9:0.7:0.8 (Figure 1B); labellum with 3 apical digitiform setae ('teeth') and one lateral setae. Thorax: pre-sutural setae continuous with supra-alares setae; anepisternal setae patch undivided; few anepimeral setae present; pteropleurite well developed, subquadrate; anepisternal suture complete but weak at apex; transversal suture on upper margin of katespisternum present. Wing (Figure 1E): Second costal node present; Sc vein short, ending at the line of base of veins Rs, M and CuA; radial fork apical to medial fork, both complete; M_{1+2} slightly expanded at base; costal cell infuscated. Legs: first tarsomere short, around 2 times longer than the second; distitarsus with short apical projection. Male terminalia: epandrial appendage long, sinuous, with globose base and a single tenaculum at the apex (Figure 1G); epandrium with one foramen (Figure 1G); posterior margin of hypoproct pointed (Figure 1G); hypandrium projected posteriorly, resembling a boomerang in shape, with an inverted V-shaped posterior margin and a concave anterior margin (Figure 1F); gonostylus around 1.4 times the length of gonocoxite, with a median finger-like projection and short sparse setae (Figure 1F); gonocoxal bridge (fused gonocoxal apodemes) bare, not projecting posteriorly; aedeagus long, around 2.2 times the length of aedeagal apodeme; paramere slender and straight in dorsal view, with a globose base and a pointed apex. Female terminalia: hypogynium bilobed, with lobes connected to the base of the hypogynium by a wide area, setae on lobes and on posterior margin of basal area (Figure 1H); genital digit present; genital chamber wider than long, without posterior apodeme; cercus long and pointed, around 1.3 times the width of female terminalia at the base.

Examined Material: holotype male Brazil, AM, São Gabriel da Cachoeira, Tigre, 20.vii.2010, M47, CRIO (INPA) (Genbank OR289961). Paratype female, same data (INPA) (Genbank OR289960).

Etymology: the name 'dactyla', from the ancient greek dáktulos, meaning finger, is an allusion to the finger-like projection of the gonostylus.

Genetics: Two specimens (one male and one female) were sequenced and the uncorrected pairwise distance between them was 0.008 (6bp) (Genbank access numbers: OR289960 and OR289961). The results of BLASTn search did not recover any sequence with a percent identity of 93% or higher.

Comments: The combination of a wide inverted-V shape (boomerang like) hypandrium, a straight and pointed paramere that runs parallel to the aedeagus and the gonostylus with a small median finger-like projection makes this species unique among *Psychoda*. The configuration of apical flagellomeres and the internal ornamentation of the female's hypogynium of *Psychoda dactyla* sp. n. resembles that of *Psychoda velita* Ibáñez-Bernal, 1993, a species known from Central Mexico, but the general shapes of the hypogynium of the two species are different, with the basal half of the hypogynium much wider in the new species. The shape of the parameres in the males of the two species is also very different, being strongly curved in *P. velita* and straight in the new species.

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