University of São Paulo
“Luiz de Queiroz” College of Agriculture

Integrative taxonomy to clarify the identity of *Anastrepha dissimilis* Stone, 1942 (Diptera: Tephritidae), with an Illustrated key to the species of *Anastrepha pseudoparallela* group recorded on passion fruit (*Passiflora* sp.) in Brazil

Alexandre Santos Araújo

Dissertation presented to obtain the degree of Master in Science. Area: Entomology

Piracicaba
2022

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Teaching degree in Biological Sciences

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versão revisada de acordo com a resolução CoPGr 6018 de 2011

Advisor:
Prof. Dr. **MARCOANDRE SAVARIS**

Dissertation presented to obtain the degree of Master in Science. Area: Entomology

Piracicaba
2022

Dados Internacionais de Catalogação na Publicação
DIVISÃO DE BIBLIOTECA – DIBD/ESALQ/USP

Araújo, Alexandre Santos

Integrative taxonomy to clarify the identity of *Anastrepha dissimilis* Stone, 1942 (Diptera: Tephritidae), with an Illustrated key to the species of *Anastrepha pseudoparallela* group recorded on passion fruit (*Passiflora* sp.) in Brazil / Alexandre Santos Araújo. - - versão revisada de acordo com a resolução CoPGr 6018 de 2011. - -.Piracicaba, 2022.

83 p.

Dissertação (Mestrado) - - USP / Escola Superior de Agricultura “Luiz de Queiroz”.

1. Moscas-das-frutas 2. Maracujá 3. Taxonomia 4. Trypetinae I. Título

DEDICATION

WITH LOVE,

I DEDICATE TO MY DEAR GRANDMOTHERS EUFROZINA AND AQUILÉIA (in memoriam)

ACKNOWLEDGMENTS

First, I would like to thank and greet my ancestors. Laroyê Esú! Ogunhê, baba mi Ogun! Atoto, baba mi Obaluae! Ora Yê Yê Iyá mi Osun. Laryoê Tranca-Rua! Laroyê Exu Caveira! Laroyê Dona Sete Encruzilhadas!

To my advisor, Dr. Marcoandre Savaris, for all the support and teachings during this incredible journey! You're such a great professor and person.

To Dr. Roberto Antonio Zucchi for helping me every time I asked him. Thank you very much!

To Dr. Sinval Silveira Neto for all the funny conversations full of knowledge. Thank you!

To Prof. Dr. Rafael Castilho (ESALQ/USP) for allowing me to use his laboratory to take photographs of the aculeus.

To Laboratório de Ecologia Química e Comportamento de Insetos (ESALQ/USP) for allowing me to use their stereomicroscope to photograph the specimens.

To Prof. Dr. Alberto Soares Corrêa and MSc. Frederico Nanini (Laboratório de Ecologia Molecular de Artrópodes – ESALQ/USP) for the support in the molecular analysis.

To Dr. Miguel Souza Filho (Instituto Biológico de São Paulo), Dra. Clarice Diniz Alvarenga (Universidade Estadual de Montes Claros) and Manoel Enéas de Carvalho Gonçalves (Agência de Defesa Agropecuária do Estado do Ceará) for sendind samples of *Anastrepha dissimilis.*

To my ex-supervisor and friend Dra. Vanessa Dias. You're such an inspiration for me. I'm grateful for everything you did for me! Thank you!

To Dra. Iara Bravo, for all the support during my career! You're such a great inspiration of professional and human for me.

To all the Professors of the Department of Entomology and Acarology. You were and are necessary for my career.

To all my coleagues from the “Laboratório de Taxonomia de Insetos – LTI” for the friendship and suport.

To my parents, Cirilo and Madalena. Your stories and love are my sources of strongness and always make me believe that I can do everything I want. It is all for both of you!

To the best Brothers and Sisters I could have. Alexsandra, Daiane, Danilo, Denilson, and Marcilio. Thank you very much for all the support and lovely words. Without all of you I couldn’t do it.

To my soul family, Ana Sueli, Desirée, Hannah, Carla, and Luis! I love you very much! Thanks for all the words, support, and friendship. Without you, I couldn’t do it.

To my loved friend Julia! Your friendship makes everything easier.

To my friends Ina and Fábio! Your friendship is essential for me! Love you! Thanks for everything!

To my friends Tais Amorim and Tainá Amorim! Love you so much! Thank you for everything.

To my incredible boyfriend, Pedro! Your company, support, and love are such important to me! Love you, baby!

To all my friends from the “Laboratório de Ecologia Comportamental de Insetos” (UFBA), Artur, Jennifer, Lorena, and Kelly! Thank you very much for everything.

To my friend Paloma Dela Giustina for all the funny moments and great conversations!

To the Departamento de Entomologia e Acarologia (ESALQ/USP) for the support during my master!
To the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior for grating the scolarship!

Ẹni tó nbẹ Òrìṣà kì í bẹ èyàn!
Whoever asks Orisa, does not need to ask a human.
(Yoruba Proverbs)

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RESUMO

Taxonomia integrativa para esclarecer a identidade de *Anastrepha dissimilis* Stone, 1942 (Diptera: Tephritidae) e chave ilustrada para as espécies brasileiras do grupo *Anastrepha pseudoparallela* registradas em maracujá (*Passiflora* sp.) no Brasil

O gênero *Anastrepha* Schiner, de moscas-das-frutas, compreende um grupo de dípteros fitófagos e algumas espécies são pragas da fruticultura, já que utilizam frutos de importância econômica como recurso nutricional para o estágio larval. Taxonomicamente, o gênero está dividido em vários grupos de espécies, de acordo com evidências morfológicas e moleculares. O grupo *pseudoparallela* compreende um total de 31 espécies descritas, sendo 11 registradas no Brasil. Geralmente, as espécies desse grupo são conhecidas por infestarem frutos de várias espécies de maracujazeiro (*Passiflora* spp.). No Brasil, o conhecimento taxonômico do grupo *pseudoparallela* ainda é limitado e não existia, até então, uma chave taxonômica para a identificação das espécies registradas em maracujá. Além disso, o estudo das espécies desse grupo é restrito àquelas que atacam frutos de espécies comerciais de *Passiflora*, como *Anastrepha consobrina* e *A. pseudoparallela*. Entre as espécies pouco conhecidas do grupo, está *Anastrepha dissimilis* Stone, 1942, que possui ampla distribuição em território brasileiro, ocorrendo em 20 estados. Comparações morfológicas entre um parátipo de *A. dissimilis* coletado em Bonito, Pernambuco, Brasil com o holótipo, coletado em Plaisance, Haiti, sugere que os espécimes brasileiros e o holótipo não se tratam da mesma espécie. Portanto, o objetivo deste trabalho foi realizar um estudo taxonômico de populações brasileiras de *A. dissimilis* sob uma abordagem integrativa e elaborar uma chave ilustrada para as espécies brasileiras do grupo *pseudoparallela* registradas em maracujá. Os objetivos específicos deste estudo foram: (1) comparar a morfologia do acúleo das fêmeas de diferentes populações brasileiras de *A. dissimilis*; (2) realizar um estudo morfométrico das asas, acúleo e mesonoto de populações brasileiras de *A. dissimilis*, utilizando a morfometria linear e geométrica; (3) investigar a variabilidade molecular entre populações brasileiras de *A. dissimilis*, utilizando a subunidade I do gene mitocondrial Citocromo Oxidase (COI); (4) elaborar uma chave ilustrada para a identificação das espécies do grupo *pseudoparallela* registradas em maracujá no Brasil e (5) incrementar e melhorar a curadoria da coleção do Museu de Entomologia Luiz de Queiroz (MELQ) da Escola Superior de Agricultura Luiz de Queiroz (ESALQ/USP) com os espécimes estudados. As análises morfológicas e moleculares dos espécimes de *A. dissimilis* oriundas de diversas localidades do Brasil sugeriram que, na verdade, se tratam de *A. chiclayae*. Além disso, o estudo morfométrico foi capaz de evidenciar uma considerável variação morfológica entre as diferentes populações, mostrando a existência de, pelo menos, duas morfoespécies. Desta forma, com a abordagem taxonômica integrativa, é registrada pela primeira vez para o Brasil, a espécie *A. chiclayae* Greene. Além disso, uma lista comentada das espécies do grupo *A. pseudoparallela*, registradas em maracujá no Brasil, foi realizada com base nos dados da literatura. Por fim, uma chave taxonômica ilustrada para a identificação dessas espécies foi elaborada.

Palavras-chave: Moscas-das-frutas, Maracujá, Taxonomia, Trypetinae

ABSTRACT

Integrative taxonomy to clarify the identity of *Anastrepha dissimilis* stone, 1942, with an illustrated key to the species of *Anastrepha pseudoparallela* group recorded on passion fruit (*Passiflora* sp.) in Brazil

The fruit flies of the genus *Anastrepha* Schiner comprise a group of phytophagous Diptera. Some species are pests of fruticulture, as they use economically significant fruits as a nutritional resource for the larval stage. Taxonomically, the genus is divided into multiple groups of species, according to morphological and molecular evidence. The *pseudoparallela* group comprises 31 described species, 11 of which are registered in Brazil. Species of the *pseudoparallela* group mainly infest passion fruits (*Passiflora* spp.). However, in Brazil, the taxonomic knowledge of these species is still limited. So far, there is no taxonomic key for identifying the species of this group recorded in passion fruit in Brazil. Furthermore, most study of species in this group has focused on species that attack fruits of commercial species of *Passiflora*, such as *Anastrepha consobrina* and *A. pseudoparallela*. Among the poorly known species of the *pseudoparallela* group, is *Anastrepha dissimilis* Stone, 1942, which has a wide distribution in Brazil, occurring in 20 states. Morphological comparisons of the *A. dissimilis* paratype from Bonito, Pernambuco, Brazil with the holotype collected in Plaisance, Haiti, suggest that more than one species is confused under a single nominal species. Therefore, this research aims to carry out a taxonomic study of Brazilian populations of *A. dissimilis* using an integrative approach and to produce an illustrated key to the Brazilian species of the *pseudoparallela* group recorded in passion fruit. The specific objectives of this thesis plan are: (1) to compare the morphology of the aculeus of *A. dissimilis* from multiple localities; (2) to carry out a morphometric study of the wing, aculeus, and mesonotum of Brazilian populations of *A. dissimilis*, using linear and geometric analysis; (3) to perform a molecular study of Brazilian populations of *A. dissimilis*, using the subunit I of the mitochondrial gene Cytochrome Oxidase (COI); (4) to develop a illustrated key to the species of the *pseudoparallela* group recorded in passion fruit in Brazil; and (5) to enhance the collection of the Museu de Entomologia Luiz de Queiroz (MELQ) of the Escola Superior de Agricultura Luiz de Queiroz (ESALQ/USP) with the specimens collected and indentified in the study. Morphological and molecular analyses of specimens from different localities in Brazil, that would be indentifed as *A. dissimilis* using the current identification key, suggested that they are *A. chiclayae* Greene. Furthermore, the morphometric study showed considerable morphological variation among several populations, suggesting the existence of at least two morphospecies. Thus, in this study, the integrative taxonomic approach allowed us to register, for the first time, the occurrence of *A. chiclayae* in Brazil. In addition, a checklist of species of the *A. pseudoparallela* group registered in passion fruit in Brazil was prepared using data from the literature. Finally, an illustrated taxonomic key for identifying these species was developed.

Keywords: Fruit flies, Passion fruit, Taxonomy, Trypetinae

1. GENERAL INTRODUCTION

The family Tephritidae comprises 5026 valid species included in about 500 genera (Norrbom et al., 1999a; Norrbom, 2010; Savaris et al., 2016; Brown et al., 2018; Borkent et al., 2018; Norrbom, pers. comm.), with occurrence known for the whole world, except on desertic and polar areas where their hosts are absent (Foote et al., 1993). In the Neotropics, this family is represented by 985 species, classified in 71 genera (Norrbom, 2010; Borkent et al., 2018; Norrbom, pers. comm.), of which 291 species are recorded in Brazil (Norrbom et al., 1999a; Uchoa, 2021; Norrbom, pers. comm.).

Phylogenetic analyses based on sequences of mitochondrial gene supported Tephritidae as a monophyletic group (Han & McPheron, 1997; Han & Ro, 2009). The morphological study of Korneyev (1999) also concluded that Tephritidae is a natural group. The latter author suggests the costal vein interrupted before the apex of the subcostal vein and the presence of two or three large and rigid costal seta near the interruption of the costal vein as synapomorphies of this family.

Many studies have supported the monophyly of the clade including *Anastrepha* Schiner and *Toxotrypana* Gerstacker. For example, Hanckock (1986), based on immunological and morphological characters, grouped these two genera in the subfamily Toxotrypaninae and tribe Anastrephini. After that, *Anastrepha* and *Toxotrypana* were placed in the tribe Toxotrypanini having *Hexachaeta* Loew as its sister group (Han & McPheron, 1997; Korneyev, 1999; Norrbom et al., 1999b).

Norrbom et al. (1999b) proposed the following synapomorphies for this tribe: eversible membrane of the oviscape basally widened with a set of dorsal teeth, presence of two lateral lobes on the base of the oviscape, glans of the male terminalia with weak sclerotization and T-shape apical sclerite, surstylus small without anterior and posterior lobes, the medial vein of the wings with a strong apical curvature, secondary connection of the subepandrial sclerite, presence of three pairs of sensilla on the aculeus tip, and dorsocentral bristle localated on the posterior region of the scutum. Recently, phylogenetic analysis using molecular data did not support *Hexachaeta* as part of Toxotrypanini and showed that *Anastrepha* is a monophyletic genus only if it includes *Toxotrypana*. Based on that, *Toxotrypana* was considered a junior synonym of *Anastrepha* (Mengual et al., 2017; Norrbom et al., 2018).

The genus *Anastrepha* is endemic to the Neotropics and is widely distributed in Central and South America (Malavasi & Zucchi, 2000; Hernandez-Órtiz, 2007; Norrbom, 2010; Norrbom et al., 2021). This group of flies is phytophagous, and the larvae of the species feed on the internal tissues of fruits, seeds, and pointers of plants. After concluding development, the larva leaves the host to pupariate in the ground (Aluja, 1994). Diagnosis of the genus is mainly based on morphological characters of the genitalia as noted above as well as wing characters, such as the curvature in the apex of the medial vein and a typical wing pattern icluding the presence of yellow to brown C, S, and V bands (Norrbom et al., 1999; Norrbom et al., 2012).

This genus currently comprises 328 species (Norrbom et al., 2021). In Brazil, 128 species of *Anastrepha* are known, and some of them are important pests of fruit crops because their larva develops inside and damage economically significant fruits (Aluja, 1994; Malavasi & Zucchi, 2000; Zucchi & Moraes, 2021).

Within *Anastrepha*, there are 25 species groups classified based on morphological, molecular, and ecological evidence: *benjamini*, *caudata*, *cryptostrepha*, *curvicauda*, *daciformis*, *dentata*, *doryphoros*, *fraterculus*, *grandis*, *hastata*, *leptozona*, *morvasi*, *mucronata*, *nigra*, *panamensis*, *pseudoparallela*, *punctata*, *ramose*, *raveni*, *robusta*, *schausi*, *serpentina*, *spatulata*, *striata*, and *tripunctata* (Norrbom and Kim, 1988; Norrbom et al., 1999b; 2012; 2018; Mengual et al., 2017; Troya et al., 2020).

The *pseudoparallela* group comprises 31 species based on the triangular shape and usually finely serrate aculeus tip. In addition, these species mainly develop inside of passion fruit (Passifloraceae) (Norrbom and Kim, 1988; Norrbom et al., 1999b; Norrbom et al., 2012; Tigrero and Norrbom, 2020; Norrbom et al., 2021; Rodriguez & Norrbom, 2021). In Brazil, this group is represented by 11 species: *Anastrepha amnis* Stone, *Anastrepha consobrina* (Loew), *Anastrepha dissimilis* Stone, *Anastrepha ethalea* (Walker), *Anastrepha glochin* Uramoto & Zucchi, *Anastrepha limae* Stone, *Anastrepha lutzi* Lima, *Anastrepha martinsi* Uramoto & Zucchi, *Anastrepha nigripalpis* Hendel, *Anastrepha pseudoparallela* (Loew), and *Anastrepha xanthochaeta* Hendel (Zucchi & Moraes, 2021).

Some species of the *Anastrepha pseudoparallela* group in Brazil include major pests of cultivated passion fruits, such as *A. consobrina* and *A. pseudoparallela* (Malavasi & Zucchi, 2000). However, the identification and taxonomy of some species of this group are poorly known, like *A. dissimilis*. This species was described based on a specimen from Plaisance (Haiti), but a paratype from Bonito, Pernambuco (Brazil) presents some morphological differences (Stone, 1942; Norrbom et al., 2012), which suggest that the specimens from Brazil identified as *A. dissimilis* might be another species.

This study was separated into two chapters, considering the following objectives of the thesis:

• Investigate the taxonomy of *A. dissimilis* from different localities of Brazil, using data from the morphology of the aculeus tip, linear morphometrics of the aculeus and mesonotum, geometric morphometrics of the wing, and sequences of the mitochondrial DNA Cytochrome Oxidase I (Chapter 1).

• Provide a checklist of the species of the *A. pseudoparallela* group breed in fruit of *Passiflora* in Brazil, with notes on the geographic distribution of each species and an illustrated key for their identification (Chapter 2).

The first chapter was written following the guidelines of the Zoological Journal of Linnean Society, while the second chapter followed the guidelines of Zootaxa.

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2. INTEGRATIVE TAXONOMY TO CLARIFY THE IDENTITY OF BRAZILIAN POPULATIONS PREVIOUSLY MISIDENTIFIED AS *Anastrepha dissimilis* STONE, 1942 (sensu lato) (DIPTERA: TEPHRITIDAE)

Abstract

In Brazil, fruit flies of the genus *Anastrepha* include important pests of fruticulture. Population previously misidentified as *Anastrepha dissimilis* are widely distributed in the Brazilian territory, occurring in 20 of 27 states. However, some morphological differences between the holotype (from Plaisance, Haiti) and one paratype (collected in Pernambuco, Brazil) suggest that the Brazilian specimens are not be *A. dissimilis*. Therefore, considering the importance of integrative taxonomy for species delimitation, we used geometric and linear morphometrics and the Cytochrome Oxidase I integrated with the morphology of aculeus tip to perform a taxonomic study of *Anastrepha dissimilis* (sensu lato) from several localities of Brazil. Morphological data show a uniform pattern among the Brazilian populations with some variation among specimens from the south and northeast. In addition, the geometric and linear morphometrics suggest considerable geographic variation among these populations, showing the existence of at least two morphospecies. The molecular identification suggested that these specimens are *Anastrepha chiclayae* Greene with a genetic distance ranging from 0.00 % to 1.30%. However, our dataset did not recover the phylogenetic relationship among the populations. Therefore, our data suggest that the specimens from Brazil indentified as *A. dissimilis* are *A. chiclayae*. We report for the first time the occurrence of this species for Brazil, clarifying the identity of this species in Brazil.

Keywords: Taxonomy; Trypetinae; Fruit flies; *Anastrepha pseudoparallela* group

2.1. Introduction

The genus *Anastrepha* Schiner, 1868 (Tephritidae) are widely distributed in Central and South America (Malavasi & Zucchi, 2000; Hernandez-Órtiz, 2007; Norrbom, 2010). This genus can be distinguished from other tephritids based on genitalic characters, but most species can be recogniezed by the combination of the following characters: apex of the medial vein of the wing usually strongly curved and wing pattern comprising yellow to brown C, S, and an V bands (Norrbom et al., 1999b; Norrbom et al., 2012). *Anastrepha* includes multiples important fruits pests, whose larva feed on internal tissues of economically important fruits (Aluja, 1994; Schutze, 2017). Currently, there are 328 species (Norrbom et al., 2021), of which 128 occur in Brazil (Zucchi and Moraes, 2021).

The *Anastrepha pseudoparallela* group comprises 31 species, most of which are specialized to develops in fruits of the family Passifloraceae (Norrbom and Kim, 1988; Norrbom et al., 1999b; Norrbom et al., 2012; Tigrero and Norrbom, 2020; Norrbom et al., 2021; Rodriguez & Norrbom, 2021). In Brazil, 11 species of this group were reported, and eight of them are recorded developing in fruits of Passifloraceae (Lima, 1934; Stone, 1942; Zucchi, 1978; Malavasi & Zucchi, 1980; Norrbom et al., 1997; Aguiar-Menezes et al., 2004; Uramoto et al., 2004; Leal, 2008; Sá et al., 2008; Garcia & Norrbom, 2011; Figueiredo et al., 2013; Marsaro Junior, 2014; Dutra et al., 2018; Almeida et al., 2019; Marinho et al., 2021; Zucchi & Moraes, 2021).

Anastrepha dissimilis Stone is recorded from Haiti, Colombia, Peru, Guyana, Trinidad, Brazil, and Argentina (Norrbom et al., 1999a). In Brazil, *A. dissimilis* has been considered widely distributed and present in all Brazilian geographic regions (Zucchi & Moraes, 2021). It has been associated with several host plants, such as *Passiflora edulis* Sims (Garcia & Norrbom, 2011), *Passiflora caerulea* L., *Passiflora elegans* L. (Marsaro Junior, 2014), *Ziziphus joazeiro* Mart (Sá et al., 2008) and *Psidium guajava* L. (Zucchi & Moraes, 2021).

Anastrepha dissimilis was described based on specimens from Plaisance, Haiti (holotype), Tumatumari, Guyana (British Guiana), and Bonito, Pernambuco, Brazil (paratypes) (Stone 1942). However, Stone (1942), in his revision of *Anastrepha*, depicted the aculeus tip of one paratype, which differs slightly from the aculeus tip of the holotype (Norrbom et al., 2012). In the aculeus tip of the paratype illustrated by Stone (1942), the serration extends about 0.77 of the aculeus tip closer to the cloacal opening (Fig. 2Q). However, in the holotype (dissected by Dr. Allen Norrbom), the serrated part is further from the cloacal opening, extending about 0.68 of the aculeus tip, and the non-serrated part shows a slight constriction (Fig. 2P; Norrbom et al., 2012).

Later, Zucchi (1978) studied the Brazilian species of *Anastrepha*, including specimens determined as *A. dissimilis*. In that study, the illustration of the aculeus tip of this species from Brazil is similar to that of the paratype from Bonito, Pernambuco.

Considering that the revisions by Stone (1942) and Zucchi (1978) were extensively used for the identification of *Anastrepha* species from Brazil for several decades, records of *A. dissimilis* were mainly based on the illustration of the aculeus tip of the paratype, which, as mentioned, differs slightly from the photograph of the aculeus tip of the holotype (Norrbom et al., 2012). Furthermore, Stone (1942) noted that the three specimens he determined as *A. dissimilis* showed considerable variation in the length of the oviscape and aculeus. However, he considered the aculeus tip and dorsobasal denticles of the eversible membrane were the same in all three specimens, with the aculeus of the holotype intermediate in length. Therefore, this morphological evidence (Stone, 1942; Zucchi, 1978; Norrbom et al., 2012) suggests that the Brazilian specimens may not be *A. dissimilis*.

Because of the importance of accurate taxonomic identification of pest insects, such as species of *Anastrepha*, to the development and establishment of pest control tactics, it is essential to provide strong species delimitation hypotheses for these groups (Rosen, 1986; Hendrichs et al., 2015; Schutze et al., 2017).

The taxonomy of Tephritidae has been quite challenging due to the difficulty in finding consistent morphological characters to guide taxonomic decisions (Schutze et al., 2017). At this point, many efforts have been made to provide more robust species delimitation hypotheses by integrating data from different areas (Dayrat, 2005; Queiroz, 2007; Schlik-Steiner et al., 2010).

In Tephritidae, Cytochrome Oxidase I (COI) has been used to detect populational, demographic, and evolutionary patterns and can also be used for molecular identification of species of different genera (Bonfim et al., 2011; Barr et al., 2018; Koohkanzadeh et al., 2019). In multidisciplinary approaches, COI has been used to identification of *Anastrepha* species (Barr et al., 2017). Furthermore, the same region of the mitochondrial DNA was part of a set of other tools, which demonstrated the absence of genetic structure among the species of the *Bactrocera dorsalis* complex, which guided the synonym of species within this complex (Schutze et al., 2012; 2015a).

Morphometrics is a set of tools used to study the effect of ecological, geographic, and biological variables on the size and shape of structures of organisms (Rholf & Marcus, 1993). Linear morphometrics study patterns and biological processes by collecting distance measurements among homologous points (Rholf & Marcus, 1993). On the other hand, geometric morphometrics quantifies shape variation by plotting landmarks (homologous points) in two- and three-dimensional structures in a Cartesian plane (Rholf & Marcus, 1993; Bookstein, 1997; Zelditch et al., 2012; Parés-Casanova et al., 2020).

Morphometric analysis can also be used to distinguish closed related species and evaluate the variability among populations (Fadda & Coeti, 2000; Baylac et al., 2003; Bubliy et al., 2007; Francuski et al., 2009; Demayo et al., 2011; Krosch et al., 2012; Jaramillo-o et al., 2015; Torres & Miranda-Esquivel, 2016; Schutze et al., 2017). In studies of the *A. fraterculus* cryptic species complex, size and shape patterns have been used to identify different morphotypes and provide information about the factors acting to determine their distribution (Hernández-Órtiz et al., 2004; 2012; 2015).

Considering the morphological variation among the type series (holotype and paratypes) of *A. dissimilis*, we integrated data from morphology, linear and geometric morphometrics, and COI sequences to investigate the taxonomy of *A. dissimilis* from multiple localities of Brazil.

2.2. Material and Methods

2.2.1. Identification

The specimens used in this study were identified based in the morphological pattern of the aculeus tip, using the taxonomic key of Zucchi (2000) and Norrbom et al. (2012).

2.2.2. Morphological study

A comparison of the morphology of the aculeus tip was conducted with 95 specimens fitting the concept of *A. dissimilis* sensu lato from Assú and Mossoró (Rio Grande do Norte - RN); Cruz das Almas, Jaguaripe and Nova Soure (Bahia - BA); Jacupiranga, Monte Alegre do Sul, Presidente Prudente and Piracicaba (São Paulo - SP); Janaúba (Minas Gerais - MG); Lages and Nova Veneza (Santa Catarina - SC); Morada Nova (Ceará - CE); Pelotas and Vacaria (Rio Grande do Sul - RS). More information about the fruit flies used in this study (locality, geographic coordinates, method of collection, and voucher number) are detailed in Table 1.

For morphological study, the abdomen of each specimen was extracted using microforceps and clarified with heated Potassium hydroxide 10% solution for 3-4 minutes. This structure was transferred to a Petri dish and washed with distilled water. The abdomen was then put into a microvial filled with glycerin and attached to the pinned specimen.

The aculeus was everted using microforceps and mounted on a temporary slide with a drop of glycerin and the morphology in ventral view was observed under an optical microscope. A photograph of the aculeus tip of one specimen from each population was taken using an SCMOS Digital Camera coupled with a Nikon Eclipse E20 with the microscope.

In addition, a Scanning Electron Microscope (SEM) was used for a more detailed observation of the morphology of one or two specimens of each. The abdomen with the aculeus everted was dried and ventrally positioned and attached to metal stub using double-sided carbon tape and sputter-coated using Balzers SCD050. The photographs of the aculeus tip were taken in a JSM-IT300 In TouchScopeTM Scanning Electron Microscope.

Voucher specimens were pinned and deposited in the Museum of Entomology Luiz de Queiroz (MELQ), Department of Entomology and Acarology, Luiz de Queiroz College of Agriculture (ESALQ/USP).

2.2.3. Linear morphometrics

The morphometric study was conducted with populations from Assú and Mossoró (RN), Cruz das Almas and Nova Soure (BA), Janaúba (MG), and Vacaria (RS). Unfortunately, other populations of *A. dissimilis* sensu lato were not included due to low sample size (< 5 specimens). Considering their geographical proximity, we considered the specimens from Mossoró and Assú as a single population (Mossoró-Assú) to increase the number of specimens in the sample.

For the linear morphometrics measurements of the aculeus and mesonotum were taken. This analysis was conducted with 48 specimens from five Brazilian populations. The aculeus was mounted on a microscopic slide with glycerin and photographed with a Moticam 2000 camera coupled with a Nikon Eclipse E200 microscope (aculeus tip) or a Nikon SMZ 168 stereomicroscope (whole aculeus). For measurements of the mesonotum, the specimens were mounted on a pin and photographed using the Moticam 2000 camera coupled to the Nikon Eclipse E2000 microscope.

For the linear analysis, a tps file containing the mesonotum and aculeus photographs was created in the software TpsUtil 32 version 2.3.1. (Rholf, 2015). The distance measurements between the homologous points (length and width) of the aculeus and mesonotum (Fig. 1) were collected in the software TpsDig2 (Rholf, 2015). The following measurements were taken: (M1) length of the mesonotum, (M2) width of the mesonotum at the level of the post-sutural supra-alar seta, (M3) length from the apex of scutellum to the left post-sutural supra-alar seta, (A0) aculeus length, (A1) length of non-serrated part of aculeus tip, (A2) length of serrated part of aculeus tip, (A3) lateral length of serrated part of aculeus tip, (A4) width of aculeus tip at the level of cloacal opening, (A5) width of the base of the serrated part, (A6) length of aculeus tip (A1+A2), (P1) ratio of length of non-serrated part and length of serrated part (A1/A2), (P2) ratio of length of the aculeus and length of aculeus tip (A0/A7), ratio of length of aculeus tip and length of serrated part (A7/A2), (P4) ratio of length and width of aculeus tip (A7/A4) (Fig 1A-B; Table 6).

The linear measurements were used to perform a Principal Component Analysis (PCA) and a Canonical Variate Analysis (CVA). The PCA condenses the variation from a multivariate dataset and creates statistical indexes, the Principal Components (P.C.s), used to understand the variability among individuals from different groups defined *a priori*. Similarly, the CVA also condenses the variation from the dataset, but this variation is used to produce Canonical Variates (C.V.s), which discriminate groups (Legendre & Legendre, 1998). Furthermore, the values of Mahalanobis distance extracted from the CVA were used to plot a dendrogram using the Unweighted Pair Group Method (UPGMA) in the software Past 4.03 (Hammer, 2020). Finally, to know which measurements and proportions were statistically significant among the populations, a Multivariate Analyses of Variance (MANOVA) was performed.

All the analyses, except the clustering plot, were done in the free software R (R Core Team, 2013) using the packages: ggfortify (Tang et al., 2016; Horikoshi & Tang, 2018), morpho (Schlager, 2020), ggplot2 (Wickham, 2011), and dplyr (Wickham, 2021).

2.2.4. Geometrics morphometrics

For the geometric morphometrics the right wing of 68 females was detached from the thorax with a microforceps and submerged in Celossolve ($C_4H_{10}O_2$) for 3-7 days. Then, the wing was mounted on a permanent slide using Euparal and dried in a laboratory oven at 35 ºC for 10-15 days. Photographs were taken with the Moticam 2000 camera coupled to the Nikon SMZ 168 stereomicroscope.

For the geometric morphometrics, a tps file was created in the software TpsUtil version 2.3.1 (Rholf, 2015). Nineteen homologous points of intersection between wing veins, apices, and maximum curvature of the veins (Bookstein, 1997; Hernandez-Órtiz et al., 2015) were marked using the software TpsDig2 version 2.26 (Rholf, 2015) (Figure 2C). Then, Procrustes Superimposition was performed using the software MorphoJ (Klingenberg, 2011) to remove the effect of size, rotation, and orientation by scaling, shifting, and aligning the set of landmarks (Klingenberg, 2013). The data from the Procrustes superimposition were submitted to a Principal Components Analysis (PCA) and a Canonical Variate Analysis (CVA) in the software MorphoJ (Klingenberg, 2011). Finally, a clustering plot was done in the software Past 4.03 (Hammer, 2020) using the Procrustes distance values extracted from the CVA.

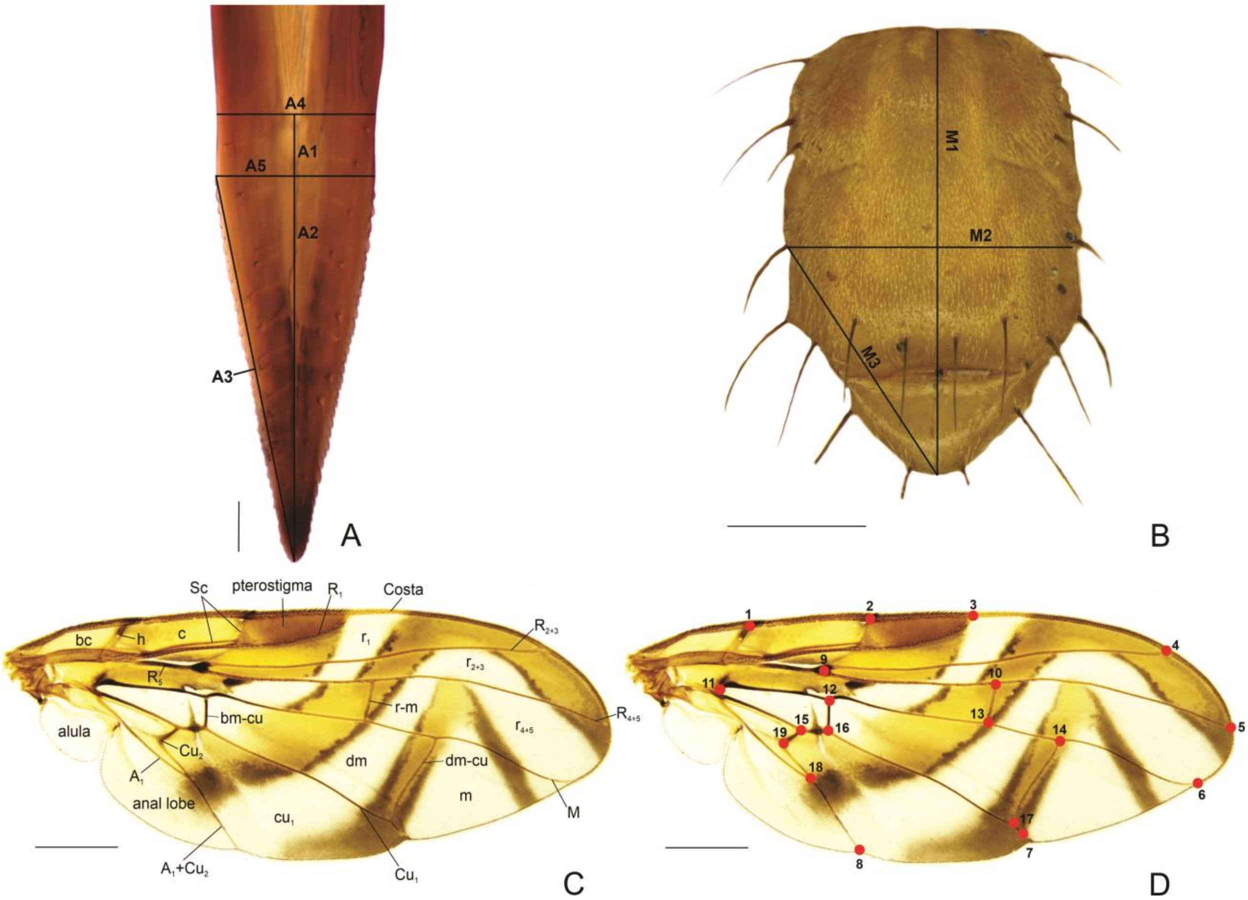

Figure 1. Measurements and landmarks collected from the aculeus tip (A), mesonotum (B), and wings (C-D) of *A. dissimilis* sensu lato for morphometric analysis. A1) Length of non-serrated part; A2) Length of serrated part; A3) Lateral length of serrated part; A4) Width of aculeus tip; A5) Width of base of serrated part; M1) Length of mesonotum; M2) Width of mesonotum at level of postsutural supra-alar seta; M3) Length from apex of scutellum to left postsutural supra-alar seta; (1) Intersection of costa and humeral crossvein; (2) intersection of subcosta and costa; (3) appex of R_1; (4) apex of R_{2+3}; (5) apex of R_{4+5}; (6) apex of M; (7) apex of Cu_1; (8) intersection of A_1 and Cu_2; (9) basal bifurcation of R_{2+3} and R_{4+5}; (10) intersection of R_{4+5} and r-m; (11) basal angle of bm cell; (12) intersection of M and bm-cu; (13) intersection of M and r-m;(14) intersection of M and dm-cu; (15) intersection of Cu_1 and Cu_2; (16) intersection of Cu_2 and bm-cu; (17) intersection of Cu_1 and dm-cu; (18) basal bifurcation of Cu_2 and A_1; (19) point of maximum curvature of Cu_1. Scale bars: A = 50 µm; B-C = 1 mm.

2.2.5. DNA Extraction

The DNA was obtained by the maceration of the pair of midlegs with attached thoracic tissue in a digestion buffer [CaCl2 (1M), SDS (2%, DTT (1M), Tris-HCl (1M; pH = 8,0), NaCl (5M), and H2O MiliQ] following the protocol adapted from Gilbert et al. (2007). First, 12,5 µl of proteinase K (20 µg/mL) was added, and the solution was incubated for 14 h at 65 ºC. Next, the extraction product was transferred to a microvial, and a solution containing chloroform and ethanol (24:1) was added and mixed for 2 min. Finally, this mixture was centrifuged at 14000 rpm for 20 min. (25 ºC).

The supernatant was transferred to a new microvial, where 0.1x of the total volume of sodium acetate, 2.5 µl of glycogen, and 0.7x of cold isopropanol 100% were added and incubated overnight at -20 ºC. It was then centrifuged at 14000 rpm for 30 min. at 4 ºC. Next, the DNA was washed in different ethanol concentrations (500 µl – 70% and 95%), and then dried in an airflow chamber and eluted in 35 µl of H2O MiliQ.

2.2.6. COI amplification and sequencing

The Polymerase Chain Reaction (PCR) was conducted using the primers LepF1/Lep R1 (Hebert et al., 2004) and LCO1490/HCO2198 (Forlmer et al., 1994). The PCR solution was composed of: 9.5µl of MilliQ-H2O (9.5 µl), 2.5 µl 10X PCR Buffer Mg2+ free (Thermo Fisher Scientific ™), 4µl MgCl2 (50mM) (Thermo Fisher Scientific ™), 0.8µl dNTP (10 mM) (Sinapse Inc®), 0.5µ of LCO1490 and HCO2198 (10µM), 1µl of LepF1 and LepR1 (5µM), 0.2 µl Platinum® Taq DNA Polymerase (5 U µl-1) (Thermo Fisher Scientific ™), and 5µl of DNA (5.0 µl).

The amplification reaction was performed with the following steps: primary denaturation for 3 min. at 94 ºC, then and 35 cycles of denaturation at 94 ºC (30 s), annealing at 53 ºC (45 s), elongation at 72 ºC (22 s), and a final extension at 72 ºC (10 min.). The PCR products' aliquots were put into an electrophorese gel (1.5% agarose) and observed under ultraviolet light.

The purification was conducted using 1µl of the enzyme Exo+Sap (Cellco Biotec) for each 10µl of PCR final product). The bidirectional sequencing followed the Sanger method performed in the Agricultural Biotechnology Laboratory (CEBTEC) at ESALQ/USP. After that, the sequences were manually edited and aligned using the software Sequencer v.4.0.1.

2.2.7. Analysis of the DNA barcode

The sequences of the specimens from Jaguaripe (BA), Janaúba (MG), Nova Veneza (SC), Monte Alegre do Sul (SP), Morada Nova (CE), and Pelotas (RS) were compared with the sequences of *Anastrepha* registered in the Barcode of Life Data System (Bold Systems). The genetic distance among these populations and the most similar sequences (extracted from Bold System) was performed using the Maximum Likelihood Model (Tamura et al., 2004) in Mega

X software (Kumar et al., 2018).

A Bayesian phylogenetic tree was constructed using the GTR nucleotides substitution model (Waddel & Steel, 1997) and the +|+ G parameters. The most suitable model and parameters were selected using the MrModeltest v2 software (Nylander, 2004). Finally, the phylogenetic analysis was performed using MrBayes (Huelsenbeck & Ronquist, 2001), and the supports for the knots were generated through ten million replicates.

2.3. Results

2.3.1. Morphological study

The morphology of aculeus tip of the samples identified as *A. dissimilis* following the concepts and keys of Stone (1942) and Zucchi (1978) revealed a uniform pattern among the Brazilian populations. They all possess a triangular and finely serrated aculeus without a strong constriction before the serrations (Figures 2-3). The serrations extend in about 0.82 to 1.0 of the aculeus tip (Figures 2-3). On the other hand, there is a constriction before the serrated part in the holotype aculeus tip and the serrations extends in about 0.63-0.72 (Norrbom, pers. Comm.; Holotype, Figure 2P).

The specimens from the Northeast (Assú, Cruz das Almas, Jaguaripe, Morada Nova, and Mossoró) and part of the southeast of Brazil (Janaúba and Monte Alegre), have an aculeus tip straighter and smoothly widened in the base of the serrations. Also, the teeth of the serrations are less conspicuous than other populations (Figures 2A-G; 3A-E; 3L-N).

In the second group, which included populations from southern Brazil (Lages, Nova Veneza, and Vacaria) and southeast (Jacupiranga, Piracicaba, and Presidente Prudente), the junction of the non-serrated and serrated parts of the aculeus tip is smoothly or moderately rounded or slightly widened, sometimes with a slight constriction before the serrations, and the serrations are more proeminent (Figures 2H-O; 3F-H; 3O-Q).

2.3.2. Linear morphometrics

The PCA generated 16 Principal Components to explain 100% of the variance contained in the dataset. The PC1 and PC2 were used for the scatter plot because they comprised more than 50% of the variance (Table 2). The PC1 separated Janaúba from the other populations (Figure 4A). The Cruz das Almas and Mossoró-Assú populations are on the same PC1 and PC2 axes region, separated from the other populations except Nova Soure (Figure 4A). Nova Soure does not precisely position the PC2 axes (Figure 4A). Finally, Vacaria was also separated from Janaúba, Mossoró-Assú, and Cruz das Almas (Figure 4A).

Table 1. Localities, geographic coordinates, collection methods, voucher numbers, and number of specimens of *A. dissimilis* sensu lato used in morphological and/or in morphometric and molecular analysis.

States	Localities	Geographic coordinates		Specimens (n)				Collection Methods	Voucher Numbers (MELQ)
		Latitude	Longitude	Morphological Study	Linear Morphometrics	Geometric Morphometrics	Molecular Analysis		
Bahia	Nova Soure	11°14'20"S	38°28'48"W	13	9	13	-	McPhail	ESALQENT000145-159
	Cruz das Almas	12°40'10"S	39°6'23"W	7	5	7	-	McPhail	ESALQENT000129-135
	Jaguaripe	13°6'45"S	38°53'34"W	1	-	-	1	McPhail	ESALQENT000580
Ceará	Morada Nova	5°6'20.2"S	38°22'1.5"W	2	-	-	2	McPhail	ESALQENT0001568-1569
Minas Gerais	Janaúba	15°48'23"S	43°18'29"W	18	11	18	4	McPhail	ESALQENT0001570-1587
Rio Grande do Norte	Assú	5°35'48"S	36°54'41"W	3	2	2	-	McPhail	ESALQENT000121-123
	Mossoró	5°11'15"S	37°20'39"W	9	7	7	-	McPhail	ESALQENT000160-168
Rio Grande do Sul	Vacaria*	28°30'7"S	50°56'14"W	39	14	21	-	McPhail	ESALQENT000180-237
	Pelotas	31º37'16.7"S	52º31'39.2"W	1	-	-	1	Fruits of *Passiflora caerulea*	ESALQENT0001588
São Paulo	Jacupiranga	24°42'13"S	48°0'29"W	4	-	-	-	McPhail	ESALQENT000238-241
	Monte Alegre do Sul	22°40'56.1"S	46°40'50.5"W	3	-	-	2	McPhail	ESALQENT0001589-1591
	Piracicaba	22°42'31"S	47°37'58"W	5	-	-	-	McPhail	ESALQENT000124-128
	Presidente Prudente	22°7'21.2"S	51°23'17.1"W	1	-	-	1	McPhail	ESALQENT0001592
Santa Catarina	Lages	27°48'55.7"S	50°19'35"W	4	-	-	-	McPhail	ESALQENT0001593-1596
	Nova Veneza	28°38'13"S	49°29'54"W	1	-	-	1	McPhail	ESALQENT000579

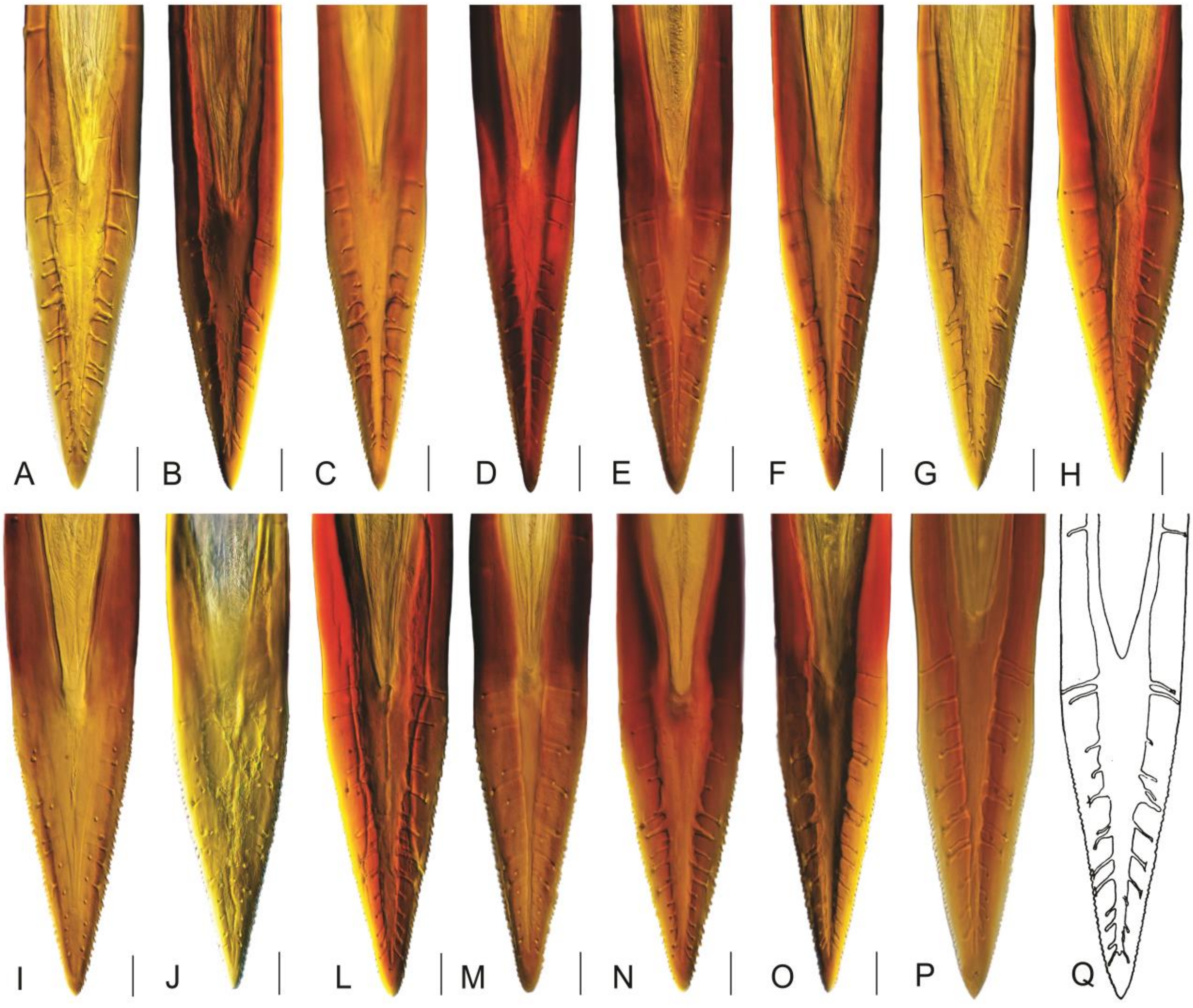

Figure 2. Aculeus tip (ventral view) of *A. dissimilis* sensu lato from Brazil A) Assú (RN); B) Cruz das Almas (BA); C) Janaúba (MG); D) Jaguaripe (BA); E) Monte Alegre do Sul (SP); F) Mossoró (RN); G) Morada Nova (CE); H) Jacupiranga (SP); I) Lages (SC); J) Pelotas (RS); L) Piracicaba (SP); M) Presidente Prudente (SP); N) Nova Veneza (SC); O) Vacaria (RS); P) Plaisance, Haiti (Holotype) (Norrbom et al., 2012); Q) Bonito, Pernambuco (Paratype) (Stone, 1942). Scale bars: 50 µm.

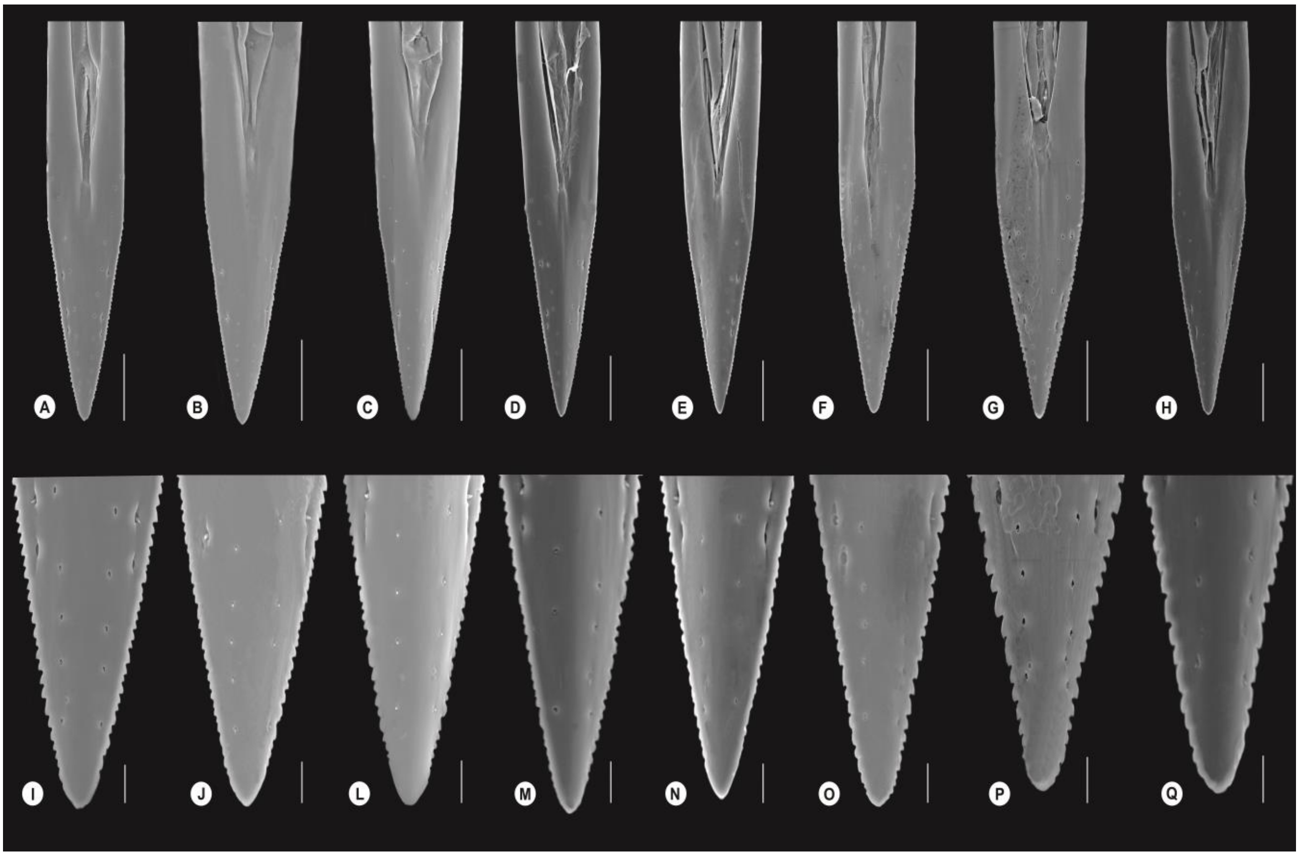

Figure 3. Scannig Electron Microscope photographs of aculeus tip (ventral view) of *A. dissimilis* sensu lato from Brazil. A) Assú (RN); B) Cruz das Almas (BA); C) Janaúba (MG); D) Mossoró (RN); E) Nova Soure (BA); F) Lages (SC); G) Piracicaba (SP); H) Vacaria (RS). Detail of tip. I) Assú; J) Cruz das Almas; L) Janaúba; M) Mossoró; N) Nova Soure; O) Lages; P) Piracicaba; Q) Vacaria. Scale bars, A-H: 100 µm; I-Q: 20 µm

The CVA generated four Canonical Variates. The CV1 and CV2 were responsible for 88.02% of the whole variance in our dataset (Table 3). Therefore, following the same criteria of PCA, CV1 and CV2 were used for the scatter plot. The Figure 4B showed that Janaúba was separated from Cruz das Almas, Mossoró-Assú, Nova Soure, and Vacaria by the CV1. On the other hand, Vacaria was separated from Cruz das Almas, Mossoró-Assú, and Nova Soure by the CV2 axis (Figure 4B). Also, the CV2 suggested that Cruz das Almas and other populations from the northeast of Brazil are from the same group (Figure 4B). The clustering plotted using the Mahalanobis distance corroborated the results observed in the CVA scatter plot (Figure 4C; Table 4).

The Multivariate Analysis of Variances was statistically significant (Table 5). The *post hoc* test indicated that only the aculeus tip width (A4) and the proportion between aculeus tip length and aculeus tip width are not statistically different among the populations (Table 6). The mean and standard deviation for each measurement and proportion are in Table 7.

Table 2. Proportion of variance and cumulative variance contained in each Principal Component (PC) from Principal Components Analyses.

Principal Component (PC)	Proportion of Variance (%)	Cumulative Variance (%)
PC1	30.02	30.02
PC2	26.91	56.92
PC3	12.77	69.69
PC4	8.92	78.61
...	...	...
PC16	0.00	100

Table 3. Proportion of variance and cumulative variance contained in each Canonical Variate (CV) from Canonical Variate Analyses.

Canonical Variate (CV)	Proportion of Variance (%)	Cumulative Variance (%)
CV1	55.35	55.35
CV2	32.67	88.02
CV3	7.71	95.74
CV4	4.25	100

Table 4. Mahalanobis distance values from Canonical Variate Analyses of Linear Morphometrics analysis.

Populations	Cruz das Almas	Janaúba	Mossoró-Assú	Nova Soure
Janaúba (MG)	5.682022			
Mossoró-Assú (RN)	2.482974	5.863150		
Nova Soure (BA)	2.685625	4.867065	2.802057	
Vacaria (RS)	4.618384	5.282119	4.087265	3.785727

Table 5. Output of Multivariate Analyses of Variance (MANOVA).

	Degrees of Freedom	**Pillai's trace**	***P***
Populations	4	28.065	<0.001*
Residuals	43	-	-

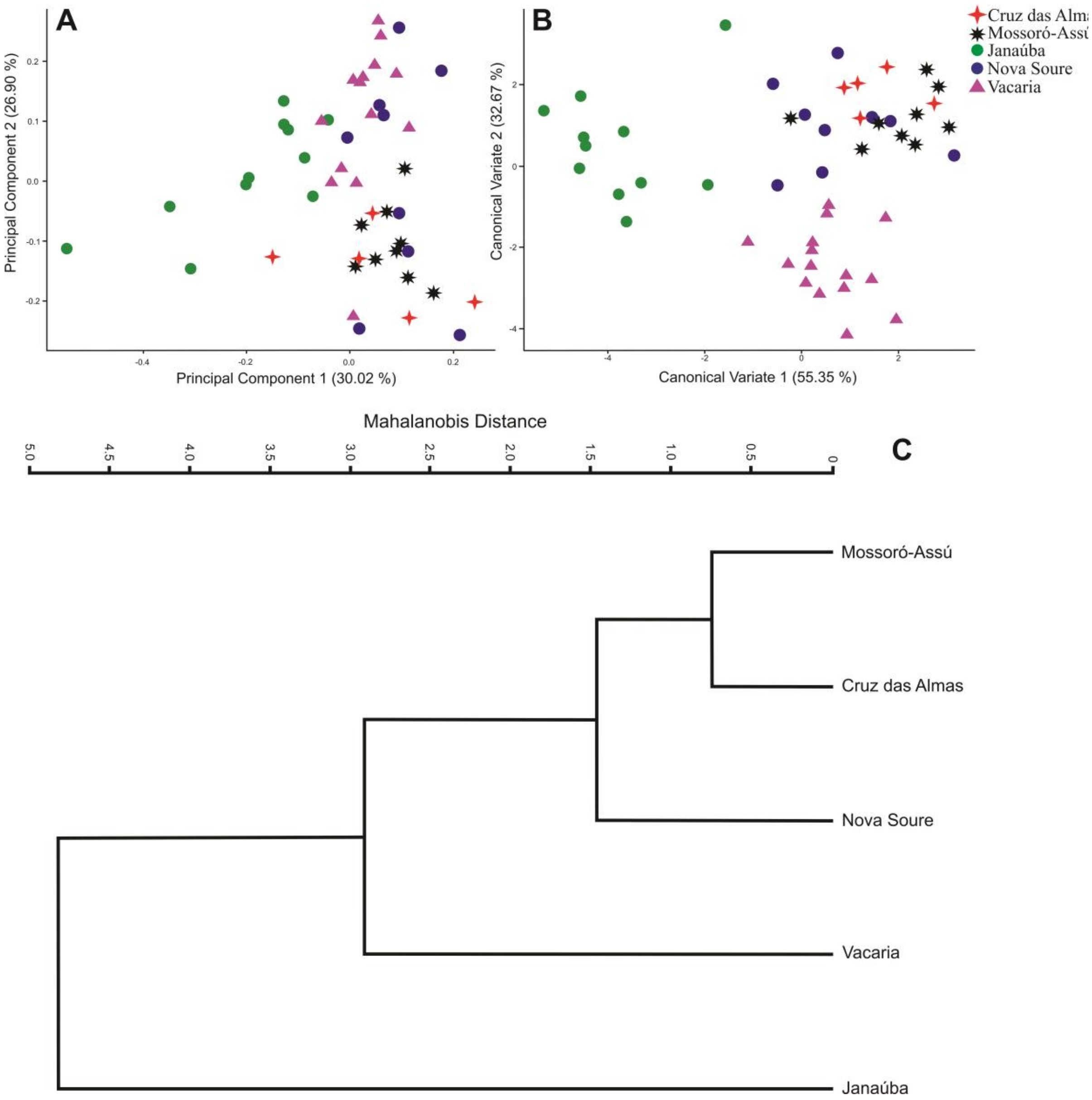

Figure 4. Graphs of linear morphometrics analysis. A) Principal Components Analysis scatter plot; C) Canonical Variate Analysis scatter plt; C) Dendrogram made with the Mahalanobis distance from canonical variate analysis.

Table 6. Linear mesurements and proportions used in MANOVA and the results from post-hoc test

Structures	**Variables**	**Descriptions**	**Degrees of freedom (Populations)**	**F**	***p***
Mesonotum	M1	Length of the mesonotum	4	9.841	<0.001*
	M2	Width of the mesonotum in the high of the postsutural supra-alar seta	4	27.675	0.039*
	M3	Length from the apex of scutellum to the left postsutural supra-alar seta	4	35.587	0.013*
Aculeus	A0	Aculeus length	4	3.473	0.015*
	A1	Length of non-serrated part	4	7.243	<0.001*
	A2	Length of serrated part	4	8.6464	<0.001*
	A3	Lateral length of serrated part	4	2.6393	0.046*
	A4	Width of aculeus tip	4	0.4547	0.768
	A5	Width of the base of serrated part	4	17.550	<0.001*
	A6	Length of aculeus tip (A1+A2)	4	11.399	<0.001*
Proportions	P1	Ratio of length of non-serrated part and length of serrated part (A1/A2)	4	6.776	<0.001*
	P2	Ratio of length of the aculeus and length of aculeus tip (A0/A7)	4	13.101	<0.001*
	P3	Ratio of length of aculeus tip and length of serrated part (A7/A2)	4	6.776	<0.001*
	P4	Ratio of length and width of aculeus tip (A7/A4)	4	0.470	0.756

P5	Ratio of length of serrated part and width of base of serrated part (A2/A5)	4	8.684	<0.001*
P6	Ratio of length of the aculeus and length of serrated part (A0/A2)	4	7.051	<0.001*
P7	Ratio of length of the mesonotum and width of the mesonotum in the high of the postsutural supra-alar seta (M1/M2)	4	3.174	0.02*

2.3.3. Geometric Morphometrics

The PCA performed with the data from Procrustes Superimposition created 34 P.C.s, with the variance distributed in the first four Principal Component (Table 8). Only the PC1 and PC2 were used to plot the graph. The landmarks that most contributed to the PC1 were: L7, L8, L10, and L17. For the PC2, the most important landmarks were: L5, L6, L7, L8, L10, L13.

The exploratory PCA showed that Vacaria differed from other populations (Figure 5). Cruz das Almas, Janaúba, Mossoró-Assú, and Nova Soure overlapped in the central and right side of the PC1 axis (Figure 5). Vacaria was separated from the other populations by the PC2 axis, which according to the outline drawn, represents shape changes in the posterior and proximal regions of the wings (Figure 5).

The Canonical Variate Analysis generated only four Canonical Variates, where most variation was concentrated in CV1 and CV2 (Table 9). The scatter plot of the CVA showed that Vacaria was separated to Cruz das Almas, Janaúba, Mossoró-Assú, and Nova Soure, suggesting the existence of two morphotypes (Figure 6).

According to Procrustes distance, the shape patterns of Vacaria were significantly different from the other populations (Permutation test, $p<0.001$). The Procrustes distance observed among the populations from the Northeast (Cruz das Almas, Mossoró-Assu, and Nova Soure) and one from the Southeast (Janaúba) of Brazil was not statistically significant (Permutation test, $p>0.001$) (Table 10). The dendrogram plotted with Procrustes distance also suggested the existence of two groups, with the first one comprising Vacaria, and the second one with Cruz das Almas, Janaúba, Mossoró-Assú, and Nova Soure (Figure 7).

Table 7. Means and standard deviation of linear measurements and proportions used in linear morphometrics analyses

Variables	Populations				
	Cruz das Almas	Janaúba	Mossoró-Assú	Nova Soure	Vacaria
M1	2.99 ± 0.19	3.27 ± 0.23	2.99 ± 0.12	2.81 ± 0.16	3.42 ± 0.35
M2	2.05 ± 0.17	2.08 ± 0.17	2.04 ± 0.21	2.00 ± 0.16	2.27 ± 0.26
M3	2.16 ± 0.57	2.21 ± 0.21	1.87 ± 0.16	1.93 ± 0.17	2.21 ± 0.25
A0	2.75 ± 0.37	3.02 ± 0.17	2.76 ± 0.17	2.82 ± 0.17	2.95 ± 0.16
A1	0.17 ± 0.03	0.07 ± 0.02	0.16 ± 0.02	0.13 ± 0.07	0.09 ± 0.04
A2	0.58 ± 0.03	0.59 ± 0.05	0.60 ± 0.02	0.67 ± 0.06	0.69 ± 0.06
A3	0.59 ± 0.03	0.58 ± 0.05	0.61 ± 0.01	0.66 ± 0.06	0.66 ± 0.11
A4	0.27 ± 0.01	0.26 ± 0.14	0.26 ± 0.01	0.26 ± 0.01	0.29 ± 0.01
A5	0.27 ± 0.01	0.23 ± 0.02	0.28 ± 0.01	0.27 ± 0.01	0.29 ± 0.01
A6	0.75 ± 0.05	0.66 ± 0.02	0.77 ± 0.07	0.80 ± 0.07	0.79 ± 0.04
P1	0.29 ± 0.06	0.12 ± 0.04	0.26 ± 0.04	0.21 ± 0.13	0.14 ± 0.08
P2	3.67 ± 0.68	4.59 ± 0.57	3.59 ± 0.21	3.51 ± 0.25	3.73 ± 0.16
P3	1.29 ± 0.06	1.12 ± 0.04	1.26 ± 0.04	1.21 ± 0.13	1.14 ± 0.08
P4	2.77 ± 0.37	3.57 ± 3.41	2.94 ± 0.17	3.11 ± 0.40	2.71 ± 0.17
P5	2.10 ± 0.08	2.47 ± 0.10	2.13 ± 0.15	2.42 ± 0.22	2.36 ± 0.15
P6	4.72 ± 0.68	5.18 ± 0.59	4.56 ± 0.33	4.24 ± 0.47	4.26 ± 0.28
P7	0.92 ± 0.12	0.93 ± 0.09	0.92 ± 0.03	1.00 ± 0.07	0.87 ± 0.13

Table 8. Proportion of variance and cumulative variance contained in each Principal Component (PC) from Principal Components Analyses.

Principal Component (PC)	Proportion of Variance (%)	Cumulative Variance (%)
PC1	14.44	14.44
PC2	10.88	25.33
PC3	10.56	35.89
PC4	9.637	45.52
...	...	...
PC34	0.05	100

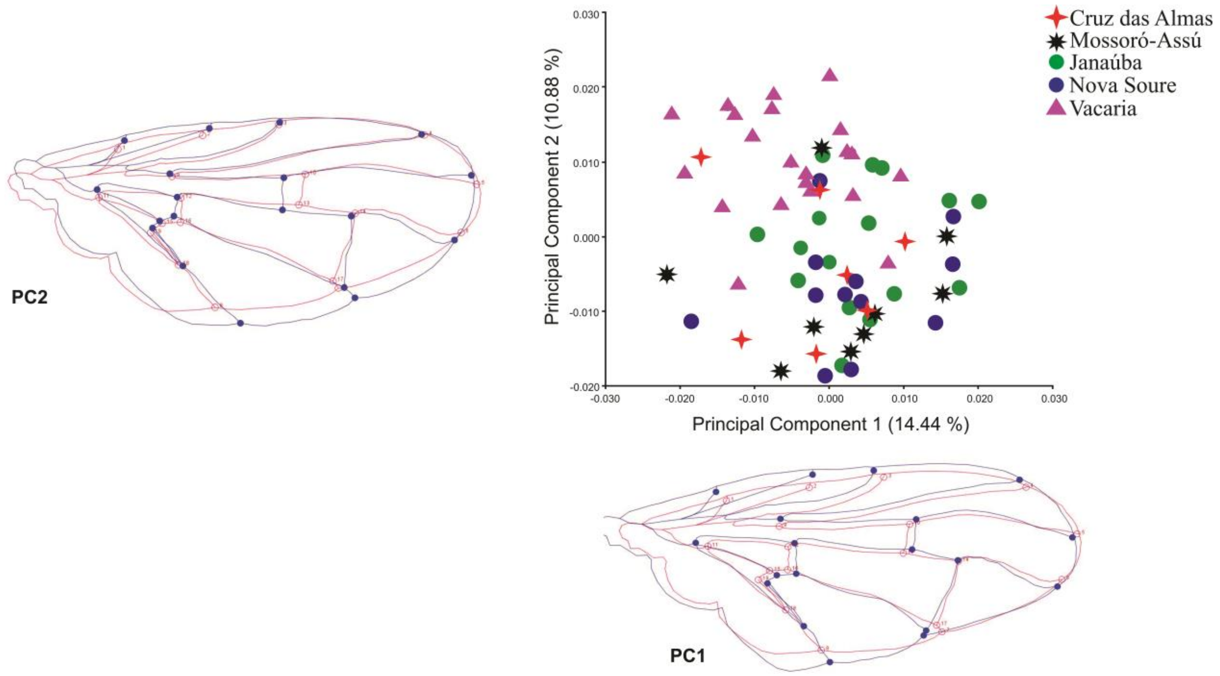

Figure 5. Scatter plot and outline drawing from Principal Component Analysis performed with the procrustes superimposition data from wing of *A. dissimilis*.

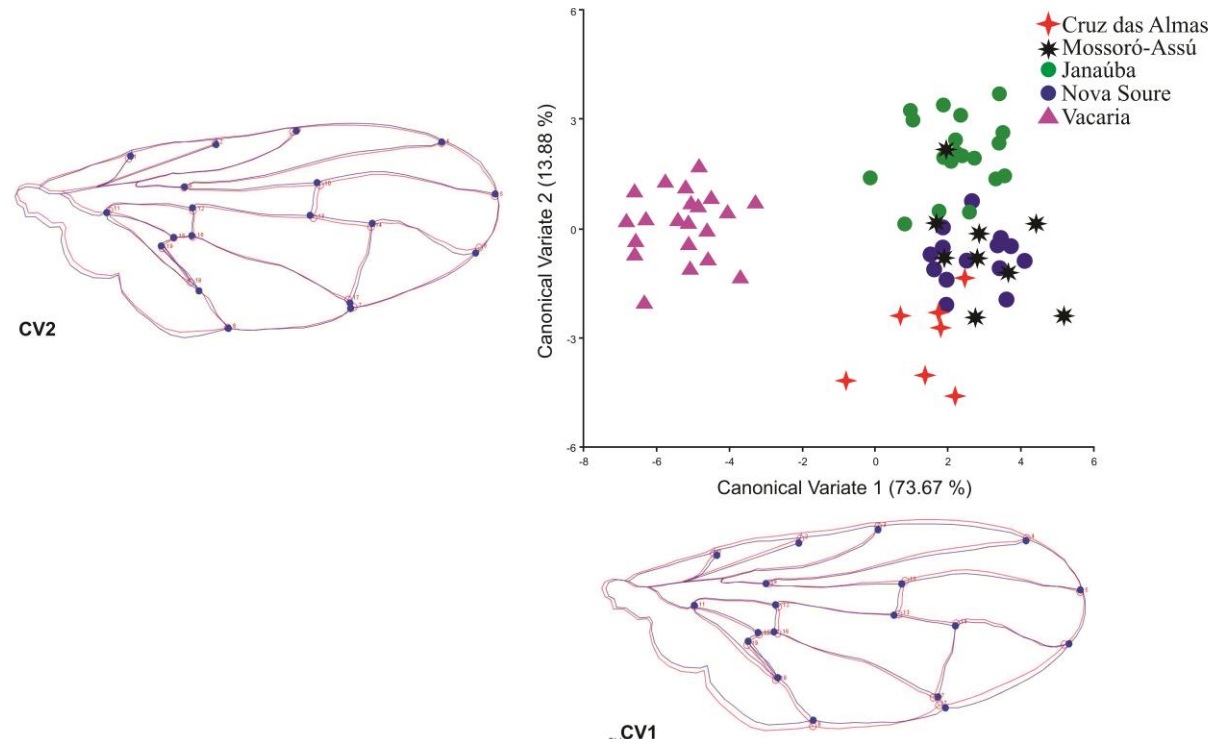

Figure 6. Scatter plot and outline drawing from Canonical Variate Analysis performed with the procrustes superimposition data from wing of *A. dissimilis*.

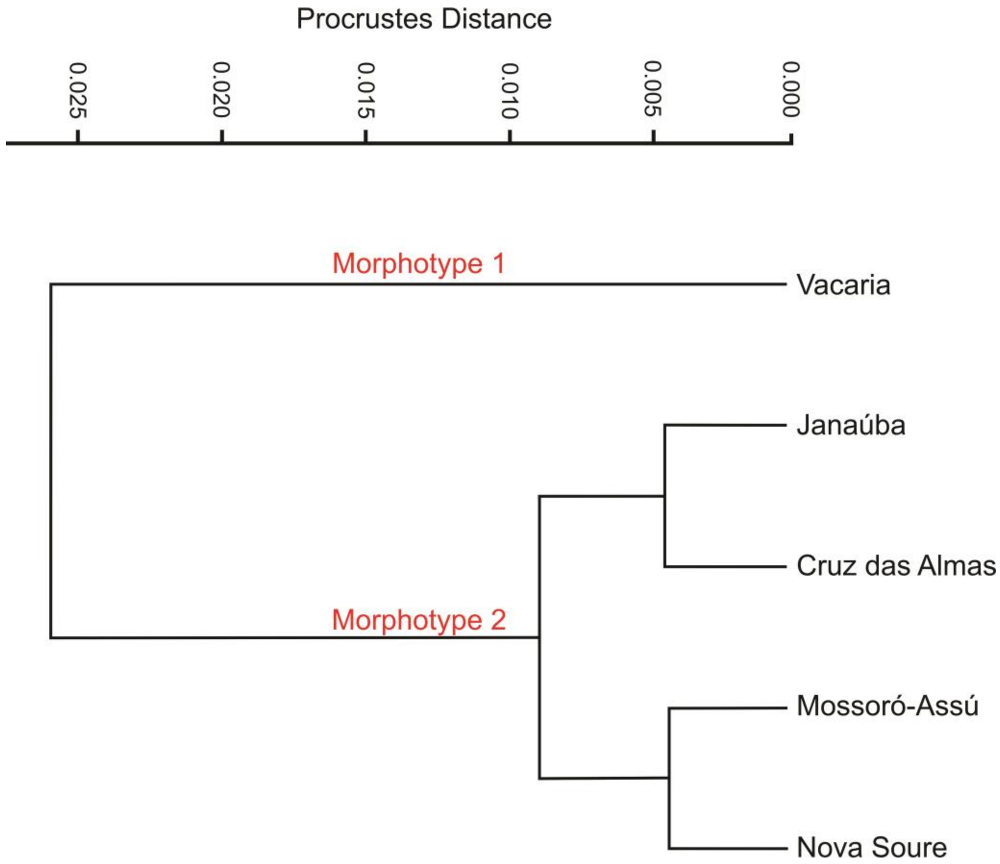

Figure 7. Dendrogram made with procrustes distance values from the Canonical Variate Analysis of Geometric Morphometrics of wing of *A. dissimilis*.

Table 9. Proportion of variance and cumulative variance contained in each canonical variate (CV) from Canonical Variate Analyses.

Canonical Variate (CV)	**Proportion of Variance (%)**	**Cumulative Variance (%)**
CV1	73.67	73.67
CV2	13.88	87.56
CV3	8.18	95.74
CV4	4.25	100

Table 10. Procrustes distance values among four populations of *A. dissimilis*, extracted from the Canonical Variate Analysis of the shape of the wings. Asterisk symbol (*) in the same column means statistical significance, provided by the Permutation tests ($p<0.001$).

Populations	Cruz das Almas	Janaúba	Mossoró-Assú	Nova Soure
Janaúba (MG)	0.0163			
Mossoró-Assú (RN)	0.0138	0.0142		
Nova Soure (BA)	0.0118	0.0132	0.0100	
Vacaria (RS)	0.0207*	0.0238*	0.0264*	0.0271*

2.3.4. Analysis of DNA Barcode

A COI fragment with 544 bp was produced from samples collected in Janaúba, Jaguaripe, Monte Alegre do Sul, Morada Nova, Nova Veneza, and Pelotas. According to the BOLD Systems, all the populations studied herein were identified as *Anastrepha chiclayae* Greene, with 99.44 to 100% similarity (Table 11). Among the sequences of Brazilian populations, the lower genetic distance was 0.00%, and the higher was 0.95% (Table 12). Considering the DNA barcoding of *A. chiclayae* from Ecuador and Peru, the genetic distance was 0.33% to 1.30% (Table 12). Unfortunately, our phylogenetic inference based on COI sequences did not recover the relationship among the populations and species within the clade formed by *A. chiclayae*, *Anastrepha* sp., and *Anastrepha consobrina* (Loew) (Figure 8).

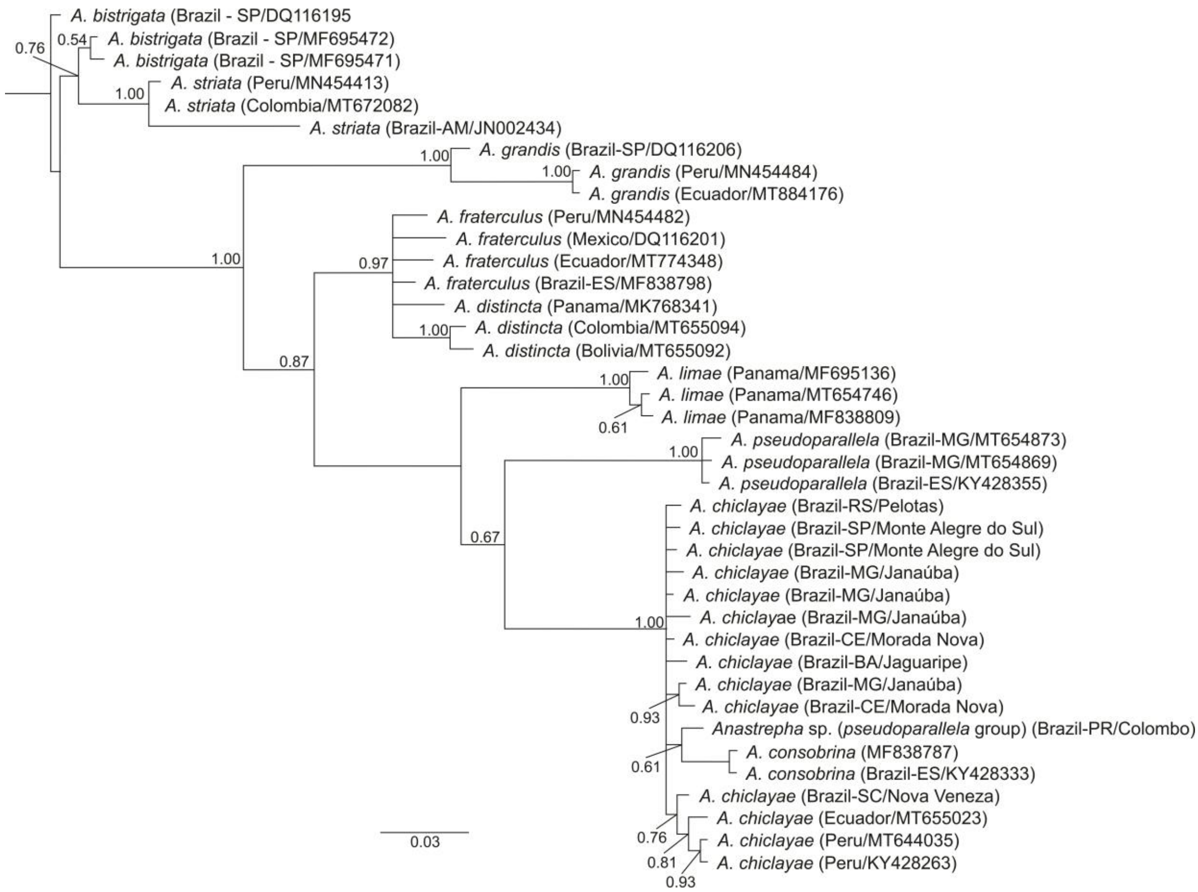

Figure 8. Phylogenetic Bayesian inference based on COI mtDNA sequences. The number on the branches represents the supporting values for the knots. The specimens of *A. chiclayae* from Brazil were previously identified as *A. dissimilis.*

Table 11. Molecular analysis of specimens previously identified as *A. dissimilis* from different populations in Brazil, based on Barcode sequences deposited on Bold System.

Samples	Bold Identifications	% ID	Best ID
Morada Nova (CE)	*Anastrepha chiclayae*	99.44	*Anastrepha chiclayae*
	Anastrepha chiclayae	99.83	*Anastrepha chiclayae*
Janaúba (MG)	*Anastrepha chiclayae*	99.39	*Anastrepha chiclayae*
	Anastrepha chiclayae	99.83	*Anastrepha chiclayae*
	Anastrepha chiclayae	99.69	*Anastrepha chiclayae*
	Anastrepha chiclayae	99.69	*Anastrepha chiclayae*
Jaguaripe (BA)	*Anastrepha chiclayae*	99.63	*Anastrepha chiclayae*
Nova Veneza (SC)	*Anastrepha chiclayae*	99.69	*Anastrepha chiclayae*
Monte Alegre do Sul (SP)	*Anastrepha chiclayae*	100	*Anastrepha chiclayae*
	Anastrepha chiclayae	100	*Anastrepha chiclayae*
Pelotas (RS)	*Anastrepha chiclayae*	99.81	*Anastrepha chiclayae*

2.4. Discussion

The morphological analysis from this study suggests that the Brazilian specimens previously considered to be *A. dissimilis* are distinct from the holotype. The Brazilian samples showed an aculeus tip without a constriction before the serrated part and the serrations beginning closer to the cloacal opening. Additionally, we noted considerable geographic variation among these populations. Specimens from Southern (Lages, Nova Veneza – SC, and Vacaria - RS) and southeast (Jacupiranga, Piracicaba, and Presidente Prudente - SP) have the aculeus tip more widened basally and the teeth more conspicuous. In contrast, the populations from the northeast (Assú and Mossoró – RN, Cruz das Almas and Jaguaripe - BA, Morada Nova - CE) and two from the southeast (Janaúba – MG and Monte Alegre do Sul - SP) have a narrower aculeus tip and less conspicuous teeth.

Table 12. Genetic distance among specimens previously identified as *A. dissimilis* sensu lato from different populations of Brazil and *A. chiclayae* from Peru and Ecuador. The genetic distances were estimated using the Maximum Likelihood Model.

<table>
<tr><th>Samples</th><th>Pelotas</th><th>Nova Veneza</th><th colspan="2">Monte Alegre do Sul</th><th colspan="4">Janaúba</th><th colspan="2">Morada Nova</th><th>Jaguaripe</th><th>A. chiclayae | Peru | KY428263</th><th>A. chiclayae | Ecuador | MT655023</th></tr>
<tr><td>Pelotas (RS)</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>Nova Veneza (SC)</td><td>0.3855</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td rowspan="2">Monte Alegre do Sul (RS)</td><td>0.0000</td><td>0.3206</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>0.0000</td><td>0.2382</td><td>0.0000</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td rowspan="4">Janaúba (MG)</td><td>0.3994</td><td>0.6444</td><td>0.0000</td><td>0.2382</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>0.5793</td><td>0.6157</td><td>0.3206</td><td>0.4774</td><td>0.6164</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>0.2005</td><td>0.3380</td><td>0.0000</td><td>0.0000</td><td>0.3380</td><td>0.5078</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>0.9690</td><td>0.9134</td><td>0.6432</td><td>0.9590</td><td>0.9230</td><td>0.5901</td><td>0.8489</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td rowspan="2">Morada Nova (CE)</td><td>0.0000</td><td>0.5221</td><td>0.0000</td><td>0.0000</td><td>0.3474</td><td>0.5219</td><td>0.0000</td><td>0.8726</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>0.8034</td><td>0.7422</td><td>0.3206</td><td>0.7177</td><td>0.7422</td><td>0.1845</td><td>0.5576</td><td>0.9294</td><td>0.5917</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>Jaguaripe (BA)</td><td>0.2520</td><td>0.7759</td><td>0.3206</td><td>0.2883</td><td>0.7384</td><td>0.5529</td><td>0.4084</td><td>0.7384</td><td>0.3962</td><td>0.4579</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>Anastrepha chiclayae | Peru | KY428263</td><td>0.8269</td><td>0.4944</td><td>0.9683</td><td>0.7180</td><td>0.7827</td><td>0.7902</td><td>0.6924</td><td>1.1042</td><td>0.6972</td><td>1.1475</td><td>0.5527</td><td>-</td><td>-</td></tr>
<tr><td>Anastrepha chiclayae | Ecuador | MT655023</td><td>0.9747</td><td>0.6288</td><td>0.9676</td><td>0.7177</td><td>0.9187</td><td>0.9063</td><td>0.8489</td><td>1.2062</td><td>0.8726</td><td>1.3056</td><td>1.1114</td><td>0.4680</td><td>-</td></tr>
<tr><td>Anastrepha chiclayae | Peru | MT644035</td><td>0.7832</td><td>0.3312</td><td>0.9683</td><td>0.7180</td><td>0.6783</td><td>0.8322</td><td>0.6818</td><td>1.1687</td><td>0.7198</td><td>1.1176</td><td>0.4107</td><td>0.0000</td><td>0.4975</td></tr>
</table>

The aculeus tip of the paratype illustrated by Stone (1942) (Figure 2Q) from Bonito, Pernambuco, is similar to the other Brazilian specimens, especially those from the northeast. These specimens share similarities including the aculeus tip with a narrowed shape without constriction before the serrations, the base of the serrated part of the aculeus tip slightly widened, and the serrations extending in 0.82-1.00 of aculeus tip. The specimen of *A. dissimilis* from Brazil illustrated by Zucchi (1978) is also similar.

The morphological findings suggest some apparent similarities among the southeastern and south Brazilian specimens with *Anastrepha correntina* Blanchard, 1961 (considered junior synonym of *A. dissimilis* by Steyskal, 1977). Furthermore, the illustrations of this species from Blanchard (1961) and Korytkowski & Ojeda (1968) show that the aculeus tip has the same morphological pattern found in Southern and southeastern Brazilian populations. Therefore, *A. correntina* may have been wrongly placed in synonym with *A. dissimilis* since it is more similar to Brazilian samples than the holotype from Haiti (Plaisance) photographed by Norrbom et al. (2012).

The data from the wing shape analysis (geometric morphometry) revealed that specimens from Vacaria are statistically different from those of Mossoró-Assú, Cruz das Almas, Nova Soure, and Janaúba, corroborating the morphological findings. Conversely, the linear morphometrics, despite showing that specimens from Vacaria are different from the other populations, indicate the existence of another group comprising only Janaúba. In *Anastrepha*, the aculeus, wing, and mesonotum have been helpful in the taxonomic distinction of related species (Hernadez-Órtiz et al., 2004; 2012; 2015; Perre et al., 2014).

Unlike the morphometric evidence, the molecular results showed a short genetic distance among the southern and northeastern Brazilian populations. Also, it was suggested that these populations are *A. chiclayae*. It corroborates our morphological findings at some point because the aculeus of *A. chiclayae* illustrated by Stone (1942) and photographed by Norrbom et al. (2012), has an aculeus tip narrower that is strongly similar to the populations studied herein. In addition, the beginning of the serrations is very close to the cloacal opening (see Norrbom et al., 2012). This morphological pattern corresponds to aculeus tip morphology found in the populations studied here and to the paratype of *A. dissimilis* illustrated by Stone (1942) (Figure 2Q).

Another point, in the description of *A. dissimilis* by Norrbom et al. (2012), it was reported the presence of microtrichia on all or almost all of the mesoscutum. However, in Brazilian populations, the mesoscutum is not entirely microtrichose, just like in *A. chiclayae* (Norrbom et al., 2012). Therefore, it is suggested here that the Brazilian populations identified as *A. dissimilis* are *A. chiclayae*.

According to Schlik-Steiner (2010), the disagreement among disciplines used in integrative approaches is a typical output. In our study, agreement only occurred among the descriptive morphological and molecular data, which suggested that these specimens are *A. chiclayae*. The morphometric results suggested the existence of at least two morphospecies among our samples, which seem to be more geographic variation in Brazil than a species-level differentiation. One morphotype is reported for the Caatinga municipalities (Assú, Cruz das Almas, Jaguaripe, Janaúba, Morada Nova, Mossoró, and Nova Soure) (Barton, 1988; Morrone, 2001) and one (Jacupiranga, Lages, Nova Veneza, Pelotas, Piracicaba, Presidente Prudente, and Vacaria) for the Paraense province (Morrone, 2001). These ecoregions probably were separated by vicariant events characterized by a Savanna corridor and mountains and valleys, suggesting the existence of geographic barriers among populations from the South and Southeast to the samples of the North of Minas Gerais and Northeast Brazil (Morrone, 2004; Pinto-da-Rocha, 2005).

Other studies have already reported the importance of environmental conditions in insect populations. For example, when Aphididae parasitoids and *Drosophila* are reared under different abiotic conditions, they show phenotypic variation. However, this variation does not represent the rise of new lineages and is probably caused by the phenotypic plasticity (Birdsall et al., 2000; Bubliy et al., 2007; Parreño et al., 2017). Geographic variation was also evident in morphometrics analysis in other tephritids fruit flies (Bonfim et al., 2011; Castañeda et al., 2015; Schutze et al., 2015b; Schutze et al., 2017).

Our results showed that the specimens identified as *A. dissimilis* in Brazil are *A. chiclayae*. Furthermore, this species is reported in Brazil for the first time, and consequently *A. dissimilis* is not actually present. Also, our morphometric results show considerable geographic variation among Brazilian populations of *A. chiclayae*. However, the phylogenetic inference based on COI sequences suggest that *A. chiclayae* is not a monophyletic group.

Acknowledgments

We thank the Departamento de Entomologia e Acarologia (ESALQ/USP) for the structural support for this study. We also thank CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for a graduate scholarship to the first author. We thank Dra. Clarice Diniz Alvarenga Corsato (Universidade Estadual de Montes Claros), Manoel Enéas de Carvalho Gonçalves (Agência de Defesa Agropecuária do Estado do Ceará) and Dr. Miguel

Souza Filho (Instituto Biológico de São Paulo) for sending specimens from Janaúba, Lages, Monte Alegre do Sul, Morada Nova, and Presidente Prudente. We thank Dr. Allen L. Norrbom (Systematic Entomology Laboratory – U.S. Department of Agriculture) and Dr. Roberto A. Zucchi (Escola Superior de Agricultura Luiz de Queiroz).

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3. NOTATED CHECKLIST AND ILLUSTRATED KEY TO THE *Anastrepha pseudoparallela* GROUP (DIPTERA: TEPHRITIDAE) RECORDED IN PASSION FRUIT (*Passiflora* spp.) IN BRAZIL

Abstract

The *Anastrepha pseudoparallela* group is widely distributed in the American tropics and subtropics and comprises 31 recognized species, commonly associated with fruits of *Passiflora* L. (Passifloraceae). In Brazil, only 11 species of this group are known, *Anastrepha amnis* Stone, *Anastrepha consobrina* (Loew), *Anastrepha chiclayae* Greene, *Anastrepha glochin* Uramoto & Zucchi, *Anastrepha limae* Stone, *Anastrepha lutzi* Lima, *Anastrepha martinsi* Uramoto & Zucchi, *Anastrepha nigripalpis* Hendel, *Anastrepha pseudoparallela* (Loew), *Anastrepha townsendi* Greene, and *Anastrepha xanthochaeta* Hendel. The host plants and distribution of these species are poorly known in Brazil, and an identification key to facilitating the recognition of these fruit flies is essential. We provide a checklist of the Brazilian *pseudoparallela* species group recorded breeding in passion fruit with notes on host plants and parasitoids and an illustrated key for these species.

Keywords: Fruit flies; Host plant; Taxonomy; Trypetinae; New world

3.1. Introduction

Tephritidae is a family of Diptera, mainly composed of phytophagous insects, known as fruit flies, with more than 500 genera and 5026 currently recognized species (Norrbom 2010; Savaris et al. 2016; Brown et al. 2018; Martinez et al. 2020; Norrbom et al. 2021; Norrbom, pers. comm), distributed in all geographic regions of the world, except polar and desert areas (Foote et al. 1993). Within Tephritidae, some of the most relevant fruit pest species belong to the genera *Anastrepha* Schiner, *Ceratitis* MacLeay, *Rhagoletis* Loew, *Dacus* Fabricius, *Bactrocera* Macquart, and *Zeugodacus* Hendel (Díaz-Fleischer and Aluja 1999; Mengual et al. 2017; Schutze et al. 2017).

The fruit flies of the genus *Anastrepha* have a geographic distribution concetrated in the Neotropical Region, and most of the species occur in Central and South America (Norrbom et al. 1999; Hernández-Ortiz 2007; Mengual et al. 2017; Norrbom et al. 2021). Currently, there are 328 described species (Norrbom et al., 2021), and in Brazil, 128 species are recorded (Zucchi and Moraes 2021).

The infrageneric classification of *Anastrepha* comprises 25 groups according to morphological and biological characters (Norrbom and Kim, 1988; Norrbom et al. 1999; 2012; 2018; Mengual et al., 2017). The *pseudoparallela* species group includes 31 species with triangular and usually finely and extensively serrated aculeus tips. Most species of this group breed in fruits of Passifloraceae (Norrbom and Kim, 1988; Norrbom et al. 1999; Norrbom et al. 2012; Tigrero and Norrbom, 2020; Norrbom et al. 2021; Rodriguez & Norrbom, 2021).

In Brazil, there are 11 species, of which seven attacking fruits of Passifloraceae: *A. chiclayae* Greene, *A. curitis* Stone, *A. consobrina* (Loew), *A. ethalea* (Walker), *A. lutzi* Lima, *A. pseudoparallela* (Loew), and *A. xanthochaeta* Hendel (Zucchi and Moraes, 2021). In this study, we provide an illustrated key for the Brazilian species of the *A. pseudoparallela* group that breed in passion fruit and information about the geographical distribution of these species based on literature records.

3.2. Material and Methods

The taxonomic key for the species of the *A. pseudoparallela* group is based on morphological characters related in the taxonomic literature (Greene, 1934, Lima, 1934; Stone, 1942; Zucchi, 1978; Norrbom et al., 2012). In addition, a morphological study was conducted to illustrate the characters used to identify these species (Zucchi, 2000; Norrbom et al., 2012).

The distribution map and table were elaborated based on an extensive search of previous records of these species in Brazil, using the Web of Science, Periodicos Capes, and Google Scholar. All papers found were checked about the presence of the host plant, locality, and information about parasitoids. Although the identification of *A. dissimilis* is probably incorrect, the distribution and host plants associated with this species were kept as originally published (Table 1).

Fruit of *Passiflora actinia* Hook (Colombo, state of Paraná), *Passiflora elegans* Mast. (Passo Fundo, state of Rio Grande do Sul), and *Passiflora edulis* Sims (Ipirá, state of Bahia) were collected and placed in plastic trays containing sand or vermiculite. Subsequently, the trays were covered and incubated at 25 ºC until the emergence of adults.

The morphological study of specimens deposited in the Entomological Collection of the Museum of Entomology Luiz de Queiroz (MELQ) was conducted with a stereomicroscope. The right wings of females were removed from the thorax using microforceps and submerged in Celossolve ($C_4H_{10}O_2$) for three days, then mounted on microscope slides using Euparal and dried for seven days at 25 ºC (Savaris et al., 2019).

The female abdomen was removed with microforceps and submerged in a hot 10% sodium hydroxide solution for 3–5 min. Subsequently, the cleared structure was transferred to a plate with water for further cleaning (removal of tissues), and the terminalia were transferred to glycerin for observation. Finally, the dissected abdomen was placed in a plastic microvial filled with glycerin and attached to the pin supporting the remainder of the insect (Savaris et al., 2019).

Aculeus measurements were made using a micrometer on a Leica Wild M10 stereomicroscope. The aculeus was photographed with a Nikon E2000 microscope with an SCMOS Digital Camera. The wing pattern of each species was photographed with a Leica DFC 450 camera mounted on an M205C stereomicroscope. Digital photographs were enhanced using Photoshop CS6 to correct the color and make minor corrections (e.g., remove debris).

The distribution map was made using Quantum GIS 1.18.24, and some adjustments were made using Corel Draw 2018 software. The distribution data of the specimens were obtained directly from labels of the samples deposited in MELQ or from the literature.

Voucher specimens are deposited in MELQ, Department of Entomology and Acarology, Luiz de Queiroz College of Agriculture (ESALQ), University of São Paulo (USP). The scientific names of the plant hosts are according to World Flora Online (2021).

3.3. Taxonomy and biology

Anastrepha pseudoparallela group

According to the infrageneric classification of *Anastrepha* proposed by Norrbom and Kim (1988), the *A. psedeudoparallela* species group comprised *A. pseudoparallela*, *Anastrepha townsendi* Greene, *A. consobrina*, and *Anastrepha amnis* Stone. Later, Norrbom (1997) increased the number of species by including *A. chiclayae*, *Anastrepha dissimilis* Stone, *Anastrepha pastranai* Blanchard, *Anastrepha munda* Schiner, *Anastrepha anduzei* Stone, *Anastrepha limae* Stone, *Anastrepha dryas* Stone, and *A. ethalea* (previously placed in the *chiclayae* group). In the same work, Norrbom (1997) also considered the species from the *A. pallidipenis* complex: *A. amnis*, *A. townsendi*, *Anastrepha pallida* Norrbom, *Anastrepha pallidipenis* Greene, and *Anastrepha velezi* Norrbom. Furthermore, Norrbom et al. (2000) included *Anastrepha mbrucuyae* Blanchard, *A. xanthochaeta*, and *A. velezi* arguing that these species also develop on passion fruits. Recently, *Anastrepha aliesae* Norrbom, *Anastrepha otongensis* Tigrero & Norrbom, and *Anastrepha arevaloi* Rodriguez & Norrbom were placed in this group (Tigrero & Norrbom, 2020; Norrbom et al. 2021; Rodriguez & Norrbom, 2021). Finally, other species such as *Anastrepha asetaocelata* Tigrero & Salas, *Anastrepha glochin* Uramoto & Zucchi, *Anastrepha lutzi* Lima, *Anastrepha martinsi* Uramoto & Zucchi, and *Anastrepha passiflorae* Greene were included (Norrbom et al. 2012).

Therefore, this species group currently comprises 31 species (Norrbom et al., 2012; Tigrero & Norrbom, 2020). However, only 11 species are recorded in Brazil: *A. amnis*, *A. consobrina*, *A. chiclayae*, *A. glochin*, *A. limae*, *A. lutzi*, *A. martinsi*, *A. nigripalpis*, *A. pseudoparallela*, *A. townsendi*, and *A. xanthochaeta* (Zucchi & Moraes, 2021; Araujo et al., in prep.). Furthermore, only *A. consobrina*, *A. chiclayae* (as *A. dissimilis*), *A. curitis*, *A. ethalea*, *A. lutzi*, *A. pseudoparallela*, and *A. xantochaeta* were reported in passion fruits. A synopsis of the Brazilian species of this group recorded in passion fruits is provided below.

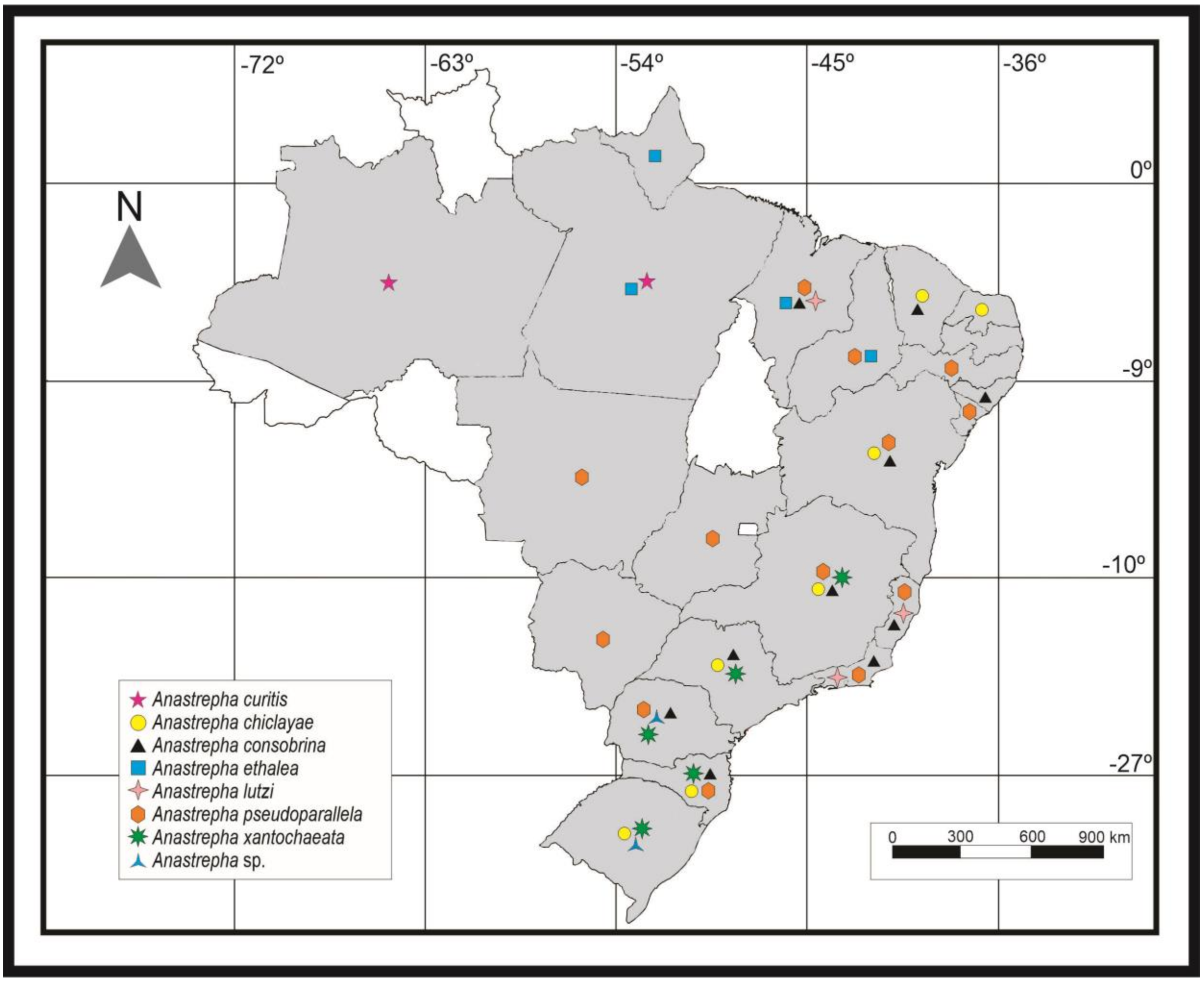

Fig. 1. Map of distribution of the eight species of the *A. pseudoparallela* group recorded in passion fruit in Brazil. The grey areas indicate the Brazilian states with records of at least one species of the group.

***Anastrepha chiclayae* Greene, 1934**

(Figs. 2A; 3C-D)

Diagnosis. Body yellow-brown. Scutum with median pale stripe. Subscutellum and mediotergite entirely yellow-orange. Macrosetae dark-brown. Wings length 6.6 to 7.6 mm; C- and S-bands separated or closely connected along vein R_{4+5} (Fig. 2A); V-band complete and separated from, or narrowly connected to S-band (Fig. 2A); Oviscape length 2.1 to 3.75 mm; aculeus length 2.0 to 3.25 mm; aculeus tip length 0.27 to 0.43 mm. Aculeus tip with fine serrations extending near the cloacal opening (Figs. 3C-D) (Stone, 1942; Norrbom et al., 2012).

Comments. In Brazil, this species used to be misidentified as *A. dissimilis.* In the original description, Stone (1942) illustrated only the aculeus tip of a paratype that differs from the aculeus tip of the holotype (see Norrbom et al., 2012). Recent molecular and morphological evidence suggests that specimens from Brazil identified as *A. dissimilis* are *A. chiclayae*. In concordance the paratype of *A. dissimilis* from Brazil (Bonito, Pernambuco) is more similar to *A. chiclayae* than the holotype of *A. dissimilis* from Plaisance, Haiti (Araujo et al. in prep.).

Biology. In Brazil, larvae of *A. chiclayae* (as *A. dissimilis*) were found feeding on fruits of *Passiflora caerulea* L., *P. elegans*, *P. edulis, Pouteria caimito* (Ruiz & Pav.) Radlk, *Psidium guajava* L., and *Ziziphus joazeiro* Mart.) (Ribeiro et al. 1997; Sá et al. 2008; Garcia and Norrbom, 2011; Marsaro Junior, 2014; Zucchi & Moraes, 2021; Araujo et al., in prep.).

Parasitoids. Unknown.

Distribution. Argentina (doubtful), Brazil (Alagoas, Amapá, Amazonas, Bahia, Ceará, Espírito Santo, Goiás, Maranhão, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Pará, Paraíba, Paraná, Pernambuco, Piauí, Rio Grande do Norte, Rio Grande do Sul, Santa Catarina, São Paulo) (Table 1; Fig. 1), Ecuador, and Peru (Norrbom et al. 1998; Ribeiro, 2005; Martins et al., 2012; Rabelo et al., 2013; Santos, 2014; Zucchi & Moraes, 2021; Araujo et al. in prep.).

***Anastrepha consobrina* (Loew, 1873)**

(Figs. 2B; 3E)

Diagnosis. Body yellow-brown. Scutum with median pale stripe. Subscutellum and mediotergite entirely yellow-orange. Macrosetae yellow-brown. Wing length 7.3 to 8.7 mm; C- and S-bands connected or separated along vein R_{4+5}; V-band complete and separated from S-band (Fig. 2B). Oviscape length 6.7 to 8.0 mm. Aculeus 6.2 to 7.5 mm; aculeus tip length 0.33

to 0.43 mm. Aculeus tip broadly widened basally and almost entirely finely serrate (Fig. 3E) (Stone, 1942; Norrbom et al., 2012).

Comments. Stone (1942) considered *Anastrepha zikani* Lima, 1934 a synonym of *A. consobrina*. Zucchi (1978) considered *A. zikani* a valid name and *A. consobrina* as *incertae sedis*. Currently, *A. consobrina* is a valid name, and *A. zikani* is a junior synonym (Norrbom et al., 1998).

Biology. *A. consobrina* larvae develop on *P. edulis, P. quadrangularis, Passiflora alata* Curtis, and *Passiflora edulis* f. *flavicarpa* Degener (Lima, 1934; Figueiredo et al. 2013; Zucchi and Moraes, 2021).

Parasitoids. *Doryctobracon areolatus* (Szépligeti) is the only parasitoid known for this species (Wharton & Marsh, 1978).

Distribution. Argentina and Brazil (Alagoas, Bahia, Ceará, Espírito Santo, Maranhão, Minas Gerais, Paraná, Rio de Janeiro, and São Paulo) (Table 1; Fig. 1) (Norrbom et al. 1998; Lampert et al. 2020; Zucchi and Moraes, 2021)

***Anastrepha curitis* Stone, 1942**

(Figs. 2C; 3A-B)

Diagnosis. Body orange-brown. Scutum without median pale stripe. Subscutellum and mediotergite and entirely yellow-orange. Macrosetae black. Wing length 7.7 to 10.9 mm; C- and S- bands separated; V- band complete and connected to or separated from S-band (Fig. 2C). Oviscape length 7.85 to 10 mm; Aculeus length 7.5 to 8.75 mm; aculeus tip length 0.45 to 0.52 mm; aculeus tip slender with fine serrations on distal two-thirds, with constriction before serrations (sometimes very slight) (Figs. 3A-B) (Stone, 1942; Zucchi, 1978; Norrbom et al. 2012).

Comments. This species is very similar to *A. pallidipennis* in the morphology of the aculeus tip. However, it differs in the distance of the spiracle of the oviscape from the base of the oviscape (Stone, 1942). Norrbom (1997) observed some variations in the morphology of the aculeus tip and suggested that this may not be a single species.

Biology. The immature stages of this species develop in fruits of *Passiflora nitida* Kunth, *Passiflora quadrangularis* L. (= *Passiflora grandiflora*), and *Passiflora* sp. (Norrbom et al. 1997; Dutra et al. 2018; Govaerts et al. 2021).

Parasitoids. Unknown.

Distribution. Brazil (Amazonas and Pará) (Table 1; Fig. 1), Bolivia, Colombia and Peru (Norrbom et al. 1998; Quisberth Ramos et al., 2021; Zucchi & Moraes, 2021).

Anastrepha ethalea **(Walker, 1849)**
(Figs. 2D; 3F)

Diagnosis. Body yellow-brown. Scutum without pale medial stripe. Subscutellum and mediotergite entirely yellow-orange. Macrosetae orange-brown. Wing length 7.9 to 8.5 mm; C- and S- bands connected along R_{4+5} (Fig. 2D); V-band complete and separated from S-band (Fig. 2D). Oviscape length 2.45 to 3.0 mm; aculeus length 2.37 to 2.39 mm; aculeus tip length 0.25 to 0.4 mm. Aculeus tip basally widened and with many serrations extending beyond distal margin of cloacal opening (Fig. 3F) (Stone, 1942; Zucchi, 1978; Norrbom et al., 2012).
Comments. This species is very similar to *A. limae* in the shape of the aculeus tip, but it differs in the color of the macrosetae and by having a shorter oviscape (Stone, 1942).
Biology. The larvae of *A. ethalea* develop on fruits of *Passiflora laurifolia* L. and *P. quadrangularis* (Stone, 1942).
Parasitoids. Unknown.
Distribution. Brazil (Maranhão, Pará, Piauí, and Roraima) (Table 1; Fig. 1), French Guiana, Guyana, Suriname, and Trinidad (Norrbom et al., 1998; Zucchi and Moraes, 2021).

Anastrepha lutzi **Lima, 1934**
(Figs. 2E; 3G)

Diagnosis. Body yellow-brown. Scutum yellow without medial and lateral pale stripes. Subscutellum and mediotergite entirely yellow-orange. Macrosetae dark-brown. Wing length 7.25 to 9.9 mm; C- and S-bands connected along R_{4+5} (Fig. 3E); V-band complete and separated from S-band (Fig. 2E). Oviscape length 2.25 to 2.7 mm. Aculeus length 2.0 to 2.22 mm; aculeus tip length 0.24 to 0.33 mm. Aculeus tip basally widened and with many serrations extending beyond distal margin of cloacal opening (Fig 3G) (Stone, 1942; Zucchi, 1978; Norrbom et al., 2012).
Comments. The specimens described by Lima (1934) have considerable variation in the wing pattern. In part of the specimens analyzed, the C- and V-band were disconnected, whereas others had these bands narrowly connected (Stone, 1942).
Biology. Larvae of *A. lutzi* develop on fruits of *Passiflora* sp. (Lima, 1934).

Parasitoids. Unknown.

Distribution. Brazil (Espírito Santo, Maranhão, and Rio de Janeiro) and Argentina (Table 1; Fig. 1) (Norrbom et al., 1998; Zucchi and Moraes, 2021).

***Anastrepha pseudoparallela* (Loew, 1873)**

(Figs. 2G; 3H)

Diagnosis. Body yellow-brown. Scutum with medial and lateral pale stripes. Subscutellum and mediotergite entirely yellow-orange. Macrosetae black. Wing length 8.0 to 9.2 mm; C- and S-bands connected along R_{4+5}; V-band complete and separated from S-band (Fig. 2F). Oviscape length 2.95 to 3.65; aculeus length 2.5 to 3.5 mm; aculeus tip length 0.35 to 0.55 mm. Aculeus tip with serrations extending in more of the apical half. In addition, the serrations begin in dorsal side of the aculeus (Fig. 3H) (Stone, 1942; Zucchi, 1978; Norrbom et al., 2012).

Comments. Loew (1873) described this species in the genus *Trypeta* based on specimens from Brazil. It was transferred to *Anastrepha* by Bezzi (1909) (Stone, 1942). According to Stone (1942), the specimens recognized by Greene (1934) as *A. pseudoparallela* are not this species.

Biology. Larvae of *A. pseudoparallela* develop on fruits of *P. alata*, *P. elegans*, *P. quadrangularis*, *P. guajava*, *Mangifera indica* L. (Zucchi, 1978; Lima, 1934; Malavasi and Zucchi, 1980; Aguiar-Menezes et al., 2004; Leal, 2008; Uramoto et al., 2004; Marsaro Junior, 2014; Almeida et al., 2019; Marinho et al., 2021).

Parasitoids. *Doryctobracon fluminensis* (Costa Lima), *Doryctobracon maculatus* Marinho, *Lopheucoila anastrephae* (Rohwer), *Odontosema anastrephae* Borgmeier (Paranhos et al., 2019; Marinho et al., 2021). The record of *Ganaspis* sp. parasitizing *A. pseudoparallela* (Aguiar-Menezes et al., 2004) is uncertain, as species of this genus parasitize drosophilids (Jorge A. Guimarães, pers. comm.).

Distribution. Argentina, Brazil (Bahia, Espírito Santo, Goiás, Maranhão, Mato Grosso do Sul, Minas Gerais, Paraná, Pernambuco, Piauí, Rio de Janeiro, Santa Catarina, São Paulo, and Sergipe) (Table 1; Fig. 1) Colombia (questionable), Ecuador (questionable) and Peru (questionable) (Norrbom et al. 1998; Zucchi and Moraes, 2021).

***Anastrepha xanthochaeta* Hendel, 1914**

(Figs. 2H; 3J)

Diagnosis. Body yellow-brown. Scutum without medial pale stripe. Subscutellum and mediotergite entirely yellow-orange. Macrosetae yellow-orange. Wing length 7.5 to 8.1 mm; C- and S-band separated (Fig. 2H); V-band complete and separated from S-band (Fig. 2H). Oviscape length 2.3 to 2.7 mm; aculeus length 2.2 to 2.8 mm; aculeus tip basally widened and with fine serrations extending in more than one-third of the aculeus tip. The serrations begin in dorsal side of the aculeus (Fig. 3J) (Stone, 1942; Zucchi, 1978; Norrbom et al., 2012).

Comments. This species was described by Hendel (1914) based on specimens from Rio Grande do Sul and included in the *pseudoparallela* group by Norrbom et al. (2000). Like other species of this group, their larvae develop in fruits of *Passiflora*, although the aculeus tip morphology differs from the general pattern of the group.

Biology. The larvae of *A. xanthochaeta* develop on fruits of *Passiflora* sp. (Zucchi, 1978; Malavasi and Zucchi, 1980).

Parasitoids. Unknown.

Distribution. Brazil (Minas Gerais, Paraná, Rio Grande do Sul, Santa Catarina, and São Paulo) (Table 1; Fig. 1), and Paraguay (Norrbom et al. 1998; Zucchi and Moraes, 2021).

Unidentified species

Anastrepha sp. Colombo and Curitiba – Paraná; Passo Fundo – Rio Grande do Sul

(Figs. 2G; 3I)

This species was found infesting fruit of *Passiflora actinia* Hook (in Colombo, Paraná) and *P. elegans* in Passo Fundo, Rio Grande do Sul (Table 1; Fig. 1). The aculeus tip morphology resembles *A. ethalea*, mainly because the serrations of both species extend to beyond the cloacal opening. However, they differ because the serrations of *A. ethalea* are more prominent and more conspicuous, while in this species, the teeth are tiny (Figs. 3F; 3I).

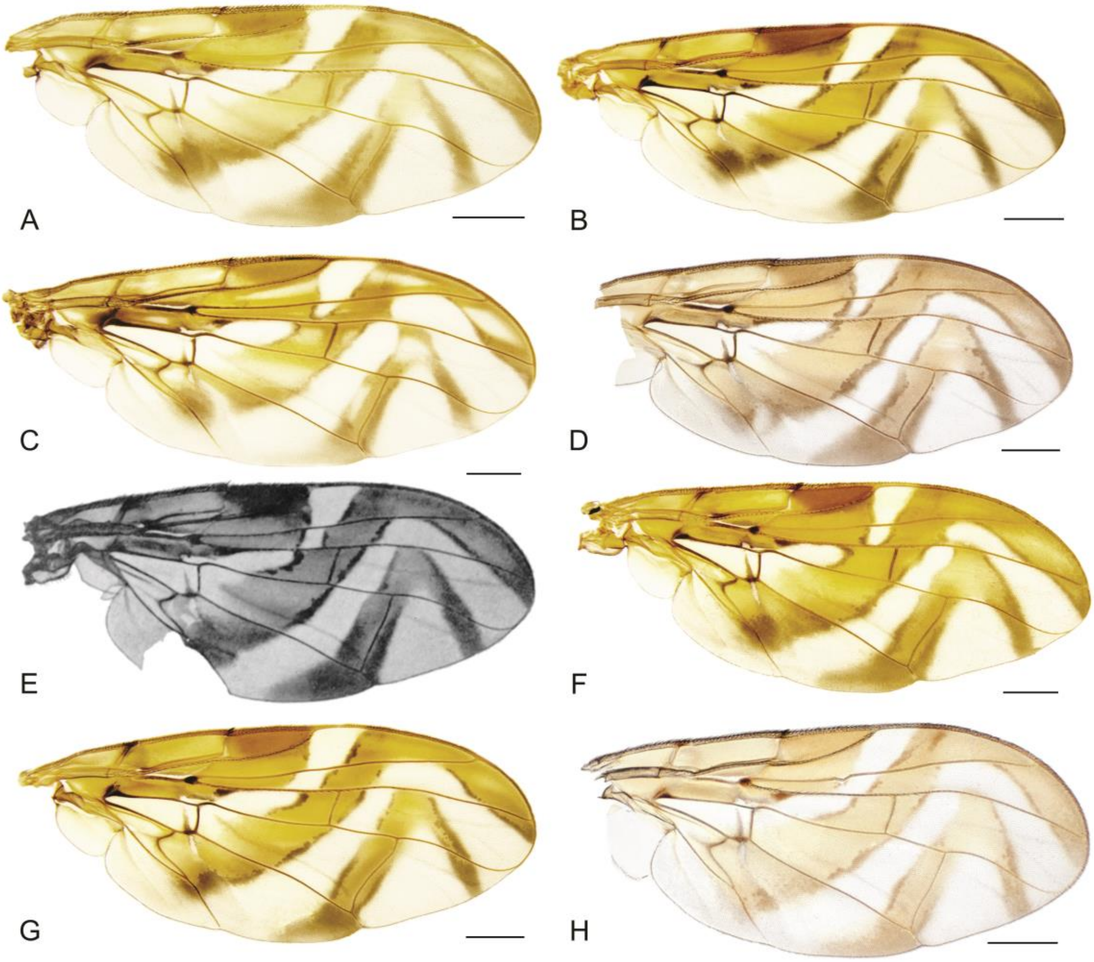

Fig. 2. Wing pattern of the Brazilian species of the *pseudoparallela* group recorded in passion fruit. A: *A. chiclayae*; B: *A. consobrina*; C: *A. curitis*; D: *A. ethalea* (from Norrbom et al., 2012); E: *A. lutzi* (from Lima, 1934); F: *A. pseudoparallela*; G: *Anastrepha* sp.; H: *A. xanthochaeta* (from Norrbom et al., 2012). Scale bars: 1 mm.

Table 1. Distribution of species of the *A. pseudoparallela* group of fruits of *Passiflora* in Brazil.

Species	States	Localities	References
Anastrepha chiclayae	Bahia	Cruz das Almas	Araujo et al. in prep.
		Jaguaripe	
		Nova Soure	
	Ceará	Morada Nova	
	Minas Gerais	Janaúba	
	Rio Grande do Norte	Assú	
		Mossoró	
	Rio Grande do Sul	Vacaria	
	Santa Catarina	Lages	
		Nova Veneza	
	São Paulo	Jacupiranga	
		Monte Alegre do Sul	
		Piracicaba	
		Presidente Prudente	

Anastrepha curitis	Amazonas	Manaus	Norrbom et al., 1997 Dutra et al., 2013
		Presidente Figueiredo	Dutra et al., 2013
	Pará	Belém	Norrbom et al., 1997
		Benevides	Norrbom et al., 1997
Anastrepha consobrina	Bahia	Ipirá*	This publication
		Wenceslau Guimarães	Melo et al., 2016
	Ceará	Jaguarauna	Araujo et al., 2009
	Espírito Santo	São Roque do Canaã	Madalon et al., 2017
	Minas Gerais	Viçosa	Pirovani et al., 2020
	Maranhão	Caxias Mirador	Holanda, 2012
	Paraná	São José dos Pinhais	Lampert et al., 2020
	Rio de Janeiro	Estrela Itatiaia	Lima, 1934
	São Paulo	Bertioga	Figueiredo et al., 2013
Anastrepha dissimilis	Alagoas	Arapiraca	Santos, 2014

	Palmeira dos Índios	
Amapá	Oiapoque	Trindade & Uchoa, 2011
Amazonas	Benjamin Constant	Ribeiro, 2015
Bahia	Anagé	Sá et al., 2008
	Brumado	Nascimento & Carvalho, 2000
	Caraíbas	Sá et al., 2012
	Cruz das Almas	Zucchi, 1978
	Juazeiro	Haji & Miranda, 2000
	Muritiba	Zucchi, 1978
	Nova Soure	Nascimento & Carvalho, 2000
Ceará	Ubajara	Sales & Gonçalves, 2000
Espírito Santo	Linhares	Martins et al., 2012
Goiás	Carmo do Rio Verde	Rabelo et al., 2013
Maranhão	Caxias	Holanda, 2012

	Mirador	Holanda, 2013
Mato Grosso	Utiariti	Zucchi, 1978
Mato Grosso do Sul	Anástacio	Uchôa-Fernandes et al., 2003
	Corumbá	Minzão & Uchôa-Fernandes, 2008
	Dourados	Oliveira et al., 2019
	Terrenos	Uchôa-Fernandes et al., 2003
Minas Gerais	Bambuí	Duarte et al., 2016
	Itacarambi	Alvarenga et al., 2000
	Jaíba	Querino et al., 2014; Camargos et al., 2015
	Janaúba	Alvarenga et al., 2000
	Matias Cardoso	Querino et al., 2014
	Nova Porteirinha	Alvarenga et al., 2000
Pará	Belém	Zucchi, 1978
Paraíba	Nova Floresta	Alves et al., 2019
Paraná	Lapa	Monteiro et al., 2018
	Porto Amazonas	

		Pinhais	
	Pernambuco	Bonito	Stone, 1942
		Petrolina	Haji & Miranda, 2000
	Piauí	Teresina	Sousa et al., 2017
	Rio Grande do Norte	Assú	Araujo et al., 2005
		Cruzeta	Araujo et al., 2013
		Mossoró	Araujo et al., 2005
	Rio Grande do Sul	Montenegro	Silva et al., 2006
		Passo Fundo	Marsaro Junior, 2014
		Porto Alegre	Zucchi, 1978
	Santa Catarina	Anchieta	Garcia et al., 2011
		Águas de Chapecó	Chiaradia et al., 2004
		Chapecó	
		Cunha Porã	Garcia et al., 2011

		Vale do Rio do Peixe	Nora et al., 2000
	São Paulo	Barueri	Zucchi, 1978
		Jaboticabal	Ribeiro et al., 1997
		Monte Alegre do Sul	Lopes et al., 2015
		Piracicaba	Zucchi, 1978
Anastrepha ethalea	Maranhão	Caxias	Holanda, 2012
	Pará	-	Stone, 1942
	Piauí	Teresina	Menezes et al., 2000
	Roraima	Boa Vista	Marsaro Junior et al., 2013
Anastrepha lutzi	Espírito Santo	Linhares	Uramoto, 2007
	Maranhão	Caxias Mirador	Holanda, 2012
	Rio de Janeiro	Manguinhos	Lima, 1934
Anastrepha pseudoparallela	Bahia	Camamu	Santos et al., 2010

	Cruz das Almas	Zucchi, 1978
	Itaberaba	
	Livramento de Nossa Senhora	
	Teixeira de Freitas	Aguiar, 2012
	Uruçuca	
	Vitória da Conquista	
Espírito Santo	North of Espírito Santo	Martins et al., 2012
	-	Zucchi & Moraes, 2021
Goiás	Distritio Federal	Icuma et al., 2001
	Goiânia	Veloso et al., 2000
Mato Grosso	Chapada dos Guimarães	
	Jaciara	Pontes, 2006
	Ouro Branco	
Mato Grosso do Sul	Dourados	Canesin & Uchôa-Fernades, 2007
	Serra da Bodoquena	Almeida et al., 2019
Minas Gerais	Belo Horizonte	Lima, 1934
	Viçosa	Pirovani et al., 2020

Paraná	Araucária	Fehn, 1981
	Campo Largo	
	Irati	
	Mandirituba	
	Ponta Grossa	Husch et al., 2012
Piauí	Teresina	Menezes et al., 2000; Sousa et al., 2017
Rio de Janeiro	Campos dos Goytacazes	Leal, 2008
	Rio de Janeiro	Lima, 1934
	São Francisco de Itabapoana	Leal, 2008
	Seropédica	Aguiar-Menezes et al., 2004
Rio Grande do Sul	Montenegro	Silva et al., 2006
	Pareci Novo	
	Quatro Irmãos	Marsaro Junior, 2014
	-	Zucchi & Moraes, 2021
Santa Catarina	Iraceminha	Alberti et al., 2009; 2012
	Vale do Rio do Peixo	Nora et al., 2000
	-	Silva et al., 1968

	São Paulo	Barueri	Zucchi, 1978
		Carapicuiba	
		Monte Alegre do Sul	Lemos et al., 2015
		Monte Alto	
		Osvaldo Cruz	Malavasi & Zucchi, 1980
		Perus	
		Piracicaba	Zucchi, 1978; Uramoto et al., 2008; Amaral et al., 2017; Araujo et al., 2018; Marinho et al., 2021
		São Paulo	Lima, 1934; Zucchi, 1978
		São Roque	
	Sergipe	São Cristovão	Barreto et al., 2020
Anastrepha xantochaeta	Minas Gerais	-	Zucchi & Moraes, 2021
	Paraná	-	Garcia, 2003
	Rio Grande do Sul	-	Norrbom et al., 1999
	Santa Catarina	Iraceminha	Alberti et al., 2009

		Nova Teutônia	Zucchi, 1978
	São Paulo	Ibiuna	Zucchi, 1978; Malavasi & Zucchi, 1980
Anastrepha sp.	Paraná	Colombo** Curitiba	This publication
	Rio Grande do Sul	Passo Fundo***	This publication

*Collected in *P. edulis*; **Collected in *P. actinia;* ***Collected in *P. elegans.*

3.4. Illustrated key to the species of *pseudoparallela* group in passion fruit in Brazil

1. Aculeus tip with serrated part as wider as or wider than long (Fig. 3J)..........*A. xanthochaeta*

1’. Aculeus tip with serrated part longer than wide...2

2(1’). Aculeus with strong constriction before serrated part (Fig. 3G).............................*A. lutzi*

2’. Aculeus with lateral margins parallel or without constriction before serrated part...............3

3(2’). Aculeus tip with serrations part extending beyond base of the aculeus tip..4

3’. Serrated part not extending beyond base of the aculeus tip..5

4(3). Aculeus tip with conspicuous teeth (Fig. 3F)...*A. ethalea*

4’. Aculeus tip with tiny teeth (Fig. 3I)..*Anastrepha* sp.

5(3’). Basal serrations of the aculeus tip extending onto dorsal side (Fig. 3H)..*A. pseudoparallela*

5’. Basal serrations of the aculeus tip not extending onto dorsal side.......................................6

6(5’). Aculeus length 7.85-10 mm; serrated part with conspicuous teeth (Fig. 3D-E)...*A. curitis*

6’. Aculeus length less than 7.8 mm; serrated part with tiny teeth...7

7(6’). Aculeus length 6.2-7.5 mm (Fig. 3C)...*A. consobrina*

7’. Aculeus length 2.0 to 3.25 mm (Fig. 3A-B)...*A. chiclayae*

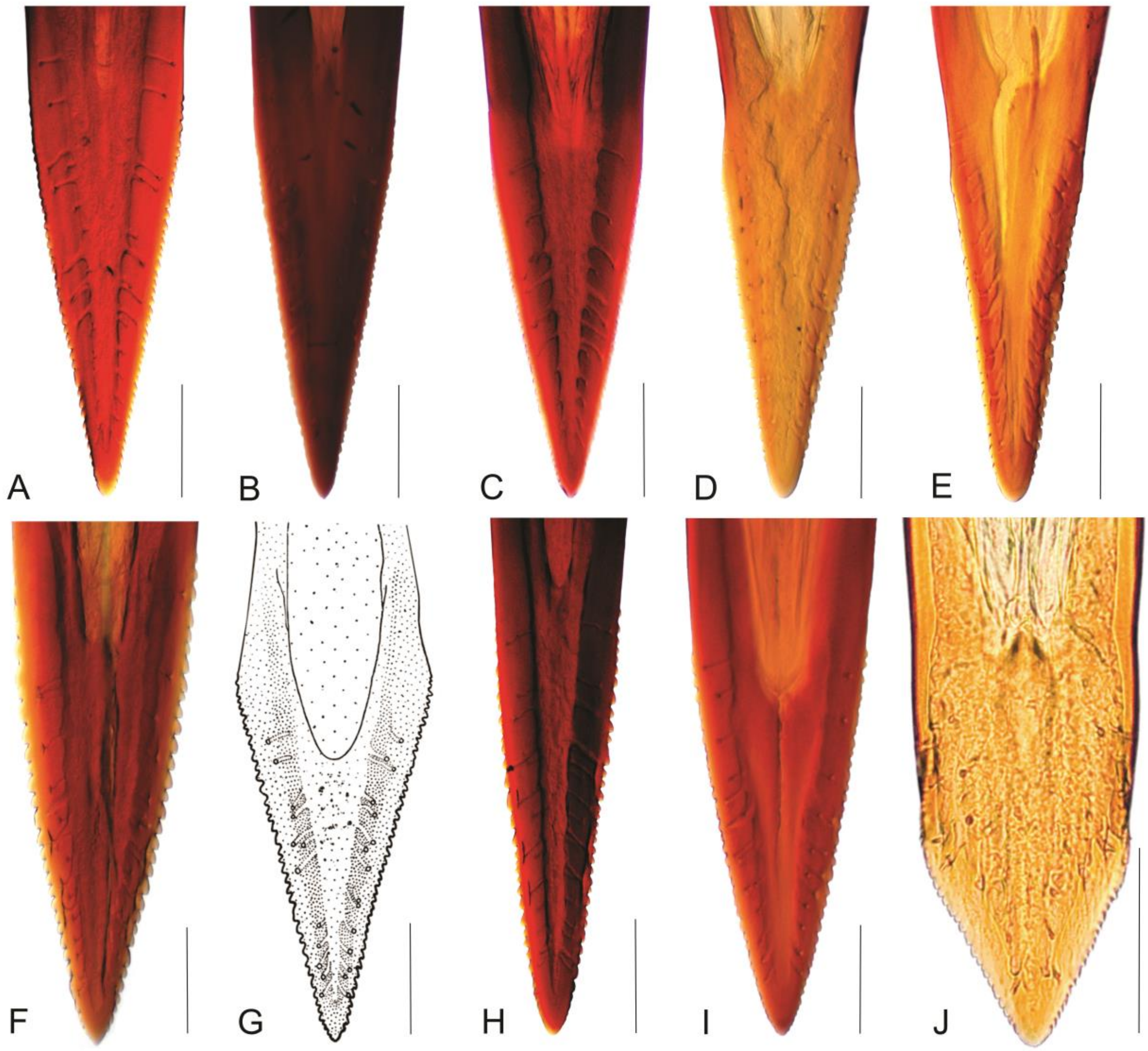

Fig. 3. Species of the *pseudoparallela* group recorded in passion fruit in Brazil, ventral view of the aculeus tip. A-B: *A. chiclayae*; C- *A. consobrina*; D-E: *A. curitis*; F: *A. ethalea*; G: *A. lutzi* (from Lima, 1934); H: *A. pseudoparallela*; I: *Anasrepha* sp.; J: *A. xanthochaeta* (from Norrbom et al., 2012). Scale bars = 0.1 mm.

Acknowledgments

We thank Artur Fiais and Jennifer Andrade (Universidade Federal da Bahia) for the support in the collections of *A. consobrina* in the municipality of Ipirá – BA, and Élinton L. Rezende for collecting samples in Passo Fundo, RS. We thank Departamento de Entomologia e Acarologia (ESALQ/USP) for supporting this study, and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for conceding the fellowship.

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